Lamin A precursor localises to the Z-disk of sarcomeres in the heart and is dynamically regulated in muscle cell differentiation

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Abstract

The lamin A precursor, prelamin A, requires extensive processing to yield mature lamin A and effect its primary function as a structural filament of the nucleoskeleton. When processing is perturbed, nuclear accumulation of prelamin A is toxic and causes laminopathic diseases such as Hutchinson-Gilford progeria syndrome and cardiomyopathy. However, the physiological role of prelamin A is largely unknown and we sought to identify novel insights about this. Utilising rodent heart tissue, primary cells and the C2C12 model of myofibrillogenesis, we investigated the expression and localisation patterns of prelamin A in heart and skeletal muscle cells. We found that endogenous prelamin A was detectable in mouse heart localised to the sarcomere in both adult mouse heart and isolated neonatal rat cardiomyocytes. We investigated the regulation of prelamin A in C2C12 myofibrillogenesis and found it was dynamically regulated and organised into striations upon myofibril formation, colocalising with the Z-disk protein α-actinin. These data provide evidence that prelamin A is a component of the sarcomere, underpinning a physiological purpose for unprocessed prelamin A.

Introduction

Prelamin A is the post-translational precursor of lamin A, a major protein of the inner nuclear membrane which alongside lamins C and B form the lattice-like structure known as the nuclear lamina [1]. The nuclear lamina is crucial for maintaining genome integrity and facilitating DNA repair [2–5]. It also enables mechanotransduction of signals to the nucleus to elicit rapid gene expression responses in reaction to mechanical stimuli [6]. In contractile tissues such as striated muscle the importance of the nuclear lamina is highlighted by the prevalence of disease-causing mutations in both skeletal muscular dystrophies and cardiomyopathies, which have been investigated at length [7]. Lamin A is unique amongst the lamins in that it is initially translated as a precursor called prelamin A and is subjected to complex post-translational processing, primarily by ZMPSTE24 metalloproteinase, in order to yield mature lamin A and allow it to effect its physiological function [8]. Many

studies have shown that this processing can become dysregulated in the face of genetic or pathological stress meaning that prelamin A can accumulate and drive disease phenotypes, such as premature ageing disorders (Hutchison Gilford Progeria) [9], in normal ageing [10] and more recently, cardiomyopathy [11]. Though most evidence suggests prelamin A is a toxic mediator of disease, some studies suggest that there is a physiological role for prelamin A, particularly in striated muscle cells, or cells destined to become so [12, 13]. Here we present evidence to support a role for endogenous prelamin A within the sarcomeres of cardiomyocytes and in a model of C2C12 myofibrillogenesis.

Methods

Animals

All procedures were performed in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (UK Home Office). Three $Lmna^{-/-}$ mice and two mice harbouring and expressing a cardiomyocyte specific uncleavable prelamin A human transgene, $L647R$-$LMNA$, were used in this study. The use of $Lmna^{-/-}$ mice and the generation of the cardiomyocyte specific prelamin A transgenic (csPLA-Tg) line was described previously [11]. Wildtype littermates were also used in this study, three from the $Lmna^{-/-}$ line and seven from the csPLA line. Neonatal rats were also used for studies involving isolated cardiomyocytes.

Isolation of neonatal rat cardiomyocytes

Isolation of neonatal rat CMs (NRCs) was performed as previously described [14]. Primary cultures of NRCs were isolated from 1 to 2-day-old neonatal Sprague–Dawley rats. Hearts were collected and atria were removed, washed, excised, minced and enzymatically digested at 37 °C with ADS buffer [116 mmol/L NaCI, 20 mmol/L HEPES, 0.8 mmol/L NaH2PO4, 5.6 mmol/L glucose, 5.4 mmol/L KCl, 0.8 mmol/L MgSO4] containing collagenase (57.5 U/mL) and pancreatin (1.5 mg/mL). The suspension was pre-plated to remove contaminating cells, before being cultured on gelatin (Sigma) pre-coated 35 mm petri dishes with a density of 2×10^5 cells/ml. Cells were allowed to adhere for 24 h, and then fixed in 4% formalin solution.

C2C12 Cell Culture

C2C12 mouse myoblasts are a cell line originally derived from dystrophic mouse skeletal muscle [15]. They were maintained in culture using DMEM with 20% fetal bovine serum (FBS) and antibiotics (Penicillin, Streptomycin, Glutamine) and kept at 37°C, 5% CO_2.

Cells were maintained and passaged at low confluency, in-line with optimised protocols for myofibril formation. To induce the formation of myotubes, cells were grown to 70% confluency and then incubated with DMEM containing 2% Horse serum at 37°C 5% CO_2 for 24, 48, 72 and 168 hours. This protocol was designed to encourage the formation of myofibrils. Cells were observed daily for the formation of myotubes and, the characteristics of which include a distinct change in morphology from rounded to multi-nucleated spindle-like cells, and subsequently myofibrils.

Antibodies

Lamin A/C - WB 1:1000, IF 1:1000 (Santa Cruz Biotechnology sc-6215), prelamin A - WB 1:250, IF 1:1000 (Santa Cruz Biotechnology sc-6214), Zmpste24 - WB 1:200, IF 1:100 (Novus Biologicals NB100-2388), Desmin - WB 1:100, (Dako M0760), Lamin B1 - WB 1:1000 (Abcam ab16048), Myomesin - WB 1:1000, IF 1:100 (Monoclonal B4 isolate) [16], α-actinin - WB 1:2500, IF 1:250 (Sigma-Aldrich EA53), GAPDH - WB 1:100 (Sigma-Aldrich G8795). Histone H3 K27me3 – WB 1:1000 (Abcam ab6002)

Subcellular Fractionation

Tissue was subjected to subcellular fractionation using the Qproteome cell compartment kit (Qiagen) as per the manufacturers instructions with the following modifications: The starting mass of tissue was 50 mg and tissue was homogenised in the first instance by pulverisation following cryopreservation.

