◉ MECHANISMS OF DISEASE

Werner and Hutchinson–Gilford progeria syndromes: mechanistic basis of human progeroid diseases

Brian A. Kudlow*¶, Brian K. Kennedy* and Raymond J. Monnat Jr‡§

Abstract | Progeroid syndromes have been the focus of intense research in part because they might provide a window into the pathology of normal ageing. Werner syndrome and Hutchinson–Gilford progeria syndrome are two of the best characterized human progeroid diseases. Mutated genes that are associated with these syndromes have been identified, mouse models of disease have been developed, and molecular studies have implicated decreased cell proliferation and altered DNA-damage responses as common causal mechanisms in the pathogenesis of both diseases.

Progeroid syndromes are heritable human disorders with features that suggest premature ageing[1]. These syndromes have been well characterized as clinical disease entities, and in many instances the associated genes and causative mutations have been identified. The identification of genes that are associated with premature-ageing-like syndromes has increased our understanding of molecular pathways that protect cell viability and function, and has provided clues to the molecular mechanisms that underlie normal human ageing. However, despite these advances, little is understood about how the molecular and cellular defects that result from mutations of progeria-associated genes lead to organismal phenotypes that resemble ageing. This lack of mechanistic understanding has also hampered efforts to determine the relationship between progeroid syndromes and normal ageing.

Many heritable human diseases are characterized by progeroid features that recapitulate some features of normal human ageing. One of the earliest compilations of genetic syndromes with potential to reveal the pathobiology of ageing was published in 1978 by Martin[1]. Martin identified 162 syndromes, in a catalogue of human genes and genetic diseases entitled *Mendelian Inheritance in Man* (see Online Mendelian Inheritance in Man in Further information), that showed different degrees of phenotypic overlap with normal ageing. These syndromes were further evaluated for the presence of 21 criteria such as chromosomal aberrations, premature greying and loss of hair, an elevated risk of cancer, diabetes mellitus or vascular disease. Notably, none of the syndromes recapitulated all of the features that are observed in normal ageing, and they were

therefore termed segmental, as opposed to global, progeroid syndromes. Among the segmental progeroid syndromes, the syndromes that most closely recapitulate the features of human ageing are Werner syndrome (WS), Hutchinson–Gilford progeria syndrome (HGPS), Cockayne syndrome, ataxia-telangiectasia, and the constitutional chromosomal disorders of Down, Klinefelter and Turner syndromes.

In this review we focus on two of the best characterized of these disorders, WS and HGPS . Both syndromes show substantial phenotypic overlap with normal ageing, and for each there has been recent progress in understanding the genetic, biochemical and cellular basis of the disease. Mutations in *WRN* and *LMNA* genes give rise to WS and HGPS, respectively. Functional roles of their encoded gene products, WRN and A-type lamins, have recently begun to emerge. We summarize new information on functional roles of these proteins, and how altered function may result in the cellular and organismal phenotypes of WS and HGPS. Important unanswered questions about the molecular pathogenesis of WS and HGPS are outlined, and we discuss whether WRN and the A-type lamins have a role in the biology of normal ageing and age-associated disease.

A better understanding of the pathogenesis of these two human progeroid syndromes is likely to improve our picture of several areas of cell biology, most notably in the areas of nuclear structure, dynamics and DNA repair, as well as how defects in these fundamental biological processes lead to cellular and organismal disease phenotypes. Other DNA-repair-deficiency syndromes that have progeroid features — such as xeroderma

Departments of
*Biochemistry, ‡Pathology,
§Genome Sciences and the
¶Molecular and Cellular
Biology Program, University
of Washington, Seattle,
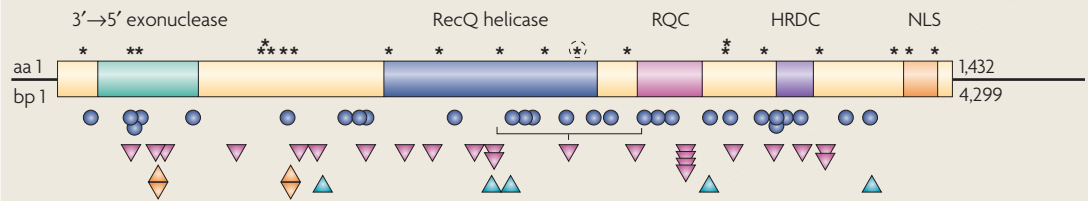Washington 98195, USA.
Correspondence to B.K.K.
and R.J.M.Jr.
e-mails:
bkenn@u.washington.edu;
monnat@u.washington.edu
doi:10.1038/nrm2161

Box 1 | Mutations and polymorphisms of WRN

The WRN protein has 1,432 amino-acid (aa) residues and contains four domains: catalytically active 3′→5′ exonuclease and ATPase-helicase domains (exonuclease and RecQ helicase segments) and two domains that are involved in DNA-substrate and protein interactions (RQC and HRDC domains). A nuclear-localization signal (NLS) is located near the C terminus of the protein.

The locations and molecular types of 58 mutations in the WRN gene that have been reported in patients with Werner syndrome (WS) are also shown[7,9]. These published examples have been assembled in the form of a web-accessible Locus-Specific Mutational Database (see Further information). All of these mutations have a common biochemical consequence: they truncate WRN and lead to loss of the WRN helicase and exonuclease activities. Patients with WS who fulfil the clinical diagnostic criteria for WS but lack WRN mutations have also been identified. These rare WS 'phenocopies' might reflect persistent silencing of WRN expression (see text) or help us to identify other genes that function with or regulate WRN. A large number of WRN single-nucleotide polymorphisms (SNPs) have also been identified (see SNP linked to WRN in Further information). The functional importance of almost all of these variants is unknown, although one coding-region non-synonymous variant (circled asterisk) has been shown to markedly reduce the catalytic activity of WRN[96]. bp, base pair.

pigmentosum and Cockayne syndrome — and defects in the response to DNA-strand breaks have been recently reviewed[2].

WS as a progeroid syndrome

WS is an autosomal recessive disease with features that are reminiscent of premature ageing. The initial description of WS by Werner in 1904 (REF. 3) emphasized four features: short stature, bilateral cataracts, early greying and loss of hair, and scleroderma-like skin changes. These phenotypes have been observed in nearly all patients, including those in a patient cohort that was recently studied by the Werner Syndrome International Registry[4–7] (see Further information). Clinical features of the syndrome appear *de novo* (often beginning during the second decade of life), are progressive and are not the consequence of another systemic disease process or the result of a primary endocrine deficiency or dysfunction[4,8].

