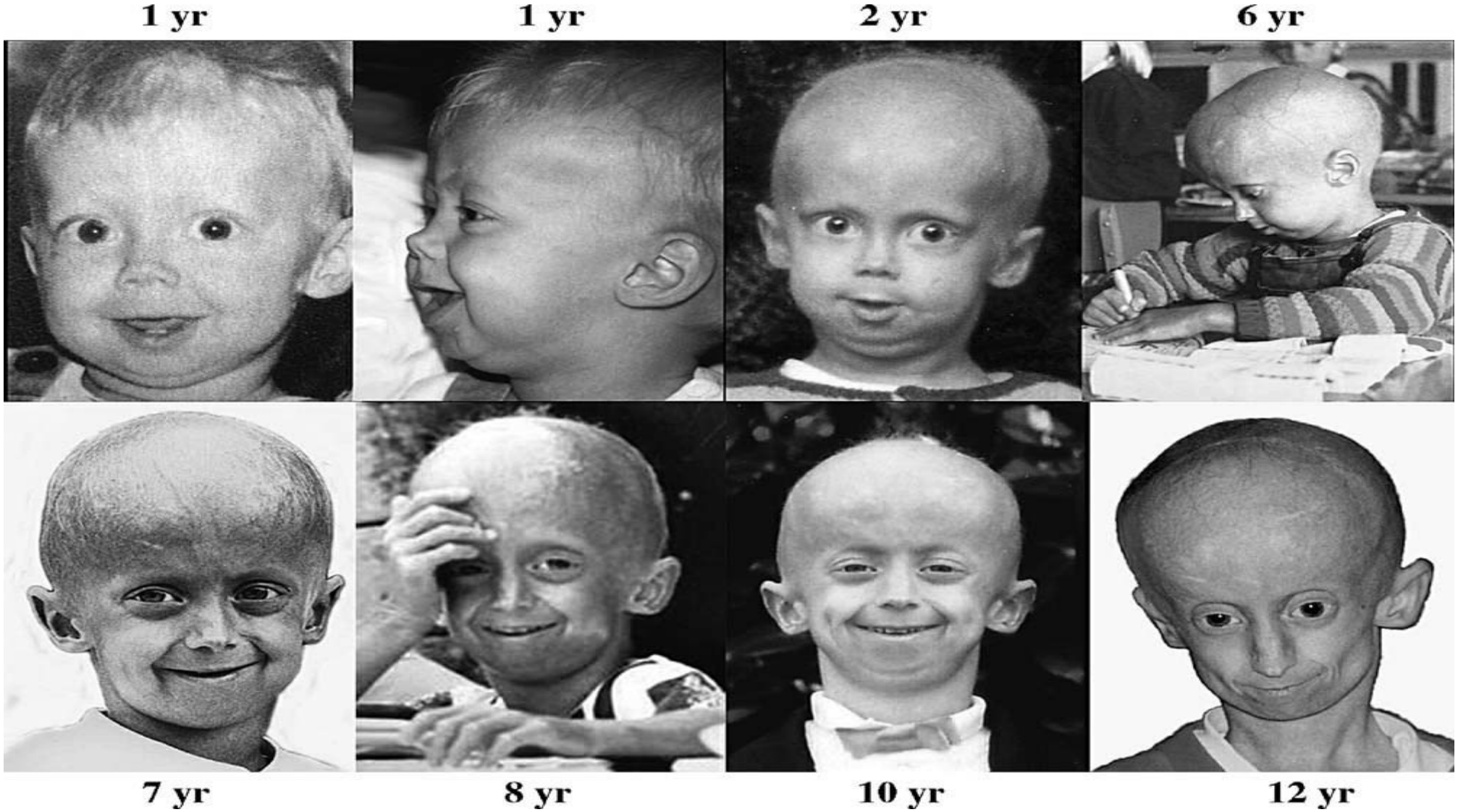
Nucleolar expansion and elevated protein translation in premature aging

Abigail Buchwalter & Martin W. Hetzer

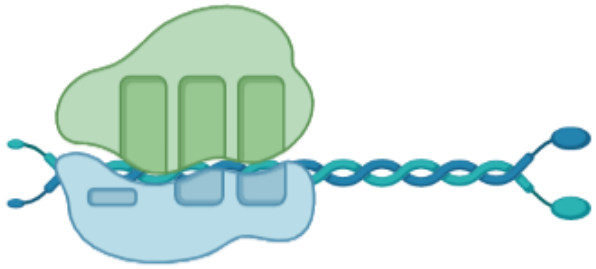
Present by Kexin Sun

What is Hutchinson-Gilford progeria syndrome（HGPS）?

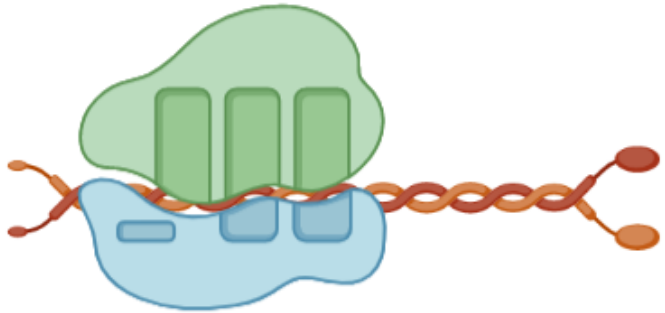


What is the genetic bases of HGPS?

Normal cells process lamin A normally



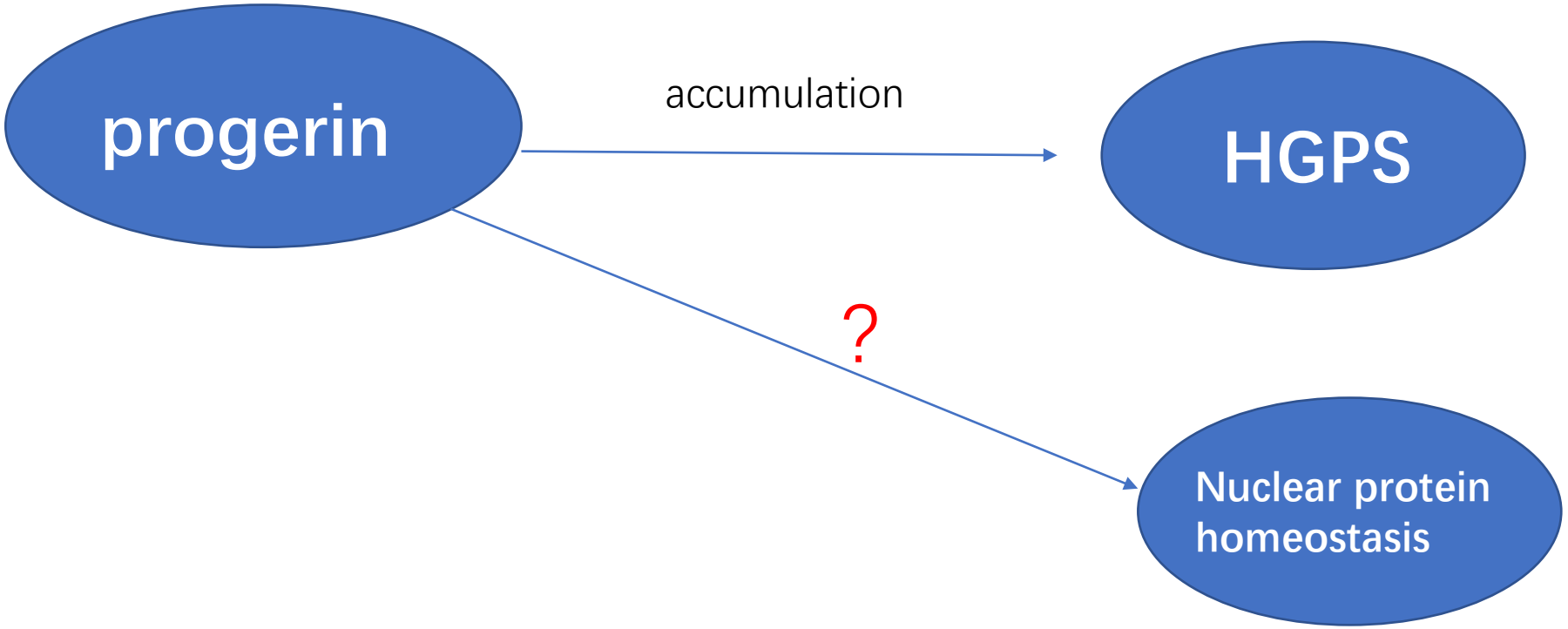
Mutant lamin A, progerin, causes HGPS in mice model



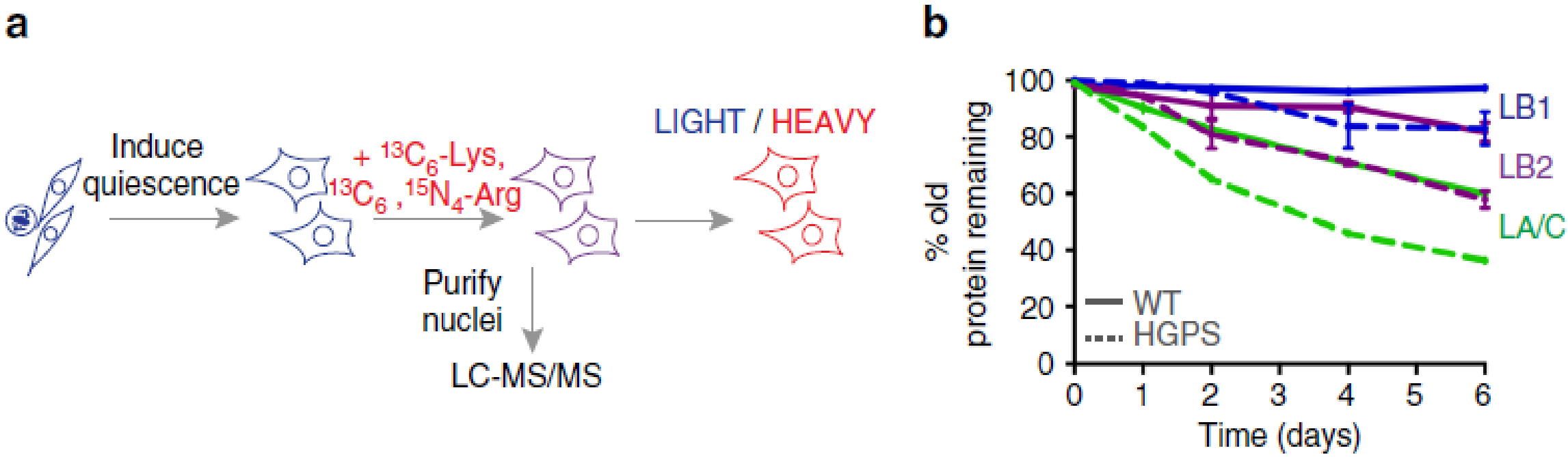
What does progerin do?

