1: Science. 1989 Nov 3;246(4930):629-34.

Checkpoints: controls that ensure the order of cell cycle events.

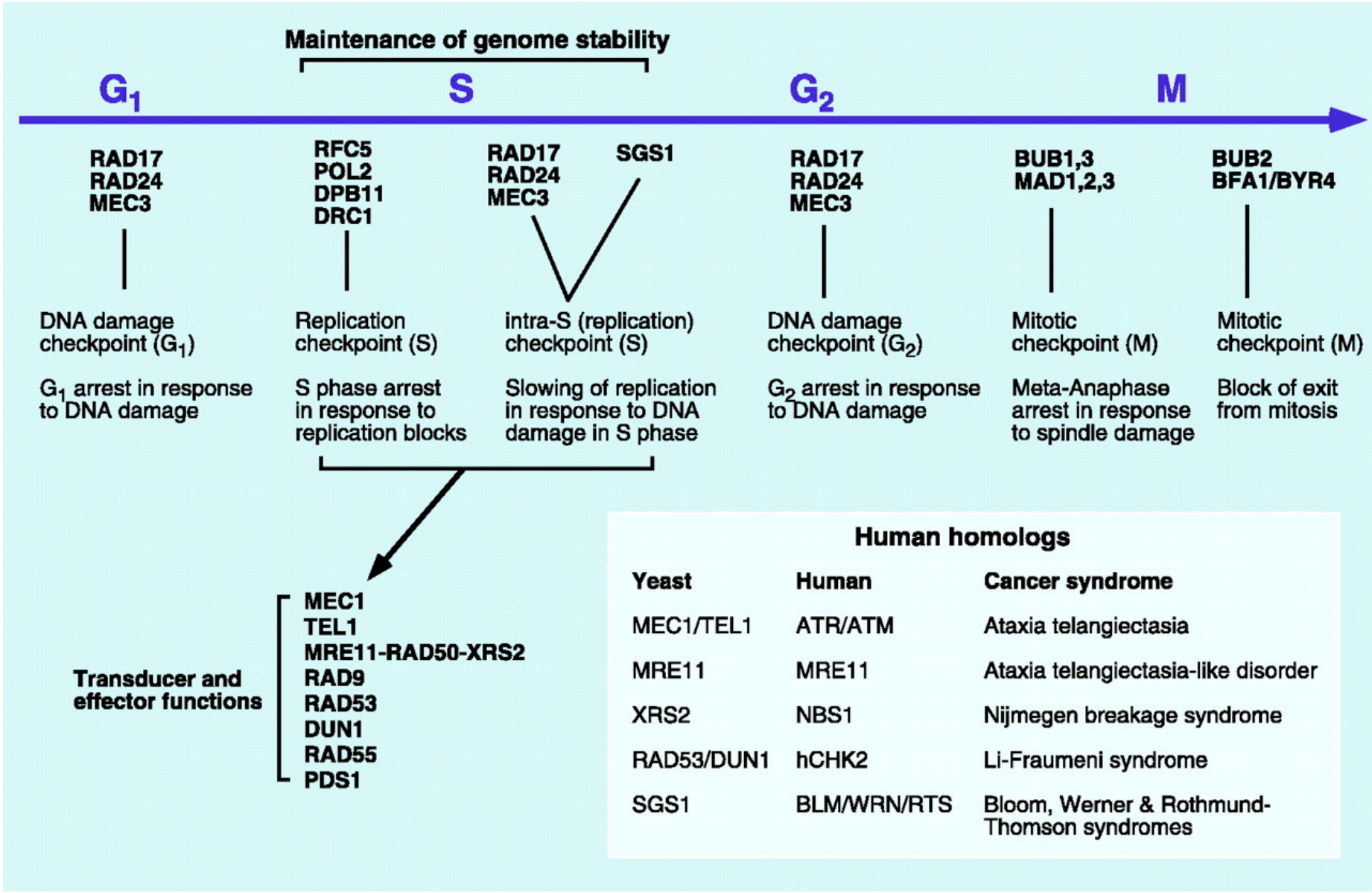
Hartwell LH, **Weinert TA**.

Department of Genetics, University of Washington, Seattle 98195.

The events of the cell cycle of most organisms are ordered into dependent pathways in which the initiation of late events is dependent on the completion of early events. In eukaryotes, for example, mitosis is dependent on the completion of DNA synthesis. Some dependencies can be relieved by mutation (mitosis may then occur before completion of DNA synthesis), suggesting that the dependency is due to a control mechanism and not an intrinsic feature of the events themselves. Control mechanisms enforcing dependency in the cell cycle are here called checkpoints. Elimination of checkpoints may result in cell death, infidelity in the distribution of chromosomes or other organelles, or increased susceptibility to environmental perturbations such as DNA damaging agents. It appears that some checkpoints are eliminated during the early embryonic development of some organisms; this fact may pose special problems for the fidelity of embryonic cell division.

