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The posttranslational processing of prelamin A and disease

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Abstract

Human geneticists have shown that some progeroid syndromes are caused by mutations that interfere with the conversion of farnesyl-prelamin A to mature lamin A. For example, Hutchinson-Gilford progeria syndrome is caused by *LMNA* mutations that lead to the accumulation of a farnesylated version of prelamin A. In this review, we discuss the posttranslational modifications of prelamin A and their relevance to the pathogenesis and treatment of progeroid syndromes.

Keywords

prenylation; ZMPSTE24; restrictive dermopathy; FTI

Introduction

Prelamin A terminates with a *CaaX* motif and like other *CaaX* proteins, undergoes farnesylation, endoproteolytic trimming of the last three amino acids, and methylation of the carboxyl-terminal farnesylcysteine (14,15,18,95,117). After these modifications are complete, prelamin A undergoes an additional processing step; the last 15 amino acids of the protein (including the farnesylcysteine α -methyl ester) are clipped off, thereby releasing mature lamin A. Over the past few years, human geneticists have shown that certain progeroid syndromes, in particular Hutchinson-Gilford progeria syndrome (HGPS) and restrictive dermopathy (RD), are caused by mutations that interfere with the conversion of farnesyl-prelamin A to mature lamin A (Figure 1). In this review, we discuss the posttranslational processing of the nuclear lamins, focusing on prelamin A. We discuss the importance of prelamin A processing to the pathogenesis of progeroid syndromes and discuss potential therapeutic approaches for these diseases.

The Posttranslational Processing of the Nuclear Lamins

Many cellular proteins, including several of the nuclear lamins, terminate with the amino acids *-C-a-a-X*, in which the “C” is a cysteine, the two “a” residues are often aliphatic amino acids, and the “X” can be many different residues (14,15). The *CaaX* motif triggers three sequential enzymatic modifications (15,118). First, the protein undergoes posttranslational lipidation; a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid is added to the thiol group of the

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cysteine by a cytosolic prenyltransferase [protein farnesyltransferase (FTase) or protein geranylgeranyltransferase type I (GGTase-I)]. When the “X” is a leucine or phenylalanine, the cysteine is geranylgeranylated; otherwise, it is farnesylated (91,118). Next, the last three amino acids of the protein (i.e., the –aaX of the *CaaX* motif) are clipped off and released by a prenylprotein-specific endoprotease of the endoplasmic reticulum. For prelamin A, it appears that both RCE1 and ZMPSTE24 can carry out this step, but for other *CaaX* proteins, this step is carried out mainly or exclusively by RCE1 (60,69,78). After this endoproteolytic cleavage step, the newly exposed isoprenylcysteine is methylated by ICMT, a prenylprotein-specific methyltransferase of the ER (19,26). The endoproteolytic cleavage and methylation steps are utterly dependent on the first step—protein isoprenylation.

The largest known group of isoprenylated *CaaX* proteins is the Ras superfamily of G proteins (91,118). This group of proteins includes the Ras proteins, which are farnesylated, and the Rho and Rap proteins, which are geranylgeranylated. Isoprenylation and the subsequent endoproteolysis and methylation steps render the carboxyl terminus of the protein more hydrophobic, facilitating their targeting to the plasma membrane or other membrane surfaces, and, in some cases, influencing protein–protein interactions (91,118).

Several nuclear lamins are *CaaX* proteins (19). The nuclear lamins are the structural proteins of the nuclear lamina, an intermediate filament network that provides a scaffolding for the cell nucleus (8,74,111). In addition to its structural role, the nuclear lamina plays important roles in DNA replication, cell division, heterochromatin organization, protein trafficking, and gene transcription (72,109). The nuclear lamins are targeted to the cell nucleus by nuclear localization motifs; however, farnesylation and methylation are thought to be important for targeting the lamins to the inner nuclear membrane (5,54,66,95), which is located immediately adjacent to the nuclear lamina.

Interest in the nuclear lamins and their posttranslational modifications has grown substantially with the discovery that mutations in *LMNA* cause a wide array of human genetic diseases (“laminopathies”). *LMNA* actually yields two protein products, prelamin A (the precursor protein to lamin A) and lamin C, as a result of alternative splicing. Lamins A and C are identical through the first 566 amino acids (encoded by exons 1–10), but diverge at the carboxyl terminus (8,41,72). Only prelamin A contains a *CaaX* motif and undergoes farnesylation and methylation. After the “*CaaX* modifications” are complete, prelamin A undergoes an additional endoproteolytic processing step (59). The last 15 amino acids of the protein, including the farnesylcysteine methyl ester, are clipped off by ZMPSTE24, thereby generating mature lamin A (Figure 1). This final endoproteolytic cleavage step is dependent on protein prenylation; blocking protein prenylation results in the accumulation of nonfarnesylated prelamin A and no mature lamin A is produced (5).

ZMPSTE24 is required for prelamin A's final endoproteolytic processing step (6,22,46,80). A deficiency of ZMPSTE24 leads to an accumulation of a farnesylated form of prelamin A and restrictive dermopathy, a severe perinatal-lethal progeroid syndrome (71,75) (Figure 1). RD fibroblasts are unable to produce any mature lamin A (6,80).

Lamins B1 and B2 (encoded by *LMNB1* and *LMNB2*, respectively) are *CaaX* proteins and undergo all of the usual *CaaX* modifications (4,16,38,110). Unlike prelamin A, however, these nuclear lamins do not undergo a second endoproteolytic cleavage reaction, so the mature versions of these proteins terminate with a farnesylcysteine α -methyl ester.

With respect to prelamin A, the obvious question is: Why has nature decided to expend energy on protein farnesylation and methylation, given that the carboxyl-terminal portion of prelamin A is eventually clipped off and degraded? Unquestionably, the posttranslational processing of prelamin A is important, as the *CaaX* motif has been conserved in vertebrate evolution (107).

If the *CaaX* modifications were functionally irrelevant, one would have expected that prelamin A would have been truncated during evolution, generating mature lamin A directly without going through the complicated series of posttranslational modifications. Most likely, the *CaaX* motif of prelamin A has been conserved because farnesylation and methylation play important roles in the assembly and function of the nuclear lamina. The *CaaX* modifications likely facilitate the association of prelamin A with the inner nuclear membrane (22,27,59), where the release of mature lamin A occurs. This explanation is plausible, as protein farnesylation and methylation are known to increase the hydrophobicity of the carboxyl terminus of the protein (94). Also, Hennekes and Nigg have demonstrated experimentally that farnesyl-prelamin A binds avidly to membranes (53). Nevertheless, the biological importance of prelamin A's *CaaX* modifications requires more study. Along those lines, Lutz et al. (66) expressed a “mature lamin A mutant” (i.e., where mature lamin A was generated directly, bypassing all *CaaX* modifications) in cultured cells, and found that the mature lamin A reached the nuclear rim and functioned normally. Hennekes and Nigg (53) also found that a “mature lamin A mutant” reached the nuclear rim, although with somewhat delayed kinetics.

