Progeria syndromes and ageing: what is the connection?

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Abstract | One of the many debated topics in ageing research is whether progeroid syndromes are really accelerated forms of human ageing. The answer requires a better understanding of the normal ageing process and the molecular pathology underlying these rare diseases. Exciting recent findings regarding a severe human progeria, Hutchinson–Gilford progeria syndrome, have implicated molecular changes that are also linked to normal ageing, such as genome instability, telomere attrition, premature senescence and defective stem cell homeostasis in disease development. These observations, coupled with genetic studies of longevity, lead to a hypothesis whereby progeria syndromes accelerate a subset of the pathological changes that together drive the normal ageing process.

Ageing studies have long shown that organismal lifespan is a modifiable parameter. The classical example is dietary restriction[1,2], which extends longevity in a growing number of organisms, including yeast (*Saccharomyces cerevisiae*)[3–5], worms (*Caenorhabditis elegans*)[6], fruit flies (*Drosophila melanogaster*)[7,8], rodents[9] and, most recently, rhesus monkeys (*Macaca mulatta*)[10]. This suggests that molecular events contributing to the pathophysiology of cellular ageing are shared to some degree among widely diverse systems, an assertion verified in a direct quantitative comparison between two disparate invertebrates[11]. Concomitant with longer lifespan, dietary restriction delays the onset of a range of age-associated pathologies in invertebrate and mammalian models[10,12–16].

Advanced age in humans is considered the largest risk factor for a range of diseases, including neurodegenerative, cardiovascular, metabolic and neoplastic syndromes, raising the possibility that targeted approaches to ageing will delay the onset of many causes of morbidity in the elderly. This approach necessitates an understanding of the molecular events that precipitate physiologic decline and underpin organismal ageing. To this end, genetic approaches have been at the forefront of discovery. Studies of invertebrate models have focused on mutations that extend lifespan, identifying numerous ageing genes and pointing to a set of highly conserved nutrient-responsive kinases that alter metabolism in a manner that affects ageing. A separate line of enquiry involves the characterization of human syndromes and mouse disease models that present with features resembling accelerated ageing. To date, the genes associated with lifespan extension in

model systems and those associated with progerias in mammals have at best limited overlap (FIG. 1).

In this Review, we combine recent developments in the branch of ageing research that focuses on lifespan extension of disease-free individuals with advancements made towards understanding the pathology of human progerias, particularly regading Hutchinson–Gilford progeria syndrome (HGPS). This Review is not exhaustive with respect to these complex topics, but rather is directed towards describing the essentials while pointing readers to sources of detailed information. We conclude by attempting to put together these different and promising branches of ageing research.

The biology of ageing

The biology of ageing perhaps has its roots in evolutionary theory, as it was evolutionary theorists, dating back to Darwin and Wallace, who first thought productively about the ageing process (BOX 1). However, many approaches have been used to understand ageing at the molecular level, including analyses of invertebrate model organisms, mammals and mammalian cells in culture. Although much progress has been made, many of the key questions remain unanswered. This section briefly addresses the approaches and findings that underlie current thoughts about the mechanistic events driving the normal ageing process.

Comparing old and young. Comparative studies largely predated genetic approaches in answering the question: what changes or 'goes wrong' during the ageing process?

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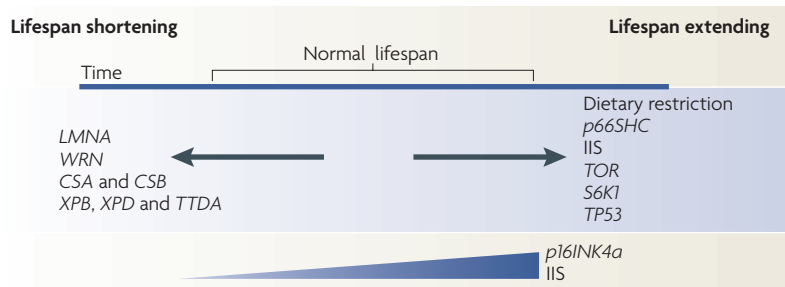e-mail:
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Figure 1 | Genetic models for progerias and enhanced longevity. Mutations in lamin A/C (*LMNA*), Werner syndrome ATP-dependent helicase (*WRN*), Cockayne syndrome protein A (*CSA*; also known as *ERCC8*) and *CSB* (also known as ERCC6), and several DNA repair genes, such as xeroderma pigmentosum group B complementing (*XPB*; also known as *ERCC3*), *XPD* (also known as ERCC2) and *TTDA* (also known as *GTF2H5*), are associated with diseases that resemble premature ageing in humans. To date, there is little, if any, known overlap of genes associated with progerias with genes that are known to extend longevity in mammals, such as target of rapamycin (*TOR*), S6 kinase 1 (*S6K1*) and tumour protein 53 (*TP53*), which are known to extend longevity in mammals. Although the genes do not overlap, growing evidence suggests that downstream molecular processes that are affected in progerias may be important for lifespan extension in mammals. Association studies have linked human longevity to the *INK4a–INK4b* locus, and to polymorphisms in components of the insulin or insulin-like growth factor 1 (IGF1) signalling (IIS) pathway.

Proteotoxicity model
A model in which the accumulation of aggregated or misfolded proteins causes cellular dysfunction that contributes partially to tissue damage and an ageing phenotype.

Forward genetic screen
A screening approach in which the phenotype is identified initially in a natural or mutagenized population, and the responsible gene is subsequently characterized.

Reverse genetics
A screening approach in which genotypic variation is identified initially in a natural or mutagenized population, and the phenotype or mutational effect is subsequently characterized.

Replicative lifespan
The lifespan of dividing cells, measured as the number of generations (or the number of daughter cells) produced by a single mother cell.

Hypomorphic mutation
A mutation that results in reduced, but not eliminated, function.

Although it is easy to underestimate the importance of these studies, they set the stage for in-depth thinking about mechanisms of ageing.

As early as 1950, oxidative damage started receiving acclaim as a causal agent in the ageing process[17]. Oxygen radicals are created during the process of respiration and acquired from external sources, and have the potential to damage nearly every cell constituent, including proteins, lipids and nucleic acids[18,19]. Damage to all of these molecules increases with age and may contribute to organismal ageing. Cells have numerous mechanisms to detoxify these agents, and several studies have been carried out to either increase or reduce the activity of these defence mechanisms and measure the effects on ageing. Results are equivocal, with clear examples of lifespan extension associated with increased activity[20,21] and decreased activity[22] of defence pathways, and numerous studies showing that either increased or reduced expression of proteins involved in these response pathways has little or no effect on longevity[23,24].