- **Distorts nuclei**
- **Disrupts the nucleoplasmic network**
- **Depletes heterochromatin marks**
- **Sequesters nuclear proteins**
- **Includes DNA damage**

GAP: The mechanistic link between progerin and aging remains unclear

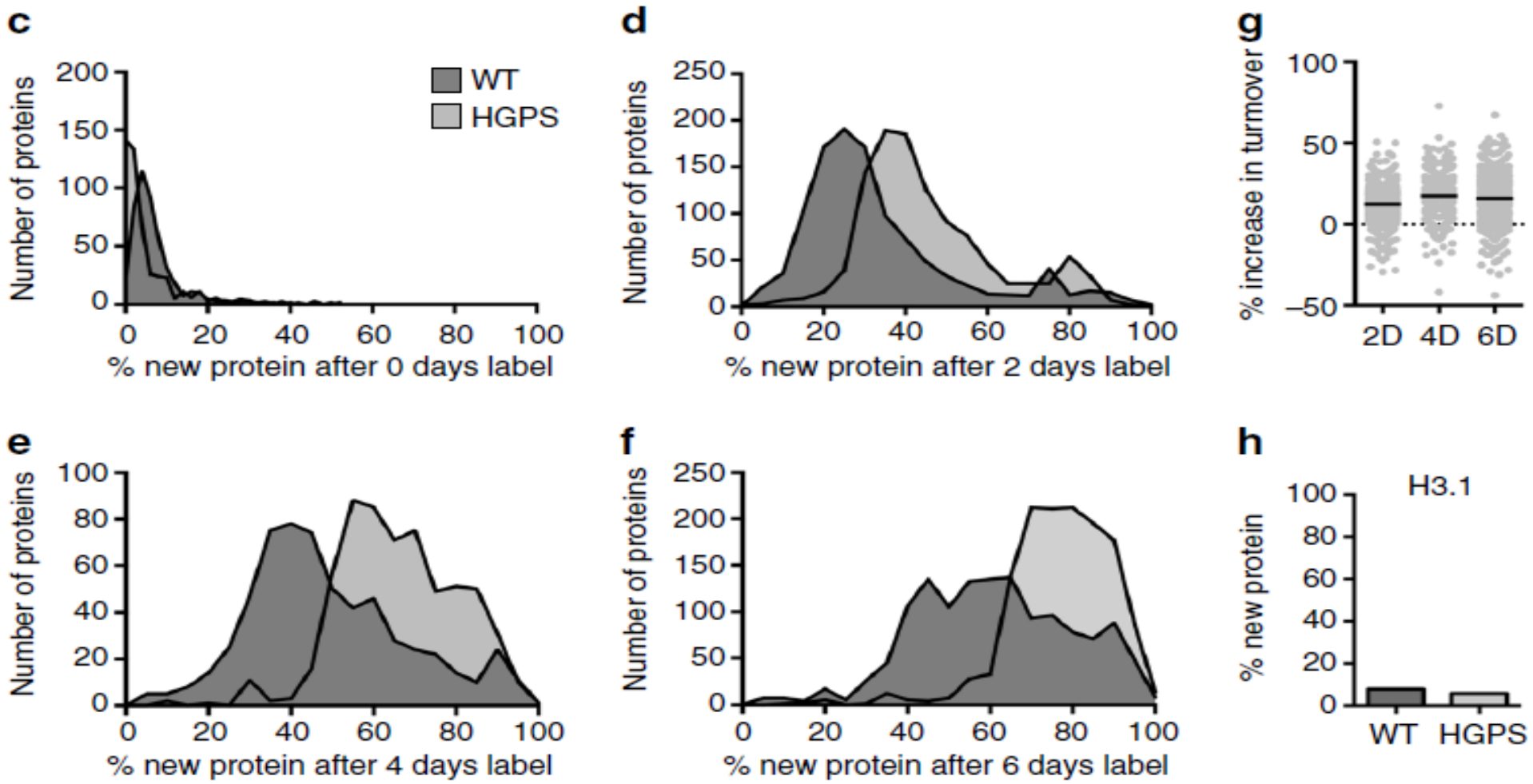


What's difference between HGPS cells and normal cells in protein turnover?

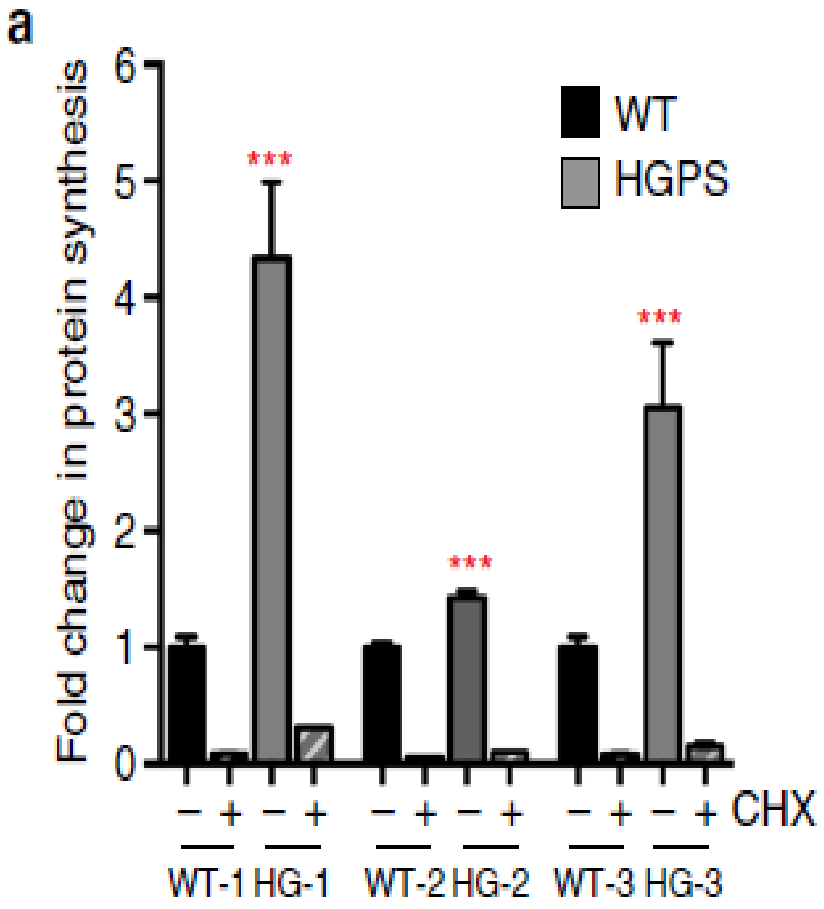


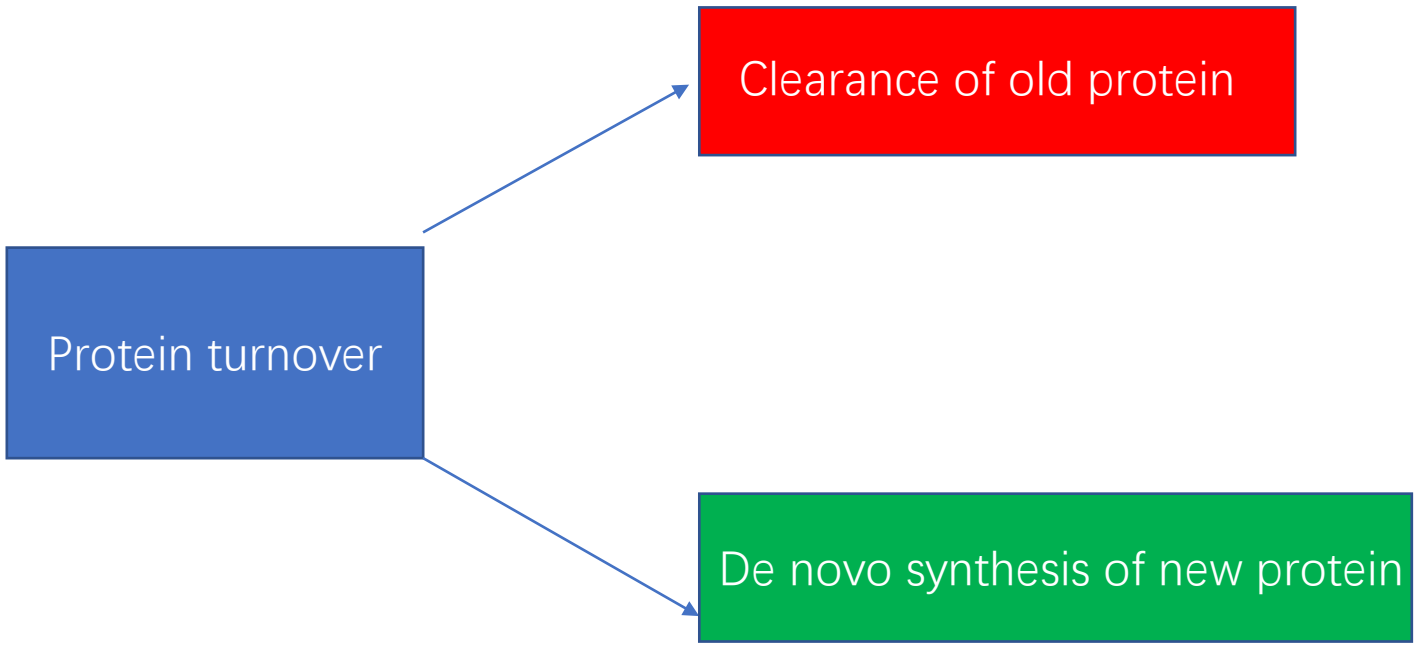
Evidence showing that increased turnover of all lamin isoforms in HGPS cells

What happens in the turnover rates for the entire nuclear proteome?



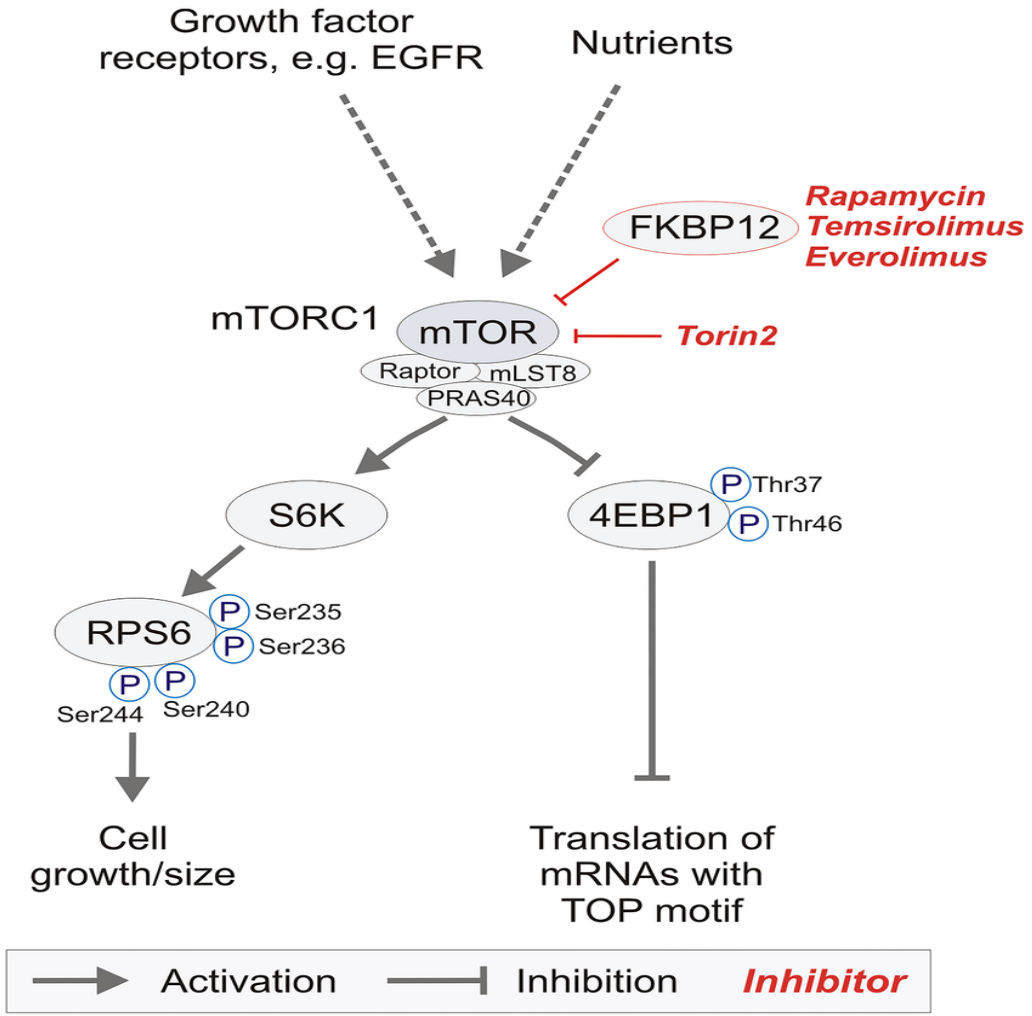
What is the biological process that accelerate the protein turnover?