If prelamin A's *CaaX* modifications were crucial for the assembly or function of the nuclear lamina, one might reasonably expect that the complete elimination of prelamin A would have dramatic consequences. However, this does not appear to be the case, at least in laboratory mice. Fong et al. (45) generated mice that synthesize exclusively lamin C and absolutely no prelamin A or lamin A (“lamin C-only” or *Lmna*^{LCO/LCO} mice). *Lmna*^{LCO/LCO} mice were phenotypically normal, and fibroblasts from these mice exhibited relatively trivial abnormalities in nuclear shape and nuclear mechanics (45,62).

Thus far, no one has developed “lamin A-only mice,” but such mice could help decipher the physiologic importance of the complicated prelamin A processing scheme. A key experiment would be to generate gene-targeted “mature lamin A-only mice” (where lamin A is generated directly, bypassing all posttranslational processing) and then compare those mice, side-by-side, with “prelamin A-only mice” (where the biogenesis of lamin A depends on the four sequential posttranslational processing steps). Given the absence of overt phenotypes in *Lmna*^{LCO/LCO} mice, we suspect that “mature lamin A-only” mice would survive. However, we would be surprised if the assembly and function of the nuclear lamina were normal in “mature lamin A-only mice.”

LMNA Mutations and Human Disease

Mutations in *LMNA* cause a wide variety of human genetic diseases, including autosomal-dominant Emery-Dreifuss muscular dystrophy (7), limb-girdle muscular dystrophy (73), Dunnigan-type familial partial lipodystrophy (47,51,52), cardiomyopathy with conduction system disease (104), atypical Werner's syndrome (17), and one type of Charcot-Marie-Tooth neuropathy (30). Most disease-causing mutations are missense mutations and most occur in exons 1–10, the region shared by lamins A and C, but a few occur in exons 11–12 (which encode the domain unique to prelamin A).

Hutchinson-Gilford progeria syndrome is caused by a mutant prelamin A that cannot be processed to mature lamin A (29,36). Affected children appear normal at birth but then manifest a number of disease phenotypes, including failure-to-thrive, dental abnormalities, micrognathia, stiff joints, alopecia, sclerodermatous skin, loss of subcutaneous fat, osteoporosis, and osteolytic lesions in bones (3,31,36,37,40,57,82). Death generally occurs by the teenage years, generally from occlusions of the coronary or cerebral arteries. The explanation for the arterial disease is not entirely clear. Pathological studies of HGPS patients are limited but at least one study revealed that the arteries contain atheroma and cholesterol clefts (97). On the other hand, a recent clinical study found that arteries of children with HGPS

had adventitial thickening and a normal intimal thickness (70)—very different from what one would expect with atherosclerosis. Also, flow-mediated dilation of the brachial artery was normal in children with HGPS, casting doubt on an atherosclerotic etiology for the arterial disease.

HGPS is caused by de novo point mutations that promote the utilization of an alternative donor splice site within exon 11 of *LMNA*, resulting in the synthesis of a mutant prelamin A, commonly called progerin, that contains an in-frame deletion of the last 50 amino acids encoded by exon 11 (residues 607–656) (29,36) (Figure 1). The internally truncated prelamin A is easily visible on western blots of cell extracts (10,98,113). The most common mutation causing HGPS is a synonymous mutation in codon 608 (G608G) (37,40,52); however, several other point mutations (including one at the exon 11–intron11 junction) promote the same alternative splicing event (52,57). The 50–amino acid deletion leaves the *CaaX* motif intact; thus, progerin undergoes farnesylation, endoproteolytic release of the *-aaX*, and carboxyl methylation (36). However, the deletion eliminates the site for endoproteolytic cleavage by ZMPSTE24; thus, progerin cannot undergo further processing and retains its carboxyl-terminal farnesylcysteine methyl ester (Figure 1) (32,112).

Much of the progerin in cultured fibroblasts is located at the nuclear rim, but some normally appears within the nucleoplasm (113). The targeting of progerin to the nuclear rim depends on the carboxyl-terminal lipid anchor, as interfering with protein farnesylation mislocalizes progerin to the nucleoplasm (49,114). HGPS fibroblasts exhibit a high percentage of misshapen nuclei, almost certainly because progerin adversely affects the integrity of the nuclear lamina (29,36).

An even more severe progeroid syndrome, restrictive dermopathy, is caused by defects in ZMPSTE24, the protease that releases mature lamin A from farnesyl-prelamin A (71,75) (Figure 1). RD is a perinatal-lethal disorder; hallmarks include sclerodermatous skin, loss of fat, prominent superficial veins, dysplastic clavicles, a reduced amount of hair, and joint contractures. Initially, Navarro et al. (76) suggested that the disease was caused by heterozygosity for *ZMPSTE24* null mutations in combination with a mutation in some other gene. Eventually, however, Moulson et al. (71) and Navarro et al. (75) showed that RD is actually caused by homozygosity for null mutations in *ZMPSTE24*.

In the absence of ZMPSTE24, the production of mature lamin A is eliminated and farnesylprelamin A accumulates in cells. Much of this farnesyl-prelamin A is located at the nuclear rim, where it leads to misshapen nuclei. *ZMPSTE24* missense mutations leading to the production of a protein with some residual enzymatic function result in progeroid syndromes less severe than typically observed in RD (2,92). In such cases, farnesyl-prelamin A accumulates in cells, but the residual ZMPSTE24 activity allows small amounts of mature lamin A to be produced (92).

An intriguing patient with homozygous *ZMPSTE24* null mutations was reported by Denecke et al. (34). Despite a complete absence of ZMPSTE24, the patient was less severely affected than typical RD patients. In this case, the milder-than-expected phenotype was due to heterozygosity for a *LMNA* mutation that truncated prelamin A, eliminating its *CaaX* motif and preventing protein farnesylation. This case highlighted the importance of farnesyl-prelamin A as the culprit molecule in the pathogenesis of disease.

While a farnesylated prelamin A is clearly the culprit in RD and in HGPS, severe progeroid syndromes can be caused by *LMNA* mutations without a prelamin A–processing defect. For example, Verstraeten et al. (103) reported a severe progeroid disorder in a child who was a compound heterozygote for T528M and M540T mutations in lamins A/C. Also, Eriksson et al. (36) identified an E145K missense mutation in a patient with a severe progeroid syndrome.

Mouse Models of Progeria

Gene-targeted mouse models have been generated for both RD and HGPS (1,6,80,88,102, 106,113,114). Inactivation of *Zmpste24* in mice (63) completely prevents the biogenesis of mature lamin A and leads to an accumulation of prelamin A (6,80). As one would predict, this prelamin A is farnesylated (21,98). Like fibroblasts from human patients with RD and HGPS, *Zmpste24*^{-/-} mouse embryonic fibroblasts exhibit prominent abnormalities in nuclear shape (46,80).

Zmpste24^{-/-} mice are normal at birth and their body weight at weaning is only slightly lower than wild-type littermates (6). However, they gain weight slowly and soon develop alopecia, kyphosis, abnormalities of their incisors, and a slow arthritic gait (6). By 10–12 weeks of age, *Zmpste24*^{-/-} mice develop a grip strength abnormality and osteolytic lesions in the ribs near the costovertebral joints. These lesions lead to fractures, which heal very poorly (6). It seems likely that the bone disease in *Zmpste24*^{-/-} mice is caused by defective formation of bone, as Bergo et al. (6) found no biomarker evidence for increased bone turnover and no histological evidence for increased osteoclast activity.