Several other theories have accrued to explain ageing. Many involve the accumulation of cellular damage and are non-exclusive with the oxidative damage hypothesis. For instance, cells isolated from ageing individuals have accumulated nuclear DNA damage, misfolded proteins and increased frequencies of mitochondrial DNA deletions, which are associated with reduced respiratory capacity. All of these events have been speculated to drive general or specific aspects of the ageing process. Chronic inflammation, both immune and non-immune mediated, may be another factor driving age-associated pathologies in a manner coupled to oxidative damage[25]. The proteotoxicity model is also currently gaining in popularity[26]. One reason is that long-lived mutants of various organisms are resistant to toxicity conferred by the expression of proteins that are linked to

neurodegeneration and prone to aggregation[27–31]. Other progressive changes occur that are not obviously related to cellular damage. Foremost among these is telomere shortening, a feature of primary human cells that do not express telomerase to levels that are sufficient for the maintenance of telomere ends[32].

It may be that several different models are partly correct. Different types of damage may accumulate at different rates in individuals of a population. When these different damaging agents reach a threshold for toxicity, they may start to precipitate aspects of the ageing process. Humans (and mice) do not die of ageing; instead, they succumb to diseases of ageing. A multifactorial model, whereby different types of cellular damage could explain the heterogeneity of diseases in the elderly (as different types of damage are likely to differentially affect individual cell types and processes), puts forward a possible explanation for divergent pathology with age. For instance, enhanced nuclear DNA damage may underlie increased cancer incidence. A related model is that some types of damage may have little impact on ageing in healthy individuals but be highly detrimental to the unhealthy. For example, obesity and the accompanying metabolic disease may increase both rates of accumulation of oxidative radicals and their capacity to cause damage.

Invertebrate genetics. Most ageing genes have been identified through studies of invertebrate models of ageing. Gene identification has resulted from educated guesswork, forward genetic screens and, more recently, reverse genetics, in which a panel of strains with reduced expression of individual genes is assayed for lifespan. Reverse genetics has opened up the possibility of genome-wide screens, leading to the identification of more than 500 ageing genes, the reduced expression of which results in lifespan extension[33–35]. In the case of yeast, this has meant screening the open reading frame deletion mutants, which represent the most viable single-gene deletion strains in an otherwise isogenic background, for modifiers of replicative lifespan or chronological lifespan[36–38]. In worms, RNA interference (RNAi) libraries expressed in arrayed bacterial strains are used to target genes in searches for extended lifespan[34]. Genome-wide screens for longevity in flies have yet to be reported, but similar RNAi strategies have recently become feasible.

Many cell signalling pathways have been linked to lifespan extension in at least one model organism. For brevity, we focus on two pathways that are linked to each other and are conserved among invertebrates and mammals: insulin or insulin-like growth factor 1 (IGF1) signalling (IIS) and target of rapamycin (TOR) signalling. The first mutant reported to extend lifespan in worms, *age-1* (REFS 39,40), encodes a phosphoinositide 3-kinase (PI3K)[41], which has a central role in IIS signalling. Similarly, hypomorphic mutations of the insulin and IGF1 receptor, DAF-2, and of the downstream components AKT-1 and serum and glucocorticoid-regulated kinase 1 (SGK-1) all lead to lifespan extension[42,43]. Receptor binding of insulin or IGF1 leads to Tyr phosphorylation of insulin receptor substrate (IRS) proteins

Box 1 | **Evolutionary theories of ageing**

Why should natural selection allow the universal decline in fitness that accompanies prolonged survival? An important theory, formulated by Medawar in 1952 (REF. 147), was based on the hypothesis that the force of natural selection declines with age, as organisms in the wild largely succumb to age-extrinsic causes of mortality. Protection of the germ line from mutations that confer detrimental effects only at increased age has little or no benefit. Several elegant theories have since been proposed that modify or alter this seminal theory. These include the concept of antagonistic pleiotropy[148], that germline mutations conferring beneficial effects early in life will be selected for even if they also convey severe costs late in life, and the disposable soma theory[149,150], which more clearly defines ageing in the context of an evolutionary trade-off of resources between reproduction and maintenance of somatic tissue. Nevertheless, they all centre on the concept (which has been acknowledged in some form by many researchers of ageing) that, unlike many developmental processes, ageing cannot be thought of as a programmed process.

The message that ageing results ultimately from the decline in natural selection with time in an organism is important to account for when molecular theories of ageing are considered. The lack of a programme calls into question whether pathways of ageing will be conserved among eukaryotic species, the extent to which stochastic events precipitate associated pathology and, perhaps most importantly, whether the pathology associated with ageing can be traced back to the same root molecular causes in all cases. In other words: is there one route to 'old' or many?

and, in turn, activation of PI3K and Akt[44]. Activated Akt phosphorylates the DAF-16 (known as FOXO in other species) transcription factor, inhibiting its migration to the nucleus; reduced Akt-dependent phosphorylation promotes nuclear FOXO localization and subsequent activation of a range of transcriptional targets, including several stress-responsive factors. Emphasizing its importance, DAF-16 is required for lifespan extension by all of the aforementioned mutants in the IIS pathway. Reduced IIS is also associated with lifespan extension in flies and mice (see below)[45].

The TOR pathway is arguably the most conserved ageing pathway, given that reduced TOR signalling leads to lifespan extension in yeast, worms, flies and mice (see below)[46]. The TOR pathway is responsive to nutrients, in the form of both amino acids and carbohdrates, and, when activated, stimulates protein synthesis and cell growth[47]. TOR kinases exist in two complexes, termed TORC1 and TORC2, which have distinct cellular functions but both are essential for viability[48,49]. TORC1, which is highly sensitive to rapamycin, is activated by nutrient availability, particularly amino acids, and coordinates protein synthesis and degradation to promote growth when nutrients are plentiful. Most studies indicate that reduced TORC1 signalling leads to lifespan extension[46], but a recent report indicates that reduced expression of a TORC2 subunit in worms also promotes longevity[50]. Reduced TOR signalling promotes mitochondrial biogenesis and cell maintenance pathways, including autophagy and stress-responsive factor-activated signalling. Studies in yeast, worms and flies have indicated that a primary benefit of dietary restriction may be reduced signalling through the TOR pathway[46]. Further emphasizing the importance of TOR, altered expression of its two primary downstream TORC1 substrates, S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), also promote lifespan extension[51].

