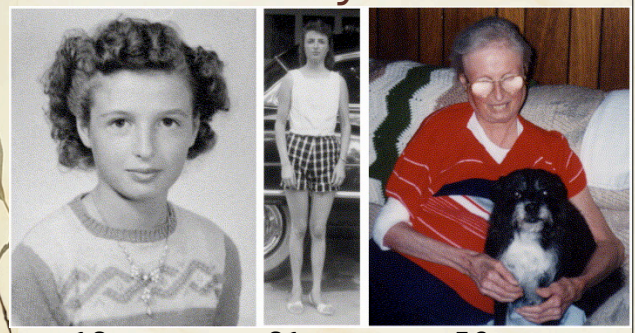
Insights from studies of premature aging

A&S300-002 Jim Lund

Werner's syndrome



12 yrs 21 yrs 56yrs

The patient had bilateral cataracts, characteristic dermatological pathology, short stature, premature graying and thinning of scalp hair, and parental consanguinity (she was the product of a second cousin marriage). She also had type 2 diabetes mellitus (not a typical Werner's syndrome symptom), hypogonadism (with menopause at age 35 years), osteoporosis, flat feet, and a characteristic high-pitched, squeaky voice (Martin 2005)

Werner's syndrome

- WRN protein
- A member of the RecQ family of DNA helicases.
- Other RecQ family helicase mutations produce genomic instability diseases with progeriod symptoms: Bloom syndrome and Rothmund–Thomson syndrome.

Werner's syndrome: cellular features

Normal human fibroblasts achieve approximately 60 population doublings in culture.

Werner syndrome cells usually achieve only about 20 population doublings.

(lower Hayflick limit).

Cell proliferation potential greater in long-lived species

Organism + L.S:
-mouse about 3 years
-human about 100
-Galapagos tortoise about 150

Hayflick Limit:
-doublings about 20
-doublings about 40-60
-doublings about 140

Species	Maximum life span (years)	Maximum doubling number
Galapagos tortoise	175	125
Man	110	60
Horse	46	82
Chicken	30	35
Cat	28	92
Kangaroo	16	46
Mink	10	34
Mouse	4	28

Cell proliferation potential lower from older donors

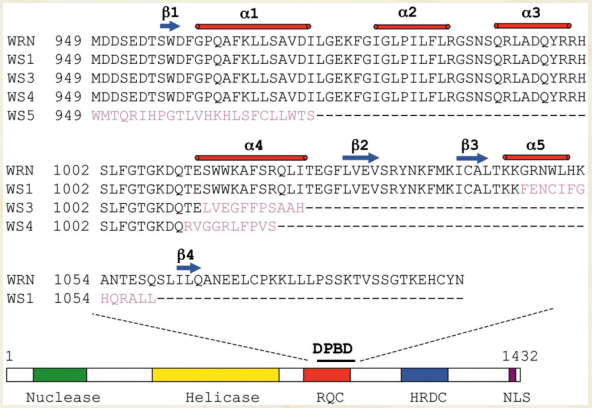
•Cells from older donors have "used up" some of doublings

	Fetal Lung		Adult Lung	
Strain	Number of population doublings	Strain	Number of population doublings	Age of donor
WI-1	51	WI-1000	29	87
WI-3	35	WI-1001	18	80
WI-11	57	WI-1002	21	69
WI-16	44	WI-1003	24	67
WI-18	53	WI-1004	22	61
WI-19	50	WI-1005	16	58
WI-23	55	WI-1006	14	58
WI-24	39	WI-1007	20	26
WI-25	41			
WI-26	50			
WI-27	41			
WI-38	48			
WI-44	63			
Average range	48 (35–63)		20 (14–29)	

Werner's syndrome: cellular features

- Sensitive to some but not all DNA damage agents:
  - Normal response: UV irradiation, ionizing radiation
  - Sensitive: carcinogen 4-nitroquinoline-1-oxide (4NQO) and to agents causing interstrand crosslinks.
- Increased rate of somatic mutations and chromosomal abnormalities such as translocations, inversions, and chromosome losses.

WRN protein domains and structure



Hu, Jin-Shan et al. (2005) Proc. Natl. Acad. Sci. USA 102, 18379-18384

PNAS

WRN homologs

Sgs1 (*S. cerevisiae*)
- Shorter replicative lifespan
- Supressed by human WRN expression in yeast!

rqh1 (*S. pombe*)

recQ (*E. coli*)

All suppress illegitimate recombination!