Fong et al. (46) suspected that farnesyl-prelamin A might account for all of the disease phenotypes in *Zmpste24*^{-/-} mice. To address this issue, they compared the phenotypes of *Zmpste24*^{-/-} mice with littermate *Zmpste24*^{-/-} mice harboring a single *Lmna* knockout allele (*Zmpste24*^{-/-}*Lmna*^{+/-}) (46). They hypothesized that the half-normal amounts of farnesyl-prelamin A in *Zmpste24*^{-/-}*Lmna*^{+/-} mice might lead to less toxicity and milder disease phenotypes than typically observed in *Zmpste24*^{-/-} mice. The result was quite stunning: *Zmpste24*^{-/-}*Lmna*^{+/-} mice were entirely free of all disease phenotypes. Their growth rate was normal; their grip strength was entirely normal; and they were free of the characteristic osteolytic lesions and rib fractures (46). Also, *Zmpste24*^{-/-}*Lmna*^{+/-} fibroblasts exhibited fewer misshapen nuclei than *Zmpste24*^{-/-} fibroblasts (46). These findings supported the idea that disease phenotypes in *Zmpste24*^{-/-} mice are due to the accumulation of farnesyl-prelamin A, and they suggested that prelamin A-associated progeroid syndromes might be cured by reducing farnesyl-prelamin A production by one-half. An independent group confirmed an absence of disease in *Zmpste24*^{-/-}*Lmna*^{+/-} mice (100).

Yang et al. (113) generated a knock-in model of HGPS. The sole product of their mutant *Lmna* allele was progerin; hence, this allele was dubbed the “progerin-only” or “Hutchinson-Gilford” (HG) allele (*Lmna*^{HG}). *Lmna*^{HG/+} fibroblasts expressed large amounts of progerin (from the mutant allele) as well as lamin A and lamin C (from the wild-type allele). *Lmna*^{HG/+} cells exhibited a high frequency of misshapen nuclei (113).

Like *Zmpste24*^{-/-} mice, *Lmna*^{HG/+} mice appeared normal for the first three weeks of life. By 6–8 weeks, however, both male and female *Lmna*^{HG/+} mice began to lose weight, and they invariably developed the same osteolytic lesions and rib fractures observed previously in *Zmpste24*^{-/-} mice (114). *Lmna*^{HG/+} mice became progressively malnourished, and most of the mice were so sick that they died or required euthanasia by 4–6 months of age. Interestingly, the intima, media, and adventitia of *Lmna*^{HG/+} aortas were free of lesions and indistinguishable from those of wild-type mice (114).

Homozygous mice (*Lmna*^{HG/HG}) expressed exclusively progerin and no lamin A or lamin C (114); they were quite small and died by 3–4 weeks of age. Several exhibited spontaneous bone fractures in the extremities (114). A very high percentage of *Lmna*^{HG/HG} fibroblasts contained misshapen nuclei (59.5% of nuclei had folds or blebs vs. 29.6% in *Lmna*^{HG/+} cells) (114).

Recently, Yang et al. (115) tested the hypothesis that compositional alterations in the nuclear lamina might modulate the phenotypes elicited by the synthesis of a fixed amount of progerin.

They compared mice harboring one *Lmna*^{HG} allele and one *Lmna*^{LCO} allele (these mice produce progerin and lamin C) with littermate *Lmna*^{HG/+} mice (which produce progerin, lamin A, and lamin C). The *Lmna*^{HG/LCO} mice had higher body weights, survived longer, and had fewer rib fractures than *Lmna*^{HG/+} mice. *Lmna*^{HG/LCO} fibroblasts also had fewer misshapen nuclei than *Lmna*^{HG/+} fibroblasts. Interestingly, the steady state levels of progerin were lower in *Lmna*^{HG/LCO} cells and tissues, providing a possible explanation for their milder disease phenotypes.

Transgenes designed to yield progerin expression have been constructed by several laboratories. Varga et al. (102) generated transgenic mice with a 164-kb human bacterial artificial chromosome (BAC) spanning four genes—*RAB25* (another isoprenylated protein), *UBQLN4*, *MAPBPIP*, and *LMNA* (engineered to contain the G608G HGPS point mutation). An RT-PCR study revealed that some progerin transcripts were produced by the transgenic mice (102). However, the amount of progerin protein produced by the transgenic mice, relative to human or mouse lamin A, was not clear from the western blot that was published (102). Of note, none of the early disease phenotypes of progeria (e.g., slow growth, bone disease) were observed in the transgenic mice. However, the mice did lose smooth muscle cells in the medial layer of large arteries. The latter pathological finding appeared to be absent or markedly reduced in a second line of transgenic mice generated from a BAC lacking the HGPS mutation, although it was not clear that the levels of *LMNA* expression were similar in the two lines of BAC transgenic mice (102).

The loss of smooth muscle cells in the BAC transgenic mice was intriguing because a similar finding has been noted in the large arteries of humans with HGPS. In human autopsy studies, prominent intimal atherosclerosis was observed in several individuals with HGPS, and in one case, intimal cholesterol clefts (1,96,97). However, a feature of the human cases was loss of medial smooth muscle cells (70). In the BAC transgenic mouse model (102), no intimal atherosclerotic lesions or arterial occlusions were observed, but loss of smooth muscle cells was detected.

It is not clear why the gene-targeted mouse model of HGPS (*Lmna*^{HG/+} mice) generated by Yang et al. (114-116) exhibit many early features of progeria, such as the slow growth, micrognathia, alopecia, osteolytic lesions in bone, but not the late phenotypes (i.e., occlusive vascular disease), while the BAC transgenic mice produced by Varga et al. (102) exhibit loss of medial smooth muscle cells but none of the other progeria-like disease phenotypes. Conceivably, these differences relate to different properties of human and mouse progerin. The BAC transgene was designed with the hope that it would yield a sufficient amount of human progerin to elicit disease, while the *Lmna*^{HG} allele clearly produced large amounts of mouse progerin. One could speculate that mouse progerin in *Lmna*^{HG/+} mice adversely affects a range of tissues, closely modeling early hallmarks of HGPS in humans, but that *Lmna*^{HG/+} mice either do not live long enough to manifest late phenotypes (vascular disease), or alternatively, that mouse progerin is intrinsically incapable of affecting smooth muscle cells in the arterial media. And one could further speculate that human progerin affects only arterial smooth muscle cells in the mouse and is entirely innocuous for all other tissues. The latter scenario is clearly a formal possibility, but perhaps somewhat unlikely, given that human progerin unequivocally affects multiple tissues in humans with HGPS. Alternatively, levels of human progerin may have varied widely in different tissues and may have been too low in some tissues to produce a phenotype. Another possibility is that the overexpression of the other proteins within the very large BAC clone, including lamins A and C, plays a role in the medial smooth muscle cell phenotype.

