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Protein farnesyltransferase inhibitors and progeria

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Genetic mutations that lead to an accumulation of farnesyl-prelamin A cause progeroid syndromes, including Hutchinson–Gilford progeria syndrome. It seemed possible that the farnesylated form of prelamin A might be toxic to mammalian cells, accounting for all the disease phenotypes that are characteristic of progeria. This concept led to the hypothesis that protein farnesyltransferase inhibitors (FTIs) might ameliorate the disease phenotypes of progeria in mouse models. Thus far, two different mouse models of progeria have been examined. In both models, FTIs improved progeria-like disease phenotypes. Here, prelamin A post-translational processing is discussed and several mutations underlying human progeroid syndromes are described. In addition, recent data showing that FTIs ameliorate disease phenotypes in a pair of mouse models of progeria are discussed.

The nuclear lamina and progeria

Several forms of progeria are caused by a defect in the conversion of farnesyl-prelamin A to mature lamin A, which is a key structural protein of the nuclear lamina [1–3]. The nuclear lamina is an intermediate filament meshwork that lies beneath the nuclear envelope; it functions as a structural scaffolding for the nucleus and interacts with proteins within the inner nuclear membrane and in the heterochromatin [3–5]. The finding that progeroid syndromes can be caused by defective processing of prelamin A to mature lamin A has awakened interest both in the post-translational processing of prelamin A and the entire topic of progeria [6,7]. Here, we review the post-translational processing of prelamin A, describe a pair of mouse models for progeria, explain the genetic basis of several human progeroid syndromes, and summarize recent studies showing that nuclear-shape abnormalities in progeria cells and disease phenotypes in progeria mice can be ameliorated by protein farnesyltransferase inhibitors (FTIs). The finding that FTIs can ameliorate progeria-disease phenotypes in mouse models suggests a promising therapeutic approach for treating humans with progeria.

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Biogenesis of lamin A from prelamin A

Lamin A and lamin C, two abundant structural proteins of the nuclear lamina, are products of the same gene, *LMNA* [1]. The transcript for lamin C terminates at the end of exon 10 of *LMNA*; the transcript for prelamin A (the lamin A precursor) involves splicing from the middle of exon 10 to exon 11, and then to exon 12 [1]. Prelamin A and lamin C are identical through the first 566 amino acids but diverge within their carboxy-terminal domains.

Prelamin A, but not lamin C, terminates with a CAAX motif; this motif triggers farnesylation of the carboxy-terminal cysteine (the C of the CAAX motif) by a cytosolic enzyme, known as protein farnesyltransferase (Figure 1). Two other nuclear lamin proteins, lamin B1 and lamin B2, also terminate with a CAAX motif and are farnesylated.

After farnesylation, the last three amino acids of prelamin A (the –AAX of the CAAX motif) are released by an endoprotease (Figure 1). In the case of prelamin A, this endoproteolytic processing step is likely to be a redundant function of a pair of membrane endoproteases of the endoplasmic reticulum (ER), the zinc metalloprotease ZMPSTE24 and prenylprotein endopeptidase RCE1 [8–10], although the involvement of both endoproteases in prelamin A processing has never been formally proven. After the release of the last three amino acids, the newly exposed farnesylcysteine is methylated by the ER isoprenylcysteine carboxyl methyltransferase (ICMT) [11,12]. Each of these CAAX modifications renders the carboxy terminus of prelamin A more hydrophobic and is probably important for targeting the protein to the inner nuclear membrane. Finally, the last 15 amino acids of prelamin A, including the farnesylcysteine methyl ester, are clipped off by ZMPSTE24, and mature lamin A is released [10,13–15] (Figure 1). This step is likely to occur at the nuclear envelope dropping off mature lamin A at the nuclear lamina.