The clinical diagnosis of WS can be challenging in light of the complex, progressive and variable nature of the WS phenotype. This is particularly true in young adults, in whom there might be few convincing signs or no family history to raise suspicion. To aid the clinical diagnosis of WS, a useful set of diagnostic criteria and a scoring system have been developed by the Werner Syndrome International Registry. These clinical criteria, together with molecular approaches to identify common WRN mutations or to document the loss of WRN protein, can usually confirm or exclude a diagnosis of WS[7,9].

The WRN *gene: mutations and epigenetic silencing.* The chromosome 8p12 WRN gene was identified in 1996 by positional cloning and encodes a 162-kDa RecQ helicase protein[10]. The human RecQ helicase family consists of 5 proteins, of which only WRN possesses 3′→5′ exonuclease activity and 3′→5′ helicase activity

(reviewed in REFS 11,12). The WRN protein seems to be ubiquitously expressed, and it can be detected by western blot in cell lines and tissue samples from normal individuals and, in reduced amounts, from heterozygous carriers of single mutant copies of the WRN gene[13–15]. However, there has been no systematic study of WRN localization in different cell types or tissues during embryonic development or in the adult.

All of the disease-associated WRN mutations[7,9] (BOX 1) confer a common biochemical phenotype: they lead to truncation or, in one instance, apparent destabilization and loss of WRN protein from patient cells[13,14]. These biochemical data are consistent with the autosomal-recessive inheritance of WS. The absence of patient-derived missense mutations that selectively inactivate the WRN helicase or exonuclease activity is notable. These findings indicate that both biochemical activities of WRN must be lost to promote WS pathogenesis[16].

Individuals who carry single mutant-WRN alleles have elevated genetic instability *in vivo*[17], and cell lines from heterozygous individuals have intermediate sensitivity to DNA-damaging agents such as DNA-crosslinking drugs and topoisomerase I inhibitors that selectively kill WRN-deficient cells[18,19]. As WRN heterozygotes are common worldwide[20], it will be important to determine whether heterozygous carriers are at increased risk of disease or treatment-related toxicity after receiving drugs such as *cis*-Pt or the topoisomerase I inhibitors camptothecin or irinotecan.

The recent report of epigenetic silencing of the WRN locus in human tumour-cell lines and tissue samples[21] identifies another mechanism by which WRN expression might be regulated. This study clearly documented the association between hypermethylation of the WRN gene and loss of the WRN protein. Moreover, it showed that WRN silencing led to increased sensitivity

RecQ helicase
A protein with a domain that contains sequence and structural homology to the *Escherichia coli* RecQ helicase. Human RecQ helicase proteins include WRN, BLM and REQ4.

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to topoisomerase I inhibitors and the DNA-crosslinking drug mitomycin-C. Therefore, cells that lack WRN protein owing to epigenetic silencing of the *WRN* gene have cellular phenotypes that resemble cells in which WRN is absent owing to germline mutation. The study is also consistent with increased cancer incidence in individuals with WS[22].

In vivo functions of WRN

The WRN helicase and exonuclease activities, first predicted on the basis of protein homologies[10,23,24], were subsequently confirmed by several groups, and these groups also defined *in vitro* substrate preferences. These preferred WRN substrates include several types of 3- and 4-way DNA junctions as well as gapped, branched or unpaired DNA and DNA overhangs. Protein-interaction studies have also identified WRN-interacting proteins, such as replication protein A (RPA), that have roles in many aspects of DNA metabolism together with proteins that are involved in specific DNA metabolic pathways such as DNA replication, recombination or repair (reviewed in REFS 11,12).

One important *in vivo* function that is consistent with these findings is a role of WRN in homology-dependent recombination repair (HDR). HDR can be used to repair DNA damage while suppressing gene loss or rearrangement[25,26] (FIG. 1). WRN seems to have a role late in HDR when recombinant molecules are topologically disentangled for segregation to daughter cells (that is, the postsynaptic or resolution phase of HDR). WRN function during recovery from replication arrest has also been postulated, largely on the basis of the functions of the budding and fission yeast RecQ homologues (see, for example, REF. 27). In mammalian cells, WRN seems to repair DNA-strand breaks that arise from replication arrest, and therefore functions to limit genetic instability and cell death. This is consistent with the role of WRN in HDR[25,26,28].

WRN also has an important role in the maintenance of telomere length and the suppression of telomere sister-chromatid exchanges (T-SCEs)[29–33]. The most likely explanation for the role of WRN in telomere maintenance is in the nature of telomeres. Telomeres are highly structured, G-rich DNA sequences that need to be partially disassembled to allow DNA replication and repair. A failure to fully replicate and reassemble telomeres at the end of S phase might lead to the generation of telomeric DNA ends that can be recognized as persistent DNA double-strand breaks to initiate a damage response that can trigger cellular senescence. Accurate telomere metabolism is also important to suppress genetic instability and chromosome rearrangements[34]. The catalytic activities of WRN could therefore be required to make telomeres accessible for replication and/or repair, or to insure the successful completion of these processes to restore telomere structure. Common substrates for WRN function at telomeres and in HDR might include 3-stranded, forked D- or T-loops, which are the preferred *in vitro* substrates for WRN[35] (reviewed in REFS 11,12). Studies in human fibroblasts and in mouse models of WS (see below) indicate that short telomeres might trigger a requirement for WRN function[30–33,36]. These experiments also indicate that WRN function at telomeres might require only the helicase activity of WRN, in contrast to HDR, for which both the helicase and exonuclease activities of WRN are required[16,32,33,37].

Various other nucleic-acid metabolic functions, including roles in non-homologous DNA-end joining, base-excision repair, DNA-damage signalling and transcription, have been proposed for WRN (reviewed in REFS 11,12,38). Although plausible, none of these claims is yet as well documented or appears to be quantitatively as important as the roles of WRN in HDR and telomere maintenance. These two functional roles for WRN (FIG. 1) are supported by concordant biochemical, cellular, genetic, patient and/or animal-modelling data.