PMID: 2683079 [PubMed - indexed for MEDLINE]

Cell cycle checkpoints in budding yeast



Kolodner RD et al. Science 2002; 297: 552-7

1: Nature. 1996 Oct 31;383(6603):840-3.

A meiotic recombination checkpoint controlled by mitotic checkpoint genes.

Lydall D, Nikolsky Y, Bishop DK, Weinert T.

Department of Molecular and Cellular Biology, University of Arizona, Tucson 85721, USA.

In budding yeast, meiotic recombination occurs at about 200 sites per cell and involves DNA double-strand break (DSB) intermediates. Here we provide evidence that a checkpoint control requiring the mitotic DNA-damage checkpoint genes RAD17, RAD24 and MEC1 ensures that meiotic recombination is complete before the first meiotic division (MI). First, RAD17, RAD24 and MEC1 are required for the meiotic arrest caused by blocking the repair of DSBs with a mutation in the recA homologue DMC1. Second, mec1 and rad24 single mutants (DMC1+) appear to undergo MI before all recombination events are complete. Curiously, the mitosis-specific checkpoint gene RAD9 is not required for meiotic arrest of dmc1 mutants. This shows that although mitotic and meiotic control mechanisms are related, they differ significantly. Rad17 and Rad24 proteins may contribute directly to formation of an arrest signal by association with single-strand DNA in mitosis and meiosis.

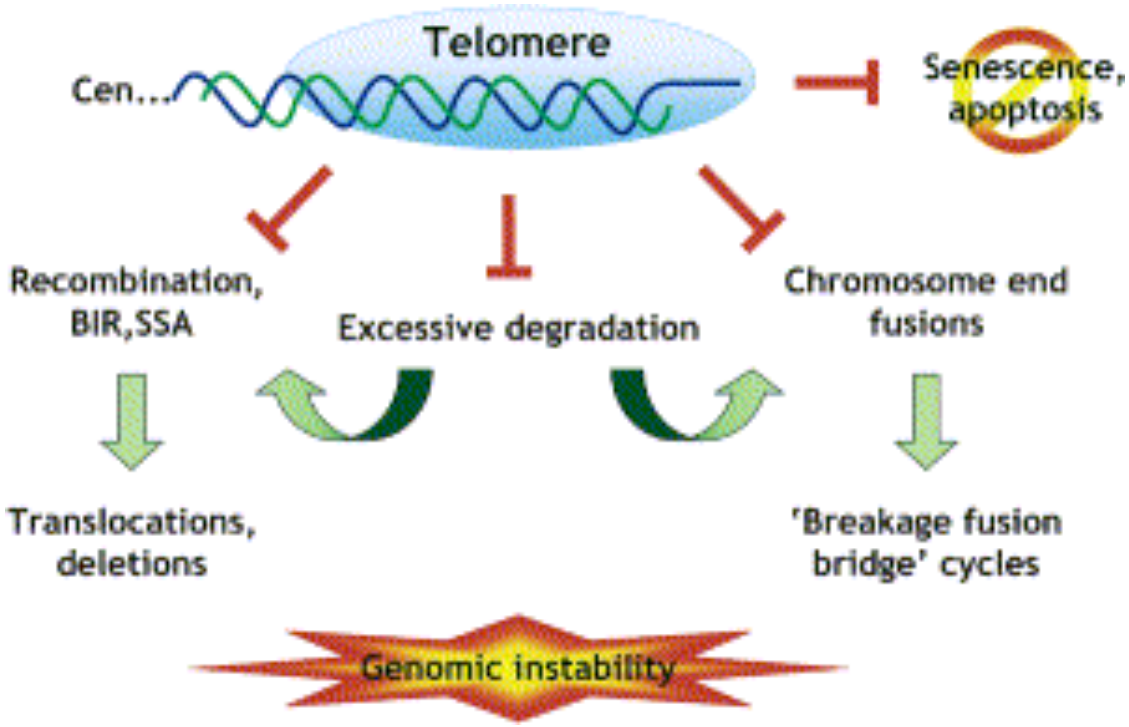
PMID: 8893012 [PubMed - indexed for MEDLINE]

“Shelterin” and “anticheckpoints” : Protecting Telomeres From DNA Damage Responses

