Nucleo-cytoskeletal interactions in the mechanical functioning of the cell

Summary and General Discussion

Not long after the first discoveries of LMNA mutations causing two different diseases [1], it was recognized that mutations in the LMNA gene cause a variety of diseases, collectively called laminopathies. While the number of described pathologies has risen exponentially, the clinical diversity of the diseases associated with LMNA mutations has raised numerous questions about the underlying disease development mechanisms at the (sub)cellular level. The first obvious question is how different mutations in a single gene can cause this variety of diseases. Extensive protein structural analyses did not solve this question yet, especially since the exact function of affected protein domains is not well known. Lamin A/C proteins form homo-and heterodimers, forming the nuclear lamina, which suggested functions are: 1) formation of a barrier between the nucleus and cytoplasm; 2) providing structural support to the nucleus; 3) aiding in nucleus reformation after mitosis; 4) interaction with several intranuclear and transmembrane proteins, forming both a mechanical scaffold, as well as a docking site for signaling proteins; 5) interaction with chromatin, suggesting several (sub-) functions: i – chromatin organisation, formation of heterochromatin; ii – chromatin replication; iii – gene regulation .
A second important question is why identical mutations lead to different disease symptoms between patients: Identical mutations can lead to FPLD or to EDMD or other clinically distinct phenotypes such as DCM. Moreover the disease penetrance can be extremely variable, even between family members. Siblings with identical mutations can either develop a severe form of laminopathy or remain without disease symptoms throughout their lifespan. This makes it virtually impossible to predict clinical outcome even if the genetic diagnosis confirms the presence of a LMNA mutation.
While several studies have focused on gene regulatory effects of LMNA mutations, this thesis describes the structural defects in lamina organisation and the resulting deficiencies in functioning, due to either absence of lamins or LMNA mutations.

In chapter 1, the impact of the absence of A-type lamins on the integrity of the cellular structural scaffold was described. This network consists of the nuclear lamina (lamins and associated proteins), the cytoskeletal proteins and cell adhesion molecules. All these proteins are interconnected through linker proteins. At the nuclear envelope, SUN-proteins and nesprins link the nuclear lamina to the different cytoskeletal elements, which in turn are connected to the cellular membrane via actinin, catenin, vinculin, plectin and other membrane linker proteins. The network as a whole forms a scaffold that acts as a tensegrity structure. This structure combines internal compression (microtubules) and tension (actin filaments) forces and protects the

cellular components (like the nucleus) during mechanical compression [2-5]. All the elements of this mechanical network are essential as they form one interacting mechanical entity, which cannot function if one of the elements fails. When the lamin structure is disturbed (as a result of a mutation or knock-out), this has a dramatic effect on the other elements of the network. Lamin-associated proteins (e.g. emerin), or linker-proteins (e.g. nesprins) are mislocalized when lamin A/C proteins are affected. Previous related studies showed that upon nuclear compression, cells without A-type lamins show nuclear rupture while these cells also showed a reduced resistance to force generated by compression [6]. Extension of these studies on isolated nuclei (with or without A-type lamins) showed that indeed the intact cellular scaffold is needed to generate cellular strength, since all types of isolated nuclei showed similar deformation upon compression.
These findings suggest that other defects in elements of the cytoskeleton and linker proteins can lead to a dysfunctional mechanical network at the cellular level, leading to comparable clinical features (see below).

In chapter 2, we were able to show that loss of the integrity of the mechanical scaffold leads to a loss of functional mechanical properties of the cell. The correct positioning of the nucleus and the MTOC is essential for the cell migration process [7, 8]. This repositioning depends on the mechanical scaffold network. As this network fails, as a result of lamin deficiency, the nucleus and the MTOC cannot reposition properly, affecting the nuclear reorientation process during wound healing. Also, defects are seen at the plasma membrane, where membrane protrusions are not formed properly during cellular migration.

These results indicate that a functional mechanical scaffold network is essential for coping with mechanical stress as well as with cell migration, and that lamin deficiency affects this network. Recent work from Brosig et al. and Lombardi et al. confirm that the LINC-complex (which connects to the nuclear lamina) is necessary for the correct nuclear repositioning and rotation [9-11].

Our data from chapter 3 shows a higher nuclear plasticity and associated increased chromatin mobility, which can affect genomic stability and thus influence premature aging.