Mechanistic origins of the WS phenotype

The identification of a role for WRN in HDR and in telomere maintenance indicates a model for how loss-of-function mutations might lead to the molecular and cellular abnormalities observed in WS (FIG. 1). WRN is proposed to function on DNA substrates that are generated during HDR repair, the stabilization or repair of replication forks, or from telomere replication, repair or remodelling. Successful resolution of these substrates suppresses genomic instability and maintains telomere
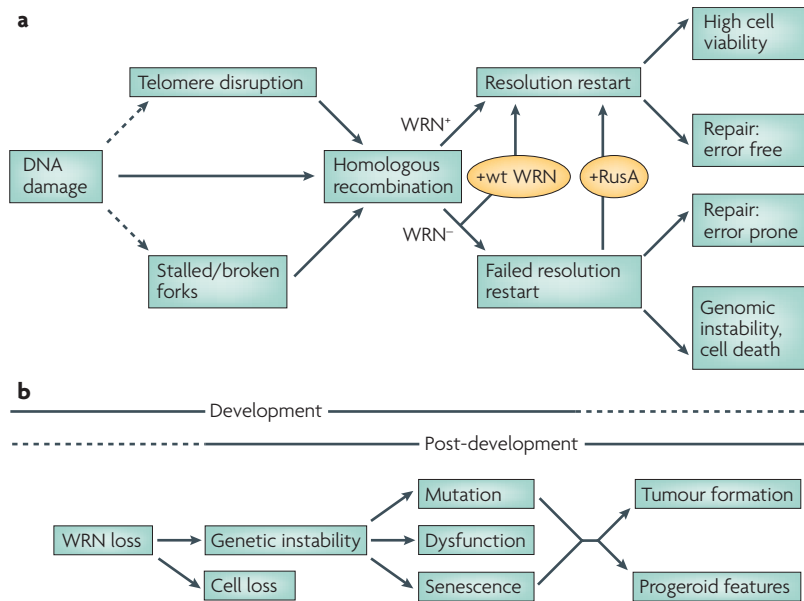
Figure 1 | **WRN function and disease pathogenesis. a** | WRN has well documented roles in homology-dependent recombinational repair (HDR) and in telomere maintenance. These roles might be functionally related; for example, WRN-dependent maintenance or recombinational repair of telomeres might ensure high cell viability and genetic stability (WRN+). In the absence of WRN (WRN−), HDR or telomere processing can fail, leading to mitotic arrest, cell death or genetic instability. Experimental tests of this model are shown by the ovals: wild-type WRN re-expression (+wt WRN) improves both cell survival and the recovery of viable mitotic recombinants, as does expression of the bacterial resolvase protein RusA (+RusA)[25,26]. WRN is likely to have a role in several other nucleic-acid metabolic and repair pathways and in HDR and telomere maintenance (see text for discussion). **b** | Model of Werner syndrome (WS) disease pathogenesis. WRN loss leads to genetic instability, mutation accumulation and cell loss during and after development in virtually all cell lineages to promote atrophic and/or progeroid changes and the emergence of neoplastic disease.

Box 2 | Therapeutic approaches for WS and HGPS

Werner syndrome (WS)

The two avenues that are being explored as therapeutic approaches to WS are suppression of the biochemical and cellular consequences of WRN loss at the cell level, and cell replacement to suppress the consequences of WRN loss at the tissue or organismal level. The most promising candidate for an intervention at the biochemical level stems from the observation that inhibitors of the mitogen-activated protein kinase (MAPK) p38, such as SB203580, can suppress the growth and senescence defects of *WRN*-deficient fibroblasts in culture[97]. This work indicates that a stress-induced, kinase-dependent, telomere-length-independent senescence mechanism might be operative in fibroblasts from patients with WS. This pathway is a target for pharmacological suppression that could be further explored in cell-culture models and in *Wrn*-knockout mice.

The second, more speculative, strategy is to suppress WS tissue or organismal phenotypes by attempting to compensate for defects in cell number or cell function. This could be done by direct transplantation, by stimulating regenerative capacity in affected tissues or organs (for example, by pharmacological inhibition of p16INK4a[98]) or by identifying how tissue-level trophic or regulatory interactions are mediated and altered in WS (see, for example, REFS 42,99). All three of these tissue-level approaches will require a better understanding of normal biology and WS pathogenesis, and therefore would greatly benefit from improved WS mouse models.

Hutchinson–Gilford progeria syndrome (HGPS)

Farnesyltransferase inhibitors (FTIs) represent a promising way to treat HGPS owing to their ability to block farnesylation of progerin and an ability to suppress development of the HGPS phenotype in mouse models. These drugs are also safe for clinical use. However, treating patients with FTIs, does not eliminate expression of progerin and prelamin A, but rather causes these proteins to accumulate in their unfarnesylated forms. Also at issue is whether the beneficial effects of FTIs are direct or secondary, because FTI treatment of HGPS-model mice results in an incomplete blockade of prelamin A maturation[72]. Therefore, significant levels of farnesylated progerin likely remain, and FTIs might function either through partial inhibition of progerin farnesylation or through effects on another CAAX-containing protein. Furthermore, although most cases of HGPS are associated with progerin expression, several HGPS-associated alleles have been identified that are not predicted to alter lamin A processing[48,50,51,100,101]. The potential efficacy of FTIs in such instances remains in question, although they might be expected to interfere with the transient prenylation of mutant proteins and target them away from the nuclear periphery, which might suppress toxicity. It would therefore be useful to determine the ability of FTIs to rescue defects in nuclear organization across the spectrum of laminopathy mutations.

Base-excision repair
A DNA-repair pathway that selectively identifies and replaces single DNA bases that have been chemically damaged.

Mesenchymal cell lineage
Cell lineages that are derived from the mesodermal germ layer. Includes many cell types that are found in connective tissue and other supporting, conducting or blood-forming lineages of the body.

length and structure to ensure high cell viability. In the absence of WRN function, cells accumulate potentially toxic DNA intermediates or critically short telomeres that can trigger genetic instability, DNA damage and apoptotic response pathways[33,36,39]. In this model, WS pathogenesis is driven by defective DNA metabolism that leads to genetic instability and mutagenesis. These consequences, together with mutation accumulation and cell loss, might drive the development of cell type, cell lineage or tissue-specific defects (FIG. 1). Compromised tissue or organ structure and function then leads to two seemingly divergent outcomes: senescence and mutation-dependent neoplastic proliferation[38,40].

Fibroblasts and other mesenchymal cell lineages might be selectively affected by the loss of WRN function because of their ability to divide throughout life and their comparative resistance to DNA-damage-induced apoptosis. Moreover, connective tissue lacks the type of compartmentalized architecture found in cell lineages such as epithelia that functions to suppress damage- or mutation-driven hyperproliferation or neoplasia[41]. Therefore, the pathogenesis of WS might reflect the progressive

accumulation of mutant or senescent cells that have lost cell-specific functions, as well as trophic or regulatory interactions with adjacent epithelial or stromal cells[42]. This model of WS pathogenesis emphasizes the importance of DNA damage and the progressive accumulation of cellular defects over time as critical determinants of WS and associated-disease pathogenesis. This model of WS pathogenesis also hints at ways in which to limit or prevent WS complications (BOX 2).

