



Models to Study Intermediate Filament Dynamics and Functions

Elin Torvaldson

Cell Biology, Faculty of Science and Engineering
Åbo Akademi University
Turku Centre for Biotechnology,
University of Turku and Åbo Akademi University

2015



Models to Study Intermediate Filament Dynamics and Functions

Elin Torvaldson

Cell Biology, Faculty of Science and Engineering
Åbo Akademi University
Turku Centre for Biotechnology,
University of Turku and Åbo Akademi University

2015

From Cell Biology, Faculty of Science and Engineering, Åbo Akademi University,
and Turku Centre for Biotechnology, University of Turku and Åbo Akademi
University

Supervised by

Professor John Eriksson

Cell Biology, Faculty of Science and Engineering,
Åbo Akademi University, and
Turku Centre for Biotechnology,
University of Turku and Åbo Akademi University
Turku, Finland

Reviewed by

Adjunct Professor Jeroen Pouwels

Turku Centre for Biotechnology,
University of Turku and Åbo Akademi University
Turku, Finland

Professor Yosef Gruenbaum

The Alexander Silberman Institute of Life Sciences
The Edmond Safra Campus
The Hebrew University of Jerusalem
Jerusalem, Israel

Opponent

Professor Roy Quinlan

School of Biological and Biomedical Sciences,
Durham University
Durham, United Kingdom

ISBN 978-952-12-3330-2

Painosalama Oy – Turku, Finland 2015

River, river have mercy
Take me down to the sea
For if I perish on these rocks
My love no more I'll see
-Philip Chevron

Table of Contents

List of Original Publications.....	6
Abbreviations	8
Svensk sammanfattning.....	10
Abstract	11
1 Introduction.....	12
2 Review of the Literature	14
2.1 Intermediate filaments.....	14
2.1.1 Cellular functions of IFs.....	16
2.1.2 Dynamics of IFs.....	20
2.1.3 Signalling properties of IFs	22
2.2 Lamin A/C.....	23
2.2.1 Splicing and processing of lamin A	23
2.2.2 Lamin PTMs	25
2.2.3 Structural and mechanical functions of lamin A/C.....	27
2.2.4 Nucleoplasmic lamin A	28
2.2.5 Laminopathies - peculiar diseases that give insights into lamin functions.....	29
2.3 Nestin	31
2.3.1 Nestin structure and polymerisation.....	31
2.3.2 Regulation of nestin gene and protein	31
2.3.3 Functions of nestin.....	34
2.3.4 Nestin in muscle.....	35
2.4 Models to study IFs	36
2.4.1 Transgenic mice as models to study IFs.....	36
2.4.2 Invertebrate models to study IFs.....	41
2.5 Cdk5 – an IF interacting kinase.....	42
2.6 Satellite cells and muscle regeneration	45
2.6.1 Models to study muscle differentiation and regeneration.....	46

3 Aims.....	48
4 Materials and Methods	49
4.1 Standard methods and Cell lines used.....	49
4.2 GFP-tagged lamin constructs	49
4.3 Primary myoblast and myofiber cultures.....	50
4.4 Additional methods	50
5 Results and Discussion.....	52
5.1 Phosphorylation determines lamin A/C localization and mobility (I)	52
5.2 The physiological relevance of lamin A/C phosphorylation	54
5.3 Cdk5 phosphorylates lamin A/C (unpublished)	57
5.4 Depletion of nestin has no obvious effect on muscle stem cell behaviour (II)	60
5.5 Nestin influences body composition (II)	61
5.6 The role of nestin in muscle differentiation and regeneration (II).....	62
5.7 Nestin affects male characteristics (unpublished)	63
5.8 Expression of human vimentin in Drosophila (III).....	66
6 Concluding Remarks.....	68
7 Acknowledgments	70
8 References	72
Original Publications	89

List of Original Publications

This thesis is based upon the following original publications and manuscripts. They are referred to in the text by their roman numerals. The original publications have been reproduced with permission of the copyright holders. In addition, this thesis includes unpublished data.

- I. Kochin V.*, Shimi T.*, **Torvaldson E.**, Adam S.A., Goldman A., Pack C.-G., Melo-Cardenas J., Imanishi S.Y., Goldman R.D. and Eriksson J.E. (2014). Interphase phosphorylation of lamin A. *J. Cell Sci.* 127, 2683–2696.
- II. Lindqvist J.*, **Torvaldson E.***, Gullmets J., Karvonen H., Taimen P. and Eriksson J.E. Nestin is required for skeletal muscle homeostasis and regeneration. *Manuscript*.
- III. Gullmets J., **Torvaldson E.**, Lindqvist J., Imanish S.Y., Taimen P., Meinander A. and Eriksson J.E. Transgenically expressed human vimentin forms a network in epithelial *Drosophila* tissues. *Manuscript*.

*Equal contribution

Related publications

Virtakoivu R., Mai A., Mattila E., De Franceschi N., Imanishi S.Y., Corthals G., Kaukonen R., Saari M., Cheng F., **Torvaldson E.**, et al. (2015). Vimentin-ERK signaling uncouples Slug gene regulatory function. *Cancer Res.* 75, 2349–2362

Lindqvist J., Imanishi S.Y.*, **Torvaldson E.***, Malinen M., Remes M., Örn F., Palvimo J., Eriksson J.E. (2015). Cyclin-dependent kinase 5 acts as a critical determinant of AKT-dependent proliferation and regulates differential gene expression by the androgen receptor in prostate cancer cells. *Mol. Biol. Cell* 26, 1971–1984.

Ferraris S.E., Isoniemi K., **Torvaldson E.**, Anckar J., Westermarck J., and Eriksson J.E. (2012). Nucleolar AATF regulates c-Jun-mediated apoptosis. *Mol Biol Cell* 23, 4323-4332.

List of Original Publications

Pallari H-M., Lindqvist J., **Torvaldson E.**, Ferraris S., He T., Sahlgren C., Eriksson J.E. (2011). Nestin as a regulator of Cdk5 in differentiating myoblasts. *Mol Biol Cell* 22, 1539-1549.

Peuhu E., Rivero-Müller A., Stykki H., **Torvaldson E.**, Holmbom T., Unkila M., Eklund P., Sjöholm R., Eriksson J.E. (2010). Inhibition of Akt signalling by the ligan matairesinol sensitizes prostate cancer cells to TRAIL- induced apoptosis. *Oncogene* 29, 898–908.

Review articles the author has contributed to that are not included in this thesis:

Torvaldson, E., Kochin, V. and Eriksson, J.E. (2015). Phosphorylation of lamins determine their structural properties and signaling functions. *Nucl. Austin Tex* 6, 166–171.

Hyder, C. L., Isoniemi, K.O.*, **Torvaldson, E.***, and Eriksson, J.E. (2011). Insights into intermediate filament regulation from development to ageing. *J. Cell Sci.* 124, 1363–1372.

Abbreviations

Cdk	Cyclin dependent kinase
CHX	Cycloheximide
CLN	Centrally located nuclei
CNS	Central nervous system
DNA	Deoxyribonucleic acid
EBS	Epidermolysis bullosa simplex
EDL	Extensor digitorum longus
EDMD	Emery-Dreifuss muscular dystrophy
ERK	Extracellular-signal-regulated kinase
FCS	fluorescence correlation spectroscopy
FRAP	fluorescence recovery after photobleaching
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
HGPS	Hutchinson-Gilford progeria syndrome
IF	Intermediate filament
Ig	Immunoglobulin
JNK	c-Jun N-terminal kinase
K	Keratin
KASH	Klarsicht, ANC-1, Syne Homology
LA	Lamin A
Lap2 α	Lamina-associated polypeptide 2 isoform alpha
LINC	Linker of Nucleoskeleton and Cytoskeleton
MAPK	Mitogen-activated protein kinases
miRNA	micro RNA
MRI	Magnetic resonance imaging
MTJ	Myotendinous junction
MyoD	Myoblast determination protein
NF	Neurofilament
NMJ	Neuromuscular junction
Pax	Paired box protein
PNS	Peripheral nervous system
PTM	Post-translational modification
RNA	Ribonucleic acid
siRNA	Small interfering RNA
SREBP1	Sterol response element binding protein 1

Abbreviations

SUMO	Small Ubiquitin-like Modifier
TA	Tibialis anterior
ULF	Unit length filament
WT	Wild type
YFP	Yellow fluorescent protein

Svensk sammanfattning

Intermediärfilament (IF) är en del av cellens stödstruktur – cellskelettet. IF bildar nätverk av repliknande strukturer som sträcker sig genom cellen. Dessa filament är uppbyggda av proteinfamiljen IF-protein, protein som kännetecknas av förmågan att bilda filament med sig själva eller andra IF-protein. Förutom att de ger cellen stöd har IF-proteiner även andra uppgifter såsom att t.ex. delta i cellsignalering och styra hur gener uttrycks. Vi känner till ungefär 70 olika IF-protein hos människan. Mutationer i de gener som kodar för IF-protein har visats orsaka flertalet obotliga sjukdomar.

IF-proteinet lamin A bygger upp ett nätverk innanför cellens kärnmembran. Detta nätverk fungerar som ett fysiskt skydd för kärninnehållet genom att ta upp stötar och förhindra att kärnan deformeras. Dessutom har lamin A även andra funktioner; t.ex. interagerar det direkt med kromosomerna och styr deras placering samt hur den genetiska koden läses. I min avhandling undersöker jag hur modifieringar av lamin A påverkar dess rörelse och lokalisering i cellkärnan.

Under musklernas utveckling och regeneration, d.v.s. läkning efter en skada, uttrycks IF-proteinet nestin. I mitt arbete har jag undersökt hur frånvaro av nestin påverkar muskelcellers differentiering och hur muskler regenererar. Resultaten visar att även om nestin inte är nödvändigt för att muskelstamceller ska omvandlas till mogna muskelceller så leder frånvaro av nestin till en rubbad muskelbalans. Möss som saknar nestin har mindre muskelmassa än motsvarande kontrollmöss. Vi visar även att i avsaknad av nestin undergår musklerna spontan regenerering utan att ha skadats, och efter en skada läker musklerna långsammare. Denna studie visar för första gången att nestin har en viktig roll i musklerna och att nestin är speciellt betydelsefullt för att skydda muskler mot stress samt för att reglera läkningsprocessen efter en skada. Jag presenterar även resultat som visar att avsaknad av nestin leder till ökad variation i testikelstorlek. Tillsammans styrker dessa resultat teorin att en av IF-proteinernas funktioner är att skydda celler mot stress.

IF-proteiner har egenskaper som gör att de är svåra att studera i däggdjursmodeller, samtidigt som cellkulturer inte kan användas för att studera alla aspekter av IF-funktioner. Vi har därför utvecklat en bananfluga som uttrycker det humana IF-proteinet vimentin, en modell som kan användas i fortsatt forskning och därmed minskar behovet av musmodeller.

Abstract

Intermediate filaments are part of the cytoskeleton and nucleoskeleton; they provide cells with structure and have important roles in cell signalling. The IFs are a large protein family with more than 70 members; each tightly regulated and expressed in a cell type-specific manner. Although the IFs have been known and studied for decades, our knowledge about their specific functions is still limited, despite the fact that mutations in IF genes cause numerous severe human diseases.

In this work, three IF proteins are examined more closely; the nuclear lamin A/C and the cytoplasmic nestin and vimentin. In particular the regulation of lamin A/C dynamics, the role of nestin in muscle and body homeostasis as well as the functions and evolutionary aspects of vimentin are investigated. Together this data highlights some less well understood functions of these IFs.

We used mass-spectrometry to identify inter-phase specific phosphorylation sites on lamin A. With the use of genetically engineered lamin A protein in combination with high resolution microscopy and biochemical methods we discovered novel roles for this phosphorylation in regulation of lamin dynamics. More specifically, our data suggests that the phosphorylation of certain amino acids in lamin A determines the localization and dynamics of the protein. In addition, we present results demonstrating that lamin A regulates Cdk5-activity.

In the second study we use mice lacking nestin to gain more knowledge of this seldom studied protein. Our results show that nestin is essential for muscle regeneration; mice lacking nestin recover more slowly from muscle injury and show signs of spontaneous muscle regeneration, indicating that their muscles are more sensitive to stresses and injury. The absence of nestin also leads to decreased over-all muscle mass and slower body growth. Furthermore, nestin has a role in controlling testicle homeostasis as nestin^{-/-} male mice show a greater variation in testicle size.

The common fruit fly *Drosophila melanogaster* lacks cytoplasmic IFs as most insects do. By creating a fly that expresses human vimentin we establish a new research platform for vimentin studies, as well as provide a new tool for the studies of IF evolution.

1 Introduction

Cells are remarkable. Although all the cells in multi-cellular organisms originate from a single cell and contain the same genetic information, the cells are incredibly different in appearance, function, and life-span. The neuronal cells, for example, are renewed at a very low rate with a life span of decades, whereas the cells of the intestinal epithelium are changed at a high rate, living only for days. These differences are a result of the process of differentiation, where a cell transforms from an un-specialised state to a specific specialised cell-type. Differentiation, and indeed all other processes in a cell, is dependent on the cell receiving and responding to signals from the outside, as well as from the inside of the cell itself. Such signalling requires the cells to have a certain degree of structure in order to be efficient.

To be able to keep its shape, to move, transport molecules to different compartments and keep its organelles organised, a cell is dependent on the cytoskeleton. The cytoskeleton is an internal cellular scaffold consisting of protein filaments. These filaments are divided into three groups; microfilaments, microtubules and intermediate filaments (IFs), which all form distinct but interacting networks throughout the cell.

The IFs, which are the subject of study in this work, constitute a diverse protein family with both structural and signalling functions in the cell. Although mutations of IFs have been proven to cause many diseases, no treatments for these often devastating syndromes have been developed. This is partly due to our lack of knowledge of the basic functions of IFs and how these proteins are regulated. The expansion of the basic research regarding IFs can, thus, have a major impact on development of novel therapies.

In this thesis, the goal has been to examine and employ three different models designed to unravel IF dynamics and functions. Phospho-proteomics in combination with site-directed mutagenesis was employed to investigate how phosphorylation affects lamin A, and we have been able to show that the movement and localization of lamin A both are dependent on the protein's phosphorylation status. The second project in this thesis looks into the roles of nestin in vivo using nestin knockout mice. More specifically, we show that nestin has an impact on muscle healing after injury at the organism level, rather than having a primary role in the differentiation of individual muscle cells. The third

study develops a new research model, a fruit fly expressing human vimentin, which can be used for research regarding both IF function and evolution. Together these studies provide new insights into the regulation and physiological impact of IFs.

2 Review of the Literature

2.1 Intermediate filaments

IFs are a large protein family consisting of more than 70 distinct proteins. All the IF-proteins are characterized by a structurally conserved rod domain flanked by variable head and tail regions. The most characteristic property of an IF-protein is the ability to assemble into filaments, either by itself to form homopolymers, or in conjunction with another IF-protein to form heteropolymers; reviewed in (Herrmann et al., 2007).

The first x-ray structure of an IF, although the concept of IFs was yet to be invented at that time, was solved from thinly sectioned wool, consisting mainly of keratins (Astbury and Street, 1932). The authors used the x-ray data as well as chemical and mechanical studies of wool to propose that keratin(s) is a filamentous structure that consists of repetitions of the same structural subunit. This hypothesis is in its essence in line with our current knowledge of IF structure.

The long, cell spanning filaments of cytoplasmic IFs are built up of protein monomers that assemble into polymers. Some of the IFs only assemble if another IF is available for co-polymerization: nestin, for example, always forms heteropolymers with vimentin, desmin or α -internexin (Sjöberg et al., 1994b; Steinert et al., 1999) while other filaments, like desmin and vimentin, also can form homopolymers. The process of turning monomeric protein units into long filaments is complex but seemingly spontaneous. The first step is the formation of subunit dimers, which then assembly into anti-parallel tetramers. The tetramers are fused into unit-length filaments, which are further longitudinally annealed into filaments. The last step is the radial compaction of the newly formed filaments (Figure 1) (Godsel et al., 2008; Herrmann et al., 2009). The assembly of lamins differs from the assembly of cytoplasmic IFs. The lamin molecules first form parallel dimmers, which then fuse head-to-tail to form tetramers, also called protofilaments. Three or four protofilaments then build up the lamin filaments (Ben-Harush et al., 2009). The lamin filaments also differ from the cytoplasmic IFs by forming a meshwork, in contrast to long filaments (Aebi et al., 1986).

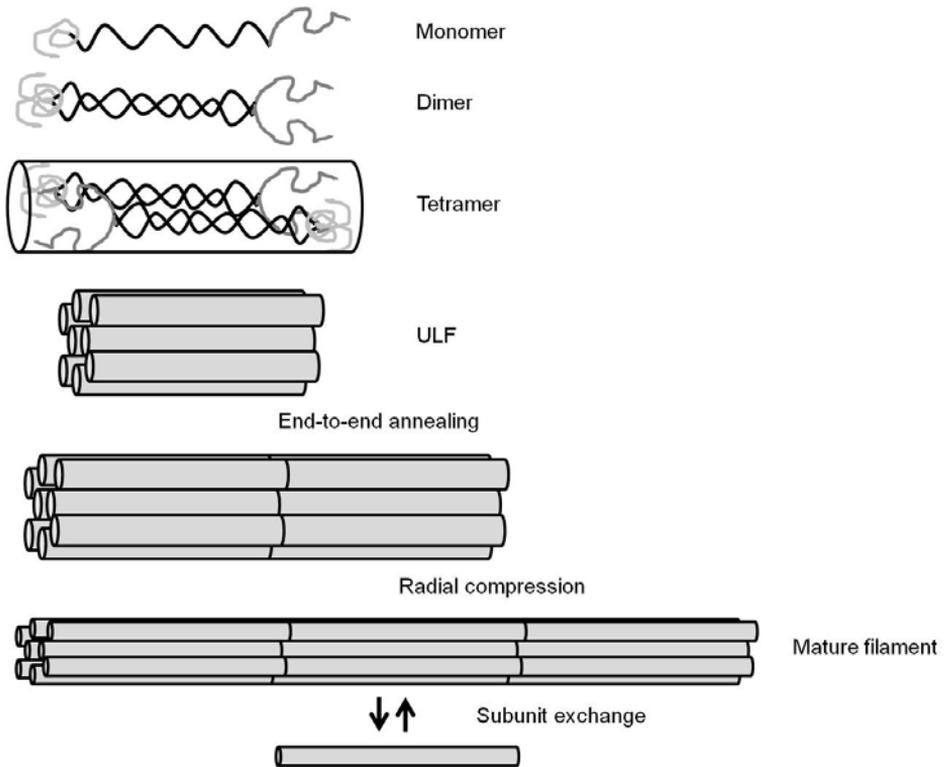


Figure 1. The cytoplasmic IF assembly process. The assembly starts with IF monomers assembling into parallel dimers. The dimers then form anti-parallel tetramers. Eight tetramers build up unit length filaments (ULF), which fuse into long filaments that are radially compressed to form the mature filaments. In the cell the filaments undergo subunit exchange, but the exact mechanisms behind this process remain unknown.

The IFs are divided into six groups based on their structural relationship, expression patterns and functions. Group I and II, the acidic and basic keratins, are the largest groups. Keratins are the typical IFs of epithelia, and they are also the building blocks of hair, nail, horn and hoofs. Due to their structure the keratins always form heterodimers consisting of one type I and one type II monomer. The third group contains vimentin, desmin, GFAP and peripherin. Group III proteins can form both homopolymers and heteropolymers with type IV IFs. Group IV in turn comprehends the neurofilaments (NFs), nestin, α -internexin, synemin and syncoilin. These proteins are very diverse, but are

grouped due to their evolutionary relationship with NFs. The lamins in group V differ from other IFs in that they localize to the nucleus. The most archaic function of the lamins was probably to protect the nucleus from physical stress; later lamin proteins have acquired a more complex function. The novel IFs in group VI, filensin and phakinin, are found in the lens.

IFs are found in all metazoan organisms (Erber et al., 1998) and, in addition, IF-like proteins have recently been identified in other eukaryotes, such as an amoeba and water mold (Kollmar, 2015). While the simpler organisms express only lamins, the numbers and complexity of the IFs increase in higher order organisms. An exception to this is the arthropods which, with only one known exception, express only lamins and no cytoplasmic IFs (Herrmann and Strelkov, 2011). In general invertebrates have only one B-type lamin, whereas A-type lamins only exist in vertebrates, reflecting lamin A as an evolutionary more recent protein (Peter and Stick, 2012). Interestingly, the cytoplasmic IFs found in lower organisms are more closely related to lamins than to cytoplasmic IFs in higher organisms (Weber et al., 1989). This indicates that a B-type lamin was the first IF to evolve, and that this founder IF gradually gave rise to cytoplasmic IFs and later to A-type lamins (Peter and Stick, 2012; Peter and Stick, 2015).

2.1.1 Cellular functions of IFs

The function of IFs has been a much debated topic. Initially, IFs were considered to be structural proteins providing the cell with mechanical stability. Over time, many other more diverse functions of IFs have been discovered. After the introduction of patient genomes sequencing in order to identify disease causing mutations, numerous diseases caused by mutations in IF genes have been discovered. This has led to a change in the view of the functions of IFs, as many of the diseases cannot be explained by mere disruption of mechanical scaffolds. The diseases are diverse and affect many different organs; even mutations in the same IF can give rise to completely different phenotypes. The largest group of IF-related diseases identified so far are the laminopathies, which are caused by mutations in the lamin genes, particularly affecting lamin A. Examples of diseases directly caused by mutated IFs and the organs affected are listed in Table 1.

Nevertheless, the structural functions of IFs are essential for everyday life. Keratins are needed for hair and nails, as reflected in the fact that mutations in the hair keratins influence hair structure. Other types of keratins have barrier

and tissue integrity related functions. Mutations in the epithelial keratins 5 or 14 give rise to the blistering skin disease epidermolysis bullosa simplex (EBS), in which epithelial cell layers do not attach firmly enough to each other (Coulombe et al., 2009).

Furthermore, IFs take part in organizing the cytoplasm and the positioning of organelles. For example both vimentin and desmin have been shown to bind to mitochondria (Milner et al., 2000; Nekrasova et al., 2011). In the absence of desmin, the mitochondria in striated muscles are disorganized and often aggregate; furthermore, they show extensive proliferation as well as degradation in heart muscle (Capetanaki, 2002; Milner et al., 2000). Vimentin has also been shown to directly modulate mitochondrial function, and other IFs are postulated to harbour similar functions (Chernoivanenko et al., 2015). Vimentin also contributes to the stiffness of the cytoplasm and to the organization of organelles by reducing random movement of particles in the cytoplasm (Guo et al., 2013). Vimentin is present at the focal adhesions, where it plays a critical role in transferring the mechanical force from the cell membrane to the cytoplasm and in activating signalling pathways in response (Gregor et al., 2014). Interestingly, vimentin also protects the nuclei from mechanical stress by decreasing the nuclear deformability in a yet un-characterized manner, which is not dependent on direct interaction with the nuclear lamins (Neelam et al., 2015).

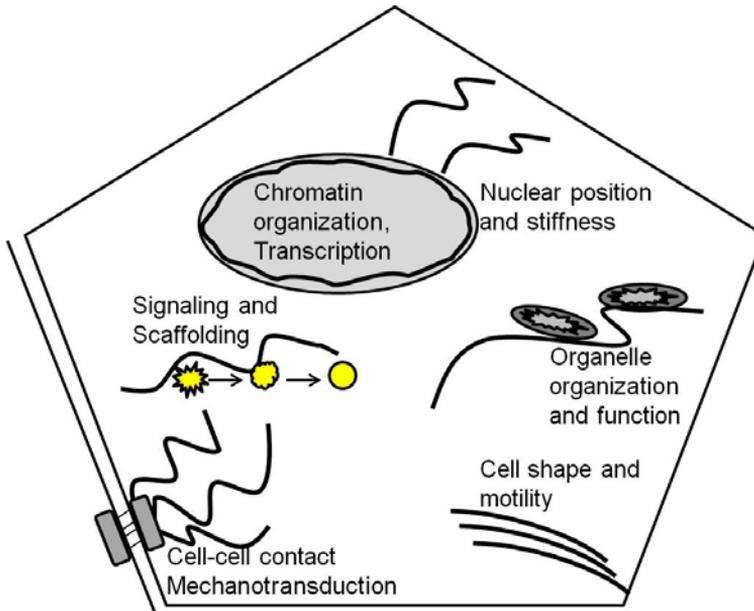
IFs also regulate the shape and motility of cells. Disruption of the vimentin network by micro-injection of a dominant negative vimentin mutant that disassembles the vimentin network leads to a dramatic change in cell shape. When the network is collapsed in mesenchymal cells, the cells lose their elongated shape and gain a round epithelial shape instead (Mendez et al., 2010). Similarly, introduction of vimentin filaments in epithelial cells leads to a mesenchymal cell shape (Mendez et al., 2010). Almost all IFs have been linked to cellular migration, depending on the filament and the cell type either promoting or inhibiting cell movement; reviewed in (Chung et al., 2013; Leduc and Etienne-Manneville, 2015). General and specific functions of IFs are outlined in Figure 2.

