1-1-2006

Farnesylated Lamins, Progeroid Syndromes and Farnesyl Transferase Inhibitors

Michael Sinensky
San Jose State University, michael.sinensky@sjsu.edu

A. E. Rusinol
East Tennessee State University

Recommended Citation

Michael Sinensky and A. E. Rusinol. "Farnesylated Lamins, Progeroid Syndromes and Farnesyl Transferase Inhibitors" *Journal of Cell Science* (2006): 3265-3272. https://doi.org/10.1242/jcs.03156

Farnesylated lamins, progeroid syndromes and farnesyl transferase inhibitors

Antonio E. Rusiñol and Michael S. Sinensky*

Department of Biochemistry and Molecular Biology, Box 70581, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37164-0581, USA

*Author for correspondence (e-mail: sinensky@etsu.edu)

Accepted 4 July 2006
Journal of Cell Science 119, 3265-3272 Published by The Company of Biologists 2006
doi:10.1242/jcs.03156

Summary

Three mammalian nuclear lamin proteins, lamin B_1, lamin B_2 and the lamin A precursor, prelamin A, undergo canonical farnesylation and processing at CAAX motifs. In the case of prelamin A, there is an additional farnesylation-dependent endoproteolysis, which is defective in two congenital diseases: Hutchinson-Gilford progeria (HGPS) and restrictive dermopathy (RD). These two diseases arise respectively from defects in the prelamin A substrate and the enzyme (ZmpSte24) that processes it. Recent work has shed light on the roles of the lamin proteins and the enzymes involved in their farnesylation-dependent maturation. Other experimental work, including mouse model studies, have examined the possibility that farnesyl transferase inhibitors can represent effective treatment for HGPS. However, there are concerns about their use for this purpose given the potential for alternative prenylation pathways.

Key words: Farnesylation, Lamins, FTIs, HGPS

The lamina, lamins and laminopathies

The lamina is a filamentous protein structure that is proximal to the inner nuclear membrane in multicellular eukaryotes. It is composed of lamin proteins, which can also be found in the nuclear interior, and lamin-associated proteins (Gruenbaum et al., 2003). There are two classes of lamin proteins: A-type and B-type, which are distinguished by whether they remain associated with membrane vesicles (B-type) or not (A-type) during mitosis. In mammalian cells there are two common B-type lamins, lamin B_1 and lamin B_2, which are encoded by two different genes, and two common A-type lamins, lamin A and lamin C, which are different mRNA splicing products of the same lamin A/C gene. Broadly speaking, the karyoskeleton formed by the lamin proteins serves to organize protein complexes within the nucleus and their interactions with chromatin, as well as providing structural support for the nucleus (Gruenbaum et al., 2005; Taddei et al., 2004). In doing so, the lamins are involved in a range of nuclear functions, including regulation of gene expression and DNA replication, although the molecular details of these functions are still to be elucidated.

Mutations in lamin A produce a range of diseases that have collectively been referred to as ‘laminopathies’ (Jacob and Garg, 2005). Two progeroid (premature aging) syndromes, Hutchinson-Gilford progeria (HGPS) (Cao and Hegele, 2003; Eriksson et al., 2003) and restrictive dermopathy (RD) (Moulson et al., 2005; Navarro et al., 2005; Navarro et al., 2004), are laminopathies that arise through defects in maturation of the lamin A precursor, prelamin A. Mandibuloacral dysplasia (MAD) (Agarwal et al., 2003), which can be considered a milder form of RD, also results in accumulation of the lamin A precursor. By contrast, the majority of laminopathies are due to point mutations in the A/C lamins; these exhibit a multitude of phenotypes depending on the site of the mutation (Gruenbaum et al., 2003).

CAAX-boxes, prenylation and the lamin proteins

The primary translation product of the lamin A mRNA, prelamin A, bears a C-terminal CA_1A_2X motif (Fig. 1) that directs farnesylation of the cysteine residue (Zhang and Casey, 1996) – A_1 and A_2 are generally aliphatic amino acid residues. As described in more detail below, direct chemical methods have confirmed farnesylation of prelamin A (Lutz et al., 1992; Sinensky et al., 1994), as well as lamin B_1 (Farnsworth et al., 1989), which also terminates in a CAAX motif.

These studies were among the first to demonstrate farnesylation of mammalian proteins. Post-translational farnesylation had previously been shown for fungal mating pheromones (Kamiya et al., 1980; Miyakawa et al., 1985), and the first direct evidence of the relationship between the CAAX motif and farnesylation was obtained by NMR (Anderegg et al., 1988) of the *Saccharomyces* a-factor. Lamin prenylation was first suggested by metabolic labeling with mevalonate (mevalonate labeling) and analysis of nuclear proteins by 1- and 2-dimensional gel electrophoresis (Beck et al., 1988; Beck et al., 1990; Wolda and Glomset, 1988). Structural verification of farnesylation of the lamin proteins was facilitated by earlier work on fungi, which implicated the thioether linkage of a polyisoprenoid to CAAX-box cysteines as the probable structure. This led investigations to use reductive chemical cleavage of the thioether linkage with Raney nickel to liberate the polyisoprenoid, which could be characterized by GLC-mass-spectrometry. This approach was first applied to lamin B_1 (Farnsworth et al., 1989). Lamin B_1 was an excellent first choice for structural determination of the polyisoprenoid because it is among the most abundant of the prenylated cellular proteins.

CAAX boxes and poly-isoprenyl transferases

The mechanisms by which CAAX boxes direct farnesylation are relevant to an understanding of laminopathies. CAAX motifs favor farnesylation, by farnesyltransferase (FTase), when X is a Ser, Gln, Met or Ala residue but geranylgeranylgeranylation, by geranylgeranyltransferase I (GGTase-I), when X is a Leu residue (Zhang and Casey, 1996). X-ray crystallography (Reid et al., 2004) has recently provided a mechanistic basis for the observed specificity of amino acid residues at the A_2 position and their contribution to the overall reactivity of the CAAX-box substrates. These studies indicate that the A_2 position can influence substrate selectivity by FTase and GGTase-I. Therefore, in the case of the lamin proteins, it is noteworthy that Ile or Val at the A_2 position is equally accepted by FTase and GGTase-I. The human lamin CAAX boxes are CSIM for prelamin A, CAIM for mammalian lamin B_1 and CYVM for lamin B_2. The preference for lamin farnesylation, over geranylgeranylation, thus derives entirely from the C-terminal Met residue. Sequences upstream of the CAAX box, as well as the A_1 residue, have the potential to alter the K_m for the protein substrate but are not mechanistic determinants of the specificity of prenylation. There are examples in which CAAX-motif prenylation occurs when the A_1 residue is not aliphatic (e.g. Gln in the case of Rap-1B and Tyr in the case of lamin B_2).

All of the above considerations strongly predict that the lamin proteins, including the mutant lamin A proteins found in the various laminopathies, as well as HGPS, should be farnesylated, and further processed at the lamin A CSIM motif. As shown in Fig. 1, CAAX-box proteins undergo endopeptidase cleavage of the – AAX residues (step B) after farnesylation followed by carboxymethylation (step C) (Zhang and Casey, 1996). The B endopeptidase activity in step B can be shown for two enzymes: Rce1 (Ras-converting enzyme 1) (Boyartchuk et al., 1997) and Zmpste24 (Zinc metalloprotease related to Ste24p) (Corrigan et al., 2005; Leung et al., 2001). The carboxymethylation is catalyzed by the enzyme isoprenylcysteine carboxyl methyltransferase (Icmt) (Winter-Vann and Casey, 2005).