Sagelius et al. (88) generated a line of transgenic mice in which a tetracycline-inducible keratin-5 promoter drove high levels of human progerin and lamin A expression in epidermal

keratinocytes. In these mice, they observed several abnormalities in the skin and teeth, including “fibrosis, loss of hypodermal adipocytes, structural defects in the hair follicles and sebaceous glands, and abnormal incisors” (88). The severity of the phenotypes in different lines of transgenic mice appeared to correlate with levels of progerin expression (88). In a separate study, Wang et al. (106) generated transgenic mice in which the keratin 14 promoter drove expression of either flag-tagged human progerin or flag-tagged human lamin A in keratinocytes. Keratinocytes from the transgenic mice had severe nuclear abnormalities, including “nuclear envelope lobulation and decreased nuclear circularity” (106).

Unfortunately, the amount of human progerin in keratinocytes, relative to the mouse lamin A and lamin C, was not clear. Despite the defects in nuclear morphology, the transgenic mice had normal wound healing and had no skin or hair abnormalities.

Reduced Numbers of Misshapen Nuclei in Progeria Cells Treated with an FTI

Fong et al. (46) hypothesized that the farnesylated form of prelamin A might be the real culprit in eliciting disease phenotypes of progeria. If this were the case, one might expect that the toxicity of prelamin A would be reduced by inhibiting protein farnesylation with a protein farnesyltransferase inhibitor (FTI). Yang et al. (113) first examined this hypothesis testing the effects of an FTI on the misshapen nuclei in *Lmna*^{HG/+} fibroblasts and otherwise identical fibroblasts lacking the targeted mutation. They found that an FTI significantly reduced the frequency of misshapen nuclei in *Lmna*^{HG/+} fibroblasts (113). Also, they found that an FTI mislocalized progerin from the nuclear envelope to the nucleoplasm in *Lmna*^{HG/HG} cells. Just a few months later, Toth et al. (98) extended these findings by showing that FTIs ameliorated the nuclear shape abnormalities in human HGPS fibroblasts, human cells lacking ZMPSTE24, and multiple lines of *Zmpste24*^{-/-} mouse fibroblasts, and several other groups reported similar findings (10,48,68). In the cell culture studies by Yang et al. (113) and Toth et al. (98), the inhibition of protein farnesylation by FTIs was impressive. Virtually all of the HDJ-2 (an unrelated *CaaX* protein) was nonfarnesylated, and significant amounts of nonfarnesylated prelamin A accumulated within cells.

The utility of the FTIs in improving nuclear shape might extend beyond ZMPSTE24-deficient and HGPS cell lines. Toth et al. (98) proposed that FTIs might also be useful for diseases caused by missense mutations in lamin A—mutations that do not lead to defective lamin A processing. They reasoned that reducing prelamin A prenylation would interfere with the biogenesis of mature lamin A and thereby limit the delivery of a mutant version of mature lamin A to the nuclear rim. To test this idea, they treated fibroblasts harboring an R644C or an E578V mutation with an FTI. Interestingly, the FTI reduced the frequency of misshapen nuclei in both cell lines (98). More recently, Verstraeten et al. (103) treated fibroblasts from a patient with two *LMNA* missense mutations (T528M and M540T) with an FTI and found that the drug reduced the frequency of nuclear honeycombing and blebs from 36 and 20%, respectively, to ~10%.

Improved Disease Phenotypes with FTI Treatment in Mouse Models of Progeria

Fong et al. (44) demonstrated that a potent FTI, ABT-100 (39), ameliorated disease phenotypes in *Zmpste24*^{-/-} mice. Treatment with an FTI improved body weight curves in both male and female *Zmpste24*^{-/-} mice, while body weight curves worsened slightly in wild-type mice that were treated with the FTI (44). FTI treatment of *Zmpste24*^{-/-} mice also led to improved survival, reduced numbers of rib fractures, and improved grip strength (44). However, the FTI treatment did not come close to achieving a cure. FTI-treated *Zmpste24*^{-/-} mice still developed rib fractures and still had abnormal body weight curves, and their survival was prolonged by only a few months. Only 10–50% of the HDJ-2 in the tissues of treated mice was nonfarnesylated. Also, while FTI treatment clearly led to the appearance of nonfarnesylated

prelamin A in tissue extracts, the amount of prelamin A accumulation was far less impressive than in FTI-treated fibroblasts (44).

Yang et al. (114) showed that treatment of *Lmna*^{HG/+} mice with ABT-100 significantly improved body weight curves, increased fat pad weight, and increased the amount of subcutaneous fat (Figure 2). Bone disease also improved in FTI-treated mice; the numbers of rib fractures were significantly decreased; the degree of kyphosis was reduced; and bone cortical thickness was improved (114). In a follow-up study, Yang et al. (116) confirmed the beneficial effects of an FTI in *Lmna*^{HG/+} mice and showed that the drugs resulted in improved survival (Figure 2). Once again, however, the FTI-induced improvements in disease phenotypes fell short of a cure. The inhibition of FTase was clearly incomplete in these mouse studies (114,116). Only 70–80% of the HDJ-2 in the livers of FTI-treated mice was nonfarnesylated, and the amount of nonfarnesylated prelamin A in tissues, although easily detectable by western blot, was small, relative to the amount of mature lamin A or lamin C (114).

Based on the favorable effects of the FTIs on disease phenotypes in *Zmpste24*^{−/−} and *Lmna*^{HG/+} mice (44,114), an FTI (lonafarnib) is now being tested in human HGPS patients in an open-label clinical trial organized by the Progeria Research Foundation (58). Thus far, the extent to which lonafarnib treatment blocks HDJ-2, progerin, and prelamin A farnesylation in children with HGPS is unclear. Clinical outcomes should be known within the next 1–2 years.

Recently, Capell et al. (11) examined the effects of tipifarnib (another FTI) in transgenic mice harboring the large BAC clone engineered to contain the HGPS G608G point mutation (102). As noted earlier, these mice did not have early hallmarks of progeria, but they have loss of smooth muscle cells in large arteries. They found that tipifarnib minimized the loss of smooth muscle cells in large arteries (11). In these studies, the FTI significantly reduced the farnesylation of HDJ-2. However, the amount of progerin in the arteries was not clear. Also, the extent to which the drug affected prelamin A farnesylation and the biogenesis of lamin A was apparently not examined.

Mice Expressing a Nonfarnesylated Version of Progerin

One potential explanation for the less-than-complete therapeutic response of the *Lmna*^{HG/+} mice to an FTI was the less-than-complete blockade of protein farnesylation (as judged by the incomplete blockade of HDJ-2 farnesylation and the modest amount of nonfarnesylated prelamin A in tissues) (44,114,116). If the incomplete inhibition of farnesylation were the explanation, the solution would obviously involve optimizing the treatment regimen (e.g., higher FTI doses, more potent drugs, higher trough concentrations of the drug). However, incomplete inhibition of FTase is certainly not the only potential explanation for suboptimal therapeutic efficacy. Another is that nonfarnesylated progerin—the molecule that accumulates with FTI treatment—might be intrinsically toxic and capable of eliciting disease. If so, optimizing FTI treatment regimens would be wasted effort.