These observations show that the IF-networks have diverse roles as organizers of cellular compartments and signalling molecules, and that they are protectors against mechanical stress and transducers of mechanical messages. This diversity in functions is also the basis for the very different phenotypes of IF-related diseases. A mutation in one region of an IF may interfere only with a very specific function of that protein, whereas a mutation in another region, or

indeed in very close proximity, can interfere with a totally different function. The most prominent example of this is the numerous disease-causing mutations in lamin A: for example the D300N mutation is associated with the premature aging disease atypical Werner syndrome, whereas the L302P mutation only two positions away gives rise to muscular dystrophy (UMD-LMNA at www.umd.be). This shows that knowledge of all the functions of a specific IF is vital for being able to design specific treatments against IF-related diseases.

Table1 Examples of diseases directly caused by mutations in IF-genes

Diseases caused by IF mutations	IF involved	Organ affected	References
Epidermolysis bullosa simplex	K5, K14	skin	(Coulombe et al., 2009)
Pachyonychia congenital	K6a, K6b, K16, K17	skin, nail	(Smith et al., 2005)
Desmin-related myopathy	desmin	skeletal muscle, heart muscle	(van Spaendonck-Zwarts et al., 2011)
Alexander disease	GFAP	CNS	(Quinlan et al., 2007)
Charcot-Marie-Tooth disease	NF-L	PNS	(Jordanova et al., 2003; Liem and Messing, 2009)
Cardiomyopathy	lamin A	heart muscle	(Lu et al., 2011)
Cataract	filensin, phakinin, vimentin	lens	(Müller et al., 2009; Perng et al., 2007)



Lamin A/C functions

Cell

- organization of chromatin
- regulation of signaling factors
- regulation of transcription
- mechanosensing
- protection from shear stress
- organization of nuclear pore complexes

Organism

- muscle homeostasis
- heart function
- fat deposition
- cellular aging
- neuronal functions
- skin function

Nestin functions

Cell

- regulation of signaling factors
- migration
- survival
- mitochondrial function

Organism

- muscle homeostasis
- organization of MTJ and NMJ
- muscle regeneration

Figure 2. Overview of IF functions. General functions of IFs are outlined (top) and some of the specific functions of lamin A/C and nestin are listed (below).

2.1.2 Dynamics of IFs

When studied in fixed cells, IF-networks give the impression of being robust and stiff. From studies of fluorescently labelled IFs in live cells it is, however, clear that the IFs are highly dynamic structures with constant exchange of subunits and rapid reorganisation of filaments in response to stimuli. The different IFs form independent, partially over-lapping or totally over-lapping networks in the cell and can have very different dynamic properties. The vimentin and keratin 8/18-networks, for example, show completely independent dynamics even within the same cell (Yoon et al., 2001). This fact supports the idea that the different IFs have different functions in the cell.

2.1.2.1 The transport and dynamics of cytoplasmic IFs

The dynamics of IF-networks are still far from fully understood. Similarly, many things remain to be learned about how the networks are formed *in vivo*. The use of fluorescently tagged proteins has made it possible to follow how IF subunits are transported in the cell, as well as how the networks themselves move and exchange subunits.

The microtubule network has been proven to be important for the movement and transport of several different IFs. IF-subunits or short filaments are transported along the microtubules throughout the cell. The microtubule-based transport is rapid and bi-directional, but also interrupted by frequent and sometimes long pausing (Wang et al., 2000; Wöll et al., 2005).

The axons of neuronal cells are rich in the different isoforms of NFs. As the NF subunits are only produced in the cell body, the content of NFs in the axon must be dependent on a transport mechanism. It has been shown that NF-particles are transported along the microtubules from the cell body to the periphery of the axon, and that the motor proteins kinesin and dynein are responsible for this microtubule dependent NF transport (Prahlad et al., 2000; Uchida et al., 2009). The transport of NFs is dependent on phosphorylation in an indirect manner; phosphorylated NF subunits have lower affinity for the motor proteins and higher affinity for other NFs; reviewed in (Shea and Chan, 2008). This gives the cell means to regulate when and where to un-couple the NF subunits from the microtubules and, thus, co-ordinate the building and specific intracellular localisation of the NFs.

In a similar manner to NFs, short vimentin filament subunits have been shown to move along microtubules at high speed (Robert et al., 2014). Still, this

transport only affects a small part of the vimentin present in a cell, as not more than 2% of the total vimentin pool was found to be associated with microtubules (Robert et al., 2014). Vimentin also interacts with the actin-network, which prevents the movement of vimentin (Robert et al., 2014).

Individual filaments in keratin 8/18-networks show a wave-like movement, as has been shown with the use of GFP-tagged keratin 18 (Yoon et al., 2001). Keratins are assembled into proto-filaments in the cell periphery and then assemble into mature filaments as they are transported towards the nucleus. Along the way some filaments are degraded, and the subunits transported back to the periphery for reuse in new filaments (Kölsch et al., 2010; Leube et al., 2011). The movement of the keratin particles, as well as the movements of the entire keratin network, is dependent on microtubules and independent of actin filaments (Windoffer and Leube, 1999). However, keratins are also moving along actin filaments in the cell periphery (Wöll et al., 2005).

Together these results show that at least some, but more probably all of the IFs are dependent on other cytoskeletal elements for network forming and for transportation of building blocks to specific parts of the cell. However, much of the details of the IF dynamics as well as how they are regulated still remain unknown.

As further discussed below in the lamin section, phosphorylation is a key post-translational modification (PTM) for nuclear IF dynamics. While understanding of the mitotic breakdown of the nuclear lamina has been a major driving force behind the studies of lamin phosphorylation, the phosphorylation of cytoplasmic IFs has been studied in a less coherent manner. It is nevertheless clear that both the mitotic and non-mitotic dynamics of cytoplasmic IFs are regulated by phosphorylation. For example, the breakdown of the vimentin network during mitosis has been shown to be driven by phosphorylation; reviewed in (Goldman et al., 2012; Sihag et al., 2007). Consistent with this, mutation of the mitotic phosphorylation sites to an un-phosphorylatable form leads to increased tetraploidy, as the intact vimentin network obstructs proper cytokinesis (Matsuyama et al., 2013; Tanaka et al., 2015). Phosphorylation is also important for IF dynamics in a non-mitotic context: lipid signalling has for example recently been shown to induce phosphorylation dependent re-organisation of both keratin 8 and vimentin networks (Busch et al., 2012; Hyder et al., 2015). The lipid- induced ERK-dependent phosphorylation of S431 in keratin 8 leads to a shift in the keratin 8/18 network, from cell spanning filaments to formation of

a perinuclear network. This in turn promotes epithelial cancer cell migration (Busch et al., 2012). In case of vimentin, the lipid signalling induces an effector Rho-associated kinase (ROCK) dependent phosphorylation of S71. The vimentin S71 phosphorylation in turn leads to retraction of the vimentin network and inhibition of cell migration (Hyder et al., 2015). That the lipid signalling induced phosphorylation of keratin 8 promotes cell migration, and that phosphorylation of vimentin induced by the same signalling inhibits migration, can be explained by the different cell-lines used in the studies. It is important to note that the cells used by Hyder et al. lack keratin 8 and, thus, cannot have a keratin 8 phosphorylation induced response (Hyder et al., 2015).

2.1.3 Signalling properties of IFs

IFs have many properties that make them excellent scaffolding molecules. They can sequester signalling molecules and keep them inactive, or active, at different parts of the cell, and they can also bring together different molecules, thus enabling them to form active complexes.

When a cell receives a signal, the message must be forwarded from the receiving part of the cell to the rest of it. This process needs to be fast and accurate, a fact especially obvious in peripheral nerve cells, where the distance between the tip of the axon and the cell body can be several centimetres or even metres. When a distal part of an axon is damaged, Erk1/2 is phosphorylated and bound to soluble vimentin fragments, which are generated by calpain-mediated cleavage of newly synthesized vimentin subunits. This complex then binds to importin β and is subjected to retrograde transport along the microtubules to the cell body (Perlson et al., 2005). When the complex reaches the cell body, phospho-Erk is released and can activate its targets. The binding of phospho-Erk to vimentin is dependent on calcium: when the complex reaches the cell body with lower calcium concentration than the periphery, Erk is released (Perlson et al., 2006). During the transport vimentin protects phospho-Erk from dephosphorylation and degradation due to physical protection of the molecule (Perlson et al., 2006). This process is an example of the signalling properties of two not yet well-studied IF-functions: the role of soluble IFs and IFs as chaperones.

Another example of an IF with signalling functions is keratin 14, which has been shown to up-regulate the JNK/MAPK-pathway (Wagner et al., 2013). Similarly, desmin has been identified as essential for the activation of JNK during mechanical muscle fiber stress (Palmisano et al., 2015) As kinases in the

JNK/MAPK-pathway are known to phosphorylate several IFs, including keratins (Toivola et al., 2002), it implicates a feedback loop, where the expression of one IF can, through JNK-signalling, regulate the structure of others and itself. These examples demonstrate how the IFs can work in cell signalling, and also show the limitations of our knowledge about cross talk between different IFs.

During cancer metastasis, as well as during physiological processes such as wound healing, cells undergo epithelial-mesenchymal transition. During this transition the cells lose their epithelial phenotype and gain a mesenchymal phenotype, including higher motility and the ability to invade. At the same time the cells lose their epithelial keratins and gain vimentin expression (Kokkinos et al., 2007). The gain of vimentin is not a mere marker of the mesenchymal state; vimentin has also a direct role in facilitating the transition. This since vimentin induces Axl, a kinase that promotes cellular motility (Vuoriluoto et al., 2011). Vimentin also regulates Erk-activity in cancer cells; vimentin sustain Erk-activity which in turn leads to expression of Slug, an EMT-inducing factor (Virtakoivu et al., 2015).

2.2 Lamin A/C

2.2.1 Splicing and processing of lamin A

In contrast to the other IFs, lamins are found in the nucleus, where they form the nuclear lamina – a thin fibrous layer inside the inner nuclear membrane. Lamin A and C are splice variants produced by the *LMNA*-gene, a gene which also gives rise to the less common splice variants lamin A Δ 10 (Machiels et al., 1996), lamin A Δ 50 or progerin (Eriksson et al., 2003), and the germ cell-specific lamin C2, that so far has only been found in rodents (Furukawa et al., 1994).

Lamin A is expressed as a precursor protein, prelamin A, which undergoes a series of modifications to become the mature protein. The C-terminus of prelamin A ends with a CAAX-box, and the cysteine residue in this motif is rapidly farnesylated. After the farnesylation step the AAX-sequence is cleaved off and the farnesylcysteine is methylated. In the last step, the 15 last amino acids of prelamin A are enzymatically cleaved by ZMPSTE24, giving rise to mature lamin A; reviewed in (Young et al., 2006). The lamin A/C processing is described in Figure 3.

In Hutchinson-Gilford progeria syndrome (HGPS), a mutation in the *LMNA*-gene leads to abnormal splicing and the production of progerin instead of lamin A, with dramatic consequences for the affected individual. Due to the differential splicing, progerin lacks the ZMPSTE24 cleavage site and thus remains constitutively farnesylated. Progerin has recently been shown to be produced in healthy individuals at certain periods during development, and during aging, although this splicing is strictly controlled in a yet unknown manner (Bökenkamp et al., 2011; Luo et al., 2013). Lamin C, in contrast to lamin A, is not processed (Dechat et al., 2008). Compared to lamin A, lamin C lacks exons 11 and 12 and has six unique amino acids in the C-terminus. In lamin C2 the N-terminal head region is replaced by a shorter sequence. Very little is known about the regulation of the differential splicing and the expression of the isoforms in vivo, or their functions for that matter. In a recent study lamin C has been identified as the predominant A-type lamin in the brain, due to the down-regulation of prelamin A by miR-9, a miRNA expressed at high levels in the brain (Yang et al., 2015). This shows that there are several mechanisms regulating the balance between lamin A and C, and that these mechanisms might play different roles in different tissues.

To elucidate the functions of the different prelamin A processing steps an array of mouse models have been created, reviewed in (Davies et al., 2011). The exact functions of the prelamin A processing still remains unknown; this as mice expressing mature lamin A directly without the prelamin A processing steps are physiologically normal (Coffinier et al., 2010). Similarly, mice expressing only lamin C, but no lamin A, are seemingly normal (Fong et al., 2006), in contrast to mice lacking both lamin A and C (Kubben et al., 2011; Sullivan et al., 1999). These results suggest that the non-farnesylated lamin C can compensate for lamin A, and that prelamin A processing is not needed for normal nuclear envelope targeting of neither lamin A nor lamin C.

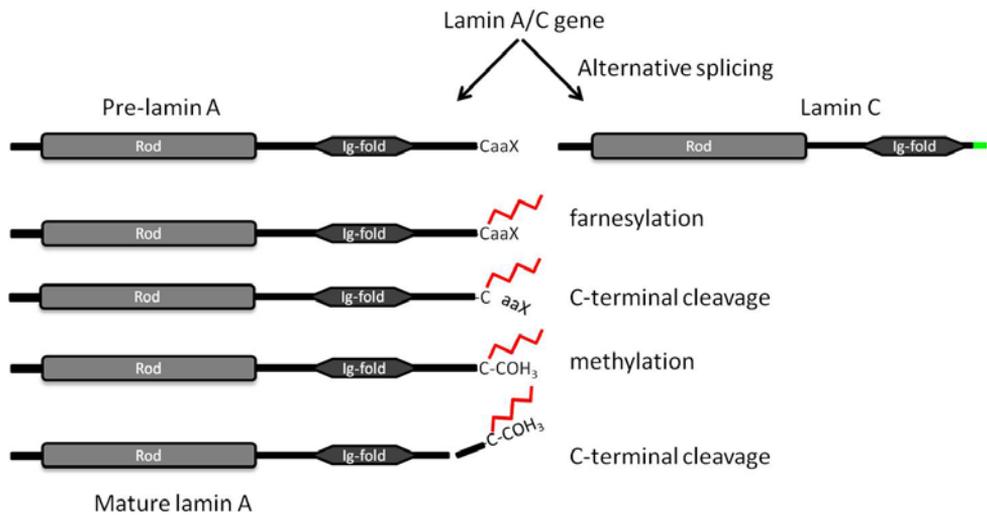


Figure 3. Lamin maturation. Alternative splicing of the lamin A/C mRNA gives rise to prelamins A and C, as well as the less common forms prelamins A $\Delta 10$ and lamin C2. Pre-lamin A undergoes a series of c-terminal modifications and cleavages, outlined in the left part of the figure, to yield mature lamin A. Lamin C, in contrast, is translated as the mature protein.

2.2.2 Lamin PTMs

Given the many diverse functions of lamin A and the numerous binding partners reported, as well as the tissue specific diseases caused by mutations in this ubiquitously expressed protein, it is clear that expression-independent ways to regulate its functions must exist. PTMs constitute one part of this regulation, offering a possibility for the cell to regulate proteins in a fast and direct manner, as an opposite to the regulation of transcription and translation. Lamin A/C is heavily posttranslationally modified, but many of the identified modifications have not been studied beyond their identification. In the following sections, I describe some of the more commonly studied lamin PTMs in more detail.

2.2.2.1 Lamin SUMOylation

Small ubiquitin-like modifier (SUMO) is a polypeptide that can be covalently attached to lysine residues on proteins by an E3-ligase. In lamin A K201, located in the rod-domain, has been identified as a SUMO2 target (Zhang and Sarge,

2008). This modification has also been shown to be clinically significant, as the cardiomyopathy-causing mutations E203G and E203K disturb the SUMO consensus sequence, resulting in un-SUMOylated lamin A (Zhang and Sarge, 2008). In the tail of lamin A, K420 and K486 have been reported to be modified by SUMO1 (Simon et al., 2013) and K520 also by SUMO3 (Galissou et al., 2011). Interestingly, the SUMOylation of the lamin A tail is also affected by mutations, in this case by the Dunnigan-type familial partial lipodystrophy-causing K486N and G465D mutations. These mutations decrease tail SUMOylation without affecting rod SUMOylation (Simon et al., 2013). Yet the function of lamin SUMOylation remains unknown. Simon et al (2013) speculate that the attachment of SUMO to the lamin A tail interferes with protein interactions dependent on these regions, and thus the signalling or scaffolding functions of lamin A.

2.2.2.2 Lamin phosphorylation

Phosphorylation is the addition of a phosphate group to a serine, threonine, tyrosine or, un-commonly, histidine residue of a protein. This process is performed by enzymes called kinases, while the reverse process, the removal of a phosphate group, is performed by phosphatases. Phosphorylation affects proteins in many ways: it can for example turn on or off an enzyme, target a protein for degradation or influence protein localisation, solubility and organisation.

Phosphorylation is one of the major PTMs of IFs, and has been shown to influence numerous IFs and their functions (Snider and Omary, 2014). Lamin A/C is no exception from this: with more than 70 unique phosphorylation sites identified, phosphorylation is the most abundant PTM of lamin A/C (Simon and Wilson, 2013). Phosphorylation of lamin A/C has mostly been studied in the context of mitosis, as the breakdown of the nuclear envelope, a requirement for mitosis, is dependent on lamin phosphorylation. During mitosis, both A- and B-type lamins are phosphorylated by Cdk1 at highly conserved sites surrounding the rod domain (Dessev et al., 1990; Dessev et al., 1991; Heald and McKeon, 1990; Peter et al., 1990). These residues are primarily S22 and S392 in human lamin A and S23 and S393 in lamin B1.

During mitosis the lamin A and lamin B networks are disrupted at different kinetics. The A-type lamins are disassociated from the inner nuclear membrane earlier than the B-type lamins (Georgatos et al., 1997). Lamin A seems to be soluble in the nucleoplasm before the breakdown of the nuclear membrane and

is later found throughout the cytoplasm, whereas B-type lamins remain attached to the nuclear envelope for a longer time (Georgatos et al., 1997). In the last stage of mitosis, the B-type lamins too are found soluble throughout the cytoplasm.

After mitosis the nuclear lamina is re-assembled. Even here, B- and A-type lamins show differences: B-type lamins associate with the still condensed chromosomes and rapidly build up a solid lamina, while A-type lamins remain in the nucleoplasm, and are slowly incorporated into the lamina (Moir et al., 2000). The phosphorylation of lamins is a dynamic process, where the different sites show different occupancy during the cell cycle (Kuga et al., 2010). The dynamics of phosphorylation can provide an explanation for the different de- and re-assembly dynamics of the lamins during mitosis.

2.2.3 Structural and mechanical functions of lamin A/C

The genetic information of cells is well protected inside the nucleus, and particularly the cage of lamins gives the nucleus strength to withstand physical deformation (Liu et al., 2000). The A- and B-type lamins work together to provide the nucleus with strength and flexibility. B-type lamins are thought to provide the nucleus with rigid but still flexible walls which will withstand physical impact. A-type lamins, on the other hand, act as a viscous fluid to absorb shock (Swift et al., 2013).

A-type lamins have been shown to be the major lamins contributing to nuclear stiffness, whereas lamin B1 seemingly does not influence nuclear stiffness (Lammerding et al., 2006). The same study also showed that lack of lamin A/C leads to nuclear blebbing and deformed nuclei at a greater rate than the loss of lamin A only or B1. The results offer yet another piece of evidence for the hypothesis that Lamin C can, at least to some extent, compensate for loss of lamin A.

Recently, the role of lamin A as a mechanosensor, sensing and responding to changes in the cells' physical environment, has been brought to attention (Isermann and Lammerding, 2013; Swift et al., 2013). Cells grown on stiffer substrates have higher lamin A-expression and stiffer nuclei than cells grown on softer substrates (Swift et al., 2013). In other words, the cells respond to their mechanical environment by regulating lamin levels and, thus, adapt their nuclear stiffness to anticipated mechanical stress. The lamin levels also correlate with differentiation pathways: mesenchymal stem cells with higher lamin A

expression are more prone to differentiate into bone, whereas the same cells with lower lamin A levels are more prone to become adipocytes (Swift et al., 2013). By adjusting the substrate stiffness it is thus possible to affect the lamin A levels and ultimately differentiation processes. The discovery that cells can react to their environment in such a direct and consequential way also gives insight into the complex factors regulating cell fate in the body. Stem cells proliferating on the hard substrate of bone are more prone to become bone, whereas stem cells proliferating in the soft environment of adipose tissue are more prone to become adipocytes.

Nuclear stiffness also has direct consequences for a migrating cell; when a cell squeezes through the tissue, for example during lymphoid metastasis of tumour cells, the cells are subjected to mechanical stress. Cells with too stiff nuclei have been shown to be unable to migrate through capillaries, due to the stiff nucleus physically hindering the migration (Harada et al., 2014). Indeed, overexpression of lamin A hampers the migration of neutrophils (Rowat et al., 2013). On the other hand, cells with too soft nuclei, due to down-regulation of lamin A, also have problems migrating, as the soft nuclei are torn by the mechanical stress, leading to apoptosis of the cells (Harada et al., 2014).

Lamin A can also influence migration and mechanosensing both by regulating transcription factors (Ho et al., 2013) and by directly influencing the cells' physical properties (Harada et al., 2014; Rowat et al., 2013; Swift et al., 2013). These results show that lamin A has a significant role as a signalling hub, receiving and forwarding signals from the environment as well as from inside the cell, but also by affecting the outcomes of the signals.

2.2.4 Nucleoplasmic lamin A

Although the majority of lamin A is found in the nuclear lamina, immunofluorescence as well as fluorescently tagged lamin A has been used to demonstrate the presence of a nucleoplasmic lamin A/C pool (Dechat et al., 2010; Gesson et al., 2014; Shimi et al., 2008). The nucleoplasmic lamin A/C fraction is more mobile than the lamina associated fraction, and can further be divided into two distinctive groups: the fast-moving and the slow-moving (Shimi et al., 2008). The functions of these different dynamics are yet unknown, but probably reflect diverse, yet undefined, functions of the nucleoplasmic lamins. There is indeed growing evidence that the nucleoplasmic pool of lamin A has important roles. For example, the nucleoplasmic lamin A interacts with lamina-associated

polypeptide 2 isoform alpha (LAP2 α) and retinoblastoma protein (pRb), which, in turn, also interacts with LAP2 α (Dechat et al., 2000). The interaction between lamin A, LAP2 α and pRb is believed to regulate the activity of pRb and cell cycle progression (Gesson et al., 2014; Naetar and Foisner, 2009). Loss of LAP2 α and nucleoplasmic lamin A impairs cell cycle exit and differentiation, whereas over-expression of LAP2 α leads to premature differentiation (Dorner et al., 2006; Naetar et al., 2008). The interaction between lamin A and LAP2 α is also essential to keep lamin A in the nucleoplasm; loss of LAP2 α has been shown to lead to significant reduction of nucleoplasmic lamin (Naetar et al., 2008). During myogenic differentiation there is also a correlation between loss of nucleoplasmic lamin A and down-regulation of LAP2 α expression (Markiewicz et al., 2005).

2.2.5 Laminopathies - peculiar diseases that give insights into lamin functions

Laminopathies, the diseases caused by mutations in the lamin genes, are the largest group of IF-associated diseases. The phenotypes of these diseases vary tremendously, reflecting the many processes which lamins influence (Table 2).

Table 2 examples of laminopathies.

Laminopathy	Organ affected	Symptom
Familial partial lipodystrophy (Dunnigan type)	Fat tissue	Abnormal body fat distribution, increased risk for type II diabetes
Emery-Dreifuss muscular dystrophy	Heart and skeletal muscle	Muscle wasting, heart failure
Dilated cardiomyopathy 1A	Heart	Sudden death, heart failure
Hutchinson-Gilford progeria syndrome	Whole body	Premature aging, atherosclerosis
Charcot-Marie-Tooth disease type 2B1	Peripheral nerves	Muscle atrophy in lower limbs

There are different theories trying to explain the effects of lamin mutations: the structural hypothesis, the gene regulation hypothesis and the impaired stem cell function hypothesis; reviewed in (Davidson and Lammerding, 2014; Gesson et

al., 2014; Ho and Lammerding, 2012). The structural hypothesis focuses, as the name suggests, on the structural properties of lamin A. As previously mentioned, one of the functions of lamin A is to provide the nucleus with physical strength. A disruption of the lamin A network due to a mutation could, thus, affect the nuclear structure and mechanics. Muscle cells, particularly cardiac muscle cells, are subjected to severe physical stress as they contract and relax during our daily life. Muscles are therefore among the first organs to show symptoms of altered structural or mechanical properties of proteins. The structural hypothesis can therefore be used to explain the symptoms of laminopathies affecting cardiac and skeletal muscles.

The gene regulation hypothesis is based upon the fact that lamin A/C interacts with DNA and regulates transcription; reviewed in (Camozzi et al., 2014; Dechat et al., 2008). Any mutation which interferes with these processes can affect downstream targets, with unexpected consequences for the cell. For example, the lamin A/C R482W mutation causing Dunnigan type familial partial lipodystrophy affects the activity of the transcription factor sterol response element binding protein 1 (SREBP1) (Vadrot et al., 2015). The mutated lamin interacts less with SREBP1, and the lost interaction in its turn leads to increased activity of SREBP1 as well as enhanced transcription of an array of its target genes.