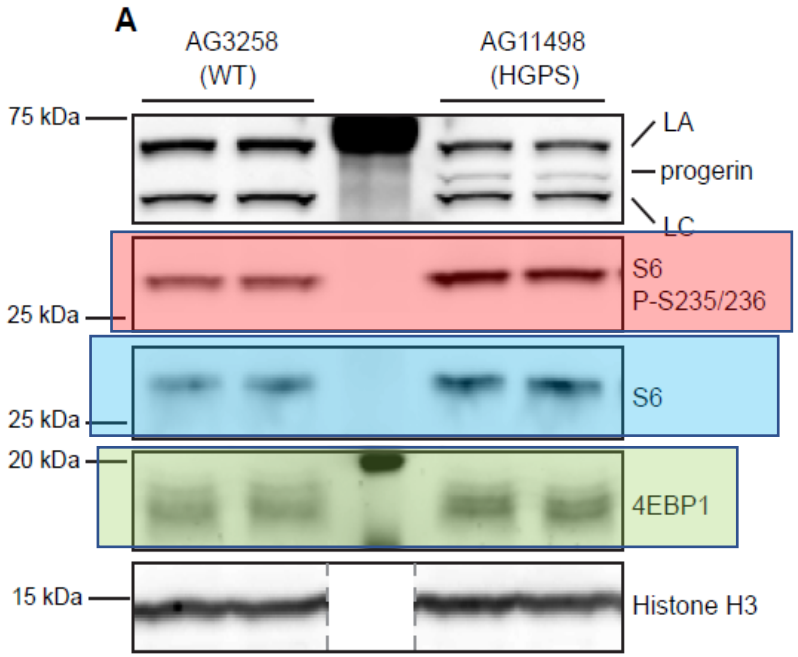
Global transition is increased and nucleoli are enlarged in HGPS

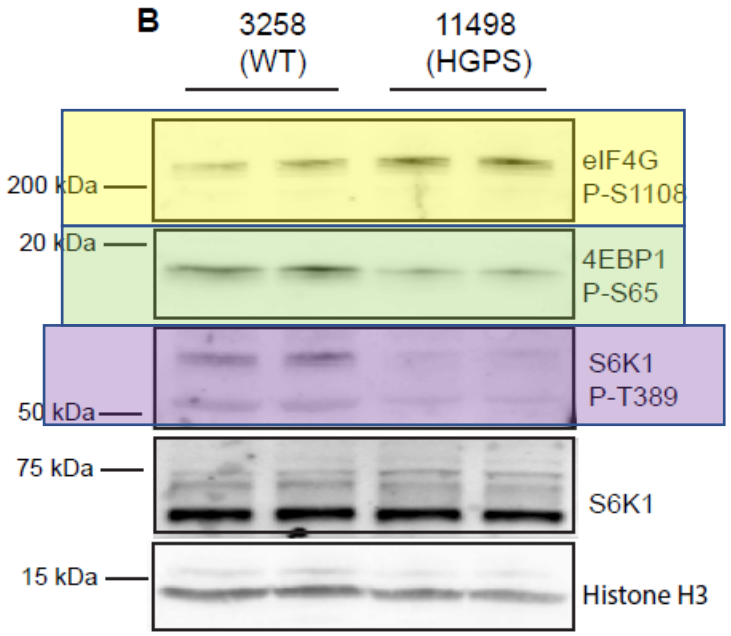
Why researchers focus on mTOR pathway?

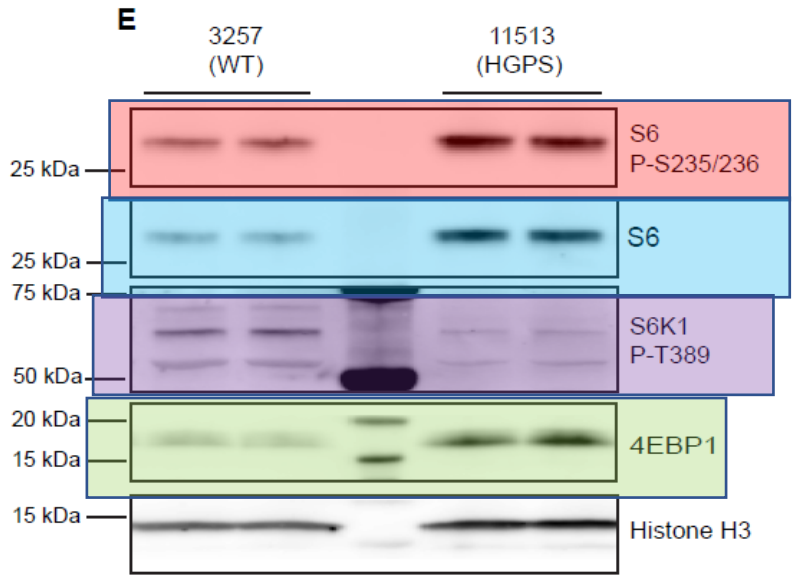


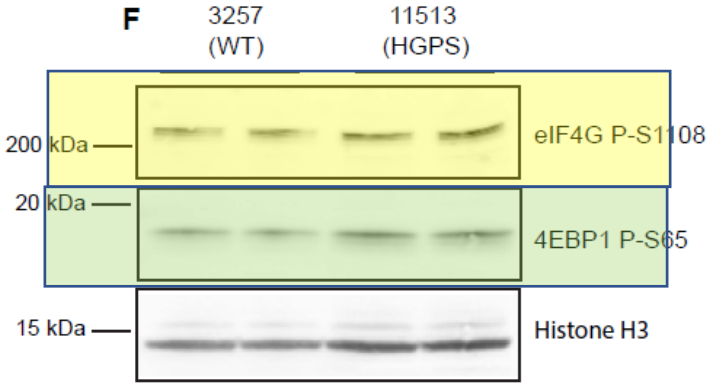
Protein synthesis is controlled by signaling through the mammalian target of rapamycin(mTOR) pathway.

How does mTOR signaling change in HGPS cultures?



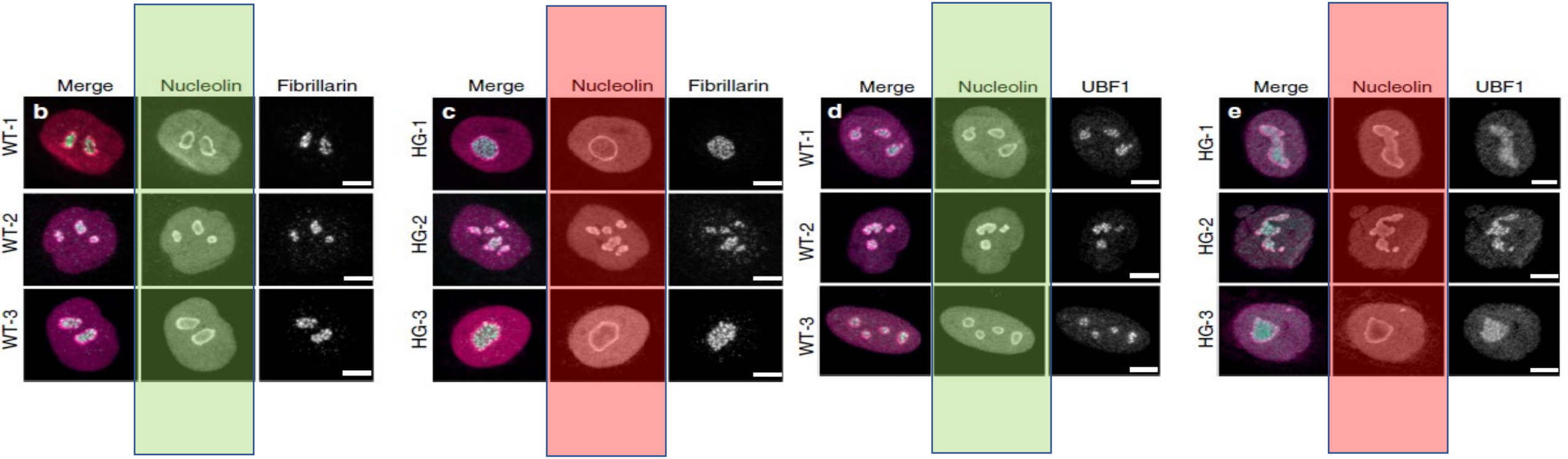






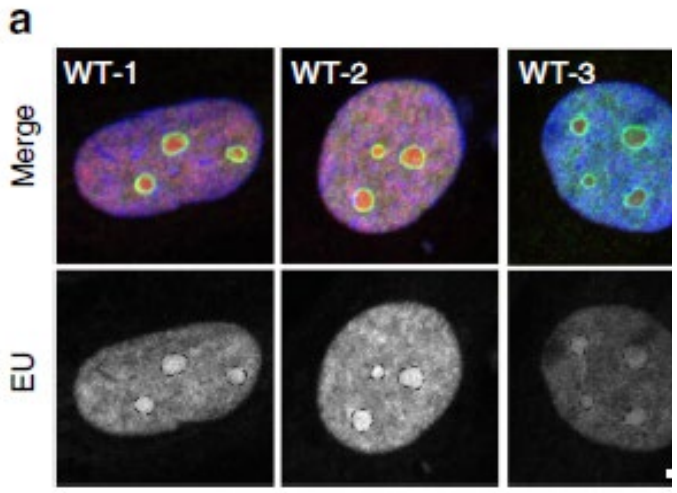
Levels of both RPS6, and phopho-RPS6 are consistently elevated in HGPS cells, but in mTOR-dependent S6 kinase, phosphorylation is decreased.