It is intriguing that ZMPSTE24 has two roles in prelamin A processing: clipping off the –AAX from the carboxy terminus and clipping the protein a second time to release mature lamin A [8]. Interestingly, the yeast ortholog of ZMPSTE24, Ste24p, also has two functions in the processing of the yeast mating pheromone **a**-factor [8,16–18]. Similar to prelamin A in mammals, **a**-factor is a CAAX protein that undergoes farnesylation, release of the –AAX and carboxylmethylation. The release of the –AAX from **a**-factor is a redundant function of Ste24p and Rce1p

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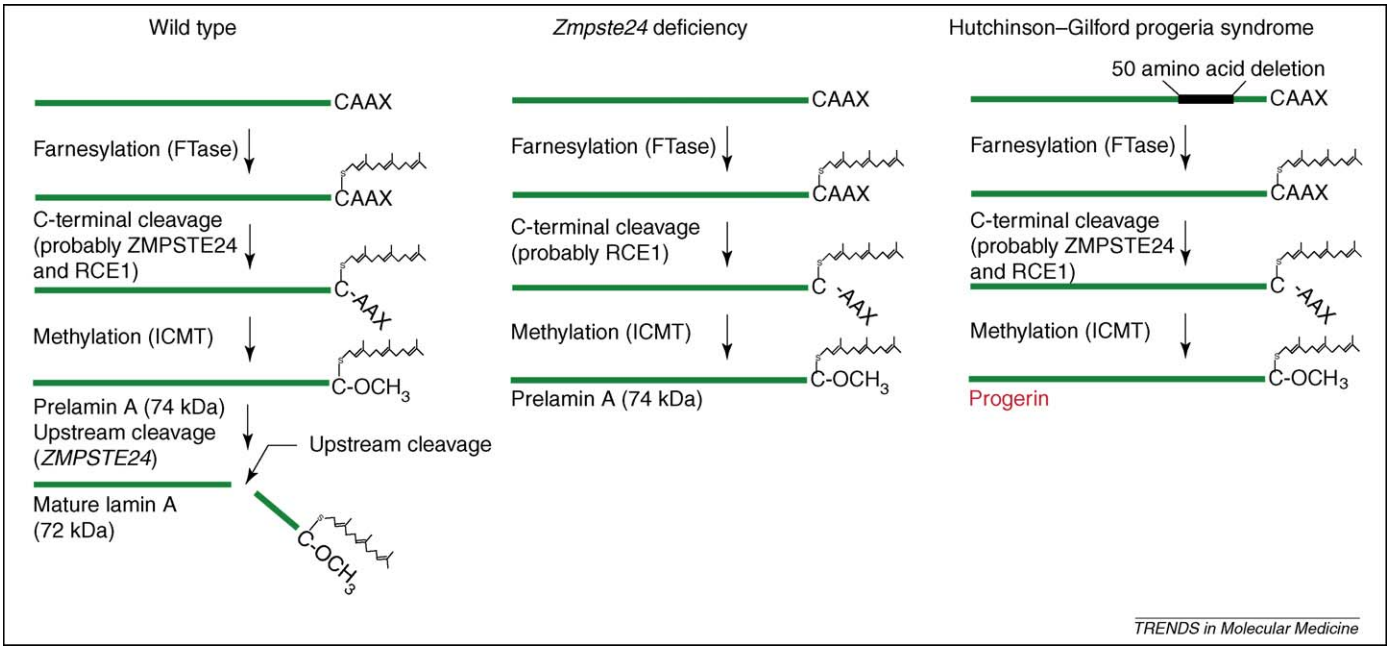