The conserved histone and protein deacetylase silent information regulator 2 (SIR2; known as SIR-2.1 in worms, SIR2 in flies and SIRT1 in mice) has also been linked to ageing and dietary restriction[52]. Increased Sir2 activity has been shown to extend yeast replicative lifespan at least in part by repressing recombination at the ribosomal DNA (rDNA) locus, reducing the accumulation of extrachromosomal rDNA circles that are toxic to dividing yeast[53]. Puzzlingly, an increased dosage of SIR2 has been reported to extend lifespan by different mechanisms in different organisms; for example, through a DAF-16-dependent mechanism in worms[54] and through unclear mechanisms in flies[55]. Although increased SIRT1 activity in mice is associated with protection from age-associated disease and many intriguing protein substrates have been identified, increased lifespan has not been reported.

Mammalian longevity studies. Dwarf mouse strains, including Ames, Snell and Little mice, have been studied for many years. Most lifespan studies indicate that these mice are long-lived[56]. All mice have defective pituitary function, resulting in reduced growth hormone signalling. Ames and Snell mice also secrete reduced levels of thyroid-stimulating hormone and prolactin, and all three mouse models display insulin sensitivity and reduced IGF1 levels. Growth hormone receptor knockout ($GHR^{-/-}$) mice are also long-lived and have reduced IGF1 signalling. IGF1 receptor-knockout mice are not viable, but heterozygous mice are reported to be long-lived. Fat- and brain-specific insulin receptor-knockout mice are also long-lived[57,58]. Together, these findings show the importance of the IIS pathway in mouse ageing.

Two studies in 2009 indicated that reduced TOR signalling results in enhanced mammalian longevity. In one report, treatment with rapamycin was found to result in enhanced lifespan, even when it was not administered to mice until 20 months of age (~70% of the mouse lifespan)[59]. Another study that implicates TOR in ageing shows that $S6K1^{-/-}$ mice, which lack one of the two S6 kinase subunits (confirmed TOR substrates), are long-lived[60].

In mice, the TOR pathway and the IIS pathway interact at several levels (FIG. 2). Activated Akt phosphorylates tuberous sclerosis complex protein 2 (TSC2), inducing the degradation of TSC1–TSC2 and thereby removing the TSC2-mediated inhibition of the small GTPase Ras
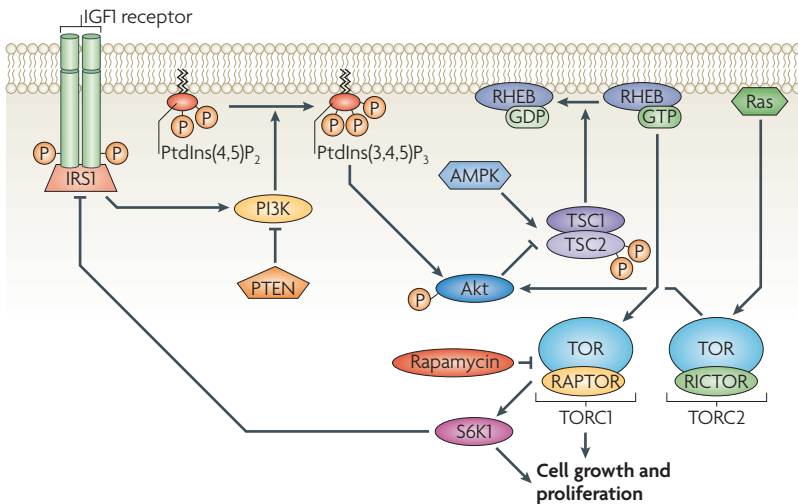
Figure 2 | **Crosstalk between insulin or IGF1 signalling and the nutrient-sensing TOR kinase pathway.** Target of rapamycin complex 1 (TORC1; mTORC1 in mammals) contains regulatory-associated protein of TOR (RAPTOR) and is sensitive to the drug rapamycin. TORC1 is responsive to nutrient availability and regulates cell growth and proliferation accordingly. The insulin or insulin-like growth factor 1 (IGF1) signalling (IIS) cascade crosstalks with TORC1 in its growth-regulating functions. The phosphoinositide 3-kinase (PI3K) pathway can be activated by IIS and in turn activate Akt, which phosphorylates tuberous sclerosis 2 (TSC2). This removes the TSC2-mediated inhibition on Ras homologue enriched in brain (RHEB), allowing RHEB to activate TORC1. In conditions of low nutrient availability, AMP-activated protein kinase (AMPK) promotes the repression on RHEB mediated by TSC1–TSC2. The downstream target of TORC1 is S6 kinase 1 (S6K1), which promotes cell growth and proliferation but can also negatively feed back to IIS. Crosstalk is also provided by TORC2, which phosphorylates Akt. IRS1, insulin receptor substrate 1; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; RICTOR, rapamycin-insensitive companion of TOR.

A-type lamin
A type V intermediate filament protein in the nucleus that is mutated in several human dystrophies and at least one severe progeria.

Cell senescence
The phenomenon in which replicatively dividing cells enter a non-dividing or quiescent phase that is accompanied by changes in gene transcription and metabolism.

homologue enriched in brain (RHEB)[61–63]. RHEB acts directly on TORC1, leading to its activation. In addition to being regulated by growth factors, TORC1 is also regulated by the energy-sensing AMP-activated protein kinase (AMPK), which promotes the repression on RHEB mediated by TSC1–TSC2 (REFS 64–66). Together with its ability to respond directly to nutrient abundance, many other input pathways place TORC1 at a key regulatory nexus that responds to nutrients, growth cues and the cellular energy status. TORC2 is responsive to growth factors, such as insulin, and promotes stress responses that are necessary for cell survival. In addition, TORC2 plays an important part in organizing the actin cytoskeleton[67,68]. TORC2 also mediates crosstalk between IIS pathways and TOR signalling by phosphorylating Akt[69]. Finally, one of the substrates of S6K1 is IRS1, the phosphorylation of which is important for feedback inhibition of IIS[70–72]. IRS1 phosphorylation by S6K1 attenuates IIS in response to sustained growth signals or under conditions of nutrient excess in a negative feedback loop. Together, these findings raise an important question: to what extent does altered TOR signalling or IIS lead to lifespan extension through enhanced FOXO activity or reduced phosphorylation of downstream targets of TOR and S6K1? One possibility is that maximum lifespan extension may result from the coordinated activation of FOXO and reduced activation of components downstream of TOR.

