Hôpital général juif
Jewish General Hospital

McGill

INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES / LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

Centre Bloomfield de recherche sur le vieillissement

The Bloomfield Centre for Research in Aging

Cancer and Aging: Two faces of the same coin

(6) Telomeres in Premature Aging and Degenerative Diseases

Diseases of Premature Aging

- Examples are Werner's syndrome, Ataxia telangiectasia, Dyskeratosis congenita

- External appearance of premature aging

- Clinical symptoms not associated with normal aging, for example in WS, there is a lack of a postadolescent growth spurt and an underdevelopment of sexual organs (segmental progerias)

- Will studies of such diseases provide keys to the understanding of normal aging?

PREMATURE AGING SYNDROMES

Table 1. Most commonly described human segmental progeroid syndromes.

Syndrome	Incidence (per live birth)	Inheritance	Mean life-span (years)	Progeroid features	Genome maintenance defect
Hutchinson-Gilford	<1/1,000,000	Unknown	~13	Alopecia, sclerosis, wrinkling, soft tissue, cachexia, arteriosclerosis, diminished fat	Unknown cause
Werner	<1/100,000	Autosomal recessive	~50	Alopecia, osteoporosis, malignancies, arteriosclerosis, diabetes, cataracts, telangiectasia, skin atrophy, graying of hair	DNA helicase (RecQ-like), exonuclease
Rothmund-Thomson	<1/100,000	Autosomal recessive	Normal?	Alopecia, malignancies, poikiloderma, cataracts, osteoporosis, graying of hair	DNA helicase (RecQ-like)
Cockayne	~1/100,000	Autosomal recessive	~20	Thin hair, cachexia, retinal degeneration, hearing loss, neurodegeneration (cerebellar ataxia), cataracts	Transcription-coupled DNA repair
Trichothiodystrophy	<1/100,000	Autosomal recessive	~10	Cachexia, osteoporosis, cataracts, fragile hair, neurodegeneration (cerebellar ataxia)	DNA repair, basal transcription
Ataxia telangiectasia	~1/60,000	Autosomal recessive	~20	Skin atrophy/sclerosis, telangiectasia, immunodeficiencies, malignancies, graying of hair, poikiloderma, neurodegeneration (cerebellar ataxia)	DNA damage signaling protein kinase
Down	~1/1,000	De novo	~60	Cataracts, graying of hair, alopecia, diminished subcutaneous fat, vision loss, neurodegeneration (Alzheimer-like), thyroid dysfunction	Unknown

Hasty, P. et al. 2003. Aging and Genome Maintenance: Lessons from the Mouse. Science 299, 1355-1359

A list of syndromes carrying defects in genome maintenance

Progeria

Syndrome	Mutated genes	Affected processes	Mouse models
Cockayne syndrome (CS)	*CSA, CSB*	TC-NER	*Csa*$^{-/-}$
			Csb$^{m/m}$
		TC-NER; GG-NER	*Csb*$^{m/m}$*Xpa*$^{-/-}$; *Csb*$^{m/m}$*/Xpc*$^{-/-}$
			Csa$^{-/-}$*Xpa*$^{-/-}$; *Csa*$^{-/-}$*Xpc*$^{-/-}$
Trichothiodystrophy (TTD)	*XPB, XPD, TTDA*	Partial GG/TC-NER	*Xpd*^{ttd}
COFS	*CSB, XPD, XPG*	GG-NER; TC-NER	*Xpg*$^{-/-}$
XPE	*XPF/ERCC1*	GG/TC-NER, ICL repair, HR	*Ercc1*$^{-/-}$
Rothmund-Thomson (RTS)	*RECQL4*	Oxidative DNA damage repair	*Recql4*$^{-/-}$
Dyskeratosis congenita	*DKC1, TERC1*	Telomere maintenance	*Dkc1*^{m}
			mTR$^{-/-}$
Hutchison-Gilford progeria syndrome (HGPS)	*LMNA*	Nuclear lamina function	*Zmpste24*$^{-/-}$
Atypical Werner syndrome			*Lmna*$^{L530P/L530P}$
Restrictive dermopathy (RD)	*LMNA, ZMPSTE24*		
Mandibuloacral dysplasia (MAD)			

Progeria + cancer

Syndrome	Mutated genes	Affected processes	Mouse models
Fanconi anaemia (FA)	*FANC, BRCA2*	DNA crosslink repair	*Fancc; Fanca; Fancg; Fancd2;Brca2*
Xeroderma pigmentosum (XP) combined with CS (XPCS)	*XPB, XPF, XPD, XPG*	NER	*Xpd*^{xpcs}
Xeroderma pigmentosum (XP)+DeSanctis-Cacchione syndrome (DSC)	*XPA, XPD*	NER	*Xpg*$^{-/-}$
Ataxia telangiectasia (AT)	*ATM*	DSB repair	*Atm*$^{-/-}$ *mTR*$^{-/-}$
Ataxia telangiectasia-like disorder (ATLD)	*MRE11*	DSB repair	*Mre11*$^{-/-}$
Nijmegen breakage syndrome (NBS)	*NBS1*	DSB and telomere maintenance	*Nbs1*^{p70}
Bloom syndrome (BLS)	*BLM*	Mitotic recombination	*Blm*$^{-/-}$
Werner syndrome (WS)	*WRN*	Telomere maintenance, DNA recombination and repair	*Wrn*$^{-/-}$ *mTR*$^{-/-}$

(Garinis et al, 2008)

Werner syndrome

Werner syndrome

- Autosomal recessive disorder
- Discovered by Otto Werner in 1904 in a family displaying symptoms similar to premature aging
- Gene affected: WRN:
  - 180 Kda protein from RecQ helicase family
  - 3’-5’ exonuclease and 3’-5’ helicase activity
- Absence of WRN protein: abnormalities in DNA repair, replication and telomere maintenance

Werner syndrome

15----------→48yo

8----------→36yo

(Muftuoglu et al 2008)

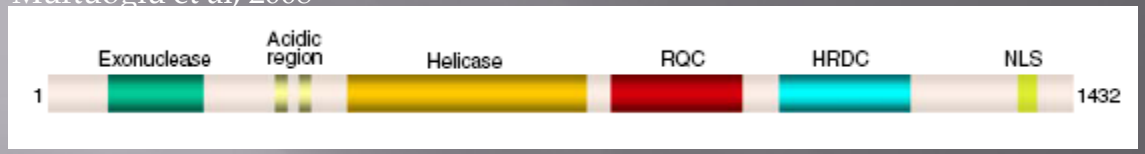
- Affects 10/million individuals
- First clinical sign: lack of growth spurt at puberty
- Short stature: patients are 13cm shorter and 20kg lighter than general population
- In 20's and 30's, manifest skin atrophy, loss of hair, early greying and cataracts
- Progressive disease

Clinical signs

Clinical diagnostic criteria
Cataracts (bilateral)
Dermatological pathology (tight, atrophic skin, pigmentary alterations, ulceration, hyperkeratosis, etc)
Short stature
Premature greying, thinning of scalp hair
Hypogonadism
Neoplasms (rare sarcomas)
Abnormal voice (high-pitches, squeaky or hoarse)
Type 2 Diabetes mellitus
Osteoporosis
Atherosclerosis (history of myocardial infarction)

WRN protein

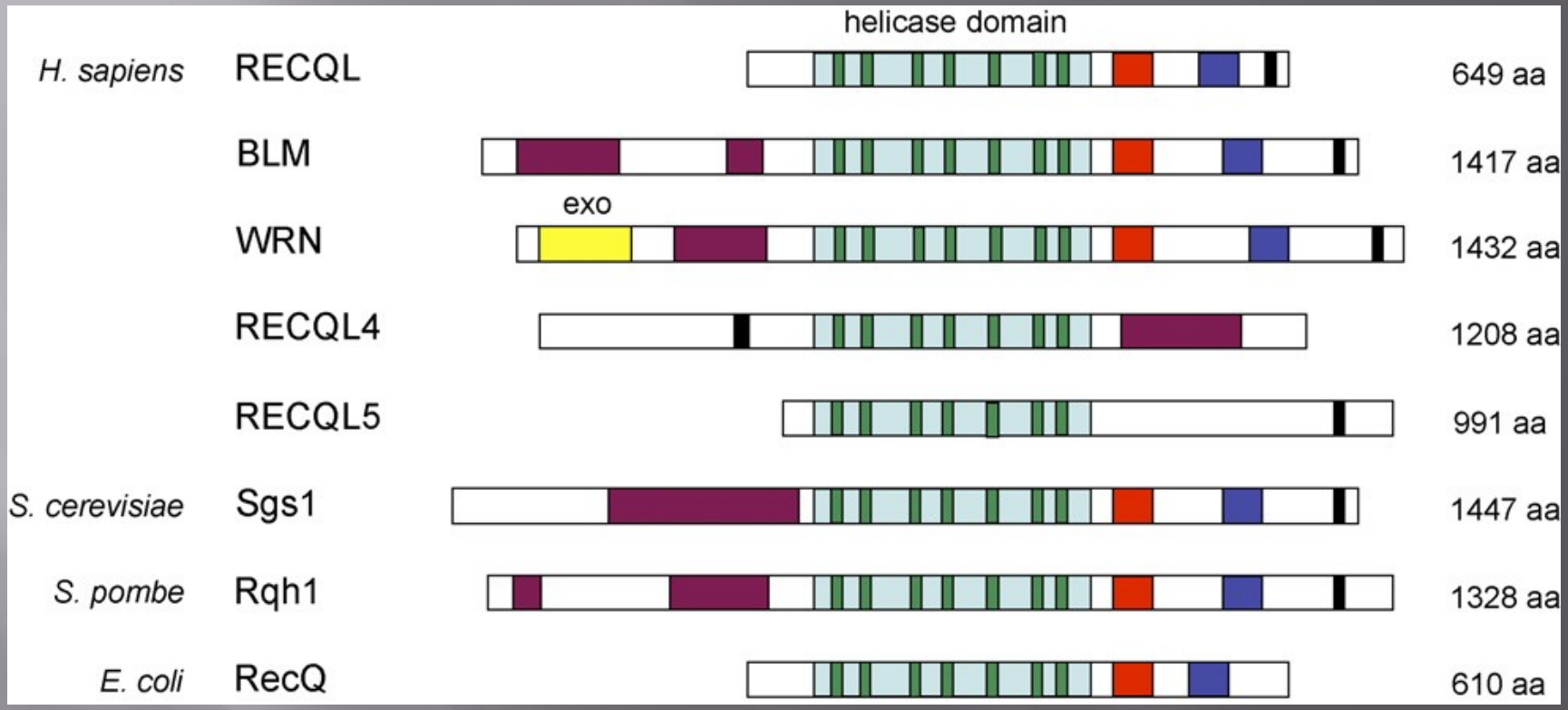
Muftuoglu et al, 2008



1432 amino acids
162 KDa

- Activities of WRN similar to other RecQ helicases except 3′-5′ exonuclease activity (proofreading)
  - Exonuclease activity: can degrade a 3′end on dsDNA or RNA-DNA duplex
- 3′-5′ helicase, coupled to ATP hydrolysis
  - Prefers G quadruplex and triple helix DNA
- RecQ C-terminal (RQC) domain
  - Prefers DNA structures resembling replication intermediates (forked and Holliday junction) and participates in protein-protein interactions (TRF2, BLM)
- Helicase and ribonuclease D C-terminal (HRDC) domain
- NLS: nuclear localization signal

The RecQ family of DNA helicases

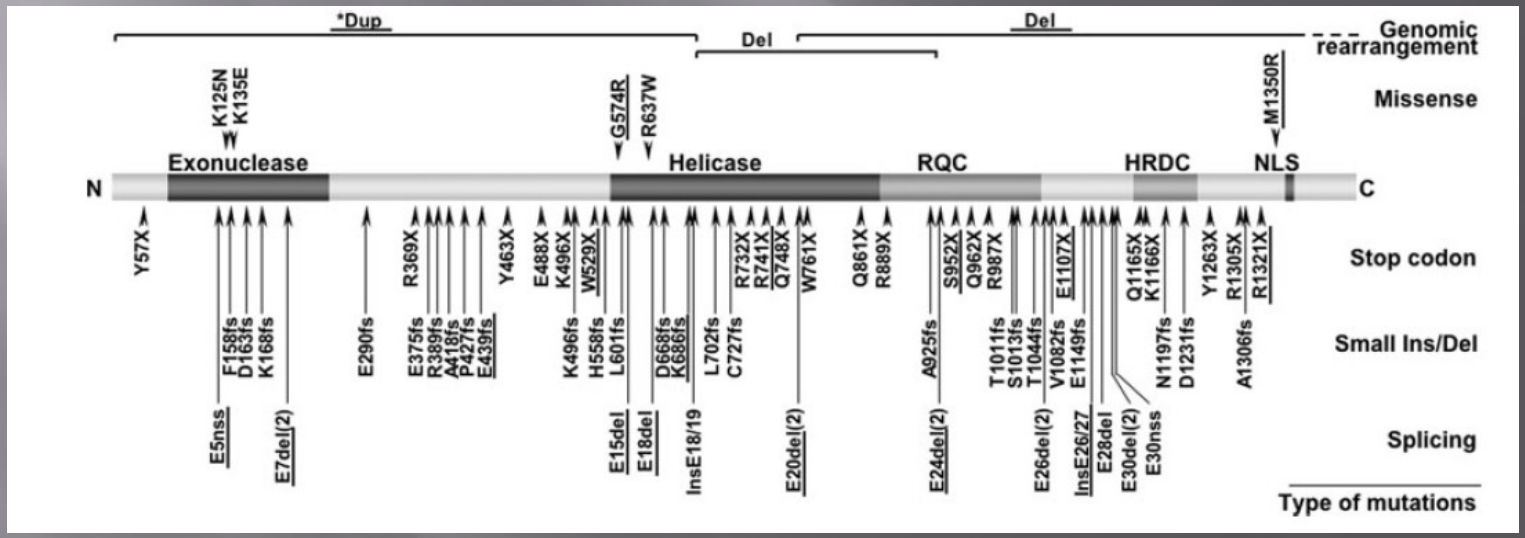


(Ouyang et al, 2008)

- conserved central helicase domain (seven helicase motifs)
- The exonuclease (exo) domain of WRN is shown in yellow

WRN Mutations

- Nonsense mutations, changes amino acid to a stop codon
- Insertion and/or deletion, leading to frameshift and subsequent termination
- Substitution at splice junction, causing skipping of exons and frameshift
- One case of missense mutation causing change in codon→protein stability affected
- Most mutations generate truncated WRN protein lacking NLS, found at the C terminal portion.