Insights from animal models of WS. Animal models of WS have been developed to provide a way to better understand WRN function and WS pathogenesis. So far, three different WS mouse models have been developed: a complete *Wrn* knockout[43], an in-frame deletion of the helicase domain (which leads to a truncated protein that retains exonuclease activity[44]) and transgenic expression of a human Lys577Met WRN variant protein that lacks helicase activity in a background of normal murine WRN protein[45].

Of these three models, only the null mutant of *Wrn* recapitulates the biochemical defects that are observed in WS patients. Although the phenotype of the *Wrn*-knockout mouse model remains to be fully characterized, these animals do not exhibit obvious premature ageing or a spontaneous cancer predisposition. One way to further explore the *Wrn*-knockout phenotype would be to systematically challenge these mice with the different types of DNA damage that selectively kill human WS cells (see above). A second route to develop more useful mouse models of WS is to generate sensitized mouse genetic backgrounds to introduce extra stress on replication, HDR or telomere-maintenance pathways. For instance, two groups crossed a murine telomerase-RNA-template-deficient (or *Terc*-deficient) mouse with a *Wrn*-knockout mouse[30]. Telomerase deficiency alone leads to progressive degenerative changes in proliferating tissues such as skin, gut and bone marrow. In $Wrn^{-/-}$ $Terc^{-/-}$ mice, both groups observed greying and loss of hair, osteoporosis, diabetes mellitus and cataracts that increased in severity in later generations and were correlated with telomeric DNA loss. Of note, laboratory mouse strains generally have longer telomeres than do wild-type mice or humans. These changes therefore depend on critically short telomeres that arise only after several generations of breeding in the absence of *Terc*[30,31]. A requirement for *Wrn* deficiency in conjunction with short telomeres to reveal a phenotype in *Wrn*-knockout mice provides an explanation for the variable and progressive appearance of the phenotype in different animals. These results are encouraging as they begin to identify mouse models in which to investigate *Wrn* function, and they highlight telomeres as an important *in vivo* substrate for *Wrn* function.

HGPS and A-type lamins

HGPS is the most severe of the progeroid syndromes; affected individuals have a mean lifespan of 13 years[46]. This disease was first described in 1886 (REF. 47), and is rare: there are currently fewer than 150 documented cases of HGPS worldwide. HGPS patients generally appear normal at birth, but prematurely develop

several features that are associated with ageing, including alopecia, atherosclerosis, rapid loss of joint mobility, osteolysis, severe lipodystrophy, scleroderma and varied skin hyperpigmentation. Death in patients with HGPS frequently results from stroke or coronary failure. HGPS patients might also have developmental defects of the clavicle, mandible and cranium as well as marked growth retardation (an extensive description of the HGPS phenotype can be found in REF. 46). The genetic basis of HGPS was uncovered in 2003, when it was found that most cases of the disease are associated with a single-nucleotide substitution that leads to aberrant splicing of *LMNA*, the gene that encodes the A-type nuclear lamins[48–50]. In most instances, HGPS is associated with dominant, *de novo* germline mutations. However, in some instances the disease seems to result from compounded heterozygous mutations or homozygous point mutations[50,51].

All A-type lamins, of which the most abundant are lamins A and C (hereafter lamin A/C), are encoded by a single *LMNA* locus through alternative splicing[52]. A-type lamins belong to the family of intermediate-filament proteins that, along with the B-type lamins, are the main constituents of the nuclear lamina in most differentiated cells. The ability of lamins to multimerize into filaments lends both rigidity and elasticity to the nuclear lamina[53]. Although the A-types lamins are highly enriched at the nuclear periphery, they can also be detected at discrete sites in the nucleus, where they might perform specific functions linked to cell proliferation[54,55]. In addition to maintaining the integrity and shape of the nuclear envelope, lamin A/C has been implicated in the regulation of transcription, DNA replication, cell-cycle control and cellular differentiation[56–59]. The involvement of A-type lamins in so many diverse cellular processes is likely to depend on both direct interactions of lamin A/C with nuclear proteins and chromatin, as well as their role in establishing a nuclear environment that is conducive to various nucleic-acid metabolic and signalling processes.

***LMNA* and laminopathies.** The identification of *LMNA* as the gene responsible for HGPS puts this disease into a broader category of laminopathies, which includes at least ten distinct diseases, each caused by mutations in *LMNA*[60]. These diseases include forms of muscular dystrophy and cardiomyopathy, a rare familial lipodystrophy, Charcot–Marie–Tooth syndrome, and several other diseases with progeroid features (described in detail below). In most instances, the laminopathies are associated with dominant missense mutations in *LMNA*. In total, over 200 disease-associated mutations in *LMNA* have been reported (see Leiden Muscular Dystrophy pages and The *LMNA* mutation database in Further information). It is not known why one *LMNA* point mutation can lead to one disease, whereas another nearby point mutation can lead to a different phenotype and disease. The phenotype of the *Lmna*-knockout mouse[61–63] indicates that mutations that lead to loss of lamin A/C function result in muscular dystrophy, dilated cardiomyopathy and possibly Charcot–Marie–Tooth

syndrome, whereas other diseases such as HGPS might result from mutations that lead to elevated or novel activities of lamin A/C.

Among the laminopathies, three might be related to HGPS at both the phenotypic and molecular level. For example, four patients diagnosed with so-called atypical WS were found to carry point mutations in *LMNA*, which indicated that these patients might be afflicted with a less severe form of HGPS[64]. All of the *LMNA* mutations that are associated with atypical WS map to the N-terminal end of the rod domain (BOX 3), where at least one HGPS-associated mutation has also been mapped[48]. By contrast, most of HGPS mutations map close to the C-terminal processing site of the lamin A protein. One possibility is that mutations at the N terminus of lamin A confer a similar, although potentially milder, toxicity to that which results from mutations proximal to the prelamin A processing site.

Mandibuloacral dysplasia (MAD) and restrictive dermopathy (RD) appear to be clinically mild and severe variants, respectively, of an HGPS-like phenotype. Both diseases can result from mutations in either *LMNA* or in *ZMPSTE24*, which encodes a protease that is required for the maturation of lamin A (see below). Along with patients with HGPS, patients with MAD and RD have some degree of progeroid features, and the underlying molecular mechanisms of the disease in each case seems to depend at least in part on defective lamin A maturation.

