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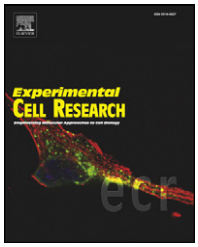

Research Article

# Accumulation of distinct prelamin A variants in human diploid fibroblasts differentially affects cell homeostasis

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ABSTRACT

Lamin A is a component of the nuclear lamina that plays a major role in the structural organization and function of the nucleus. Lamin A is synthesized as a prelamin A precursor which undergoes four sequential post-translational modifications to generate mature lamin A. Significantly, a large number of point mutations in the *LMNA* gene cause a range of distinct human disorders collectively known as laminopathies. The mechanisms by which mutations in lamin A affect cell function and cause disease are unclear. Interestingly, recent studies have suggested that alterations in the normal lamin A pathway can contribute to cellular dysfunction. Specifically, we and others have shown, at the cellular level, that in the absence of mutations or altered splicing events, increased expression of wild-type prelamin A results in a growth defective phenotype that resembles that of cells expressing the mutant form of lamin A, termed progerin, associated with Hutchinson–Gilford Progeria syndrome (HGPS). Remarkably, the phenotypes of cells expressing elevated levels of wild-type prelamin A can be reversed by either treatment with farnesyltransferase inhibitors or overexpression of ZMPSTE24, a critical prelamin A processing enzyme, suggesting that minor increases in the steady-state levels of one or more prelamin A intermediates is sufficient to induce cellular toxicity. Here, to investigate the molecular basis of the lamin A pathway toxicity, we characterized the phenotypic changes occurring in cells expressing distinct prelamin A variants mimicking specific prelamin A processing intermediates. This analysis demonstrates that distinct prelamin A variants differentially affect cell growth, nuclear membrane morphology, nuclear distribution of lamin A and the fundamental process of transcription. Expression of prelamin A variants that are constitutively farnesylated induced the formation of lamin A aggregates and dramatic changes in nuclear membrane morphology, which led to reduced levels of the basal transcription factor TATA-binding protein (TBP) and global transcription, and severely limited cell growth. Expression of a prelamin A variant that cannot be farnesylated, although did not appreciably influence cell growth, resulted in the formation of lamin A nucleoplasmic foci and caused, in a minor subpopulation of cells, changes in nuclear morphology that were accompanied by reduced levels of TBP and transcription. In contrast, expression of mature lamin A did not affect any of these parameters. These data demonstrate that accumulation

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of any partially processed prelamin A protein alters cellular homeostasis to some degree, even though the most dramatic effects are caused by variants with a permanently farnesylated carboxyl-terminal tail.



## Introduction

Lamin A, a member of the family of intermediate filaments, is synthesized in the cytosol as a precursor molecule (prelamin A), which is then posttranslationally modified to generate a mature lamin A lacking the carboxyl-terminal tail [1]. Prelamin A is first modified by a farnesyltransferase that adds a 15-carbon farnesyl isoprenoid to the carboxyl-terminal cysteine (**C**SIM). Farnesylated prelamin A is then modified by a prenyl-CAAX-specific endoprotease (AAX = aliphatic-aliphatic-any amino acid) known as RAS converting enzyme 1 (RCE1) that removes the last three carboxyl-terminal amino acids. ZMPSTE24, the enzyme required for the last proteolytic step, has also been proposed to perform this cleavage reaction. This prelamin A intermediate is then the substrate for a second ER-resident enzyme, isoprenylcysteine carboxyl methyltransferase (ICMT), which methylesterifies the carboxyl-terminal farnesylcysteine. Lastly, farnesylated prelamin A is then processed by ZMPSTE24, which cleaves the carboxyl-terminal 15 amino acids releasing the mature, unfarnesylated lamin A. The processing of prelamin A into mature lamin A in normal human cells has been determined to take approximately three hours [2,3].