It is not farfetched to suggest that nonfarnesylated progerin could be toxic, given that subtle amino acid substitutions in mature lamin A and lamin C—neither of which is farnesylated—can cause progeroid syndromes very similar to HGPS (36,103). Also, treatment of *Lmna*^{HG/HG} cells with an FTI led to the appearance of aggregates in the nucleoplasm (10,68,113), and it seems at least possible that these collections of nonfarnesylated progerin could have adverse consequences for cells.

To examine the possibility that nonfarnesylated progerin might be toxic to mammalian tissues, Yang et al. (112) created knock-in mice expressing a nonfarnesylated version of progerin (*Lmna*^{nHG/+}). The *Lmna*^{nHG/+} mice were genetically identical to *Lmna*^{HG/+} mice, except that

the cysteine in the *CaaX* motif was replaced with a serine (a mutation that eliminates protein prenylation). The progerin in *Lmna*^{nHG/+} cells migrated more slowly on SDS-polyacrylamide gels, and metabolic labeling studies revealed that the progerin in these mice could not be farnesylated. It appeared that a lower percentage of the progerin in *Lmna*^{nHG/+} fibroblasts was localized to the nuclear rim, and the frequency of misshapen nuclei in *Lmna*^{nHG/+} fibroblasts was somewhat lower than in *Lmna*^{HG/+} fibroblasts (112).

The disease phenotypes in *Lmna*^{nHG/+} mice were unquestionably milder than in *Lmna*^{HG/+} mice. The body weight curves were improved; the number of rib fractures was reduced; body fat was increased, and the mice survived longer (Figure 2). However, even though the disease phenotypes in *Lmna*^{nHG/+} mice were milder, it is important to emphasize that the *Lmna*^{nHG/+} mice were severely affected and all of these mice died from their disease. Thus, nonfarnesylated progerin is intrinsically capable of causing severe disease.

The milder phenotypes in *Lmna*^{nHG/+} mice are entirely consistent with the results of FTI treatment in *Lmna*^{HG/+} mice (Figure 2), and together, the pharmacologic and genetic studies support the concept that lonafarnib treatment of humans with HGPS might be partially efficacious. On the other hand, these findings also suggest that the therapeutic benefit of any FTI will be limited, given that nonfarnesylated progerin also yields severe progeria-like disease phenotypes. However, the latter suggestion is subject to some caveats. One is that the *Lmna*^{nHG/+} studies deal with the effects of mouse progerin, not human progerin. This consideration may be quite important, given that subtle differences in lamin structure can have major consequences. Also, the mutant lamin protein in *Lmna*^{nHG/+} mice contains a cysteine-to-serine substitution in the *CaaX* motif, so the structure of that molecule is different from the nonfarnesylated progerin that would accumulate in the presence of an FTI. It is conceivable that the single amino acid substitution in the progerin of *Lmna*^{nHG/+} mice is relevant to the toxicity elicited by the nonfarnesylated progerin. In other words, there is a formal possibility that the nonfarnesylated progerin in *Lmna*^{nHG/+} mice is quite toxic, while the nonfarnesylated progerin in FTI-treated *Lmna*^{HG/+} mice is not.

The more severe disease in *Lmna*^{HG/+} mice, compared with *Lmna*^{nHG/+} mice, might lead one to conclude that the farnesyl lipid anchor on progerin adds to the toxicity of the molecule. However, Yang et al. (112) uncovered another potential mechanism—higher steady-state levels of progerin in *Lmna*^{HG/+} mice. As judged by quantitative western blots, *Lmna*^{HG/+} mice had significantly more progerin (relative to actin) in embryonic fibroblasts and in various mouse tissues (heart, liver, kidney, skull) than *Lmna*^{nHG/+} mice. Thus, it is possible that the farnesyl group elicits more toxicity by increasing levels of progerin, rather than by changing the intrinsic toxicity of the molecule. The lower levels of progerin in *Lmna*^{nHG/+} mice suggested that FTIs might lower steady-state levels of progerin in *Lmna*^{HG/+} mice. Indeed, this was the case. Treatment of *Lmna*^{HG/+} fibroblasts with an FTI reduced progerin levels, relative to actin, and treatment of *Lmna*^{HG/+} mice with an FTI resulted in lower levels of progerin in the heart, relative to actin, compared with *Lmna*^{HG/+} mice treated with vehicle alone (112). Several lines of evidence suggested that an FTI might lower progerin levels in *Lmna*^{HG/+} mice by accelerating the turnover of progerin (112).

The improvement in disease phenotypes in *Lmna*^{HG/+} mice with an FTI obviously suggests that reducing protein farnesylation is beneficial. One possibility is that these drugs reduce severity of disease by lowering steady-state levels of progerin (112). However, at this point, no one has proven that the benefits of FTI treatment are a direct consequence of their effects on progerin and prelamin A processing. Indeed, given the incomplete blockade of lamin A biogenesis in FTI-treated *Lmna*^{HG/+} mice, one might argue that the beneficial effects of the FTIs on bones and body weight could be indirect, perhaps an unexpected physiologic consequence of inhibiting the farnesylation of some other FTase substrate. In the future, it

ought to be possible to address this issue. One approach would be to test the effects of an FTI in *Lmna*^{HG/+} mice, which express nonfarnesylated progerin. If the FTI had absolutely no effect on disease phenotypes in *Lmna*^{HG/+} mice, one could probably conclude that the beneficial effects of the FTIs in *Lmna*^{HG/+} mice were due to effects on the farnesylation of progerin. On the other hand, if FTIs significantly improved disease phenotypes both in *Lmna*^{HG/+} and *Lmna*^{HG/+} mice, it would suggest that the mechanism is indirect, independent of progerin prenylation.

What do the *Lmna*^{HG/+} mice mean for the pathogenesis of disease in *Zmpste24*^{-/-} mice? *Zmpste24*^{-/-} mice have an accumulation of farnesyl-prelamin A and severe progeria-like disease phenotypes, but they do not have the 50-amino acid deletion that underlies disease in *Lmna*^{HG/+} and *Lmna*^{HG/+} mice. Is prelamin A's farnesyl lipid anchor the culprit in *Zmpste24*^{-/-} mice? Or is it the presence of the extra 15 amino acids at the carboxyl terminus of prelamin A (the segment that is absent from mature lamin A)? Would a nonfarnesylated prelamin A also be toxic to cells (analogous to nonfarnesylated progerin)? The answers to these questions are not known, but could be addressed by creating a mouse that expresses a nonfarnesylated version of prelamin A (by changing the cysteine within the carboxyl terminus of prelamin A to a serine).