Biochemical studies on the endoproteolytic maturation of prelamin A

The initial characterization of the processing pathway of prelamin A presented some challenges. Early work by Gerace and co-workers (Gerace et al., 1984) had demonstrated the likelihood of a higher-molecular-weight precursor for lamin A. Later protein-sequencing studies by Weber on the mature lamin A molecule (Weber et al., 1989), coupled with comparison to the prelamin A sequence predicted from its cDNA, demonstrated that lamin A undergoes the loss of 18 amino acid residues from the C-terminus during the formation of mature lamin A, which terminates in a Tyr residue. Metabolic radiolabeling and pulse-chase studies with mevalonate demonstrated likely prenylation of prelamin A and a requirement for the putative prenylation for further maturation of the protein (Beck et al., 1990).

The transient nature of prelamin A does not allow accumulation of sufficient material for one to characterize the putative isoprenoid by mass-spectrometry. Radiolabeling of the polyisoprenoid with mevalonate is also difficult because of the poor uptake of mevalonate by most cells. The problem of obtaining enough radio-labeled material to characterize, was eventually solved by the use of inducible prelamin A constructs expressed in a cell line with an activated mevalonate transporter (Faust and Krieger, 1987). Characterization of the prelamin A poly-isoprenoid substituent as farnesyl could then

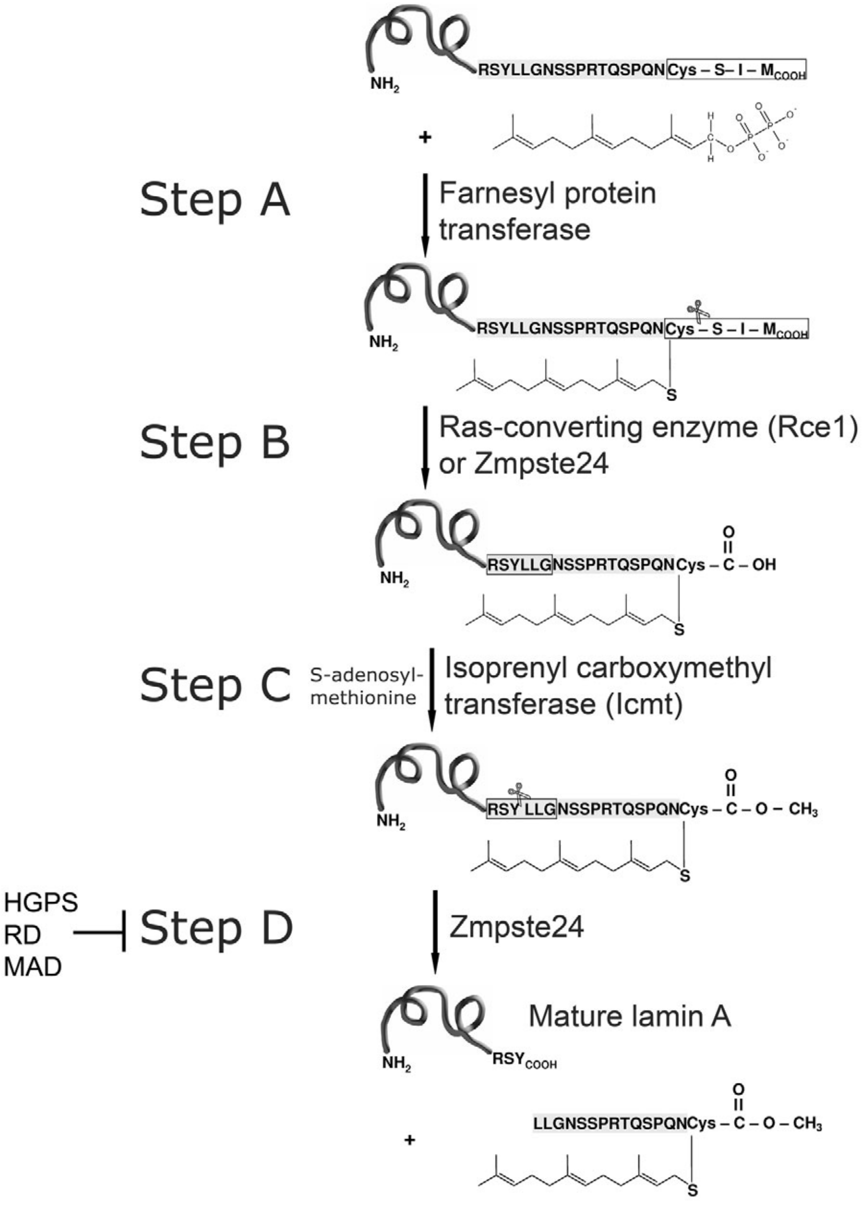

Fig. 1. Maturation pathway of prelamin A. Steps A-C are common to all CAAX proteins. Notice that there are two endoproteolytic cleavages for prelamin A – Step A and Step D.

proceed through the same Raney-nickel cleavage method that had been successfully employed for lamin B_1 but utilizing co-chromatography on GLC with synthetic standards and in-line radiodetection to identify the product as being derived from farnesyl thioether.

The inducible prelamin A constructs were utilized to verify carboxymethylation of prelamin A (Sinensky et al., 1994). Since carboxymethylation can only occur after endoproteolysis, this was consistent with prelamin processing proceeding through the canonical CAAX-box processing pathway (Fig. 1). In vitro studies with recombinant enzymes directly demonstrate that both Rce 1 or Zmpste24 can use prelamin A as a substrate in step B (Corrigan et al., 2005).

In vitro studies with a model polypeptide, as well as studies on CAAX-box mutants expressed in cells (Kilic et al., 1997), showed that a farnesylated, carboxymethylated prelamin A is the required substrate for a final endoproteolytic cleavage after Tyr646 (step D in Fig. 1) by an enzyme that recognizes a conserved hexapeptide cleavage motif RSY↓LLG, and gives rise to mature lamin A. No other farnesylated mammalian protein is known to undergo a second, upstream endoproteolysis, and the highly conserved cleavage sequence is unique to prelamin A in vertebrate protein sequence databases, even allowing for conserved substitutions (Kilic et al., 1997). Thus, the second endoproteolysis currently appears to be unique to prelamin A processing.

In vitro studies with recombinant Zmpste24 and a recombinant fragment of prelamin A demonstrated that Zmpste24 has step D endoprotease activity (Corrigan et al., 2005). In vivo studies (data not shown) indicate that Zmpste24 is not essential for the first endoproteolytic cleavage, which can also be performed by Rce1, but is essential for the second (our unpublished data).

Genetic studies on farnesylation-dependent lamin maturation

Knockout studies, in fact, first suggested that Zmpste24 is the step D endoprotease. As mentioned above, farnesylation was first studied in fungal pheromones, particularly *Saccharomyces* a-factor. This polypeptide, like prelamin A, undergoes N-terminal endoproteolysis during maturation, which is mediated by two enzymes: Ste24p and Ste23p. Discovery of a mammalian orthologue of Ste24p, Zmpste24, suggested that Zmpste24 plays a similar role in prenylation-dependent post-translational processing in mammalian cells (Young et al., 2005). Zmpste24-knockout mice (Bergo et al., 2002; Pendas et al., 2002) accumulate prelamin A, and the later biochemical studies (Corrigan et al., 2005) showed that Zmpste24 can mediate both of the endoproteolytic steps in prelamin A maturation.