Immunoblotting

Immunoblotting was performed on whole heart tissue lysates as previously described [11] and on whole cell lysates of C2C12 myoblasts as previously described [14]. Semi-quantitative densitometric analysis was performed on images acquired by an Odyssey scanner (LiCor, USA) from C2C12 cell differentiation experiments using image-studio software (LiCor, USA).

Indirect immunofluorescence

Cryosections were briefly air dried without chemical fixation prior to blocking. Cells were fixed in 4% paraformaldedhyde (PFA) for 10 min or in methanoacetone for 2 min, and then washed three times in Tris-Buffered Saline (TBS). Cells fixed in formaldehyde were then permeabilised in 0.2% NP40 for 2 minutes and washed three times in TBS. Samples were then incubated for 1 h with 1% Bovine Serum Albumin (BSA) in TBS at room temperature to prevent non-specific binding. The sections/coverslips were incubated with primary antibodies overnight 4°C or for 1 hour at room temperature and then washed in TBS. They were then incubated with fluorescent secondary antibodies for 1 hour at room temperature and then 4',6-diamidino-2-phenylindole (DAPI) diluted 1:10,000 for 5 minutes at room temperature to visualise nucleic structures. The sections/coverslips were then washed and mounted with mowiol mounting media.

Peptide competition assay

Antibody was incubated for 30 minutes at room temperature in blocking buffer and 5 times concentration of blocking peptide to which the epitope of the antibody was generated (Santa Cruz Biotechnology sc-6214 P).

Microscopy

Immunofluorescence was visualised using widefield fluorescence microscopy (Olympus IX-8). Imaging for prelamin A in figure 1B was performed using widefield fluorescence microscopy at fixed exposure and gain settings for direct comparison. All other images

for publication were captured using a Leica SP5 confocal microscope (LeicaMicrosystems, UK).

Statistics

One-way ANOVA with unrepeated measures between all groups was performed on densitometry experiments concerning C2C12 myoblast differentiation. Tukey's post-test was applied to datasets which were deemed significant ($p < 0.05$). Data were analysed and graphed in GraphPad Prism 7.

Data availability

The underlying data for this study are available from the Figshare data repository: https://doi.org/10.5522/04/17135936.

Results

Unprocessed prelamin A is present at the Z-disk of sarcomeres in heart

Prelamin A intermediates are rapidly converted into mature lamin A for insertion into the nuclear lamina [17, 18]. As such, prelamin A is considered minimally detectable in normal or healthy cells [19]. Western blot of mouse myocardium probed for lamin A/C showed the 74kDa prelamin A band was clearly detectable in normal heart tissue when subjected to long exposure times alongside heart tissue overexpressing prelamin A (Fig. 1A). Immunofluorescence staining of heart tissue sections confirmed this finding and suggested prelamin A was abundant in sarcomeres (Fig. 1B). These initial findings were surprising. To determine the veracity of this observation we performed a peptide competition assay to block the epitope of the antibody and observed loss of staining when the antibody was used to probe heart tissue, thus confirming the specificity of sarcomere localisation. To further interrogate this finding we used the prelamin A antibody to probe and stain heart tissue sections from a $Lmna^{-/-}$ mouse line, which yielded little to no sarcomeric staining further supporting the initial finding that prelamin A localises to sarcomeres (Fig. 1B). To investigate further still, subcellular fractionation of mouse heart tissue was performed to resolve proteins abundant in cytosol, membrane, nucleus and cytoskeleton/sarcomere. Prelamin A was evident only in the sarcomere fraction. Probing for lamin A/C showed lamin A and C expression in the nuclear fraction whilst in the sarcomere fraction of the same membrane prelamin A was detected at a higher molecular weight providing compelling evidence for the localisation of prelamin A in sarcomeres (Fig. 1C). Intriguingly Zmpste24 was also present in the sarcomeric fraction, and a faint band was evident in the membrane fraction (Fig. 1C). Whilst GAPDH, β-1-integrin and Histone H3 confirmed the cytosolic, membranous and nuclear fractions were successfully obtained (Fig. 1C). However, whilst we expected α-actinin to be present only in the sarcomeric fraction it was detectable to some degree in all fractions though the greatest abundance was in the sarcomere (Fig. 1C). Since α-actinin specifically localised to sarcomeres in cryosections (Fig. 1B) we believe this to be a technical issue related to the fact that to detect low endogenous levels of prelamin A it was necessary to increase the starting mass of tissue in the fractionation protocol, which can lead to carry over of highly abundant proteins into unexpected fractions. Immunocytochemical staining of isolated cardiomyocytes (CMs) from neonatal rat hearts showed that lamin A/C

strongly stained the nuclear rim of CMs, whilst prelamin A stained sarcomeric structures and co-localised with the Z-disk protein α-actinin, and alternated with the M-band protein myomesin which was supported by line profiling analysis of images. (Fig 2).