In chapter 4, we show that the loss of integrity of the nuclear membrane is a common finding in isolated dermal fibroblasts from a variety of laminopathy patients. We

stipulate that the loss of lamin organization in laminopathies leads to a weakening of the nuclear scaffold. Weak spots in the nuclear envelope can lead to dynamic, reversible ruptures of the nuclear membrane at these locations. These ruptures are non-lethal to the cell, and result in a temporary loss of compartmentalization between the nucleus and the cytoplasm, resulting in a redistribution of signalling proteins and transcription factors that are normally transported actively across the nuclear membrane. While nuclear components that are associated with chromatin or other intranuclear structures will not diffuse out of the nucleus during the ruptures, (temporary) soluble proteins can diffuse out or into the nucleus. Similar results were seen in HIV-infected cells [12]. It is known that viruses modulate the nuclear lamina, inducing similar weak spots in the nuclear envelope, which can lead to the dynamic ruptures of the nuclear membrane [13-23].
In chapter 5, we concentrate on the presence of cytoplasmic PML nuclear body staining in a range of dermal fibroblasts from laminopathy patients. In general, patients with the most severe disease symptoms suffered from the highest numbers of cytoplasmic PLM-Nabs. Although this can partially be explained by the previous described phenomenon of nuclear ruptures, other mechanisms are believed to be involved in the mislocalization of PML bodies.

Over the years, many hypotheses concerning laminopathies have been formulated throughout the scientific community [24]. These hypotheses originate from different research fields in cell biology: cellular mechanics, signalling pathways and gene expression regulation.
The described hypotheses include:

1. The structural hypothesis
2. The gene expression hypothesis
3. The premature aging hypothesis
4. The cell proliferation hypothesis
5. The nuclear integrity hypothesis, as formulated in chapter 4 and 5

The structural hypothesis, stating that loss of structural strength causes laminopathies, is supported by our findings described in chapter 1 and 2. We were able to demonstrate that the structure of physical connections between the different elements of the cellular scaffold (elements of the cytoskeleton, nuclear lamins and linker proteins) is compromised in diseased cells, leading to a disturbed nuclear reorientation upon scratch wound healing and cell migration. Apparently, if one of the cellular scaffold elements is aberrant, both structural and functional effects can be seen.

Next to the pure mechanical function of the cellular scaffold network, this network also provides the infrastructure for response upon mechanical stress, through the activation of mechanosensitive signalling proteins [25]. This is called mechanosignalling. Mechanosignalling can become disturbed in case of a lamin mutation, which in turn can affect gene regulation. Lammerding et al. [25] showed that expression of mechanosensitive genes *egr-1* and *iex-1*in response to mechanical stimulation was impaired in *Lmna–/–* cells.

The second hypothesis states that these diseases arise from the loss of gene regulatory functions of lamin complexes While nuclear lamins are no transcription factors, they can act as a scaffold for gene regulation complexes [26]. An aberration in the nuclear lamina structure could thus affect the formation, localization and functioning of the complexes. Indications for impaired scaffold function come from studies examining the phosphorylation state of the transcription factor ERK.
Data from Muchir et al [27-30] and Emerson [31], as well as from our own research (see chapter 2) clearly indicate a constitutive increase in the amount of phosphorylated ERK in diseased cells. ERK1 and ERK2 interact with A-type lamins at the nuclear periphery and participate in the rapid regulation of activator protein 1 (AP-1) activity. Initially, it was suggested that the NE directly modulates ERK1/2 activity and downstream signaling, and that alterations in lamin A/C expression might perturb NE structure sufficiently to directly affect these processes (see González et al [32]). However, a more recent study by Warren et al. [33] shows that nesprin-2 tethers ERK at PML-nuclear bodies,. As the nuclear localization of nesprin-2 depends on lamin A/C (chapter 2), it is conceivable that ERK can be retained in a lamin A/C/ERK/PML NB complex. This suggestion is even more interesting because of our discovery that PML NBs are often lost from the nucleus in laminopathies (chapter 4 and 5).
Other studies, which show a role of A-type lamins in transcription regulation, suggest that lamin A/C deficiency abolishes TGF-ß1 induced pRB dephosphorylation through its interaction with PP2A. As a result, LMNA deficient cells display increased proliferation and decreased terminal differentiation [34]. Other pathways that were disturbed in case of lamin deficiency are the NFκB pathway and the activation of mechanosensitive genes iex-1 and egr-1, leading to an impaired mechanotransduction (see above) [25]. Expression of several other genes was suggested to be (negatively) regulated by lamins A/C in specific tissues, such as SREBP1 in adipocyte development [35], After treatment with protease inhibitors, prelamin A binds to SREBP1, which will lead to acquired lipodystrophy [36]. For reviews on the role of A-type lamins in the control gene expression see Andres et al [26] and Maraldi et al [37].