In mouse embryo fibroblasts lacking Icmt or Rce1 (but not those lacking Zmpste24) a fluorescent construct expressing the 40 C-terminal residues of lamin B_1 is mislocalized and the nuclear lamina has structural defects (Maske et al., 2003). The apparent lack of activity of Zmpste24 in B-lamin processing is surprising because the yeast orthologues appear to have overlapping substrate specificities (Trueblood et al., 2000). Notice, however, that the Rce1 and Icmt knockouts will have defective processing of numerous farnesylated and geranylgeranylated proteins, which could disrupt lamina structure indirectly.

Prenylated proteins, membranes and farnesylated lamins

Biophysical studies using model farnesylated and carboxymethylated peptides indicate that farnesylated proteins should be predominantly, but not entirely, membrane associated (Silvius and l'Heureux, 1994). The hydrophobic contribution of carboxymethylation is also significant and a substantial fraction of a protein modified only by a farnesyl group would not be expected to be associated with membranes. Even with both farnesylation and methylation, the dissociation constants of peptides from lipid bilayers can vary from 10 μM to several hundred micromolar, depending on the bilayer composition. Thus, CAAX-box-mediated association with membrane bilayers is inherently reversible. Stable membrane association of a farnesylated protein (e.g. Ras) requires a second lipid-bilayer-binding moiety (Hancock et al., 1990; Schroeder et al., 1997; Shahinian and Silvius, 1995). For Ras proteins, this is either palmitoylation (N-Ras and H-Ras) or a polybasic domain (K-Ras). Both carboxymethylation and palmitoylation are chemically reversible modifications, making it theoretically feasible to dissociate even doubly modified proteins from membranes. In the case of Ras proteins, both palmitoylation status and subcellular localization are highly dynamic (Magee and Seabra, 2005).

This reversibility of membrane association of farnesylated proteins is consistent with the hypothesis that CAAX modifications, in addition to targeting proteins to membranes, also facilitate protein-protein interactions. Indeed, there is considerable evidence that this is the case (Basso et al., 2006; Sinensky, 2000; Zhang and Casey, 1996).

Prelamin A mutants that cannot be processed at step D by Zmpste24 (Corrigan et al., 2005; Hennekes and Nigg, 1994; Mallampalli et al., 2005) are localized to the nuclear membrane. Since these mutants are expected to be farnesylated and carboxymethylated, these observations support a role for these CAAX modifications of prelamin A in nuclear membrane targeting (Nigg et al., 1992). Similar observations exist for B-lamins. Mutations of the lamin B_1 (Mical and Monteiro, 1998) or lamin B_2 (Kitten and Nigg, 1991) CAAX box that block prenylation, also block incorporation of the lamin B proteins into the nuclear envelope, leading to their accumulation in the nucleoplasm. Lamin proteins do not have secondary lipid-bilayer-binding regions analogous to those of the Ras proteins. Membrane association of prenylated lamin proteins might therefore be stabilized by their binding to a membrane receptor, and a body of evidence supports binding of lamin B_1 to a lamin B receptor (Smith and Blobel, 1994; Worman et al., 1990; Worman et al., 1988). The binding site appears to map to the C-terminus of the B-lamins (Dreger et al., 2002; Maske et al., 2003).

Since the CAAX-motif modifications of the B-lamins are typical of CAAX proteins, their role as mediators of nuclear envelope association has a strong rationale. By contrast, the functional significance of prelamin A processing, in which the farnesylated and carboxymethylated C-terminal peptide is ultimately removed, is unknown and intriguing. It is clearly not required for assembly of lamin A into the lamina – this has been shown by expression studies of the mature protein (Lutz et al., 1992) and, of course, mature lamin A is disassembled and reassembled into the lamina routinely during the course of mitosis (Gerace et al., 1984). However, mutations or reagents that block farnesylation, thereby preventing removal of the C-terminal 18 residues of prelamin A, block its incorporation into the lamina (Holtz et al., 1989; Lutz et al., 1992; Sasseville and Raymond, 1995), which suggests that the sequence inhibits this.

One hypothesis is that farnesylated and carboxymethylated prelamin A has a distinct function and that the processing pathway regulates its levels. In this regard, yeast two-hybrid studies have revealed a specific binding partner of farnesylated prelamin A: Narf (Barton and Worman, 1999). The function of Narf is unknown. However, epitope-tagged Narf localizes to both the nuclear envelope and the nucleoplasm, which suggests it interacts with farnesylated and carboxymethylated prelamin A in both locations.

RD and HGPS: diseases of prelamin A maturation

In both RD and HGPS, there is strong circumstantial evidence that step D of the prelamin A maturation pathway is defective, and we have confirmed this chemically (unpublished data).

RD (OMIM: 275210) is a neonatally fatal autosomal recessive disease that arises from homozygous loss of expression of Zmpste24 (Levy et al., 2005; Moulson et al., 2005; Navarro et al., 2005; Navarro et al., 2004). It is characterized by very tight, thin easily eroded skin, rocker-bottom feet and joint contractures. In addition, prelamin A accumulates in cells from RD patients (Moulson et al., 2005; Navarro et al., 2005).

HGPS is a complex autosomal dominant disease (OMIM: 176660) that arises from a mutation in the prelamin A gene that leads to low-level expression of a prelamin A mRNA (Δ150 LMNA) that has a deletion of 150 bp of exon 11 arising from a variably used new splice site (Eriksson et al., 2003). Δ150 LMNA constitutes approximately 40% of the transcripts found in cells from HGPS patients (Reddel and Weiss, 2004). This mutant mRNA encodes a protein (known as LAΔ50 or progerin) that has an in-frame deletion of 50 amino acid residues of the prelamin A sequence. Progerin can readily be separated from wild-type prelamin A and lamin A on SDS-PAGE and visualized by anti-lamin-A antibody (Cadinanos et al., 2005; Pollex and Hegele, 2004). The predicted amino acid sequence of progerin (Eriksson et al., 2003) is missing the sequence necessary for step D, RSY↓LLG, which is consistent with the hypothesis that there is a block in maturation at this step (Fig. 2). Progerin, as is the case for artificially generated Step-D-resistant mutants, localizes to the nuclear envelope (Goldman et al., 2004).

Patients with HGPS exhibit physical features reminiscent of aging, including bone fragility, loss of hair and lipodystrophy (Pollex and Hegele, 2004). They also have extensive vascular problems, which lead to premature atherosclerosis and stroke, common causes of death (Gordon et al., 2005; Ha et al., 1993; McClintock et al., 2006). Cells from HGPS patients senesce prematurely in culture (Liu et al., 2005; Wallis et al., 2004). This appears to arise from DNA-repair defects, which results in accumulation of DNA double-strand breaks and p53 activation (Liu et al., 2005). Similar events are associated with senescence of cultured human cells and cells from aging mice (Sedelnikova et al., 2004). HGPS may thus, at least in some respects, reflect normal aging. Indeed, it has recently been reported (Scaffidi and Misteli, 2006) that progerin accumulates in tissues from aged individuals.