Hanada et al., 1997

WRN protein

- Location: nucleolar
- WRN co-purifies with a 17S DNA replication complex.
- WRM binds proteins involved in DNA and RNA processing including DNA repair.

Loss of WRN:
- Transcriptional changes
- RNA pol II transcription is reduced by 40–60%.
- Deletion of telomeres from single sister chromatids.

CELLULAR DEFECTS IN WS

- Genomic Instability
  - Chromosomal rearrangements
  - Large spontaneous deletions
- Telomere maintenance
  - Shortened telomeres
  - WRN co-localizes with telomeric DNA in some cells
- Recombination
  - Hyper-recombination
  - Recombination defects
- Transcription
  - RNA polymerase II transcription
    - RNA pol I
- Apoptosis
  - Attenuated apoptosis
    - p53 mediated
- Replication
  - Reduced replicative lifespan
  - Extended S-phase
  - Reduced frequency of initiation sites
  - WRN is part of replication complex
- DNA Repair (BER, NHEJ)
  - Hypersensitivity to 4-NQO
  - Hypersensitivity to DNA cross linking agents
  - Reduced repair of psoralen crosslinks (shuttle vector)
  - Hypersensitivity to camptothecin
  - Reduced telomeric repair
  - Reduced transcription coupled repair

Opresko et al., 2003

Mouse models of Werner's syndrome

Deleted of helicase domain of WRN.

Cells had DNA repair defects.

Fibroblasts derived from homozygous *Wrn* -/- embryos showed premature loss of proliferative capacity.

Classic Werner's syndrome features not seen.

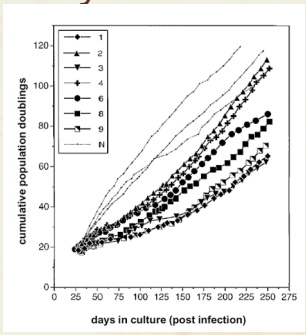
Lebel and Leder, 1998

Lab strains of mice have long telomeres.

Wrn, *Terc* (telomerase RAN component) double KO mice have a classic Werner's syndrome phenotype when telomeres grew short.

Chang et al., 2004

Werner's cellular phenotype reversed by telomerase expression



Dermal fibroblasts transformed with TERT (telomerase) continue dividing, Werner's cells typically stop dividing at 20 population doublings.

Effects of WRN mutations

- Loss of proliferation of cell populations that replace themselves by cell division cause by cell death or early senescence.
- Renewing cell populations fail to replenish themselves and this gives rise to the Werner's syndrome phenotype.

Rothmund–Thomson syndrome

- RECQL4, a RecQ DNA helicase
- Is regulated by SIRT1
- SIRT1 deacetylates RECQL4, deactivating it and causing it to localize to the nucleolus.
- SIRT1 appears to be a regulator of several proteins involved in aging processes.

Hutchison-Gilford syndrome

- Lamin A / LMNA protein.
- Lamins are structural protein components of the nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size.
- The lamins constitute a class of intermediate filaments

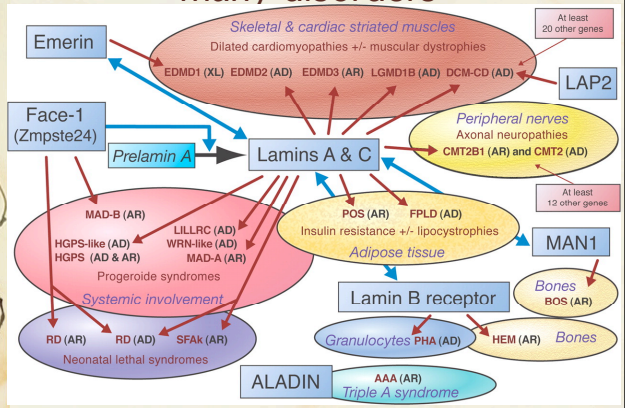
Lamin A

- Expressed in some but not all cell types:
  - Epithelial HeLa cells
  - Not in T lymphoblasts.
  - No comprehensive survey done yet.
  - There are 3 lamins (A/B/C) different cells express different types or combinations of types.