(Friedrich et al 2010)

Pathogenesis

- WS pathogenesis driven by defective DNA metabolism, leading to genetic instability
- In absence of WRN, cells accumulate toxic DNA intermediates and/or critically short telomeres that lead to DNA damage and apoptotic responses

WRN Cells

- Cells from WS patients display accelerated aging characteristics
- Increased chromosomal instability
- Abnormal telomere maintenance
- Premature replicative senescence in culture
- 70% reduction in mean population doublings
- Prolonged S phase
- Sensitivity to certain genotoxic drugs
- Apoptotic response attenuated

CELLULAR DEFECTS IN WS

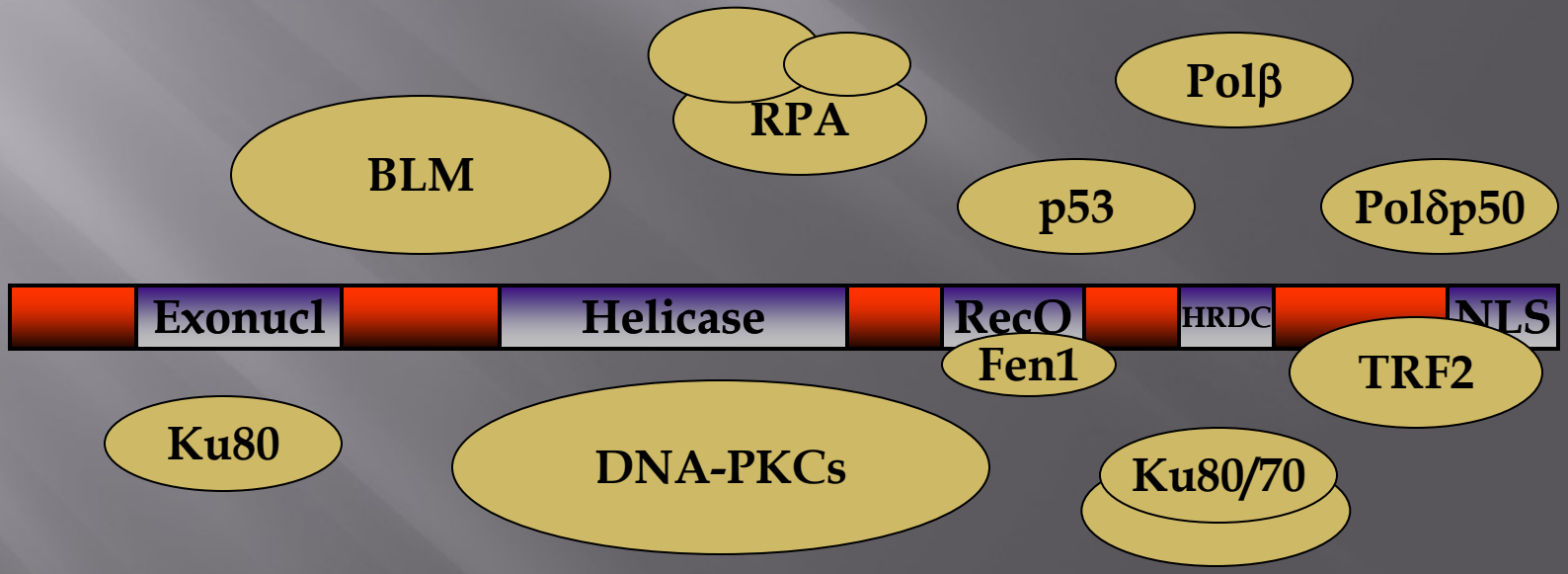
- Genomic Instability
  - Chromosomal rearrangements
  - Large spontaneous deletions
- Telomere maintenance
  - Shortened telomeres
  - WRN co-localizes with telomeric DNA in some cells
- Recombination
  - Hyper-recombination
  - Recombination defects
- Transcription
  - RNA polymerase II transcription
    - 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

Evidence suggesting that WRN functions to resolve aberrant DNA structures resulting from DNA metabolic processes, thus maintaining the genetic integrity of cells

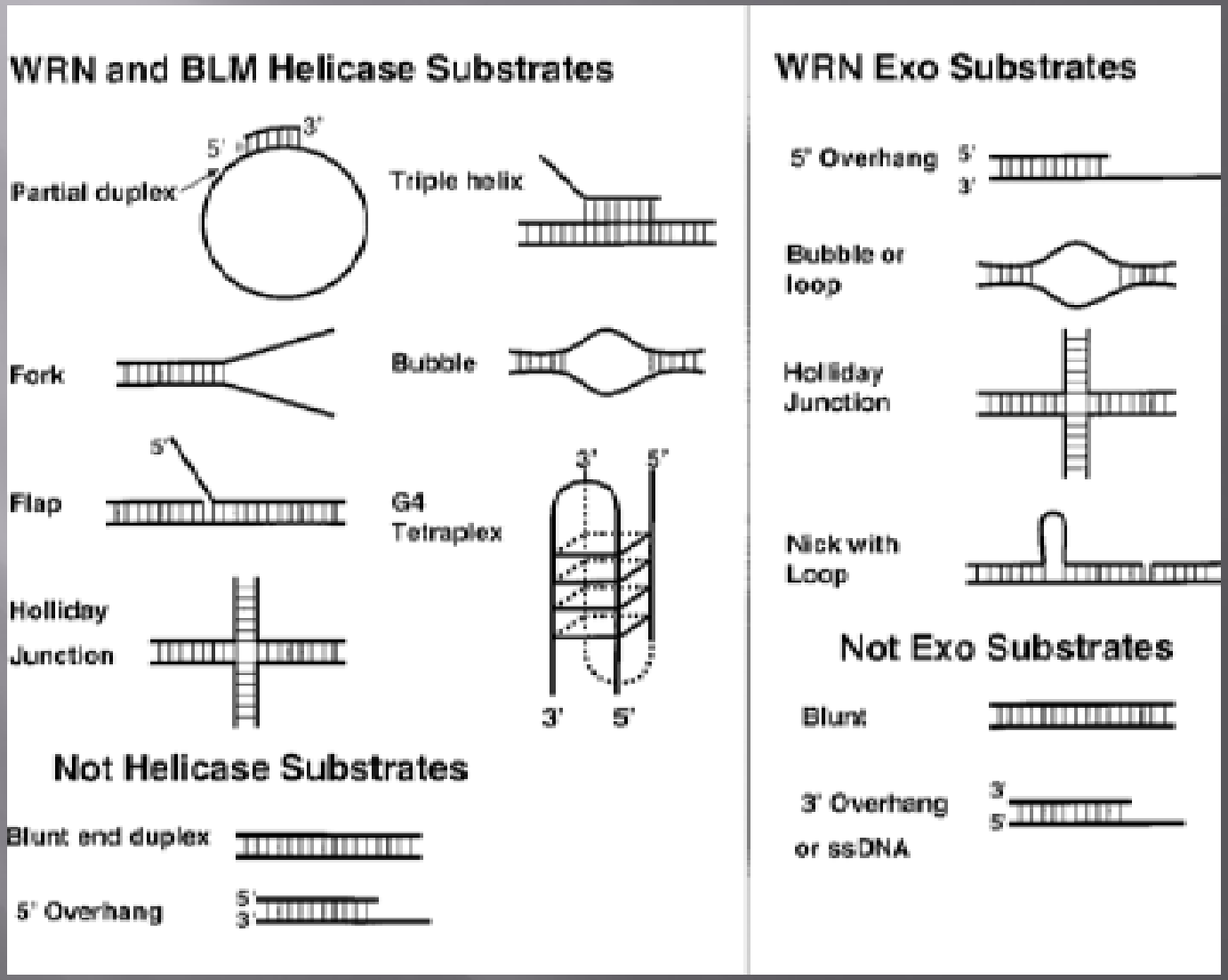
Opresko, P.L. et al. 2003. Carcinogenesis 24, 791-802

Biological Roles of WRN

- **Direct protein-protein interactions, IPs, Y2H, immunostaining**
  - Nuclear proteins → cooperate in DNA interactions during replication, repair (recombination), etc
  - Shelterin proteins→Telomere maintenance ie during replication of telomeres



Substrate specificity of WRN helicase *in vitro* includes DNA replication, recombination and repair intermediates

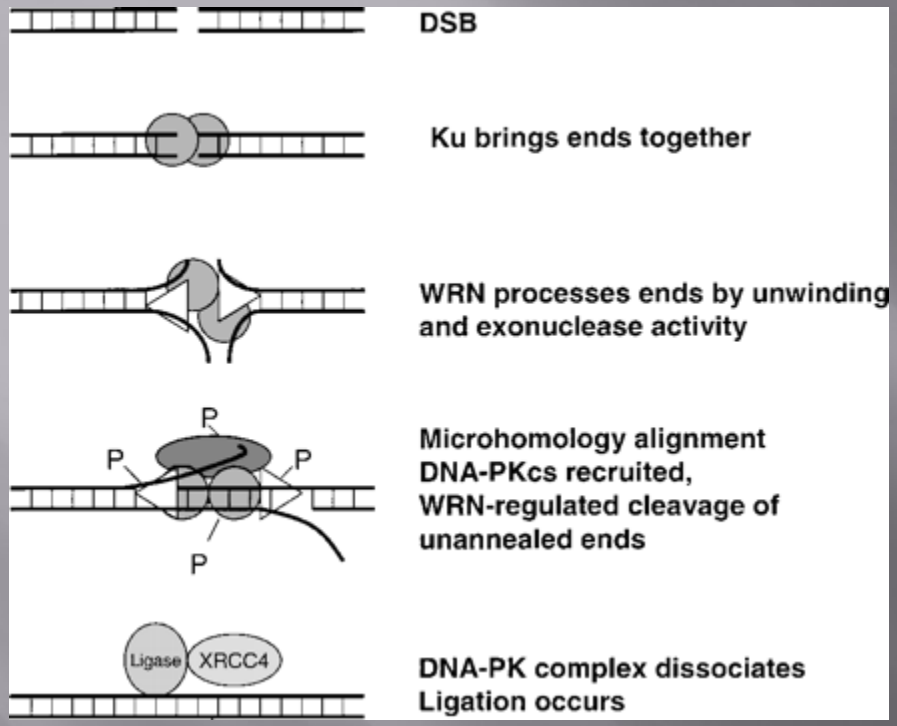


(Opresko et al. 2003)

Apoptosis

- WRN binds to C terminus of p53 in vivo
- WS fibroblasts display attenuated p53-mediated apoptotic response, rescued by expression of wild type WRN
- Increased cancer incidence due to
  - Inability to suppress genomic instability
    - Disruption of p53-mediated apoptotic pathway
    - Wrn/p53 double knockout mice: increased rate in mortality and increased rate of tumor development

Function of WRN in DNA repair pathways



Opresko et al. 2003

Ku and DNA-PK are components of the NHEJ pathway for repair of DSBs

Ku stimulates WRN 3′ to 5′ exonuclease

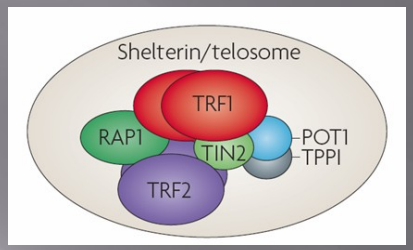
Generation of 5′ ss flaps

Phosphorylation of WRN by DNA-PK limits the extent of end degradation?