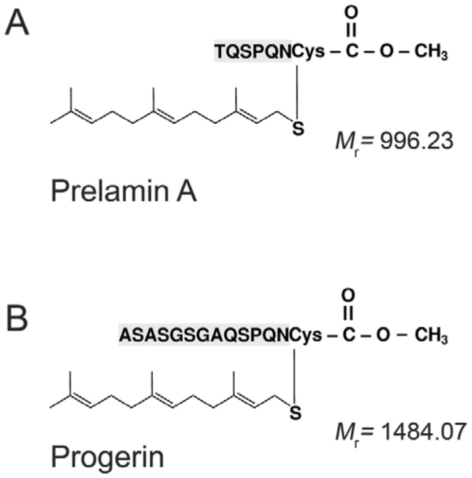

Fig. 2. (A,B) Expected C-terminal fragments of (A) wild-type prelamin A and (B) progerin after trypsin digestion. Relative molecular masses were calculated using average masses of the occurring residues and giving masses as [M+H]+.

Homozygous loss of Zmpste24 in RD should also result in the generation of a farnesylated and carboxymethylated prelamin A, Rce1 performing the first endoproteolysis. No wild-type lamin A is produced in this case (Toth et al., 2005). A compound-heterozygous loss-of-function of Zmpste24 (OMIM: 608612) causes mandibuloacral dysplasia characterized by 'skeletal abnormalities including hypoplasia of the mandible and clavicles, acro-osteolysis, cutaneous atrophy and lipodystrophy' (Agarwal et al., 2003). This condition might reflect incomplete loss of Zmpste24, because the neonate is viable. Mouse embryo fibroblasts from Zmpste24$^{-/-}$ mice show the same molecular and cellular characteristics of premature senescence similar to those seen in patients with HGPS (Liu et al., 2005; Varela et al., 2005). Farnesylated, carboxymethylated prelamin is associated with the nuclear membrane, including invaginations of this membrane into the nucleus (Holtz et al., 1989; Moulson et al., 2005; Toth et al., 2005). By contrast, non-prenylated CAAX-box cysteine mutants of prelamin A accumulate in nucleoplasmic aggregates (Capell et al., 2005; Holtz et al., 1989; Lutz et al., 1992), which are not membrane associated (Fig. 3).

Farnesyl transferase inhibitors, lamin maturation and HGPS

Shortly after lamin proteins were shown to be farnesylated, the mammalian Ras proteins were shown to be similarly modified (Casey et al., 1989; Leonard et al., 1990). A body of yeast genetic studies (Goodman et al., 1990; Powers et al., 1986; Schafer et al., 1990) had previously suggested that post-translational modification of Ras was required for its signaling and subsequent work showed Ras farnesylation is required for the transforming activity of oncogenic Ras mutants (Casey et al., 1989; Hancock et al., 1989; Schafer et al., 1989). This raised the possibility that inhibitors of farnesyl transferase (FTIs) could be used in the treatment of cancer. A number of such compounds were developed, and their current status has recently been reviewed (Basso et al., 2006). Surprisingly, studies from several laboratories revealed that although the

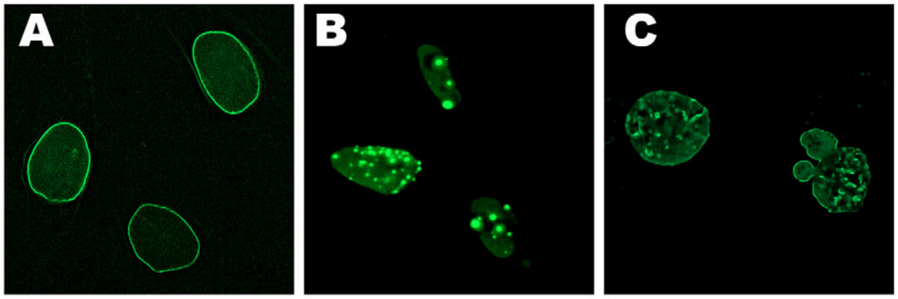

Fig. 3. (A-C) Subnuclear distribution of wild-type and mutant prelamin A. (A) Wild-type EGFP-prelamin A, (B) prenylation-incompetent mutant prelamin A (^{661}C→M) and, (C) step-D-incompetent prelamin-A–EGFP–progerin were transiently expressed in HeLa cells and imaged live (A,C) or after indirect immunofluorescence with anti-prelamin A antibodies (B). Digital images taken by light-microscopy and digital deconvolution are shown.

prenylation, maturation and function of H-Ras are inhibited by the FTIs, this is not the case for N-Ras and K-Ras (Fiordalisi et al., 2003; James et al., 1996; Rowell et al., 1997; Whyte et al., 1997). The explanation is that they become geranylgeranylated in the presence of FTIs. This is because of the kinetic basis of specificity towards CAAX substrates of FTase and GGTase1. In the case of K-Ras, there is an ~400-fold higher K_m for K-Ras for GGTase 1 compared with FTase (Roskoski, Jr and Ritchie, 1998; Zhang et al., 1997). However, the k_{cat} values for the two enzymes for K-Ras are virtually identical. Thus, at high cellular levels of non-prenylated K-Ras, as would be the case when farnesylation is blocked, geranylgeranylation by GGTase1 can occur (James et al., 1995). By contrast, GGTase1 exhibits not only a higher K_m for H-Ras than does FTase but the k_{cat} is >100 times lower, which accounts for the effectiveness of FTIs in blocking H-Ras prenylation. Signaling, including oncogenic signaling, by mutant K-Ras (Kato et al., 1992), and by implication N-Ras, can be supported by geranylgeranylation.

These observations should sound a cautionary note on how biological readouts can be overinterpreted in explanations of biochemical events. FTIs can, indeed, block the growth of numerous N-Ras- and K-Ras-transformed cells in soft agar, nude mice or transgenic mouse models (End et al., 2001; Liu et al., 1999; Nagasu et al., 1995; Omer et al., 2000). They just do not necessarily do it by affecting the prenylation of the Ras proteins. Other farnesylated proteins have been implicated in the antitumor activity of FTIs (Basso et al., 2006)

FTIs have also been proposed as a treatment for HGPS. RD is not a candidate for treatment since it is neonatally lethal. The rationale for such an approach is that the pathological phenotypes seen in HGPS arise from a farnesylated, mutant prelamin A and that inhibition of its farnesylation might reverse these phenotypes. Encouragingly, the blebs that have been observed in the nuclear membranes of cultured fibroblasts from patients with HGPS can be eliminated by treatment with FTIs (Capell et al., 2005; Glynn and Glover, 2005; Mallampalli et al., 2005; Toth et al., 2005). Although, at this time, no direct demonstration of the farnesylation of progerin has been reported, mevalonate labeling studies by one laboratory (Glynn and Glover, 2005) are certainly consistent with this prediction. The loss of mevalonate labeling of progerin upon treatment of HGPS cells with an FTI is also consistent with inhibition of farnesylation but can be misleading (see below).

FTI treatment has been shown to ameliorate the pathology exhibited by Zmpste24$^{-/-}$ mice (Fong et al., 2006) although the responses vary depending on the defect. For example, the effects of treatment on the mice are very dramatic in the case of reduction of rib fractures but less so in the case of growth (as measured by body weight). Although there are structural differences between progerin and the prelamin A molecule that accumumulates in the absence of Zmpste24, there are obviously shared structural features, especially the farnesylated and carboxymethylated C-terminal cysteine residue. Thus, these results are consistent with the concept that FTIs could be useful in the treatment of HGPS.