The third hypothesis states that the structure of the nuclear envelope is essential for the repair of damaged DNA. If the lamin structure is altered as a result of lamin deficiency or mutation, DNA repair fails and cells and tissues will age prematurely [38, 39]. Of course, extreme examples of premature aging can be found in the group of systemic diseases, including HGPS [40, 41]. However also in other laminopathies premature ageing of myoblasts can be observed. Also, an effect on proteasome inactivation (which is associated with cellular aging) was seen in the absence of lamins [42].
Another clue to the mechanism underlying premature ageing was found by Gonzalez-Suarez et al. [43, 44], who demonstrated that lamins are essential for the maintenance of telomeres. Loss of lamins leads to shortening of the telomeres and increased genomic instability [45, 46]. In addition, a dramatic increase in telomere mobility within LMNA deficient cells suggest destabilization of chromatin organisation (Chapter 3)

The fourth hypothesis, the cell proliferation hypothesis, as described in [24], states that the loss of Rb function leads to an impaired satellite stem cell differentiation [47-49]. In normal cells A-type lamins form complexes with LAP2alfa to keep Rb in a dephosphorylated state. Absence of A-type lamins or lamin mutations disturb these complexes, leading to disturbance of the G1-S cell checkpoint control mechanism [50]. The inappropriate G1-S phase transition, allowing DNA duplication not preceded by DNA repair, could explain why in laminopathy cells so often DNA abnormalities arise during mitosis, as seen as a delay in the onset en progression of cytokinesis, and an impaired targeting of nuclear lamins into the nuclei of daughter cells [51]. Mutated lamins can also mislocalize during mitosis, leading to abnormal chromosome segregation and binucleation [52]. Disruption of mitosis leads to loss of proliferative capacity and premature aging [53].

Finally, the new hypothesis described by us, is based on our findings of nuclear ruptures in laminopathy cells. These ruptures, causing a sudden loss of compartmentalization between the nucleus and the cytoplasm, will disturb all existing gradients between the nucleus and the cytoplasm and allow proteins, which are under normal circumstances kept out of the nucleus, to diffuse into the nuclear interior and exert an untimely action. Not only unwanted transcription factors, but also protein modifying complexes (proteases, kinases, phosphatases etc.) and even organelles can enter into the ruptured nucleus (chapter 4 and 5) An overview of eukaryotic functions that involve a regulated nucleo-cytoplasmic compartmentalization is given in a review

article from Carmo-Fonseca. These functions include the control of the cell cycle, signal transduction pathways (like the MAPK-pathway), stress response, muscle differentiation, and many others [54]. While it remains to be shown that nuclear ruptures indeed have a detrimental effect on tissues in patients, the occurrence of intranuclear inclusions of organelles in nuclei of patient's muscle and heart tissues indicates that indeed nuclear ruptures take place *in vivo,* and may be a major cause of disease development.

Given the large variety of laminopathies, it is well possible that not all hypotheses apply to all types of laminopathies, some of which affect one specific type of tissue (muscle, heart, adipose tissue), while other have a more systemic effect.
In laminopathies that affect muscle cells and other tissues that are under constant mechanical stress, the cellular pathology is probably mainly associated with the structural hypothesis, as the cells do no longer have a functional mechanical scaffold to withstand the constant tension and compression. In contrast, the premature ageing hypothesis applies mainly to progeroid diseases, although also in muscular dystrophies premature ageing of muscle cells has been encountered [42].
The gene expression hypothesis might be very important in lipodystrophies The inhibition of pre-adipocyte differentiation is the most probable cause by disturbed interaction with the transcription factors SREBP1 which is critically important for the differentiation of adipose tissue [35, 55-58].

When we carefully examine all the hypotheses formulated over the last years, most of which can be supported by solid evidence, we must conclude that lamins have multiple functions within the mammalian cells, and are linked to both structural and regulatory elements. When the nuclear lamina becomes dysfunctional, because of a structural defect, the connected networks (structural network, gene regulatory network, etc) are affected. When the nuclear membrane ruptures, it will affect both the gene regulation and the mechanical functioning of the cell.
Therefore, integration of the different hypotheses to form a holistic approach for the cellular mechanisms that underlie the several pathologies in the group of laminopathies will be necessary.
It is the combination of the different pathways, together with the specific features of the different affected tissues (skeletal muscle, adipose tissue, heart muscle and other tissues), that leads to the wide spectrum of diseases and clinical features ranging from very mild (no clinical symptoms) to very severe (death *in utero*), that are linked to a mutation in the lamin proteins.

While this thesis mainly has shed light on the structural abnormalities in laminopathy cells, further research should focus on the link between cellular abnormalities, tissue damage, and prevention of or even treatment of disease development, based on our new knowledge on lamin function.

References

[1] D. Fatkin, C. MacRae, T. Sasaki, M.R. Wolff, M. Porcu, M. Frenneaux, J. Atherton, H.J. Vidaillet, Jr., S. Spudich, U. De Girolami, J.G. Seidman, C. Seidman, F. Muntoni, G. Muehle, W. Johnson and B. McDonough, Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease, N Engl J Med 341 (1999) 1715-1724.

[2] D.E. Ingber, Tensegrity I. Cell structure and hierarchical systems biology, J Cell Sci 116 (2003) 1157-1173.

[3] D.E. Ingber, Tensegrity II. How structural networks influence cellular information processing networks, J Cell Sci 116 (2003) 1397-1408.