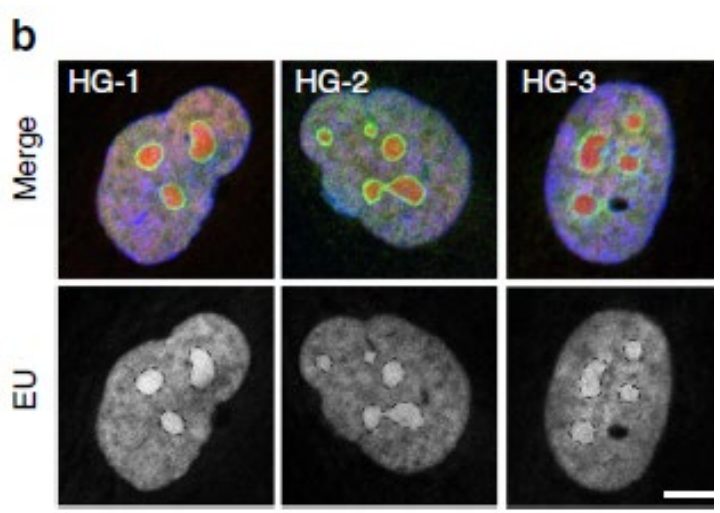
Is there any change in nuclear structures of HGPS cells?

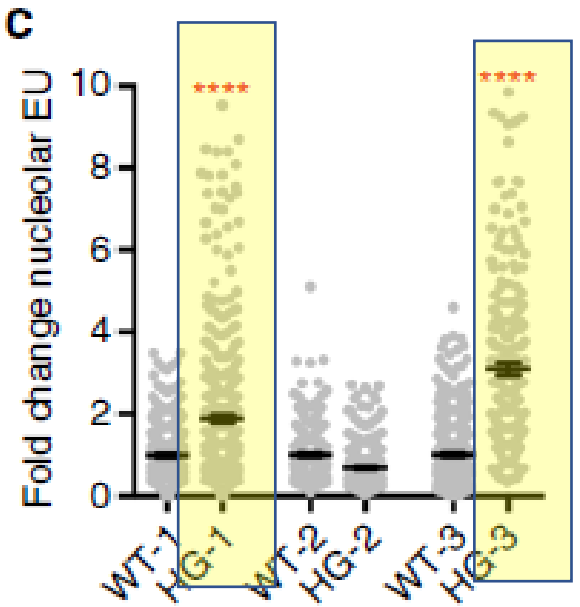


A general increase in total nucleolar area in HGPS cells is observed.

What happens to the transcription activity of rRNA in HGPS?

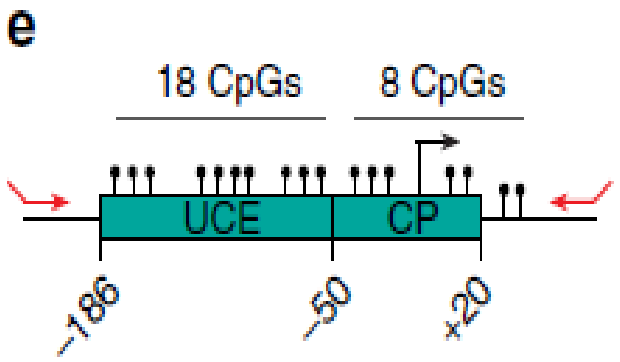


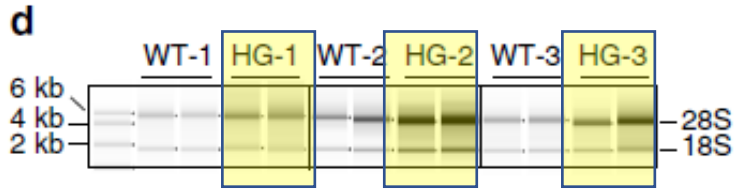




The researchers found increased transcription of rRNA in HGPS lines

Is nucleolar expansion in HGPS caused by de-repression of rDNA loci?





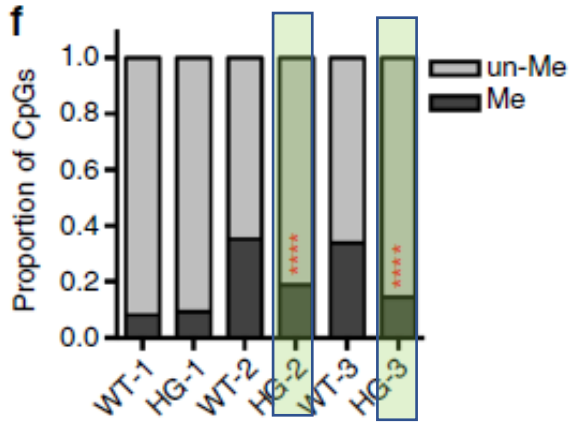


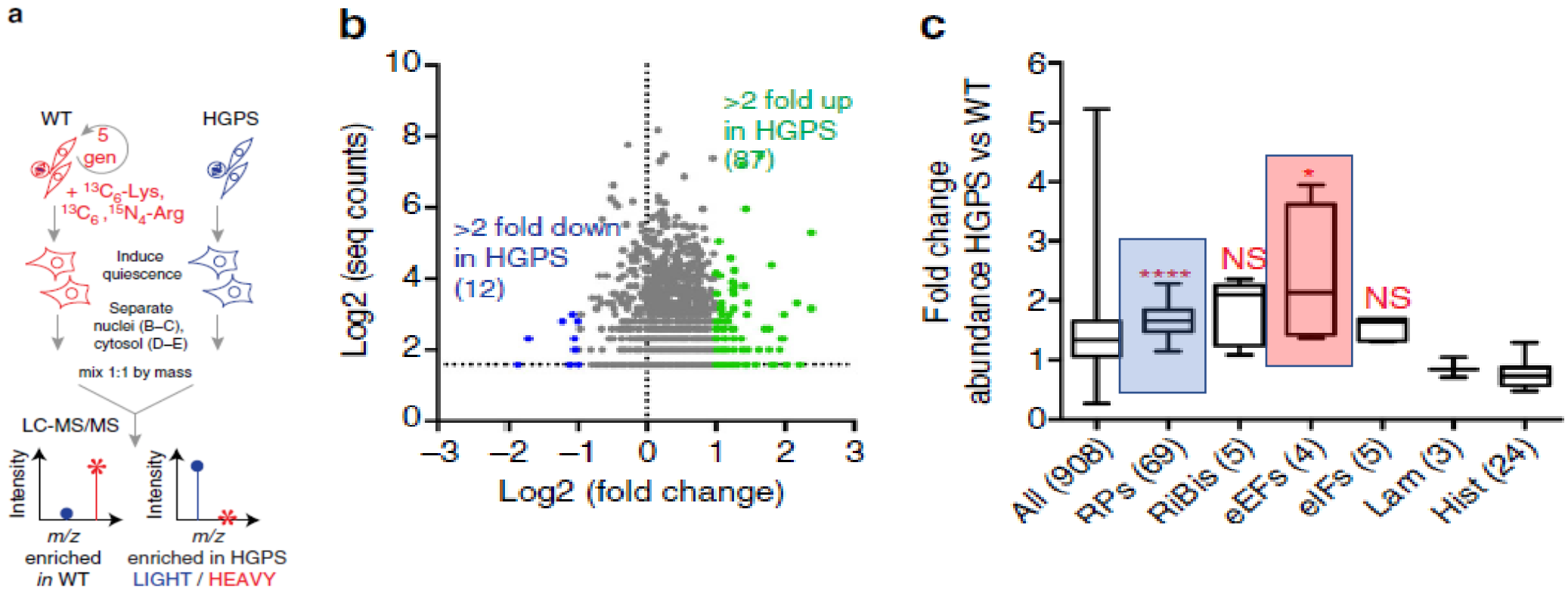
Table 1 Analysis of rDNA promoter methylation

Sample	No. of loci Me	No. of loci un-Me	% loci Me
WT-1	5	5	50.0
HG-1	3	7	30.0
WT-2	15	4	78.9
HG-2	14	6	70.0
WT-3	11	5	68.8
HG-3	7	8	46.7

Summary of number of rDNA loci analyzed and percentage of loci with any CpG methylation in WT and HGPS fibroblasts

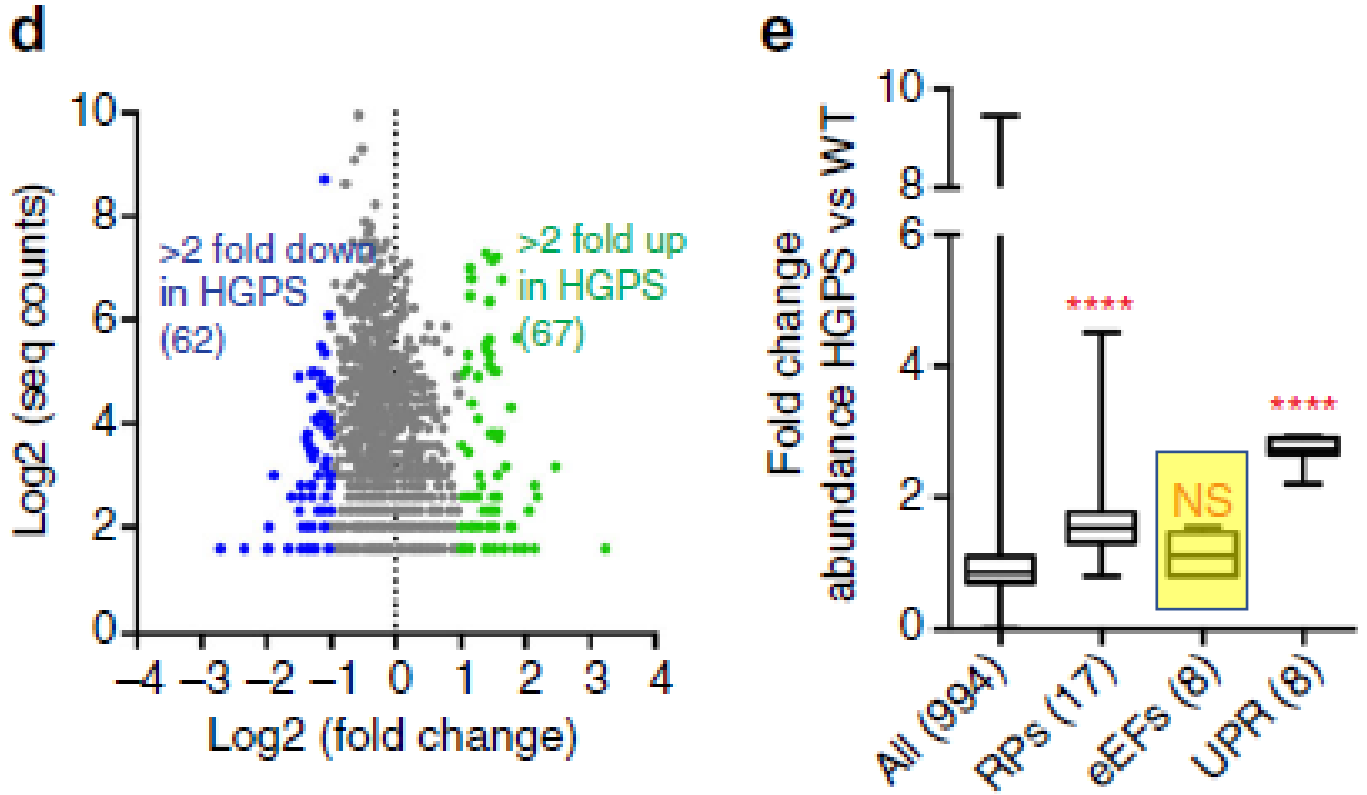
The research data suggests that a larger proportion of rDNA loci that retain methylation.