Examining the effects of the FTI BZA-5B, on prelamin A maturation (Dalton et al., 1995) in CHO-K1 cells expressing an activated mevalonate transporter, we observed specific inhibition of protein farnesylation. Considering one recent database search revealed ~70 potential farnesylated proteins (Fiordalisi et al., 2003), it was surprising that growth of CHO-K1 and HeLa cells is not inhibited by treatment with BZA-5B. Although prelamin A accumulates, its processing still occurs, as does assembly of lamin A and lamin B into the nuclear lamina. Farnesylation-dependent protein processing appears to occur even in the absence of detectable farnesylation. The lack of mevalonate labeling of N-Ras, lamin B_1 or prelamin A in these cells in the presence of BZA-5B was not consistent with processing by alternative prenylation. Other work, however, has suggested that mevalonate labeling of geranylgeranylated CAAX proteins can be difficult to detect (Kato et al., 1992; Vestal et al., 1996). Furthermore, in vitro studies on the prenylation of lamin A and lamin B CAAX polypeptides (Moores et al., 1991) demonstrate that both can be a substrate for GGTase 1, as do studies of in vitro translated prelamin A mRNA (our unpublished data). Expression of prelamin A containing a CVLL geranylgeranylation CAAX box in mammalian cells results in a geranylgeranylated protein that is processed to mature lamin A, albeit inefficiently, such that prelamin A accumulates (Kilic et al., 1997).

FTI treatment could result in inefficient processing of prelamin A at two steps. Since the K_m of GGTase 1 for the prelamin A CAAX of should be considerably higher than that of FTase, non-prenylated prelamin A should accumulate; since the K_m of Zmpste24 for the geranylgeranylated prelamin A is higher than for farnesylated prelamin A, the geranylgeranylated precursor might accumulate as well. Prelamin A has been detected with specific anti-prelamin-A antibody in patients treated with FTIs (Adjei, 2003), but the prenylation status of the accumulated protein was not determined.

Thus, those investigating treatment of HGPS with FTIs face the same questions that concern investigators developing them for treatment of malignancy. (1) Given the spectrum of farnesylated proteins, can FTIs be relied upon to target the protein of interest specifically? (2) Can alternative prenylation produce sufficient levels of biologically active prenylated protein to result in inadequate reversal of the pathological phenotype? As in the case of the Ras proteins, these issues are exacerbated by the desirability of blocking farnesylation of the mutant protein but not the wild-type.

Possible methods for characterization of progerin in cells treated with FTIs

Could progerin become a substrate for GGTase 1 in the presence of an FTI? This seems quite possible and it certainly should be tested if these compounds are to be considered for use in the treatment of HGPS. In our hands, characterization of progerin by mass spectrometry does not give information on the putative farnesylated and carboxymethylated C-terminal cysteine residue. Since this is generally done after trypsin digestion of the sample protein, the peptide fragment expected from progerin is larger than that produced from normal human prelamin A (Fig. 2) (Corrigan et al., 2005). The reasons it has not been detected are not immediately obvious.

Alternative methods for analysis of the polyisoprenoid substituents include labeling of the polyisoprenoids with mevalonate followed by radiochemical analysis of the cleavage product (Dalton and Sinensky, 1995). As mentioned above, labeling of prenylated proteins with mevalonate is greatly facilitated by using cell lines that express an activated mevalonate transporter (Faust and Krieger, 1987; Whyte et al., 1997) and incubating cells with lovastatin to block endogenous mevalonate biosynthesis. High-specific activity [^{3}H]mevalonate is incorporated into proteins either as farnesyl or geranylgeranyl substituents. These can be released by cleavage of the thioether linkage by either Raney nickel to yield the corresponding alkenes or methyl iodide to yield the corresponding alcohols (Casey et al., 1989). The alkenes are best resolved by GLC; the alcohols are best resolved by reverse-phase HPLC. The latter has the advantage of not requiring in-line radiodetection if this apparatus is not available. Specific labeling of farnesylated proteins can also be achieved with tritiated farnesol (Andres et al., 1999).

Another useful method, which gives an indirect measurement of the extent of prenylation but not the polyisoprenoid specificity, is a base-release assay of the prenylated protein after labeling of the lamin protein with [tritiated CH_3]-methionine. This method has been successfully applied to the lamin proteins (Chelsky et al., 1987; Dalton and Sinensky, 1995). Typically, the results are normalized with respect to the total incorporation of the methionine into protein relative to the number of methionines, and compared with lamin B_1 for a theoretical 100% CAAX-modification. This methodology should also be useful for evaluation of alternative processing because a true block to prenylation would also block carboxymethylation.

A recent novel approach to determining farnesylation has been described by Spielmann, Andres and colleagues (Troutman et al., 2005). They grow cells in medium supplemented with the farnesol analogue anilinogeraniol. This compound incorporates into proteins in place of farnesol, and its incorporation can be measured by antibodies directed against *S*-anilinogeranyl cysteine methyl ester. This technique is particularly directed at demonstrating the action of an FTI, because the signal would disappear upon FTI treatment. It would not, however, detect alternative prenylation because that would not be distinguishable from the absence of prenylation. However, it could be combined with another method that tests for whether prenylation occurs in the presence of the FTI.

Conclusions and perspectives

To the cell biologist, the most interesting aspect of the lamin CAAX-box post-translational modifications is their functional significance. As pointed out above, these modifications generally seem to function to create reversible membrane associations and heterodimeric protein-protein interactions. The B-lamins appear to represent examples of this common behavior of farnesylated proteins.

The functional role of the CAAX-box modification of prelamin A is, by contrast, quite puzzling, because it is eliminated during the course of prelamin A maturation and thus unique among mammalian CAAX proteins. In the dominant progeroid syndrome, HGPS, prelamin A accumulates to only very low levels and wild-type lamin A levels are not dramatically affected. Prelamin A itself may thus have a functional role and Zmpste24 could serve to regulate its levels. That cellular senescence accompanies accumulation of farnesylated prelamin A in HGPS and RD may provide a clue to that function.

These considerations should stimulate further investigation into the role of farnesylation in the function of prelamin A. The idea that inhibition of prelamin A farnesylation is a potential therapeutic approach, warrants further biochemical analysis of the effects of FTIs on post-translational modification not only of progerin but also of prelamin A and the B-lamins.

References

Adjei, A. A. (2003). Immunohistochemical assays of farnesyltransferase inhibition in patient samples. *Methods Mol. Med.* **85**, 141-145.

Agarwal, A. K., Fryns, J. P., Auchus, R. J. and Garg, A. (2003). Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. *Hum. Mol. Genet.* **12**, 1995-2001.

Anderegg, R. J., Betz, R., Carr, S. A., Crabb, J. W. and Duntze, W. (1988). Structure of Saccharomyces cerevisiae mating hormone a-factor. Identification of S-farnesyl cysteine as a structural component. *J. Biol. Chem.* **263**, 18236-18240.

Andres, D. A., Crick, D. C., Finlin, B. S. and Waechter, C. J. (1999). Rapid identification of cysteine-linked isoprenyl groups by metabolic labeling with [3H]farnesol and [3H]geranylgeraniol. *Methods Mol. Biol.* **116**, 107-123.

Barton, R. M. and Worman, H. J. (1999). Prenylated prelamin A interacts with Narf, a novel nuclear protein. *J. Biol. Chem.* **274**, 30008-30018.