Prelamin A is dynamically regulated during C2C12 differentiation and becomes striated at the point of myofibrillogenesis

Having discovered that prelamin A localised to the sarcomere in CMs, we sought to assess the regulation of prelamin A in a C2C12 model of myofibrillogenesis. To achieve myofibrils cycling myoblasts were seeded and differentiation induced at relatively low confluency (Fig. 3A). They were allowed to differentiate for 168 hours and timepoints of 0, 48, 72 and 168 hours were studied. Western blotting showed that expression of prelamin A in C2C12 cycling myoblasts was observed in whole cell lysates (Fig. 3B, C) and this remained the case at 48 hours post-induction of differentiation. At 72 and 168 hours, prelamin A was detected, at 150 kDa and not at 74 kDa. Though unusual, this is not without precedent as 150 kDa prelamin A was detected in C2C12 cells in a previous study [13]. Immunofluorescence showed that the distribution of prelamin A appeared to be within peri-nuclear regions or the endoplasmic reticulum in cycling myoblasts and in cells differentiated for 48 hours (Fig. 4A). The distribution became more diffuse in cells differentiated for 72 hours and, after 168 hours, it was possible to see prelamin A organized in a striated pattern, in close proximity to α-actinin (Fig. 4B), whilst desmin protein abundance showed a nominal but steady increase during differentiation in line with lamins A/C (Figs. 3B, C, D). According to Western blotting, Zmpste24 underwent a steady and significant decrease over the course of differentiation (Figs. 3B, C, D) and did not localise to distal and sarcomeric regions (Fig. 4D).

Discussion

In this study we have provided evidence that the lamin A precursor, prelamin A, is present at low levels in its unprocessed form localised at the sarcomere of striated muscle cells. Since *Lmna*^{-/-} mice are viable [20, 21] it is unlikely that sarcomeric prelamin A plays a strong role in heart muscle development *in utero*. However, clues as to the potential importance of this unique finding perhaps lie in a study using a mouse genetically engineered to express non-farnesylated prelamin A only, which described a late onset cardiomyopathy in aged mice [22]. Farnesylation is a critical step in the pathway to yield mature lamin A and preventing farnesylation via farnesyl-transferase inhibitors (FTIs) is a target of therapies aiming to treat prelamin A accumulating diseases, an approach proven to benefit experimental animals and patients exhibiting prelamin A/progerin accumulation [23–25]. Importantly, nuclear morphology defects are rescued by inhibition of farnesylation. That we discovered prelamin A abundant in sarcomeres implies that late onset cardiomyopathy in non-farnesylated prelamin A only mice may be caused by sarcomere dysfunction rather than nucleus disruption. As such, we hypothesise that prelamin A potentially plays a subtle supporting role in myofilament structure and/or function in fully developed sarcomeres. One confusing finding was that Zmpste24 was also present in the sarcomeric fraction suggesting it may localise in close proximity to prelamin A in sarcomeres. This would suggest that it

should be able to locally cleave prelamin A to yield lamin A. Further investigation is needed in this area.

Previously, it has been shown that prelamin A is dynamically regulated during C2C12 myotube differentiation [13]. Contrary to the current study, Capanni et al focused primarily on the nuclear domain and did not describe the formation of myofibrils and did not study timepoints later than 120 hours. Intriguingly, Capanni et al also described the presence of a 150 kDa band during differentiation and speculated that this was a possible prelamin A dimer. In the current study reducing conditions were employed for Western blotting, therefore we argue that if it is a prelamin A dimer it must be covalently linked or a non-specific antibody detection, which would be unusual since our experience is that this antibody is highly specific for prelamin A. Nevertheless, taken together there is clear evidence for dynamic regulation of prelamin A in myotube/myofiber differentiation. Uniquely, our study has shown that upon the development of (pre)myofibrils, when sarcomeres begin to form, prelamin A localised to the Z-disc along with α-actinin, coinciding with the observation of the 150 kDa protein band. Hypothetically, dimerisation of prelamin A to form a homo- or hetero-dimer could provide a signal which facilitates the localisation of prelamin A from the nuclear rim/peri-nuclear region to more remote regions of the cell to form part of the z-disc. Indeed, to speculate further this process may serve to intentionally prevent the processing of prelamin A by Zmpste24. Taken in isolation, the fact that Zmpste24 expression and localisation in C2C12 myofibrillogenesis opposes prelamin A expression and localisation, suggests that the spatial regulation observed during differentiation of prelamin A and Zmpste24 is mutually co-operative. Disruption of this process may explain the observation that prelamin A accumulates in muscle during disease [10, 11]. That we noted Zmpste24 in the sarcomeric and membrane fractions is also indicative of differential regulation of Zmpste24 localisation in muscle. Future investigations should aim to determine whether this becomes dysregulated in disease.

Conclusions

These data provide evidence that prelamin A is subjected to spatial regulation of processing and is a component of the sarcomere in striated muscle cell types. The question of the evolutionary purpose of prelamin A processing has previously been discussed [26], and these novel insights point towards a potential physiological role. Further our data suggest that in striated muscle there may be a complex role for prelamin A in physiology and pathogenesis separately. The mechanical properties of unprocessed prelamin A, known as a 'stiff' molecule due to the presence of a farnesylated C-terminus perhaps suggest that prelamin A may play a role in determining the stiffness of myofilaments. Further *in vivo* physiological characterisation and biophysical investigation of isolated myofilaments [27, 28] from 'lamin A only' mice, which bypass prelamin A processing to yield lamin A and 'non-farnesylated prelamin A only' mice [22, 29] would provide an appropriate starting point to investigate this question. For example, investigating cardiac functional changes in lamin A only mice subjected to cardiac stress via isoproterenol infusion or transverse aortic constriction would yield insight as to the importance of sarcomeric prelamin A for cardioprotection in the face of excessive loading of myofilaments in vivo. In these same mice, isolation of 'skinned' myofilaments followed by measurements of passive force and

calcium activated force will provide information on the fundamental role of sarcomeric prelamin A in stiffness and contractility respectively. Further, the use of pull-down [30] or proximity [31] proteomics will enable identification of crucial protein binding partners to enable mechanistic understanding of the purpose and regulation of sarcomeric prelamin A.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