Altering lamin A processing

The lamin A protein is synthesized as a 664-amino-acid precursor protein, called prelamin A (reviewed in REF. 65). Prelamin A contains a C-terminal CAAX amino-acid motif that undergoes farnesylation at the Cys residue. Farnesylated prelamin A undergoes two cleavages; the first takes place C-terminal to the modified Cys, whereas the second removes 15 C-terminal residues, including the farnesyl–Cys. The mature lamin A protein contains no farnesyl modification (FIG. 2). Deletion of the endopeptidase-encoding gene *Zmpste24* blocks both prelamin A cleavage events and the production of mature lamin A. The resulting protein that accumulates is farnesylated, uncleaved prelamin A[66]. Due to differential splicing of the exons that encode the C terminus, lamin C is synthesized without the CAAX motif, and therefore does not undergo proteolytic processing.

The most frequent HGPS-associated mutation, Gly608Gly, is a silent base substitution that activates a cryptic splice donor in exon 11 of *LMNA* (BOX 3). Use of this anomalous splice donor leads to the loss of 150 nucleotides from the 3′ end of exon 11 in the mature lamin A mRNA, and internal deletion of 50 amino-acid residues from the C terminus of lamin A. The resulting mutant protein is called progerin. Progerin retains its C-terminal CAAX motif, and therefore is farnesylated. However, it lacks the recognition site for the second cleavage event and it therefore accumulates in farnesylated form, which localizes exclusively to the nuclear periphery[67] owing to the association of the hydrophobic prenyl group of the protein with the nuclear envelope.

Nuclear lamina
The proteinacious meshwork that underlies the inner nuclear membrane.

Box 3 | Progeria-associated *LMNA* mutations
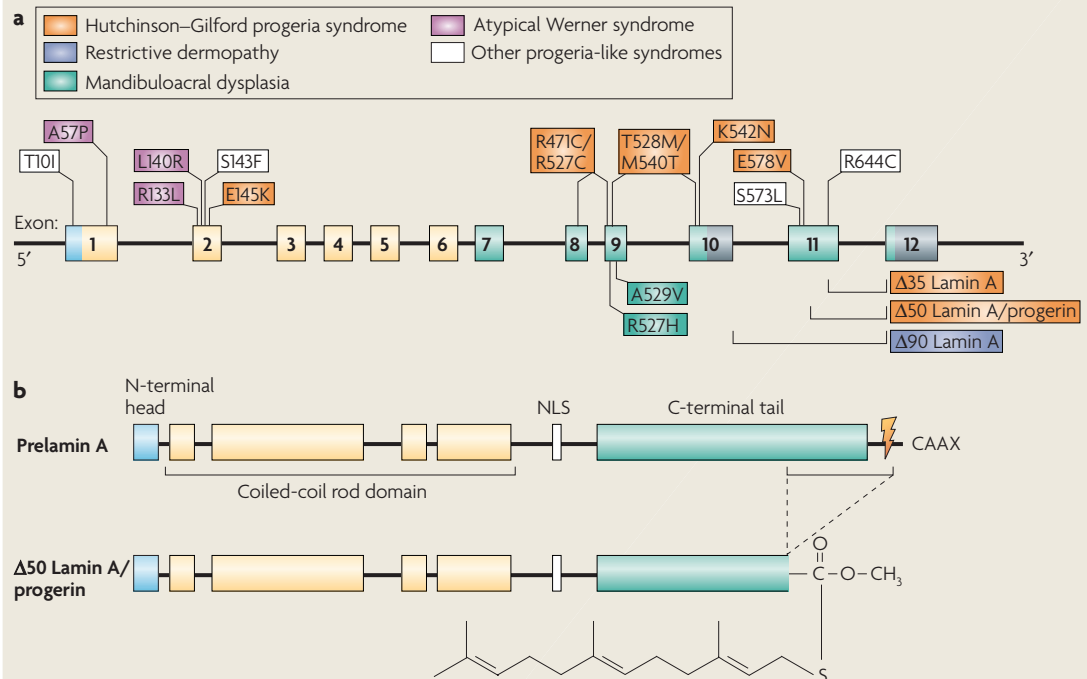
In addition to Hutchinson–Gilford progeria syndrome (HGPS), several other progeria-like syndromes, including atypical Werner syndrome (WS), mandibuloacral dysplasia (MAD) and restrictive dermopathy (RD), are also associated with mutations in *LMNA* or *ZMPSTE24* (REFS 102–105). Known progeria-associated mutations are shown against the exon structure of human *LMNA* (panel **a**). Mutations such as Arg471Cys and Arg527Cys[50] were identified in individuals who are compound heterozygous for these alleles.

MAD, like HGPS, features lipodystrophy, alopecia and short stature as well as developmental defects of the mandible and clavicle, and a mild progeroid appearance, and might represent a mild form of HGPS. MAD can result from homozygous mutation in *LMNA* or compound heterozygous mutation in *ZMPSTE24* (REFS 102,103,105). By contrast, RD might be an exaggerated form of HGPS. RD is a perinatally lethal disease with marked dermal hypoplasia, abnormal epidermal structure and joint contractures. In most cases, cells from RD fetuses are devoid of ZMPSTE24 activity owing to nonsense mutations in both copies of *ZMPSTE24* (REF. 106), and therefore they fail to process prelamin A. So, prelamin A processing is central to the pathogenesis of MAD, HGPS and RD. These syndromes, therefore, might represent a continuum of increasingly severe, mechanistically related phenotypes.

The most common HGPS-associated mutation, Gly608Gly, causes 150 nucleotides encoded in exon 11 to be spliced out of the final mRNA and results in a protein that lacks 50 amino acids (panel **b**). This protein, progerin, retains its C-terminal CAAX motif but lacks sequences that are required for complete processing and is, therefore, stably farnesylated. Similarly, RD can result from heterozygous mutations altering the splicing of exon 11 of *LMNA*[104], and in one case this causes the complete removal of exon 11 and produces a protein that is similar to progerin but that lacks 90 amino acids at the C terminus. By contrast, a similar but smaller C-terminal deletion (35 amino acids) has been identified in a patient with HGPS who survived more than 30 years longer than the average disease-associated life expectancy[107]. In this case, the causative *LMNA* mutation activated a cryptic splice donor 3′ to the site that leads to progerin production from the Gly608Gly allele. This succession of progerin-like deletions, removing 35, 50 and 90 amino acids in, mild HGPS, canonical HGPS and RD, respectively, seems to represent an allelic series of increasing toxicity. However, the effect of each of these deletions on lamin A function is unclear. NLS, nuclear-localization signal.