Basso, A. D., Kirschmeier, P. and Bishop, W. R. (2006). Thematic review series: lipid posttranslational modifications. farnesyl transferase inhibitors. *J. Lipid Res.* **47**, 15-31.

Beck, L. A., Hosick, T. J. and Sinensky, M. (1988). Incorporation of a product of mevalonic acid metabolism into proteins of Chinese hamster ovary cell nuclei. *J. Cell Biol.* **107**, 1307-1316.

Beck, L. A., Hosick, T. J. and Sinensky, M. (1990). Isoprenylation is required for the processing of the lamin A precursor. *J. Cell Biol.* **110**, 1489-1499.

Bergo, M. O., Gavino, B., Ross, J., Schmidt, W. K., Hong, C., Kendall, L. V., Mohr, A., Meta, M., Genant, H., Jiang, Y. et al. (2002). Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. *Proc. Natl. Acad. Sci. USA* **99**, 13049-13054.

Boyartchuk, V. L., Ashby, M. N. and Rine, J. (1997). Modulation of Ras and a-factor function by carboxyl-terminal proteolysis. *Science* **275**, 1796-1800.

Cadinanos, J., Varela, I., Lopez-Otin, C. and Freije, J. M. (2005). From immature lamin to premature aging: molecular pathways and therapeutic opportunities. *Cell Cycle* **4**, 1732-1735.

Cao, H. and Hegele, R. A. (2003). LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). *J. Hum. Genet.* **48**, 271-274.

Capell, B. C., Erdos, M. R., Madigan, J. P., Fiordalisi, J. J., Varga, R., Conneely, K. N., Gordon, L. B., Der, C. J., Cox, A. D. and Collins, F. S. (2005). Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. USA* **102**, 12879-12884.

Casey, P. J., Solski, P. A., Der, C. J. and Buss, J. E. (1989). p21ras is modified by a farnesyl isoprenoid. *Proc. Natl. Acad. Sci. USA* **86**, 8323-8327.

Chelsky, D., Olson, J. F. and Koshland, D. E., Jr (1987). Cell cycle-dependent methyl esterification of lamin B. *J. Biol. Chem.* **262**, 4303-4309.

Corrigan, D. P., Kuszczak, D., Rusinol, A. E., Thewke, D. P., Hrycyna, C. A., Michaelis, S. and Sinensky, M. S. (2005). Prelamin A endoproteolytic processing in vitro by recombinant Zmpste24. *Biochem. J.* **387**, 129-138.

Dalton, M. and Sinensky, M. (1995). Expression systems for nuclear lamin proteins: farnesylation in assembly of nuclear lamina. *Meth. Enzymol.* **250**, 134-148.

Dalton, M. B., Fantle, K. S., Bechtold, H. A., DeMaio, L., Evans, R. M., Krystosek, A. and Sinensky, M. (1995). The farnesyl protein transferase inhibitor BZA-5B blocks farnesylation of nuclear lamins and p21ras but does not affect their function or localization. *Cancer Res.* **55**, 3295-3304.

Dreger, C. K., Konig, A. R., Spring, H., Lichter, P. and Herrmann, H. (2002). Investigation of nuclear architecture with a domain-presenting expression system. *J. Struct. Biol.* **140**, 100-115.

End, D. W., Smets, G., Todd, A. V., Applegate, T. L., Fuery, C. J., Angibaud, P., Venet, M., Sanz, G., Poignet, H., Skrzat, S. et al. (2001). Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer Res.* **61**, 131-137.

Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., Erdos, M. R., Robbins, C. M., Moses, T. Y., Berglund, P. et al. (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* **423**, 293-298.

Farnsworth, C. C., Wolda, S. L., Gelb, M. H. and Glomset, J. A. (1989). Human lamin B contains a farnesylated cysteine residue. *J. Biol. Chem.* **264**, 20422-20429.

Faust, J. and Krieger, M. (1987). Expression of specific high capacity mevalonate transport in a Chinese hamster cell variant. *J. Biol. Chem.* **262**, 1996-2004.

Fiordalisi, J. J., Johnson, R. L., 2nd, Weinbaum, C. A., Sakabe, K., Chen, Z., Casey, P. J. and Cox, A. D. (2003). High affinity for farnesyltransferase and alternative prenylation contribute individually to K-Ras4B resistance to farnesyltransferase inhibitors. *J. Biol. Chem.* **278**, 41718-41727.

Fong, L. G., Frost, D., Meta, M., Qiao, X., Yang, S. H., Coffinier, C. and Young, S. G. (2006). A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. *Science* **17**, 1621-1623.

Gerace, L., Comeau, C. and Benson, M. (1984). Organization and modulation of nuclear lamina structure. *J. Cell Sci. Suppl.* **1**, 137-160.

Glynn, M. W. and Glover, T. W. (2005). Incomplete processing of mutant lamin A in Hutchinson-Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition. *Hum. Mol. Genet.* **14**, 2959-2969.

Goldman, R. D., Shumaker, D. K., Erdos, M. R., Eriksson, M., Goldman, A. E., Gordon, L. B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R. et al. (2004). Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. USA* **101**, 8963-8968.

Goodman, L. E., Judd, S. R., Farnsworth, C. C., Powers, S., Gelb, M. H., Glomset, J. A. and Tamanoi, F. (1990). Mutants of Saccharomyces cerevisiae defective in the farnesylation of Ras proteins. *Proc. Natl. Acad. Sci. USA* **87**, 9665-9669.

Gordon, L. B., Harten, I. A., Patti, M. E. and Lichtenstein, A. H. (2005). Reduced adiponectin and HDL cholesterol without elevated C-reactive protein: clues to the biology of premature atherosclerosis in Hutchinson-Gilford Progeria Syndrome. *J. Pediatr.* **146**, 336-341.

Gruenbaum, Y., Goldman, R. D., Meyuhas, R., Mills, E., Margalit, A., Fridkin, A., Dayani, Y., Prokocimer, M. and Enosh, A. (2003). The nuclear lamina and its functions in the nucleus. *Int. Rev. Cytol.* **226**, 1-62.

Gruenbaum, Y., Margalit, A., Goldman, R. D., Shumaker, D. K. and Wilson, K. L. (2005). The nuclear lamina comes of age. *Nat. Rev. Mol. Cell Biol.* **6**, 21-31.

Ha, J. W., Shim, W. H. and Chung, N. S. (1993). Cardiovascular findings of Hutchinson-Gilford syndrome–a Doppler and two-dimensional echocardiographic study. *Yonsei Med. J.* **34**, 352-355.

Hancock, J. F., Magee, A. I., Childs, J. E. and Marshall, C. J. (1989). All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell* **57**, 1167-1177.

Hancock, J. F., Paterson, H. and Marshall, C. J. (1990). A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. *Cell* **63**, 133-139.

Hennekes, H. and Nigg, E. A. (1994). The role of isoprenylation in membrane attachment of nuclear lamins. A single point mutation prevents proteolytic cleavage of the lamin A precursor and confers membrane binding properties. *J. Cell Sci.* **107**, 1019-1029.

Holtz, D., Tanaka, R. A., Hartwig, J. and McKeon, F. (1989). The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. *Cell* **59**, 969-977.

Jacob, K. N. and Garg, A. (2005). Laminopathies: multisystem dystrophy syndromes. *Mol. Genet. Metab.* **87**, 289-302.