The stem cell hypothesis is based upon the fact that lamin A is known to regulate stem cell populations. Mice expressing progerin, the HGPS-causing form of lamin A, in their keratinocytes have fewer stem cell-like keratinocytes (Rosengardten et al., 2011). Similarly, over-expression of progerin in human bone marrow derived stem cell-like cells leads to decreased proliferation and decreased expression of stem cell marker proteins (Pacheco et al., 2014). Apart from affecting stem cell populations, lamin A also affects differentiation and is for example critical for myogenic differentiation (Frock et al., 2006). Disturbances in stem cell numbers and differentiation will affect both specific tissues and the whole organism, explaining why the laminopathies have such dramatic phenotypes.

These hypotheses do not exclude each other: impaired differentiation can for example be due to altered gene expression, which in turn can be due to altered physical lamina properties. Most probably a combination of the proposed hypotheses and yet unknown factors is the causative element of laminopathies.

2.3 Nestin

2.3.1 Nestin structure and polymerisation

Nestin is a less studied IF and little is known about its functions, although it is commonly used as a stem cell marker. Nestin was first identified in radial glial cells with the use of the monoclonal antibody rat-401 (Hockfield and McKay, 1985). Later nestin was discovered to be expressed in, and became used as a marker for neuroepithelial stem cells, from which the protein also got its name (*neuroepithelial stem cell protein*) (Lendahl et al., 1990).

The nestin gene consists of 4 (human) or 5 (mouse) exons encoding a 1621 (human) or 1864 (mouse) amino acids long protein with a molecular mass of around 200 kDa. Nestin is characterized by its unusually long tail, consisting of sequence repeats. The longer murine form is due to loss of a number of the repeats in human (Dahlstrand et al., 1992). Three different isoforms of nestin have been identified so far: apart from the full length protein two truncated forms lacking either the repeats or the repeats and part of the rod domain have been described (Su et al., 2013; Wong et al., 2013).

Like the keratins, nestin cannot form filaments on its own. It has been shown to form heteropolymers with vimentin and α -internexin in vitro, and although nestin forms homo-oligomers, in vitro these cannot form filaments (Steinert et al., 1999). In vivo nestin co-localizes with desmin, and these IFs form an indistinguishable network (Sjöberg et al., 1994b). In vimentin-negative SW13 cells, the expression of nestin leads to diffuse cytoplasmic localisation of the protein, whereas expression of nestin in corresponding vimentin positive cells gives rise to a nestin-vimentin network with clearly distinguishable filaments (Marvin et al., 1998). The loss of a nestin network in the absence of vimentin has also been confirmed in cells from vimentin^{-/-} mice, underscoring the importance of binding partners for the formation of nestin filament (Eliasson et al., 1999).

2.3.2 Regulation of nestin gene and protein

During development, nestin is expressed in numerous tissues; apart from the developing CNS, nestin is also found in developing skeletal and cardiac muscle cells, in the developing eye and in the blood vessels; reviewed in (Wiese et al., 2004). In the healthy adult nestin expression is limited to very few, specific regions such as the podocytes (Chen et al., 2006) and the pancreatic islets (Hunziker and Stein, 2000). Following injury nestin is re-expressed in muscle

(Vaittinen et al., 2001) and brain (Lin et al., 1995). This expression pattern hints that nestin expression is tightly regulated.

The regulation of IF expression is a very rarely studied subject, and nestin is no exception to this with only few studies regarding its expression existing. A simplified overview of the regulation of nestin, as described below, is outlined in Figure 4. The introns in the nestin gene have been proven to be essential for its expression patterns. Elements within the first intron regulate the expression in muscle, and elements within the second intron regulate expression in brain (Zimmerman et al., 1994). However, the nestin GFP mice, where the second nestin intron is utilized for driving a GFP transgene, have a more widespread expression pattern, leaving the exact mechanisms behind nestin gene regulation still unsolved (Walker et al., 2011). The nestin promoter is unusually short and lacks a functional TATA-box, but contains two SP1-sites that are needed for transcription (Cheng et al., 2004). The tumour suppressor p53 targets nestin expression by preventing the binding of Sp1 and Sp3 to the nestin promoter (Tschaharganeh et al., 2014). Mutation of p53, as is common in many cancers, interferes with this process and enables nestin expression (Tschaharganeh et al., 2014). The nestin gene has also been shown to be regulated by epigenetic factors; histone acetylation is needed for activation of nestin expression, whereas demethylation is not strictly needed but plays a role in proper regulation of expression (Han et al., 2009).

The transcription of nestin has been more thoroughly characterized in neurons, but some information about the regulation of nestin in muscle exists. In the mouse myoblast cell line C2C12, the first intron of nestin has been shown to regulate the transcription; furthermore, the transcription factor MyoD binds this intron and enhances the protein expression (Zhong et al., 2008). Forced expression of MyoD in the nestin negative mouse pre-adipocyte cell line 3T3-L1 leads to nestin expression, even though myoblasts without MyoD-expression do express nestin, indicating that additional elements regulate the nestin gene (Kachinsky et al., 1994). The fact that nestin gene activity has been detected in quiescent satellite cells (Day et al., 2007), where MyoD is not detectable, confirms the complexity of nestin regulation in muscle.

Recently it has become well accepted that not only the transcription, but also the degradation of mRNA is a heavily regulated and important part of the cell's protein production control. Several studies show that nestin mRNA is targeted by miRNAs. In nasopharyngeal carcinoma cells, nestin is targeted by miR-940

(Ma et al., 2014). The miR-940-induced down-regulation of nestin correlates with apoptosis and cell cycle arrest, reinforcing the finding that nestin protects cells against apoptosis (Sahlgren et al., 2006). Nestin mRNA has been shown to be a target for miR-125b in neuronal stem and progenitor cells where up-regulation of miR-125b correlates with down-regulation of nestin and differentiation (Cui et al., 2012). In addition to this, nestin mRNA has also been shown to be targeted by miR-432 in differentiating neuroblastoma cells (Das and Bhattacharyya, 2014). As more miRNA sequences are discovered, our understanding of this form of regulation of nestin, as well as other IFs, its complexity and co-ordination with other types of gene- and protein-regulation will without a doubt expand.

Apart from the gene expression, protein stability and degradation are important regulatory steps. This is especially apparent when it comes to IFs, as these proteins normally are very stable; the half-life of NFs in axons has been suggested to be in the time frame of weeks (Nixon and Logvinenko, 1986). During differentiation of neuronal stem cells nestin expression is rapidly lost, and there is evidence that this is through notch-dependent targeting of nestin for proteosomal degradation (Mellodew et al., 2004). The tail region of nestin is important for driving the protein-degradation, as mutants lacking various parts of the C-terminal region have a significantly longer half-life (Sahlgren et al., 2006).

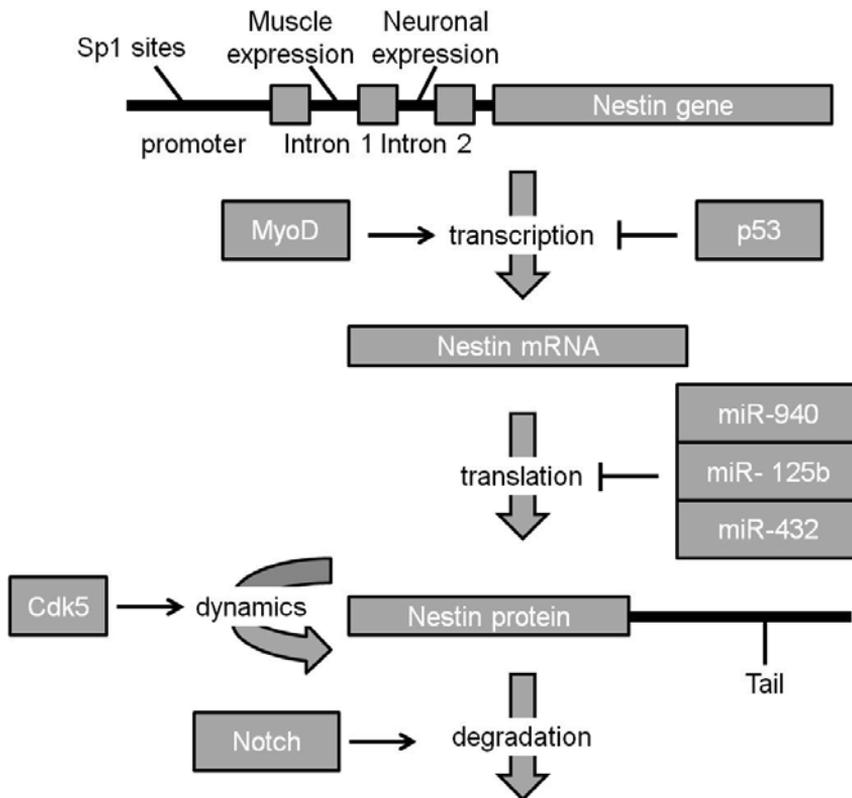


Figure 4. Simplified overview of nestin regulation. The short nestin promoter contains two Sp1-sites needed for transcription. Regulatory elements in the first and second intron of the nestin gene drive tissue specific expression. Several microRNA-molecules have been shown to promote the degradation of nestin mRNA. The tail region of nestin protein is needed for the ubiquitin-induced proteasomal degradation of nestin.

2.3.3 Functions of nestin

Still, three decades after the discovery of nestin, its molecular and physiological functions remain mainly unclear. Genetic depletion of nestin revealed that whereas nestin is not essential for survival of mice, it plays a role in proper organization of neuromuscular junctions (Mohseni et al., 2011). Using an in vivo knockdown strategy Yang et al. showed that nestin is needed for organization of acetylcholin receptors at the postsynaptic membrane of neuromuscular junctions (NMJ) (Yang et al., 2011). These studies did not, however, look into the role of nestin outside the NMJs. Apart from these studies a second knockout mouse has been reported (Park et al., 2010). The phenotype of this mouse strain differs

from the other reported strains as these mice undergo embryonic lethality at a high rate, with only rare knockout animals surviving until birth (Park et al., 2010). The lethality was reported to be due to extensive apoptosis of cells in the CNS. In both knockout models the first exon of nestin is targeted, but the targeting vectors differ. As knockdown of nestin (Yang et al., 2011) gives an identical phenotype as one of the knockout models (Mohseni et al., 2011), the embryonic lethality reported in the second knockout model (Park et al., 2010) is more probably the result of an off-target effect of the recombination vector used.

During myogenic differentiation, nestin regulates Cdk5 activity by forming a scaffold for Cdk5 and p35 (Sahlgren et al., 2003). Nestin in turn is regulated by Cdk5 phosphorylation: when nestin is phosphorylated by Cdk5 it loses its filamentous structure and becomes more soluble (Sahlgren et al., 2003). The phosphorylation also leads to decreased interaction between nestin and p35, showing the ability of Cdk5 to regulate its own activity through regulating nestin's scaffolding capacity.

It has been shown that nestin facilitates the disassembly of vimentin filaments during mitosis, but the importance of this process remains unclear, as vimentin positive, nestin negative cells undergo mitosis without problem (Chou, 2003). During mitosis nestin filaments are rearranged due to phosphorylation of nestin by Cdk1 (Sahlgren et al., 2001). This rearrangement also affects other proteins binding to nestin; for example, the binding between nestin and phosphatase and actin regulator 4 is disrupted during mitosis due to the phosphorylation of nestin (Cho et al., 2014). The interactions between nestin/vimentin and insulin degrading enzyme are also dependent on filament phosphorylation (Chou et al., 2009).

2.3.4 Nestin in muscle

Soon after the initial discovery of nestin its expression was identified in skeletal muscle during development (Sejersen and Lendahl, 1993) and in regenerating muscle fibers of Duchenne/Becker muscular dystrophy patients (Sjöberg et al., 1994a). During the early stages of limb bud development nestin is found in mesenchymal cells, but as development proceeds nestin becomes restricted to differentiating muscle cells (Wroblewski et al., 1997). After birth nestin is down-regulated and desmin becomes the primary IF in muscle fibers (Carlsson et al., 1999; Sejersen and Lendahl, 1993). In the normal adult muscle nestin expression is limited to NMJ and myotendinous junctions (MTJ) (Carlsson et al., 1999;

Vaittinen et al., 1999). In mouse models that express GFP under the control of the second intron of the nestin promoter satellite cells are GFP labelled *in vivo*, indicating that at least the nestin locus is active in these cells (Day et al., 2007). When a muscle injury occurs, the expression of nestin is initiated, and nestin protein is found both in newly differentiated myoblasts and in regenerating myofibers (Vaittinen et al., 2001). This up-regulation of nestin has been identified in several different injury models, such as denervation (Vaittinen et al., 1999), cut injury and *in situ* necrosis (Vaittinen et al., 2001). In a transplantation model, which reflects ischemic injury due to transient loss of blood supply, it was shown that newly formed myotubes and fibers express nestin (Čížková et al., 2009). This suggests that expression of nestin is a general feature of muscle regeneration, regardless of the type of injury initiating the regeneration.

Despite the fact that nestin expression has been detected in muscle disease (Sjöberg et al., 1994a) and that nestin is found in regenerating muscle (Vaittinen et al., 1999), almost nothing is known about the functions of nestin in muscle *in vivo*. *In vitro* nestin has been shown to function as a regulator of the muscle differentiation process. Down-regulation of nestin by siRNA leads to accelerated differentiation of the C2C12 myoblast cell line. Conversely over-expression of nestin inhibits the differentiation process (Pallari et al., 2011). This effect is due to nestin's ability to regulate Cdk5 activity through modulation of p35 turnover; blockage of Cdk5 activity overrides the nestin over-expression effect (Pallari et al., 2011).

2.4 Models to study IFs

2.4.1 Transgenic mice as models to study IFs

Genetic engineering of mice has led to many breakthroughs in the biological sciences. The functions of IFs have, thus, been studied with the use of knockout and knock-in mice. The mouse as a model organism as well as some of the properties of IFs have made these studies difficult and, in some cases, unsuccessful. Despite these shortcomings, knockout and knock-in mice as well as mice expressing fluorescently tagged proteins are invaluable tools in the IF-field.

2.4.1.1 Knockout approaches

The knockout of a protein is the disruption of the corresponding gene by recombinant DNA technology, which makes all cells in the animal lack the

protein. In conditional knockouts the gene of interest is disrupted in specific cells constitutively or after external induction; this is achieved by first producing animals with the gene of interest flanked by loxP-sites, and then by crossing these with animals expressing Cre-recombinase in a cell-specific manner or under an inducible promoter. The recombinase will then cleave the DNA at the loxP-sites, leading to knockout of the gene only in the specific cells.

Although knockout mice have given much information about IF functions, many questions have not been answered with these animal models. One of the main problems with IF-knockout mice is that many IFs have partly overlapping functions and, thus, can compensate for each other in vivo. Another problem is that many phenotypes are subtle and require detailed investigation to be identified. Thirdly, the phenotypes caused by lack of an IF may become apparent only after stress and are therefore not detected in laboratory animals at normal circumstances. A few examples of IF-knockout mice are discussed in more detail below to give insights into some of the challenges with the knockout approaches.

Epidermolysis bullosa simplex is a devastating human disease caused by mutations in keratin 5 or 14. Thus it was a great advance that the knockout of keratin 14 led to a blistering phenotype resembling the human disease, and that these mice could be used for enhancement of the understanding of the disease (Lloyd et al., 1995). Normally, keratin 14 forms heterofilaments with keratin 5: the knockout of keratin 14 would, thus, also disturb the function of keratin 5. However, investigation of the mice lacking keratin 14 led to the discovery that keratin 5 can bind to keratin 15 in the absence of its normal binding partner, and thus still form filaments. This unusual keratin 5-15 filament partly dampens the effect of keratin 14 knockout, as shown by the early postnatal death of keratin 5 knockout mice which lack both 5-14 and 5-15 filaments (Peters et al., 2001).

In contrast to the keratin 14 knockouts, mice lacking vimentin were first reported to develop without a phenotype (Colucci-Guyon et al., 1994). However, later studies have shown that vimentin is important for many cellular functions, and that the mice develop phenotypes in response to various stresses such as wound healing (Eckes et al., 2000) or reduction of kidney mass (Terzi et al., 1997). The combination of GFAP and vimentin knockout has a more profound effect on the healing of injured CNS than the single knockouts (Pekny et al., 1999). This shows that vimentin and GFAP partially can compensate for each other in the CNS.

Lamin A/C was first knocked out in 1999 by disruptions of exons 8-11 of the lamin gene (Sullivan et al., 1999). Later the original lamin knockout mouse was shown to express a truncated form of lamin (lamin A Δ 8-11) (Jahn et al., 2012). A lamin A knockout constructed with a gene trap strategy has several phenotypic differences compared to the original mouse (Kubben et al., 2011). Both these mice strains show reduced weight gain, skeletal and cardiac muscle myopathy, and early death (Kubben et al., 2011; Sullivan et al., 1999). Mice homozygous for the genetrapp allele, however, die at an age of 16-19 days, whereas the lamin A Δ 8-11 mice survive until 8 weeks after birth. Interestingly, a third lamin A null mice strain, a conditional knockout, shows an identical life span to the gene trap model (Kim and Zheng, 2013). Furthermore, mice heterozygous for the original lamin A knockout develop cardiac myopathy, whereas genetrapp heterozygous mice appear to be healthy (Kubben et al., 2011; Wolf et al., 2008). These results indicate that the truncated lamin A Δ 8-11 has retained at least some of the functions of full length lamin A, and that the truncated form even can be harmful (Jahn et al., 2012). This shows that even a successful disruption of an IF gene can be unsuccessful in deleting the protein function due to the structural features of IFs.

2.4.1.2 Knock-in approaches

Deleting a protein does not always give much information about the functions of that particular protein. In these situations, the expression of a mutated or truncated version of the protein, instead of the normal form, can give greater insights into its roles. In knock-in mice, a normal copy of the gene of interest is exchanged to a mutated form using recombinant DNA technology.

This strategy has been employed to study disease-causing IF mutations as well as the function of certain regions of IFs *in vivo*. The laminopathies are the most studied IF-caused diseases, and several mice models for various laminopathies have been created (Zhang et al., 2013). These models have given new insight into the phenotypes of many laminopathies, though some mouse models do not phenocopy the human disease caused by the corresponding mutation. For example, the mouse overexpressing lamin A M371K develops acute cardiac damage that is much more severe than the corresponding human Emery-Dreifuss muscular dystrophy (EDMD), where patients develop cardiac damage gradually (Wang et al., 2006).

The deletion of vimentin did not reveal much about the molecular functions of this abundant protein (Colucci-Guyon et al., 1994). As vimentin is heavily

phosphorylated with numerous identified but so far sparsely studied phosphorylation sites, a different strategy to gain insight into both the functions of vimentin and the role of phosphorylation was employed. Matsuyama et al (2013) created mice expressing vimentin where all the sites known to be phosphorylated during mitosis were mutated to mimic an un-phosphorylated state (Matsuyama et al., 2013). In the lens (the only organ studied this far) of these mice the cells fail to undergo proper cytokinesis at the end of mitosis. This leads to an accumulation of bi-nucleated cells, aneuploid cells and the development of cataract. This underscores the importance of phosphorylation for breakdown of IFs during mitosis (Izawa and Inagaki, 2006; Sahlgren et al., 2001).

The knockout of lamin B1 and lamin B2 generated the question of how and if these B-type lamins have overlapping functions. The approach to investigate this issue was to develop knock-in mice which express lamin B1 in the lamin B2 locus and mice that express lamin B2 in lamin B1 locus (Lee et al., 2014). These mice made it possible to prove that lamin B1 and B2 cannot functionally be exchanged for each other during brain development, although the extra amount of one B-type lamin somewhat reduced the phenotypes of lack of the other. The strategy to exchange one of the lamin B genes for the other offers a solution for the ever present problem regarding compensatory effects of other IFs in lack of function approaches. A similar strategy has also been used to create mice lacking all type II keratins, and thus all keratin filaments (Bär et al., 2014). In reality it is of course not possible to exchange all the different IFs for just one, although it would be an interesting approach, but for some of the closely related genes this strategy can offer new insights.

As discussed earlier, the devastating accelerated aging disease HGPS is caused by a mutation of lamin A that gives rise to a truncated protein form named progerin (Eriksson et al., 2003). To study the mechanisms behind this disease several mouse models have been created (Jung et al., 2014; Osorio et al., 2011; Varga et al., 2006; Yang et al., 2005); reviewed in (Zhang et al., 2013). The different approaches used to create mice expressing progerin, ranging from introduction of point mutations in the mouse *LMNA* gene to knock-in of the mutated human form, have given rise to surprisingly different phenotypes (Zhang et al., 2013). The phenotype dispersion cannot be easily explained, but most probably it arises from the different strategies and vectors used. Although all the progerin mice show some resemblance with the human patients, they are

not a perfect phenocopy of the human disease. Nevertheless, these mice are still useful for aging studies.

2.4.1.3 Fluorescence to study IF gene activity and protein dynamics

One of the challenges in visualizing IFs in tissue is the cumbersome labelling processes relying on antibodies. To be able to detect IFs in tissue sections, as well as in live mice, fluorescent proteins have been utilized.

There are several different mice strains with nestin promoter driven GFP-expression. These mice are often, perhaps misleading, referred to as nestin-GFP mice. The original strain expresses GFP under the nestin promoter and the enhancers from the second intron of the rat nestin gene (Yamaguchi et al., 2000). In another strain a similar approach was utilized, but a longer part of the promoter was used (Mignone et al., 2004). Furthermore, a mouse which expresses GFP under the control of a hsp68 promoter and the nestin second intron enhancer element has been generated (Kawaguchi et al., 2001). The mice developed by Kawaguchi et al (2001) were later further developed when the GFP was exchanged for dVenus, a fluorescent protein with short half-life (Sunabori et al., 2008). The fast degradation of dVenus made it possible to follow the activity of the nestin enhancer element in real-time during the different phases of the cell cycle (Sunabori et al., 2008). Yet a different strategy was employed to create mice expressing GFP under the second intron enhancer and thymidine kinase promoter (Walker et al., 2011). The expression patterns of GFP vary between the different strains (Walker et al., 2011; Yamaguchi, 2005), and some are reported to express GFP in cells where no nestin could be detected at protein level (Mignone et al., 2004).

Although mice expressing GFP under the nestin promoter and/or enhancers do not give any information about nestin protein levels, these mice give invaluable information about where and when the nestin gene is expressed. These mice have also become a valuable tool for identifying nestin positive cells, as well as processes relying on these cells. Apart from identifying nestin positive cells in the central nervous system (Kawaguchi et al., 2001; Mignone et al., 2004; Walker et al., 2011; Yamaguchi et al., 2000), these mice have also been used for identification and isolation of satellite cells from muscle (Day et al., 2007), and to identify and follow stem cells in various tissues (Jiang et al., 2014; Li et al., 2003). More recently the nestin GFP mice have been crossed with nude mice (Amoh et al., 2005) and used for live imaging of angiogenesis in tumour bearing mice (Aki et al., 2014).

Apart from the mice expressing fluorescent proteins under direct control of the nestin promoter and enhancer elements, mice that express tamoxifen inducible cre-recombinase under the nestin promoter have been created (Lagace et al., 2007). These mice have been crossed with mice having fluorescent proteins with loxP-sites to create mice where nestin positive cells can be induced to become fluorescent (Chow et al., 2015; Lagace et al., 2007). This setup allows for fate tracking of certain cells as well as identification of nestin positive cells in adults.

Lately, mice that can be used in a more direct way to study IF dynamics have been created. The keratin 8-YFP mice carry an YFP sequence inserted in frame with the endogenous keratin 8 gene, an approach which gives rise to a keratin 8-YFP fusion protein expressed and regulated as the endogenous keratin 8 (Schwarz et al., 2015). In contrast to the nestin-GFP mice the keratin 8-YFP mice do not only give information about protein expression, but can also be utilized for live imaging of keratin dynamics. The advantages of the keratin 8-YFP mice compared to classical staining methods are obvious, and the mouse model has already provided enhanced knowledge about the intercellular arrangements and dynamics of keratin 8 (Schwarz et al., 2015).

2.4.2 Invertebrate models to study IFs

Although the studies of genetically engineered mice have given many valuable insights into the functions of IFs, mice have several drawbacks as animal models. Most apparent are the cost and long generation turnover. As IFs are evolutionary conserved and present in all animals, invertebrates can be used to study IFs. Most commonly the nematode *Caenorhabditis elegans* (*C. elegans*) has been employed.

C. elegans has 11 genes coding for IFs, four of which are essential for development and survival (Karabinos et al., 2001). Given that the cytoplasmic IFs in *C. elegans* are more closely related to the nuclear lamins than to mammalian cytoplasmic IFs, insights from the nematode cytoplasmic IFs cannot be directly translated to human findings. Nevertheless, the nematode offers a splendid platform for studying general aspects of IFs. Furthermore, the nematode lamin has direct human counterparts, and corresponding mutations have been proven to be a valuable tool for laminopathy research (Bank et al., 2012).