WRN stimulation of FEN1 flap cleavage

WRN’s role at telomeres

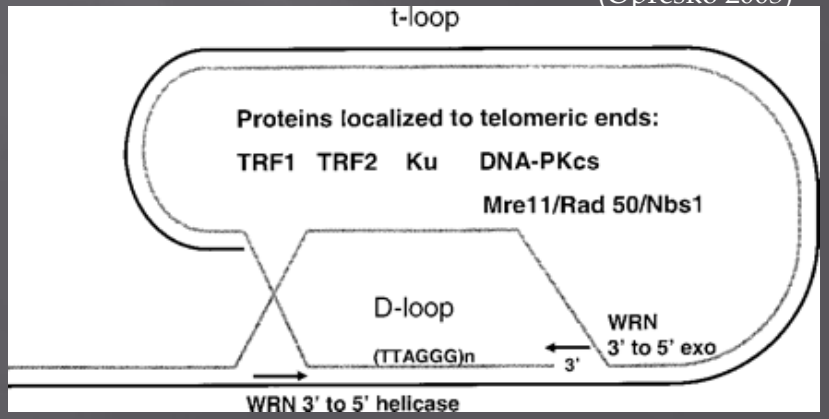
WRN's role at telomeres

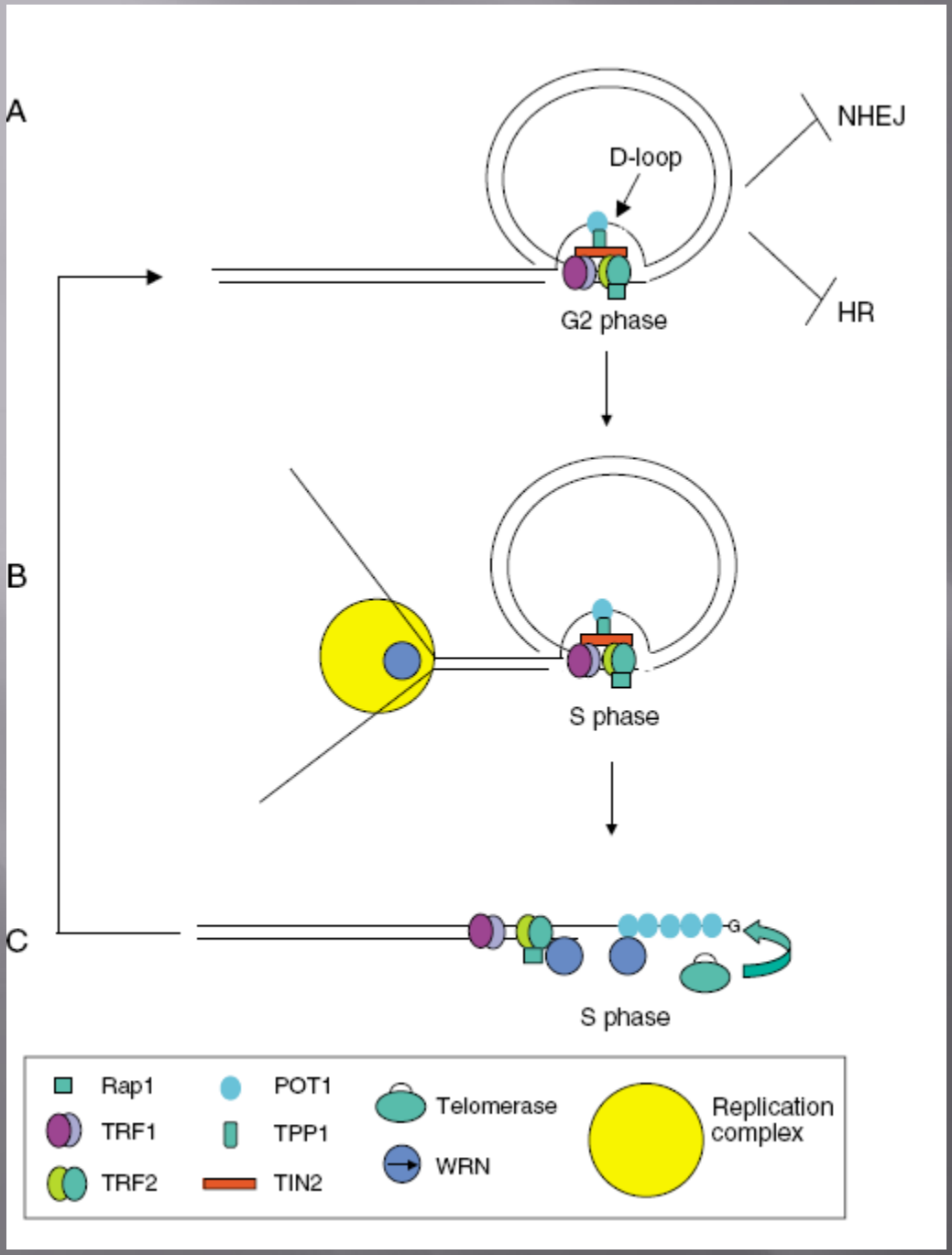


Blasco 2007

- Telomeres protect ends of linear chromosomes
  - Shelterin proteins remodel chromosome end so 3' ssDNA tail is tucked into the D-loop
  - Prevent recognition as DS DNA breaks
  - Protects ends from enzymatic attack to avoid loss of genetic information

(Opresko 2003)



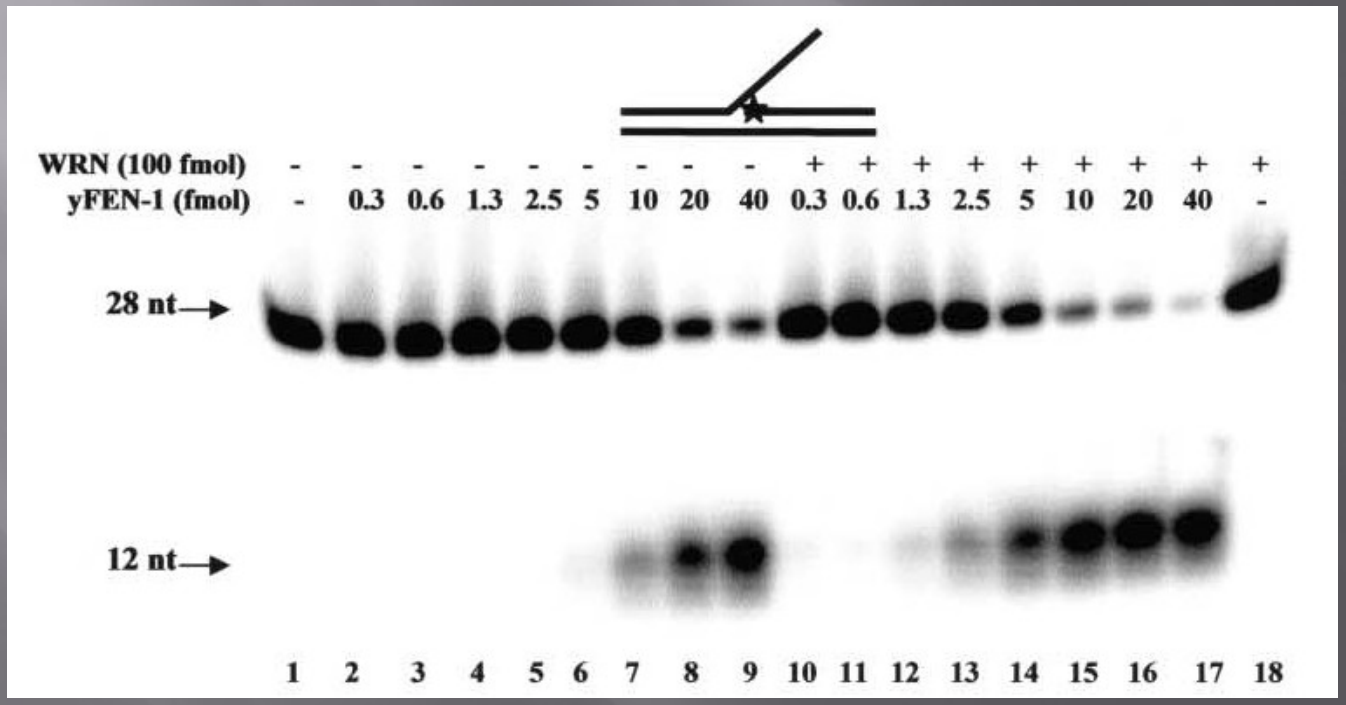


- During telomere replication, the presence of WRN at the replication fork is postulated to enable the replication complex to efficiently replicate telomeric DNA
- The presence of WRN at telomeres may facilitate unwinding of the D-loop, enabling telomerase to extend telomeres
- TRF1, TRF2 and Pot1 stimulate and modulate WRN's activity

(Multani and Chang, 2007)

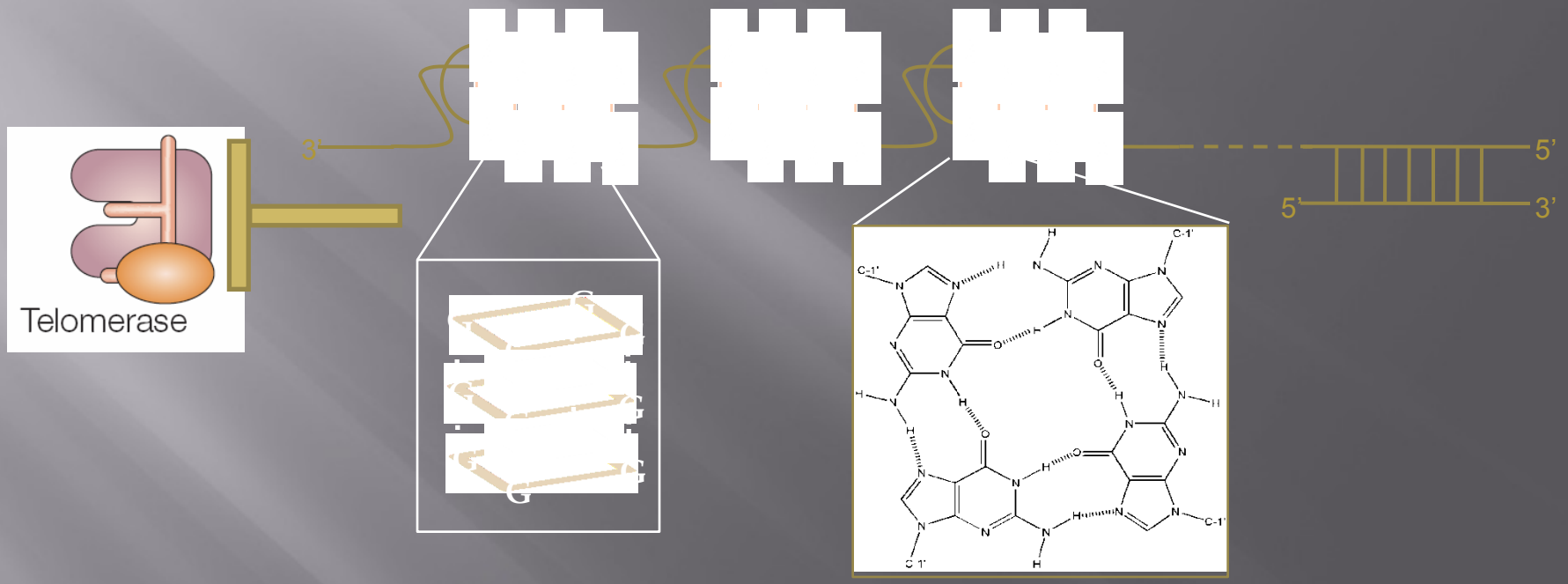
WRN's role in telomere replication

- When WRN is inhibited : loss of G-rich lagging strand
- WRN interacts with FEN-1 flap endonuclease, which helps process and join Okazaki fragments on the lagging strand. In WRN null cells this interaction with FEN-1 may be compromised



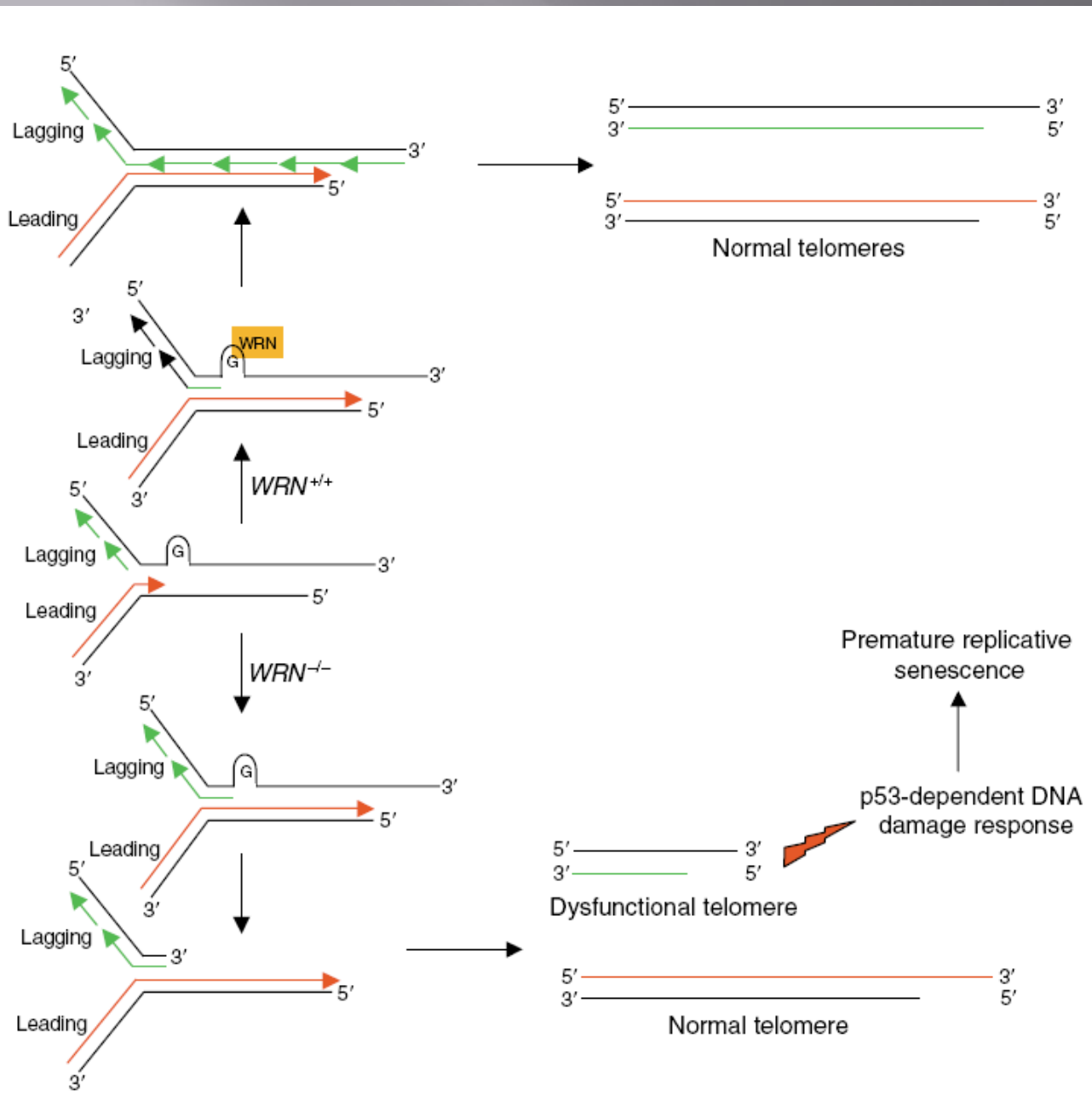
(Sharma et al 2004)