[4] D.E. Ingber, Cellular mechanotransduction: putting all the pieces together again, Faseb J 20 (2006) 811-827.

[5] D.E. Ingber, Tensegrity and mechanotransduction, J Bodyw Mov Ther 12 (2008) 198-200.

[6] J.L. Broers, E.A. Peeters, H.J. Kuijpers, J. Endert, C.V. Bouten, C.W. Oomens, F.P. Baaijens and F.C. Ramaekers, Decreased mechanical stiffness in LMNA-/- cells is caused by defective nucleo-cytoskeletal integrity: implications for the development of laminopathies, Hum Mol Genet 13 (2004) 2567-2580.

[7] J.S. Lee, C.M. Hale, P. Panorchan, S.B. Khatau, J.P. George, Y. Tseng, C.L. Stewart, D. Hodzic and D. Wirtz, Nuclear lamin A/C deficiency induces defects in cell mechanics, polarization, and migration, Biophys J 93 (2007) 2542-2552.

[8] M. Schneider, W. Lu, S. Neumann, A. Brachner, J. Gotzmann, A.A. Noegel and I. Karakesisoglou, Molecular mechanisms of centrosome and cytoskeleton anchorage at the nuclear envelope, Cell Mol Life Sci.

[9] M. Brosig, J. Ferralli, L. Gelman, M. Chiquet and R. Chiquet-Ehrismann, Interfering with the connection between the nucleus and the cytoskeleton affects nuclear rotation, mechanotransduction and myogenesis, Int J Biochem Cell Biol 42 (2010) 1717-1728.

[10] M.L. Lombardi and J. Lammerding, Altered mechanical properties of the nucleus in disease, Methods Cell Biol 98 (2010) 121-141.

[11] M.L. Lombardi, D.E. Jaalouk, C.M. Shanahan, B. Burke, K.J. Roux and J. Lammerding, The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton, J Biol Chem (2011).

[12] C.M. de Noronha, M.P. Sherman, H.W. Lin, M.V. Cavrois, R.D. Moir, R.D. Goldman and W.C. Greene, Dynamic disruptions in nuclear envelope

architecture and integrity induced by HIV-1 Vpr, Science 294 (2001) 1105-1108.
[13] W. Muranyi, J. Haas, M. Wagner, G. Krohne and U.H. Koszinowski, Cytomegalovirus recruitment of cellular kinases to dissolve the nuclear lamina, Science 297 (2002) 854-857.
[14] A.E. Reynolds, L. Liang and J.D. Baines, Conformational changes in the nuclear lamina induced by herpes simplex virus type 1 require genes U(L)31 and U(L)34, J Virol 78 (2004) 5564-5575.
[15] M. Marschall, A. Marzi, P. aus dem Siepen, R. Jochmann, M. Kalmer, S. Auerochs, P. Lischka, M. Leis and T. Stamminger, Cellular p32 recruits cytomegalovirus kinase pUL97 to redistribute the nuclear lamina, J Biol Chem 280 (2005) 33357-33367.
[16] R. Park and J.D. Baines, Herpes simplex virus type 1 infection induces activation and recruitment of protein kinase C to the nuclear membrane and increased phosphorylation of lamin B, J Virol 80 (2006) 494-504.
[17] S.L. Bjerke and R.J. Roller, Roles for herpes simplex virus type 1 UL34 and US3 proteins in disrupting the nuclear lamina during herpes simplex virus type 1 egress, Virology 347 (2006) 261-276.
[18] D. Camozzi, S. Pignatelli, C. Valvo, G. Lattanzi, C. Capanni, P. Dal Monte and M.P. Landini, Remodelling of the nuclear lamina during human cytomegalovirus infection: role of the viral proteins pUL50 and pUL53, J Gen Virol 89 (2008) 731-740.
[19] C.P. Lee, Y.H. Huang, S.F. Lin, Y. Chang, Y.H. Chang, K. Takada and M.R. Chen, Epstein-Barr virus BGLF4 kinase induces disassembly of the nuclear lamina to facilitate virion production, J Virol 82 (2008) 11913-11926.
[20] S. Hamirally, J.P. Kamil, Y.M. Ndassa-Colday, A.J. Lin, W.J. Jahng, M.C. Baek, S. Noton, L.A. Silva, M. Simpson-Holley, D.M. Knipe, D.E. Golan, J.A. Marto and D.M. Coen, Viral mimicry of Cdc2/cyclin-dependent kinase 1 mediates disruption of nuclear lamina during human cytomegalovirus nuclear egress, PLoS Pathog 5 (2009) e1000275.
[21] G.L. Cano-Monreal, K.M. Wylie, F. Cao, J.E. Tavis and L.A. Morrison, Herpes simplex virus 2 UL13 protein kinase disrupts nuclear lamins, Virology 392 (2009) 137-147.
[22] N.J. Buchkovich, T.G. Maguire and J.C. Alwine, Role of the endoplasmic reticulum chaperone BiP, SUN domain proteins, and dynein in altering nuclear morphology during human cytomegalovirus infection, J Virol 84 7005-7017.
[23] N.R. Leach and R.J. Roller, Significance of host cell kinases in herpes simplex virus type 1 egress and lamin-associated protein disassembly from the nuclear lamina, Virology 406 127-137.
[24] J.L. Broers, F.C. Ramaekers, G. Bonne, R.B. Yaou and C.J. Hutchison, Nuclear lamins: laminopathies and their role in premature ageing, Physiol Rev 86 (2006) 967-1008.