Cells from patients with HGPS display several phenotypes that indicate that their general nuclear organization and dynamics are compromised. For example, cells from patients with HGPS display irregular nuclear morphology, a phenotype that has been considered diagnostic of HGPS[48,49] and consistent with altered lamin A/C function. However, cells from patients with most, if not all, of the other laminopathies also exhibit this phenotype. Cells from patients with HGPS also show relocalization or decreased expression of the heterochromatin marker heterochromatin protein-1 (HP1) and of members of the lamina-associated polypeptide-2 (LAP2) family of proteins[68], altered patterns of histone modification and evidence of DNA damage[69]. Similar cellular phenotypes have been observed in fibroblasts that have undergone physiological ageing *in vivo* or that have been continuously passaged *in vitro*[70].

Interfering with lamin A processing in the mouse, either by deleting *Zmpste24* or by expressing progerin, results in an HGPS-like phenotype[66,71,72]. Treating these mice with farnesyltransferase inhibitors (FTIs) markedly ameliorates many of the HGPS-like phenotypes such as lack of adipose tissue, growth retardation and skeletal pathology[72–74] (BOX 2). FTI treatment is also correlated
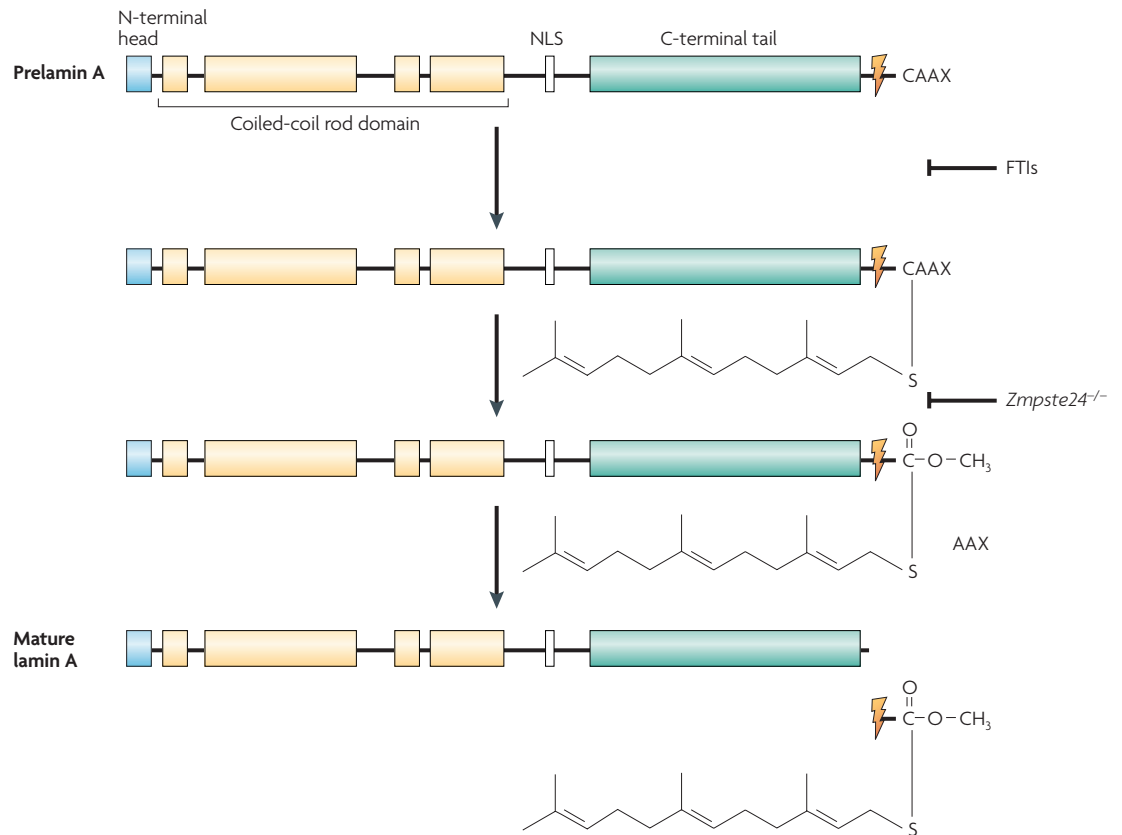
Figure 2 | **The maturation of lamin A.** Lamin A is synthesized as the 664-amino-acid precursor protein prelamin A. Prelamin A contains a C-terminal CAAX motif that directs farnesylation by farnesyl protein transferase. This step can be blocked by farnesyltransferase inhibitors (FTIs), which inhibit all subsequent processing reactions. Following farnesylation, the three C-terminal amino acids of prelamin A are cleaved and the new C terminus is methylated. This cleavage event requires the endopeptidase ZMPSTE24. Subsequently, the maturing lamin A molecule rapidly undergoes a second cleavage event, also mediated by ZMPSTE24, removing the 15 C-terminal residues, including the farnesylated Cys residue. This process results in mature lamin A that contains no farnesyl modification. Like other intermediate-filament proteins, lamin A contains N- and C-terminal globular domains that flank a coiled-coil rod domain. This central rod domain mediates the protein–protein interaction between lamin A molecules that allows for filament formation. The nuclear localization of lamin A is driven by a nuclear-localization signal (NLS) that is adjacent to the C-terminal globular domain.

with the relocalization of the lamin A protein away from the nuclear periphery and partially rescues the nuclear morphology phenotype[73,75–78]. These data strongly indicate that stably farnesylated lamin A at the nuclear periphery is toxic owing to elevated or novel lamin A function at this subnuclear location. This is consistent with the dominant inheritance of HGPS.

Mechanistic origins of the HGPS phenotype

How does expression of progerin lead to HGPS? The genetics of HGPS strongly indicate that HGPS-associated mutations are dominant neomorphic or hypermorphic alleles of *LMNA*. By contrast, as noted above, deletion of *Lmna* in the mouse results in a muscular dystrophy phenotype that is distinct from the phenotype that results from the expression of progerin. On the basis of FTI data, stable farnesylation of progerin also seems to be associated with the pathogenesis of HGPS. This hypothesis is further supported by the finding that deletion of *Zmpste24*, the endopeptidase that is responsible for both prelamin A cleavage events, results in an HGPS-like phenotype in

the mouse[66,71]. Despite these observations, it is unlikely that stable farnesylation of progerin alone accounts for its toxicity. As noted above, several HGPS-associated mutations are not predicted to alter prelamin A processing. Moreover, in some assays progerin behaves differently from lamin A mutants that are stably prenylated owing to site-directed mutation of the cleavage sites[59].