Are RPs significantly unregulated in the nuclei of HGPS cells?



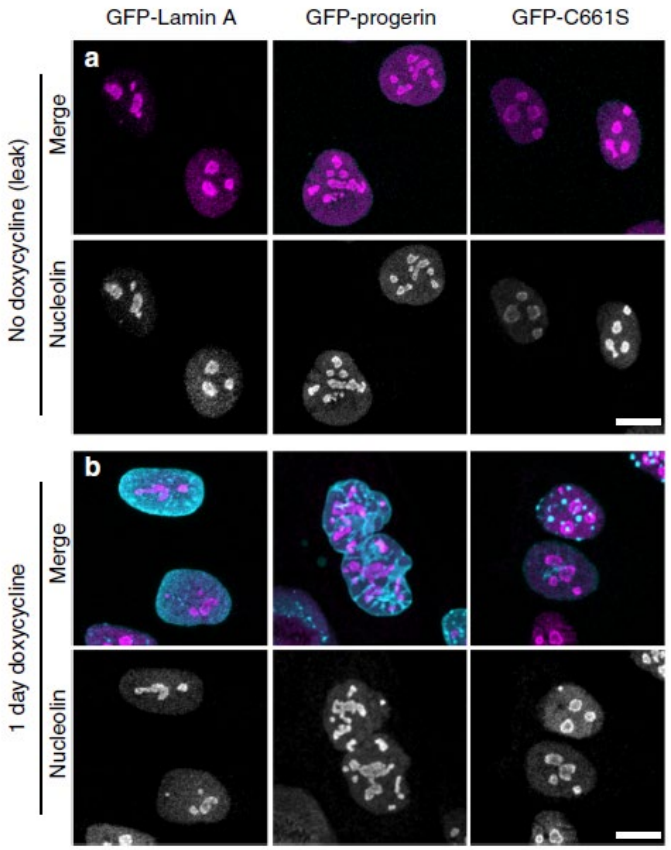
As a group, RPs are significantly upregulated in the nuclei of HGPS cells.

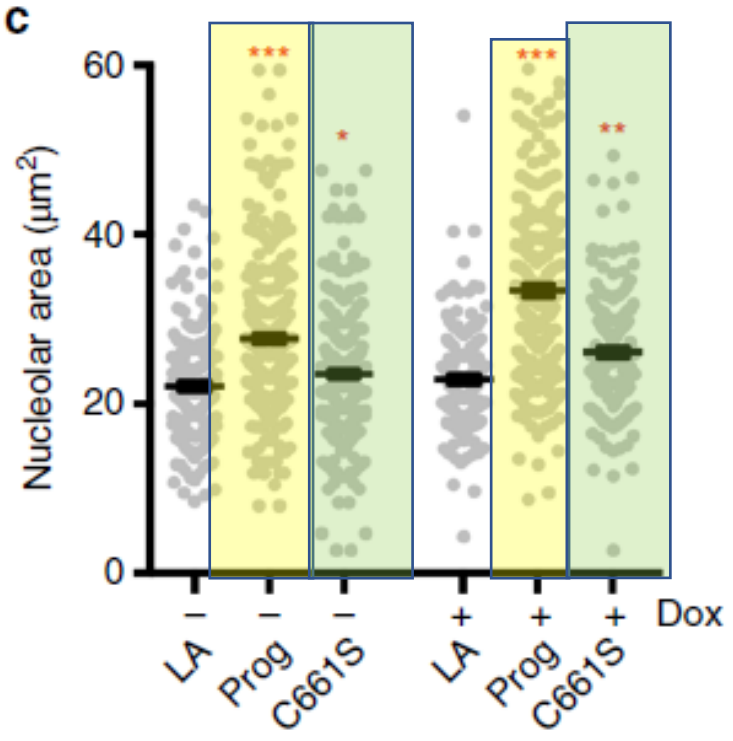
Are eEFs significantly upregulated in the cytosol of HGPS?

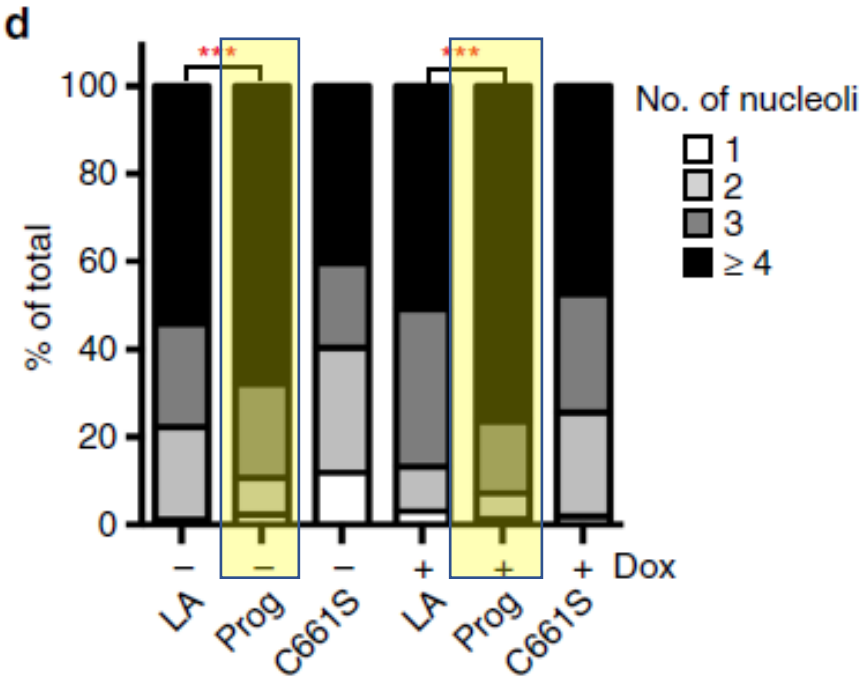


eEFs were not significantly upregulated in the cytosol of HGPS cells

Does progerin expression promote nucleolar expansion?

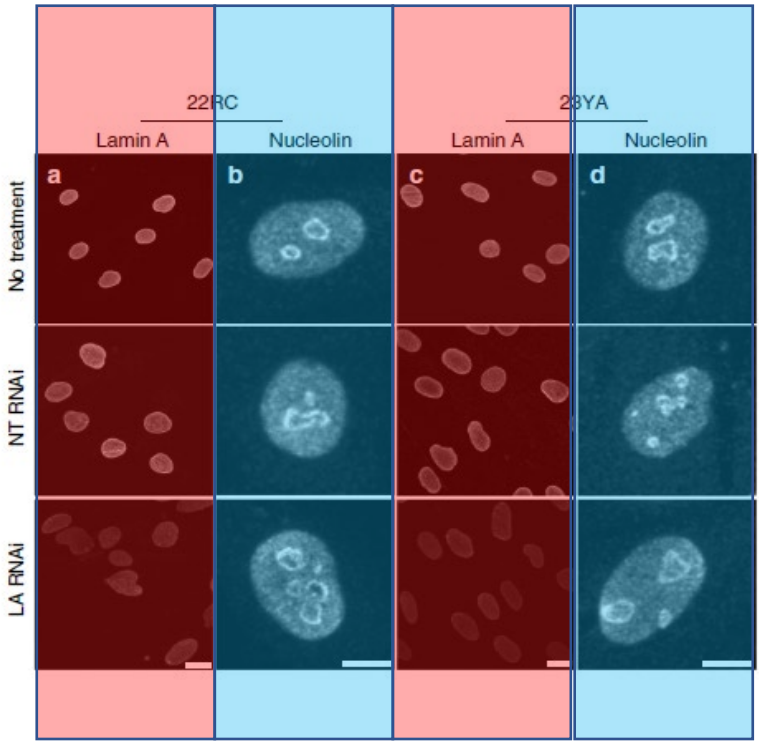


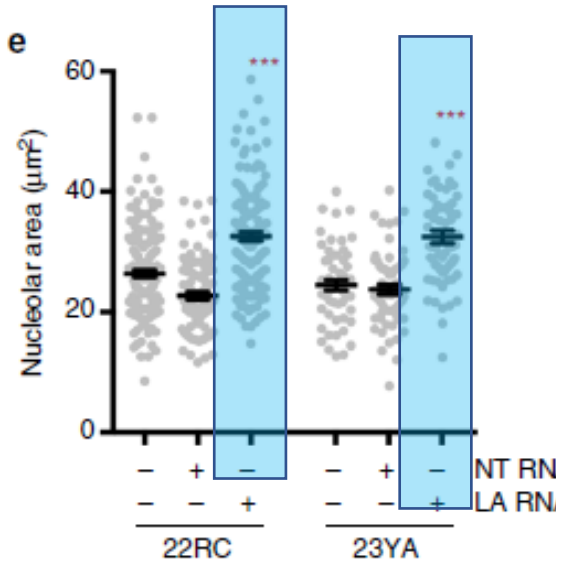


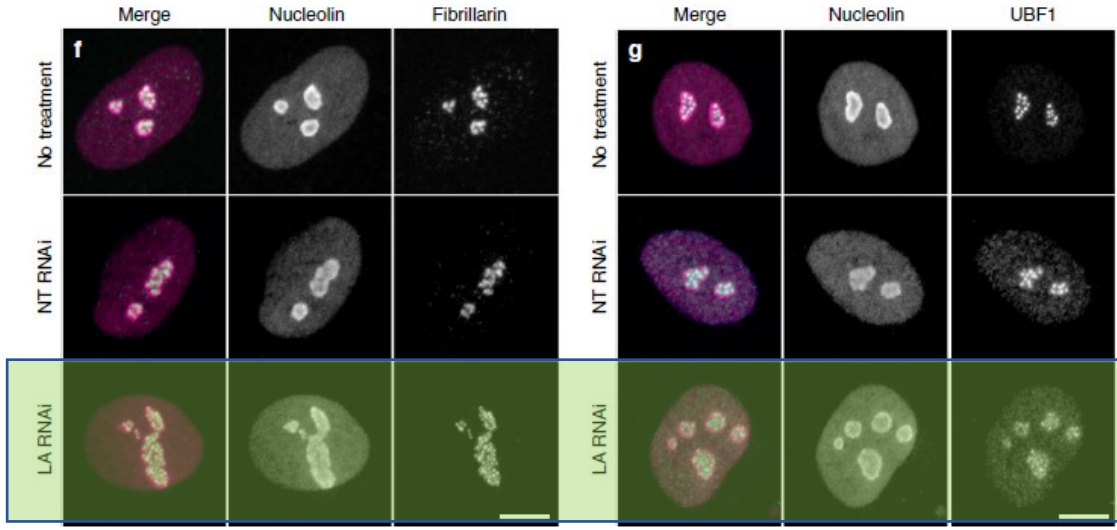


Progerin expression is sufficient to promote nucleolar expansion

Does lamin depletion cause nucleolar expansion?