The fruit fly, *Drosophila melanogaster* (*D. melanogaster*) belongs to the arthropods, animals with exoskeletons, a phylum that has long been considered to lack cytoplasmic IFs (Bartnik and Weber, 1989). *D. melanogaster* has two lamins resembling the human A- and B-type lamins, lamin C and lamin Dm₀, respectively. Knockout of lamin C in *Drosophila*, as well as expression of mutant human lamin, has successfully been employed for studies of lamin function (Lyakhovetsky and Gruenbaum, 2014). Knockout of lamin C, the *Drosophila* A-type lamin, leads to lethality in the larval or pupal stages of development (Schulze et al., 2005; Schulze et al., 2009; Uchino et al., 2013). More specifically, the lack of lamin C affects muscle function by causing disorganization of the tendon nuclei and faulty MTJs (Uchino et al., 2013). This finding is interesting as it offers a new explanation for the mechanisms causing the muscle laminopathies. Unfortunately, tendon cells have not yet been investigated in human laminopathy patients.

Recently, a *Drosophila* model expressing the human keratins 5 and 14 has been established (Bohnekamp et al., 2015). The authors also introduce an EBS causing mutant to the flies. While the wild type keratins seemingly do not affect the flies, the mutant keratins give rise to a phenotype surprisingly similar to the human disease with blistering of the wings. This research not only shows that the expression of human cytoplasmic IFs in flies is possible, but also that disease-causing mutations can be studied in this system.

In summary, these examples prove that invertebrates can be utilized for IF-research, but also that, like mice, they have certain shortcomings. Most of the problems with invertebrate models for human IF-research stems from the evolutionary distance, but for basic research invertebrates remain an interesting alternative.

2.5 Cdk5 – an IF interacting kinase

Cdk5 belongs to the class of cyclin dependent kinases, but unlike other cyclin dependent kinases Cdk5 is not regulated in a cell cycle dependent manner, and until recently it was thought to be regulated only by non-cyclin proteins (Arif, 2012; Contreras-Vallejos et al., 2012; Dhariwala and Rajadhyaksha, 2008). The most studied Cdk5 activators are the non-cyclin proteins p35 and p39 and their cleavage products p25 and p29. In addition, Cdk5 has been shown to interact with cyclin D, cyclin E, cyclin G₁ and cyclin I; reviewed in (Arif, 2012). The

different activators can both influence Cdk5 localization and target range (Brinkkoetter et al., 2009; Hagmann et al., 2015).

The most well-known Cdk5 activators, p35 and p39, are myristoylated, a PTM that targets them, and the Cdk5 activity they induce, to the plasma membrane (Patrick et al., 1999). When p35 is cleaved to p25 by calpains the myristoylation is lost, leading to de-attachment from the membrane and a localization shift in the Cdk5 activity. The cleavage of p35 does not only permit a shift in localization; p25 is a more potent Cdk5 activator as it has a substantially longer half-life due to being protected from proteasomal degradation (Patrick et al., 1998). The idea that localization of the different activators drives Cdk5 activity to certain parts of the cell has recently been brought to attention (Asada et al., 2012; Hagmann et al., 2015). With the use of p35^{-/-}, cyclin I^{-/-} and p35^{-/-} cyclin I^{-/-} mice it has been demonstrated that cyclin I sequesters Cdk5 in the nucleus, whereas p35 drives a cytoplasmic localization (Hagmann et al., 2015). Interestingly, not only the activators but also their Cdk5-induced phosphorylation regulate Cdk5 localization, at least in the case of p35 and p39 (Asada et al., 2012). Furthermore, the localization of Cdk5 is cell type-dependent, as the different Cdk5 activators have distinct expression patterns in different tissues.

Cdk5 was initially thought to harbour kinase activity only in the brain and in nervous tissue (Tsai et al., 1993). Its presence is essential for brain development, as disruption of the Cdk5-gene leads to embryonic or early post-natal death due to under-development of the brain (Ohshima et al., 1996). Deregulated Cdk5 has also been linked to several neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases; reviewed in (Cheung and Ip, 2012). The deregulated neuronal Cdk5 activity is associated with production of protein inclusions; in Alzheimer's, for example, the excess phosphorylation of Tau and NFs by Cdk5 drives the formation of neurofibrillary tangles (Shukla et al., 2012).

Nevertheless, more recent studies have detected Cdk5 activity and showed functions for Cdk5 outside the nervous system in tissues such as kidney, pancreas and muscle; reviewed in (Arif, 2012; Contreras-Vallejos et al., 2012; Rosales and Lee, 2006). Cdk5 has received attention in conjugation to diabetes, as it regulates both insulin secretion (Lee et al., 2008) and glucose uptake (Lalioi et al., 2009), as well as in cancers, as it has functions in processes such as migration and angiogenesis; reviewed in (Liebl et al., 2011), but the non-neuronal functions of Cdk5 have remained less studied than the neuronal functions. In models of muscle differentiation Cdk5 activity has been shown to

be important for regulation of differentiation, both in mouse and rat cell-lines as well as in *Xenopus* embryos (Lazaro et al., 1997; Philpott et al., 1997). More specifically p25-driven Cdk5 activity has been determined as a critical factor for the onset of differentiation (de Thonel et al., 2010).

The relationship between IFs and Cdk5 is complex; NFs (Pant et al., 1997; Sharma et al., 1998), vimentin (Lee et al., 2012), nestin (Sahlgren et al., 2003) and lamin (Chang et al., 2011) have all been identified as Cdk5 substrates. As noted above, at least nestin also works as a scaffold for Cdk5 and regulates its localization as well as its activity by regulating p35 turn-over (Figure 5) (Sahlgren et al., 2003; Sahlgren et al., 2006). The interplay between Cdk5 and nestin has been shown to regulate myogenic differentiation in vitro (Pallari et al., 2011; Sahlgren et al., 2003). The nestin-driven regulation of Cdk5 also underlies the organisation of NMJ in vivo, as mice with down-regulated nestin show clustering of NMJs, underscoring the role of nestin as a Cdk5 scaffold (Yang et al., 2011).

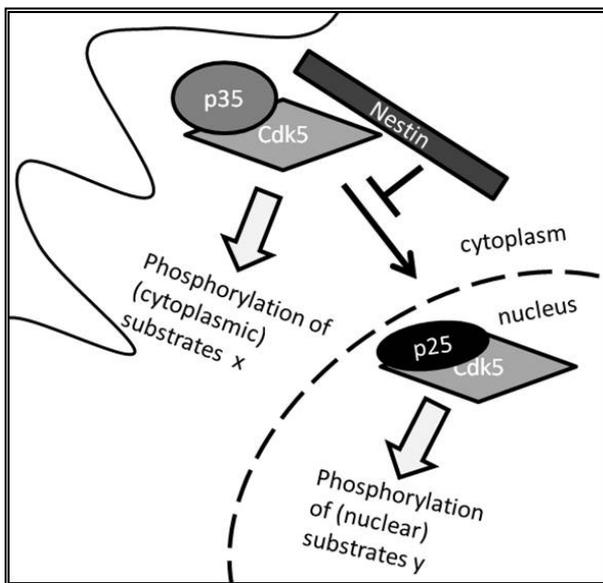


Figure 5. Nestin as a regulator of Cdk5 activity and localization. In the cytoplasm, nestin forms a scaffold for p35 and Cdk5. This scaffold prevents the cleavage of p35 to p25 and the transport of p25/Cdk5 into the nucleus.

2.6 Satellite cells and muscle regeneration

The long multinucleated cells of the muscle, the muscle fibers, do not divide, but yet the number of nuclei in a fiber can increase and the fibers can grow in size (and when injured, re-grow). These processes depend on stem cells that reside outside of the muscle fiber.

These satellite cells are the primary stem cells of muscle; under normal conditions they reside in a quiescent state under the basal lamina of myofibers. The satellite cells are identified by their expression of certain marker proteins: mostly used is pax7, a transcription factor expressed by quiescent and early activated satellite cells (Yin et al., 2013). Although mostly mitotically inactive under normal conditions, satellite cells can divide in a symmetrical fashion, giving rise to two stem cells, to keep the stem cell population size intact. Activated satellite cells can also divide asymmetrically, giving rise to one stem cell and one cell committed to differentiation (Yin et al., 2013).

Muscle regeneration after injury is a distinct process, although it resembles and shares some mechanisms with developmental muscle growth. The first regenerative processes start immediately after a muscle injury: the damaged myofibers undergo necrosis and an inflammatory response starts. Neutrophils and macrophages invade the injury site and take part in the clearing process; the later macrophages also secrete factors that stimulate myoblast proliferation and differentiation; reviewed in (Ciciliot and Schiaffino, 2010; Yin et al., 2013). Soon after an injury the satellite cells become activated and start proliferating. The activation of satellite cells is a complex process regulated by numerous secreted factors such as growth factors, interleukins and nitric oxide; reviewed in (Chargé and Rudnicki, 2004; Yin et al., 2013). The satellite cells differentiate and fuse with existing myotubes to rebuild the muscle (Figure 6). The last step of the regeneration process is to re-obtain muscle homeostasis, this step includes re-building of NMJs and MTJs as well as re-growth of blood vessels (Ciciliot and Schiaffino, 2010).

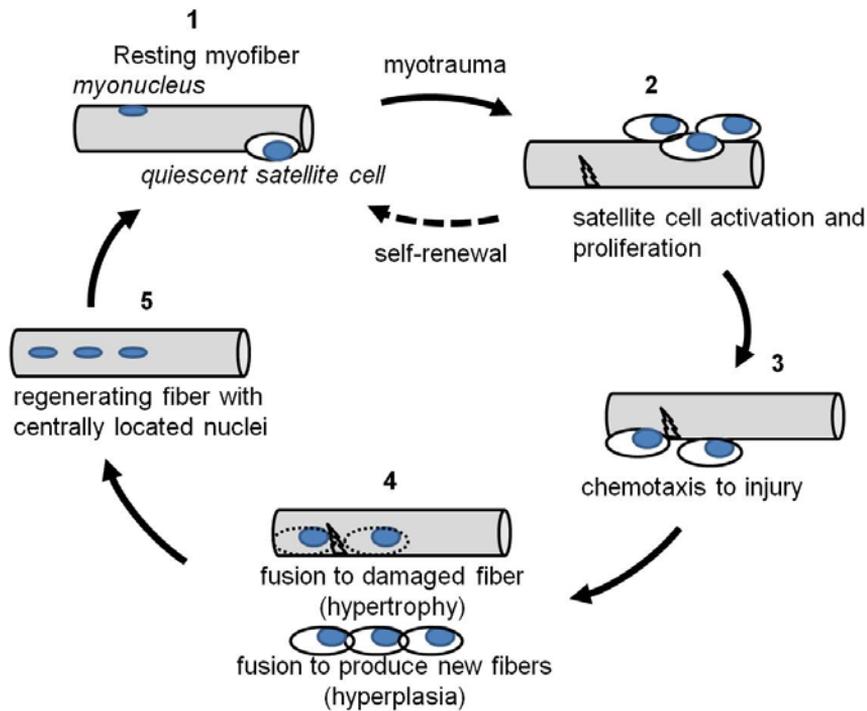


Figure 6. Muscle regeneration. Under normal conditions (1) the myofibers are resting and the satellite cells reside under the basal lamina in a quiescent state. Upon myotrauma (2) the satellite cells get activated and start to proliferate. Some of the new cells will become new quiescent stem cells (self-renewal), whereas most will migrate to the injured muscle fiber (3), undergo differentiation and fuse either with an existing fiber or with each other to form a new fiber (4). The regenerating myofiber (5) is characterised by centrally located nuclei, but over time the nuclei will migrate to the periphery of the fiber as the myofiber enters a resting state (1). Figure adapted from (Hawke and Garry, 2001).

2.6.1 Models to study muscle differentiation and regeneration

The most commonly used cell line to study muscle differentiation *in vitro* is the C2C12 line. These cells originate from an adult, dystrophic, female mouse and readily form myotubes in culture in response to high confluency and serum withdrawal (Yaffe and Saxel, 1977). Although, or maybe because, this cell line has been used for several decades it has some shortcomings. To gain a more physiologically relevant cell culture model, primary myoblasts can be isolated from mouse muscle. There are many, slightly different, protocols for primary myoblast isolation, but they are all based on physical disruption and enzymatic

digestion of whole muscles, followed by culture of the cells under conditions promoting myoblast survival. The downside with primary myoblast culture, apart from the need of animals and the workload, is that they are, to a greater or lesser extent, contaminated by other cell types such as adipocytes and fibroblasts. To achieve pure satellite cell cultures, cell sorting of muscles digests can be used. Commonly the nestin-GFP and Pax7-GFP mice strains, as well as sequential antibody labelling of satellite cell specific proteins, have been used for this (Bosnakovski et al., 2008; Day et al., 2007; Pasut et al., 2012). Another method to achieve pure satellite cell cultures is the isolation of intact myofibers with their associated satellite cells; overviewed in (Keire et al., 2013).

Multiple injury models have been established to study the regeneration of injured muscle *in vivo*. To mimic common sport-type injuries, physical force such as blunt impact, crush, tear or local incisions have been used; reviewed in (Carlson, 2008; Souza and Gottfried, 2013). In addition, models that take advantage of myotoxins such as snake venoms and local anaesthetics are often used (Carlson, 2008). These injuries are cleaner than the physically induced injuries, but have the disadvantage of being less clinically relevant. The model system to be used has to be chosen depending on which of the regeneration processes one intends to study.

3 Aims

The aim of this work was to study IFs at the molecular, cellular and organism level to gain more basic knowledge of these proteins. Lately the IFs have emerged as important regulators of cell signalling events. This has led to a shift in the IF-research from structural properties to functional aspects of IFs. When this work began, the phosphorylation of lamins had mainly been studied in the context of mitotic reorganisation of the lamina (Dessev et al., 1990; Dessev et al., 1991; Heald and McKeon, 1990; Peter et al., 1990). As phosphorylation had been shown to influence many IFs in more than just structural manners, we wanted to explore the effects of lamin A phosphorylation outside mitosis and investigate how phosphorylation regulates the localization and dynamics of lamin A.

Nestin had previously been identified in many cell types and tissues and there were limited studies on nestin in myogenic differentiation. The role and function of nestin *in vivo*, however, was unknown. As the nestin^{-/-} mice became available, we wanted to expand our previous work, where nestin had been identified to influence muscle cell differentiation *in vitro* and to be expressed in muscle regeneration *in vivo* (Pallari et al., 2011; Sahlgren et al., 2003; Vaittinen et al., 2001). We aimed at studying how the absence of nestin influences cell and body homeostasis, with emphasis on the functions of nestin during muscle regeneration and homeostasis

The absence of cytoplasmic IFs in arthropods has long baffled the IF-field. At the same time the need for new model organisms to study IFs has become apparent, both for ethical and economic reasons, and in order to avoid the risk of compensation by a related IF. We wanted to tackle both of these questions by exploring the possibility to express a human cytoplasmic IF in *Drosophila melanogaster*.

4 Materials and Methods

More detailed information on the materials and methods used can be found in the original publications and manuscripts.

4.1 Standard methods and Cell lines used

A summary of standard methods and cell lines that I have used in the indicated manuscripts is found bellow.

HeLa human cervical cancer cell line (I)

ST15A rat CNS progenitor cell line (unpublished) (Frederiksen et al., 1988)

Primary mouse myoblasts and satellite cells (II)

Schneider 2 (S2) Drosophila cells (III)

Cell culture (I, II, III, unpublished)

Microscopy (I, II, III)

Site-directed mutagenesis (I)

Western blot (I, II, III, unpublished)

Induction of muscle injury (II)

4.2 GFP-tagged lamin constructs

The full-length human prelamin A cDNA cloned into pEGFP-C1 was cloned in Dr. Robert Goldman's lab (Moir et al., 2000). When expressed in cells, this plasmid gives rise to prelamin A with GFP fused to the N-terminal end, the protein undergoes the same posttranslational processing as endogenous prelamin A. Mutations in the original plasmid were induced using QuikChange site-directed mutagenesis kit (Stratagene). In order to create plasmids which mimic an un-phosphorylated state, the targeted serine residues were changed to alanine. Alanine has a non-reactive, neutral side chain and is therefore ideal to use for a non-phosphorylated substitute. Aspartate was used to mimic constitutive phosphorylation; aspartate has a negatively charged side chain that mimics the charge of a phosphate group.

4.3 Primary myoblast and myofiber cultures

Primary myoblasts were isolated from adult mice and from pups at postnatal day 3 as previously described (Danoviz and Yablonka-Reuveni, 2012). This protocol gives a good yield of myogenic precursor cells, but the cultures are frequently contaminated with other cell types, like fibroblasts. These cells do not affect the analysis of molecules specific to the myogenic lineage; they do, however, influence the non-myogenic transcriptome, which can be problematic in certain analyses. As the yield from this protocol is very high, it was used for western blot analyses of myoblast differentiation as well as for molecular studies of Cdk5.

To obtain cleaner satellite cell cultures, intact myofibers from adult mice were isolated as previously described (Keire et al., 2013). This method results in whole live myofibers cultured floating in media. Activated satellite cells then migrate out from the fibers and start proliferating and differentiating. This method gives rise to very clean cultures, but the yield is low. The myofiber cultures were used for microscopic imaging of satellite cell numbers and their differentiation. These kinds of analyses give very specific data in contrast to the bulk data acquired from myoblast cultures.

4.4 Additional methods

To identify lamin residues phosphorylated by Cdk5, an IF-preparation done according to (Sahlgren et al., 2001) was phosphorylated *in vitro* with recombinant Cdk5/p25 complex as described previously (Lindqvist et al., 2015). The phosphorylated samples were separated by SDS-PAGE and the bands corresponding to lamins were cut out and analyzed by mass-spectrometry as in Kochin et al., (2014).

In order to investigate the influence of lamin A on p35 localization, ST15A cells transfected with p35 and GFP or GFP-lamin A were fractionated. The fractionation protocol from Markiewicz et al., (2005) allows for separation of the nuclei from the cytoplasm; the nuclei are then further extracted with buffers containing increasing amounts of salt for separation into low salt soluble, high salt soluble and insoluble fractions.

Cycloheximide (CHX) is an inhibitor of protein synthesis which can be utilized for determination of protein degradation rate. ST15A cells were transfected with p35 and GFP or GFP-lamin A. 15 to 20 h after the transfection the cells were treated with 30µg/ml CHX, then lysed after 1 h and 2 h, and p35 content was analysed using western blotting.

To determine Cdk5 activity ST15A cells were transfected with GFP-lamin A or GFP, lysed, and then Cdk5 was immunoprecipitated and used to phosphorylate histone in presence of ³²P-ATP as described in (Lindqvist et al., 2015). The amount of incorporated ATP was analyzed with Phosphoimager.

To analyse cell death ST15A cells transfected with GFP-lamin A or GFP were re-suspended in propidium iodine buffer (40 mM Na-citrate, 0.3% Triton-X 100, 50µM propidium iodine) and analysed with LSRII flow cytometer (BD Biosciences). Each sample was run in triplicate. The fraction of sub-G1 events (nuclear fragmentation) was gated as a measure of apoptotic cell death.

Serum testosterone was determined from serum collected from 3-month-old *nestin*^{-/-} and wild type male mice by cardiac punctation. The serum was analysed with liquid chromatography–tandem mass spectrometry at the Department of Pharmacy, University of Eastern Finland. The detection limit for testosterone was 0.08 nM.

5 Results and Discussion

5.1 Phosphorylation determines lamin A/C localization and mobility (I)

Lamin A/C has previously been shown to be heavily phosphorylated, and many of the phosphorylation sites have been identified (Simon and Wilson, 2013). Yet few of the identified sites have been studied in detail, as most of the identification has been in high-throughput mass-spectrometry analyses. Furthermore, many sites have been detected in mitotic or mixed cell populations, leaving the role of steady-state interphase phosphorylation of lamin A/C open. To address these questions we performed a study combining mass-spectrometry results from interphase cells with site directed mutagenesis and detailed imaging based studies.

We identified 20 phosphorylation sites from interphase HeLa-cells. (Figure 7, I Table 1). In order to study how the phosphorylation of these sites affects primal lamin properties such as mobility and localization, GFP-tagged lamin constructs were generated. By site directed mutagenesis we introduced mutations of the most occupied phosphorylation sites that we identified. To mimic the un-phosphorylated state serine was mutated to alanine, and to mimic phosphorylation mutation of serine to aspartic acid was utilised.

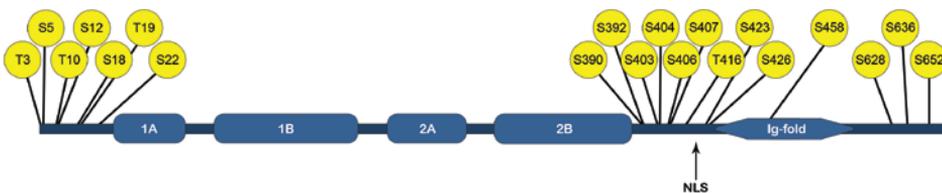


Figure 7. Lamin A/C phosphorylation sites. Summary of the phosphorylation sites identified in (I). Figure from (Torvaldson et al., 2015).

To determine the effect of the mutations, which are indicative of the effect of phosphorylation, on lamin A localization, mobility and solubility, confocal microscopy in conjunction with triton-X extraction, and fluorescence recovery after photobleaching (FRAP) were used. In addition fluorescence correlation spectroscopy (FCS) was utilised. The results from these experiments are summarized in Tables 3-5 (I Figures 2-7). The previously identified mitotic

phosphorylation sites S22 and S392 (Dessev et al., 1990; Dessev et al., 1991; Heald and McKeon, 1990; Peter et al., 1990) clearly affect lamin A also in interphase. Our results indicate that S22 is dominant over S392, but that phosphorylation of both these sites work together to regulate lamin A properties.

The S22D, S392D doublemutation was further combined with a S628D mutation, to create a triple phosphomimetic construct. This construct unexpectedly localizes to the cytoplasm in part of the cells (I Figure 8). This suggests that sequential phosphorylation could lead to the exclusion of lamin A from the nucleus. In the light of the recently discovered cytoplasmic function of lamin B2 (Yoon et al., 2012), it is tempting to speculate about a similar role for cytoplasmic lamin A, but to date there is no in vivo evidence for triple phosphorylation of lamin A nor a confirmed cytoplasmic role has been shown. S628 is also interesting because this residue is located in the part missing from progerin, the pathological form of lamin A expressed in Hutchinson-Gilford progeria syndrome (Eriksson et al., 2003). The loss of the S628 phosphorylation, with yet unknown functions, offers a further explanation for the toxicity of progerin.

Table 3 Effect of mutations on lamin A localization

Site	S to A (phosphodeficient)	S to D (phosphomimetic)
S12	no effect	no effect
S22	no effect	nucleoplasm
S390	lamina	no effect
S392	lamina	nucleoplasm
S404 S407	no effect	nucleoplasm
S423	no effect	no effect
S628	no effect	no effect
S22, S392	lamina	nucleoplasm
S22D, S392A	-	nucleoplasm
S22A, S392D	-	no effect

Table 4 Effect of mutations on lamin A mobility

Site	Lamina	Nucleoplasm
S22D, S392D	++	+
S22D, S392A	+	+
S22A, S392A	--	no effect
S22A, S392D	-	+

Table 5 Effect of mutations on lamin A solubility

Site	Lamina	Nucleoplasm
S22D, S392D	+	+
S22D, S392A	+	+
S22A, S392A	-	-
S22A, S392D	-	-

5.2 The physiological relevance of lamin A/C phosphorylation

With very few exceptions, the laminopathy causing mutations does not affect known phosphorylation sites. The fact that the lamin A/C phosphorylation sites show high evolutionary conservation (I, Figure 1) reflects their low tolerance for mutations and can explain why the phosphorylation sites are so rarely mutated. Any mutation affecting these sites would probably be lethal at an early developmental stage due to a dominant negative effect.

The only reported lamin mutation directly affecting an identified phosphorylation site is a heterozygous lamin A S22L mutation (Pethig et al., 2005). The patient with this mutation suffered from dilated cardiomyopathy requiring a heart transplant. As there is no detailed description of this case, it is difficult to know what effects such a mutation has on the cellular level.

Proline 4, which resides between T3 and S5, both of which were identified in our study of interphase phosphorylation (I), has been reported to be mutated in a mild progeria like syndrome (Garg et al., 2009; Guo et al., 2014). The mutation

of this proline will most likely influence the phosphorylation of the surrounding amino acids as the proline is part of a SP-motif.