R3 Journal Club
Daniel Pankratz, Ph.D.
1/2007

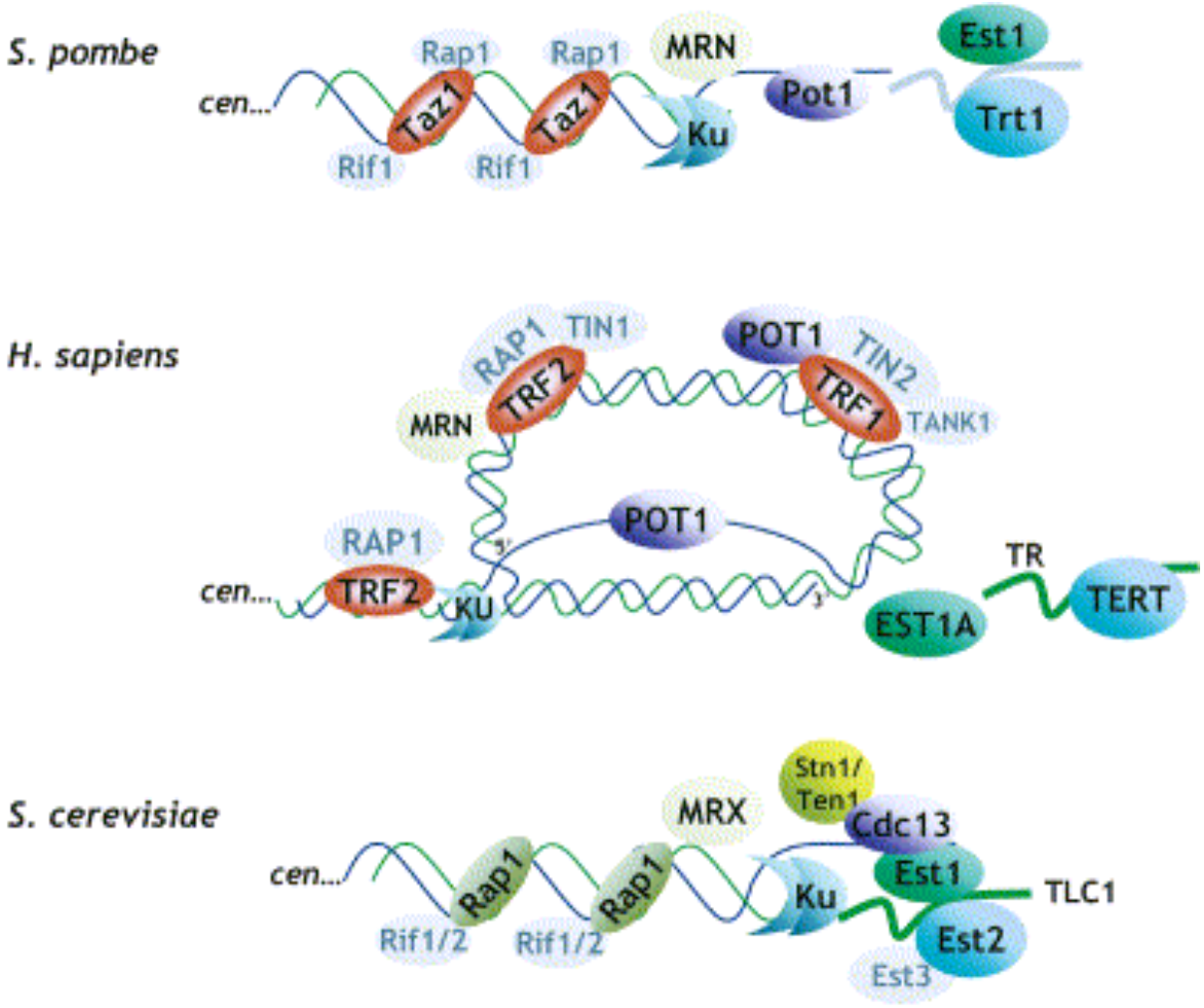
Telomeres

- solve the end replication problem
- senescence “clock”
- prevent end of chromosomes from behaving like a DSB
- Compromised telomeres result in resected or expanded chromosome ends, recombination “repair”, chromosome fusions