James, G. L., Goldstein, J. L. and Brown, M. S. (1995). Polylysine and CVIM sequences of K-RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic in vitro. *J. Biol. Chem.* **270**, 6221-6226.

James, G., Goldstein, J. L. and Brown, M. S. (1996). Resistance of K-RasBV12 proteins to farnesyltransferase inhibitors in Rat1 cells. *Proc. Natl. Acad. Sci. USA* **93**, 4454-4458.

Kamiya, Y., Sakurai, A. and Takahashi, N. (1980). Metabolites of mating pheromone, rhodotorucine A, by a cells of Rhodosporidium toruloides. *Biochem. Biophys. Res. Commun.* **94**, 855-860.

Kato, K., Cox, A. D., Hisaka, M. M., Graham, S. M., Buss, J. E. and Der, C. J. (1992). Isoprenoid addition to Ras protein is the critical modification for its membrane association and transforming activity. *Proc. Natl. Acad. Sci. USA* **89**, 6403-6407.

Kilic, F., Dalton, M. B., Burrell, S. K., Mayer, J. P., Patterson, S. D. and Sinensky, M. (1997). In vitro assay and characterization of the farnesylation-dependent prelamin A endoprotease. *J. Biol. Chem.* **272**, 5298-5304.

Kitten, G. T. and Nigg, E. A. (1991). The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B2. *J. Cell Biol.* **113**, 13-23.

Leonard, S., Beck, L. and Sinensky, M. (1990). Inhibition of isoprenoid biosynthesis and the post-translational modification of pro-p21. *J. Biol. Chem.* **265**, 5157-5160.

Leung, G. K., Schmidt, W. K., Bergo, M. O., Gavino, B., Wong, D. H., Tam, A., Ashby, M. N., Michaelis, S. and Young, S. G. (2001). Biochemical studies of Zmpste24-deficient mice. *J. Biol. Chem.* **276**, 29051-29058.

Levy, N., Lopez-Otin, C. and Hennekam, R. C. (2005). Defective prelamin A processing resulting from LMNA or ZMPSTE24 mutations as the cause of restrictive dermopathy. *Arch. Dermatol.* **141**, 1473-1474.

Liu, B., Wang, J., Chan, K. M., Tjia, W. M., Deng, W., Guan, X., Huang, J. D., Li, K. M., Chau, P. Y., Chen, D. J. et al. (2005). Genomic instability in laminopathy-based premature aging. *Nat. Med.* **11**, 780-785.

Liu, M., Bryant, M. S., Chen, J., Lee, S., Yaremko, B., Li, Z., Dell, J., Lipari, P., Malkowski, M., Prioli, N. et al. (1999). Effects of SCH 59228, an orally bioavailable farnesyl protein transferase inhibitor, on the growth of oncogene-transformed fibroblasts and a human colon carcinoma xenograft in nude mice. *Cancer Chemother. Pharmacol.* **43**, 50-58.

Lutz, R. J., Trujillo, M. A., Denham, K. S., Wenger, L. and Sinensky, M. (1992). Nucleoplasmic localization of prelamin A: implications for prenylation-dependent lamin A assembly into the nuclear lamina. *Proc. Natl. Acad. Sci. USA* **89**, 3000-3004.

Magee, T. and Seabra, M. C. (2005). Fatty acylation and prenylation of proteins: what's hot in fat. *Curr. Opin. Cell Biol.* **17**, 190-196.

Mallampalli, M. P., Huyer, G., Bendale, P., Gelb, M. H. and Michaelis, S. (2005). Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. USA* **102**, 14416-14421.

Maske, C. P., Hollinshead, M. S., Higbee, N. C., Bergo, M. O., Young, S. G. and Vaux, D. J. (2003). A carboxyl-terminal interaction of lamin B1 is dependent on the CAAX endoprotease Rce1 and carboxymethylation. *J. Cell Biol.* **162**, 1223-1232.

McClintock, D., Gordon, L. B. and Djabali, K. (2006). Hutchinson-Gilford progeria mutant lamin A primarily targets human vascular cells as detected by an anti-Lamin A G608G antibody. *Proc. Natl. Acad. Sci. USA* **103**, 2154-2159.

Mical, T. I. and Monteiro, M. J. (1998). The role of sequences unique to nuclear intermediate filaments in the targeting and assembly of human lamin B: evidence for lack of interaction of lamin B with its putative receptor. *J. Cell Sci.* **111**, 3471-3485.

Miyakawa, T., Tabata, M., Tsuchiya, E. and Fukui, S. (1985). Biosynthesis and secretion of tremerogen A-10, a polyisoprenyl peptide mating pheromone of Tremella mesenterica. *Eur. J. Biochem.* **147**, 489-493.

Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O'Hara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L. and Gibbs, J. B. (1991). Sequence dependence of protein isoprenylation. *J. Biol. Chem.* **266**, 14603-14610.

Moulson, C. L., Go, G., Gardner, J. M., van der Wal, A. C., Smitt, J. H., van Hagen, J. M. and Miner, J. H. (2005). Homozygous and compound heterozygous mutations in ZMPSTE24 cause the laminopathy restrictive dermopathy. *J. Invest. Dermatol.* **125**, 913-919.

Nagasu, T., Yoshimatsu, K., Rowell, C., Lewis, M. D. and Garcia, A. M. (1995). Inhibition of human tumor xenograft growth by treatment with the farnesyl transferase inhibitor B956. *Cancer Res.* **55**, 5310-5314.

Navarro, C. L., De Sandre-Giovannoli, A., Bernard, R., Boccaccio, I., Boyer, A., Genevieve, D., Hadj-Rabia, S., Gaudy-Marqueste, C., Smitt, H. S., Vabres, P. et al. (2004). Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy. *Hum. Mol. Genet.* **13**, 2493-2503.

Navarro, C. L., Cadinanos, J., De Sandre-Giovannoli, A., Bernard, R., Courrier, S., Boccaccio, I., Boyer, A., Kleijer, W. J., Wagner, A., Giuliano, F. et al. (2005). Loss of ZMPSTE24 (FACE-1) causes autosomal recessive restrictive dermopathy and accumulation of Lamin A precursors. *Hum. Mol. Genet.* **14**, 1503-1513.

Nigg, E. A., Kitten, G. T. and Vorburger, K. (1992). Targeting lamin proteins to the nuclear envelope: the role of CaaX box modifications. *Biochem. Soc. Trans.* **20**, 500-504.

Omer, C. A., Chen, Z., Diehl, R. E., Conner, M. W., Chen, H. Y., Trumbauer, M. E., Gopal-Truter, S., Seeburger, G., Bhimnathwala, H., Abrams, M. T. et al. (2000). Mouse mammary tumor virus-Ki-rasB transgenic mice develop mammary carcinomas that can be growth-inhibited by a farnesyl:protein transferase inhibitor. *Cancer Res.* **60**, 2680-2688.

Pendas, A. M., Zhou, Z., Cadinanos, J., Freije, J. M., Wang, J., Hultenby, K., Astudillo, A., Wernerson, A., Rodriguez, F., Tryggvason, K. et al. (2002). Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice. *Nat. Genet.* **31**, 94-99.

Pollex, R. L. and Hegele, R. A. (2004). Hutchinson-Gilford progeria syndrome. *Clin. Genet.* **66**, 375-381.

Powers, S., Michaelis, S., Broek, D., Santa Anna, S., Field, J., Herskowitz, I. and Wigler, M. (1986). RAM, a gene of yeast required for a functional modification of RAS proteins and for production of mating pheromone a-factor. *Cell* **47**, 413-422.