[25] J. Lammerding, P.C. Schulze, T. Takahashi, S. Kozlov, T. Sullivan, R.D. Kamm, C.L. Stewart and R.T. Lee, Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction, J Clin Invest 113 (2004) 370-378.
[26] V. Andres and J.M. Gonzalez, Role of A-type lamins in signaling, transcription, and chromatin organization, J Cell Biol 187 (2009) 945-957.
[27] A. Muchir, P. Pavlidis, G. Bonne, Y.K. Hayashi and H.J. Worman, Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery Dreifuss muscular dystrophy, Hum Mol Genet 16 (2007) 1884-1895.
[28] A. Muchir, P. Pavlidis, V. Decostre, A.J. Herron, T. Arimura, G. Bonne and H.J. Worman, Activation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy, J Clin Invest 117 (2007) 1282-1293.
[29] A. Muchir, J. Shan, G. Bonne, S.E. Lehnart and H.J. Worman, Inhibition of extracellular signal-regulated kinase signaling to prevent cardiomyopathy caused by mutation in the gene encoding A-type lamins, Hum Mol Genet 18 (2009) 241-247.
[30] A. Muchir, W. Wu and H.J. Worman, Reduced expression of A-type lamins and emerin activates extracellular signal-regulated kinase in cultured cells, Biochim Biophys Acta 1792 (2009) 75-81.
[31] L.J. Emerson, M.R. Holt, M.A. Wheeler, M. Wehnert, M. Parsons and J.A. Ellis, Defects in cell spreading and ERK1/2 activation in fibroblasts with lamin A/C mutations, Biochim Biophys Acta 1792 (2009) 810-821.
[32] J.M. Gonzalez, A. Navarro-Puche, B. Casar, P. Crespo and V. Andres, Fast regulation of AP-1 activity through interaction of lamin A/C, ERK1/2, and c-Fos at the nuclear envelope, J Cell Biol 183 (2008) 653-666.
[33] D.T. Warren, T. Tajsic, J.A. Mellad, R. Searles, Q. Zhang and C.M. Shanahan, Novel nuclear nesprin-2 variants tether active extracellular signal-regulated MAPK1 and MAPK2 at promyelocytic leukemia protein nuclear bodies and act to regulate smooth muscle cell proliferation, J Biol Chem 285 (2010) 1311-1320.
[34] J.H. Van Berlo, J.W. Voncken, N. Kubben, J.L. Broers, R. Duisters, R.E. van Leeuwen, H.J. Crijns, F.C. Ramaekers, C.J. Hutchison and Y.M. Pinto, A-type lamins are essential for TGF-beta1 induced PP2A to dephosphorylate transcription factors, Hum Mol Genet 14 (2005) 2839-2849.
[35] C. Capanni, E. Mattioli, M. Columbaro, E. Lucarelli, V.K. Parnaik, G. Novelli, M. Wehnert, V. Cenni, N.M. Maraldi, S. Squarzoni and G. Lattanzi, Altered pre-lamin A processing is a common mechanism leading to lipodystrophy, Hum Mol Genet 14 (2005) 1489-1502.
[36] C.N. Goulbourne and D.J. Vaux, HIV protease inhibitors inhibit FACE1/ZMPSTE24: a mechanism for acquired lipodystrophy in patients on highly active antiretroviral therapy?, Biochem Soc Trans 38 (2010) 292-296.

[37] N.M. Maraldi, C. Capanni, R. Del Coco, S. Squarzoni, M. Columbaro, E. Mattioli, G. Lattanzi and F.A. Manzoli, Muscular laminopathies: role of prelamin A in early steps of muscle differentiation, Adv Enzyme Regul 51 (2011) 246-256.

[38] A. Vaughan, M. Alvarez-Reyes, J.M. Bridger, J.L. Broers, F.C. Ramaekers, M. Wehnert, G.E. Morris, W.G.F. Whitfield and C.J. Hutchison, Both emerin and lamin C depend on lamin A for localization at the nuclear envelope, J Cell Sci 114 (2001) 2577-2590.