Ferreira MG et al. Mol. Cell 2004; 13: 7-18

Telomeres are complex nucleoprotein structures



Ferreira MG et al. Mol. Cell 2004; 13: 7-18

Telomeres and Checkpoints

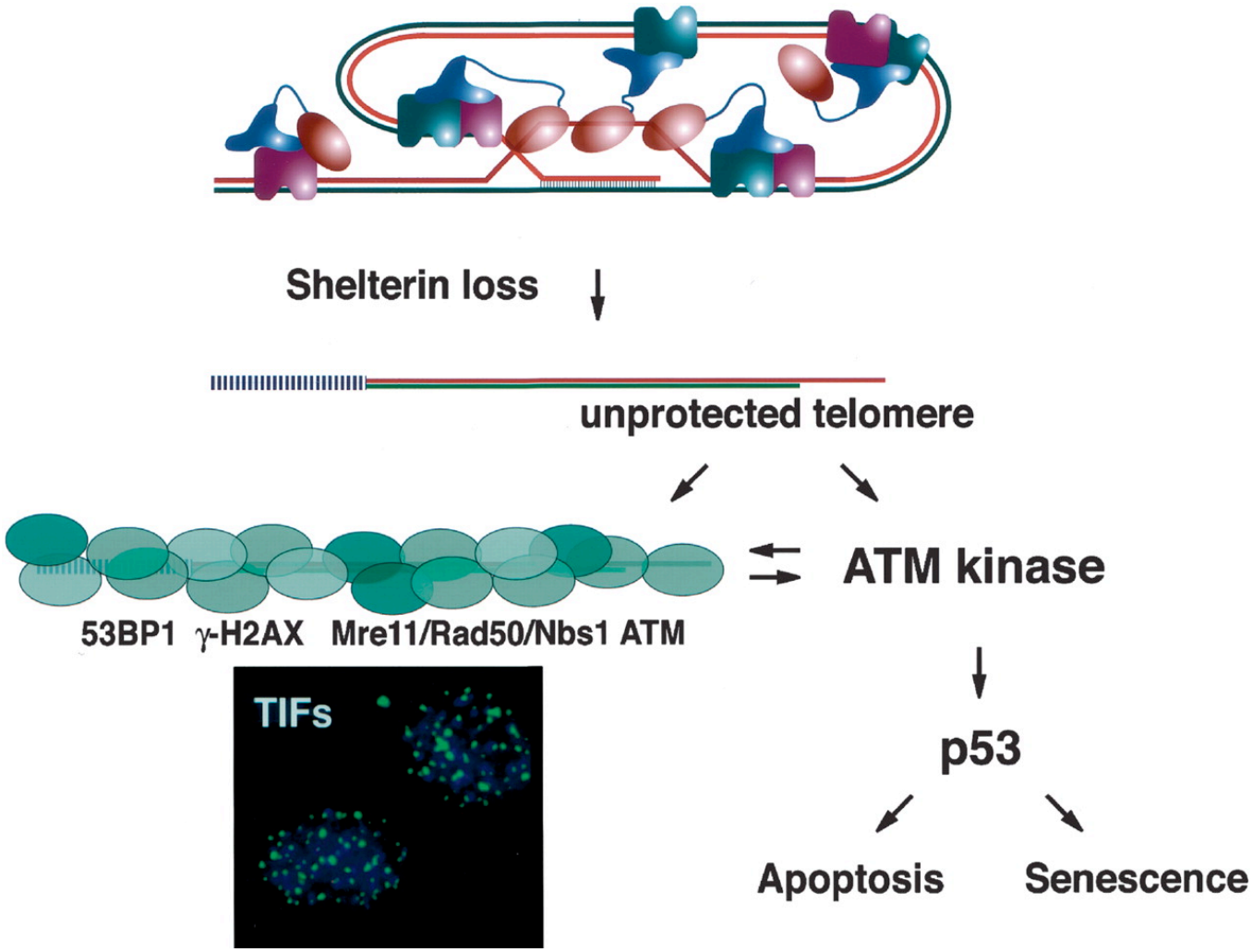
Checkpoint proteins Tel1 and Mec1 (ATM and ATR homologs) are found at normal telomeres at different times in the cell cycle.

Loss of Tel1/MRX and Mec1 results in unstable telomeres end fusion and senescence.

Checkpoints are transiently and locally activated at exposed telomeres during late S-G2; local ATM phosphorylates local Nbs1 (Verdun, 2005)

Telomeric protein TRF2 inhibits ATM in mammals (no TRF2 in yeast). “Shelterin” complex likely protects telomeres from checkpoint activation (de Lange, 2005).

Shelterin, telomere deprotection and checkpoint activation



Titia de Lange Genes Dev. 2005; 19: 2100-2110

Cell cycle checkpoints must not recognize normal telomeres as DSBs, otherwise checkpoint arrest and repair would occur.

What mechanisms protect normal telomeres from activating the checkpoint?

A telomeric repeat sequence adjacent to a DNA double-stranded break produces an anticheckpoint