[1]. de Leeuw R, Gruenbaum Y, Medalia O. Nuclear Lamins: Thin Filaments with Major Functions. Trends in cell biology. 2017; doi: 10.1016/j.tcb.2017.08.004

[2]. Cho S, Vashisth M, Abbas A, Majkut S, Vogel K, Xia Y, Ivanovska IL, Irianto J, Tewari M, Zhu K, et al. Mechanosensing by the Lamina Protects against Nuclear Rupture, DNA Damage, and Cell-Cycle Arrest. Dev Cell. 2019; 49: 920–935. e925 doi: 10.1016/j.devcel.2019.04.020 [PubMed: 31105008]

[3]. Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 8963–8968. DOI: 10.1073/pnas.0402943101 [PubMed: 15184648]

[4]. Liu Y, Rusinol A, Sinensky M, Wang Y, Zou Y. DNA damage responses in progeroid syndromes arise from defective maturation of prelamin A. J Cell Sci. 2006; 119: 4644–4649. DOI: 10.1242/jcs.03263 [PubMed: 17062639]

[5]. Xanthis I, Iskratsch T. Lamin-A Mechano-Protects the Heart. Dev Cell. 2019; 49: 821–822. DOI: 10.1016/j.devcel.2019.05.041 [PubMed: 31211988]

[6]. Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL, Lee RT. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. The Journal of clinical investigation. 2004; 113: 370–378. DOI: 10.1172/JCI19670 [PubMed: 14755334]

[7]. Brayson D, Shanahan CM. Current insights into LMNA cardiomyopathies: Existing models and missing LINCs. Nucleus. 2017; 8: 17–33. DOI: 10.1080/19491034.2016.1260798 [PubMed: 28125396]

[8]. Sinensky M, Fantle K, Trujillo M, McLain T, Kupfer A, Dalton M. The processing pathway of prelamin A. J Cell Sci. 1994; 107 (Pt 1) 61–67. [PubMed: 8175923]

[9]. Shackleton S, Smallwood DT, Clayton P, Wilson LC, Agarwal AK, Garg A, Trembath RC. Compound heterozygous ZMPSTE24 mutations reduce prelamin A processing and result in a severe progeroid phenotype. Journal of medical genetics. 2005; 42 e36 doi: 10.1136/jmg.2004.029751 [PubMed: 15937076]

[10]. Ragnauth CD, Warren DT, Liu Y, McNair R, Tajsic T, Figg N, Shroff R, Skepper J, Shanahan CM. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation. 2010; 121: 2200–2210. DOI: 10.1161/CIRCULATIONAHA.109.902056 [PubMed: 20458013]

[11]. Brayson D, Frustaci A, Verardo R, Chimenti C, Russo MA, Hayward R, Ahmad S, Vizcay-Barrena G, Protti A, Zammit PS, et al. Prelamin A mediates myocardial inflammation in dilated and HIV-associated cardiomyopathies. JCI Insight. 2019; 4 doi: 10.1172/jci.insight.126315

[12]. Brodsky GL, Bowersox JA, Fitzgerald-Miller L, Miller LA, Maclean KN. The prelamin A pre-peptide induces cardiac and skeletal myoblast differentiation. Biochem Biophys Res Commun. 2007; 356: 872–879. DOI: 10.1016/j.bbrc.2007.03.062 [PubMed: 17389141]

[13]. Capanni C, Del Coco R, Squarzoni S, Columbaro M, Mattioli E, Camozzi D, Rocchi A, Scotlandi K, Maraldi N, Foisner R, et al. Prelamin A is involved in early steps of muscle differentiation. Experimental cell research. 2008; 314: 3628–3637. DOI: 10.1016/j.yexcr.2008.09.026 [PubMed: 18951892]

[14]. Zhou C, Li C, Zhou B, Sun H, Koullourou V, Holt I, Puckelwartz MJ, Warren DT, Hayward R, Lin Z, et al. Novel nesprin-1 mutations associated with dilated cardiomyopathy cause nuclear envelope disruption and defects in myogenesis. Hum Mol Genet. 2017; 26: 2258–2276. DOI: 10.1093/hmg/ddx116 [PubMed: 28398466]

[15]. Yaffe D, Saxel O. A myogenic cell line with altered serum requirements for differentiation. Differentiation; research in biological diversity. 1977; 7: 159–166. [PubMed: 558123]

[16]. Grove BK, Kurer V, Lehner C, Doetschman TC, Perriard JC, Eppenberger HM. A new 185,000-dalton skeletal muscle protein detected by monoclonal antibodies. The Journal of cell biology. 1984; 98: 518–524. DOI: 10.1083/jcb.98.2.518 [PubMed: 6537951]

[17]. Barrowman J, Hamblet C, George CM, Michaelis S. Analysis of prelamin A biogenesis reveals the nucleus to be a CaaX processing compartment. Mol Biol Cell. 2008; 19: 5398–5408. DOI: 10.1091/mbc.E08-07-0704 [PubMed: 18923140]