Several reports have indicated that progerin alters the composition and mechanical properties of the nuclear lamina. HGPS cells in culture show age- and passage-dependent changes in nuclear morphology that are correlated with increasing levels of progerin[79]. One potential explanation for these changes in morphology is altered interaction between A- and B-type lamins. Fluorescence resonance energy transfer (FRET) analyses have demonstrated that although A- and B-type lamins can interact *in vitro*, they segregate into distinct homopolymers *in vivo*[80]. Progerin disrupts this segregation, possibly because its high affinity for the nuclear envelope causes it to juxtapose more tightly to the membrane, a location normally solely occupied by constitutively prenylated

Neomorphic allele
An allele of a gene that confers to the encoded gene product a new activity not normally found in the product encoded by the wild-type allele.

Hypermorphic allele
An allele of a gene that confers to the encoded gene product an increase in the activity that is normally associated with the product encoded by the wild-type allele.
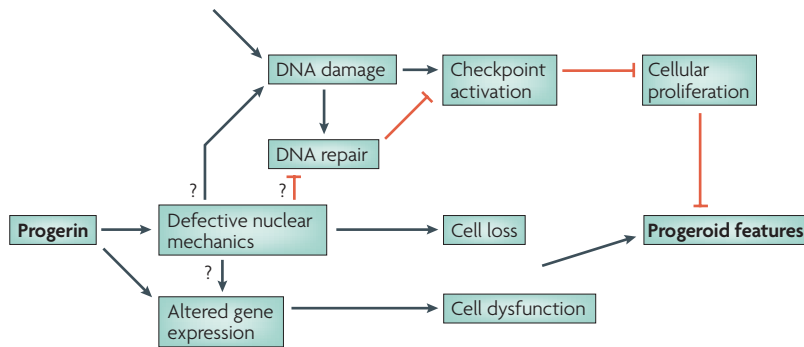
Figure 3 | **Model for the pathogenesis of HGPS in the presence of progerin.** Progerin disrupts the composition and dynamics of the nuclear lamina, which might lead to cell death or dysfunction. These changes might also generate DNA damage or interfere with DNA repair in cells from patients with Hutchinson–Gilford progeria syndrome (HGPS), leading to the accumulation of DNA damage and the constitutive activation of a p53-dependent checkpoint response. Similarly, changes in gene expression might contribute to the overall HGPS cellular phenotype. Cell dysfunction, proliferative defects and cell death that result from progerin expression contribute to generate the progeroid phenotype.

B-type lamins. Biochemical fractionation and live-cell imaging experiments have also shown that progerin expression induces immobilization of A-type lamins in the nuclear lamina[67]. This is correlated with distinct changes in the mechanical properties of progerin-expressing cells that differ from $Lmna^{-/-}$ cells. How these changes in nuclear structure and dynamics lead to HGPS is unclear, although one intriguing and testable hypothesis is that these progerin-dependent defects trigger a structural checkpoint that suppresses both cell proliferation and survival.

An activated DNA-damage response. Cells derived from patients with HGPS and HGPS mouse models display several indicators of an activated DNA-damage response, including enhanced phosphorylation of histone H2AX and markedly increased transcription of p53 target genes[69,81]. Cells from patients with HPGS might also display aneuploidy and chromosome instability. These cells show delayed recruitment of DNA-repair proteins to sites of DNA damage, sensitivity to double-strand breaks and reduced cell proliferation. That these cellular phenotypes are causally linked to the HGPS phenotype is suggested by evidence that deletion of *p53* in the $Zmpste24^{-/-}$ mouse model of HGPS partially mitigates some of the progeroid phenotypes and extends lifespan[81]. Although tantalizing, the cohort size of the $Zmpste24^{-/-}$ $p53^{-/-}$ mice in this study was limited and further studies of the relationship between progerin and p53 are warranted. It is unknown how progerin causes these phenotypes, although these observations are consistent with the idea that progerin interferes with DNA repair while permitting damage detection and signalling through p53. This could constitute a positive-feedback loop, leading to the accumulation of DNA damage over time and constitutive activation of a damage response. These observations identify the role of lamin A in DNA repair and the DNA-damage response as important areas for further research.

Ultimately, changes in the function of the nuclear lamina that result from the expression of progerin must cause changes in cellular viability, function and gene expression. Characterization of cells from patients with HGPS in culture has revealed decreased proliferative capacity[82] and several changes in gene expression, especially of extracellular matrix proteins[83–85]. It remains unclear how directly these changes result from progerin expression. For example, the altered mechanical properties of HGPS nuclei probably result directly from the ability of progerin to interfere with the composition of the nuclear lamina. Similarly, the DNA-repair defects and altered gene-expression patterns in HGPS cells might result from the direct interference of progerin with the protein complexes that are involved in these processes. Alternatively, the faulty nuclear structure imparted by progerin might be responsible for defective targeting of repair complexes to damage sites or of transcription factors to their sites of activity. Furthermore, the altered dynamics of the nuclei might result in changes in mechanosensitive gene expression[86]. Each of these changes has the potential to disrupt the proliferative capacity, viability and function of cells, and taken together all these perturbations might contribute to the progeroid features that are characteristic of HGPS (FIG. 3).

Insights from mouse models of HGPS. Several murine models of HGPS have been developed, providing insight into disease mechanisms and a way to test the efficacy of potential therapies. The development of the first HGPS-like mouse model was largely serendipitous: Mounkes *et al.* generated a mouse in which the *Lmna* gene had been altered to express a muscular-dystrophy-associated *Lmna* allele[87]. However, they were surprised to find a mouse that developed severe HGPS-like phenotypes, including growth retardation, decreased hair-follicle density, and skin and muscle defects. Analysis of the products derived from the altered *Lmna* gene revealed that the targeted mutation unexpectedly altered *Lmna* splicing.