[39] B. Liu, J. Wang, K.M. Chan, W.M. Tjia, W. Deng, X. Guan, J.D. Huang, K.M. Li, P.Y. Chau, D.J. Chen, D. Pei, A.M. Pendas, J. Cadinanos, C. Lopez-Otin, H.F. Tse, C. Hutchison, J. Chen, Y. Cao, K.S. Cheah, K. Tryggvason and Z. Zhou, Genomic instability in laminopathy-based premature aging, Nat Med 11 (2005) 780-785.

[40] B. Liu and Z. Zhou, Lamin A/C, laminopathies and premature ageing, Histol Histopathol 23 (2008) 747-763.

[41] S. Pereira, P. Bourgeois, C. Navarro, V. Esteves-Vieira, P. Cau, A. De Sandre-Giovannoli and N. Levy, HGPS and related premature aging disorders: from genomic identification to the first therapeutic approaches, Mech Ageing Dev 129 (2008) 449-459.

[42] S. Kandert, M. Wehnert, C.R. Muller, B. Buendia and M.C. Dabauvalle, Impaired nuclear functions lead to increased senescence and inefficient differentiation in human myoblasts with a dominant p.R545C mutation in the LMNA gene, Eur J Cell Biol 88 (2009) 593-608.

[43] I. Gonzalez-Suarez, A.B. Redwood and S. Gonzalo, Loss of A-type lamins and genomic instability, Cell Cycle 8 (2009) 3860-3865.

[44] I. Gonzalez-Suarez and S. Gonzalo, Nurturing the genome: A-type lamins preserve genomic stability, Nucleus 1 (2010) 129-135.

[45] P. Therizols, C. Fairhead, G.G. Cabal, A. Genovesio, J.C. Olivo-Marin, B. Dujon and E. Fabre, Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region, J Cell Biol 172 (2006) 189-199.

[46] I. Gonzalez-Suarez, A.B. Redwood, S.M. Perkins, B. Vermolen, D. Lichtensztejin, D.A. Grotsky, L. Morgado-Palacin, E.J. Gapud, B.P. Sleckman, T. Sullivan, J. Sage, C.L. Stewart, S. Mai and S. Gonzalo, Novel roles for A-type lamins in telomere biology and the DNA damage response pathway, Embo J 28 (2009) 2414-2427.

[47] C.J. Hutchison and H.J. Worman, A-type lamins: guardians of the soma?, Nat Cell Biol 6 (2004) 1062-1067.

[48] I. Mariappan and V.K. Parnaik, Sequestration of pRb by cyclin D3 causes intranuclear reorganization of lamin A/C during muscle cell differentiation, Mol Biol Cell 16 (2005) 1948-1960.

[49] E. Markiewicz, M. Ledran and C.J. Hutchison, Remodelling of the nuclear lamina and nucleoskeleton is required for skeletal muscle differentiation in vitro, J Cell Sci 118 (2005) 409-420.
[50] N. Naetar and R. Foisner, Lamin complexes in the nuclear interior control progenitor cell proliferation and tissue homeostasis, Cell Cycle 8 (2009) 1488-1493.
[51] T. Dechat, T. Shimi, S.A. Adam, A.E. Rusinol, D.A. Andres, H.P. Spielmann, M.S. Sinensky and R.D. Goldman, Alterations in mitosis and cell cycle progression caused by a mutant lamin A known to accelerate human aging, Proc Natl Acad Sci U S A 104 (2007) 4955-4960.
[52] K. Cao, B.C. Capell, M.R. Erdos, K. Djabali and F.S. Collins, A lamin A protein isoform overexpressed in Hutchinson-Gilford progeria syndrome interferes with mitosis in progeria and normal cells, Proc Natl Acad Sci U S A 104 (2007) 4949-4954.
[53] T.V. Cohen, L. Hernandez and C.L. Stewart, Functions of the nuclear envelope and lamina in development and disease, Biochem Soc Trans 36 (2008) 1329-1334.
[54] M. Carmo-Fonseca, The contribution of nuclear compartmentalization to gene regulation, Cell 108 (2002) 513-521.
[55] D.J. Lloyd, R.C. Trembath and S. Shackleton, A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies, Hum Mol Genet 11 (2002) 769-777.
[56] R.L. Boguslavsky, C.L. Stewart and H.J. Worman, Nuclear lamin A inhibits adipocyte differentiation: implications for Dunnigan-type familial partial lipodystrophy, Hum Mol Genet 15 (2006) 653-663.
[57] N.M. Maraldi, C. Capanni, G. Lattanzi, D. Camozzi, A. Facchini and F.A. Manzoli, SREBP1 interaction with prelamin A forms: a pathogenic mechanism for lipodystrophic laminopathies, Adv Enzyme Regul 48 (2008) 209-223.
[58] V.L. Verstraeten, J. Renes, F.C. Ramaekers, M. Kamps, H.J. Kuijpers, F. Verheyen, M. Wabitsch, P.M. Steijlen, M.A. van Steensel and J.L. Broers, Reorganization of the nuclear lamina and cytoskeleton in adipogenesis, Histochem Cell Biol 135 (2011) 251-261.