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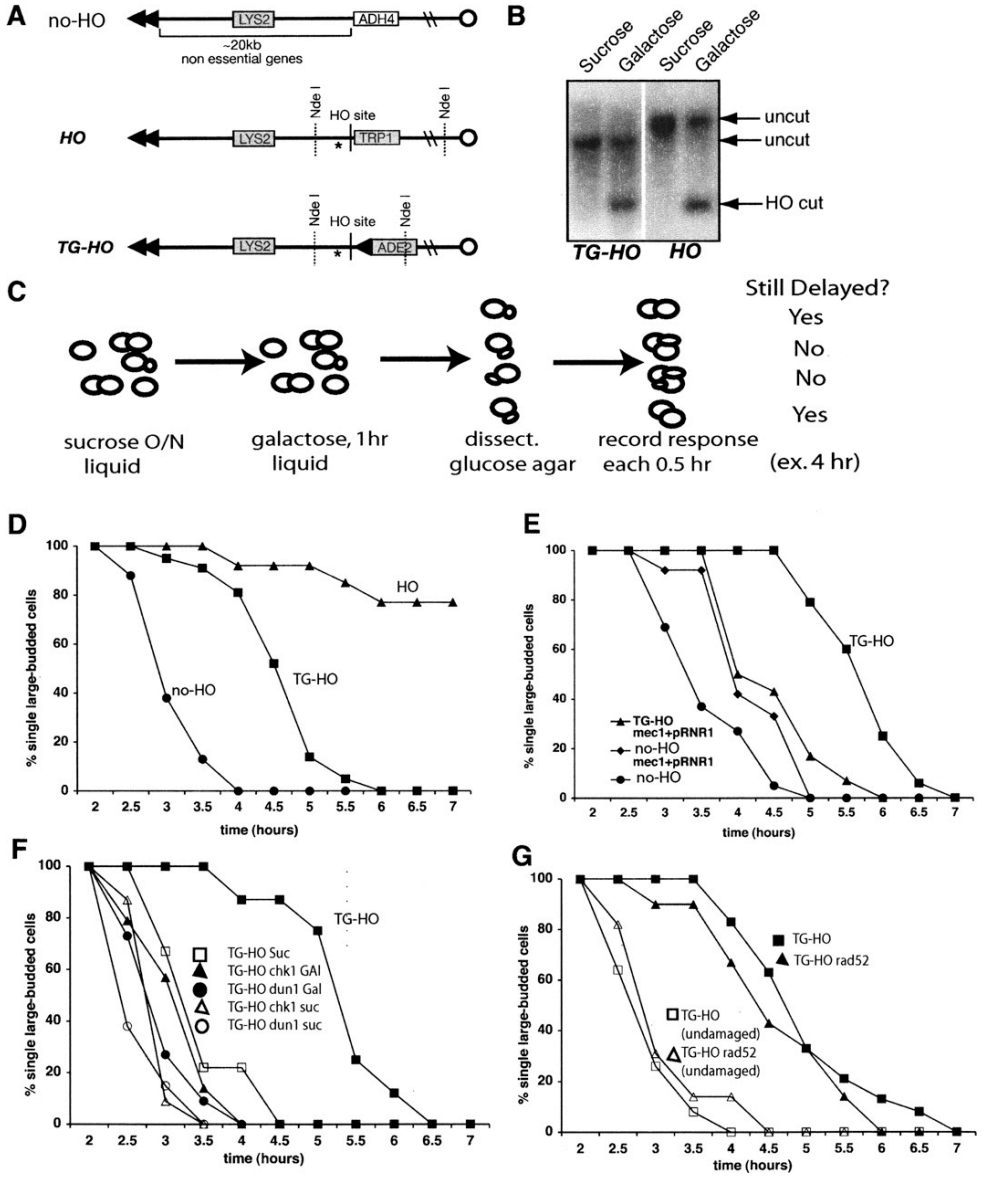
The HO inducible break system in budding yeast

The HO endonuclease generate DSBs at a target sequence

galactose: HO expression induced
glucose: HO expression repressed

HO cleavage of a chromosome was previously shown to generate a telomere *de novo* (Diede and Gottschling, 1999, 2001) in haploid yeast

Figure 1. A DSB adjacent to telomeric repeat sequences results in an abridged G2/M arrest