[18]. Barrowman J, Hamblet C, Kane MS, Michaelis S. Requirements for efficient proteolytic cleavage of prelamin A by ZMPSTE24. PLoS One. 2012; 7 e32120 doi: 10.1371/journal.pone.0032120 [PubMed: 22355414]

[19]. Casasola A, Scalzo D, Nandakumar V, Halow J, Recillas-Targa F, Groudine M, Rincon-Arano H. Prelamin A processing, accumulation and distribution in normal cells and laminopathy disorders. Nucleus. 2016; 7: 84–102. DOI: 10.1080/19491034.2016.1150397 [PubMed: 26900797]

[20]. Nikolova V, Leimena C, McMahon AC, Tan JC, Chandar S, Jogia D, Kesteven SH, Michalicek J, Otway R, Verheyen F, et al. Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice. The Journal of clinical investigation. 2004; 113: 357–369. DOI: 10.1172/JCI19448 [PubMed: 14755333]

[21]. Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. The Journal of cell biology. 1999; 147: 913–920. [PubMed: 10579712]

[22]. Davies BS, Barnes RH, Tu Y, Ren S, Andres DA, Spielmann HP, Lammerding J, Wang Y, Young SG, Fong LG. An accumulation of non-farnesylated prelamin A causes cardiomyopathy but not progeria. Hum Mol Genet. 2010; 19: 2682–2694. DOI: 10.1093/hmg/ddq158 [PubMed: 20421363]

[23]. Fong LG, Frost D, Meta M, Qiao X, Yang SH, Coffinier C, Young SG. A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. Science. 2006; 311: 1621–1623. DOI: 10.1126/science.1124875 [PubMed: 16484451]

[24]. Gordon LB, Kleinman ME, Miller DT, Neuberg DS, Giobbie-Harder A, Gerhard-Herman M, Smoot LB, Gordon CM, Cleveland R, Snyder BD, et al. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeria syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 16666–16671. DOI: 10.1073/pnas.1202529109 [PubMed: 23012407]

[25]. Yang SH, Meta M, Qiao X, Frost D, Bauch J, Coffinier C, Majumdar S, Bergo MO, Young SG, Fong LG. A farnesyltransferase inhibitor improves disease phenotypes in mice with a Hutchinson-Gilford progeria syndrome mutation. The Journal of clinical investigation. 2006; 116: 2115–2121. DOI: 10.1172/JCI28968 [PubMed: 16862216]

[26]. Davies BS, Coffinier C, Yang SH, Barnes RH, Jung HJ, Young SG, Fong LG. Investigating the purpose of prelamin A processing. Nucleus. 2011; 2: 4–9. DOI: 10.4161/nucl.2.1.13723 [PubMed: 21647293]

[27]. Brayson D, Holohan SJ, Bardswell SC, Arno M, Lu H, Jensen HK, Tran PK, Barallobre-Barreiro J, Mayr M, Dos Remedios CG, et al. Right Ventricle Has Normal Myofilament Function But Shows Perturbations in the Expression of Extracellular Matrix Genes in Patients With Tetralogy

of Fallot Undergoing Pulmonary Valve Replacement. J Am Heart Assoc. 2020; 9 e015342 doi: 10.1161/JAHA.119.015342 [PubMed: 32805183]

[28]. Levy Y, Ross JA, Niglas M, Snetkov VA, Lynham S, Liao CY, Puckelwartz MJ, Hsu YM, McNally EM, Alsheimer M, et al. Prelamin A causes aberrant myonuclear arrangement and results in muscle fiber weakness. JCI Insight. 2018; 3 doi: 10.1172/jci.insight.120920

[29]. Fong LG, Ng JK, Lammerding J, Vickers TA, Meta M, Cote N, Gavino B, Qiao X, Chang SY, Young SR, et al. Prelamin A and lamin A appear to be dispensable in the nuclear lamina. The Journal of clinical investigation. 2006; 116: 743–752. DOI: 10.1172/JCI27125 [PubMed: 16511604]

[30]. Wang H, Marrosu E, Brayson D, Wasala NB, Johnson EK, Scott CS, Yue Y, Hau KL, Trask AJ, Froehner SC, et al. Proteomic analysis identifies key differences in the cardiac interactomes of dystrophin and micro-dystrophin. Hum Mol Genet. 2021; 30: 1321–1336. DOI: 10.1093/hmg/ddab133 [PubMed: 33949649]