Figure 1. Biogenesis of lamin A in normal cells and the failure to generate mature lamin A in *Zmpste24* deficiency or HGPS. Formation of lamin A from prelamin A in wild-type cells (left). Prelamin A (664 amino acids) undergoes four post-translational processing steps. First, the cysteine of the CAAX motif is farnesylated by FTase. Second, the –AAX is released. Third, the newly exposed farnesylcysteine is methylated. Fourth, the carboxy-terminal 15 amino acids, including the farnesylcysteine methyl ester, are clipped off (by ZMPSTE24) and then degraded, leaving mature lamin A (646 amino acids) [13–15]. Production of a mutant prelamin A, progerin, in HGPS (right). The 50-amino acid deletion in prelamin A (amino acids 607–656) removes the site for the second endoproteolytic cleavage. Thus, no mature lamin A is formed. For reasons discussed elsewhere [8], it seems probable that the –AAX of progerin is cleaved by either RCE1 or ZMPSTE24 and that the farnesylcysteine is methylated by ICMT. Defective prelamin A processing in *Zmpste24* deficiency (middle). By analogy to **a**-factor biogenesis in yeast [16,18,19,51], it seems probable that the –AAX is released by RCE1 in *Zmpste24*$^{-/-}$ cells. Another reason to believe that RCE1 might release the –AAX is the observation that RCE1 cleaves the –AAX from lamin B1 [52] and from a mutant **a**-factor that contains the –AAX from prelamin A [8]. Blocking farnesylation with an FTI would mean that the carboxy terminus of progerin terminates with an α-carboxylate anion rather than a farnesylcysteine methyl ester; this change would reduce the hydrophobicity of the carboxy terminus of the protein [53] and would be expected to reduce the avidity of the molecule for the inner nuclear membrane. Reproduced, with permission, from [8].

[19]. After the CAAX modifications of the **a**-factor precursor are complete, the biogenesis of mature **a**-factor requires two additional N-terminal endoproteolytic processing steps; the first of these is carried out by Ste24p [16].

The production of mature lamin A from prelamin A (Figure 1) is entirely dependent on the first post-translational processing step, farnesylation. When farnesylation is blocked, mature lamin A cannot be generated and non-farnesylated prelamin A accumulates within cells [9,13]. Similarly, the production of mature lamin A is entirely dependent on ZMPSTE24. In the absence of ZMPSTE24, the second and final endoproteolytic processing step cannot occur and a farnesylated version of prelamin A accumulates in cells (Figure 1). The farnesyl-prelamin A in *ZMPSTE24*-deficient cells is almost certainly carboxylmethylated because there is no reason to believe that RCE1 would not release the three carboxy-terminal amino acids from the protein, thereby rendering the protein susceptible to methylation by ICMT [8]. However, conclusive proof that progerin is actually methylated has not yet been published. The presence of the farnesyl lipid anchor on prelamin A has important functional consequences. In the absence of the farnesyl lipid anchor (i.e. when wild-type cells are treated with an FTI), the non-farnesylated prelamin A still reaches the nucleus, but the protein is mislocalized to the nucleoplasm and does not reach the nuclear rim. When prelamin A is farnesylated (i.e. in *ZMPSTE24* deficiency), farnesyl-prelamin A accumulates at the rim of the nucleus, presumably along the inner nuclear membrane and the nuclear lamina [20].

A few years ago, in early studies of *Icmt*$^{-/-}$ mouse fibroblasts, Bergo *et al.* [9] presented evidence for a profound defect in lamin A biogenesis in *Icmt*$^{-/-}$ cells, with a striking accumulation of prelamin A [9]. At that time, this finding made sense because Sinensky and colleagues [15] had previously published data indicating that the carboxy-terminal methyl ester was important for the final endoproteolytic processing step. However, the experiments with *Icmt*$^{-/-}$ cells have recently been repeated, and it now seems clear that the defect in lamin A biogenesis is not complete [21]. In *Icmt*$^{-/-}$ cells, farnesyl-prelamin A accumulates but substantial amounts of mature lamin A are also generated. Thus, the carboxy-terminal methyl ester on prelamin A is not absolutely required for the biogenesis of mature lamin A.

ZMPSTE24 deficiency in mice

To define the physiological importance of ZMPSTE24 in mammals, Bergo *et al.* [9] created *Zmpste24*$^{-/-}$ mice [9]. *Zmpste24*$^{-/-}$ mice appear normal until about three weeks of age. At that time, their body weight is reduced compared with wild-type littermates. By 16 weeks of age, *Zmpste24*$^{-/-}$ mice manifest incisor abnormalities, alopecia, kyphosis of the spine, muscle weakness and a slow arthritic gait. The bone disease and the muscle weakness are progressive, and the mice either die or need to be euthanized by 24–32 weeks of age.

Micro-computed tomography (μCT) scans of 3-week-old *Zmpste24*$^{-/-}$ mice revealed normal-appearing bones and normal bone density. By 8–12-weeks of age, however, the