Point mutations in the *LMNA* gene have been shown to be associated with a distinct set of human disorders collectively known as laminopathies [4]. The most severe form of laminopathy is Hutchinson Gilford Progeria Syndrome (HGPS), which is a rare premature aging disease that affects children [5,6]. The most frequent mutation in HGPS patients is a *de novo* heterozygous C to T transition at nucleotide 1824 of the *LMNA* gene, which causes the production of a mutant lamin A protein termed progerin with a 50 amino acids internal deletion that lacks the internal proteolytic cleavage site used to remove the last 18 carboxyl-terminal amino acids for generating mature lamin A [7,8]. Progeria cells and Hela cells expressing ectopic progerin are characterized by poor growth and display atypical nuclear membrane morphology (blebs), two phenotypes that can be reversed by inhibition of protein farnesylation by treatment with farnesyl transferase inhibitors (FTIs) [9–13]. Significantly, recent studies have indicated that small amounts of progerin are present in cells from non-progeria individuals, suggesting that this lamin A variant may play a role in normal human aging [14,15].

We and others have shown that alterations in the lamin A metabolism triggered by increased expression of wild-type lamin A result in a cellular dysfunction phenotype that resembles that of cells expressing progerin [13,16]. Significantly, our study demonstrated that increases in the expression of wild-type lamin A that are within the physiological range of lamin A levels are sufficient to induce, although with slower kinetics, the alterations observed in HGPS cells including growth defects, dysmorphic nuclei, cell death and premature senescence [13]. Since these alterations are reversed by overexpression of ZMPSTE24, it is likely that abnormal prelamin A processing, leading to increased steady-state levels of specific prelamin A intermediates, is responsible for these phenotypic changes. To directly test the role of specific prelamin A intermediates in the onset of the progeroid phenotype induced by increased lamin A expression, we have ectopically expressed a set of prelamin A variants lacking specific processing determinants in normal human fibroblasts and examined their effects on nuclear membrane morphology and cell growth over time. Moreover, we examined whether accumulation of these prelamin A variants leads to altered lamin A localization and affects a nuclear process such as transcription. Our data show that distinct prelamin A variants, depending on their post-translational modification, localize differentially in the cell and differentially affect growth and nuclear membranes morphology. Prelamin A variants that are constitutively farnesylated have a major inhibitory effect on cell growth, significantly altered nuclear membrane morphology and induced the formation of lamin A aggregates at the nuclear membrane. In contrast, expression of prelamin A ′MSIM, a variant that cannot be farnesylated, minimally influenced cell growth, although it leads to the formation of small nucleoplasmic foci and induces a small, but significant number of cells with dismorphic nuclei. Significantly, dismorphic nuclei and formation of lamin A fold-like aggregates, but not foci, are accompanied by a significant decrease in the levels of the basal transcription factor TBP and global transcription. This suggests that changes in nuclear morphology and lamin A assembly caused by accumulation of specific prelamin A intermediates alter fundamental aspects of nuclear function. An implication of these findings is that, in the absence of lamin A mutations, any age-associated decrease in the levels or activity of enzymes involved in the prelamin A processing pathway will be accompanied by an increase in the steady-state levels of intermediates that will result in cell dysfunction and ultimately lead to the development of age onset-pathologies.

## Materials and methods

### *Cell culture*

Primary dermal fibroblast cell lines from healthy newborn (GM00038) were obtained from the Coriell Cell Repository. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum, 2 mM L-glutamine, 100 U $mL^{-1}$ penicillin, and 100 μg $mL^{-1}$ streptomycin at 37 °C in 5% $CO_2$ and 3% $O_2$. Cells seeded at $2.5 \times 10^4$ per 30 mm diameter dish were passaged once cultures reached 85% confluency. Cell growth was measured by calculation of accumulated population doublings using the formula (logH − logS)/log2.0, where log H is the logarithm of the number of cells harvested and log S is the logarithm of the number of cells seeded on the first day of each passage, as described in Bridger and Kill [17].

### *Construction of prelamin A variants vectors and generation of stably transduced cell lines*

Human lamin A cDNA was purchased from ATCC (7517636) and cloned into the pCR4-topo vector (Invitrogen) by PCR using the following primers modified to create and amplify the