Mice Expressing a Geranylgeranylated Version of Progerin

A geranylgeranyl lipid is ~45-fold more potent than a farnesyl lipid in anchoring proteins to lipid membranes (94). Because the targeting of progerin to the nuclear rim is often assumed to be crucial in the pathogenesis of progeria, and because a geranylgeranyl lipid is a much stronger membrane anchor than a farnesyl lipid, Davies et al. (28) hypothesized that a geranylgeranylated version of progerin might cause far more severe disease than a farnesylated progerin. To explore this issue, Davies et al. (28) used gene targeting to change the carboxyl-terminal amino acid of progerin to a leucine, thereby generating mice expressing a geranylgeranylated version of progerin (*Lmna*^{ggHG/+}). Aside from this single amino acid substitution within the *CaaX* motif, these mice were identical to *Lmna*^{HG/+} mice. The fact that the progerin in *Lmna*^{ggHG/+} fibroblasts was geranylgeranylated and the progerin in *Lmna*^{HG/+} fibroblasts was farnesylated was documented with metabolic labeling experiments. Contrary to a priori expectations, the *Lmna*^{ggHG/+} mice actually survived longer than *Lmna*^{HG/+} mice, and they also had fewer of the characteristic rib fractures (28). Thus, a stronger lipid anchor at the carboxyl terminus of progerin did not worsen disease phenotypes. Interestingly, the steady-state levels of progerin, relative to lamin C, were somewhat lower in the tissues of *Lmna*^{ggHG/+} mice (28), providing a possible explanation for the milder disease phenotypes.

Alternative Strategies for Inhibiting the Prenylation of Progerin

The nitrogen-containing bisphosphonates, commonly prescribed drugs for osteoporosis in humans (43,81), block farnesyl diphosphate synthase, an enzyme that produces farnesyl diphosphate (23,35,77,83,84). Farnesyl diphosphate is a cosubstrate for FTase and therefore is required for protein farnesylation, but it is also required for the formation of geranylgeranyldiphosphate, which in turn is essential for protein geranylgeranylation and sterol synthesis. Because of their ability to block farnesyl diphosphate formation, and because bone abnormalities are prominent features of progeria, Toth et al. (98) reasoned that bisphosphonates might conceivably be useful for treating progeria. To explore this idea, they tested the ability of a bisphosphonate, alendronate, to inhibit protein prenylation and block the processing of prelamin A. They found that alendronate, very much like an FTI, blocked lamin A biogenesis and resulted in the accumulation of prelamin A—both in wild-type and HGPS fibroblasts. The drug also ameliorated nuclear shape abnormalities in human ZMPSTE24-deficient fibroblasts

(63). These findings led Toth et al. (98) to suggest that the drug potentially might be useful for the treatment of progeria. However, the studies by Toth et al. (98) were exclusively cell culture studies; as far as we are aware, no one has yet examined the effects of bisphosphonates on prelamin A processing in the bones of mice (or any other tissue).

Bisphosphonates are taken up by osteoclasts (cells that resorb bone during remodeling) and are thought to be useful for osteoporosis because they interfere with bone resorption by those cells (43,85). The extent to which bisphosphonates directly perturb the function of osteoblasts is not clear. The cell type underlying the osteoporosis and osteolytic lesions in progeria requires much more investigation, but the available data point against overactive bone resorption by osteoclasts (42,43). At this point, it seems more likely that the bone disease in progeria is due to defective osteoblasts. If the bone disease in progeria is due to dysfunctional osteoblasts, and if bisphosphonates achieve high concentrations in those cells, bisphosphonates might be useful in reducing prelamin A prenylation and improving osteoblast function. However, even if bisphosphonates were inactive in blocking protein prenylation in osteoblasts, the drugs might still be useful in progeria (as they are for run-of-the-mill osteoporosis), simply by interfering with bone resorption by osteoclasts. Clinical trials would be required to assess this issue.

Alternative Prenylation and Progeria

As noted earlier, treatment with a highly specific FTI, ABT-100, ameliorated disease phenotypes in *Lmna*^{HG/+} and *Zmpste24*^{-/-} mice, but the treatment fell far short of a cure. At least in part, the less-than-complete therapeutic response is probably due to the intrinsic toxicity of nonfarnesylated progerin (112). However, another potential explanation for the less-than-complete response to FTIs would be alternate prenylation of lamins by GGTase-I. Some *CaaX* proteins that terminate with a methionine and which are normally farnesylated, such as K-Ras, are substrates for GGTase-I when farnesylation is blocked with an FTI (108). The nuclear lamins also terminate with a methionine; thus, it is plausible that the lamin proteins might be geranylgeranylated when FTase activity is inhibited (87). This type of alternate prenylation might conceivably blunt any beneficial affects of FTIs.

In the absence of an FTI, prelamin A clearly undergoes farnesylation (95). Consistent with that result, Coffinier et al. (20,21) found that an FTI led to an accumulation of nonfarnesylated prelamin A in cells, while an inhibitor of GGTase did not. Toth et al. (98) found that the electrophoretic migration of the prelamin A was slightly retarded in FTI-treated ZMPSTE24-deficient cells, suggesting that little alternate prenylation occurred in the setting of an FTI. Similarly, Capell et al. (10) found that the electrophoretic migration of progerin was retarded in FTI-treated cells, again suggesting that progerin was not alternately prenylated. More recently, Varela et al. (101) examined the effects of an FTI and a GGTase-I inhibitor (GGTI) on prelamin A processing in HeLa cells. Surprisingly, the FTI that they tested led to very little accumulation of prelamin A. However, when a GGTI was added to the FTI, significant amounts of prelamin A accumulation were observed. They interpreted these studies as showing evidence for alternate prenylation (101), although it was unclear whether their GGTI was specific for GGTase-I or perhaps inhibited another enzyme in prelamin A processing, for example ZMPSTE24 or FTase.

Varela et al. (101) also used mass spectrometry to look for alternate prenylation in FTI-treated fibroblasts. In the absence of an FTI, they found that the carboxyl termini of prelamin A in *Zmpste24*^{-/-} cells and progerin in HGPS cells were farnesylated. However, in the presence of an FTI, a new peak emerged, corresponding in size to a carboxyl-terminal peptide containing a geranylgeranyl lipid. These studies suggested that geranylgeranylation may indeed occur in cultured cells. In the future, it would be desirable to verify the geranylgeranylation of prelamin A with a direct biochemical approach (108) or with metabolic labeling studies (28).

The mass spectrometry findings in FTI-treated *Zmpste24*^{-/-} and HGPS fibroblasts led Varela et al. (101) to consider the possibility that *Zmpste24*^{-/-} mice might be improved with a combination of statins (HMG-CoA reductase inhibitors) and bisphosphonates. They reasoned that this combination might be particularly useful since it might block both farnesylation by FTase and alternate prenylation by protein GGTase-I (101). In cultured cells, they found that a combination of 1 μ M zoledronate (a potent bisphosphonate) and 1 μ M pravastatin (a commonly used statin) blocked prelamin A farnesylation and geranylgeranylation in *Zmpste24*^{-/-} cells, and also improved nuclear shape. Next, they treated cohorts of *Zmpste24*^{-/-} mice with zoledronate (100 μ g/kg/d) and pravastatin (100 mg/kg/d). The drug combination reduced rib fractures and significantly extended the survival of *Zmpste24*^{-/-} mice (101), very similar to the results obtained by Fong et al. (44) with an FTI. In contrast to the FTI experiments, however, the authors did not test whether the bisphosphonate/statin combination inhibited the geranylgeranylation or farnesylation of prelamin A or any other protein in vivo (101). Interestingly, the authors found that zoledronate alone or pravastatin alone had no effect on the survival of *Zmpste24*^{-/-} mice, although the number of mice in these experiments ($n = 5$) made it very difficult to be confident about those conclusions. Once again, no studies of protein prenylation were performed in the latter animals. Thus, while the studies by Varela et al. (101) were intriguing and pointed out potential complexities in prelamin A processing, key questions remain unanswered, for example: Is there evidence of prelamin A geranylgeranylation in the tissues of FTI-treated mice? Was there any effect of the bisphosphonate/statin combination on protein farnesylation in vivo? Was there any effect on protein geranylgeranylation? Was lamin A biogenesis affected in any cell type or tissue? Did the drug combination reverse pathology in the cell types most affected by progeria, or were all of the benefits due to the nonspecific poisoning of osteoclasts and the inhibition of bone resorption? Were the results obtained in *Zmpste24*^{-/-} mice on the bisphosphonate/statin combination any different from those described earlier with FTIs? Answers to these questions will undoubtedly emerge over the next few years.