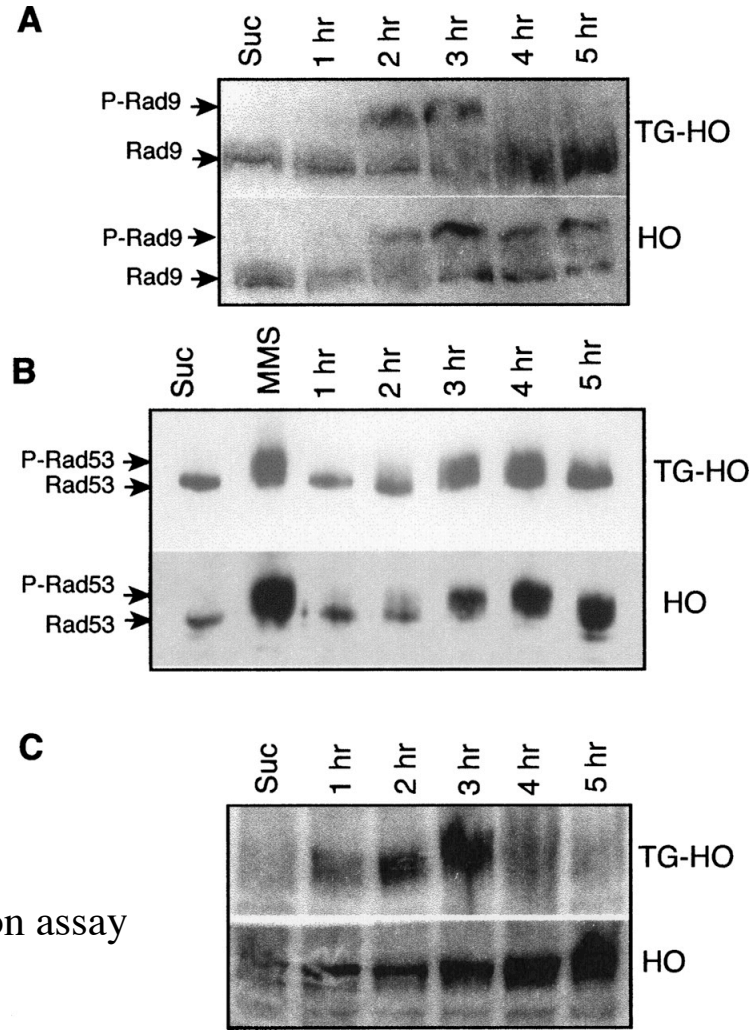
cut 1h -> dissect S-ph cells (small bud) -> examine every 30 min for M exit (new bud).

"abridged arrest" in response to an early-S break when telo repeat present.

is *mec1, chk1, rad53, rad9* dependent, *rad52, ku70* independent (not repair dependent)

Rhett J. Michelson et al. Genes Dev. 2005; 19: 2546-2559

Figure 2. The anticheckpoint correlates with transient Rad53 and Rad9 phosphorylation and Rad53 kinase activity



Rad53 autophosphorylation assay

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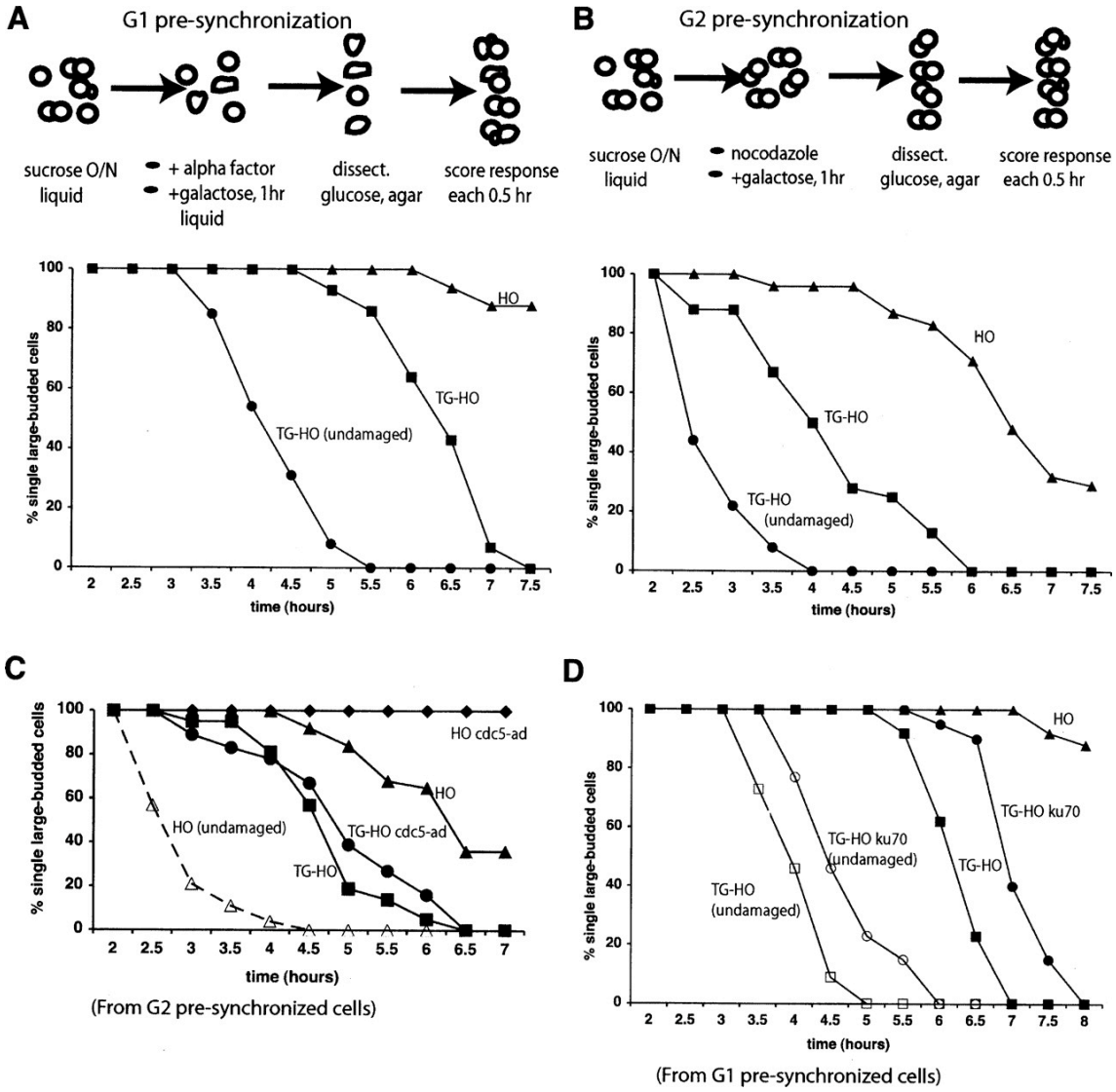
Figure 3. The abridged arrest is independent of the cell cycle stage and is not the result of early adaptation