More recently, mouse models have been generated by either transgenic expression of human progerin[88] or by the replacement of a 3′ region of the *Lmna* gene with a single exon that encodes the C terminus of progerin[73]. The $Zmpste24^{-/-}$ mouse has also been used to model HGPS in light of its HGPS-like phenotype, which is dependent on defective lamin A processing and the presence of functional p53 (REFS 81,89). Whereas the transgenic HGPS mouse shows phenotypes that are largely restricted to the vascular system, including progressive loss of vascular smooth-muscle cells and thickening of the walls of large vessels, the other two models show a broader progeria-like phenotype with severe growth retardation, fragile bones, alopecia, skin defects and dramatically reduced viability. The phenotype of the HGPS C-terminal progerin-replacement mouse can be made more severe by crossing the mutant allele to homozygosity, or by crossing a single copy of the HGPS allele into a null background. Of note, both the C-terminal progerin replacement mouse and the $Zmpste24^{-/-}$ mouse show considerable phenotypic improvement in growth and survival when treated with FTIs[72,74] (BOX 2).

Progeroid syndromes and normal ageing

The striking, progressive phenotypes of WS and of HGPS have suggested that one or both syndromes might represent a form of premature or accelerated ageing that is mechanistically linked to normal ageing. However, careful clinical and pathological examination of patients with either WS or HGPS has pointed out differences in the nature or degree of changes between patients and normally aged individuals. For example, WS is commonly referred to as a premature-ageing syndrome despite both quantitative and qualitative differences with normal ageing, including the greater extent or severity of the loss of hair or hair colour and the development of an unusual type of ocular cataract or unusual neoplasms[4]. These differences led Epstein and colleagues to conclude that WS was neither precocious nor accelerated ageing, but "may be better considered a 'caricature' of aging, exaggerating, although not necessarily by the same mechanisms, some of the clinical and pathologic changes which connote aging[4]."

If this conclusion is correct, how likely is it that the knowledge gained studying WS or HGPS will provide general insights into the biology of ageing or the pathogenesis of age-associated disease? One view is that progeroid syndromes represent phenocopies of what we are really interested in: the mechanisms that underlie normal ageing or disease pathogenesis[90]. Therefore the underlying biology of WS or HGPS might be fascinating in its own right, but unlikely to reveal broader mechanistic aspects of ageing or disease pathogenesis. A more optimistic view, to which we subscribe, is that many progeroid syndromes might have partial mechanistic overlap with normal ageing and therefore might provide uniquely informative opportunities to formulate and test hypotheses regarding the biology of ageing and age-dependent disease[40,91].

How can we identify mechanistic links? Three different avenues of research might aid the identification of mechanistic links between the progeroid syndromes and normal ageing or disease pathogenesis. These links include better definition of the underlying normal biology, the use of genetic and environmental modifiers to confirm suspected links and establish causation, and the linkage of cellular changes to tissue or organismal phenotypes.

For example, there is considerable interest in the role of DNA damage and altered DNA-damage responses as mediators of ageing and age-associated disease. This notion is further supported by the characterization of a number of other human and animal mutations that result in progeroid features[60]. The mutated genes in these instances have been associated with various functions, among which the most common is DNA metabolism. Two recent reports[92,93] describe the analyses of DNA-repair-deficient patients and mice that provide new information on this question, and identify a common mechanism by which exogenous and endogenous DNA damage might trigger the development of progeroid changes. This mechanism entails DNA-damage-dependent suppression of growth hormone (GH)–insulin-like growth-factor-1 (IGF1) somatotroph signalling, a deeply conserved, important regulator of metabolism and longevity in many organisms[94]. Therefore, it would be useful to know whether lamin A/C function and WRN function converge on DNA repair and the response to DNA damage, what roles they have in these processes, and how these roles are modified or disrupted by WS- or HGPS-associated mutations.

A second strategy focuses on *in vivo* analyses of cellular phenotypes that accompany disease-associated mutations in *WRN* and *LMNA*. There are now a growing number of morphological and molecular tools to identify and quantify persistent DNA-damage signalling, cell death and cellular senescence in tissue. The application of these tools to patients and to animal models should give us a better sense of the extent to which impaired cell proliferation, excessive cell death or the accumulation of senescent cells contribute to the pathogenesis of WS and HGPS. It will also be important to determine whether these cellular changes are selectively targeted to specific cell lineages or tissues.

A converse strategy to analyse progeroid syndromes has been to search for genes or pathways that delay ageing or extend lifespan when they are mutated. These longevity mutations have been proposed to be enriched in genes that modulate normal ageing[95]. It is of interest to note that the genes and pathways identified so far by these two mutation-driven approaches show little overlap. To this end, efforts are underway to identify *WRN* and *LMNA* polymorphisms that are associated with enhanced longevity.

Recently, a potential link between *LMNA* and cellular senescence was reported. Human cells with normal *LMNA* genes were found to produce small amounts of progerin protein by using the same splice sites that are used by HGPS-associated *LMNA* alleles[70]. The ability of morpholino oligonucleotide-mediated ablation of progerin production to reverse some of the nuclear structural defects associated with cellular ageing indicates that progerin expression might have a role in normal cell senescence. If endogenous progerin production also promotes cellular senescence in the mouse, it might be possible to extend lifespan by engineering the *Lmna* locus to block progerin production. This would establish a strong mechanistic link between the pathogenesis of HGPS and normal ageing.

Conclusions

The 'greying' of many human populations has provided a strong stimulus to better understand ageing and the pathogenesis of age-related disease. New knowledge will have considerable practical, as well as conceptual, importance. Recent progress to understand the molecular basis of WS and HGPS, two well characterized, genetically defined human progeroid syndromes, has led to the suggestion that different primary nuclear metabolic defects might accelerate ageing. The likely mechanism is by directly or indirectly promoting mutation accumulation, together with persistent activation of DNA-damage-dependent cell-signalling pathways. The most exciting consequence of identifying common mechanistic features

of progeroid syndromes, such as WS or HGPS, and of normal ageing or age-associated disease pathways is the prospect of identifying new ways to improve quality of life and to treat or prevent age-associated disease.

Note added in proof

Two recent papers by Cao et al.[108] and Dechat et al.[109] demonstrate that the constitutive farnesylation of progerin causes it to associate with membrane structures throughout the cell cycle, even in mitosis when lamin A normally becomes soluble. The mitotic membrane association of progerin is akin to that of the B-type lamins. Cells expressing progerin appear to have delayed and defective mitotic progression, suggesting that the inappropriate association of an A-type lamin with mitotic membrane structures interferes with some processes in mitosis. How these findings relate to the progeroid phenotype of HGPS remains to be elucidated.

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The following terms in this article are linked online to:
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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
LMNA | *WRN* | *ZMPSTE24*
OMIM:
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Ataxia-telangiectasia | Down syndrome | Hutchinson–Gilford progeria syndrome | Werner syndrome

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Ray Monnat's laboratory:
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SNP linked to *WRN*: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7486&chooseRs=all
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