Reversibility of Established Disease Phenotypes

In their FTI treatment studies, Fong et al. (44) and Yang et al. (114,116) initiated drug therapy in *Zmpste24*^{-/-} and *Lmna*^{HG/+} mice at 4–5 weeks of age, before disease phenotypes were obvious. Thus, these FTI experiments simply analyzed emergence of disease, not reversal of disease. In the ongoing trial, lonafarnib is being given to children with established disease. Are advanced disease phenotypes reversible? The answer is not yet known for humans, of course, but a recent transgenic mouse study suggests that progerin-induced pathology is at least potentially reversible. Sagelius et al. (88) generated transgenic mice in which human progerin and lamin A were expressed in epidermal keratinocytes in a tetracycline-dependent manner. Expression of the transgene led to hyperplasia in the interfollicular epidermis, along with dystrophic changes in the hair follicles (88,89). When doxycycline was added to the drinking water, human lamin A and progerin expression were silenced and the skin pathology largely disappeared (89).

This same issue was approached by Capell et al. (11). They examined the effects of an FTI on the smooth muscle cell phenotype in transgenic mice harboring a 164-kb BAC engineered to contain the G608G HGPS mutation (102). Starting an FTI in older mice appeared to ameliorate the smooth muscle cell phenotype after it was well established.

Alternative Approaches to Treat HGPS

Given the persistent disease in *Lmna*^{nHG/+} mice and the possibility that FTIs may not be a panacea for this disease, new therapeutic strategies are needed. Scaffidi and Misteli (90) reasoned that it might be possible to attack the root cause of HGPS—abnormal splicing of the

prelamin A transcript—with a morpholino antisense oligonucleotide directed against the alternative splice donor site in exon 11. When this oligonucleotide was transfected into HGPS fibroblasts, it reduced levels of the progerin transcript (as well as the levels of progerin protein). Moreover, the oligonucleotide largely rescued the abnormal nuclear shape phenotype in HGPS fibroblasts and normalized the expression of several genes found to be misregulated in HGPS.

An RNA interference approach to reduce progerin expression was explored by Huang et al. (55). They screened several short hairpin RNA (shRNA) molecules against the exon 11–exon 12 junction region of the progerin transcript and identified one that reduced progerin transcript and protein levels in HGPS cells by about 26%. Although the magnitude of the reduction was not dramatic, there was an improvement in cellular phenotypes (i.e., improved nuclear shape, increased cell proliferation).

The problem with targeting the splice donor site or the exon 11–exon 12 junction is that the selection of antisense or shRNA molecules is highly restricted. However, recent studies by Fong et al. (45) may have opened the door to a wider range of possible target sequences. As noted earlier, Fong et al. (45) found that *Lmna*^{LCO/LCO} mice are entirely healthy and that the nuclei of *Lmna*^{LCO/LCO} cells display only miniscule alterations in nuclear shape (45,62). The finding that mature lamin A and prelamin A are apparently dispensable raised the possibility that one might be able to treat HGPS effectively (and with impunity) with antisense strategies designed to target all prelamin A transcripts (both prelamin A and progerin transcripts). To explore this possibility, Fong et al. (45) screened a series of oligonucleotides and identified one that effectively reduced prelamin A transcripts and lamin A protein levels in wild-type fibroblasts. When transfected into *Zmpste24*^{−/−} fibroblasts, prelamin A levels fell, and there was an improvement in nuclear morphology (45).

None of the antisense or shRNA approaches have yet been tested in mouse models of progeria, so it is not yet known whether the cell culture studies will translate into efficacious treatments. However, we believe that these approaches are attractive and deserve further study.

Molecular Mechanisms Underlying Disease Phenotypes

No single mechanism for progeria has been identified, but most studies point to an impaired ability to maintain the genome (i.e., accumulation of DNA damage, altered gene expression, defective repair of DNA) (50,61,86). The challenge is to identify the primary pathogenic event (s) from the myriad of metabolic changes that are likely to be perturbed as a result of the disease. Some progress has been made. Liu et al. (64) showed that *Zmpste24*^{−/−} fibroblasts are more sensitive to DNA-damaging agents and proposed that defective DNA repair promotes genome instability and early senescence. Interestingly, FTI treatment of HGPS fibroblasts did not reduce DNA double-strand breaks and damage checkpoint signaling, although the treatment did reduce numbers of misshapen cell nuclei (65). Varela et al. (100) performed microarray analyses and identified a possible link between p53 activation and *Zmpste24* deficiency. The disease phenotypes of ZMPSTE24 deficiency appeared to be partially ameliorated by homozygous loss of *p53*, a result that is consistent with the notion that p53 is activated in aging (99). Shumaker et al. (93) showed that HGPS alters histone methylation patterns, providing evidence that the lamin network interacts in some fashion with chromatin to modulate heterochromatin in cells (and presumably gene transcription). These changes preceded the appearance of misshapen cell nuclei. Dahl et al. (25) examined the mechanical properties of HGPS cell nuclei. They expected that the nuclei would be fragile, but found that they actually had normal or increased rigidity. Verstraeten et al. (103) found that fibroblasts from HGPS patients developed progressively stiffer nuclei with increasing passage number. They also found that these cells had decreased viability under repetitive mechanical strain, as well as attenuated wound healing. Treating fibroblasts with an FTI accelerated wound healing and

restored nuclear stiffness, but the FTI did not improve cellular sensitivity to mechanical strain. Cao et al. (9) found that progerin mislocalizes during mitosis and that this leads to abnormalities in chromosome segregation and binucleation. They also found that this phenotype was at least partially rescued with an FTI. Delbarre et al. (33) found that lamin A and lamin B1 polymerize as homopolymers, but progerin polymerizes with both lamin A and lamin B1, resulting in mixed heteropolymers. It is unclear exactly why this happens, but their study provides a possible mechanism of progerin toxicity. They did not determine whether prenylation of progerin was necessary for altered polymerization.