Several other genetic interventions promote mouse longevity, including deletion of p66SHC (also known as SHC1), which mediates signals involved in the production of intracellular reactive oxygen species (ROS)[73], and deletion of the RIIβ subunit of protein kinase A[74]. Another molecule linked to longevity is the tumour suppressor p53. Interestingly, aberrant activation of p53 promotes accelerated ageing[75], whereas increased p53 activity at the appropriate time contributes to lifespan extension[76]. p53 function has also been associated with HGPS and is discussed below in this context.

Human ageing studies. Ageing in humans has mainly been studied by identifying polymorphisms associated with long lifespan using various genome-wide or targeted approaches. Early studies proved difficult to replicate, and many genes and loci identified in one study were not seen in others. A second issue is whether the genes identified alter the risk of specific age-related diseases or the basic biology of ageing itself. Although both are interesting, only variants that alter basic biology are likely to be informative about the underlying mechanisms of ageing.

In recent years, gene association studies using both whole-genome screening and targeted approaches for subsets of longevity genes are becoming much more informative[77]. An increasing number of longevity studies are being carried out on different populations, more individuals in each study are being analysed, improved study designs are being used, and more sensitive technology is available. A large amount of data has accumulated, and suspected (as well as unexpected) longevity genes are starting to be identified. For example, polymorphisms in the *INK4a–INK4b* locus have been identified in many age-associated disease studies[78]. p16INK4A (also known as CDKN2A), also discussed below in the context of A-type lamins, is a known tumour suppressor that inhibits G1 cyclin–cyclin-dependent kinase (CDK) function, leading to activation of retinoblastoma protein (RB). p16INK4A has also been implicated recently in mouse ageing, particularly in the context of the maintenance of stem cell function[79]. In addition, polymorphisms have been identified in components of the IIS pathway and in other genes associated with longevity in animal models[80]. In the near future, these studies are likely to have more influence in shaping studies of animal models as they indicate which pathways are conserved in humans.

Another approach to examining human longevity has been to study cell senescence in culture. Many human cell types (fibroblasts have generally been the experimental cell type of choice) have finite replicative capacity in culture, eventually achieving a stable growth-arrested state that can be defined by certain molecular markers[81]. Recent studies have confirmed that senescent cells can be seen *in vivo*, and cell stress can drive the rapid onset of a senescent phenotype. Senescent cells also secrete a unique set of factors that can influence normal cells in their vicinity[82]. Cell senescence is discussed below in the context of A-type lamin function and HGPS.

Clavicular agenesis
The incomplete development of the clavicle.

Diseases resembling premature ageing

Among the several human syndromes in which pathology associated with normal ageing seems to accumulate at an accelerated rate, Werner syndrome and HGPS stand out. In these diseases, many tissues show age-related phenotypes. However, they are both segmental in nature, as only a subset of tissues seems to age prematurely[83]. Humans with these syndromes also show phenotypes that are not common during normal ageing, including clavicular agenesis in HGPS and an abnormal tumour spectrum in Werner syndrome. Nevertheless, many researchers have speculated that knowledge of the molecular pathology of these diseases will also provide insight into normal ageing.

We focus on HGPS, as rapid progress has been made in the past 7 years following the identification of disease-associated lamin A/C (*LMNA*) mutations as causal elements. Mouse models of HGPS have proven to be immensely powerful research tools for understanding the pathology of progerias. Although a mouse strain that completely recapitulates all of the disease symptoms of HGPS has yet to be developed, several mouse models are available that interfere with the carboxy-terminal processing of lamin A and display a range of phenotypes that resemble aspects of the human syndrome[84]. Werner syndrome is discussed briefly in BOX 2, with emphasis on mechanistic insights that may overlap with HGPS and normal ageing. In BOX 3, we describe findings regarding a set of childhood-onset progeria-like diseases, including Cockayne syndrome and trichothiodystrophy, in which links have been made between DNA damage and altered metabolism. These findings potentially connect processes that have been long-speculated to be involved in the normal ageing process.

Hutchinson–Gilford progeria syndrome. In all metazoans examined to date, the inner nuclear membrane is in contact with a protein network called the nuclear lamina[85], which is composed of type V intermediate filaments termed lamins and several lamina-associated proteins. All A-type lamins, including the two predominant forms, lamin A and lamin C, are derived from alternative splicing of *LMNA*, whereas the two B-type lamins are encoded by two genes: *LMNB1* and *LMNB2* (REF. 86). Although the presence of at least one B-type lamin is thought to be essential for viability, expression of *LMNA* is developmentally timed, and a knockout mouse model is viable[87,88].

Both A- and B-type lamins undergo a sequence of post-translational modifications at the C terminus, a topic that has been covered extensively in other reviews[89]. Briefly, the nuclear lamins contain a Cys-Ala-Ala-X (CAAX) prenylation motif at the C terminus, which drives farnesylation of the Cys residue by thioether linkage. The Ala-Ala-X tripeptide of the C terminus is then cleaved, and the farnesylated Cys is carboxymethylated. Finally, the endopeptidase zinc metalloproteinase Ste24 homologue (ZMPSTE24) proteolytically cleaves nuclear lamins at Lys647 to liberate a 15 amino acid product, which contains the Cys-bound isoprenyl lipid. This second cleavage site is directed by a six-peptide sequence that is unique to lamin A maturation (lamin B permanently retains the isoprenoid[90]).

Mutations in *LMNA* give rise to specific genetic disorders with tissue specificity, including Emery–Dreifuss muscular dystrophy, limb girdle muscular dystrophy, familial partial lipodystrophy, mandibuloacral dysplasia and Charcot–Marie–Tooth neuropathy[86,91]. Collectively termed laminopathies, these diseases are, with notable exceptions, the result of heterozygous missense mutations resulting in single amino acid substitutions. Although most mutations are dominant, they are likely to have different consequences on A-type lamin function, with some mutations acting in a dominant-negative manner and others conferring increased or new functions. Among these laminopathies, mutation of *LMNA* is responsible for a severe autosomal dominant human progeria, HGPS, which occurs in approximately 1 in 4 million live births[92]. The most common HGPS mutation (at Gly608) is silent with respect to the coding sequence, but introduces a cryptic donor splice site that drives the production of a protein isoform containing

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Box 2 | Werner syndrome

Although the onset is slower than Hutchinson–Gilford progeria syndrome (HGPS), the pathology that accompanies Werner syndrome resembles (but also differs from) premature ageing to an extent that is comparable to HGPS. Clinical pathology starting at 10–20 years of age includes short stature, bilateral cataracts, early greying, hair loss and scleroderma-like skin changes[151]. Other age-related pathologies include osteoporosis, hypogonadism, type I diabetes, atherosclerosis and neoplasms, with the median age of death at 54 years of age. The neoplasms are interesting in that they do not overlap with neoplasms that commonly occur in normal ageing individuals.