Although few cases of direct mutations of lamin A/C phosphorylation sites have been reported in laminopathies, there are indications that phosphorylation is indirectly affected by several mutations. In EDMD, the level of N-terminal lamin A/C phosphorylation is decreased in muscle cells, but not in fibroblasts. The exact site for this phosphorylation has, however, not been determined (Cenni et al., 2005). In another study, the phosphorylation of S458 in patients with lamin A mutation-associated myopathies was investigated (Mitsuhashi et al., 2010). The authors found that S458 was specifically phosphorylated in cells from myopathy patients with lamin A/C Ig-domain mutations, but not in cells from patients with other myopathies or mutations in other parts of lamin A/C. Furthermore, Akt1 was shown to specifically phosphorylate S458 in mutated, but not in wild type lamin A. This specific phosphorylation could be due to the Ig-fold mutation, which may open up the lamin structure and expose the otherwise hidden S458 (Mitsuhashi et al., 2010). In contrast, we identified the phosphorylation of S458 from interphase HeLa-cells (Kochin et al., 2014), which would indicate that S458 also can be phosphorylated in wild type lamin A/C, and that this phosphorylation possibly has a non-pathological function. Although the phosphorylation of specific sites is altered in disease, the effect of this PTM change is difficult to distinguish from the effect of the disease-causing mutation.

Lamin A/C interacts with numerous signalling molecules (Simon and Wilson, 2013). Even though we did not investigate the effects of phosphorylation on any of these interactions, it will most likely affect the formation of contacts. For example the binding of Lap2 α has been mapped to residues 319-566 on lamin A (Dechat et al., 2000), a region where we identified numerous phosphorylation sites. Furthermore, the interaction between Lap2 α and lamin A/C takes place in the nucleoplasm, and is thus dependent on lamin A/C localization, which in turn is dependent on phosphorylation. As the cell-cycle regulatory function of pRb is influenced by the lamin A/C and Lap2 α binding, phosphorylation of lamin A/C can have consequences for cell-cycle progression.

The specific phosphorylation of lamin A/C could, thus, have many different effects: it affects protein localization and mobility, it could affect protein structure and accessibility of buried amino acid residues, and it could affect protein-protein interactions and downstream signalling. We propose a model where extracellular factors, such as stress-signals or substrate stiffness, act

through kinases and phosphatases to induce changes in lamin A dynamics. The lamin A dynamics in turn affect the interactions and functions of lamin A resulting in signalling output, such as altered transcription or cell motility (Figure 8) (Torvaldson et al., 2015). Our study, as well as previous work, has concentrated on the mechanical functions of lamin A/C phosphorylation, while the signalling induced by this PTM remains little studied. Future studies emphasizing the physiological functions of lamin A/C phosphorylation will have a great impact on our understanding of the many diverse roles these proteins have.

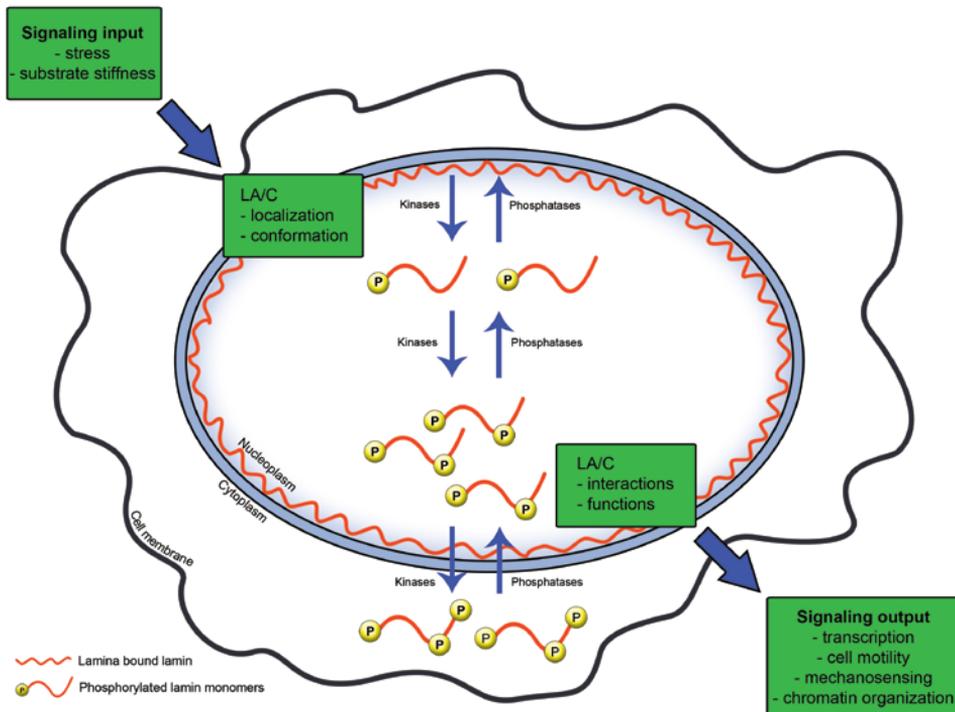


Figure 8. Lamin A/C phosphorylation as a signalling mediator. Information received from the environment influences lamin A/C localization and conformation. This, in turn, will influence lamin A/C functions and interactions, and in the end the cellular response to the stimuli. Kinases and phosphatases determine the lamin A/C phosphorylation status, which is the basis for lamin localization, conformation, function and interactions. Image from (Torvaldson et al., 2015).

5.3 Cdk5 phosphorylates lamin A/C (unpublished)

It has previously been reported that Cdk5 can phosphorylate lamins during neurotoxic stress (Chang et al., 2011). To confirm these findings, we used an *in vitro* phosphorylation strategy. Isolated IF-preparation from rat ST15A-cells was phosphorylated with recombinant Cdk5/p25 complex *in vitro*. The results clearly show a specific phosphorylation of the lamins (Figure 9). To investigate the nature of this phosphorylation the samples were analysed with mass-spectrometry (Figure 9). The analyses confirmed that lamin A/C indeed is phosphorylated by Cdk5. Two highly phosphorylated Cdk5-dependent sites were identified: T19 and S22, furthermore phosphorylation of S212, S390, S392 and S637 were observed at low rates. In the same sample phosphorylation of lamin B1, T5 was detected too, showing that B-type lamins also can be phosphorylated by Cdk5.

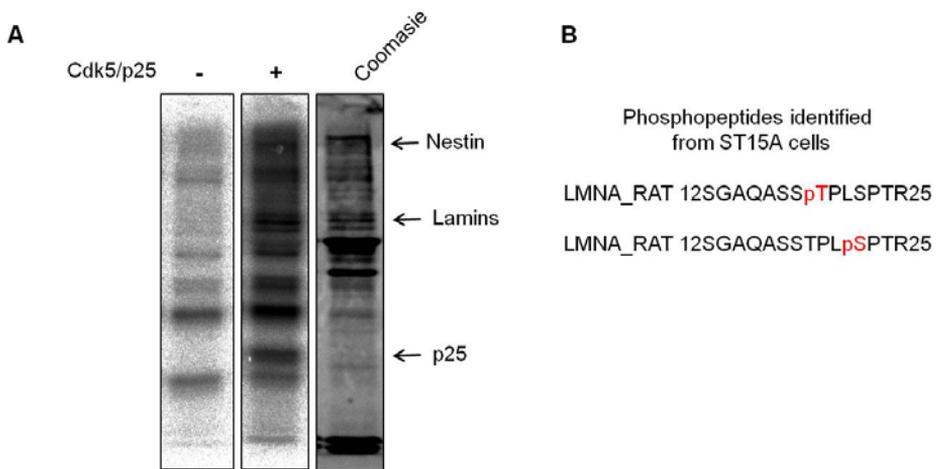


Figure 9. Lamin A is a Cdk5 substrate *in vitro*. A, The intermediate filament fraction was isolated from ST15A cells and phosphorylated *in vitro* with recombinant Cdk5/p25-complex in presence of ^{32}p -ATP. The amount of incorporated ATP was analyzed with phosphorimager. B, The lamin bands were extracted from the gel and analyzed with mass-spectrometry to identify phospho-peptides. Two lamin A sites were found to be specifically phosphorylated in the Cdk5 treated samples, T19 and S22.

As previous studies have showed that the IF nestin interacts with Cdk5 and the Cdk5-activator p35, targeting Cdk5 activity to the insoluble cellular fraction (Sahlgren et al., 2006), we wanted to find out if Cdk5 regulation is a common IF

feature. To study this we used over-expression of lamin A in the ST15A cell-line, in combination with a cell fractionation protocol, where the nuclear fraction is extracted with buffers with increasing concentrations of salt to gain soluble and less soluble nuclear fractions (Markiewicz et al., 2005). Using this protocol, we show that lamin A sequesters p35 into the nucleus, and further to the insoluble nuclear fraction (Figure 10 A). This would indicate that insoluble lamin A acts as a scaffold for p35 in the nuclear lamina.

To investigate if the localisation of p35 to the insoluble nuclear fraction can protect p35 from degradation, we examined the stability of p35 using the protein synthesis inhibitor cycloheximide. By inhibiting protein synthesis, and then following the degradation of proteins, it is possible to estimate the protein turnover rate. In the presence of over-expressed lamin A, p35 is degraded at a slower pace than in mock transfected cells (Figure 10 B). These results indicate that over-expression of lamin A both changes the localisation and stabilizes p35, possibly by protecting p35 from proteasomal degradation.

Next we investigated if the elevated p35 levels affect Cdk5 activity. To do this, ST15A cells over-expressing GFP-lamin A or GFP alone were used for a Cdk5 activity assay. As expected, the lamin A over-expression induced p35 stabilisation, which clearly increases Cdk5 activity (Figure 10 C). The physiological importance of the lamin A over-expression was investigated in a stress situation. ST15A cells were treated with 100 μ M H₂O₂ for 6 h to induce oxidative stress; the cells were then subjected to propidium iodine staining and FACS-analysis. The results show that cells over-expressing lamin A undergo apoptosis at a higher rate than control cells, as more cells have fragmented nuclei (cells in sub-G1 phase) (Figure 10 D). This indicates that the lamin A induced Cdk5 activity has pro-apoptotic function. To prove that the increased apoptosis is due to Cdk5 activity further experiments where Cdk5 activity is inhibited by use of kinase dead Cdk5 or with Cdk5 down-regulation will be needed.

Previously, nestin too has been reported to stabilise p35 and sequester it in the cytoplasm in ST15A cells, and to protect the cells from oxidative stress (Sahlgren et al., 2006). When our results are combined with the previous results, it is clear that both a cytoplasmic and a nuclear IF have the ability to stabilize and scaffold p35, indicating that the balance between these IFs would be crucial for the amount and localization of available p35 in the cell. As lamin A and nestin turned out to have the opposite effects in terms of cell survival, it is also clear that the sequestration of p35 has a physiological role. In addition to nestin and

lamin A, also NFs and vimentin are phosphorylated by Cdk5. It would therefore be interesting to look into which roles these IFs have on Cdk5 activity and localization. As previously noted, the different IFs are expressed in a highly regulated manner in different cell types and during differentiation. Cdk5 activity also differs in different cellular compartments and at different rates during the same processes. It is thus very tempting to speculate that the expression-profile of IFs could regulate localisation of Cdk5 activity in cells.

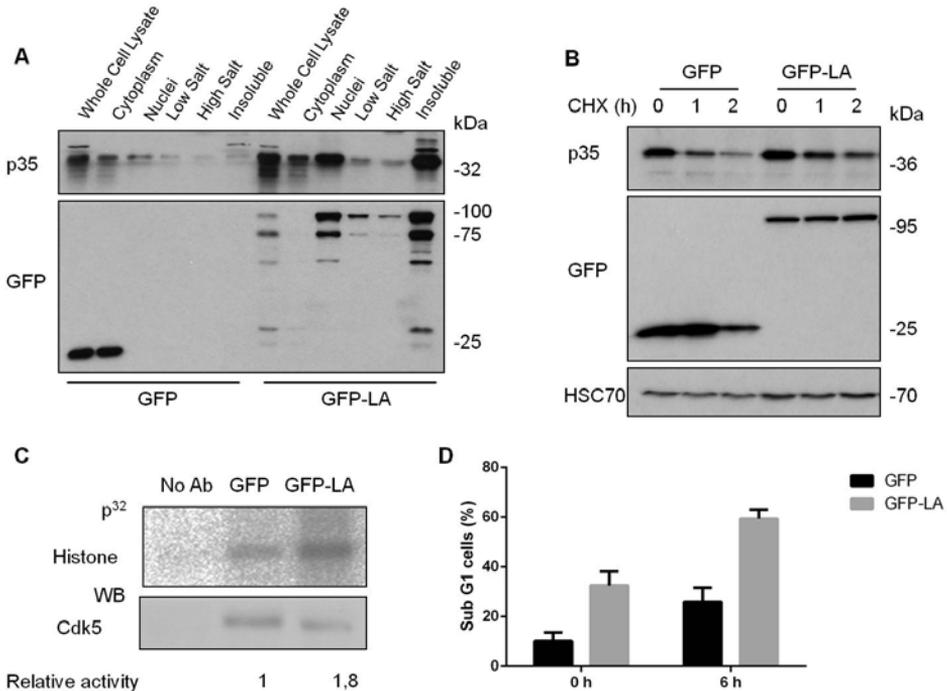


Figure 10. Lamin A influences p35 localisation and stability. ST15A cells were transfected with GFP or GFP-lamin A and, in addition, p35 (A, B). A, The cells subjected to cell fractionation. The cells over-expressing lamin A have a higher p35 content, and furthermore more p35 in the nuclear and especially the insoluble nuclear fraction. This suggests that lamin A confines part of the p35-pool to the lamina. B, The cells were treated with the protein synthesis inhibitor cycloheximide for indicated time, lysed and subjected to western blotting with indicated antibodies. The half-life of p35 is prolonged in the presence of lamin A; this indicates that lamin A protects p35 from degradation by sequestering it in the lamina. ST15A cells were transfected with GFP or GFP-lamin A (C, D). C, Cdk5 was immunoprecipitated from the cells and used to phosphorylate histone in

presence of ^{32}P -ATP. The amount of incorporated ATP was analyzed with phosphoimager. The results show that Cdk5 activity is increased in cells over-expressing lamin A. D, Cells were treated with $100\mu\text{M}$ H_2O_2 for 6h to induce oxidative stress and then subjected to propidium iodine staining followed by flowcytometry analysis. The GFP-lamin A over-expressing cells have decreased survival potential as shown by an increased amount of cells in the sub-G1 fraction. The bars represent average \pm SEM of two independent experiments. The western blots are representative from three independent experiments.

5.4 Depletion of nestin has no obvious effect on muscle stem cell behaviour (II)

Nestin is considered a stem cell protein, and nestin gene activity has been identified as a specific marker of quiescent satellite cells (Day et al., 2007). The down-regulation of nestin with siRNA in C2C12 myoblasts leads to a remarkable increase in myogenic differentiation (Pallari et al., 2011), showing that nestin plays an important role in regulating the differentiation pace. To investigate the stem cell number and behaviour following genetic ablation of nestin was thus the outset for our study of the nestin^{-/-} mice.

In primary myoblast isolated from nestin^{-/-} mice, no changes in differentiation were observed compared to corresponding wild type cultures. The protein levels of the Cdk5 activator p35 were, however, remarkably up-regulated in nestin^{-/-} myoblast cultures (II Figure 4). This was in line with elevated Cdk5 activity as measured by Cdk5 assay (II Figure 4). In previous studies, we have shown that Cdk5 activity, and in particular p25-driven Cdk5 activity, is needed for differentiation of myoblasts (de Thonel et al., 2010; Pallari et al., 2011). As p35 is localized to the plasma membrane, whereas p25 to the cytoplasm and the nucleus, the elevated p35 levels in the nestin^{-/-} myoblasts may not influence the Cdk5 activity in the right cellular compartment for driving differentiation, and hence, no difference in differentiation was observed.

The numbers of satellite cells were assessed from myofibers fixed immediately after isolation and stained against pax7 (II Figure 3). The number of satellite cells did not differ between nestin^{-/-} and wt myofibers, indicating that although nestin normally is expressed in these cells, its absence does not influence satellite cell numbers. To further characterize the behaviour of satellite cells, single myofibers were kept floating in culture for 72 hours. Under these conditions, the satellite

cells associated with the fibers will become activated and start to differentiate. This system allows for quantification of single cells expressing stem cell and differentiation markers, and thus gives a more clean view than myoblast cultures analyzed by western blotting. By staining the myofiber cultures with the satellite cell marker pax7 and the early differentiation marker MyoD, we found no significant difference in differentiation between nestin^{-/-} satellite cells and wt satellite cells. This reflects the unaltered differentiation observed in the myoblast cultures. The divergence between these and previous results obtained from nestin siRNA treated C2C12 cells (Pallari et al., 2011) is not surprising, considering the different cell systems and the different modes of nestin depletion. The floating myofiber cultures reflect a more natural environment with signalling from the myofiber as well as from the activated satellite cell. The myoblast cultures are not as clean as the cell line or the myofiber cultures due to the isolation process, and minor changes can be masked by the presence of other cell types. The different modes of nestin depletion may also affect the results; downregulation with siRNA (Pallari et al., 2011) is not as effective as genetic depletion (Mohseni et al., 2011), and remaining levels of nestin can have a profound effect on myoblast differentiation.

5.5 Nestin influences body composition (II)

Although nestin is a minor IF in the adult, it could potentially influence developmental growth as well as tissue homeostasis; to examine this, we determined the body weight of a large set of mice, as well as food intake and spontaneous activity from a subset. Although there was no difference in activity or eating between the genotypes (II Supplementary Figure 1), we found that mice lacking nestin weigh significantly less than their wild type counterparts. This is true for both 3-month old male and female mice, and particularly for 15-month-old male mice (II Figure 1, Supplementary Figure 2). Younger mice did not show any weight differences. This means that the nestin^{-/-} mice do not accumulate weight in the same manner as wild type mice. To further investigate the source of the mass difference, the animals were scanned with the EcoMRI system. This body composition analyzer allows for the differentiation of fat and lean mass of live mice. The results clearly showed that the lean mass of nestin^{-/-} mice was less, whereas the average fat mass was unaltered compared to wild type mice. II Figure 1. To confirm the decreased muscle mass in nestin^{-/-} mice, muscles from the hind legs were dissected and weighed (II Figure 2). The tibialis anterior (TA) and extensor digitorum longus (EDL) muscles from nestin^{-/-} mice

were significantly lighter than the wild type muscles, whereas the mass of soleus was not changed. Although the *nestin*^{-/-} muscles were lighter, the myofiber size, as assessed from sections of soleus and EDL muscles, does not differ. Taken together, these results show that *nestin*^{-/-} mice have less muscle mass and that this phenotype is gender independent, becoming more prominent with age.

Several other IF-knockout mice have been shown to have decreased body mass. Mice lacking the major muscle IF desmin weigh less than wild type mice (Wede et al., 2002), but given the severe myopathy and cardiopathy these mice show (Milner et al., 1996), the weight difference is not surprising. Mice lacking the nestin-like IF synemin have less body mass, too (Garcia-Pelagio et al., 2015). As nestin is a very minor IF in the muscle, the effect of its absence on muscle and body mass points towards a novel and rather surprising role for nestin in muscle homeostasis.

5.6 The role of nestin in muscle differentiation and regeneration (II)

The results from the EcoMRI and muscle mass studies showed *nestin*^{-/-} mice to have less muscle mass. To investigate if this loss of muscle mass is accompanied with morphological changes in the muscles, histological samples from several skeletal muscles were examined. Sections of soleus, EDL and TA muscles showed that although *nestin*^{-/-} muscle does not show any gross abnormality, the number of regenerating myofibers, as determined by the presence of centrally located nuclei (CLN) in myofibers, is higher in the absence of nestin (II Figure 2). The increased amount of CLN is most prominent in the TA and soleus muscles, which reflects their weight bearing function. Although the trend for more regenerating myofibers is strong in the *nestin*^{-/-} animals, it is important to notice that the variation between animals is large; some of the knockouts show seemingly normal muscles, whereas others have a prominent regenerative phenotype. Our results show that in the absence of nestin, muscle fibers undergo degradation and regeneration more frequently, and that regeneration is triggered by normal stress like the everyday use of the muscles.

In previous studies, the dynamics of nestin expression after muscle injury has been addressed (Čížková et al., 2009; Vaitinen et al., 2001), but the functions of nestin in muscle regeneration have not yet been investigated. Utilizing the *nestin*^{-/-} mice, we looked into the function of nestin during *in vivo*-muscle

regeneration. The TA muscle of 2-month-old mice was injured by an incision through half of the muscle thickness: this cutting type injury mimics a traumatic muscle injury. By staining tissue sections harvested at different time points after the injury against nestin, we confirmed that nestin is upregulated after muscle injury (II Figure 5). We also showed that the dynamics in mice differ slightly from the previously published results acquired from rats (Vaattinen et al., 1999). Furthermore, we prove that nestin is an important part of the muscle regeneration process. In the absence of nestin, the regeneration is prolonged, as evident from more regenerating myofibers being detected 28 days after the muscle injury.

Interestingly, the *synemin*^{-/-} mice show a similar increase in CLN, but also in cell membrane defects (Li et al., 2014). The membrane injuries in the *synemin*^{-/-} mice could be due to the fact that synemin binds to several proteins important for membrane structure, such as plectin (Li et al., 2014). In the case of nestin, little is known about its binding partners, but as nestin specifically localizes to the MTJ (Vaattinen et al., 1999), nestin might take part in the organization of these, or in their functions. The formation of new MTJs, and the attachment of newly formed myotubes to the connective tissue, is part of the last steps of the regeneration process. As our study shows that the absence of nestin prolongs the regeneration process, it is indeed tempting to speculate that the formation of functional MTJs in the *nestin*^{-/-} mice is impaired, and that this affects the regeneration. Both nestin and synemin are minor IFs in the skeletal muscle, and yet these proteins play important roles in preserving muscle homeostasis and in protecting from stress.

In summary, our results demonstrate that although nestin is not needed for normal baseline muscle function, nestin plays important roles in protecting the muscles from stress. This is evident, as the phenotypes of the *nestin*^{-/-} mice worsen with age and injury induced stress.

5.7 Nestin affects male characteristics (unpublished)

Nestin has been detected in the Leydig and Sertoli cells of the testis during development and in neoplasia (Davidoff et al., 2004; Fröjdman et al., 1997; Lobo et al., 2004). Although nestin expression has been identified in specific testicular cells, nothing is known about nestin function in the testis. We used a lack of function-approach to improve the understanding of the role of nestin in the

testis. Therefore, we analyzed various parameters of the testes from *nestin*^{-/-} animals.

Firstly, we detected that the individual testis weight variation was slightly enhanced between animals and significantly enhanced in individual animals in mice lacking nestin (Figure 11). Interestingly, there is no difference in the combined testis weight between wt and *nestin*^{-/-} mice, nor is the fertility affected (data not shown). As nestin has been reported to be expressed in Leydig cells, the main testosterone-producing cells in the male, we wanted to investigate if nestin affects the testosterone processing. Serum testosterone levels were measured from 3-month-old wild type and *nestin*^{-/-} mice (Figure 12). Although no significant differences were detected, *nestin*^{-/-} mice tended to have lower serum testosterone levels. However, as the variation between animals is very large also in wt mice, measurements of serum testosterone levels give limited information about gonad function.

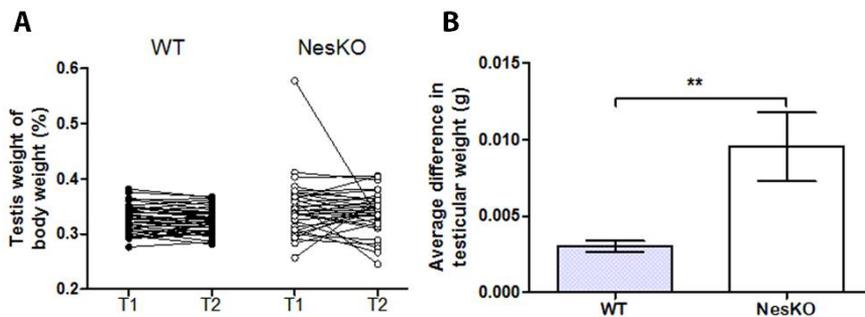


Figure 11. *Nestin*^{-/-} mice have greater variance in testicular weight. A, Single testicle weight from 3-month-old wild type and *nestin*^{-/-} mice expressed as percent of body weight. Each circle represents one testicle and the line connects the testes from the same animal. B, Average inter-animal difference in testicular weight. The *nestin*^{-/-} mice have a greater size variance between their testes, indicating that nestin has a role in building or maintaining testicular weight in mice. WT, n=41, KO n=33, ** p=0.0072 using unpaired t test with Welch's correction.