G-Quadruplex Stabilization Leads to Telomerase Repression



The G-rich strand may fold into G quadruplex structure which can stall the replication fork

Fakhoury, J, Nimmo, G, Autexier, C. Anticancer Agents in Medicinal Chemistry, 2007



- -G-quadruplex formation on the lagging telomeric DNA is normally resolved by WRN
- -In absence of WRN, G-quadruplex formation on the lagging telomere leads to replication fork stalling and deletion of lagging strand telomeres

(Multani and Chang, 2007)

Telomere length in WS patients

- The resultant dysfunctional telomeres in absence of WRN can initiate a DNA-damage response, leading to premature onset of replicative senescence
- Cells from WS patients undergo premature replicative senescence
- However telomeres in WS cells erode at rates similar to normal control cells (in some studies, telomere length of senescent WS-derived cells are longer than normal)
- WS cells may be sensitive to presence of few dysfunctional telomeres-one may even be sufficient to limit replicative potential
  - (one dysfunctional telomere signals to cell that it is time to enter replicative senescence)

WS Animal Model

- WRN knockout
- WRN deletion of helicase domain (retains exonuclease activity)
- Transgenic expression of human Lys577Met WRN variant, lacks helicase domain

- None of these mice display obvious premature aging or spontaneous cancer predisposition
- Murine WRN might be functionally redundant with other RecQhelicases

Late generation mice with short telomeres exhibit nearly the full spectrum of WS syndromes

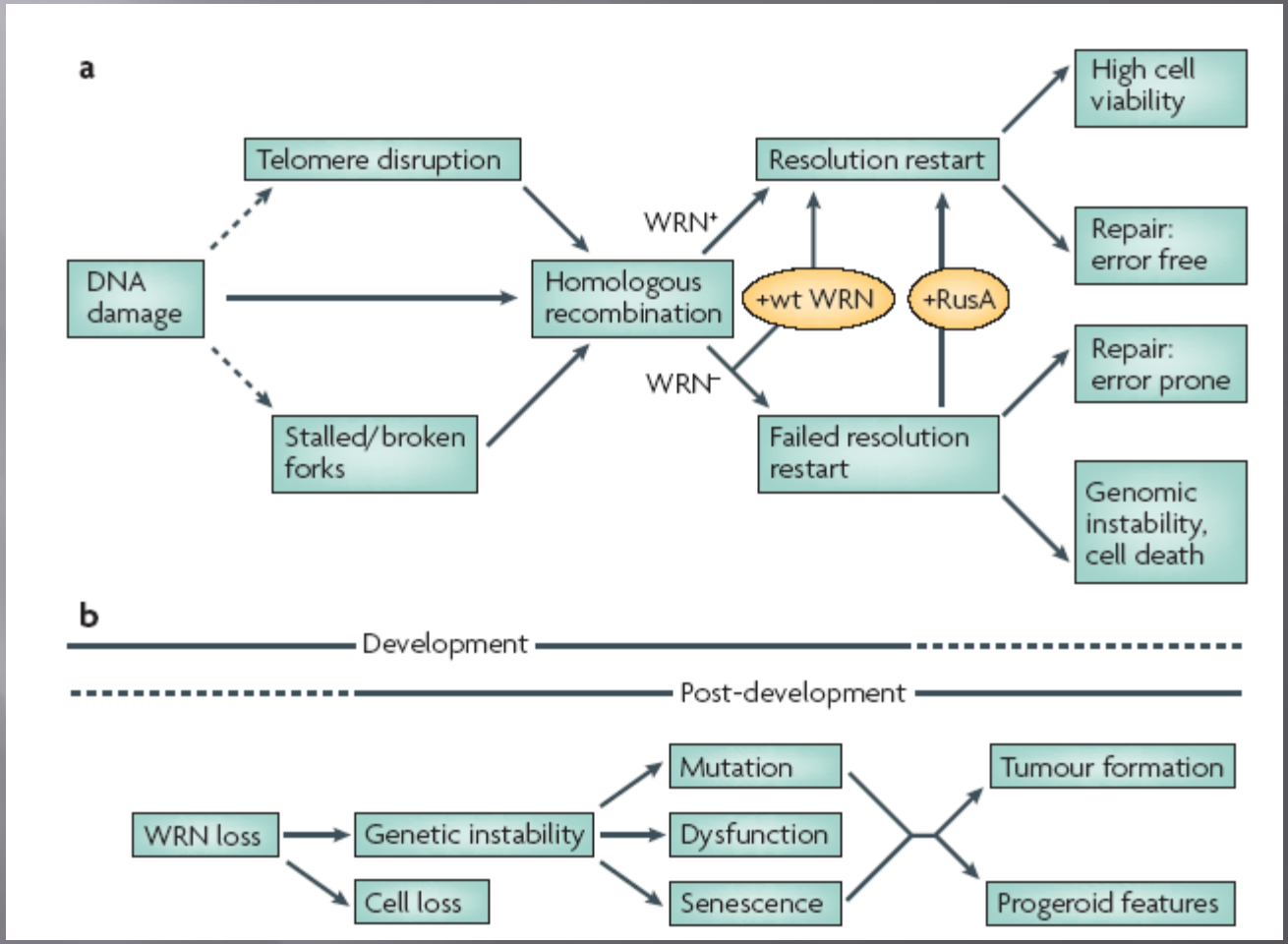
Table 1
Summary of phenotypes observed in the *WRN*$^{-/-}$, *mTerc*$^{-/-}$, and G4-6 *mTerc*$^{-/-}$ *WRN*$^{-/-}$ mouse models

Human WS	Mouse models		
	WRN$^{-/-}$	G4-6 *WRN*$^{+/+}$	G4-6 *WRN*$^{-/-}$
Osteoporosis	No	No	++++
Cataracts	No	No	++++
Type II diabetes	No	No	++++
Skin defects	No	++	++++
Hypogonadism	No	++	++++
Atherosclerosis	No	No	No
Genome instability	No	+++	++++
Mesenchymal tumors	+	+	++++

Phenotypes characteristic of WS patients are observed only in *mTerc*$^{-/-}$ *WRN*$^{-/-}$ mouse model with dysfunctional telomeres.

Chang, S. 2005. IJBCB 37: 991-999

WRN function and disease pathogenesis



Kudlow, B.A. et al. 2007. Werner and Hutchinson-Gilford progeria syndromes: mechanistic basis of human progeroid diseases. Nature Reviews Mol. Cell Biol. 8: 394-404.

Ataxia-telangiectasia

ATAXIA TELANGIECTASIA

Pleiotropic, autosomal recessive inherited disease with a complex clinical phenotype

Phenotypes typically appear in the second year of life

Frequency of ATM gene carriers 1/100; estimated frequency of affects 1/40000

Clinical signs

Clinical diagnostic criteria
Early onset progressive cerebral ataxia
Oculocutaneous telangiectasia: angioma of skin of face, brain
Susceptibility to bronchopulmonary disease
Susceptibility to lymphoid tumors
Absence of or rudimentary thymus
Immunodeficiency
Progressive apraxia of eye movements: inability to move eyes voluntarily
Insulin resistant diabetes
Clinical/cellular radiosensitivity
Cell cycle checkpoint defects
Chromosomal instability

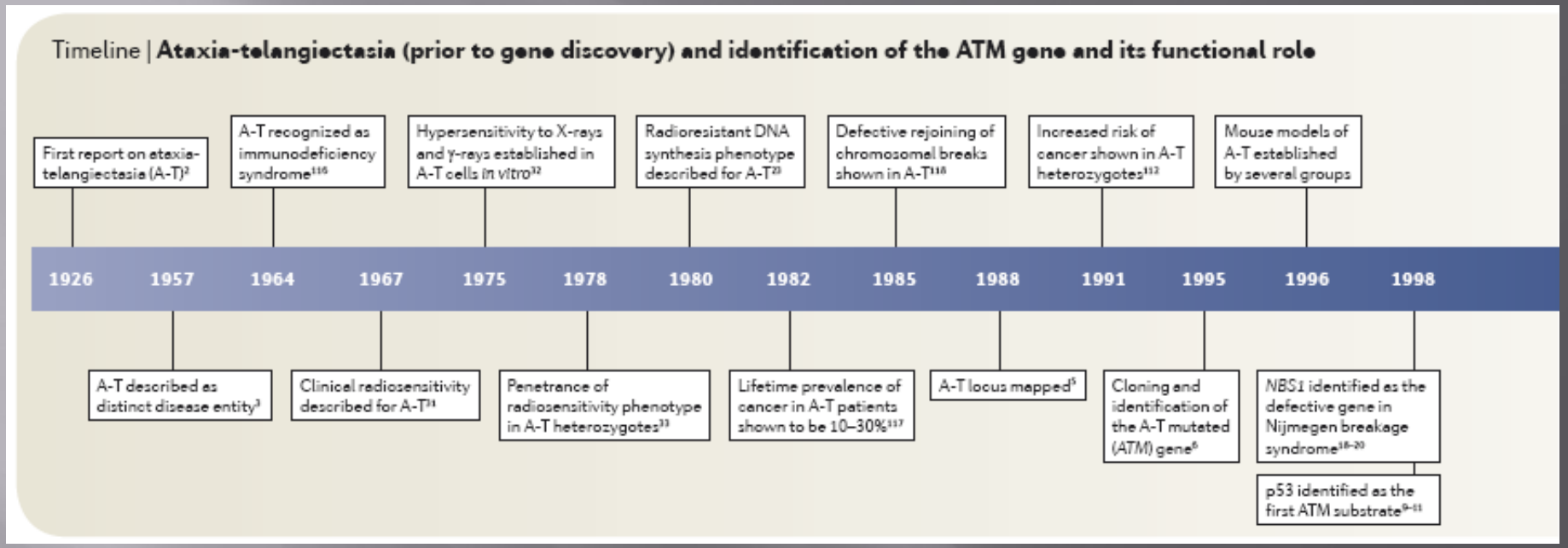
DNA damage recognition/repair syndromes defective in DNA double-strand break repair

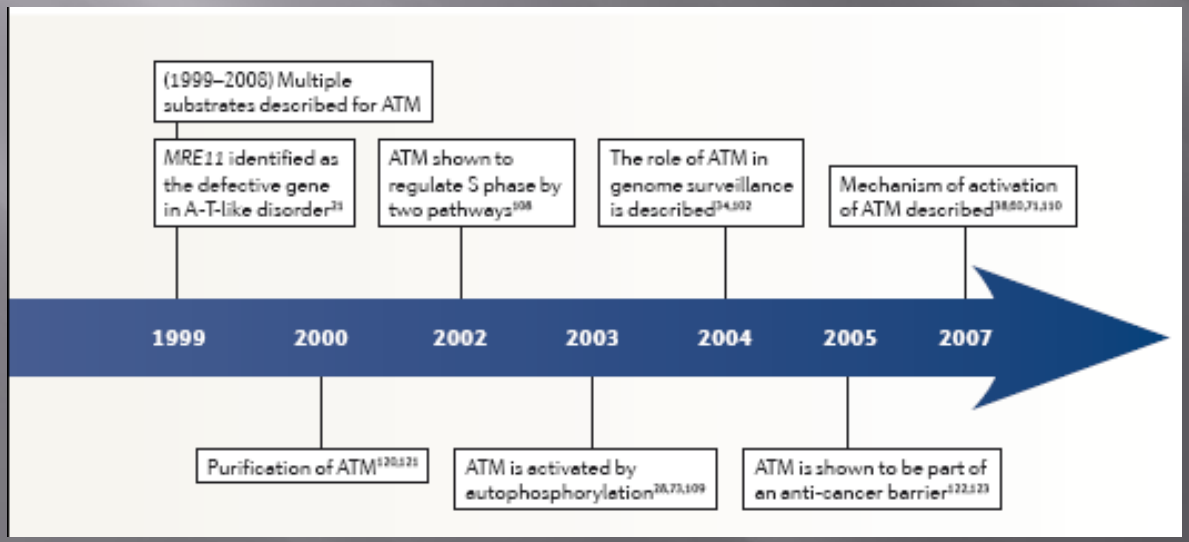
Table 1 DNA damage recognition/repair syndromes defective in DNA double-strand break repair

Syndrome	*Defective gene*	*Mutant protein*	*Cancer susceptibility*	*Neurological changes*	*Developmental/ growth delay*	*Agent/sensitivity*
Ataxia-telangiectasia (A-T)	*ATM*	ATM	Yes	Neurodegeneration	No	Ionizing radiation
A-T-like disorder (ATLD)	*Mre11*	Mre11	No	Neurodegeneration	No	Ionizing radiation
Nijmegen breakage syndrome (NS)	*Nbs1*	Nbs1	Yes	Microcephaly	Yes	Ionizing radiation
Rad50-deficient patient	*Rad50*	Rad50	?	Microcephaly	?	Ionizing radiation
Ataxia oculomotor apraxia type 1 (AOA1)	*Aptx*	Aprataxin	No	Neurodegeneration	No	H_2O_2
Ataxia oculomotor apraxia type 2	*Setx*	Senataxin	No	Neurodegeneration	No	H_2O_2
Spinocerebellar ataxia with axonal neuropathy (SCAN1)	*Tdp1*	TDP1	No	Neurodegeneration	No	H_2O_2
A-T and Rad30 related disorder (Seckels)	*ATR*	ATR	No	Neurodegeneration	No	HU, UV
DNA ligase	*Lig IV*	DNA Ligase IV	No	Microcephaly	Yes	Ionizing radiation

Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia mutated and Rad3 related; NBS, Nijmegen breakage syndrome.