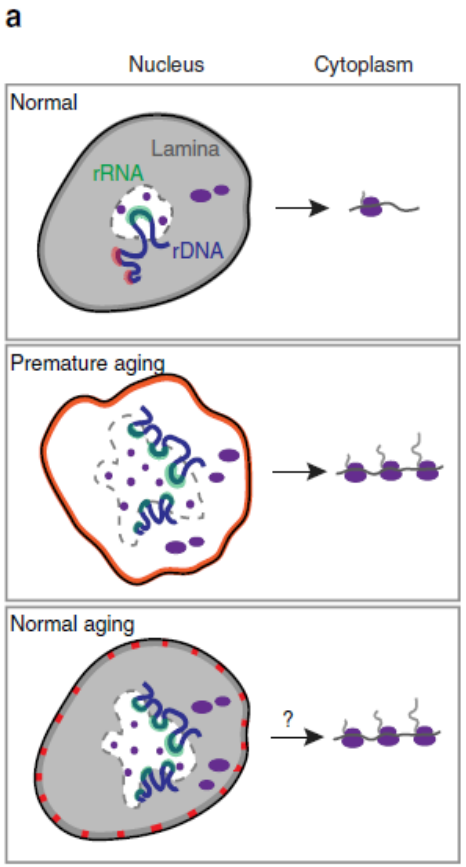


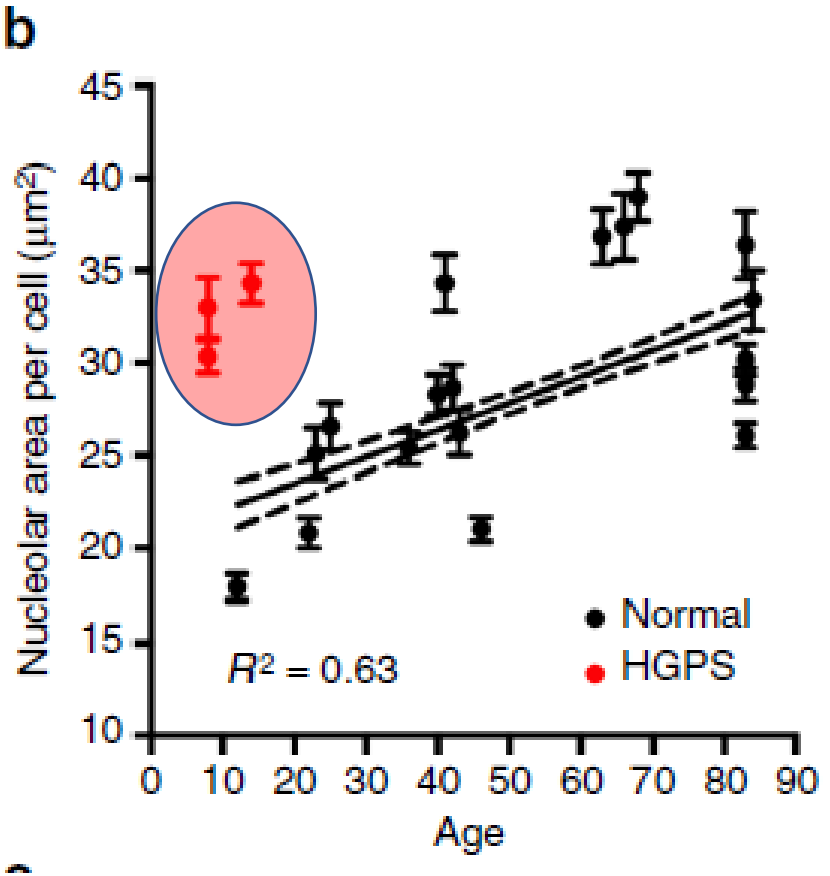




Nucleoli remains functional even there is no Lamin A exists.

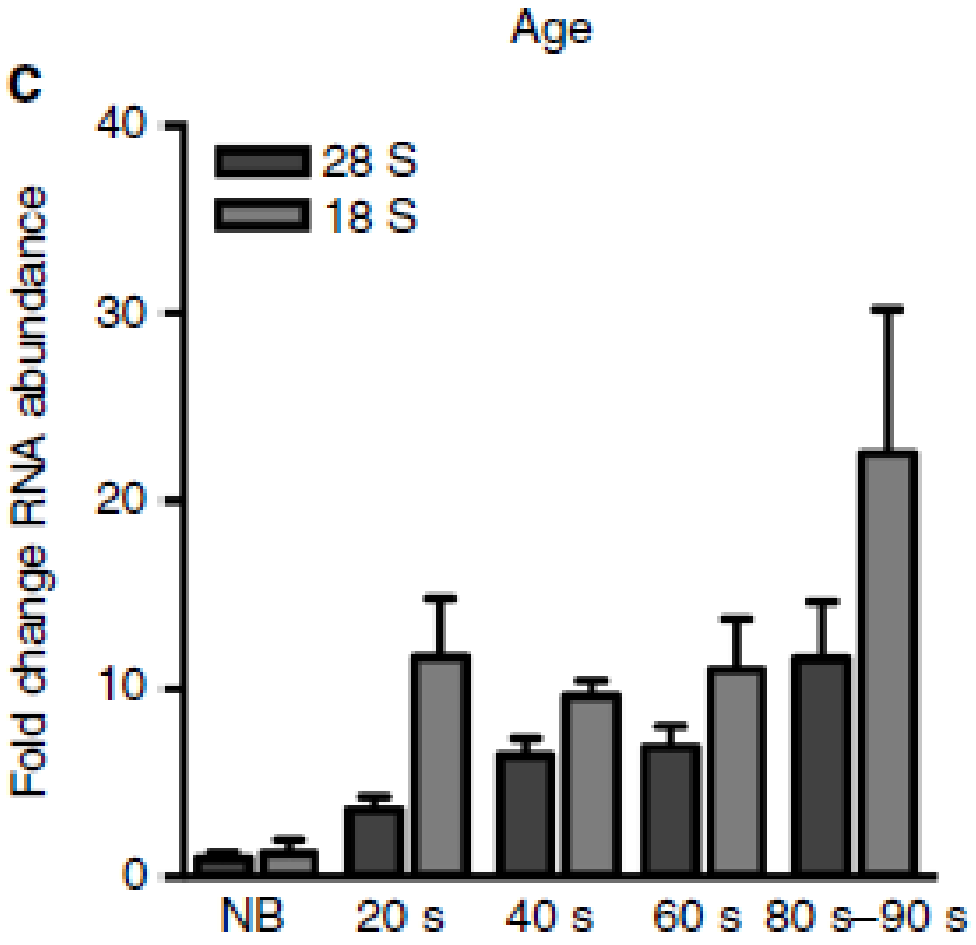
What's the relationship between nucleolar size increasing and aging?





There is a significant relation between aging and nucleolar size

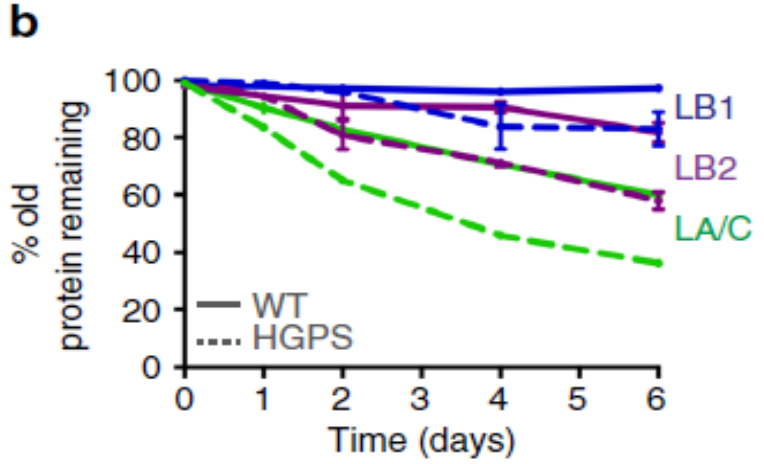
Can increased nucleolar size be considered as a hallmark of aging?



The researchers' data indicate that increased nucleolar size and activity are hallmarks of aging in both pathological and physiological context.

Conclusions

First, from the research, we know that progerin acts as a dominant negative mutant to prevent the previously unappreciated role of lamin A in organizing nucleoli and limiting ribosome biogenesis.



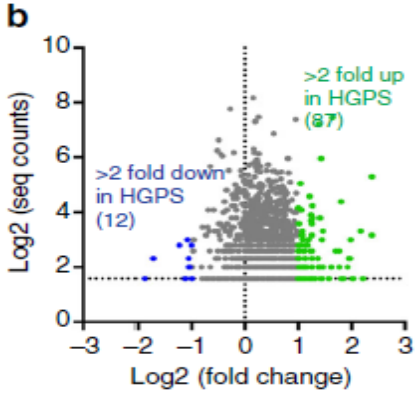
Next, the research has shown that a larger proportion of rDNA promoters are un-methylated in HGPS, and thus are in the chromatin state that allows POL I binding.

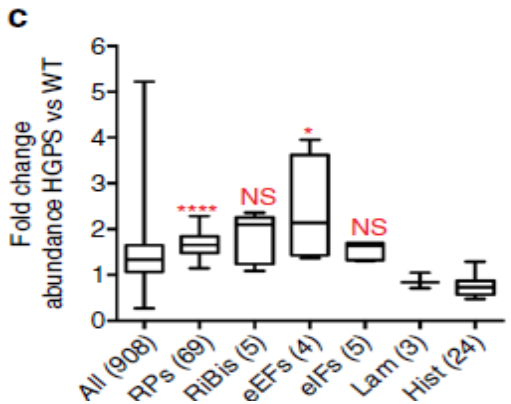
Table 1 Analysis of rDNA promoter methylation

Sample	No. of loci Me	No. of loci un-Me	% loci Me
WT-1	5	5	50.0
HG-1	3	7	30.0
WT-2	15	4	78.9
HG-2	14	6	70.0
WT-3	11	5	68.8
HG-3	7	8	46.7

Summary of number of rDNA loci analyzed and percentage of loci with any CpG methylation in WT and HGPS fibroblasts

The researchers also observed elevated production of RPS in HGPS, which assemble into functional polysomes in the end increasing global translation rates. They surmise that overproduction of protein in HGPS may promote premature aging by depleting cellular energy stores.





Reference

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- Harter, Patrick & Jennewein, Lukas & Baumgarten, Peter & Ilina, Elena & Burger, Michael & Luger, Anna-Luisa & Tichy, Julia & Zörnig, Martin & Senft, Christian & Steinbach, Joachim & Mittelbronn, Michel & Ronellenfitsch, Michael. (2015). Immunohistochemical Assessment of Phosphorylated mTORC1-Pathway Proteins in Human Brain Tumors. PloS one. 10. e0127123. 10.1371/journal.pone.0127123.

Questions?

Questions： Are there any small molecules or drugs available or known currently that could inhibit rRNA production?