Werner syndrome results from mutations in Werner syndrome ATP-dependent helicase (WRN), which belongs to the RecQ protein family and has several enzymatic functions linked to DNA metabolism, including helicase, ATPase, exonuclease and single-strand annealing activities[152,153]. Mutations are loss of function in nature. The cellular phenotypes associated with Werner syndrome show significant overlap with laminopathies even though WRN function has not been linked directly to that of lamin A. For instance, cells lacking WRN have defects in the repair of DNA double-strand breaks, particularly those linked to DNA replication fork arrest. WRN-mutant cells also have enhanced telomere attrition, uncovering a role for WRN in promoting the repair of chromosome ends. Increased cell death and/or senescence (as well as incidence of neoplasms) may result from defects in DNA damage repair and telomere end maintenance. Another commonality between Werner syndrome and laminopathies is that they predominantly affect mesenchymal cell lineages. Together, these findings implicate enhanced DNA damage and proliferation defects due to impaired telomere maintenance in the onset of pathology that is associated with Werner syndrome.

Box 3 | Progeria-associated syndromes linked to deficient DNA repair

A range of diseases, such as Cockayne syndrome, tricothiodystrophy and ataxia telangiectasia, are characterized by a subset of progeria phenotypes, including cachexia, kyphosis, retinal degeneration, shortened lifespan, neurodevelopmental delay and deafness[154,155]. Cockayne syndrome is linked to mutations in either *CSA* (also known as *ERCC8*) or *CSB* (also known as *ERCC6*) and tricothiodystrophy to mutations in xeroderma pigmentosum group B complementing *XPB* (also known as *ERCC3*), *XPD* (also known as ERCC2) or *TTDA* (also known as *GTF2H5*), all leading to deficient transcription-coupled repair (TCR). Mutation in *XPB* and *XPD* can also cause xeroderma pigmentosum, a cancer-prone syndrome with only minor links to progeria. These mutations differ from those associated with progerias in that they more broadly affect nucleotide excision repair (NER). A possible explanation is that defective NER leads to a rate of mutations that is sufficient to stimulate high levels of cancer incidence, whereas defects in TCR affect a smaller region of the genome (transcriptionally active regions) and the accrued mutations are insufficient for cancer progression[154]. Ataxia telangiectasia is caused by defects in DNA repair and cell cycle checkpoint control, implicating these processes in the ageing process.

Although the disease mechanisms for these syndromes are not fully resolved, recent findings have provided a link with metabolism[154]. A mouse model of severe Cockayne syndrome shows accompanying defects in insulin or insulin-like growth factor 1 (IGF1) signalling (IIS), hypoglycaemia associated with hepatic glycogen and fat accumulation, reduced oxidative metabolism and increased antioxidant responses[156]. A broader comparison at the genome-wide transcriptional level showed that mouse models of DNA repair-deficient progeroid syndromes have extensive phenotype similarities across a range of tissues with long-lived dwarf mice[157]. The authors speculate that defective DNA repair pathways may trigger survival responses in an attempt to reduce cellular production of DNA-damaging agents. The fact that a similar response is associated with long lifespan suggests that the triggering agent, namely DNA lesions, may be a causal agent in normal ageing. One concern is that many of these mouse models of progerias have severe defects that result in markedly reduced lifespan, leading to speculation over what extent other short-lived mouse models that are unrelated to progerias show a similar response. Is reduced IIS a common response to a range of stress-inducing events experienced at the organismal level? Concerns aside, studies like these are important in that they attempt to connect divergent molecular models of ageing, potentially leading to unifying theories.

a 50 amino acid deletion in the C-terminal portion of lamin A[93]. This aberrantly spliced lamin A isoform is termed progerin[94]. Progerin retains the CAAX pre-nylation motif but lacks the second proteolytic cleavage site, resulting in a permanently farnesylated lamin A protein.

A-type lamins participate in several important nuclear functions, as they localize both in the periphery of the nucleus between chromatin and the nuclear envelope and in a nucleoplasmic pool. Consistent with the structural properties of intermediate filaments, lamin A/C is crucial for nuclear integrity. In *LMNA*^{−/−} fibroblasts, nuclear morphology is grossly perturbed, resulting in loss of lamin B localization, clustering of nuclear pore complexes and translocation of emerin from the inner nuclear membrane to the cytosol[88]. Lamin A/C also coordinates the activity of several transcription factors, helping them stably associate with the nuclear substructure and maintain activity[95]. This has consequences that can affect tissue function. For instance, lamin A/C function in adult muscle stem cells is required for proper levels of myogenic transcription factors, which are in turn important for proper differentiation into myofibres[96]. Lamin A/C-dependent recruitment of FOS to the nuclear periphery is required for FOS function[97]. Improper lamin A/C activity may contribute to inappropriately increased mitogen-activated protein kinase (MAPK) activity, a possible determinant in dilated cardiomyopathy, which is associated with at least three laminopathies[98]. The activity of several other transcription factors is linked to A-type lamins and their associated proteins, including β-catenin[99] and the Notch signalling cascade[100]. Proper epigenetic control also requires A-type lamins, as markers for both constitutive and facultative heterochromatin are markedly altered in cells expressing progerin[101–103], and a two-fold loss of heterochromatin protein 1α (HP1α), which mediates attachment of heterochromatin to the nuclear lamina, is observed in HGPS fibroblasts[104]. Finally, lamin A/C has a poorly defined role in coordinating DNA replication. Amid the many functions of A-type lamins, how does one identify those that are altered in laminopathies and which of these alterations drive disease progression?

Mechanisms underlying HGPS: recent advances

Since the identification of *LMNA* as the target gene for mutations driving HGPS, research into disease mechanisms has markedly increased. Several possible, often non-exclusive, hypotheses have been generated, which are described below, emphasizing possible links to normal ageing. The efficacy of farnesyltransferase inhibitors as a therapeutic for HGPS is discussed in BOX 4.

Enhanced DNA damage and defective repair. Reactive oxygen species accumulate at a higher rate in HGPS fibroblasts[105], an interesting feature that is shared with normally aged fibroblasts. This may contribute to increased levels of DNA damage and may underlie defects in proliferation or early senescence associated with HGPS cells[106,107]. Although the link between ROS accumulation and short lifespan in individuals with HGPS remains correlative and indirect, further studies are likely to yield promising results.