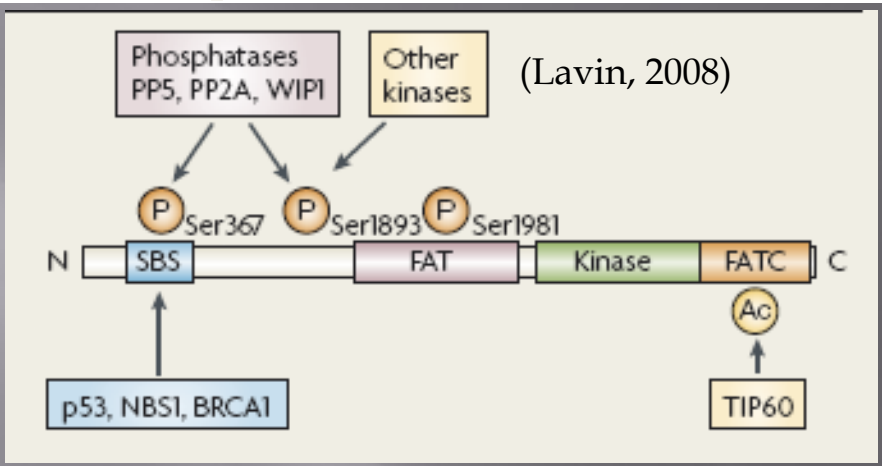
Lavin, MF. 2007. Oncogene 26, 7749-7758.


Timeline | Ataxia-telangiectasia (prior to gene discovery) and identification of the ATM gene and its functional role
First report on ataxia-telangiectasia (A-T)[2]
A-T recognized as immunodeficiency syndrome[115]
Hypersensitivity to X-rays and γ-rays established in A-T cells in vitro[32]
Radioresistant DNA synthesis phenotype described for A-T[20]
Defective rejoining of chromosomal breaks shown in A-T[116]
Increased risk of cancer shown in A-T heterozygotes[112]
Mouse models of A-T established by several groups
1926
1957
1964
1967
1975
1978
1980
1982
1985
1988
1991
1995
1996
1998
A-T described as distinct disease entity[3]
Clinical radiosensitivity described for A-T[31]
Penetrance of radiosensitivity phenotype in A-T heterozygotes[33]
Lifetime prevalence of cancer in A-T patients shown to be 10–30%[117]
A-T locus mapped[5]
Cloning and identification of the A-T mutated (ATM) gene[6]
NBS1 identified as the defective gene in Nijmegen breakage syndrome[18–20]
p53 identified as the first ATM substrate[9–11]


(1999–2008) Multiple substrates described for ATM
MRE11 identified as the defective gene in A-T-like disorder[21]
ATM shown to regulate S phase by two pathways[108]
The role of ATM in genome surveillance is described[34,102]
Mechanism of activation of ATM described[38,60,71,110]
1999
2000
2002
2003
2004
2005
2007
Purification of ATM[120,121]
ATM is activated by autophosphorylation[28,73,109]
ATM is shown to be part of an anti-cancer barrier[122,123]

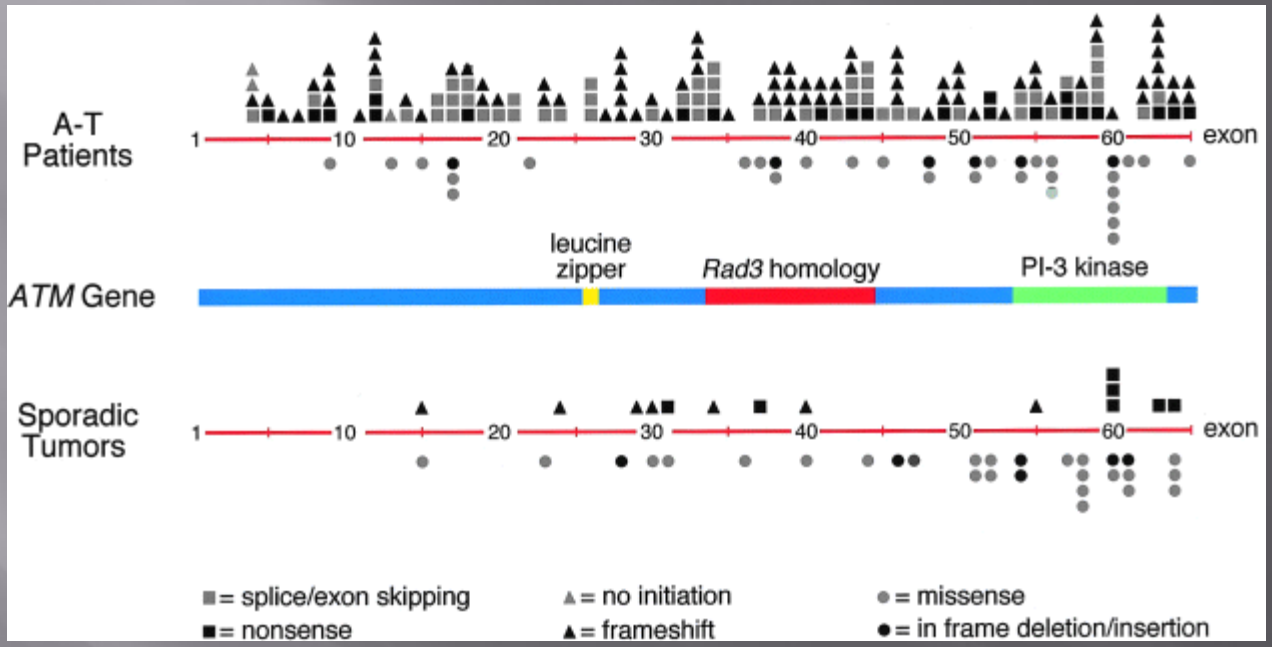
(Lavin, 2008)

Ataxia-telangiectasia mutated (ATM)



- Serine-threonine protein kinase
- Member of the PIKK family (Phospho-inositide-3-kinase-related protein kinase family)
- Kinase domain includes the ATP binding site and the catalytic residues
- FAT domain function unknown; contains the serine 1981 that is autophosphorylated during ATM activation
- FATC domain C-terminal domain conserved in those proteins that also have FAT domain
- Leucine zipper usually involved in forming helices involved in protein-protein interactions; thus far this region in ATM doesn't interact with other proteins or mediate ATM dimerization
- Proline-rich region mediates interaction with SH3 domain of c-Abl tyrosine kinase
- N-terminal substrate-binding site: p53, BRCA1, BLM binding

Spectra of *ATM* mutations found in patients



Approximately 85% are predicted to truncate the protein-unstable
Missense cause loss of protein kinase activity or destabilization (potential for dominant effect of mutant ATM on wild-type in heterozygote)

Meyn, SM. 1999. Clin.Genet. 55, 289-304.

ATM maintains integrity of the genome

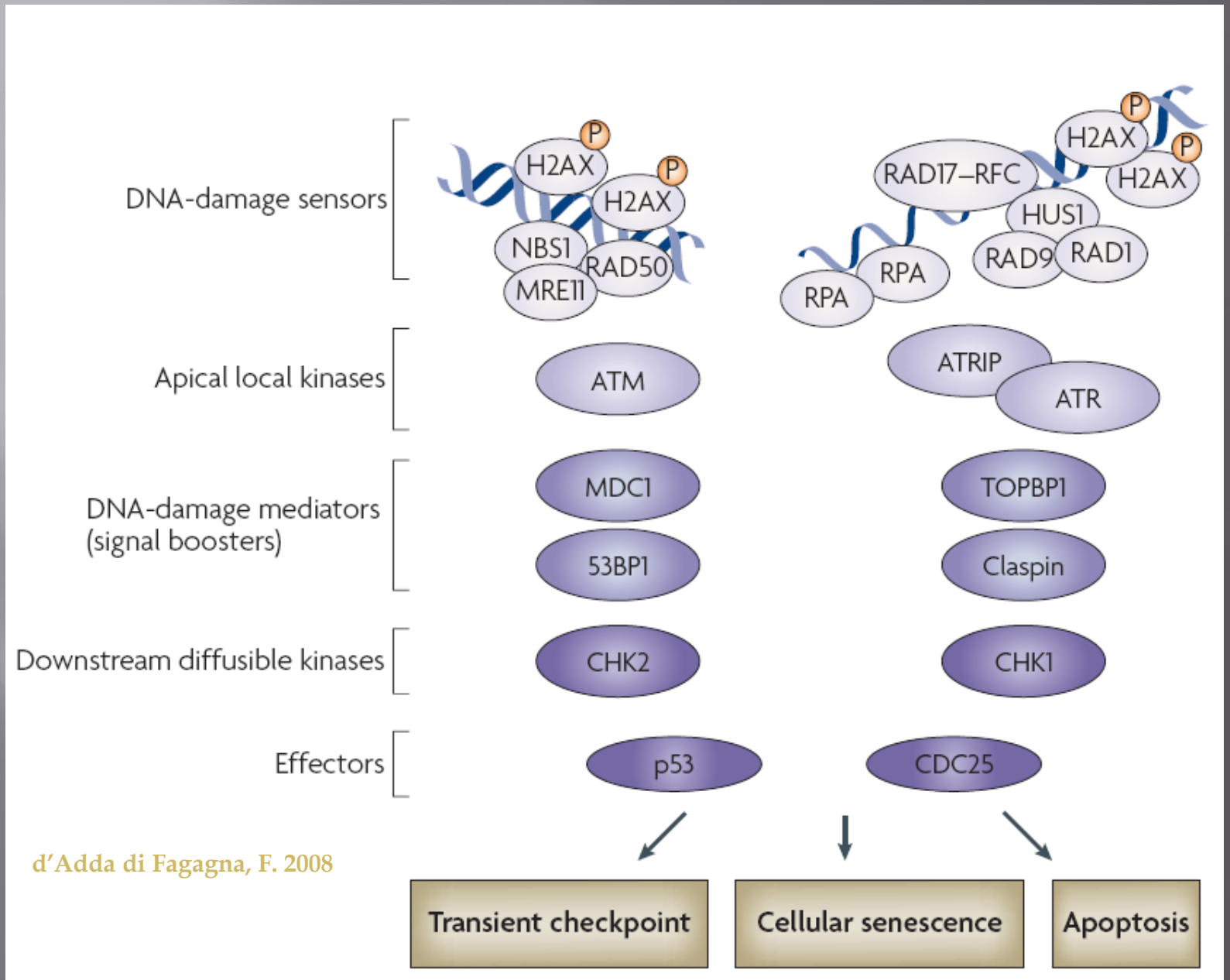
- ATM plays crucial role in cellular response to DNA damage
- ATM recognizes and responds to double stranded DNA breaks

- Once activated, ATM signals to
  - cell cycle checkpoints to slow passage of the cell through the cell cycle to facilitate repair
  - DNA repair machinery to protect against DNA insults

AT cells

- Exhibit various abnormalities:
  - Defects in cell cycle checkpoints
  - Increased radiation sensitivity
  - Chromosome instability
  - Defective telomere maintenance
  - Cells derived from AT patients show an elevated frequency of chromosomal aberrations such as end-to-end fusions
  - Primary fibroblasts both from human patients and Atm-/-mice undergo premature senescence in culture