[31]. Liu G, Papa A, Katchman AN, Zakharov SI, Roybal D, Hennessey JA, Kushner J, Yang L, Chen BX, Kushnir A, et al. Mechanism of adrenergic CaV1.2 stimulation revealed by proximity proteomics. Nature. 2020; 577: 695–700. DOI: 10.1038/s41586-020-1947-z [PubMed: 31969708]
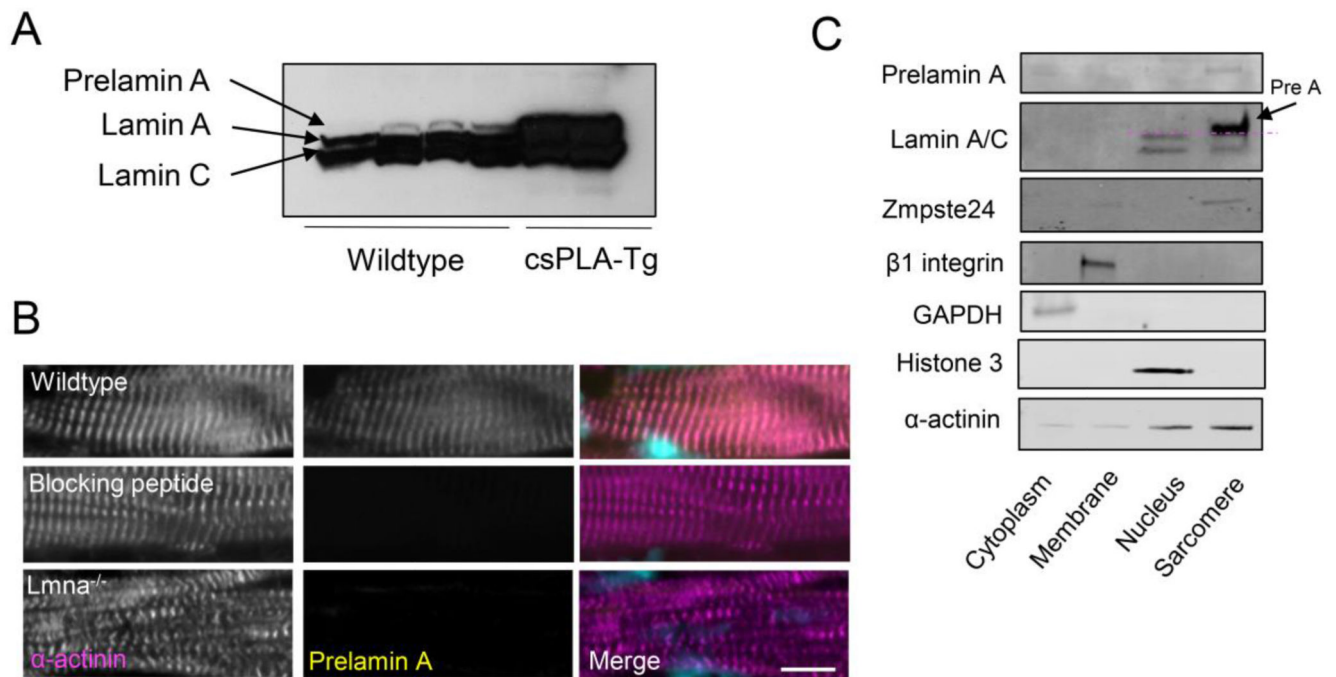
Figure 1. Prelamin A is detectable in whole heart lysate, heart tissue sections and is abundant in the cytoskeleton in biochemically fractionated heart tissue.