HGPS cells display persistent markers of increased basal DNA damage, such as nuclear ataxia telangiectasia mutated (ATM) and ATM- and RAD3-related (ATR) foci[108]; activation of these protein kinases is characteristic of genomic instability. This is consistent with

observations in HGPS cells and mouse cells lacking the lamin A-processing endopeptidase ZMPSTE24, which have increased chromosomal aberrations and increased sensitivity to DNA-damaging agents[107]. Fibroblasts from patients with HGPS also show increased amounts of basal phosphorylated histone variant H2AX (γH2AX) and increased levels of phosphorylated checkpoint kinase 1 (CHK1) and CHK2, compared with unaffected fibroblasts[108]. Interestingly, γH2AX in HGPS cells with DNA double-strand breaks (DSBs) co-localized with xeroderma pigmentosum group A (XPA) foci, an essential factor of nucleotide excision repair (NER), and not with DSB repair proteins[109]. This co-localization was specific to XPA and not to other recognition enzymes involved in NER, suggesting that either the type of basal damage that exists in HGPS cells may be qualitatively different from damage accrued by other exogenous genotoxic stresses or the recruitment process to DSBs differs. However, it is unknown to what extent ROS contribute to increased DNA damage in these cells. In either case, persistent, unrepaired DNA damage can promote permanent forms of cell cycle arrest, including apoptosis and senescence.

Studies of fibroblasts isolated from individuals with HGPS show that lamin A plays an important part in the efficiency of repair of DNA lesions. Fibroblasts from individuals affected by HGPS, or from mice lacking ZMPSTE24, show a marked delay in the recruitment of p53 binding protein 1 (53BP1) to sites of DNA repair on exposure to DSB-inducing irradiation[110]. The delay in 53BP1 recruitment to DSBs in these cells preceded the presence of unresolved DNA damage foci, and the accumulation of irreparable damage may be a potent physiological genotoxic stress in individuals with HGPS. Increased levels of DNA damage may have important consequences *in vivo*. The homozygous knockout of *Zmpste24* in mice results in the accumulation of prelamin A and a progeroid phenotype [111].

Progeria is clearly linked to unprocessed lamin A, as knockout of one copy of *Lmna* in the *Zmpste24⁻/⁻* background ameliorates disease phenotypes[112]. Interestingly, this mouse strain shows increased levels of p53 transcriptional targets, such as growth arrest and DNA damage-inducible protein 45α (GADD45α), p21 and activating transcription factor 3 (ATF3)[112], which are not accompanied by a detectable increase in levels of activated p53. Furthermore, some features of the *Zmpste24⁻/⁻* phenotype, including an increase in the onset of cell senescence, are ameliorated in the *Zmpste24⁻/⁻ Tp53⁻/⁻* double mutant, indicating that the premature ageing phenotype in this mouse strain is at least partially dependent on p53 hyperactivation. In a separate report, expression of progerin in normal diploid fibroblasts was not found to activate p53 target genes[113], so the role of p53 activation in promoting HGPS pathology remains unclear.

Altered cell proliferation and senescence. Several studies have addressed the senescent properties of HGPS cells. HPGS fibroblasts are reported to undergo premature senescence[106,114]. Moreover, exogenous expression of progerin in cultured cells is sufficient to induce some of the progeroid features seen in HPGS fibroblasts, such as abnormal nuclear morphology and reduced proliferation, and the expression of markers of premature cell senescence[113,115], such as senescence-associated β-galactosidase activity.

There are intriguing parallels between early-passage HGPS primary fibroblasts and aged fibroblasts with regard to chromatin changes. Notably, aged human fibroblasts show reduced nuclear staining of lamina-associated polypeptide 2 (LAP2), HP1α, H3 Lys9 trimethylation (H3K9me3) and nucleoplasmic lamin A/C compared with young control cells, all of which resemble changes seen in HGPS fibroblasts[116]. In addition, progerin can be detected at low levels in normal human

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Box 4 | Farnesyltransferase inhibitors as therapeutics for HGPS

Given that farnesylated lamin A (known as progerin) exerts a dominant effect on the pathophysiology of Hutchinson–Gilford progeria syndrome (HGPS), it was reasonable to test the effect of farnesyltransferase inhibitors (FTIs) on progerin-expressing cells. Treatment of HGPS fibroblasts with FTIs has proven useful in reversing the morphology of HGPS nuclei[158] and modestly delays mortality in progerin-expressing mice[159]. These findings indicate that stable farnesylation is a contributing factor to nuclear defects. Cells expressing progerin have altered localization of A-type lamins (both progerin and lamin A/C) such that peripheral localization is enhanced and internal localization is diminished. At the periphery, the assembled lamin structure is rigidified, altering nuclear dynamics. In addition, progerin can assemble into filaments with lamin B, generating a more homogenous intermediate filament assembly instead of the parallel lamin B and lamin A/C assemblies thought to occur in normal cells[160]. However, it cannot be excluded that a subset of phenotypes in progerin-expressing cells arises from loss of lamin A/C in the nuclear interior.

These findings have led to the initiation of a clinical trial with patients with HGPS, using a combination of agents designed to inhibit lamin A isoprenylation. In addition to FTIs, a combination of statins and aminobisphosphonates are included in the trial, as lamin A can also be geranylgeranylated. These agents are designed to block both pathways simultaneously. This is clearly warranted, as statins and aminobisphosphonates together have been shown to improve several phenotypes of mice lacking zinc metalloproteinase Ste24 (*Zmpste24*), including survival[161]. The results from this trial remain unknown, but initial findings should be available in the near future. In the progeria mouse model, the modest delay in mortality can be explained by the observation that mice expressing a non-farnesylatable progerin still develop progeroid phenotypes, but at a slower rate[162]. This indicates that stable farnesylation contributes to, but does not completely account for, the toxicity of progerin. Nevertheless, even delay in the onset with pathology by FTIs would be of high value to patients with HGPS.

fibroblasts[116,117], indicating that the cryptic donor splice site that is active in HGPS cells drives a low level of aberrant splicing in healthy normal cells. Expression of progerin can also be detected in tissue isolated from aged healthy individuals[116,118]. Although a role for progerin in normal healthy ageing is largely based on correlative observations, there is certainly cause for further inquiries into a possible role for A-type lamins in normal ageing.