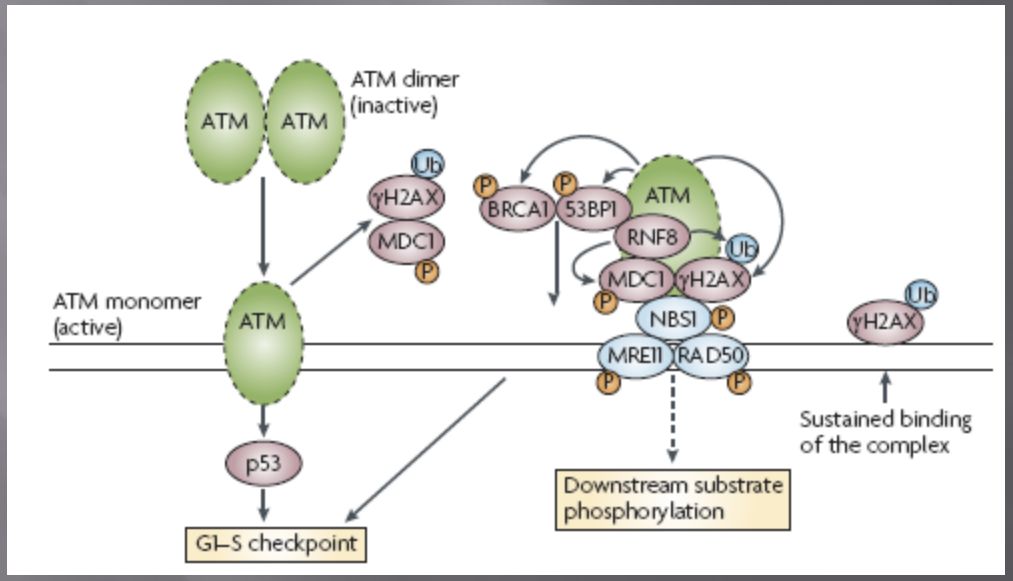
DDR activation by double or single stranded DNA and activation of ATM or ATR



d'Adda di Fagagna, F. 2008

ATM substrate: p53

- First substrate to be identified; phosphorylated on ser15
- ATM need only be partially activated to phosphorylate p53
- ATM also phosphorylates MDM2 and Chk2, which also help to stabilize p53

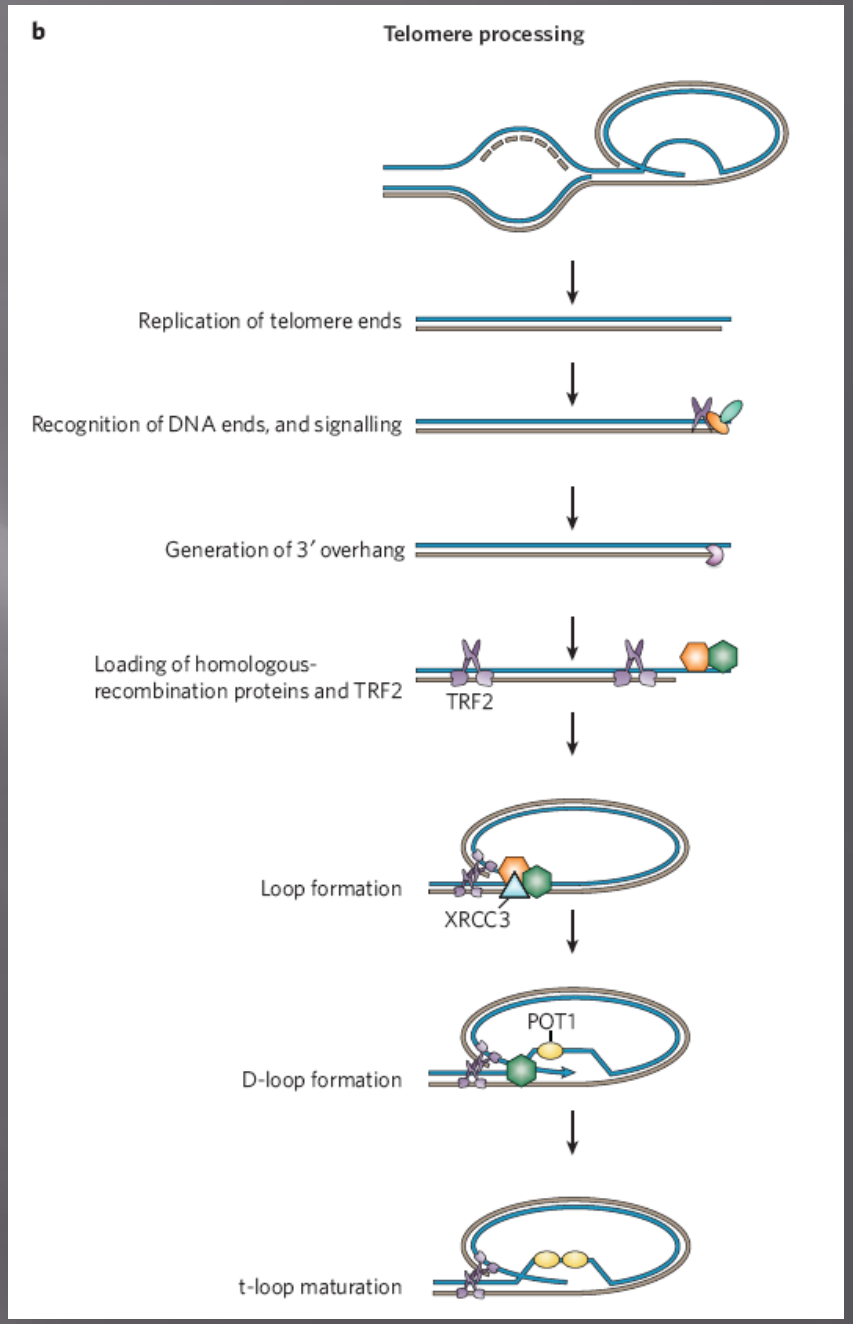


(Lavin, 2008)

Processing of telomeres

(Verdun and Karlseder 2007)

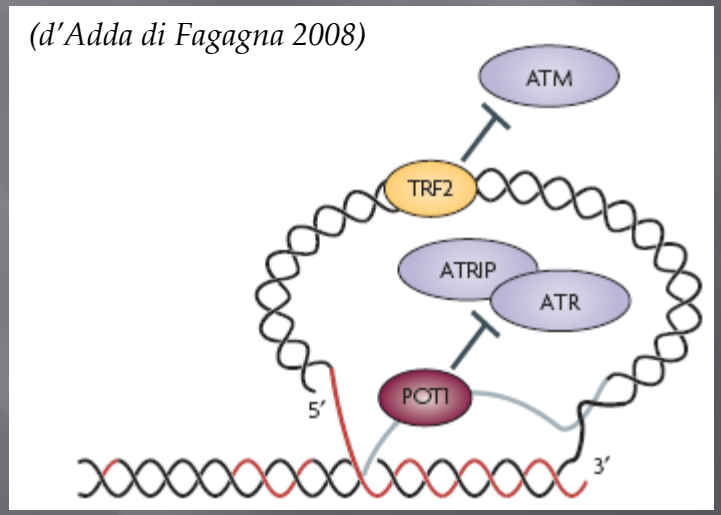
- MRN and ATM localize to telomeres from late S phase until G2 phase
- In a manner analogous to that of DSB processing, telomeres recruit repair proteins resulting in a search for homologous DNA sequences followed by strand invasion-->T-loop and D loops are formed
- TRF2 keeps the telomere end and the duplex DNA of the same telomere in proximity so that invasion of another chromosome does not occur



Telomere integrity

- Telomeres do not activate the DNA damage response despite resembling a break because of T loop
- TRF2 inhibits the checkpoint activity of ATM
- When a cell undergoes replicative senescence, the telomere reaches a critical length, resulting in loss of shelterin proteins such as TRF2
- The loss of proteins negative regulators such as TRF2 leads to DDR; one critically short telomere is sufficient to send a cell into replicative senescence

(d'Adda di Fagagna 2008)



Laminopathies including Hutchinson-Gilford progeria syndrome (HGPS)

Table 1
Diseases caused by mutations in genes encoding lamins and associated proteins

Disease	Mutation	Major disease phenotypes
Striated muscle diseases		
Autosomal dominant EDMD	*LMNA*	Muscle weakness and wasting in scapulohumeral-peroneal distribution; early joint contractures; dilated cardiomyopathy
Autosomal recessive EDMD	*LMNA*	Muscle weakness and wasting in scapulo-humeral peroneal distribution; early joint contractures; dilated cardiomyopathy
Cardiomyopathy dilated 1A	*LMNA*	Cardiomyopathy with minimal to no skeletal muscle involvement
Limb-girdle muscular dystrophy type 1B	*LMNA*	Muscle weakness and wasting in limb-girdle distribution; dilated cardiomyopathy
Congenital-type muscular dystrophy	*LMNA*	Severe relatively diffuse myopathy presenting in first year of life; later cardiomyopathy
"Heart-hand" syndrome (with limb defects)	*LMNA*	Brachydactyly with mild hand and more severe foot involvement; cardiomyopathy
X-linked EDMD	*EMD*	Muscle weakness and wasting in scapulo-humeral peroneal distribution; early joint contractures; and dilated cardiomyopathy
Partial lipodystrophy syndromes		
FPLD2	*LMNA*	Loss of subcutaneous fat from the extremities at puberty, followed by increased fat accumulation in the face and neck; insulin resistance; diabetes mellitus; hyptertriglyceridemia; hepatic steatosis
Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules	*LMNA*	Generalized fat loss; insulin-resistant diabetes, hypertriglyceridemia, hepatic steatosis, hypertrophic cardiomyopathy; disseminated whitish papules
Mandibuloacral dysplasia (also has features of progeria)	*LMNA*	Hypoplastic mandible with dental crowding, acroosteolysis, stiff joints, atrophy of the skin over hands and feet, hypoplastic clavicles; "Andy Gump" appearance; persistently wide cranial sutures and multiple wormian bones; alopecia and short stature; and partial lipodystrophy
Acquired partial lipodystrophy (Barraquer-Simons syndrome)	*LMNB2*	Progressive, sporadic lipodystrophy with phenotype similar to FPLD2 (above)
Progeria		
HGPS	*LMNA*	Children appear aged; retarded growth; micrognathia; reduced subcutaneous fat; alopecia; skin mottling; osteoporosis; and premature occlusive vascular disease
Atypical Werner syndrome	*LMNA*	Various combinations of signs and symptoms including an aged appearance; short stature; cataracts; sclerodermatous skin; osteoporosis; vascular disease
Mandibuloacral dysplasia (also has partial lipodystrophy)	*LMNA*	Partial lipodystrophy features along with osteolytic lesions in bone similar to those found in HGPS
RD	*ZMPSTE24*	Perinatal lethal; tight skin; loss of fat; prominent superficial vasculature; dysplastic clavicles; sparse hair; and multiple joint contractures
Peripheral neuropathy		
Charcot-Marie-Tooth disorder type 2B1	*LMNA*	Wasting and weakness of the lower distal limbs; and lower limb areflexia
Other diseases		
Adult-onset autosomal dominant leukodystrophy	*LMNB1*	Symmetrical widespread myelin loss in the CNS; phenotype similar to that of chronic progressive multiple sclerosis
Pelger-Huet anomaly (heterozygous)/ HEM-Greenberg skeletal dysplasia (homozygous)	*LBR*	Pelger-Huet anomaly: benign blood disorder of hyposegmented neutrophil nuclei; HEM: generally prenatal/perinatal lethal with fetal hydrops; short limbs; and abnormal chondroosseous calcification
Osteopoikilosis, Buschke-Ollendorff syndrome, nonsporadic melorheostosis	*LEMD3*	Hyperostosis of cortical bone; dermatofibrosis in Buschke-Ollendorff syndrome
Autosomal recessive cerebellar ataxia	*SYNE1*	Dysarthria and ataxia; dysmetria; and brisk lower-extremity tendon reflexes
DYT1 dystonia	*TOR1A*	Early onset symptoms variably including twisted postures; turning in of the foot or arm; muscle spasms; and jerking movements
Dilated cardiomyopathy	*TMPO*	Dilated cardiomyopathy

HEM, hydrops-ectopic calcification motheaten; *LBR*, lamin B receptor; *LEMD3*, LEM domain–containing protein 3, also known as MAN1; *SYNE1*, spectrin repeat containing nuclear envelope 1, also known as nesprin-1; *TOR1A*, torsin family 1, member A; TMPO, thymopoietin, also known as lamina-associated polypeptide 2.