Reddel, C. J. and Weiss, A. S. (2004). Lamin A expression levels are unperturbed at the normal and mutant alleles but display partial splice site selection in Hutchinson-Gilford progeria syndrome. *J. Med. Genet.* **41**, 715-717.

Reid, T. S., Terry, K. L., Casey, P. J. and Beese, L. S. (2004). Crystallographic analysis of CaaX prenyltransferases complexed with substrates defines rules of protein substrate selectivity. *J. Mol. Biol.* **343**, 417-433.

Roskoski, R., Jr and Ritchie, P. (1998). Role of the carboxyterminal residue in peptide binding to protein farnesyltransferase and protein geranylgeranyltransferase. *Arch. Biochem. Biophys.* **356**, 167-176.

Rowell, C. A., Kowalczyk, J. J., Lewis, M. D. and Garcia, A. M. (1997). Direct demonstration of geranylgeranylation and farnesylation of Ki-Ras in vivo. *J. Biol. Chem.* **272**, 14093-14097.

Sasseville, A. M. and Raymond, Y. (1995). Lamin A precursor is localized to intranuclear foci. *J. Cell Sci.* **108**, 273-285.

Scaffidi, P. and Misteli, T. (2006). Lamin A-dependent nuclear defects in human aging. *Science* **312**, 1059-1063.

Schafer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S. H. and Rine, J. (1989). Genetic and pharmacological suppression of oncogenic mutations in ras genes of yeast and humans. *Science* **245**, 379-385.

Schafer, W. R., Trueblood, C. E., Yang, C. C., Mayer, M. P., Rosenberg, S., Poulter, C. D., Kim, S. H. and Rine, J. (1990). Enzymatic coupling of cholesterol intermediates to a mating pheromone precursor and to the ras protein. *Science* **249**, 1133-1139.

Schroeder, H., Leventis, R., Rex, S., Schelhaas, M., Nagele, E., Waldmann, H. and Silvius, J. R. (1997). S-Acylation and plasma membrane targeting of the farnesylated carboxyl-terminal peptide of N-ras in mammalian fibroblasts. *Biochemistry* **36**, 13102-13109.

Sedelnikova, O. A., Horikawa, I., Zimonjic, D. B., Popescu, N. C., Bonner, W. M. and Barrett, J. C. (2004). Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. *Nat. Cell Biol.* **6**, 168-170.

Shahinian, S. and Silvius, J. R. (1995). Doubly-lipid-modified protein sequence motifs exhibit long-lived anchorage to lipid bilayer membranes. *Biochemistry* **34**, 3813-3822.

Silvius, J. R. and l'Heureux, F. (1994). Fluorimetric evaluation of the affinities of isoprenylated peptides for lipid bilayers. *Biochemistry* **33**, 3014-3022.

Sinensky, M. (2000). Functional aspects of polyisoprenoid protein substituents: roles in protein-protein interaction and trafficking. *Biochim. Biophys. Acta* **1529**, 203-209.

Sinensky, M., Fantle, K., Trujillo, M., McLain, T., Kupfer, A. and Dalton, M. (1994). The processing pathway of prelamin A. *J. Cell Sci.* **107**, 61-67.

Smith, S. and Blobel, G. (1994). Colocalization of vertebrate lamin B and lamin B receptor (LBR) in nuclear envelopes and in LBR-induced membrane stacks of the yeast Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* **91**, 10124-10128.

Taddei, A., Hediger, F., Neumann, F. R. and Gasser, S. M. (2004). The function of nuclear architecture: a genetic approach. *Annu. Rev. Genet.* **38**, 305-345.

Toth, J. I., Yang, S. H., Qiao, X., Beigneux, A. P., Gelb, M. H., Moulson, C. L., Miner, J. H., Young, S. G. and Fong, L. G. (2005). Blocking protein farnesyltransferase improves nuclear shape in fibroblasts from humans with progeroid syndromes. *Proc. Natl. Acad. Sci. USA* **102**, 12873-12878.

Troutman, J. M., Roberts, M. J., Andres, D. A. and Spielmann, H. P. (2005). Tools to analyze protein farnesylation in cells. *Bioconjug. Chem.* **16**, 1209-1217.

Trueblood, C. E., Boyartchuk, V. L., Picologlou, E. A., Rozema, D., Poulter, C. D. and Rine, J. (2000). The CaaX proteases, Afc1p and Rce1p, have overlapping but distinct substrate specificities. *Mol. Cell. Biol.* **20**, 4381-4392.

Varela, I., Cadinanos, J., Pendas, A. M., Gutierrez-Fernandez, A., Folgueras, A. R., Sanchez, L. M., Zhou, Z., Rodriguez, F. J., Stewart, C. L., Vega, J. A. et al. (2005). Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. *Nature* **437**, 564-568.

Vestal, D. J., Buss, J. E., Kelner, G. S., Maciejewski, D., Asundi, V. K. and Maki, R. A. (1996). Rat p67 GBP is induced by interferon-gamma and isoprenoid-modified in macrophages. *Biochem. Biophys. Res. Commun.* **224**, 528-534.

Wallis, C. V., Sheerin, A. N., Green, M. H., Jones, C. J., Kipling, D. and Faragher, R. G. (2004). Fibroblast clones from patients with Hutchinson-Gilford progeria can senesce despite the presence of telomerase. *Exp. Gerontol.* **39**, 461-467.

Weber, K., Plessmann, U. and Traub, P. (1989). Maturation of nuclear lamin A involves a specific carboxy-terminal trimming, which removes the polyisoprenylation site from the precursor; implications for the structure of the nuclear lamina. *FEBS Lett.* **257**, 411-414.

Whyte, D. B., Kirschmeier, P., Hockenberry, T. N., Nunez-Oliva, I., James, L., Catino, J. J., Bishop, W. R. and Pai, J. K. (1997). K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J. Biol. Chem.* **272**, 14459-14464.

Winter-Vann, A. M. and Casey, P. J. (2005). Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat. Rev. Cancer* **5**, 405-412.

Wolda, S. L. and Glomset, J. A. (1988). Evidence for modification of lamin B by a product of mevalonic acid. *J. Biol. Chem.* **263**, 5997-6000.

Worman, H. J., Yuan, J., Blobel, G. and Georgatos, S. D. (1988). A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA* **85**, 8531-8534.

Worman, H. J., Evans, C. D. and Blobel, G. (1990). The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. *J. Cell Biol.* **111**, 1535-1542.

Young, S. G., Fong, L. G. and Michaelis, S. (2005). Prelamin A, Zmpste24, misshapen cell nuclei, and progeria–new evidence suggesting that protein farnesylation could be important for disease pathogenesis. *J. Lipid Res.* **46**, 2531-2558.

Zhang, F. L. and Casey, P. J. (1996). Protein prenylation: molecular mechanisms and functional consequences. *Annu. Rev. Biochem.* **65**, 241-269.

Zhang, F. L., Kirschmeier, P., Carr, D., James, L., Bond, R. W., Wang, L., Patton, R., Windsor, W. T., Syto, R., Zhang, R. et al. (1997). Characterization of Ha-ras, N-ras, Ki-Ras4A, and Ki-Ras4B as in vitro substrates for farnesyl protein transferase and geranylgeranyl protein transferase type I. *J. Biol. Chem.* **272**, 10232-10239.