Inhibition of translation and bacterial growth by peptide nucleic acid targeted to ribosomal RNA

Liam Good and Peter E. Nielsen
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PNAS March 3, 1998 95 (5) 2073-2076; https://doi.org/10.1073/pnas.95.5.2073

Edited by Leslie Orgel, Salk Institute for Biological Studies, San Diego, CA, and approved December 24, 1997 (received for review November 10, 1997)

Abstract

Peptide nucleic acid (PNA) is a DNA mimic that has shown considerable promise as a lead compound for developing gene therapeutic drugs. We report that PNAs targeted to functional and accessible sites in ribosomal RNA can inhibit translation in an *Escherichia coli* cell-free transcription/translation system, with 50% reductions caused by nanomolar PNA concentrations. The effect *in vitro* is quantitatively similar to that of the known translation inhibitor and antibiotic tetracycline. Also, the targeted PNAs inhibited bacterial growth on agar plates and in liquid culture. A strain of *E. coli* (AS19) that is more permeable to antibiotics was approximately 10-fold more sensitive to the active PNAs, suggesting that the effect on growth indeed was caused by PNAs that entered cells. Inhibition was not observed when using control PNAs of similar composition but with an unrelated or mismatched sequence. The results demonstrate that ribosomal RNA is a possible target for sequence-designed novel antibiotics based on DNA analogues or mimics.

Question: In the paper they discuss how GFP-tagged lamin A variants exhibited enlarged and more numerous nucleoli. On the other hand they show that when lamin A is knocked down in primary human fibroblasts using RNAi there was expansion in nucleoli but not an increase in the number of nucleoli. What is the reason for this difference and how is the number of nucleoli significant?

Review

Nuclear lamin functions and disease

Veronika Butin-Israeli ✉, Stephen A. Adam, Anne E. Goldman, Robert D. Goldman

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Recent studies have shown that premature cellular senescence and normal organ development and function depend on the type V intermediate filament proteins, the lamins, which are major structural proteins of the nucleus. This review presents an up-to-date summary of the literature describing new findings on lamin functions in various cellular processes and emphasizes the relationship between the lamins and devastating diseases ranging from premature aging to cancer. Recent insights into the structure and function of the A- and B- type lamins in normal cells and their dysfunctions in diseased cells are providing novel targets for the development of new diagnostic procedures and disease intervention. We summarize these recent findings, focusing on data from mice and humans, and highlight the expanding knowledge of these proteins in both healthy and diseased cells.

Previous article in issue Next article in issue

Question: Why lamin B is not as important as lamin A in the generation of progeria?

Nucleus

Nucleus. 2013 Jul 1; 4(4): 283–290.
Published online 2013 Jul 18. doi: 10.4161/nucl.25808

PMCID: PMC3810336
PMID: 23873483

The contrasting roles of lamin B1 in cellular aging and human disease

Oliver Dreesen, [1,*] Peh Fern Ong, [1] Alexandre Chojnowski, [2] and Alan Colman [1,*]

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See the article "Lamin B1 fluctuations have differential effects on cellular proliferation and senescence" in *J Cell Biol*, volume 200 on page 605.

This article has been cited by other articles in PMC.

Abstract

Go to:

The nuclear lamina underlies the inner nuclear membrane and consists of a proteinaceous meshwork of intermediate filaments: the A- and B-type lamins. Mutations in *LMNA* (encoding lamin A and C) give rise to a variety of human diseases including muscular dystrophies, cardiomyopathies and the premature aging syndrome progeria (HGPS). Duplication of the *LMNB1* locus, leading to elevated levels of lamin B1, causes adult-onset autosomal dominant leukodystrophy (ADLD), a rare genetic disease that leads to demyelination in the central nervous system (CNS). Conversely, reduced levels of lamin B1 have been observed in HGPS patient derived fibroblasts, as well as fibroblasts and keratinocytes undergoing replicative senescence, suggesting that the regulation of lamin B1 is important for cellular physiology and disease. However, the causal relationship between low levels of lamin B1 and cellular senescence and its relevance in vivo remain unclear. How do elevated levels of lamin B1 cause disease and why is the CNS particularly susceptible to lamin B1 fluctuations? Here we summarize recent findings as to how perturbations of lamin B1 affect cellular physiology and discuss the implications this has on senescence, HGPS and ADLD.

Keywords: lamin B1, ADLD, senescence, telomeres, telomerase, p53

Questions: **What are some of the side effects from prolonged mTOR inhibition treatment? Why wouldn't rRNA inhibition treatment have the same side effects as mTOR inhibition treatment?**

Hot Topics in Aging Research: Protein Translation, 2009

Brian K. Kennedy[1] and Matt Kaeberlein[2]

Summary

Go to:

In the last few years, links between regulation of mRNA translation and aging have been firmly established in invertebrate model organisms. This year, a possible relationship between mRNA translation and aging in mammals has been established with the report that rapamycin increases life span in mice. Other significant findings have connected translation control with other known longevity pathways and provided fodder for mechanistic hypotheses. Here we summarize advances in this emerging field and raise questions for future studies.

Keywords: proteotoxicity, longevity, dietary restriction, rapamycin, TOR, ribosome, translation, degradation

Introduction

Go to:

It is clear from a number of prior studies that reduced or altered protein translation can promote longevity in invertebrate model organisms (Kaeberlein & Kennedy 2007; Syntichaki *et al.* 2007b). Life span extending mutations have been identified in a growing number of genes that code for translation-related proteins, including kinases that signal to promote mRNA translation, translation initiation factors, structural components of the ribosome, and ribosomal RNA processing factors (Table 1). The two foremost questions arising from these studies address (1) what are the mechanistic principles linking translational control pathways to aging in invertebrates? and (2) do similar mechanisms of life span control exist in mammals? Reports in the last year have shed light on both of these questions. It is now clear that inhibition of the mammalian target of rapamycin (mTOR), a key regulator of mRNA translation in response to nutrient and growth cues, can result in life span extension in mice (Harrison *et al.* 2009). Moreover, findings from several groups have provided mechanistic insight into how factors that regulate translation could also modulate aging.

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Antimicrob Agents Chemother. 2012 Aug; 56(8): 4046–4051.
doi: 10.1128/AAC.00678-12
PMCID: PMC3421593
PMID: 22615289

Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components

Alison E. Barnhill, Matt T. Brewer, and Steve A. Carlson✉

ABSTRACT

Go to:

This minireview explores mitochondria as a site for antibiotic-host interactions that lead to pathophysiologic responses manifested as nonantibacterial side effects. Mitochondrion-based side effects are possibly related to the notion that these organelles are archaic bacterial ancestors or commandeered remnants that have co-evolved in eukaryotic cells; thus, this minireview focuses on mitochondrial damage that may be analogous to the antibacterial effects of the drugs. Special attention is devoted to aminoglycosides, chloramphenicol, and fluoroquinolones and their respective single side effects related to mitochondrial disturbances. Linezolid/oxazolidinone multisystemic toxicity is also discussed. Aminoglycosides and oxazolidinones are inhibitors of bacterial ribosomes, and some of their side effects appear to be based on direct inhibition of mitochondrial ribosomes. Chloramphenicol and fluoroquinolones target bacterial ribosomes and gyrases/topoisomerases, respectively, both of which are present in mitochondria. However, the side effects of chloramphenicol and the fluoroquinolones appear to be based on idiosyncratic damage to host mitochondria. Nonetheless, it appears that mitochondrion-associated side effects are a potential aspect of antibiotics whose targets are shared by prokaryotes and mitochondria—an important consideration for future drug design.

Question: Figure 1 shows that there is an obvious elevated protein synthesis rates in non-dividing cells. Do you think this faster proteins synthesis could be related to an elevated metabolism rate?

Question: The progeria paper suggests that enlarged nucleoli generate more rRNAs. I'm curious as to how these two things are related. Do the nucleoli expand because rRNA production increases? Do they expand because of decreased stability AND increased production? In other words, which of these factors (rRNA production and nucleolar size) is causal of the other, or are both effected by the same cause?

Question: In the paper, it says that non-dividing cells are especially vulnerable to the accumulation of protein and DNA damage over time, and that changes to the function of non-dividing and proliferating cell populations both contribute to age-associated decline of tissues and organs. Since both non-dividing cells and proliferating cells can be affected, which would contribute more to organismal aging?

Question: **The paper mentioned that larger nucleolar size leads to premature aging because they activate rRNA generation. They then found in C elegans that smaller nucleoli was correlated with longevity. I was wondering if they had found a point in which the nucleoli got too small and had damaging effects for the cell. If so, is there a determined size range that balances these two extremes?**

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Small nucleoli are a cellular hallmark of longevity

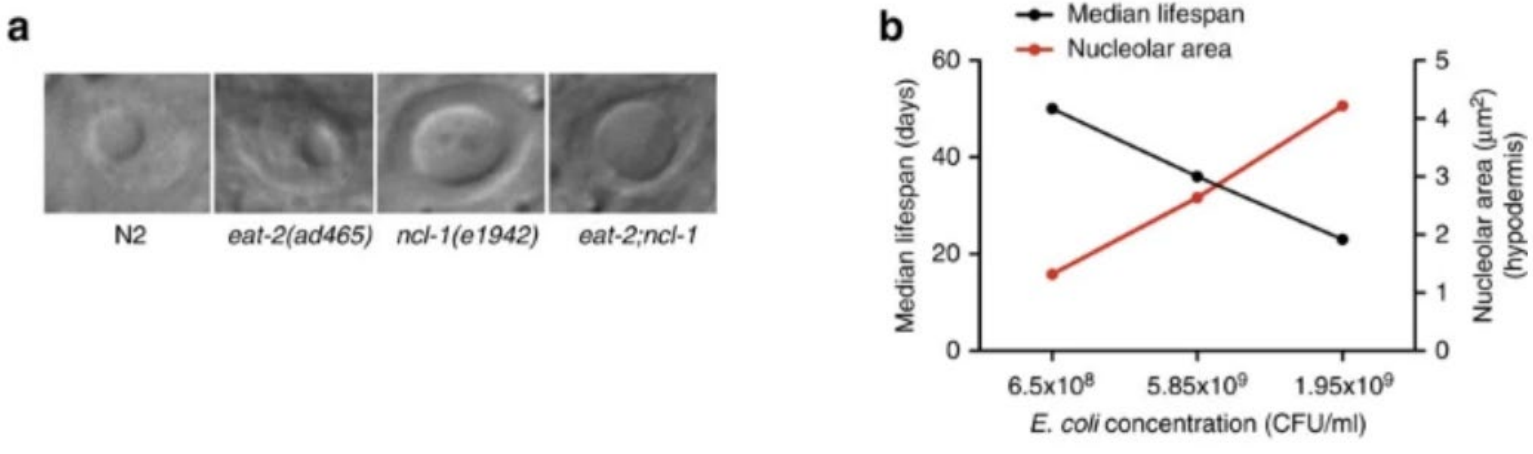
Varnesh Tiku, Chirag Jain, Yotam Raz, Shuhei Nakamura, Bree Heestand, Wei Liu, Martin Späth, H. Eka. D. Suchiman, Roman-Ulrich Müller, P. Eline Slagboom, Linda Partridge & Adam Antebi

Nature Communications **8**, Article number: 16083 (2017) | Cite this article

Abstract

Animal lifespan is regulated by conserved metabolic signalling pathways and specific transcription factors, but whether these pathways affect common downstream mechanisms remains largely elusive. Here we show that NCL-1/TRIM2/Brat tumour suppressor extends lifespan and limits nucleolar size in the major *C. elegans* longevity pathways, as part of a convergent mechanism focused on the nucleolus. Long-lived

Figure 2: Nucleolar size inversely correlates with longevity.