Worman et al 2009

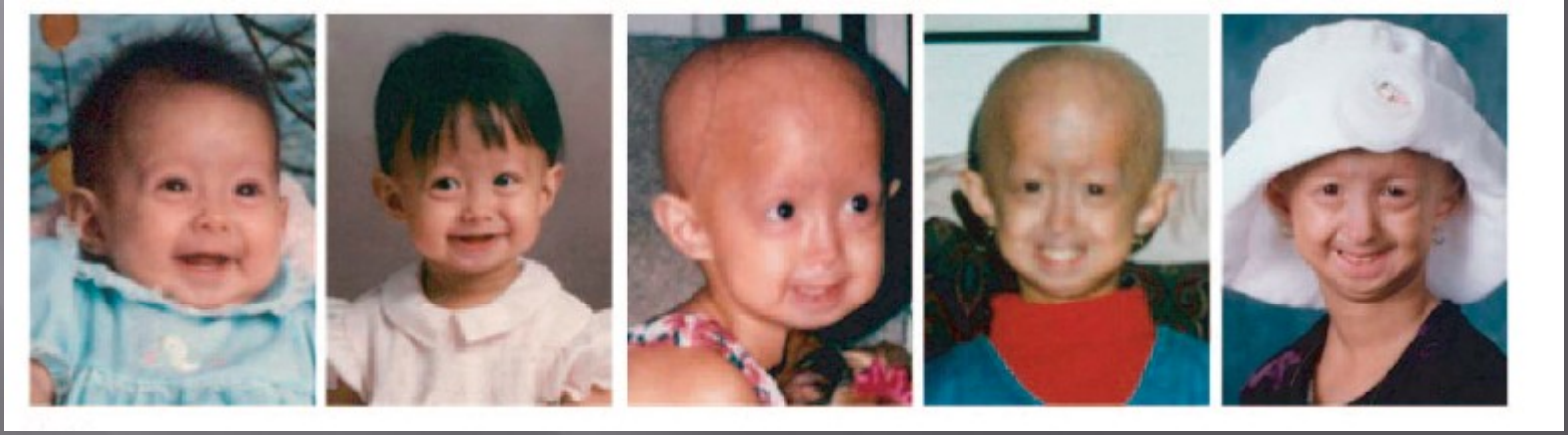
HGPS

Premature aging syndrome which affects 1 in 4-8 million children

Symptoms: thin skin, loss of subcutaneous fat, alopecia, stiff joints, osteoporosis, and heart disease

Age of onset within 2 years, with death at mean age of 13 due to heart attack or stroke

Physical characteristics of progeria with age. A, A girl with progeria at ages 3 months, 13 months, 3 years 11 months, 6 years 6 months, and 9 years.



Mutation in Lamin A

G608G mutation which exposes a cryptic splice site in exon 11 that leads to a 50 amino acid deletion resulting in lack of prelamin A processing and the translation of an aberrant protein called progerin

Lamins function in

- supporting the nuclear envelope and play a role in
  - mitosis
  - DNA synthesis and repair
  - RNA transcription and processing
  - apoptosis
  - organization of chromatin structure
  - regulation of gene expression

Nuclear Lamina Function

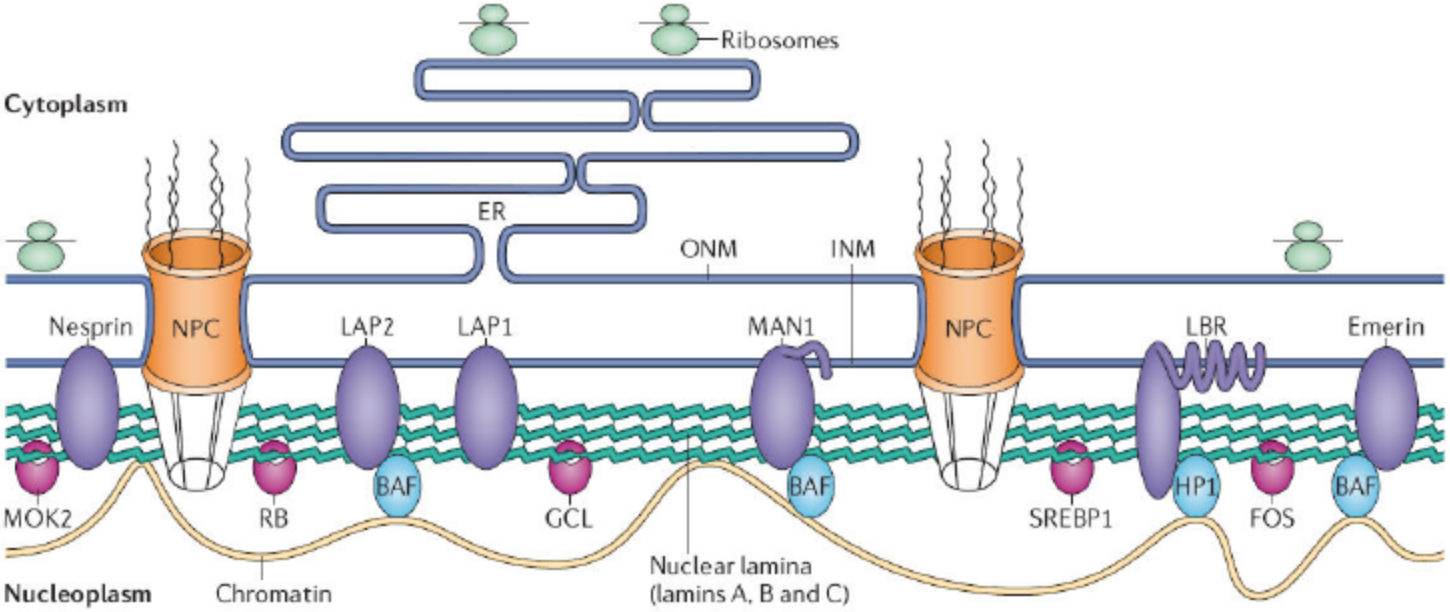
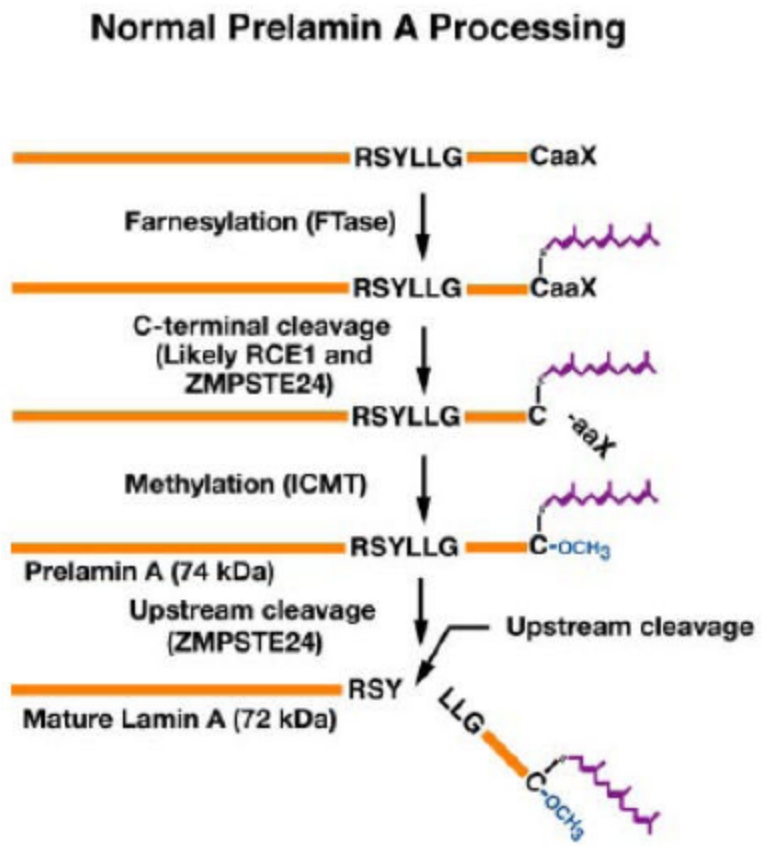


Figure 1
Structure and function of the nuclear lamina. The nuclear lamina lies on the inner surface of the inner nuclear membrane (INM), where it serves to maintain nuclear stability, organize chromatin and bind nuclear pore complexes (NPCs) and a steadily growing list of nuclear envelope proteins (purple) and transcription factors (pink). Nuclear envelope proteins that are bound to the lamina include nesprin, emerin, lamina-associated proteins 1 and 2 (LAP1 and LAP2), the lamin B receptor (LBR) and MAN1. Transcription factors that bind to the lamina include the retinoblastoma transcriptional regulator (RB), germ cell-less (GCL), sterol response element binding protein (SREBP1), FOS and MOK2. Barrier to autointegration factor (BAF) is a chromatin-associated protein that also binds to the nuclear lamina and several of the aforementioned nuclear envelope proteins. Heterochromatin protein 1 (HP1) binds both chromatin and the LBR. ONM, outer nuclear membrane [9].

Coutinho et al 2009

Lack of mature lamin A in HGPS



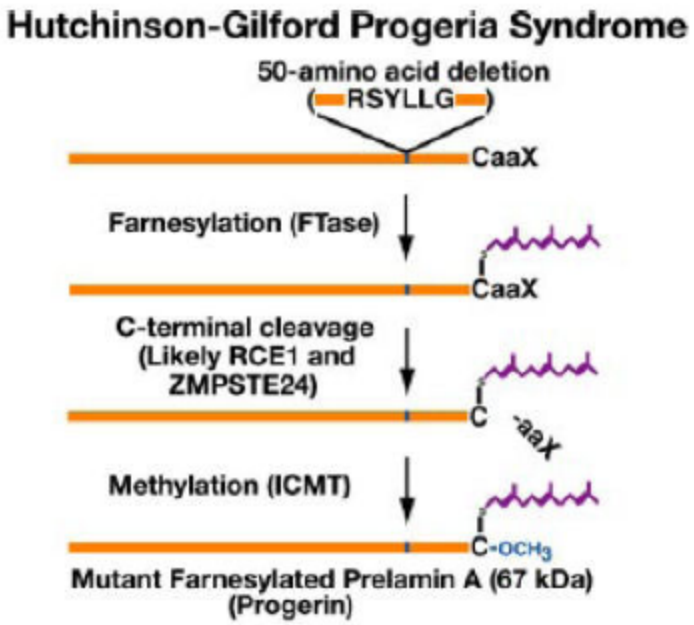


Figure 3
Biogenesis of lamin A in normal cells and the failure to generate mature lamin A in HGPS. In the setting of ZMPSTE24 deficiency, the final step of lamin processing does not occur, resulting in an accumulation of farnesyl-prelamin A. In HGPS, a 50-amino acid deletion in prelamin A (amino acids 607–656) removes the site for the second endoproteolytic cleavage. Consequently, no mature lamin A is formed, and a farnesylated mutant prelamin A (progerin) accumulates in cells [25].

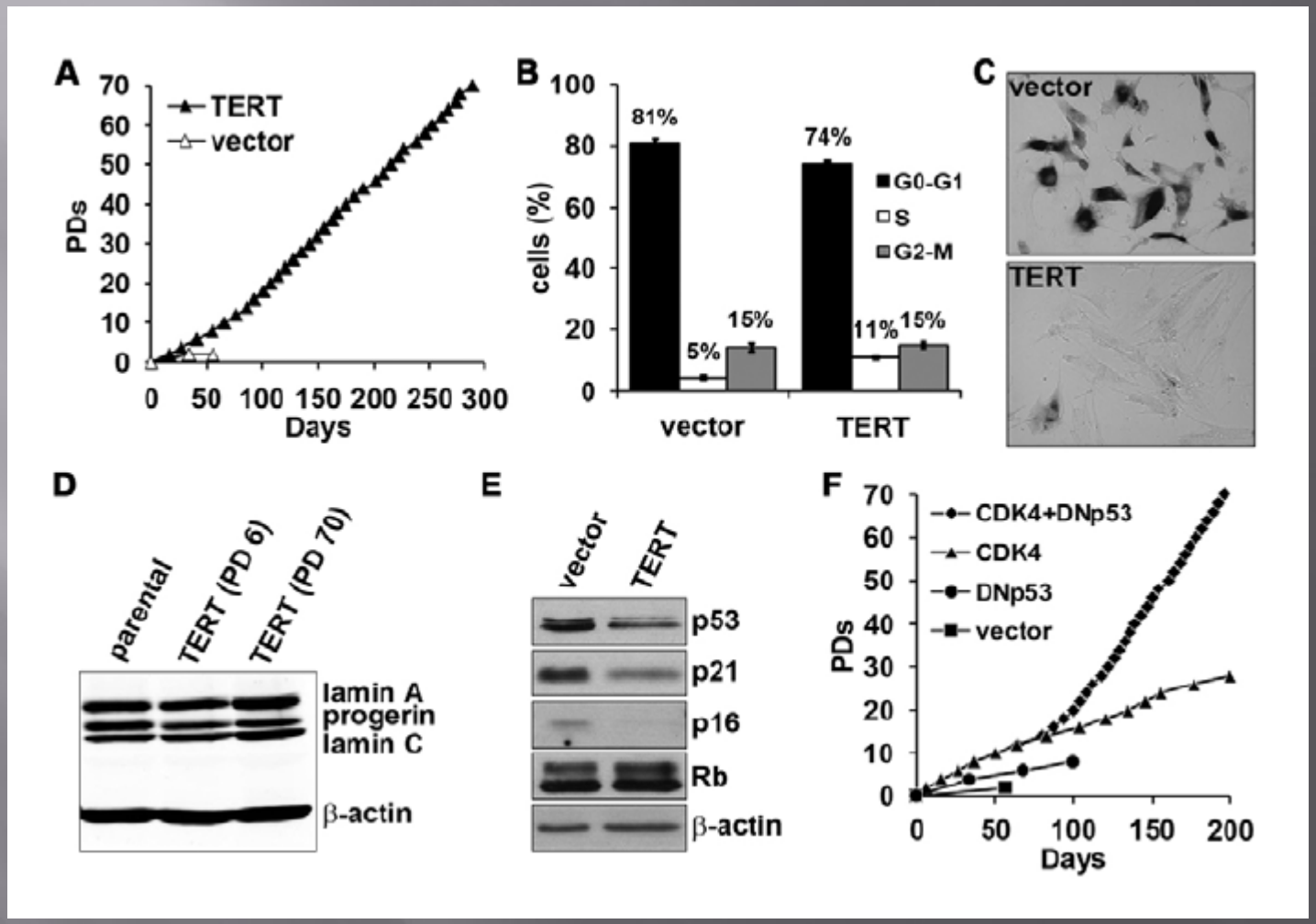
Coutinho et al 2009; see also Kieran et al 2009