In contrast to the aged, individuals with HGPS are not predisposed to a higher incidence of neoplasia[119], possibly because death due to severe atherosclerotic disease precludes the onset of malignancies. Malignancy has been documented in only two subjects with HGPS, one of which was heterozygous for an atypical mutation (Cys1868Gly) that, similarly to the progerin mutation, interferes with C-terminal processing and lamin A maturation[120–122]. Both patients developed osteosarcoma, a malignancy often associated with hereditary retinoblastoma in young adolescents[123]. Retinoblastoma is most often associated with loss of heterozygosity of *RB* in otherwise heterozygous individuals. RB, a tumour suppressor that functions in cell cycle regulation, has been known to localize to lamin A/C-associated internal nuclear foci, and the affinity of this interaction depends on the RB phosphorylation state[124,125]. Interestingly, lamin A/C and LAP2α have been shown to interact with RB *in vitro*, suggesting that the nuclear lamina has an important role in anchoring hypophosphorylated RB to the nuclear matrix during G1 phase[126,127]. In support of this hypothesis, fibroblasts from *Lmna*^{−/−} mice show markedly reduced levels of RB and the related protein, p107, compared with wild-type controls[128]. This has functional consequences, as *Lmna*^{−/−} fibroblasts are unresponsive to cell cycle arrest mediated by another tumour suppressor, p16INK4A[129]. It is intriguing that osteosarcoma was identified in a non-canonical progeria mutant. A study of lamin A disease mutants reported that expression of progerin in *Lmna*^{−/−} mouse fibroblasts is sufficient to restore RB stability[129]. Thus, individuals expressing progerin may be refractory to tumorigenesis owing to RB dysfunction.

The expression of lamin A is downregulated in several malignant and benign neoplasms, such as small cell lung carcinoma, colorectal carcinoma and adenomatous polyps of the colon[130–132], leading to speculation that lamin A/C functions as a tumour suppressor itself. Further evidence for the tumour suppressor activity of lamin A comes from the recent report of a direct interaction between the rod domain of lamin A/C and the epigenetic regulator inhibitor of growth protein 1 (ING1)[133]. The Ing protein family interacts with histone acetyltransferases and histone deacetylase complexes to modify chromatin; these complexes are also inactivated in many cancers. Like RB, ING1 is stabilized by lamin A, and, interestingly, this interaction is compromised by the expression of progerin in HGPS fibroblasts[133]. In summary, these reports establish a role for lamin A in stabilizing tumour suppressors, although the functional consequences with regard to laminopathies and progerias are an ongoing area of investigation.

Mesenchymal stem cell
A pluripotent progenitor cell of mesenchymal origin that gives rise to adult tissues such as bone, cartilage and adipose.

Altered telomere dynamics. Telomere shortening occurs as cells approach replicative senescence[134]. The loss of telomeric DNA that occurs as the result of both *in vitro* and *in vivo* ageing initiates a DNA damage checkpoint response, with the appearance of cellular markers characteristic of DSBs, such as co-localization of 53BP1 and nijmegen breakage syndrome protein 1 (NBS1; also known as NBN) with γH2AX foci[135,136]. Enhanced telomere attrition is seen in fibroblasts from individuals with HGPS[114,137,138]. This is probably a cell-autonomous effect, as telomere length in cells from patients with HGPS that do not express A-type lamins (granulocytes and T cells) was found to be normal[138].

However, telomere effects are not specific for progerin expression, as fibroblasts from *Lmna*^{−/−} mice are reported to have shorter steady-state telomere lengths, and knockdown of *Lmna* results in progressive telomere shortening. Telomere length changes may reflect a direct role for A-type lamins in coordinating telomere function. Consistent with this, one study[103] recently reported that antibodies specific for lamin A/C immunoprecipitate telomeric DNA, and A-type lamins regulate the subnuclear position of telomeres. In addition, another group[139,140] reported that telomeres from human mesenchymal stem cells (MSCs) cluster in intranuclear foci containing lamin A/C specifically during senescence or apoptosis.

Finally, there is evidence that cell proliferation defects associated with progerin expression may be linked to telomere dysfunction, as human diploid fibroblasts stably engineered to express progerin exhibited a proliferation defect that could be rescued either by expression of the catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), or by expression of the human papillomavirus E6 protein, which inactivates p53 (REF. 113). One intriguing and yet to be tested possibility is that the enhanced basal levels of DNA damage in progerin-expressing cells may be located directly at telomeres, reflecting a defect in telomere dynamics. This idea has not been directly investigated; however, it has been reported that the loss of A-type lamins leads to defects in the telomere end-to-end fusions that are promoted by unprotected chromosomal ends[103]. This phenotype is probably linked to the observation that lamin A/C is required for normal expression of 53BP1, which plays an active part in this process.

Mesenchymal stem cells. Damaged and non-functional tissue leads to physiological decline and must be replenished to retain tissue functionality. Homeostasis is accomplished through the action of a self-renewing population of stem cells with the capacity to differentiate into new tissue. Stem cell populations in adults decline as a function of age, and several lines of evidence suggest that MSCs are affected. Human MSCs are multipotent progenitors that drive regeneration by differentiating along lineages into cell types that can contribute to specialized tissue in adults[141]. In terms of homeostasis, tissue degeneration can result from either an increase in cellular damage or a decrease in regeneration potential, or from a combination of both. Evidence in support of the combination hypothesis
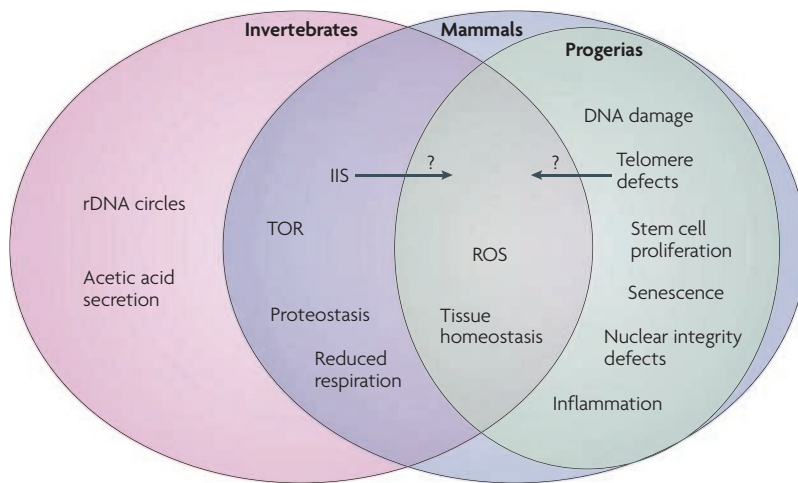
Figure 3 | **Pathways shared between ageing in invertebrate model organisms, mammals and human progerias.** Several features of ageing, which have been elucidated by genetics and biochemistry in invertebrate and mammalian model organisms, are also characteristic features in human progerias such as Hutchinson–Gilford progeria syndrome. Although some identified molecular pathways of ageing are likely to be specific, we suggest that many are likely to be conserved across species. The alteration of a subset of mammalian pathways affecting longevity may be linked to the development of progerias. IIS, insulin or IGF1 signalling; rDNA, ribosomal DNA; TOR, target of rapamycin; ROS, reactive oxygen species.