arrest from HO break is shorter in G2, and adaptation dependent in G2 (*cdc5-ad*).

no *cdc5-ad* or *ku70* effect on TG-HO breaks: neotelomere abridged arrest is not adaptation or NHEJ-dependent

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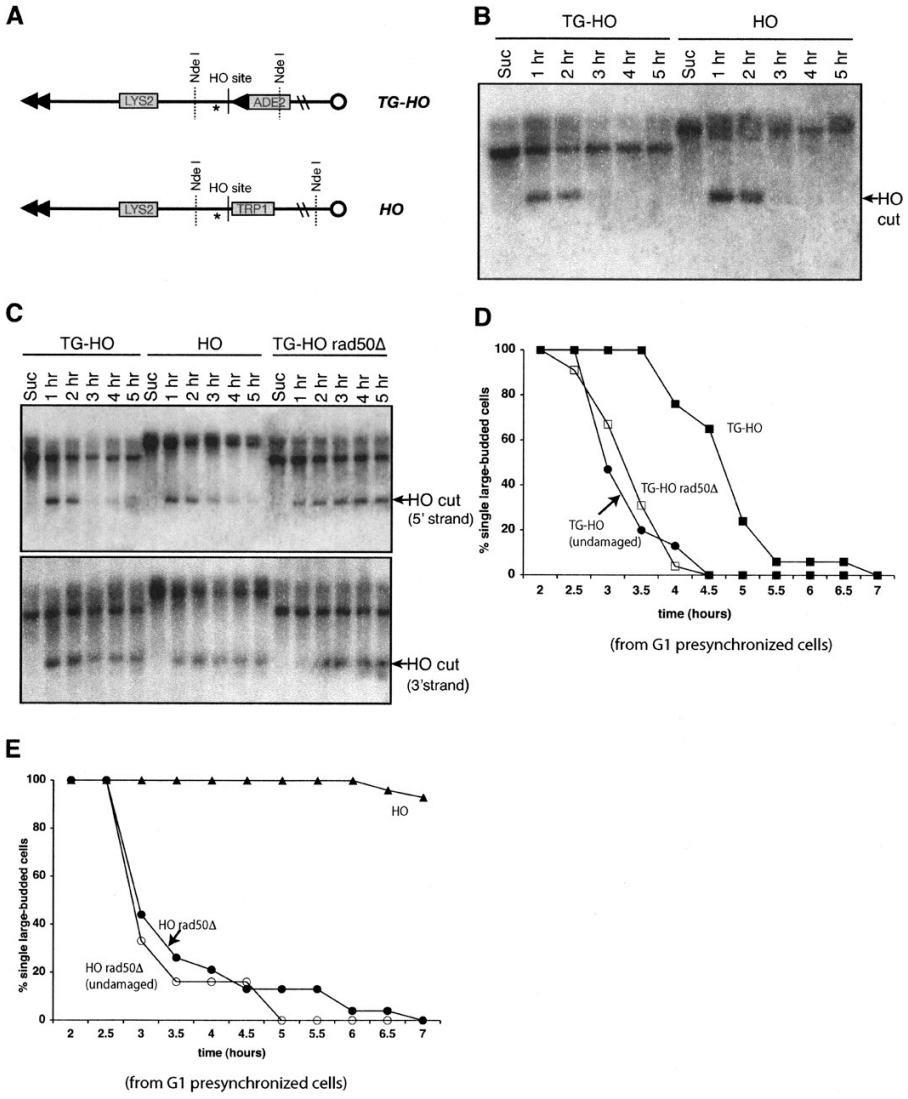
Figure 4. DSB resection rate is not reduced in the TG-HO strain relative to a normal HO site, and a DSB-induced arrest requires RAD50



riboprobe to degraded 5'-3' "Crick" strand: resection (ssDNA formation) at non-repeat side of DSB is similar in TG-HO vs. HO