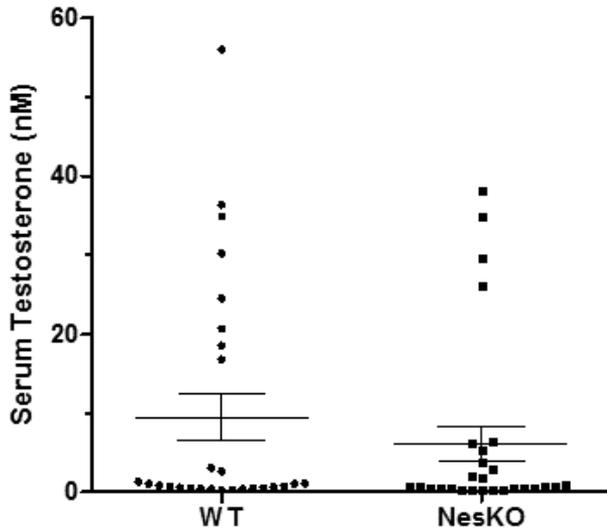


Figure 12. Serum testosterone is not significantly altered in *nestin*^{-/-} mice. Serum testosterone levels were determined from 3-month-old male mice. Although the *nestin*^{-/-} mice tend to have lower testosterone levels (average 6.171 ± 2.185 nM, $N=27$) than wild type mice (average 9.474 ± 2.888 nM, $N=27$), the difference is not significant.

Deletion of vimentin from mice has been shown to affect steroidogenesis in ovaries and adrenal cortex, but not in testis (Shen et al., 2012). The *vimentin*^{-/-} mice did not show any changes in serum testosterone levels or in testosterone production from isolated Leydig cells (Shen et al., 2012). However, no data is available on testis size in the absence of vimentin. As vimentin is strongly expressed in the Leydig and Sertoli cells (Lydka et al., 2011; Ortega et al., 2004), a comparison of the testes from mice lacking vimentin, and thus nestin filaments (Eliasson et al., 1999), with the testes from mice lacking nestin would be of interest.

In summary, our results indicate that the highly regulated processes controlling testicular weight and appearance are more prone to failure in the absence of nestin, but also that this imbalance does not have dramatic effects on testicular function. The source of this, however, remains unidentified; given that nestin has been identified in the Leydig stem cells (Jiang et al., 2014), a depletion of them or malfunction in their recruitment or proliferation seems plausible. A detailed study of the testis and its different cell types in absence of nestin is needed to

understand the different functions of nestin in this tissue. As elevated expression of nestin has been reported in human testicular cancer (Lobo et al., 2004), deeper knowledge of the function of nestin and the processes it controls in the testis can indeed have clinical significance.

5.8 Expression of human vimentin in *Drosophila* (III)

As noted earlier, the use of mice in IF-research has its limitations. Apart from the IF-specific shortcomings, cost, generation length and ethics are major considerations for the use of mice. The fruit fly *D. melanogaster* is a widely used laboratory animal, which has a short generation turnover and is cost-efficient to use. As noted earlier, arthropods lack cytoplasmic IFs, a trait that makes them ideal for the study of intrinsic IF functions in the absence of interacting and compensatory mechanisms. Furthermore, the expression of a cytoplasmic IF in an arthropod could give insight into why these animals lack cytoplasmic IFs, and if any compatibility between IFs and the *Drosophila* cytoskeleton exists. The formation of an IF-network in *Drosophila* and/or interactions between the introduced IF and the endogenous *Drosophila* proteins could indicate that close ancestors of the *Drosophila* have harboured functional IFs later lost during evolution.

Flies ubiquitously expressing vimentin were constructed using recombinant DNA technology. The vimentin flies were apparently normal, with a weight and lifespan indistinguishable from control flies (III Figure 3). This finding shows that vimentin is not toxic to *Drosophila*, as also keratin 5/14 has been proven non-toxic to flies (Bohnekamp et al., 2015). Taken together, this strongly suggests that cytoplasmic IFs are not harmful to *Drosophila*. After establishing this, we looked further into how vimentin is behaving in a *Drosophila* environment. We showed that although the mRNA expression levels of vimentin are the same in the whole fly, vimentin, surprisingly, appears to be stabilised at the protein level in certain parts of the body (III Figure 4). This stabilisation is difficult to explain, and its confirmation requires more detailed biochemical analyses to be performed.

In mammals vimentin has important functions during wound healing, as shown by the delayed wound healing in mice lacking vimentin (Eckes et al., 2000). *Drosophila* can be used to study wound healing, although some of the mechanisms of healing differ between mammals and flies; reviewed in (Belacortu

and Paricio, 2011). To investigate if the introduction of vimentin could affect wound healing in the *Drosophila* model, as well as related processes, such as cell migration and differentiation, could be of interest.

The establishment of the keratin 5/14-expressing fly and the *Drosophila* model of EBS (Bohnekamp et al., 2015), as well as our vimentin fly, has opened up a new path in the IF-field. These proof-of-concept studies offer a new way of studying both structure and function of IFs, and especially IF mutations. These studies can also give insights into the evolution of IFs and the remaining question of why arthropods lack cytoplasmic IFs.

6 Concluding Remarks

IFs function as cellular scaffolds, signalling transducers and organisers; IFs are needed for proper healing after injury and protect against stress; in line with this, mutations in IFs give rise to a wide variety of diseases. Still, many of the exact cellular roles of IFs and how they are regulated remain unknown. The work in this thesis gives insight into the diverse roles of IFs.

Lamin A/C is present in almost all differentiated cell types, yet mutations in it give rise to tissue specific diseases. To understand why this is and how we can relieve these diseases, we have to gain deeper knowledge about the functions of lamin A/C under normal conditions, as well as the processes that regulate these functions. Phosphorylation is the predominant PTM of lamin A/C. Even though many phosphorylation sites had been identified previously, our work offers a new understanding of how phosphorylation affects lamin A in interphase cells. By looking at how mutations of phosphorylation sites affect lamin A dynamics, we were able to define a new mechanism that regulates the ratio of lamina-bound and nucleoplasmic lamin A, as well as lamin A movements. This opens up for future studies of the roles of lamin A localization and dynamics in e.g. cell signalling and transcription.

Nestin has been known to be expressed in muscle during development and regeneration; the roles of nestin in these contexts have, however, remained unknown. Our study of the nestin^{-/-} mouse is the first thorough investigation of the functions of nestin in healthy and injured muscle. We also show that nestin regulates body weight and composition. As we looked further into the effect of nestin ablation on animals, we discovered that nestin^{-/-} mice have a great variation in testicle size. The indications that nestin has a role as a protector of tissue homeostasis in several different tissues are reinforced when these results are combined. Plausibly, nestin acts as a stress protector by regulating signalling cascades. The findings are in line with previous reports of other IF knockout mice, underlining the role of IFs as stress protectors and regulators of tissue homeostasis.

We show that it is possible to express human vimentin in *Drosophila*, and that vimentin is not harmful to the flies. The vimentin expressing *Drosophila* model is a new tool in IF research and can be utilized for further studies both of vimentin function and structure as well as arthropod evolution.

The work in this thesis highlights how small changes at protein level can have profound effects on cells and cell signalling. Furthermore, my work shows that even obscure proteins with limited expression patterns can influence the whole body, and have unexpected functions.

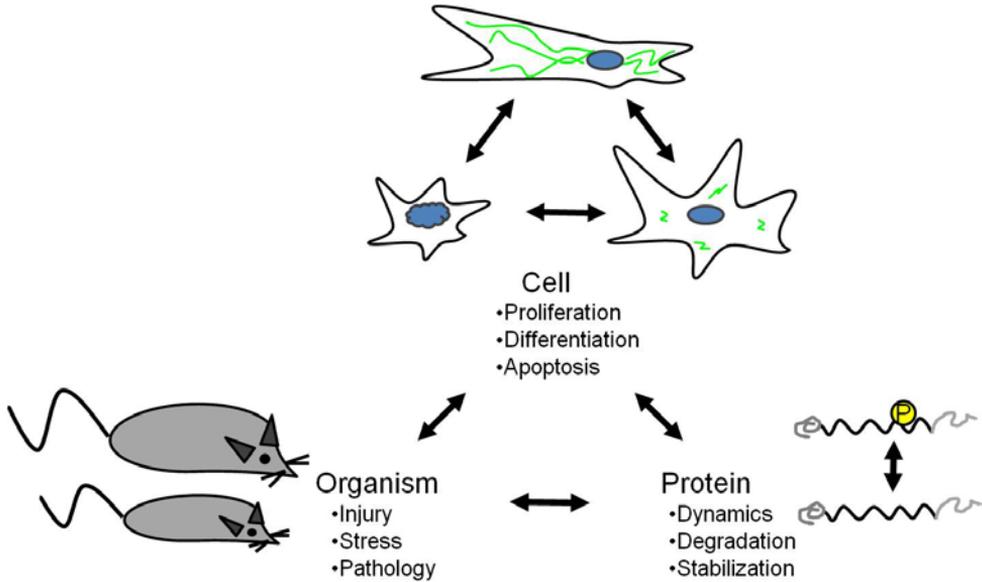


Figure 13. Multilevel impact of IFs. The presence or absence as well as the form of IFs have profound effects on cellular processes, such as proliferation, migration, differentiation and survival. These processes also affect the IFs by, for example, changing their phosphorylation. Phosphorylation regulates IF dynamics, localisation, and degradation; this in turn influences cellular decisions such as whether to migrate, proliferate or differentiate. An organism without an IF, or with a faulty IF, can develop a distinct phenotype, such as a disease. These phenotypes are dependent on the cellular changes that IFs induce, but may also induce changes in other IFs such as the compensatory expression of an otherwise non-expressed IF.

7 Acknowledgments

The work with this thesis was carried out at the Department of Biosciences at Åbo Akademi University and at the Turku Centre for Biotechnology (CBT), Åbo Akademi University and University of Turku. I am grateful for the excellent working facilities and infrastructure provided, and the management who keep working for developing and expanding these institutions. I want to express my special gratitude to the technical personnel at ÅA Cell Biology, the CBT, the Cell Imaging Core, Turku Bioimaging, and the Central Animal Laboratory; your work, assistance, and help have been invaluable.

I am thankful to Professor John Eriksson for letting me join the lab as a young and naive graduate student and for providing an environment to grow and mature in. Adjunct Professor Jeroen Pouwels and Professor Yosef Gruenbaum are warmly thanked for accepting to review my thesis. I am so, so grateful for your valuable comments and helpful suggestions. I also want to thank Professor Roy Quinlan for accepting to be my opponent, and for a friendly attitude and a great sense of humor whenever our paths have crossed.

I am grateful to all my co-authors for the collaborations that are the basis for this thesis. Being part of the truly international lamin phosphorylation study was a great learning experience. The number of people, time zones, serines and plasmids, not to mention the number of missing spaces, involved in this study made it a very special collaboration. The nestin projects have been lined with disappointments and struggle, but also some highly rewarding moments; I am thankful to have had Julia, Josef and the other nestin people to share the frustration and occasional cheerfulness with. You know that it wouldn't have been without you. Josef also developed the vimentin fly and managed to inspire me about IF evolution, thank you for all the hard work. Apart from the projects presented in this thesis, I have been involved in several other studies. I am grateful also for these collaborations and the publications they resulted in.

During my PhD-studies I have been working among and along a great amount of diverse people, although you are too many to mention, I do remember you all and I am happy for all the things you have taught me. The Eriksson lab is a melting pot of different subjects, techniques, outsets and personalities; this makes it a unique place filled with unexpected learning possibilities.

I have been privileged to have a succession of great students. I have learned so much from each and every one of you and I am grateful for your contributions to my projects and for testing my ideas. Special thanks to my Österbottnian trio: Josef, for managing me inside and outside the lab; Carolina, for being such an inspirational woman and Num, for all the laughter and all the randomness and for talking, or at least listening to me talking, about knitting and sheep.

During my studies I have been fortunate to travel to conferences and courses at distant places. I would like to thank my travel companions for being lost, and found, with me at exotic places including, but not limited to, the slippery hills of Turku, the muddy fields of Prague and seismologically active islands across the world. Somehow we always managed to get both there and back home again. And on the way we saw whales and glaciers and Alps and Frankfurt Airport and woodchucks and freaky insects and occasionally a sheep or two.

I am also grateful for my family who provides me with a safe haven far away from the stormy seas of science.

Lastly, the financial support from Magnus Ehrnrooths stiftelse, Otto A. Malms donationsfond, Svenska Kulturfonden, Waldemar von Frenckells stiftelse, K. Albin Johanssons stiftelse, Medicinska understödsföreningen Liv och Hälsa, Stiftelsens för Åbo Akademi forskningsinstitut, Ålands självstyrelses 75-årsjubileumsfond, Alfred Kordelinin säätiö, Oskar Öflunds stiftelse and Runar Erikssons fond is gratefully acknowledged.

Det fanns en tid när jorden var platt
Det fanns en tid när allt var nytt
-Leo Lundberg

8 References

- Aebi, U., Cohn, J., Buhle, L. and Gerace, L. (1986). The nuclear lamina is a meshwork of intermediate-type filaments. *Nature* **323**, 560–564.
- Aki, R., Amoh, Y., Bouvet, M., Katsuoka, K. and Hoffman, R. M. (2014). Color-coded fluorescence imaging of lymph-node metastasis, angiogenesis, and its drug-induced inhibition. *J. Cell. Biochem.* **115**, 457–463.
- Amoh, Y., Yang, M., Li, L., Reynoso, J., Bouvet, M., Moossa, A. R., Katsuoka, K. and Hoffman, R. M. (2005). Nestin-Linked Green Fluorescent Protein Transgenic Nude Mouse for Imaging Human Tumor Angiogenesis. *Cancer Res.* **65**, 5352–5357.
- Arif, A. (2012). Extraneuronal activities and regulatory mechanisms of the atypical cyclin-dependent kinase Cdk5. *Biochem. Pharmacol.* **84**, 985–993.
- Asada, A., Saito, T. and Hisanaga, S. (2012). Phosphorylation of p35 and p39 by Cdk5 determines the subcellular location of the holokinase in a phosphorylation-site-specific manner. *J. Cell Sci.* **125**, 3421–3429.
- Astbury, W. T. and Street, A. (1932). X-Ray Studies of the Structure of Hair, Wool, and Related Fibres. I. General. *Philos. Trans. R. Soc. Lond. Ser. Contain. Pap. Math. Phys. Character* **230**, 75–101.
- Bank, E. M., Ben-Harush, K., Feinstein, N., Medalia, O. and Gruenbaum, Y. (2012). Structural and physiological phenotypes of disease-linked lamin mutations in *C. elegans*. *J. Struct. Biol.* **177**, 106–112.
- Bär, J., Kumar, V., Roth, W., Schwarz, N., Richter, M., Leube, R. E. and Magin, T. M. (2014). Skin Fragility and Impaired Desmosomal Adhesion in Mice Lacking All Keratins. *J. Invest. Dermatol.* **134**, 1012–1022.
- Bartnik, E. and Weber, K. (1989). Widespread occurrence of intermediate filaments in invertebrates; common principles and aspects of diversion. *Eur. J. Cell Biol.* **50**, 17–33.
- Belacortu, Y. and Paricio, N. (2011). *Drosophila* as a model of wound healing and tissue regeneration in vertebrates. *Dev. Dyn.* **240**, 2379–2404.
- Ben-Harush, K., Wiesel, N., Frenkiel-Krispin, D., Moeller, D., Soreq, E., Aebi, U., Herrmann, H., Gruenbaum, Y. and Medalia, O. (2009). The Supramolecular Organization of the *C. elegans* Nuclear Lamin Filament. *J. Mol. Biol.* **386**, 1392–1402.
- Bohnekamp, J., Cryderman, D. E., Paululat, A., Baccam, G. C., Wallrath, L. L. and Magin, T. M. (2015). A *Drosophila* model of Epidermolysis Bullosa Simplex. *J. Invest. Dermatol.*
- Bökenkamp, R., Raz, V., Venema, A., DeRuiter, M. C., van Munsteren, C., Olive, M., Nabel, E. G. and Gittenberger-de Groot, A. C. (2011). Differential Temporal and Spatial Progerin Expression during Closure of the Ductus Arteriosus in Neonates. *PLoS ONE* **6**.
- Bosnakovski, D., Xu, Z., Li, W., Thet, S., Cleaver, O., Perlingeiro, R. C. R. and Kyba, M. (2008). Prospective isolation of skeletal muscle stem cells with a Pax7 reporter. *Stem Cells Dayt. Ohio* **26**, 3194–3204.
- Brinkkoetter, P. T., Olivier, P., Wu, J. S., Henderson, S., Krofft, R. D., Pippin, J. W., Hockenbery, D., Roberts, J. M. and Shankland, S. J. (2009). Cyclin I activates Cdk5 and regulates expression of Bcl-2 and Bcl-XL in postmitotic mouse cells. *J. Clin. Invest.* **119**, 3089–3101.

References

- Busch, T., Armacki, M., Eiseler, T., Joodi, G., Temme, C., Jansen, J., von Wichert, G., Omary, M. B., Spatz, J. and Seufferlein, T. (2012). Keratin 8 phosphorylation regulates keratin reorganization and migration of epithelial tumor cells. *J. Cell Sci.* **125**, 2148–2159.
- Camozzi, D., Capanni, C., Cenni, V., Mattioli, E., Columbaro, M., Squarzoni, S. and Lattanzi, G. (2014). Diverse lamin-dependent mechanisms interact to control chromatin dynamics. Focus on laminopathies. *Nucl. Austin Tex* **5**, 427–440.
- Capetanaki, Y. (2002). Desmin Cytoskeleton: A Potential Regulator of Muscle Mitochondrial Behavior and Function. *Trends Cardiovasc. Med.* **12**, 339–348.
- Carlson, B. M. (2008). Muscle Regeneration in Animal Models. In *Skeletal Muscle Repair and Regeneration*, pp. 163–180. Springer Netherlands.
- Carlsson, L., Li, Z., Paulin, D. and Thornell, L. E. (1999). Nestin is expressed during development and in myotendinous and neuromuscular junctions in wild type and desmin knock-out mice. *Exp. Cell Res.* **251**, 213–223.
- Cenni, V., Sabatelli, P., Mattioli, E., Marmioli, S., Capanni, C., Ognibene, A., Squarzoni, S., Maraldi, N., Bonne, G., Columbaro, M., et al. (2005). Lamin A N-terminal phosphorylation is associated with myoblast activation: impairment in Emery-Dreifuss muscular dystrophy. *J. Med. Genet.* **42**, 214–220.
- Chang, K.-H., Multani, P. S., Sun, K.-H., Vincent, F., de Pablo, Y., Ghosh, S., Gupta, R., Lee, H.-P., Lee, H., Smith, M. A., et al. (2011). Nuclear envelope dispersion triggered by deregulated Cdk5 precedes neuronal death. *Mol. Biol. Cell* **22**, 1452–1462.
- Chargé, S. B. P. and Rudnicki, M. A. (2004). Cellular and Molecular Regulation of Muscle Regeneration. *Physiol. Rev.* **84**, 209–238.
- Chen, J., Boyle, S., Zhao, M., Su, W., Takahashi, K., Davis, L., Decaestecker, M., Takahashi, T., Breyer, M. D. and Hao, C.-M. (2006). Differential expression of the intermediate filament protein nestin during renal development and its localization in adult podocytes. *J. Am. Soc. Nephrol. JASN* **17**, 1283–1291.
- Cheng, L., Jin, Z., Liu, L., Yan, Y., Li, T., Zhu, X. and Jing, N. (2004). Characterization and promoter analysis of the mouse nestin gene. *FEBS Lett.* **565**, 195–202.
- Chernoivanenko, I. S., Matveeva, E. A., Gelfand, V. I., Goldman, R. D. and Minin, A. A. (2015). Mitochondrial membrane potential is regulated by vimentin intermediate filaments. *FASEB J.* **29**, 820–827.
- Cheung, Z. H. and Ip, N. Y. (2012). Cdk5: a multifaceted kinase in neurodegenerative diseases. *Trends Cell Biol.* **22**, 169–175.
- Cho, H. M., Kim, J. Y., Kim, H. and Sun, W. (2014). Phosphatase and actin regulator 4 is associated with intermediate filaments in adult neural stem cells and their progenitor astrocytes. *Histochem. Cell Biol.*
- Chou, Y.-H. (2003). Nestin Promotes the Phosphorylation-dependent Disassembly of Vimentin Intermediate Filaments During Mitosis. *Mol. Biol. Cell* **14**, 1468–1478.
- Chou, Y.-H., Kuo, W.-L., Rosner, M. R., Tang, W.-J. and Goldman, R. D. (2009). Structural changes in intermediate filament networks alter the activity of insulin-degrading enzyme. *FASEB J.* **23**, 3734–3742.
- Chow, C. L., Guo, W., Trivedi, P., Zhao, X. and Gubbels, S. P. (2015). Characterization of a Unique Cell Population Marked by Transgene Expression in the Adult Cochlea of Nestin-CreERT2/tdTomato-Reporter Mice. *J. Comp. Neurol.* n/a–n/a.

References

- Chung, B.-M., Rotty, J. D. and Coulombe, P. A.** (2013). Networking galore: Intermediate filaments and cell migration. *Curr. Opin. Cell Biol.* **25**, 600–612.
- Ciciliot, S. and Schiaffino, S.** (2010). Regeneration of Mammalian Skeletal Muscle: Basic Mechanisms and Clinical Implications. *Curr. Pharm. Des.* **16**, 906–914.
- Čížková, D., Soukup, T. and Mokřý, J.** (2009). Nestin Expression Reflects Formation, Revascularization and Reinnervation of New Myofibers in Regenerating Rat Hind Limb Skeletal Muscles. *Cells Tissues Organs* **189**, 338–347.
- Coffinier, C., Jung, H.-J., Li, Z., Nobumori, C., Yun, U. J., Farber, E. A., Davies, B. S., Weinstein, M. M., Yang, S. H., Lammerding, J., et al.** (2010). Direct synthesis of lamin A, bypassing prelamin A processing, causes misshapen nuclei in fibroblasts but no detectable pathology in mice. *J. Biol. Chem.* **285**, 20818–20826.
- Colucci-Guyon, E., Portier, M. M., Dunia, I., Paulin, D., Pournin, S. and Babinet, C.** (1994). Mice lacking vimentin develop and reproduce without an obvious phenotype. *Cell* **79**, 679–694.
- Contreras-Vallejos, E., Utreras, E. and Gonzalez-Billault, C.** (2012). Going out of the brain: Non-nervous system physiological and pathological functions of Cdk5. *Cell. Signal.* **24**, 44–52.
- Coulombe, P. A., Kerns, M. L. and Fuchs, E.** (2009). Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. *J. Clin. Invest.* **119**, 1784–1793.
- Cui, Y., Xiao, Z., Han, J., Sun, J., Ding, W., Zhao, Y., Chen, B., Li, X. and Dai, J.** (2012). MiR-125b orchestrates cell proliferation, differentiation and migration in neural stem/progenitor cells by targeting Nestin. *BMC Neurosci.* **13**, 116.
- Dahlstrand, J., Zimmerman, L. B., McKay, R. D. and Lendahl, U.** (1992). Characterization of the human nestin gene reveals a close evolutionary relationship to neurofilaments. *J. Cell Sci.* **103** (Pt 2), 589–597.
- Danoviz, M. E. and Yablonka-Reuveni, Z.** (2012). Skeletal muscle satellite cells: background and methods for isolation and analysis in a primary culture system. *Methods Mol. Biol. Clifton NJ* **798**, 21–52.
- Das, E. and Bhattacharyya, N. P.** (2014). MicroRNA-432 contributes to dopamine cocktail and retinoic acid induced differentiation of human neuroblastoma cells by targeting NESTIN and RCOR1 genes. *FEBS Lett.* **588**, 1706–1714.
- Davidoff, M. S., Middendorff, R., Enikolopov, G., Riethmacher, D., Holstein, A. F. and Müller, D.** (2004). Progenitor cells of the testosterone-producing Leydig cells revealed. *J. Cell Biol.* **167**, 935–944.
- Davidson, P. M. and Lammerding, J.** (2014). Broken nuclei – lamins, nuclear mechanics, and disease. *Trends Cell Biol.* **24**, 247–256.
- Davies, B. S., Coffinier, C., Yang, S. H., Barnes, R. H., Jung, H.-J., Young, S. G. and Fong, L. G.** (2011). Investigating the purpose of prelamin A processing. *Nucleus* **2**, 4–9.
- Day, K., Shefer, G., Richardson, J. B., Enikolopov, G. and Yablonka-Reuveni, Z.** (2007). Nestin-GFP reporter expression defines the quiescent state of skeletal muscle satellite cells. *Dev. Biol.* **304**, 246–259.
- Dechat, T., Korbei, B., Vaughan, O. A., Vlcek, S., Hutchison, C. J. and Foisner, R.** (2000). Lamina-associated polypeptide 2alpha binds intranuclear A-type lamins. *J. Cell Sci.* **113** Pt 19, 3473–3484.