is provided by recent investigations in immortalized human MSCs, in which exogenous expression of progerin was shown to induce changes in differentiation potential[100]. Interestingly, progerin reduced the potential to differentiate along the adipogenic lineage, which may be linked to the loss of subcutaneous fat that is characteristic of individuals with HGPS. However, in mouse preadipocytes, accumulation of prelamin A, expression of dominant lipodystrophy-associated *Lmna* mutations or small interfering RNA-mediated knockdown of *Lmna* do not affect differentiation[142]. Cell type differences may play a part in these apparent disparities, so further investigation into the role of lamin A/C in differentiation and tissue homeostasis is necessary. Lamin A/C is absent from undifferentiated mouse and human embryonic stem cells[143], consistent with the findings that development of the *Lmna*^-/- mouse embryo seems largely unhindered.

In addition to altered differentiation potential, a defective nuclear lamina influences stem cell number and replicative potential. Using the hair follicle as a model, one group examined stem cell dynamics in the *Zmpste24*^-/- mouse strain and reported an increase in the number of epidermal stem cells with reduced replicative potential[144]; the phenotype was linked to defective Wnt signalling. Together, these findings suggest a disease model for the laminopathies in which defective tissue homeostasis culminates from a loss of regeneration potential in damaged tissues.

Unifying the approaches: a framework for ageing
In the past few decades, researchers of ageing have moved along several parallel tracks to define the molecular secrets of the ageing process, and many models

have centred on hypotheses involving unimodal causes. Nevertheless, it has become clear that although supportive evidence for many different molecular causes of ageing has been generated, the proverbial 'definitive experiment' for any of these models has yet to emerge. This has led researchers of ageing to think in terms of multiple molecular factors driving the ageing process. A multifactoral model must, however, account for genetic findings in which manipulation of the activity of a single gene leads to significant lifespan extension.

Evaluating the genes that influence ageing across divergent model organisms has provided support for a multifactoral model. For instance, comparison of pathways modulating ageing between worms and yeast was notable for providing quantitative evidence of conserved longevity pathways[11]. However, the extent of overlap was limited, and the study also provided further evidence for private (that is, specific to a particular evolutionary lineage) mechanisms of ageing. For yeast, private mechanisms of ageing have already been proposed[53,145]. In an effort to include many exciting veins of ageing research, we put forward a multifactoral model (FIG. 3), which, when viewed from the pathways affecting ageing, has elements that are conserved in invertebrates and others that are specific to progerias. This model represents a 'best guess' of the level of conservation for each pathway but is not fully inclusive, so it will be further elaborated as new findings emerge. An advantage is that it could account for why certain individuals are more susceptible to specific diseases with increasing age, as each individual is likely to experience different levels of contribution from specific ageing pathways. We think that the bulk of evidence supports multiple causes of ageing and that the inclusiveness of this thinking is likely to encourage findings that bridge different lines of research.

How does a multifactoral ageing model relate to progerias such as HGPS? The obvious benefit to this way of thinking is that a segmental progeria, which seems to have only a subset of premature ageing phenotypes, can be affected only for a subset of pathways that combine to promote ageing. HGPS and Werner syndrome, for example, may have altered nuclear mechanics leading to the reduced maintenance of specific types of stem cell that impair the ability to replenish tissues, leading to the accelerated appearance of ageing and early mortality. Cockayne syndrome and tricthiodystrophy may have an altered endocrine axis and increased susceptibility to DNA damage. Other syndromes that have a more modest resemblance to premature ageing may alter only one ageing pathway (for example, dyskeratosis congenita, which affects telomere dynamics[146]). Given that ageing pathways may interact at least indirectly, enhanced contribution from some but not all ageing pathways may have different consequences to a slow progressive contribution from many or all that accompany normal ageing. If this model is correct, it provides a sound argument for studying progerias to understand the normal ageing process, while keeping in mind that they are not a complete phenocopy.

REVIEWS

Conclusions

Humans have been interested in slowing the ageing process for thousands of years, but the complexity of the phenomenon has made it resistant to intervention. In a time when diseases of ageing have taken over as the leading killers in the first world, the mandate for targeting ageing to slow age-related disease is clearly evident. The fact that genes in many important biological pathways can affect the rate of ageing in model organisms lends credence to the idea that there are multiple causes of ageing. Research in the basic biology of ageing has focused on interventions that extend the maximal longevity of an organism because this means that the affected pathway must impinge on the ageing process. In addition to studying long-lived models of ageing, we think that progerias such as HGPS are a unique 'window' into a subset of normal ageing mechanisms and that the view from this window may provide a 'scene' that is hard to see using invertebrate models.

The finding that the TOR inhibitor rapamycin extended longevity, even when administered late in the lifespan of a mouse, is the first clear example of targeting ageing to address age-related disease. This raises an interesting question: what would a drug that extends normal mouse lifespan do to a progeroid mouse? Would rapamycin, for instance, help? If the molecular pathology that drives HGPS resembles an accelerated form of normal ageing, then drugs that slow normal ageing may have similar efficacy in these models or in human patients, in whom the severity of the disease may increase the probability of potential side effects. Rapamycin has also been extensively tested in the clinic and even approved for a range of uses. Although its role as an anti-ageing medicine is unclear, rapamycin is probably only the first of many candidates that will emerge in the near future. With every new candidate, the likelihood increases that one will be efficacious, leading to a realization of the medical promise of ageing research.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
age-1 | LMNA | LMNB1 | LMNB2
OMIM: http://www.ncbi.nlm.nih.gov/omim
Charcot–Marie–Tooth neuropathy | Cockayne syndrome | Emery–Dreifuss muscular dystrophy | familial partial lipodystrophy | HGPS | limb girdle muscular dystrophy | mandibuloacral dysplasia | trichothiodystrophy | Werner syndrome
UniProtKB: http://www.uniprot.org
AKT-1 | ATF3 | 53BP1 | DAF-2 | DAF-16 | 4EBP1 | GADD45α | ING1 | LAP2 | NBS1 | p21 | p53 | p16INK4A | p66SHC | RB | RHEB | SGK-1 | TSC2 | ZMPSTE24

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