Lamin A mutations

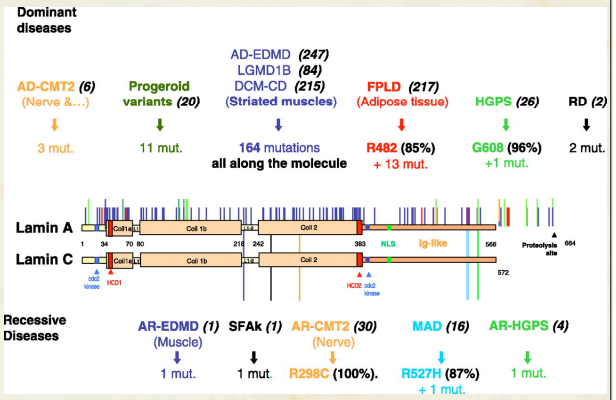
- Truncations/missense mutations produce Hutchinson-Gilford syndrome.
- Other mutations in this gene give other disorders: muscular dystrophy, dilated cardiomyopathy, lipodystrophy

Lamin A mutations give rise to many disorders



Broers et al., 2006

Map of LMNA mutations



Broers et al., 2006

Lamin A mutations

- In a myoblast-to-myotube differentiation model, lamin A (-/-) cells fail to differentiate. (Favreau et al., 2004)
- Lamin A (-/-) cells under mechanical strain have impaired viability under mechanical strain compared to wildtype cells (Lammerding et al., 2004)

Lamin A mutation cellular phenotype

- Disrupted nuclear lamina, intranuclear architecture, and macromolecular interactions.
- Fibroblasts from individuals with HGPS have severe morphologic abnormalities in nuclear envelope structure.
- Heterochromatin-specific histone modifications
- Transcriptional changes.

Hutchison-Gilford worm model

- Functional knock-out of lamin protein
  - Halted process of cell division, resulting in a static "bridge" structure between cells that should have separated.
  - Gross defects in chromosome segregation, chromatin decondensation, and mitotic progression as early as the two-cell stage, and embryos died at the ≈100-cell stage
  - Damage to the gonad cell structure

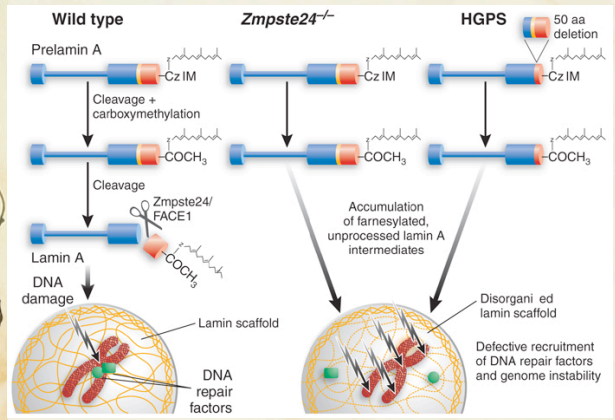
Margalit et al., 2005

Hutchison-Gilford mouse model

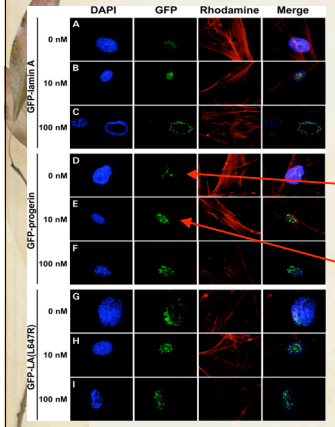
- Introduced a mutation in Lamin A that causes autosomal dominant Emery-Dreifuss muscular dystrophy in humans.
- Normal at birth
- At 4 to 6 days developed severe growth retardation, dying within 4 to 5 weeks.
  - slight waddling gait, suggesting immobility of joints.
  - Loss of subcutaneous fat
  - Reduced numbers of eccrine and sebaceous glands
  - Increased collagen deposition in skin
  - Decreased hair follicle density
- Nuclear envelope abnormalities
- Decreased fibroblast Hayflick limit

Mounkes et al., 2003

A lamin A protein maturation defect disrupts the nuclear lamina



A farnesyltransferase inhibitor reverses nuclear defects in Hutchison-Gilford



Normal LMNA

Protein aggregates

Disease mutation in LMNA

More normal

LMNA where the protein can't be cut to remove the farnesyl

The effect of farnesyltransferase inhibitor (FTI) on the distribution of GFP signal in normal fibroblasts expressing GFP–lamin A (A, B and C), GFP–progerin (D, E and F) and GFP–LA (L647R) (G, H and I) with various FTI concentrations. Fibroblasts were maintained in medium with 0 nM (A, D and G), 10 nM (B, E and H) or 100 nM (C, F and I) FTI for six days.

Glynn, M. W. et al. Hum. Mol. Genet. 2005 14:2959-2969; doi:10.1093/hmg/ddi326

Remaining questions

- How do the mutations affect cell division and proliferation?
- How does this cellular defect lead to the disease features?
- What causes the loss of cell proliferation in normal aging, and how significant is it?