Samenvatting

De celkern speelt een centrale rol in het functioneren van de cel. Het bevat het DNA, waaruit alle onderdelen van de cel gevormd en gereguleerd worden. Het DNA wordt omgeven door het kernmembraan.
De laatste jaren is echter duidelijk geworden dat de celkern geen losliggend vat vol DNA is, maar ook zowel fysiek als functioneel direct communiceert met haar omgeving. Een heel scala aan eiwitten is recent ontdekt die direct of indirect aan de celkern binden. Hierbij speelt de nucleaire lamina een belangrijke rol. De lamina ligt net onder het kernmembraan en bestaat uit een fijnmazig netwerk van lamine filamenten. Samen met het membraan en een aantal geassocieerde eiwitten vormt ze de nucleaire envelop.

Lamines zijn type V intermediaire filamenten. Ze kunnen worden onderverdeeld in twee hoofdtypes, de A-type lamines: lamines A, AΔ10, C en C2, gecodeerd door het lamine A/C gen en de B-type lamines: lamines B1, B2 en B3, gecodeerd door de lamine B1 en lamine B2 genen. De nucleaire lamina zorgt ervoor dat het kernmembraan een zekere stijfheid krijgt, waardoor de kern beter bestand is tegen externe mechanische krachten.

De kritische rol van lamines komt tot uiting wanneer de nucleaire lamina verstoord wordt door mutaties in het lamine A/C gen. De pathologieën die dan ontstaan, worden laminopathieën genoemd. Op basis van ziekteverschijnselen die ze veroorzaken, kunnen we ze verdelen in verschillende groepen:
- Spierdystrofieën;
- Vetdystrofieën;
- Perifere neuropathieën;
- Progeroïde aandoeningen (vroegtijdige veroudering);
- Laminopathieën die andere weefsels aantasten.

In de loop der jaren zijn vele hypotheses over laminopathieën geformuleerd. Deze hypotheses zijn afkomstig uit verschillende onderzoeksgebieden in de celbiologie: cellulaire mechanica, signaaltransductie en genregulatie en –expressie.
De beschreven hypotheses zijn onder andere:
1. De structurele hypothese
2. De genexpressie hypothese
3. De voortijdige veroudering hypothese
4. De cel proliferatie hypothese
5. De nucleaire integriteit hypothese, zoals geformuleerd in hoofdstuk 4 en 5

Het doel van dit proefschrift was om de structurele en de functionele gevolgen van lamine A/C mutaties te onderzoeken in celkweken, afkomstig van huidfibroblasten van patiënten. Zo willen we de ontstaansmechanismen van laminopathieën beter leren begrijpen.

In hoofdstuk 1 beschrijven we de gevolgen van de afwezigheid van A-type lamines op de integriteit van de cellulaire mechanische structuur. Dit netwerk bestaat uit de nucleaire lamina (lamines en de bijbehorende eiwitten), het cytoskelet en cel adhesie moleculen. Normaal zijn al deze eiwitten met elkaar verbonden door middel van verbindingseiwitten. In de kernenvelop verbinden SUN-eiwitten en nesprines de nucleaire lamina met de verschillende elementen van het cytoskelet, die op hun beurt zijn verbonden met de buitenkant van de cel via catenine, vinculine, plectine en andere verbindingseiwitten. Het geheel vormt een netwerk dat fungeert als een z.g. tensigrity. Deze structuur is een combinatie van interne compressie (microtubuli) en spanningselementen (actine filamenten) en beschermt de cellulaire componenten (zoals de kern) tijdens externe mechanische krachten (bijvoorbeeld compressie). Alle elementen van dit mechanisch netwerk zijn essentieel, ze vormen samen één mechanische entiteit. Deze entiteit wordt in zijn geheel verstoord als een van de onderdelen verstoord is. Wanneer de lamina structuur wordt verstoord (als gevolg van een lamine A/C mutatie of lamine verlies), heeft dit een dramatisch effect op de andere elementen van het netwerk. De lokalisatie van lamine geassocieerde eiwitten (bijvoorbeeld emerine), of verbindingseiwitten (bijvoorbeeld nesprines) is verstoord als de nucleaire lamina aangetast is.
Voorgaande studies toonden aan dat bij compressie kernen van cellen zonder lamine A/C kunnen openscheuren. Tegelijkertijd vertoonden deze cellen ook een verminderde weerstand tegen de compressie. Een uitbreiding van dit experiment op geïsoleerde kernen (niet langer verbonden met het cytoskelet) toonde aan dat een intact mechanisch netwerk nodig is om voldoende weerstand te genereren aangezien alle geïsoleerde kernen (met of zonder A-type lamines) vergelijkbare vervorming vertoonden bij de compressie.