Question: In the paper they mentioned increased nucleolar size and activity to be hallmarks of aging. Has there been any work done in the aging to field to counteract this to help humans live longer?

Question: The paper mentions that there are already some rRNA inhibition drugs in the trial pipeline, since the papers release, have any of these drugs shown promising results for treatment of HGPS or lifespan extension?

Cancer Cell. 2012 Jul 10;22(1):51-65. doi: 10.1016/j.ccr.2012.05.019.

Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53

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Affiliations + expand

PMID: 22789538 PMCID: PMC3749732 DOI: 10.1016/j.ccr.2012.05.019

Free PMC article

Abstract

Increased transcription of ribosomal RNA genes (rDNA) by RNA Polymerase I is a common feature of human cancer, but whether it is required for the malignant phenotype remains unclear. We show that rDNA transcription can be therapeutically targeted with the small molecule CX-5461 to selectively kill B-lymphoma cells in vivo while maintaining a viable wild-type B cell population. The therapeutic effect is a consequence of nucleolar disruption and activation of p53-dependent apoptotic signaling. Human leukemia and lymphoma cell lines also show high sensitivity to inhibition of rDNA transcription that is dependent on p53 mutational status. These results identify selective inhibition of rDNA transcription as a therapeutic strategy for the cancer specific activation of p53 and treatment of hematologic malignancies.

Epub 2016 Apr 7.

Regulation of ribosomal RNA expression across the lifespan is fine-tuned by maternal diet before implantation

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Affiliations + expand

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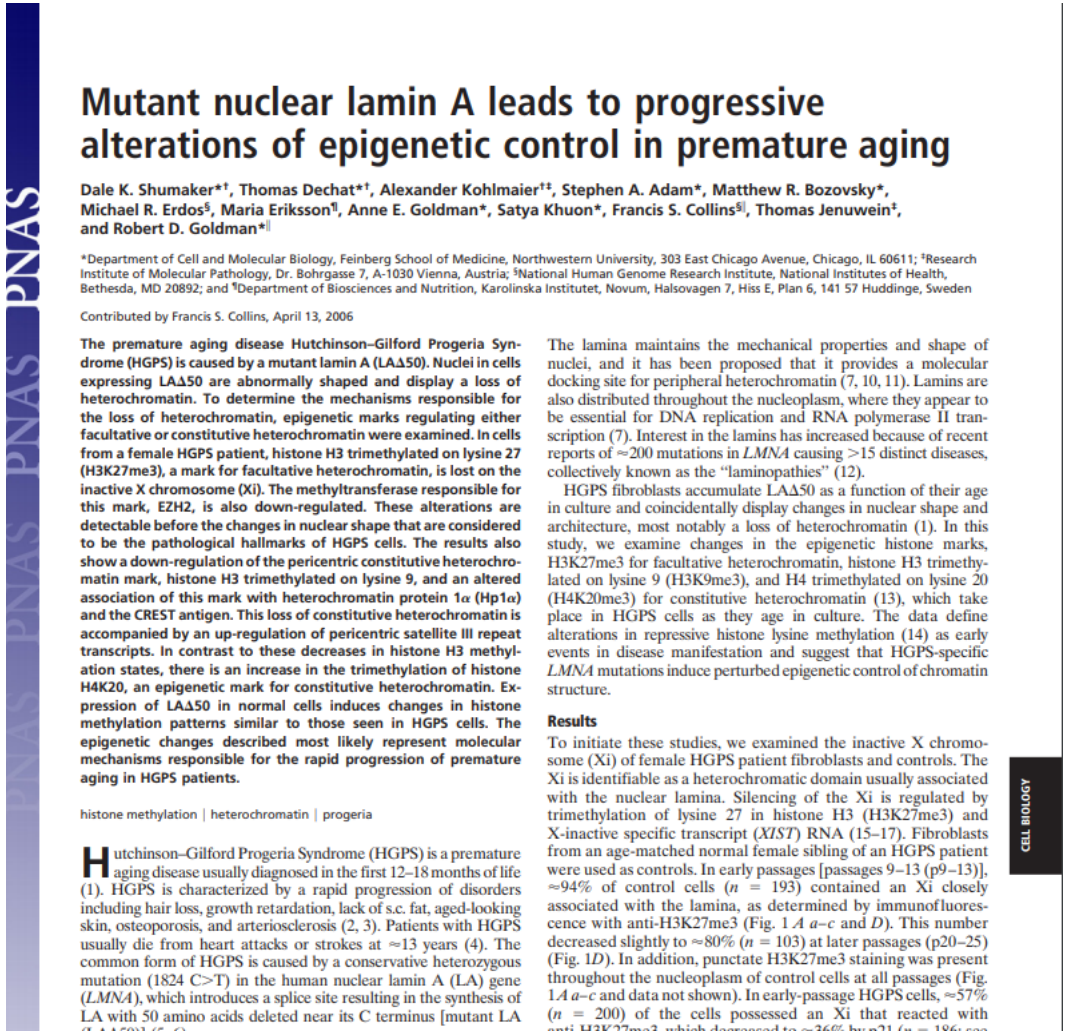
Free PMC article

Abstract

Cells and organisms respond to nutrient deprivation by decreasing global rates of transcription, translation and DNA replication. To what extent such changes can be reversed is largely unknown. We examined the effect of maternal dietary restriction on RNA synthesis in the offspring. Low protein diet fed either throughout gestation or for the preimplantation period alone reduced cellular RNA content across fetal somatic tissues during challenge and increased it beyond controls in fetal and adult tissues after challenge release. Changes in transcription of ribosomal RNA, the major component of cellular RNA, were responsible for this phenotype as evidenced by matching alterations in RNA polymerase I density and DNA methylation at ribosomal DNA loci. Cellular levels of the ribosomal transcription factor Rrn3 mirrored the rRNA expression pattern. In cell culture experiments, Rrn3 overexpression reduced rDNA methylation and increased rRNA expression; the converse occurred after inhibition of Rrn3 activity. These observations define novel mechanism where poor nutrition before implantation irreversibly alters basal rates of rRNA transcription thereafter in a process mediated by rDNA methylation and Rrn3 factor.

Question: The progeria paper talked about the effects of progerin disrupting the lamin A nucleoplasmic meshwork, which disrupts the interaction of lamin A with heterochromatin and euchromatin. I was wondering if there are any epigenetic treatments that might be effective in maintaining these important repressive marks.



Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging

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Contributed by Francis S. Collins, April 13, 2006

The premature aging disease Hutchinson–Gilford Progeria Syndrome (HGPS) is caused by a mutant lamin A (LAΔ50). Nuclei in cells expressing LAΔ50 are abnormally shaped and display a loss of heterochromatin. To determine the mechanisms responsible for the loss of heterochromatin, epigenetic marks regulating either facultative or constitutive heterochromatin were examined. In cells from a female HGPS patient, histone H3 trimethylated on lysine 27 (H3K27me3), a mark for facultative heterochromatin, is lost on the inactive X chromosome (Xi). The methyltransferase responsible for this mark, EZH2, is also down-regulated. These alterations are detectable before the changes in nuclear shape that are considered to be the pathological hallmarks of HGPS cells. The results also show a down-regulation of the pericentric constitutive heterochromatin mark, histone H3 trimethylated on lysine 9, and an altered association of this mark with heterochromatin protein 1α (Hp1α) and the CREST antigen. This loss of constitutive heterochromatin is accompanied by an up-regulation of pericentric satellite III repeat transcripts. In contrast to these decreases in histone H3 methylation states, there is an increase in the trimethylation of histone H4K20, an epigenetic mark for constitutive heterochromatin. Expression of LAΔ50 in normal cells induces changes in histone methylation patterns similar to those seen in HGPS cells. The epigenetic changes described most likely represent molecular mechanisms responsible for the rapid progression of premature aging in HGPS patients.

histone methylation | heterochromatin | progeria

Hutchinson–Gilford Progeria Syndrome (HGPS) is a premature aging disease usually diagnosed in the first 12–18 months of life (1). HGPS is characterized by a rapid progression of disorders including hair loss, growth retardation, lack of s.c. fat, aged-looking skin, osteoporosis, and arteriosclerosis (2, 3). Patients with HGPS usually die from heart attacks or strokes at ≈13 years (4). The common form of HGPS is caused by a conservative heterozygous mutation (1824 C>T) in the human nuclear lamin A (LA) gene (*LMNA*), which introduces a splice site resulting in the synthesis of LA with 50 amino acids deleted near its C terminus [mutant LA

The lamina maintains the mechanical properties and shape of nuclei, and it has been proposed that it provides a molecular docking site for peripheral heterochromatin (7, 10, 11). Lamins are also distributed throughout the nucleoplasm, where they appear to be essential for DNA replication and RNA polymerase II transcription (7). Interest in the lamins has increased because of recent reports of ≈200 mutations in *LMNA* causing >15 distinct diseases, collectively known as the "laminopathies" (12).

HGPS fibroblasts accumulate LAΔ50 as a function of their age in culture and coincidentally display changes in nuclear shape and architecture, most notably a loss of heterochromatin (1). In this study, we examine changes in the epigenetic histone marks, H3K27me3 for facultative heterochromatin, histone H3 trimethylated on lysine 9 (H3K9me3), and H4 trimethylated on lysine 20 (H4K20me3) for constitutive heterochromatin (13), which take place in HGPS cells as they age in culture. The data define alterations in repressive histone lysine methylation (14) as early events in disease manifestation and suggest that HGPS-specific *LMNA* mutations induce perturbed epigenetic control of chromatin structure.

Results

To initiate these studies, we examined the inactive X chromosome (Xi) of female HGPS patient fibroblasts and controls. The Xi is identifiable as a heterochromatic domain usually associated with the nuclear lamina. Silencing of the Xi is regulated by trimethylation of lysine 27 in histone H3 (H3K27me3) and X-inactive specific transcript (*XIST*) RNA (15–17). Fibroblasts from an age-matched normal female sibling of an HGPS patient were used as controls. In early passages [passages 9–13 (p9–13)], ≈94% of control cells (n = 193) contained an Xi closely associated with the lamina, as determined by immunofluorescence with anti-H3K27me3 (Fig. 1 *A–c* and *D*). This number decreased slightly to ≈80% (n = 103) at later passages (p20–25) (Fig. 1*D*). In addition, punctate H3K27me3 staining was present throughout the nucleoplasm of control cells at all passages (Fig. 1*A a–c* and data not shown). In early-passage HGPS cells, ≈57% (n = 200) of the cells possessed an Xi that reacted with

Question: How do you anticipate oxidative stress could impact the rate of metabolism? We know that it impacts processes and morphology of mitochondria but I am curious how this would impact the nucleus with metabolism as well.

Questions: For these studies, they used fibroblasts. Do you think there would be any differences in their results if they used other types of cells? Also, do think these results could (or should) be used to look at aging as a whole outside of this disease and what would be the implications for doing that?