- Dechat, T., Pflieger, K., Sengupta, K., Shimi, T., Shumaker, D. K., Solimando, L. and Goldman, R. D. (2008). Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev.* **22**, 832–853.
- Dechat, T., Gesson, K. and Foisner, R. (2010). Lamina-Independent Lamins in the Nuclear Interior Serve Important Functions. *Cold Spring Harb. Symp. Quant. Biol.* **75**, 533–543.
- Dessev, G. N., Iovcheva-Dessev, C. and Goldman, R. D. (1990). Lamin dimers. Presence in the nuclear lamina of surf clam oocytes and release during nuclear envelope breakdown. *J. Biol. Chem.* **265**, 12636–12641.
- Dessev, G., Iovcheva-Dessev, C., Bischoff, J. R., Beach, D. and Goldman, R. (1991). A complex containing p34cdc2 and cyclin B phosphorylates the nuclear lamin and disassembles nuclei of clam oocytes in vitro. *J. Cell Biol.* **112**, 523–533.
- de Thonel, A., Ferraris, S. E., Pallari, H.-M., Imanishi, S. Y., Kochin, V., Hosokawa, T., Hisanaga, S., Sahlgren, C. and Eriksson, J. E. (2010). Protein kinase Czeta regulates Cdk5/p25 signaling during myogenesis. *Mol. Biol. Cell* **21**, 1423–1434.
- Dhariwala, F. A. and Rajadhyaksha, M. S. (2008). An unusual member of the Cdk family: Cdk5. *Cell. Mol. Neurobiol.* **28**, 351–369.
- Dorner, D., Vlcek, S., Foeger, N., Gajewski, A., Makolm, C., Gotzmann, J., Hutchison, C. J. and Foisner, R. (2006). Lamina-associated polypeptide 2alpha regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. *J. Cell Biol.* **173**, 83–93.
- Eckes, B., Colucci-Guyon, E., Smola, H., Nodder, S., Babinet, C., Krieg, T. and Martin, P. (2000). Impaired wound healing in embryonic and adult mice lacking vimentin. *J. Cell Sci.* **113**, 2455–2462.
- Eliasson, C., Sahlgren, C., Berthold, C. H., Stakeberg, J., Celis, J. E., Betsholtz, C., Eriksson, J. E. and Pekny, M. (1999). Intermediate filament protein partnership in astrocytes. *J. Biol. Chem.* **274**, 23996–24006.
- Erber, A., Riemer, D., Bovenschulte, M. and Weber, K. (1998). Molecular phylogeny of metazoan intermediate filament proteins. *J. Mol. Evol.* **47**, 751–762.
- Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., Erdos, M. R., Robbins, C. M., Moses, T. Y., Berglund, P., et al. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Jordan progeria syndrome. *Nature* **423**, 293–298.
- Fong, L. G., Ng, J. K., Lammerding, J., Vickers, T. A., Meta, M., Coté, N., Gavino, B., Qiao, X., Chang, S. Y., Young, S. R., et al. (2006). Prelamin A and lamin A appear to be dispensable in the nuclear lamina. *J. Clin. Invest.* **116**, 743–752.
- Frederiksen, K., Jat, P. S., Valtz, N., Levy, D. and McKay, R. (1988). Immortalization of precursor cells from the mammalian CNS. *Neuron* **1**, 439–448.
- Frock, R. L., Kudlow, B. A., Evans, A. M., Jameson, S. A., Hauschka, S. D. and Kennedy, B. K. (2006). Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation. *Genes Dev.* **20**, 486–500.
- Fröjdman, K., Pelliniemi, L. J., Lendahl, U., Virtanen, I. and Eriksson, J. E. (1997). The intermediate filament protein nestin occurs transiently in differentiating testis of rat and mouse. *Differ. Res. Biol. Divers.* **61**, 243–249.
- Furukawa, K., Inagaki, H. and Hotta, Y. (1994). Identification and Cloning of an mRNA Coding for a Germ Cell-Specific A-Type Lamin in Mice. *Exp. Cell Res.* **212**, 426–430.

References

- Galisson, F., Mahrouche, L., Courcelles, M., Bonneil, E., Meloche, S., Chelbi-Alix, M. K. and Thibault, P.** (2011). A Novel Proteomics Approach to Identify SUMOylated Proteins and Their Modification Sites in Human Cells. *Mol. Cell. Proteomics MCP* **10**.
- Garcia-Pelagio, K. P., Muriel, J., O'Neill, A., Desmond, P. F., Lovering, R. M., Lund, L., Bond, M. and Bloch, R. J.** (2015). Myopathic Changes in Murine Skeletal Muscle Lacking Synemin. *Am. J. Physiol. Cell Physiol.* ajpcell.00331.2014.
- Garg, A., Subramanyam, L., Agarwal, A. K., Simha, V., Levine, B., D'Apice, M. R., Novelli, G. and Crow, Y.** (2009). Atypical Progeroid Syndrome due to Heterozygous Missense LMNA Mutations. *J. Clin. Endocrinol. Metab.* **94**, 4971–4983.
- Georgatos, S. D., Pyrpasopoulou, A. and Theodoropoulos, P. A.** (1997). Nuclear envelope breakdown in mammalian cells involves stepwise lamina disassembly and microtubule-drive deformation of the nuclear membrane. *J. Cell Sci.* **110**, 2129–2140.
- Gesson, K., Vidak, S. and Foisner, R.** (2014). Lamina-associated polypeptide (LAP)2? and nucleoplasmic lamins in adult stem cell regulation and disease. *Semin. Cell Dev. Biol.* **29**, 116–124.
- Godsel, L. M., Hobbs, R. P. and Green, K. J.** (2008). Intermediate filament assembly: dynamics to disease. *Trends Cell Biol.* **18**, 28–37.
- Goldman, R. D., Cleland, M. M., Murthy, P., Mahammad, S. and Kuczmariski, E. R.** (2012). Inroads into the Structure and Function of Intermediate Filament Networks. *J. Struct. Biol.* **177**, 14–23.
- Gregor, M., Osmanagic-Myers, S., Burgstaller, G., Wolfram, M., Fischer, I., Walko, G., Resch, G. P., Jörgl, A., Herrmann, H. and Wiche, G.** (2014). Mechanosensing through focal adhesion-anchored intermediate filaments. *FASEB J.* **28**, 715–729.
- Guo, M., Ehrlicher, A. J., Mahammad, S., Fabich, H., Jensen, M. H., Moore, J. R., Fredberg, J. J., Goldman, R. D. and Weitz, D. A.** (2013). The Role of Vimentin Intermediate Filaments in Cortical and Cytoplasmic Mechanics. *Biophys. J.* **105**, 1562–1568.
- Guo, H., Luo, N., Hao, F. and Bai, Y.** (2014). p.Pro4Arg mutation in LMNA gene: a new atypical progeria phenotype without metabolism abnormalities. *Gene* **546**, 35–39.
- Hagmann, H., Taniguchi, Y., Pippin, J. W., Kauerz, H.-M., Benzing, T., Shankland, S. J. and Brinkkoetter, P. T.** (2015). Cyclin I and p35 determine the subcellular distribution of Cdk5. *Am. J. Physiol. - Cell Physiol.* **308**, C339–C347.
- Han, D. W., Do, J. T., Araúzo-Bravo, M. J., Lee, S. H., Meissner, A., Lee, H. T., Jaenisch, R. and Schöler, H. R.** (2009). Epigenetic hierarchy governing Nestin expression. *Stem Cells Dayt. Ohio* **27**, 1088–1097.
- Harada, T., Swift, J., Irianto, J., Shin, J.-W., Spinler, K. R., Athirasala, A., Diegmiller, R., Dingal, P. C. D. P., Ivanovska, I. L. and Discher, D. E.** (2014). Nuclear lamin stiffness is a barrier to 3D migration, but softness can limit survival. *J. Cell Biol.* **204**, 669–682.
- Hawke, T. J. and Garry, D. J.** (2001). Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* **91**, 534–551.
- Heald, R. and McKeon, F.** (1990). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* **61**, 579–589.
- Herrmann, H. and Strelkov, S. V.** (2011). History and phylogeny of intermediate filaments: now in insects. *BMC Biol.* **9**, 16.
- Herrmann, H., Bär, H., Kreplak, L., Strelkov, S. V. and Aebi, U.** (2007). Intermediate filaments: from cell architecture to nanomechanics. *Nat. Rev. Mol. Cell Biol.* **8**, 562–573.

References

- Herrmann, H., Strelkov, S. V., Burkhard, P. and Aebi, U. (2009). Intermediate filaments: primary determinants of cell architecture and plasticity. *J. Clin. Invest.* **119**, 1772–1783.
- Ho, C. Y. and Lammerding, J. (2012). Lamins at a glance. *J. Cell Sci.* **125**, 2087–2093.
- Ho, C. Y., Jaalouk, D. E., Vartiainen, M. K. and Lammerding, J. (2013). Lamin A/C and emerin regulate MKL1-SRF activity by modulating actin dynamics. *Nature* **497**, 507–511.
- Hockfield, S. and McKay, R. D. (1985). Identification of major cell classes in the developing mammalian nervous system. *J. Neurosci. Off. J. Soc. Neurosci.* **5**, 3310–3328.
- Hunziker, E. and Stein, M. (2000). Nestin-expressing cells in the pancreatic islets of Langerhans. *Biochem. Biophys. Res. Commun.* **271**, 116–119.
- Hyder, C. L., Kemppainen, K., Isoniemi, K. O., Imanishi, S. Y., Goto, H., Inagaki, M., Fazeli, E., Eriksson, J. E. and Törnquist, K. (2015). Sphingolipids inhibit vimentin-dependent cell migration. *J. Cell Sci.* **128**, 2057–2069.
- Isermann, P. and Lammerding, J. (2013). Nuclear Mechanics and Mechanotransduction in Health and Disease. *Curr. Biol.* **23**, R1113–R1121.
- Izawa, I. and Inagaki, M. (2006). Regulatory mechanisms and functions of intermediate filaments: A study using site- and phosphorylation state-specific antibodies. *Cancer Sci.* **97**, 167–174.
- Jahn, D., Schramm, S., Schnölzer, M., Heilmann, C. J., de Koster, C. G., Schütz, W., Benavente, R. and Alsheimer, M. (2012). A truncated lamin A in the Lmna^{-/-} mouse line. *Nucleus* **3**, 463–474.
- Jiang, M. H., Cai, B., Tuo, Y., Wang, J., Zang, Z. J., Tu, X. 'an, Gao, Y., Su, Z., Li, W., Li, G., et al. (2014). Characterization of Nestin-positive stem Leydig cells as a potential source for the treatment of testicular Leydig cell dysfunction. *Cell Res.* **24**, 1466–1485.
- Jordanova, A., Jonghe, P. D., Boerkoel, C. F., Takashima, H., Vriendt, E. D., Ceuterick, C., Martin, J.-J., Butler, I. J., Mancias, P., Papasozomenos, S. C., et al. (2003). Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot–Marie–Tooth disease. *Brain* **126**, 590–597.
- Jung, H.-J., Tu, Y., Yang, S. H., Tatar, A., Nobumori, C., Wu, D., Young, S. G. and Fong, L. G. (2014). New Lmna knock-in mice provide a molecular mechanism for the “segmental aging” in Hutchinson–Gilford progeria syndrome. *Hum. Mol. Genet.* **23**, 1506–1515.
- Kachinsky, A. M., Dominov, J. A. and Miller, J. B. (1994). Myogenesis and the intermediate filament protein, nestin. *Dev. Biol.* **165**, 216–228.
- Karabinos, A., Schmidt, H., Harborth, J., Schnabel, R. and Weber, K. (2001). Essential roles for four cytoplasmic intermediate filament proteins in *Caenorhabditis elegans* development. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 7863–7868.
- Kawaguchi, A., Miyata, T., Sawamoto, K., Takashita, N., Murayama, A., Akamatsu, W., Ogawa, M., Okabe, M., Tano, Y., Goldman, S. A., et al. (2001). Nestin-EGFP Transgenic Mice: Visualization of the Self-Renewal and Multipotency of CNS Stem Cells. *Mol. Cell. Neurosci.* **17**, 259–273.
- Keire, P., Shearer, A., Shefer, G. and Yablonka-Reuveni, Z. (2013). Isolation and culture of skeletal muscle myofibers as a means to analyze satellite cells. *Methods Mol. Biol. Clifton NJ* **946**, 431–468.
- Kim, Y. and Zheng, Y. (2013). Generation and Characterization of a Conditional Deletion Allele for Lmna in Mice. *Biochem. Biophys. Res. Commun.* **440**,

References

- Kochin, V., Shimi, T., Torvaldson, E., Adam, S. A., Goldman, A., Pack, C.-G., Melo-Cardenas, J., Imanishi, S. Y., Goldman, R. D. and Eriksson, J. E. (2014). Interphase phosphorylation of lamin A. *J. Cell Sci.* **127**, 2683–2696.
- Kokkinos, M. I., Wafai, R., Wong, M. K., Newgreen, D. F., Thompson, E. W. and Waltham, M. (2007). Vimentin and Epithelial-Mesenchymal Transition in Human Breast Cancer – Observations in vitro and in vivo. *Cells Tissues Organs* **185**, 191–203.
- Kollmar, M. (2015). Polyphyly of nuclear lamin genes indicates an early eukaryotic origin of the metazoan-type intermediate filament proteins. *Sci. Rep.* **5**.
- Kölsch, A., Windoffer, R., Würflinger, T., Aach, T. and Leube, R. E. (2010). The keratin-filament cycle of assembly and disassembly. *J. Cell Sci.* **123**, 2266–2272.
- Kubben, N., Voncken, J. W., Konings, G., van Weeghel, M., van den Hoogenhof, M. M., Gijbels, M., van Erk, A., Schoonderwoerd, K., van den Bosch, B., Dahlmans, V., et al. (2011). Post-natal myogenic and adipogenic developmental: defects and metabolic impairment upon loss of A-type lamins. *Nucl. Austin Tex* **2**, 195–207.
- Kuga, T., Nozaki, N., Matsushita, K., Nomura, F. and Tomonaga, T. (2010). Phosphorylation statuses at different residues of lamin B2, B1, and A/C dynamically and independently change throughout the cell cycle. *Exp. Cell Res.* **316**, 2301–2312.
- Lagace, D. C., Whitman, M. C., Noonan, M. A., Ables, J. L., DeCarolis, N. A., Arguello, A. A., Donovan, M. H., Fischer, S. J., Farnbauch, L. A., Beech, R. D., et al. (2007). Dynamic Contribution of Nestin-Expressing Stem Cells to Adult Neurogenesis. *J. Neurosci. Off. J. Soc. Neurosci.* **27**, 12623–12629.
- Lalioti, V., Muruais, G., Dinarina, A., Damme, J. van, Vandekerckhove, J. and Sandoval, I. V. (2009). The atypical kinase Cdk5 is activated by insulin, regulates the association between GLUT4 and E-Syt1, and modulates glucose transport in 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci.* **106**, 4249–4253.
- Lammerding, J., Fong, L. G., Ji, J. Y., Reue, K., Stewart, C. L., Young, S. G. and Lee, R. T. (2006). Lamins A and C but Not Lamin B1 Regulate Nuclear Mechanics. *J. Biol. Chem.* **281**, 25768–25780.
- Lazaro, J. B., Kitzmann, M., Poul, M. A., Vandromme, M., Lamb, N. J. and Fernandez, A. (1997). Cyclin dependent kinase 5, cdk5, is a positive regulator of myogenesis in mouse C2 cells. *J. Cell Sci.* **110** (Pt 10), 1251–1260.
- Leduc, C. and Etienne-Manneville, S. (2015). Intermediate filaments in cell migration and invasion: the unusual suspects. *Curr. Opin. Cell Biol.* **32**, 102–112.
- Lee, H. Y., Jung, H., Jang, I. H., Suh, P.-G. and Ryu, S. H. (2008). Cdk5 phosphorylates PLD2 to mediate EGF-dependent insulin secretion. *Cell. Signal.* **20**, 1787–1794.
- Lee, K.-Y., Liu, L., Jin, Y., Fu, S.-B. and Rosales, J. L. (2012). Cdk5 mediates vimentin Ser56 phosphorylation during GTP-induced secretion by neutrophils. *J. Cell. Physiol.* **227**, 739–750.
- Lee, J. M., Tu, Y., Tatar, A., Wu, D., Nobumori, C., Jung, H.-J., Yoshinaga, Y., Coffinier, C., de Jong, P. J., Fong, L. G., et al. (2014). Reciprocal knock-in mice to investigate the functional redundancy of lamin B1 and lamin B2. *Mol. Biol. Cell* **25**, 1666–1675.
- Lendahl, U., Zimmerman, L. B. and McKay, R. D. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell* **60**, 585–595.
- Leube, R. E., Moch, M., Kolsch, A. and Windoffer, R. (2011). Panta rhei. *Bioarchitecture* **1**, 39–44.

References

- Li, L., Mignone, J., Yang, M., Matic, M., Penman, S., Enikolopov, G. and Hoffman, R. M. (2003). Nestin expression in hair follicle sheath progenitor cells. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 9958–9961.
- Li, Z., Parlakian, A., Coletti, D., Alonso-Martin, S., Hourdé, C., Joanne, P., Gao-Li, J., Blanc, J., Ferry, A., Paulin, D., et al. (2014). Synemin acts as a regulator of signalling molecules in skeletal muscle hypertrophy. *J. Cell Sci.* jcs.143164.
- Liebl, J., Fürst, R., Vollmar, A. M. and Zahler, S. (2011). Twice switched at birth: Cell cycle-independent roles of the “neuron-specific” cyclin-dependent kinase 5 (Cdk5) in non-neuronal cells. *Cell. Signal.* **23**, 1698–1707.
- Liem, R. K. H. and Messing, A. (2009). Dysfunctions of neuronal and glial intermediate filaments in disease. *J. Clin. Invest.* **119**, 1814–1824.
- Lin, R. C., Matesic, D. F., Marvin, M., McKay, R. D. and Brüstle, O. (1995). Re-expression of the intermediate filament nestin in reactive astrocytes. *Neurobiol. Dis.* **2**, 79–85.
- Lindqvist, J., Imanishi, S. Y., Torvaldson, E., Malinen, M., Remes, M., Örn, F., Palvimo, J. J. and Eriksson, J. E. (2015). Cyclin-dependent kinase 5 acts as a critical determinant of AKT-dependent proliferation and regulates differential gene expression by the androgen receptor in prostate cancer cells. *Mol. Biol. Cell* **26**, 1971–1984.
- Liu, J., Ben-Shahar, T. R., Riemer, D., Treinin, M., Spann, P., Weber, K., Fire, A. and Gruenbaum, Y. (2000). Essential Roles for *Caenorhabditis elegans* Lamin Gene in Nuclear Organization, Cell Cycle Progression, and Spatial Organization of Nuclear Pore Complexes. *Mol. Biol. Cell* **11**, 3937–3947.
- Lloyd, C., Yu, Q. C., Cheng, J., Turksen, K., Degenstein, L., Hutton, E. and Fuchs, E. (1995). The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. *J. Cell Biol.* **129**, 1329–1344.
- Lobo, M. V. T., Arenas, M. I., Alonso, F. J. M., Gomez, G., Bazán, E., Paíno, C. L., Fernández, E., Fraile, B., Paniagua, R., Moyano, A., et al. (2004). Nestin, a neuroectodermal stem cell marker molecule, is expressed in Leydig cells of the human testis and in some specific cell types from human testicular tumours. *Cell Tissue Res.* **316**, 369–376.
- Lu, J. T., Muchir, A., Nagy, P. L. and Worman, H. J. (2011). LMNA cardiomyopathy: cell biology and genetics meet clinical medicine. *Dis. Model. Mech.* **4**, 562–568.
- Luo, Y.-B., Mitrpant, C., Johnsen, R., Fabian, V., Needham, M., Fletcher, S., Wilton, S. D. and Mastaglia, F. L. (2013). Investigation of splicing changes and post-translational processing of LMNA in sporadic inclusion body myositis. *Int. J. Clin. Exp. Pathol.* **6**, 1723–1733.
- Lyakhovetsky, R. and Gruenbaum, Y. (2014). Studying lamins in invertebrate models. *Adv. Exp. Med. Biol.* **773**, 245–262.
- Lydka, M., Kotula-Balak, M., Kopera-Sobota, I., Tischner, M. and Bilińska, B. (2011). Vimentin expression in testes of Arabian stallions. *Equine Vet. J.* **43**, 184–189.
- Ma, J., Sun, F., Li, C., Zhang, Y., Xiao, W., Li, Z., Pan, Q., Zeng, H., Xiao, G., Yao, K., et al. (2014). Depletion of intermediate filament protein Nestin, a target of microRNA-940, suppresses tumorigenesis by inducing spontaneous DNA damage accumulation in human nasopharyngeal carcinoma. *Cell Death Dis.* **5**, e1377.
- Machiels, B. M., Zorenc, A. H. G., Endert, J. M., Kuijpers, H. J. H., Eys, G. J. J. M. van, Ramaekers, F. C. S. and Broers, J. L. V. (1996). An Alternative Splicing Product of the Lamin A/C Gene Lacks Exon 10. *J. Biol. Chem.* **271**, 9249–9253.

References

- Markiewicz, E., Ledran, M. and Hutchison, C. J.** (2005). Remodelling of the nuclear lamina and nucleoskeleton is required for skeletal muscle differentiation in vitro. *J. Cell Sci.* **118**, 409–420.
- Marvin, M. J., Dahlstrand, J., Lendahl, U. and McKay, R. D.** (1998). A rod end deletion in the intermediate filament protein nestin alters its subcellular localization in neuroepithelial cells of transgenic mice. *J. Cell Sci.* **111 (Pt 14)**, 1951–1961.
- Matsuyama, M., Tanaka, H., Inoko, A., Goto, H., Yonemura, S., Kobori, K., Hayashi, Y., Kondo, E., Itoharu, S., Izawa, I., et al.** (2013). Defect of Mitotic Vimentin Phosphorylation Causes Microphthalmia and Cataract via Aneuploidy and Senescence in Lens Epithelial Cells. *J. Biol. Chem.* **288**, 35626–35635.
- Mellodew, K., Suhr, R., Uwanogho, D. A., Reuter, I., Lendahl, U., Hodges, H. and Price, J.** (2004). Nestin expression is lost in a neural stem cell line through a mechanism involving the proteasome and Notch signalling. *Brain Res. Dev. Brain Res.* **151**, 13–23.
- Mendez, M. G., Kojima, S.-I. and Goldman, R. D.** (2010). Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *FASEB J.* **24**, 1838–1851.
- Mignone, J. L., Kukekov, V., Chiang, A.-S., Steindler, D. and Enikolopov, G.** (2004). Neural stem and progenitor cells in nestin-GFP transgenic mice. *J. Comp. Neurol.* **469**, 311–324.
- Milner, D. J., Weitzer, G., Tran, D., Bradley, A. and Capetanaki, Y.** (1996). Disruption of muscle architecture and myocardial degeneration in mice lacking desmin. *J. Cell Biol.* **134**, 1255–1270.
- Milner, D. J., Mavroidis, M., Weisleder, N. and Capetanaki, Y.** (2000). Desmin Cytoskeleton Linked to Muscle Mitochondrial Distribution and Respiratory Function. *J. Cell Biol.* **150**, 1283–1298.
- Mitsuhashi, H., Hayashi, Y. K., Matsuda, C., Noguchi, S., Wakatsuki, S., Araki, T. and Nishino, I.** (2010). Specific phosphorylation of Ser458 of A-type lamins in LMNA-associated myopathy patients. *J. Cell Sci.* **123**, 3893–3900.
- Mohseni, P., Sung, H.-K., Murphy, A. J., Laliberte, C. L., Pallari, H.-M., Henkelman, M., Georgiou, J., Xie, G., Quaggin, S. E., Thorner, P. S., et al.** (2011). Nestin is not essential for development of the CNS but required for dispersion of acetylcholine receptor clusters at the area of neuromuscular junctions. *J. Neurosci. Off. J. Soc. Neurosci.* **31**, 11547–11552.
- Moir, R. D., Yoon, M., Khuon, S. and Goldman, R. D.** (2000). Nuclear lamins A and B1: different pathways of assembly during nuclear envelope formation in living cells. *J. Cell Biol.* **151**, 1155–1168.
- Müller, M., Bhattacharya, S. S., Moore, T., Prescott, Q., Wedig, T., Herrmann, H. and Magin, T. M.** (2009). Dominant cataract formation in association with a vimentin assembly disrupting mutation. *Hum. Mol. Genet.* **18**, 1052–1057.
- Naetar, N. and Foisner, R.** (2009). Lamin complexes in the nuclear interior control progenitor cell proliferation and tissue homeostasis. *Cell Cycle Georget. Tex* **8**, 1488–1493.
- Naetar, N., Korbei, B., Kozlov, S., Kerenyi, M. A., Dorner, D., Kral, R., Gotic, I., Fuchs, P., Cohen, T. V., Bittner, R., et al.** (2008). Loss of nucleoplasmic LAP2 α -lamin A complexes causes erythroid and epidermal progenitor hyperproliferation. *Nat. Cell Biol.* **10**, 1341–1348.