Over the past few years, several groups have performed microarray profiling of HGPS fibroblasts (24,67,79,105). Csoka and coworkers (24) examined three different HGPS fibroblast cell lines and identified 361 genes that were differentially expressed, including extracellular matrix proteins and many transcription factors. The most affected gene in their studies, *MEOX2/GAX*, is a transcription factor that regulates mesodermal tissue proliferation. More recently, Wang et al. (105) identified 66 genes that were differentially expressed in HGPS fibroblasts, including many known to be associated with cell growth and morphology. The overexpression of *ANK3* in HGPS cells was verified by quantitative PCR, western blotting, and immunofluorescence microscopy. Several gene perturbations identified in the microarray studies have been confirmed (90) but very few have been verified in multiple laboratories or validated in mouse models where genetic background issues are better defined. It would be very desirable to identify a marker of HGPS that is so robust that it overshadows other differences, such as those due to passage number, gender, genetic background, or the site of the skin biopsy.

A Broader Relevance of Prelamin A Processing

Most of the recent work on prelamin A has centered on progeroid disorders in humans, or on mouse models of those diseases. Relatively little work has been performed on how metabolic or pharmacologic factors might affect prelamin A processing. During the past year, however, Coffinier et al. (20,21) showed that HIV protease inhibitor drugs, for example lopinavir, inhibit ZMPSTE24 and lead to an accumulation of farnesyl-prelamin A. The finding that HIV-protease inhibitors lead to the accumulation of prelamin A was initially noted by Caron et al. (13), who were intrigued by the similarity between the lipodystrophy syndromes occurring in HIV-treated patients and patients harboring lamin A/C mutations. Using a western blotting approach, Caron et al. (13) found trace levels of prelamin A in differentiated 3T3-F442A preadipocytes that had been treated with indinavir or nelfinavir (HIV protease inhibitors). More recently, the same group found prelamin A in the adipose tissue of patients treated with the same drugs (12). However, the extent of prelamin A accumulation was not clear from those early studies, nor was the biochemical explanation for these findings. In 2007, Coffinier et al. (20,21) showed that therapeutic concentrations of lopinavir led to a large amount of prelamin A accumulation in cultured fibroblasts. In fact, the levels of prelamin A accumulation in lopinavir-treated cells were almost as great as those observed with an FTI. The prelamin A in HIV-PI-treated cells was shown to be farnesylated (21), resulting in a more rapid electrophoretic mobility than the nonfarnesylated prelamin A in FTI-treated cells (20,21). Biochemical assays of ZMPSTE24 enzymatic activity revealed that the HIV-PIs inhibited ZMPSTE24 in mammalian cell membranes (20,21). Interestingly, these drugs also inhibited the enzymatic activity of purified preparations of Ste24p, the yeast orthologue of ZMPSTE24 (56). HIV-PIs had no effect on any of the other enzymes involved in the processing of farnesylated proteins, for example FTase, RCE1, or ICMT (20,21). The amount of ZMPSTE24 inhibition varied among different HIV-PIs. A new and very potent HIV-PI, darunavir, had no effect on ZMPSTE24 activity and did not lead to prelamin A accumulation in cultured cells (21).

These observations with the HIV-PIs were intriguing, given that the farnesylated form of prelamin A is toxic (6,80) and capable of eliciting disease phenotypes (71,75,92). However, at this point, it is unclear whether the level of prelamin A accumulation that occurs with HIV-PI treated patients is sufficient to explain any of the side effects of these drugs.

SUMMARY OF MAIN POINTS

1. Prelamin A, one of the gene products of *LMNA*, contains a *CaaX* motif and undergoes the typical *CaaX* modifications: prenylation, endoproteolytic cleavage, and methylation. It also undergoes an additional endoproteolytic cleavage that removes the final 15 amino acids including the modified C-terminus
2. Mutations that disrupt prelamin A processing underlie severe human genetic diseases, including restrictive dermopathy and Hutchinson-Gilford progeria syndrome.
3. Mouse models have been created for both restrictive dermopathy and Hutchinson-Gilford Progeria Syndrome and these mice display many, but not all, of the disease phenotypes observed in the human diseases
4. Treatment with an FTI ameliorates disease phenotypes in mouse models, and reduces the frequency of misshapen nuclei in cultured fibroblasts. However, FTI treatment falls short of a cure
5. Mice that produce a nonfarnesylated version of progerin have milder disease phenotypes, but still have severe disease, suggesting that blocking progerin farnesylation with an may help, but may not completely cure the disease.
6. Approaches that target progerin or prelamin A expression levels (rather than prenylation of these molecules) have been tested with some success in cultured cells, but have not been tested in mice.
7. HIV protease inhibitors inhibit ZMPSTE24 and disrupt prelamin A processing in cells. The *in vivo* consequences of this inhibition is not yet known.

FUTURE ISSUES

1. Are the beneficial effects of FTIs (or statins/bisphosphonates) a direct result of blocking prelamin A prenylation, or do they act indirectly by affecting the prenylation of some other protein substrate?
2. Is the farnesyl lipid anchor of progerin or prelamin A intrinsically toxic, or does it simply render these molecules more stable, thus increasing toxicity.
3. Will the treatments studied in mice (FTIs, a statin/bisphosphonate combination) be effective in humans?
4. Will other approaches to treating progeria (shRNA, antisense oligonucleotides) be effective in mouse models, and eventually in humans?
5. Are perturbations in prelamin A processing from HIV protease inhibitors responsible for any side effects of those drugs?

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ACRONYMS/ABBREVIATIONS

BAC	bacterial artificial chromosome
ER	endoplasmic reticulum
FTase	protein farnesyltransferase
FTI	protein farnesyltransferase inhibitor
GGTase-I	protein geranylgeranyltransferase, type I
GGTI	protein geranylgeranyltransferase type I inhibitor
HGPS	Hutchinson-Gilford progeria syndrome
HIV	human immunodeficiency virus
HIV-PI	HIV protease inhibitor
RD	restrictive dermopathy

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Figure 1.

Biogenesis of lamin A and the failure to generate mature lamin A in the setting of ZMPSTE24 deficiency (restrictive dermopathy) and HGPS. Prelamin A (664 amino acids) normally undergoes four posttranslational processing steps (left panel). 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 carboxyl-terminal 15 amino acids, including the farnesylcysteine methyl ester, are clipped off by ZMPSTE24 and degraded, releasing mature lamin A (646 amino acids). In the setting of ZMPSTE24 deficiency (middle panel), the last endoproteolytic processing step does not occur, resulting in the accumulation of the farnesylated form of prelamins A. In the setting of HGPS (right panel), a point mutation results in a 50-amino acid deletion in prelamins A (amino acids 607–656), which removes the site for the second endoproteolytic cleavage. Thus, the farnesylated mutant prelamins A (progerin) accumulates in cells, and no mature lamin A is formed. Modified, with permission, from the *Journal of Lipid Research* (117).

Figure 2.

Phenotypic comparison of *Lmna*^{nHG/+} mice and FTI-treated *Lmna*^{HG/+} mice. When compared to *Lmna*^{+/+} and *Lmna*^{HG/+} mice, FTI-treated *Lmna*^{HG/+} mice (left, green) and *Lmna*^{nHG/+} mice (right, red) have very similar body weight curves (a), survival patterns (b), number of rib fractures (c), and body fat (d). Modified, with permission, from *The Journal of Clinical Investigation* (112,114) and *Biochimica et Biophysica Acta* (116).