A. Long exposure of membrane to film revealed low level expression of prelamin A in whole heart tissue lysates of wildtype mice, positive controls are from csPLA-Tg mice. N=4 wildtype and 2 csPLA-Tg mice B. Prelamin A localised to sarcomeres in cryosectioned mouse heart but was not detectable after incubation of the prelamin A antibody with its blocking peptide or in *Lmna*⁻/⁻ heart tissue. Scale =10 μm. N=3 wildtype and 3 *Lmna*⁻/⁻ mice. C. Subcellular fractionation of wildtype mouse heart tissue revealed prelamin A was present in the sarcomere fraction whilst lamin A was only abundant in the nuclear fraction according to both lamin A/C and prelamin A specific antibodies. N=3 wildtype mice.
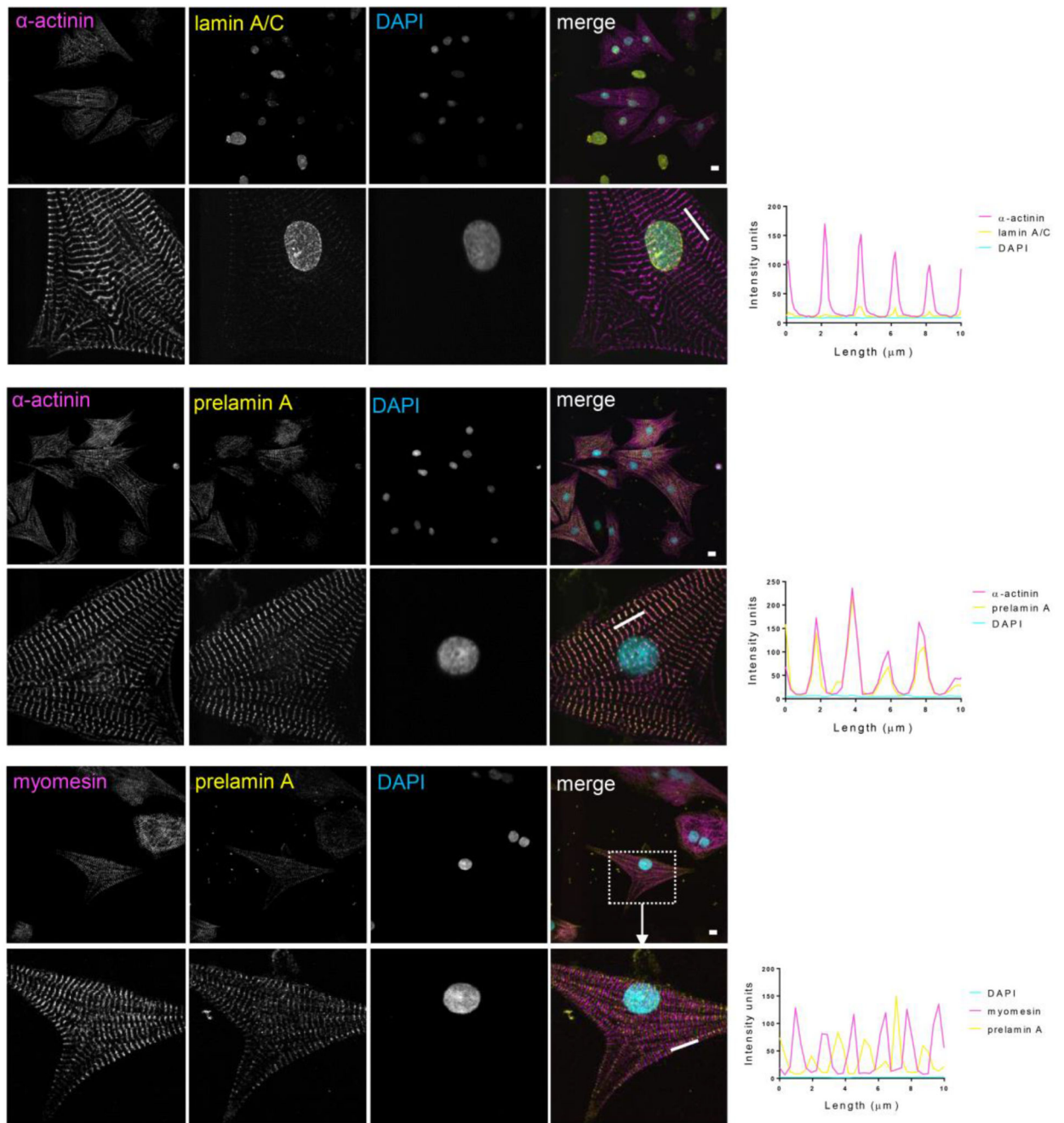
Figure 2. Prelamin A localises to Z-disks of sarcomeres in isolated cardiomyocytes.
Immunofluorescence staining and corresponding profiling of isolated neonatal rat
cardiomyocytes showing lamin A/C localising to the nuclear envelope and nucleoplasm.
Prelamin A localises with α-actinin and alternates with myomesin suggesting that prelamin
A localises to the Z-disk region of the sarcomere. Scale = 10 μm. Scales denote profiled
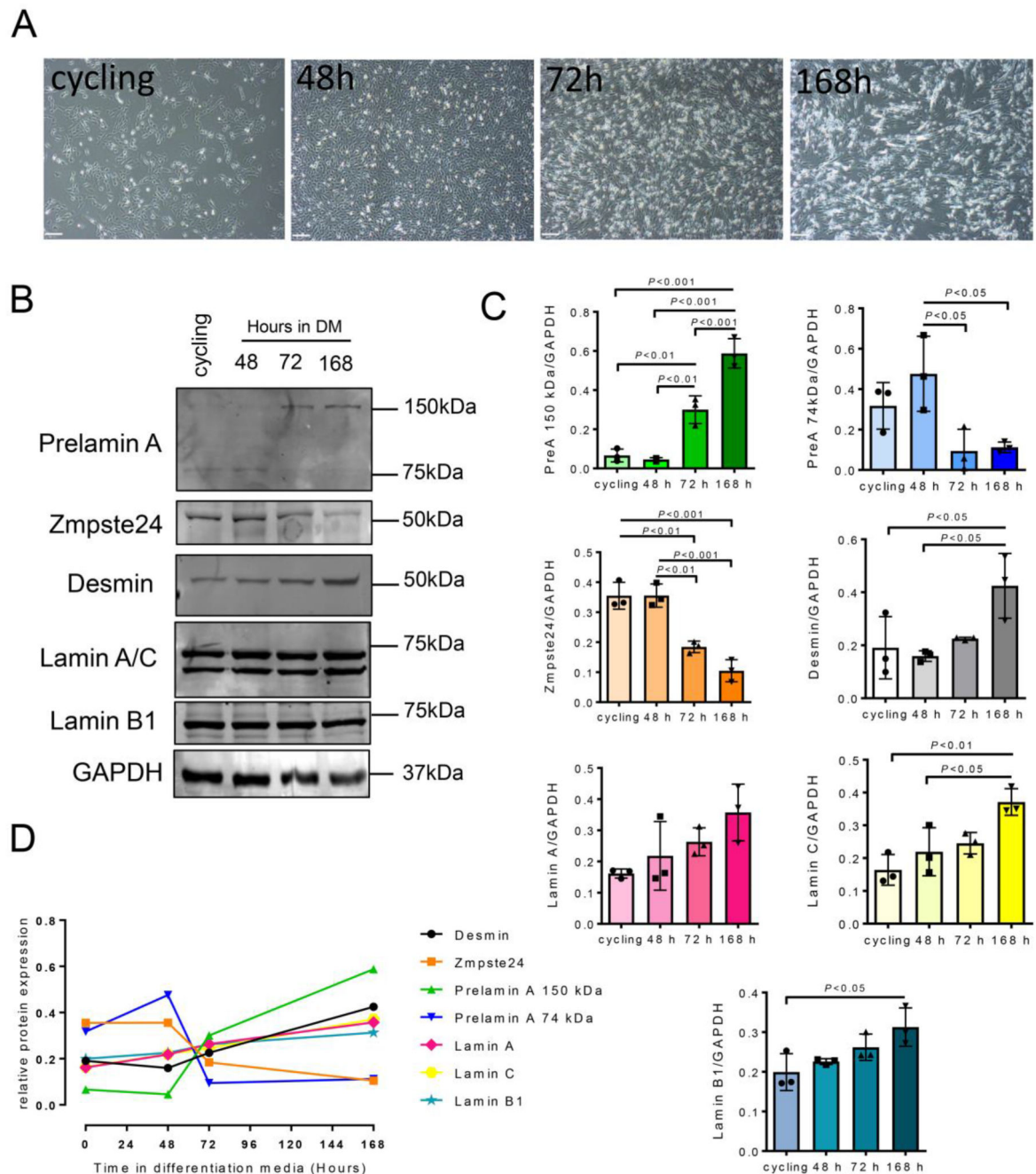regions corresponding to graphs. N=9 rat pup hearts pooled into one primary isolation
procedure.