References

- Neelam, S., Chancellor, T. J., Li, Y., Nickerson, J. A., Roux, K. J., Dickinson, R. B. and Lele, T. P. (2015). Direct force probe reveals the mechanics of nuclear homeostasis in the mammalian cell. *Proc. Natl. Acad. Sci. U. S. A.*
- Nekrasova, O. E., Mendez, M. G., Chernoiivanenko, I. S., Tyurin-Kuzmin, P. A., Kuczmariski, E. R., Gelfand, V. I., Goldman, R. D. and Minin, A. A. (2011). Vimentin intermediate filaments modulate the motility of mitochondria. *Mol. Biol. Cell* **22**, 2282–2289.
- Nixon, R. A. and Logvinenko, K. B. (1986). Multiple fates of newly synthesized neurofilament proteins: evidence for a stationary neurofilament network distributed nonuniformly along axons of retinal ganglion cell neurons. *J. Cell Biol.* **102**, 647–659.
- Ohshima, T., Ward, J. M., Huh, C. G., Longenecker, G., Veeranna, Pant, H. C., Brady, R. O., Martin, L. J. and Kulkarni, A. B. (1996). Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 11173–11178.
- Ortega, H. H., Lorente, J. A. and Salvetti, N. R. (2004). Immunohistochemical Study of Intermediate Filaments and Neuroendocrine Marker Expression in Leydig Cells of Laboratory Rodents. *Anat. Histol. Embryol.* **33**, 309–315.
- Osorio, F. G., Navarro, C. L., Cadiñanos, J., López-Mejía, I. C., Quirós, P. M., Bartoli, C., Rivera, J., Tazi, J., Guzmán, G., Varela, I., et al. (2011). Splicing-Directed Therapy in a New Mouse Model of Human Accelerated Aging. *Sci. Transl. Med.* **3**, 106ra107–106ra107.
- Pacheco, L. M., Gomez, L. A., Dias, J., Ziebarth, N. M., Howard, G. A. and Schiller, P. C. (2014). Progerin expression disrupts critical adult stem cell functions involved in tissue repair. *Aging* **6**, 1049–1063.
- Pallari, H.-M., Lindqvist, J., Torvaldson, E., Ferraris, S. E., He, T., Sahlgren, C. and Eriksson, J. E. (2011). Nestin as a regulator of Cdk5 in differentiating myoblasts. *Mol. Biol. Cell* **22**, 1539–1549.
- Palmisano, M. G., Bremner, S. N., Hornberger, T. A., Meyer, G. A., Domenighetti, A. A., Shah, S. B., Kiss, B., Kellermayer, M., Ryan, A. F. and Lieber, R. L. (2015). Skeletal muscle intermediate filaments form a stress-transmitting and stress-signaling network. *J. Cell Sci.* **128**, 219–224.
- Pant, A. C., Veeranna, null, Pant, H. C. and Amin, N. (1997). Phosphorylation of human high molecular weight neurofilament protein (hNF-H) by neuronal cyclin-dependent kinase 5 (cdk5). *Brain Res.* **765**, 259–266.
- Park, D., Xiang, A. P., Mao, F. F., Zhang, L., Di, C.-G., Liu, X.-M., Shao, Y., Ma, B.-F., Lee, J.-H., Ha, K.-S., et al. (2010). Nestin is required for the proper self-renewal of neural stem cells. *Stem Cells Dayt. Ohio* **28**, 2162–2171.
- Pasut, A., Oleynik, P. and Rudnicki, M. A. (2012). Isolation of muscle stem cells by fluorescence activated cell sorting cytometry. *Methods Mol. Biol. Clifton NJ* **798**, 53–64.
- Patrick, G. N., Zhou, P., Kwon, Y. T., Howley, P. M. and Tsai, L. H. (1998). p35, the neuronal-specific activator of cyclin-dependent kinase 5 (Cdk5) is degraded by the ubiquitin-proteasome pathway. *J. Biol. Chem.* **273**, 24057–24064.
- Patrick, G. N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. and Tsai, L. H. (1999). Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* **402**, 615–622.

References

- Pekny, M., Johansson, C. B., Eliasson, C., Stakeberg, J., Wallen, ?sa, Perlmann, T., Lendahl, U., Betsholtz, C., Berthold, C.-H. and Frisen, J.** (1999). Abnormal Reaction to Central Nervous System Injury in Mice Lacking Glial Fibrillary Acidic Protein and Vimentin. *J. Cell Biol.* **145**, 503–514.
- Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R. and Fainzilber, M.** (2005). Vimentin-Dependent Spatial Translocation of an Activated MAP Kinase in Injured Nerve. *Neuron* **45**, 715–726.
- Perlson, E., Michaelevski, I., Kowalsman, N., Ben-Yaakov, K., Shaked, M., Seger, R., Eisenstein, M. and Fainzilber, M.** (2006). Vimentin binding to phosphorylated Erk sterically hinders enzymatic dephosphorylation of the kinase. *J. Mol. Biol.* **364**, 938–944.
- Perng, M.-D., Zhang, Q. and Quinlan, R. A.** (2007). Insights into the beaded filament of the eye lens. *Exp. Cell Res.* **313**, 2180–2188.
- Peter, A. and Stick, R.** (2012). Evolution of the lamin protein family: what introns can tell. *Nucl. Austin Tex* **3**, 44–59.
- Peter, A. and Stick, R.** (2015). Evolutionary aspects in intermediate filament proteins. *Curr. Opin. Cell Biol.* **32**, 48–55.
- Peter, M., Nakagawa, J., Dorée, M., Labbé, J. C. and Nigg, E. A.** (1990). In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. *Cell* **61**, 591–602.
- Peters, B., Kirfel, J., Bussow, H., Vidal, M. and Magin, T. M.** (2001). Complete Cytolysis and Neonatal Lethality in Keratin 5 Knockout Mice Reveal Its Fundamental Role in Skin Integrity and in Epidermolysis Bullosa Simplex. *Mol. Biol. Cell* **12**, 1775–1789.
- Pethig, K., Genschel, J., Peters, T., Wilhelmi, M., Flemming, P., Lochs, H., Haverich, A. and Schmidt, H. H.-J.** (2005). LMNA Mutations in Cardiac Transplant Recipients. *Cardiology* **103**, 57–62.
- Philpott, A., Porro, E. B., Kirschner, M. W. and Tsai, L. H.** (1997). The role of cyclin-dependent kinase 5 and a novel regulatory subunit in regulating muscle differentiation and patterning. *Genes Dev.* **11**, 1409–1421.
- Prahlad, V., Helfand, B. T., Langford, G. M., Vale, R. D. and Goldman, R. D.** (2000). Fast transport of neurofilament protein along microtubules in squid axoplasm. *J. Cell Sci.* **113**, 3939–3946.
- Quinlan, R. A., Brenner, M., Goldman, J. E. and Messing, A.** (2007). GFAP and its role in Alexander Disease. *Exp. Cell Res.* **313**, 2077–2087.
- Robert, A., Herrmann, H., Davidson, M. W. and Gelfand, V. I.** (2014). Microtubule-dependent transport of vimentin filament precursors is regulated by actin and by the concerted action of Rho- and p21-activated kinases. *FASEB J.* **28**, 2879–2890.
- Rosales, J. L. and Lee, K.-Y.** (2006). Extraneuronal roles of cyclin-dependent kinase 5. *BioEssays* **28**, 1023–1034.
- Rosengarden, Y., McKenna, T., Grochová, D. and Eriksson, M.** (2011). Stem cell depletion in Hutchinson–Gilford progeria syndrome. *Aging Cell* **10**, 1011–1020.
- Rowat, A. C., Jaalouk, D. E., Zwerger, M., Ung, W. L., Eydelnant, I. A., Olins, D. E., Olins, A. L., Herrmann, H., Weitz, D. A. and Lammerding, J.** (2013). Nuclear Envelope Composition Determines the Ability of Neutrophil-type Cells to Passage through Micron-scale Constrictions. *J. Biol. Chem.* **288**, 8610–8618.

References

- Sahlgren, C. M., Mikhailov, A., Hellman, J., Chou, Y. H., Lendahl, U., Goldman, R. D. and Eriksson, J. E. (2001). Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. *J. Biol. Chem.* **276**, 16456–16463.
- Sahlgren, C. M., Mikhailov, A., Vaittinen, S., Pallari, H.-M., Kalimo, H., Pant, H. C. and Eriksson, J. E. (2003). Cdk5 regulates the organization of Nestin and its association with p35. *Mol. Cell. Biol.* **23**, 5090–5106.
- Sahlgren, C. M., Pallari, H.-M., He, T., Chou, Y.-H., Goldman, R. D. and Eriksson, J. E. (2006). A nestin scaffold links Cdk5/p35 signaling to oxidant-induced cell death. *EMBO J.* **25**, 4808–4819.
- Schulze, S. R., Curio-Penny, B., Li, Y., Imani, R. A., Rydberg, L., Geyer, P. K. and Wallrath, L. L. (2005). Molecular Genetic Analysis of the Nested *Drosophila melanogaster* Lamin C Gene. *Genetics* **171**, 185–196.
- Schulze, S. R., Curio-Penny, B., Speese, S., Dialynas, G., Cryderman, D. E., McDonough, C. W., Nalbant, D., Petersen, M., Budnik, V., Geyer, P. K., et al. (2009). A Comparative Study of *Drosophila* and Human A-Type Lamins. *PLoS ONE* **4**, e7564.
- Schwarz, N., Windoffer, R., Magin, T. M. and Leube, R. E. (2015). Dissection of keratin network formation, turnover and reorganization in living murine embryos. *Sci. Rep.* **5**.
- Sejersen, T. and Lendahl, U. (1993). Transient expression of the intermediate filament nestin during skeletal muscle development. *J. Cell Sci.* **106** (Pt 4), 1291–1300.
- Sharma, P., Barchi, J. J., Huang, X., Amin, N. D., Jaffe, H. and Pant, H. C. (1998). Site-specific phosphorylation of Lys-Ser-Pro repeat peptides from neurofilament H by cyclin-dependent kinase 5: structural basis for substrate recognition. *Biochemistry (Mosc.)* **37**, 4759–4766.
- Shea, T. B. and Chan, W. K.-H. (2008). Regulation of neurofilament dynamics by phosphorylation. *Eur. J. Neurosci.* **27**, 1893–1901.
- Shen, W.-J., Zaidi, S. K., Patel, S., Cortez, Y., Ueno, M., Azhar, R., Azhar, S. and Kraemer, F. B. (2012). Ablation of Vimentin Results in Defective Steroidogenesis. *Endocrinology* **153**, 3249–3257.
- Shimi, T., Pflieger, K., Kojima, S., Pack, C.-G., Solovei, I., Goldman, A. E., Adam, S. A., Shumaker, D. K., Kinjo, M., Cremer, T., et al. (2008). The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. *Genes Dev.* **22**, 3409–3421.
- Shukla, V., Skuntz, S. and Pant, H. C. (2012). Deregulated Cdk5 Activity Is Involved in Inducing Alzheimer's Disease. *Arch. Med. Res.* **43**, 655–662.
- Sihag, R. K., Inagaki, M., Yamaguchi, T., Shea, T. B. and Pant, H. C. (2007). Role of Phosphorylation on the Structural Dynamics and Function of Types III and IV Intermediate Filaments. *Exp. Cell Res.* **313**, 2098–2109.
- Simon, D. N. and Wilson, K. L. (2013). Partners and post-translational modifications of nuclear lamins. *Chromosoma* **122**, 13–31.
- Simon, D. N., Domaradzki, T., Hofmann, W. A. and Wilson, K. L. (2013). Lamin A tail modification by SUMO1 is disrupted by familial partial lipodystrophy-causing mutations. *Mol. Biol. Cell* **24**, 342–350.
- Sjöberg, G., Edström, L., Lendahl, U. and Sejersen, T. (1994a). Myofibers from Duchenne/Becker muscular dystrophy and myositis express the intermediate filament nestin. *J. Neuropathol. Exp. Neurol.* **53**, 416–423.

References

- Sjöberg, G., Jiang, W.-Q., Ringertz, N. R., Lendahl, U. and Sejersen, T.** (1994b). Colocalization of Nestin and Vimentin/Desmin in Skeletal Muscle Cells Demonstrated by Three-Dimensional Fluorescence Digital Imaging Microscopy. *Exp. Cell Res.* **214**, 447–458.
- Smith, F. J. D., Liao, H., Cassidy, A. J., Stewart, A., Hamill, K. J., Wood, P., Joval, I., van Steensel, M. A. M., Björck, E., Callif-Daley, F., et al.** (2005). The genetic basis of pachyonychia congenita. *J. Investig. Dermatol. Symp. Proc. Soc. Investig. Dermatol. Inc Eur. Soc. Dermatol. Res.* **10**, 21–30.
- Snider, N. T. and Omary, M. B.** (2014). Post-translational modifications of intermediate filament proteins: mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* **15**, 163–177.
- Souza, J. de and Gottfried, C.** (2013). Muscle injury: Review of experimental models. *J. Electromyogr. Kinesiol.* **23**, 1253–1260.
- Steinert, P. M., Chou, Y. H., Prahlad, V., Parry, D. A., Marekov, L. N., Wu, K. C., Jang, S. I. and Goldman, R. D.** (1999). A high molecular weight intermediate filament-associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. Limited co-assembly in vitro to form heteropolymers with type III vimentin and type IV alpha-internexin. *J. Biol. Chem.* **274**, 9881–9890.
- Su, P.-H., Chen, C.-C., Chang, Y.-F., Wong, Z.-R., Chang, K.-W., Huang, B.-M. and Yang, H.-Y.** (2013). Identification and cytoprotective function of a novel nestin isoform, Nes-S, in dorsal root ganglia neurons. *J. Biol. Chem.* **288**, 8391–8404.
- Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C. L. and Burke, B.** (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* **147**, 913–920.
- Sunabori, T., Tokunaga, A., Nagai, T., Sawamoto, K., Okabe, M., Miyawaki, A., Matsuzaki, Y., Miyata, T. and Okano, H.** (2008). Cell-cycle-specific nestin expression coordinates with morphological changes in embryonic cortical neural progenitors. *J. Cell Sci.* **121**, 1204–1212.
- Swift, J., Ivanovska, I. L., Buxboim, A., Harada, T., Dingal, P. C. D. P., Pinter, J., Pajerowski, J. D., Spinler, K. R., Shin, J.-W., Tewari, M., et al.** (2013). Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**, 1240104.
- Tanaka, H., Goto, H., Inoko, A., Makihara, H., Enomoto, A., Horimoto, K., Matsuyama, M., Kurita, K., Izawa, I. and Inagaki, M.** (2015). Cytokinetic Failure-induced Tetraploidy Develops into Aneuploidy, Triggering Skin Aging in Phosphovimentin-deficient Mice. *J. Biol. Chem.* **290**, 12984–12998.
- Terzi, F., Henrion, D., Colucci-Guyon, E., Federici, P., Babinet, C., Levy, B. I., Briand, P. and Friedlander, G.** (1997). Reduction of renal mass is lethal in mice lacking vimentin. Role of endothelin-nitric oxide imbalance. *J. Clin. Invest.* **100**, 1520–1528.
- Toivola, D. M., Zhou, Q., English, L. S. and Omary, M. B.** (2002). Type II Keratins Are Phosphorylated on a Unique Motif during Stress and Mitosis in Tissues and Cultured Cells. *Mol. Biol. Cell* **13**, 1857–1870.
- Torvaldson, E., Kochin, V. and Eriksson, J. E.** (2015). Phosphorylation of lamins determine their structural properties and signaling functions. *Nucl. Austin Tex* **6**, 166–171.
- Tsai, L. H., Takahashi, T., Caviness, V. S. and Harlow, E.** (1993). Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse nervous system. *Development* **119**, 1029–1040.

- Tschaharganeh, D. F., Xue, W., Calvisi, D. F., Evert, M., Michurina, T. V., Dow, L. E., Banito, A., Katz, S. F., Kasthuber, E. R., Weissmueller, S., et al. (2014). p53-Dependent Nestin Regulation Links Tumor Suppression to Cellular Plasticity in Liver Cancer. *Cell* **158**, 579–592.
- Uchida, A., Alami, N. H. and Brown, A. (2009). Tight Functional Coupling of Kinesin-1A and Dynein Motors in the Bidirectional Transport of Neurofilaments. *Mol. Biol. Cell* **20**, 4997–5006.
- Uchino, R., Nonaka, Y., Horigome, T., Sugiyama, S. and Furukawa, K. (2013). Loss of Drosophila A-type lamin C initially causes tendon abnormality including disintegration of cytoskeleton and nuclear lamina in muscular defects. *Dev. Biol.* **373**, 216–227.
- UMD-LMNA at www.umd.be
- Vadrot, N., Duband-Goulet, I., Cabet, E., Attanda, W., Barateau, A., Vicart, P., Gerbal, F., Briand, N., Vigouroux, C., Oldenburg, A. R., et al. (2015). The p.R482W substitution in A-type lamins deregulates SREBP1 activity in Dunnigan-type familial partial lipodystrophy. *Hum. Mol. Genet.* **24**, 2096–2109.
- Vaittinen, S., Lukka, R., Sahlgren, C., Rantanen, J., Hurme, T., Lendahl, U., Eriksson, J. E. and Kalimo, H. (1999). Specific and innervation-regulated expression of the intermediate filament protein nestin at neuromuscular and myotendinous junctions in skeletal muscle. *Am. J. Pathol.* **154**, 591–600.
- Vaittinen, S., Lukka, R., Sahlgren, C., Hurme, T., Rantanen, J., Lendahl, U., Eriksson, J. E. and Kalimo, H. (2001). The expression of intermediate filament protein nestin as related to vimentin and desmin in regenerating skeletal muscle. *J. Neuropathol. Exp. Neurol.* **60**, 588–597.
- van Spaendonck-Zwarts, K. Y., van Hessem, L., Jongbloed, J. D. H., de Walle, H. E. K., Capetanaki, Y., van der Kooi, A. J., van Langen, I. M., van den Berg, M. P. and van Tintelen, J. P. (2011). Desmin-related myopathy. *Clin. Genet.* **80**, 354–366.
- Varga, R., Eriksson, M., Erdos, M. R., Olive, M., Harten, I., Kolodgie, F., Capell, B. C., Cheng, J., Faddah, D., Perkins, S., et al. (2006). Progressive vascular smooth muscle cell defects in a mouse model of Hutchinson–Gilford progeria syndrome. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 3250–3255.
- Virtakoivu, R., Mai, A., Mattila, E., De Franceschi, N., Imanishi, S. Y., Corthals, G., Kaukonen, R., Saari, M., Cheng, F., Torvaldson, E., et al. (2015). Vimentin-ERK Signaling Uncouples Slug Gene Regulatory Function. *Cancer Res.* **75**, 2349–2362.
- Vuoriluoto, K., Haugen, H., Kiviluoto, S., Mpindi, J.-P., Nevo, J., Gjerdrum, C., Tiron, C., Lorens, J. B. and Ivaska, J. (2011). Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. *Oncogene* **30**, 1436–1448.
- Wagner, M., Trost, A., Hintner, H., Bauer, J. W. and Onder, K. (2013). Imbalance of intermediate filament component keratin 14 contributes to increased stress signalling in epidermolysis bullosa simplex. *Exp. Dermatol.* **22**, 292–294.
- Walker, A. S., Goings, G. E., Kim, Y., Miller, R. J., Chenn, A. and Szele, F. G. (2011). Nestin Reporter Transgene Labels Multiple Central Nervous System Precursor Cells. *Neural Plast.* **2010**, e894374.
- Wang, L., Ho, C., Sun, D., Liem, R. K. H. and Brown, A. (2000). Rapid movement of axonal neurofilaments interrupted by prolonged pauses. *Nat. Cell Biol.* **2**, 137–141.

References

- Wang, Y., Herron, A. J. and Worman, H. J.** (2006). Pathology and nuclear abnormalities in hearts of transgenic mice expressing M371K lamin A encoded by an LMNA mutation causing Emery–Dreifuss muscular dystrophy. *Hum. Mol. Genet.* **15**, 2479–2489.
- Weber, K., Plessmann, U. and Ulrich, W.** (1989). Cytoplasmic intermediate filament proteins of invertebrates are closer to nuclear lamins than are vertebrate intermediate filament proteins; sequence characterization of two muscle proteins of a nematode. *EMBO J.* **8**, 3221–3227.
- Wede, O. K., Löfgren, M., Li, Z., Paulin, D. and Arner, A.** (2002). Mechanical function of intermediate filaments in arteries of different size examined using desmin deficient mice. *J. Physiol.* **540**, 941–949.
- Wiese, C., Rolletschek, A., Kania, G., Blyszczuk, P., Tarasov, K. V., Tarasova, Y., Wersto, R. P., Boheler, K. R. and Wobus, A. M.** (2004). Nestin expression--a property of multi-lineage progenitor cells? *Cell. Mol. Life Sci. CMLS* **61**, 2510–2522.
- Windoffer, R. and Leube, R. E.** (1999). Detection of cytokeratin dynamics by time-lapse fluorescence microscopy in living cells. *J. Cell Sci.* **112**, 4521–4534.
- Wolf, C. M., Wang, L., Alcalai, R., Pizard, A., Burgon, P. G., Ahmad, F., Sherwood, M., Branco, D. M., Wakimoto, H., Fishman, G. I., et al.** (2008). Lamin A/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease. *J. Mol. Cell. Cardiol.* **44**, 293–303.
- Wöll, S., Windoffer, R. and Leube, R. E.** (2005). Dissection of keratin dynamics: different contributions of the actin and microtubule systems. *Eur. J. Cell Biol.* **84**, 311–328.
- Wong, Z.-R., Su, P.-H., Chang, K.-W., Huang, B.-M., Lee, H. and Yang, H.-Y.** (2013). Identification of a rod domain-truncated isoform of nestin, Nes- $\Delta_{107-254}$, in rat dorsal root ganglia. *Neurosci. Lett.* **553**, 181–185.
- Wroblewski, J., Engström, M., Edwall-Arvidsson, C., Sjöberg, G., Sejersen, T. and Lendahl, U.** (1997). Distribution of nestin in the developing mouse limb bud in vivo and in micro-mass cultures of cells isolated from limb buds. *Differ. Res. Biol. Divers.* **61**, 151–159.
- Yaffe, D. and Saxel, O.** (1977). Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature* **270**, 725–727.
- Yamaguchi, M.** (2005). Analysis of neurogenesis using transgenic mice expressing GFP with nestin gene regulatory regions. *Chem. Senses* **30**, i117–i118.
- Yamaguchi, M., Saito, H., Suzuki, M. and Mori, K.** (2000). Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice. *Neuroreport* **11**, 1991–1996.
- Yang, S. H., Bergo, M. O., Toth, J. I., Qiao, X., Hu, Y., Sandoval, S., Meta, M., Bendale, P., Gelb, M. H., Young, S. G., et al.** (2005). Blocking protein farnesyltransferase improves nuclear blebbing in mouse fibroblasts with a targeted Hutchinson–Gilford progeria syndrome mutation. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 10291–10296.
- Yang, J., Dominguez, B., de Winter, F., Gould, T. W., Eriksson, J. E. and Lee, K.-F.** (2011). Nestin negatively regulates postsynaptic differentiation of the neuromuscular synapse. *Nat. Neurosci.* **14**, 324–330.
- Yang, S. H., Procaccia, S., Jung, H.-J., Nobumori, C., Tatar, A., Tu, Y., Bayguinov, Y. R., Hwang, S. J., Tran, D., Ward, S. M., et al.** (2015). Mice that express farnesylated versions of prelamin A in neurons develop achalasia. *Hum. Mol. Genet.* **24**, 2826–2840.

References

- Yin, H., Price, F. and Rudnicki, M. A.** (2013). Satellite cells and the muscle stem cell niche. *Physiol. Rev.* **93**, 23–67.
- Yoon, K. H., Yoon, M., Moir, R. D., Khuon, S., Flitney, F. W. and Goldman, R. D.** (2001). Insights into the Dynamic Properties of Keratin Intermediate Filaments in Living Epithelial Cells. *J. Cell Biol.* **153**, 503–516.
- Yoon, B. C., Jung, H., Dwivedy, A., O'Hare, C. M., Zivraj, K. H. and Holt, C. E.** (2012). Local Translation of Extranuclear Lamin B Promotes Axon Maintenance. *Cell* **148**, 752–764.
- Young, S. G., Meta, M., Yang, S. H. and Fong, L. G.** (2006). Prelamin A Farnesylation and Progeroid Syndromes. *J. Biol. Chem.* **281**, 39741–39745.
- Zhang, Y.-Q. and Sarge, K. D.** (2008). Sumoylation regulates lamin A function and is lost in lamin A mutants associated with familial cardiomyopathies. *J. Cell Biol.* **182**, 35–39.
- Zhang, H., Kieckhafer, J. E. and Cao, K.** (2013). Mouse models of laminopathies. *Aging Cell* **12**, 2–10.
- Zhong, H., Jin, Z., Chen, Y., Zhang, T., Bian, W., Cui, X. and Jing, N.** (2008). First intron of nestin gene regulates its expression during C2C12 myoblast differentiation. *Acta Biochim. Biophys. Sin.* **40**, 526–532.
- Zimmerman, L., Parr, B., Lendahl, U., Cunningham, M., McKay, R., Gavin, B., Mann, J., Vassileva, G. and McMahon, A.** (1994). Independent regulatory elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. *Neuron* **12**, 11–24.



ISBN 978-952-12-3330-2
Painosalama Oy – Turku, Finland 2015