Cellular defects in HGPS

Reduced lifespan in culture
Irregular nuclear phenotypes such as blebbing of nuclear envelope
Altered chromatin organization
Reduced telomere lengths
Chronic DNA-damage response

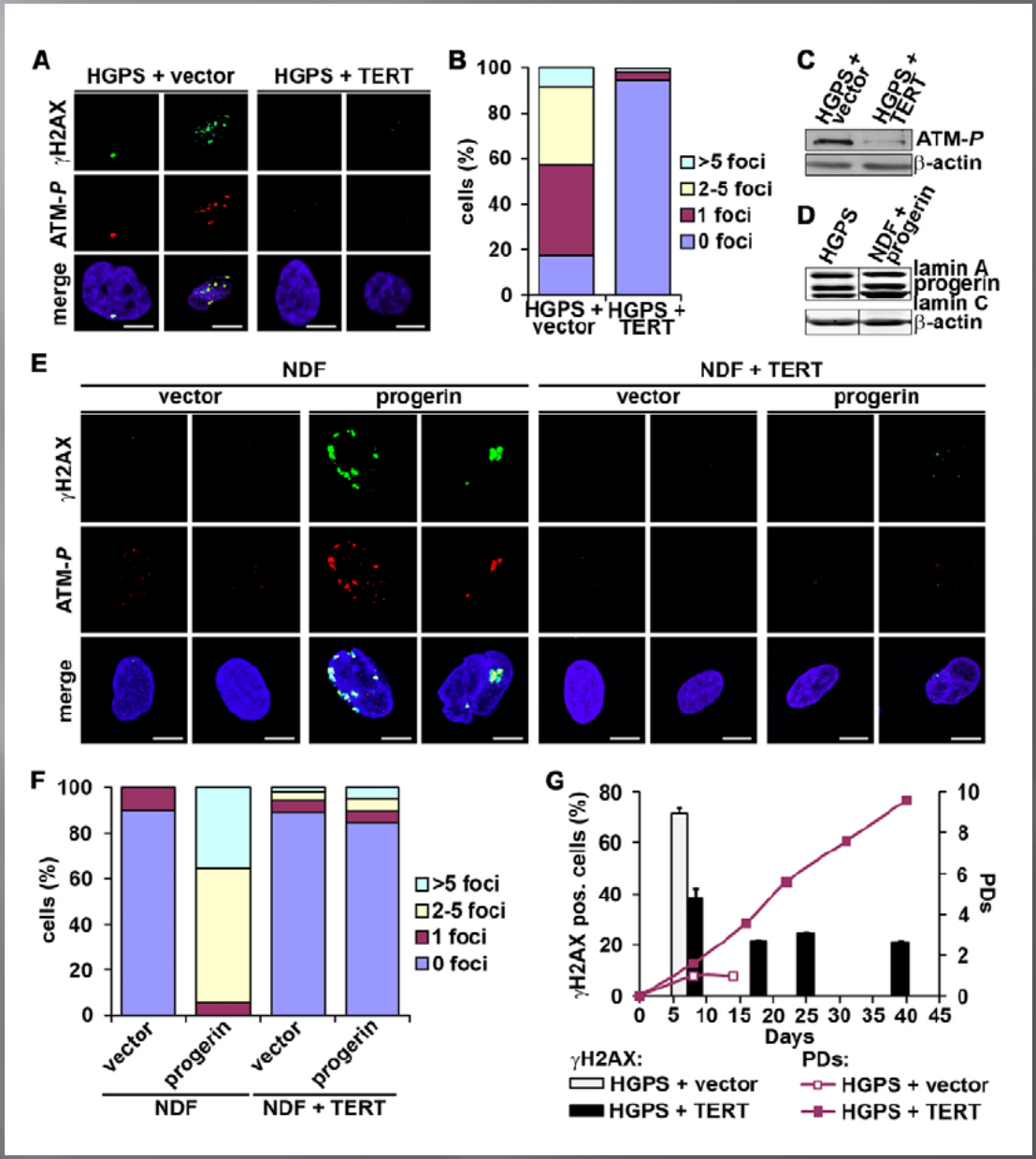
hTERT extends HGPS cellular lifespan
hTERT rescues proliferative defects associated with progerin

TERT rescues HGPS premature senescence through inhibition of tumor-suppressor pathway activation



Benson, E.K. et al. 2010. J. Cell Science 123, 2605-2612.

TERT blocks progerin-induced DNA damage signaling



Benson, E.K. et al. 2010. J. Cell Science 123, 2605-2612.

Duchenne Muscular Dystropy (DMD)

Mutation in dystrophin leads to progressive lethal skeletal muscle degeneration

Dystrophin deficiency does not recapitulate DMD in mice (mdx)

Mdx mice has mild skeletal defects and potent regenerative capacity

Is human DMD progression a loss of functional muscle stem cells?

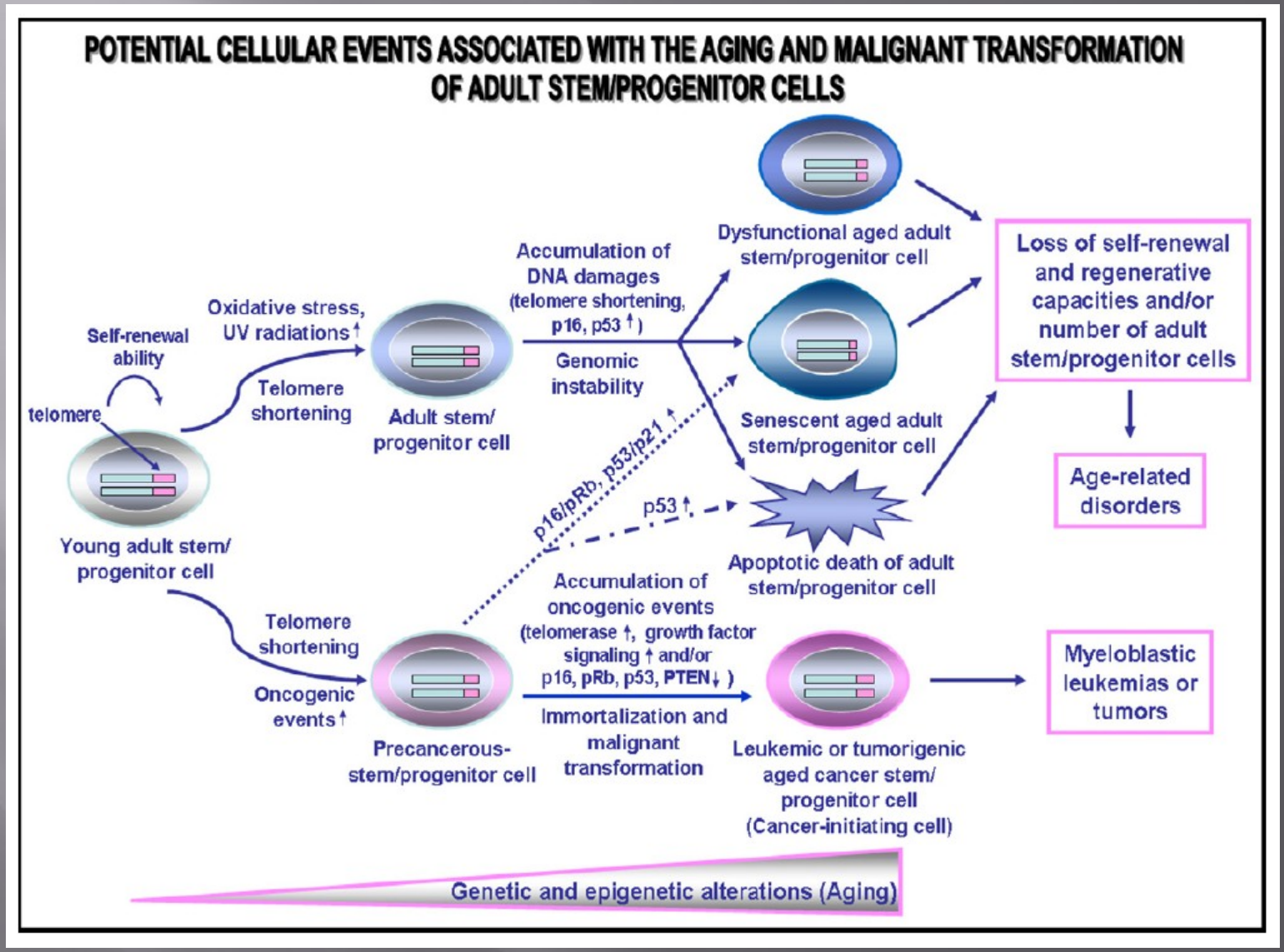
Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice

Alessandra Sacco,[1,4,5] Foteini Mourkioti,[1,4] Rose Tran,[1] Jinkuk Choi,[2] Michael Llewellyn,[3] Peggy Kraft,[1] Marina Shkreli,[2] Scott Delp,[3] Jason H. Pomerantz,[1,6,*] Steven E. Artandi,[2] and Helen M. Blau[1,*]

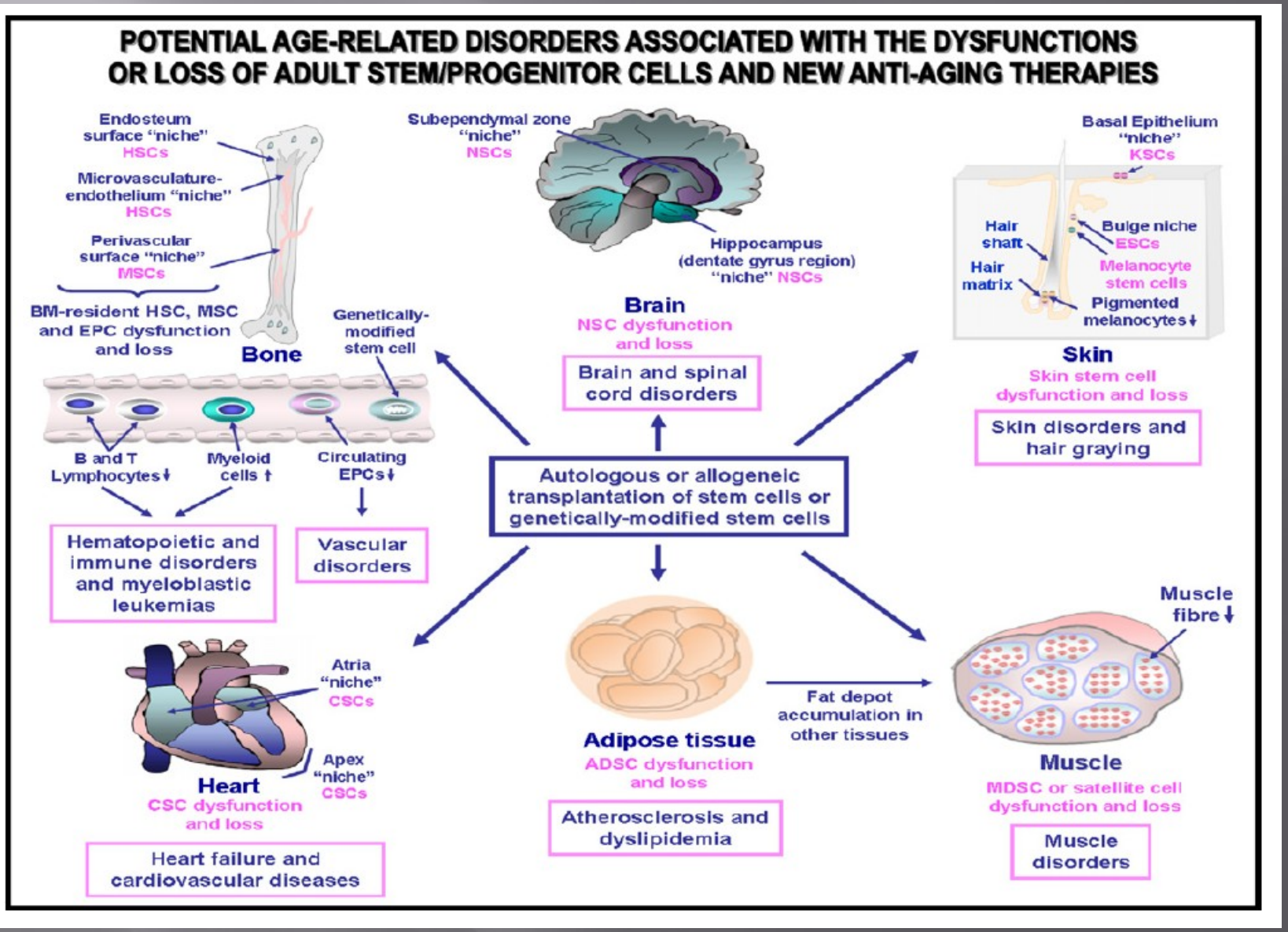
Mdx/mTR mice have shortened telomeres in muscle cells and severe muscular dystrophy that progressively worsens with age

Muscle wasting severity parallels a decline in muscle stem cell regenerative capacity

Sacco, A. et al. 2010. Cell 143, 1059-1071.



Mimeau and Batra, 2009



Mimeau and Batra, 2009