Figure 3. Prelamin A is dynamically regulated during C2C12 differentiation.
A. phase contrast images of C2C12 differentiation occurring over 168 hours. Scale = 30 μm.
B & C. Western blotting revealed a dynamic and potentially co-operative relationship of prelamin A and ZMPSTE24 abundance during C2C12 differentiation to myofibrillogenesis.
D. summary of time dependent protein abundance changes in differentiating C2C12 cells.
N=3 independent myotube differentiation experiments. *$P<0.05$ **$P<0.01$, ***$P<0.001$ based on one-way ANOVA with Tukey post-hoc test for multiple comparisons.
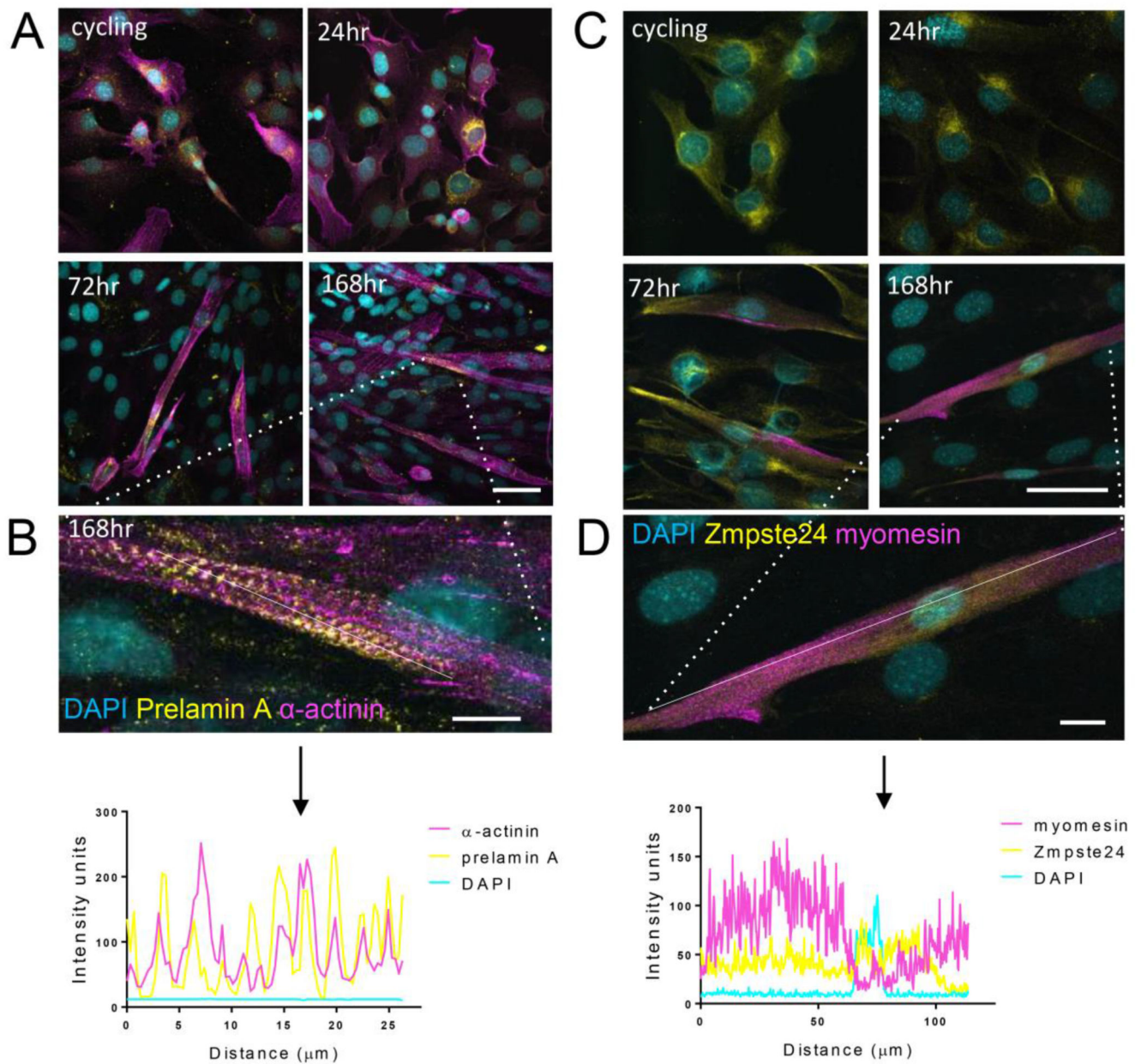
Figure 4. Prelamin A localises with α-actinin in pre-myofibrils.
A. Prelamin A expression occurred at the nuclear periphery in cycling C2C12 cells and cells induced to differentiate for 24hrs. Scale = 30 μm. **B**. Prelamin A localised with α-actinin in C2C12 pre-myofibrils. Scale = 10 μm. **C**. Zmpste24 localised to the nuclear periphery in cycling C2C12 cells and cells differentiated for 24hrs. Scale = 30 μm **D**. In myotubes Zmpste24 appeared to be expressed more in regions where myomesin was low and vice versa. Scale = 10 μm. Thin white lines indicate profiled regions corresponding to illustrative graphs from N=3 independent myotube differentiation experiments.