In hoofdstuk 2 konden we aantonen dat het verlies van de integriteit van het mechanisch netwerk kan leiden tot een verlies van de functionele mechanische eigenschappen van de cel, zoals de reoriëntatie van de kern. De juiste positionering van de kern en het MTOC is essentieel voor het proces van cellulaire migratie. Deze herpositionering hangt af van het mechanisch netwerk. Als het netwerk verstoord

wordt door een verstoring van de lamina, kan de kern en de MTOC zich niet correct herpositioneren tijdens cellulaire migratie. Dit leidt ook tot het niet correct hervormen van de plasma membraan tijdens cellulaire migratie (vorming van filopodia en plooien in het plasmamembraan.
Deze resultaten geven aan dat een functioneel mechanisch netwerk essentieel is voor de cel om te gaan met mechanische belasting tijdens cellulaire migratie, en dat een tekort aan functioneel lamine een invloed heeft op dit netwerk.

Onze gegevens uit hoofdstuk 3 tonen een hogere nucleaire plasticiteit van kernen zonder functioneel lamine A/C, wat leidt tot een toegenomen telomeer mobiliteit.
Progeria cellen, die progerine bevatten (gemuteerd lamine A/C), zijn juist minder elastisch en vertonen ook een lagere telomeer mobiliteit.
Een afwijkende genomische mobiliteit kan de stabiliteit van het genoom beïnvloeden, wat kan leiden tot vroegtijdige veroudering.

In hoofdstuk 4 beschrijven we een omkeerbaar, niet-lethaal verlies van integriteit van het kernmembraan in fibroblasten van verschillende laminopathie patiënten. We stellen vast dat het verlies van lamine organisatie in laminopathieën leidt tot een verzwakking van de mechanische structuur van de kern. Zwakke plekken in de nucleaire enveloppe kunnen leiden tot dynamische, omkeerbare scheuren in het kernmembraan op de plaats van de verzwakking. Deze breuken leiden niet tot celdood, maar resulteren in een tijdelijk verlies van de compartimentalisatie tussen kern en cytoplasma. Dit resulteert in een in- en uitstroom van signaaleiwitten en transcriptiefactoren die normaal gesproken enkel op een actieve manier door het kernmembraan heen getransporteerd kunnen worden. Andere nucleaire componenten die vasthangen aan chromatine of andere intranucleaire structuren diffunderen dan weer (bijna) niet uit de kern bij het optreden van een tijdelijke scheur in het kernmembraan. Een vergelijkbaar fenomeen werd ook al gezien bij HIV-geïnfecteerde cellen. Het is bekend dat virussen de nucleaire lamina moduleren en zwakke plekken induceren in de nucleaire envelop, wat kan leiden tot dynamische scheuren in het kernmembraan.

In hoofdstuk 5 concentreren we ons op de aanwezigheid van PML lichaampjes in het cytoplasma van fibroblasten van laminopathie patiënten. Normaal gezien komen deze lichaampjes enkel voor in de celkern. In het algemeen hebben patiënten met de meest ernstige ziekteverschijnselen de hoogste aantallen cytoplasmatische PML lichaampjes. Hoewel dit gedeeltelijk kan worden verklaard door het fenomeen van de nucleaire

scheuren, zijn mogelijk ook andere mechanismen betrokken bij de mislokalisatie van PML lichaampjes.

Wanneer we zorgvuldig alle hypothesen onderzoeken die de afgelopen jaren geformuleerd zijn, en de verschillende onderzoeksresultaten die deze hypothesen ondersteunen, moeten we concluderen dat lamines meerdere functies hebben, en dat ze gekoppeld zijn aan zowel structurele als regulerende elementen. Wanneer de nucleaire lamina disfunctioneel wordt als gevolg van een mutatie in het lamine A/C gen, beïnvloedt dit de verbonden netwerken (structureel netwerk, genregulerend netwerk, etc). Wanneer de kernmembraan scheurt, zal dit van invloed zijn op zowel de genregulatie en de mechanische werking van de cel.
Daarom is de integratie noodzakelijk van de verschillende hypotheses tot één holistische benadering voor de cellulaire mechanismen die ten grondslag liggen aan de verschillende pathologieën in de groep van laminopathieën.
Het is de combinatie van de verschillende trajecten, samen met de specifieke kenmerken van de verschillende aangetaste weefsels (spier, vetweefsel, hartspier e.a.), die leidt tot het brede spectrum van ziekten en klinische kenmerken, van heel mild (klinisch geen meetbare verschijnselen) tot heel dramatisch, (leidend tot voortijdige veroudering of plotseling hartfalen).

Hoewel dit proefschrift zich vooral richtte op de structurele afwijkingen in laminopathie cellen, is er meer inzicht verkregen in het mogelijk verband tussen cellulaire afwijkingen, en de beschadiging van het weefsel bij patiënten, waardoor er een betere voorspelling van de ziekteontwikkeling mogelijk is, en nieuwe wegen geopend worden voor de behandeling van deze ziekten.