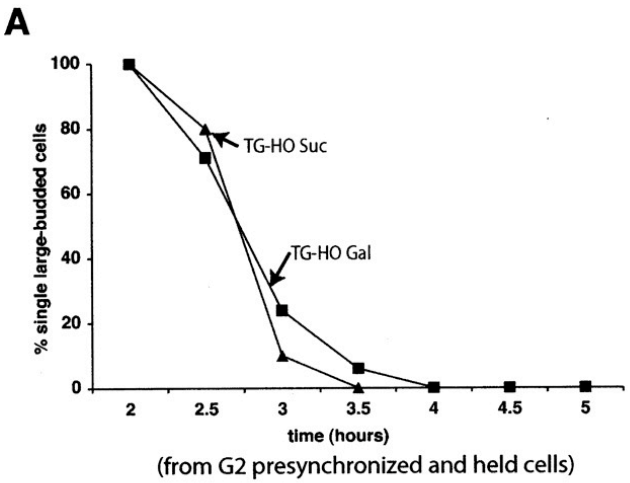
TG-HO resume cell cycle *before* break resection (buds form 1-2h but resection doesn't occur until 3h), ∴ "anticheckpoint" inactivates the checkpoint before ssDNA formation

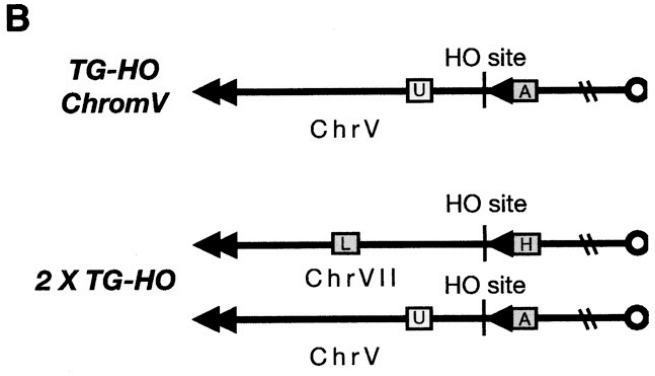
(telomerase dep. add'n to neotelomere is detectable @~2h & ~125bp by 4h (Diede & Gottschling, 1999)

(TG-HO & HO delay is *rad50* dependent)

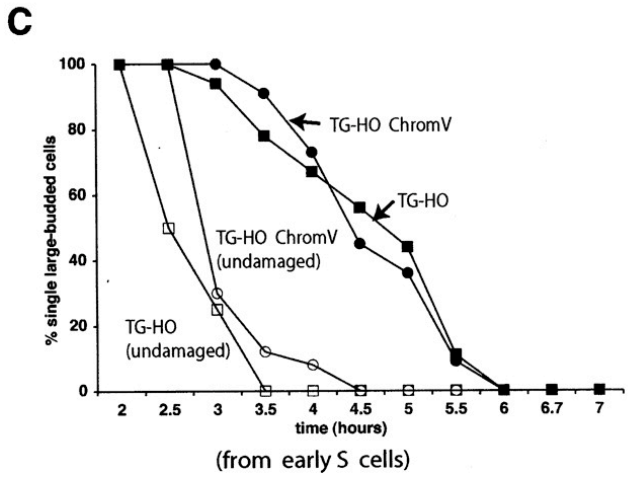
Rhett J. Michelson et al. Genes Dev. 2005; 19: 2546-2559

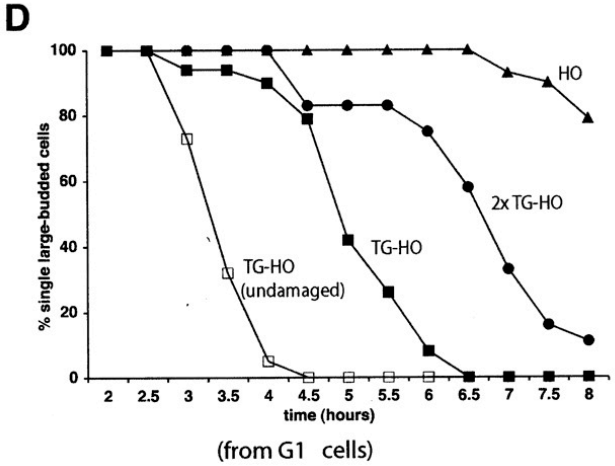
Figure 5. Enhanced ssDNA production does not prevent the anticheckpoint, and two HO cuts adjacent to C1-3A/TG1-3 repeats do not produce a permanent arrest phenotype





"anticheckpoint" can inactivate the checkpoint after ssDNA formation as well





breaks at two telomere sites generate a longer delay than a single telomere break, but shorter than a single nontelomere break (not a permanent arrest)

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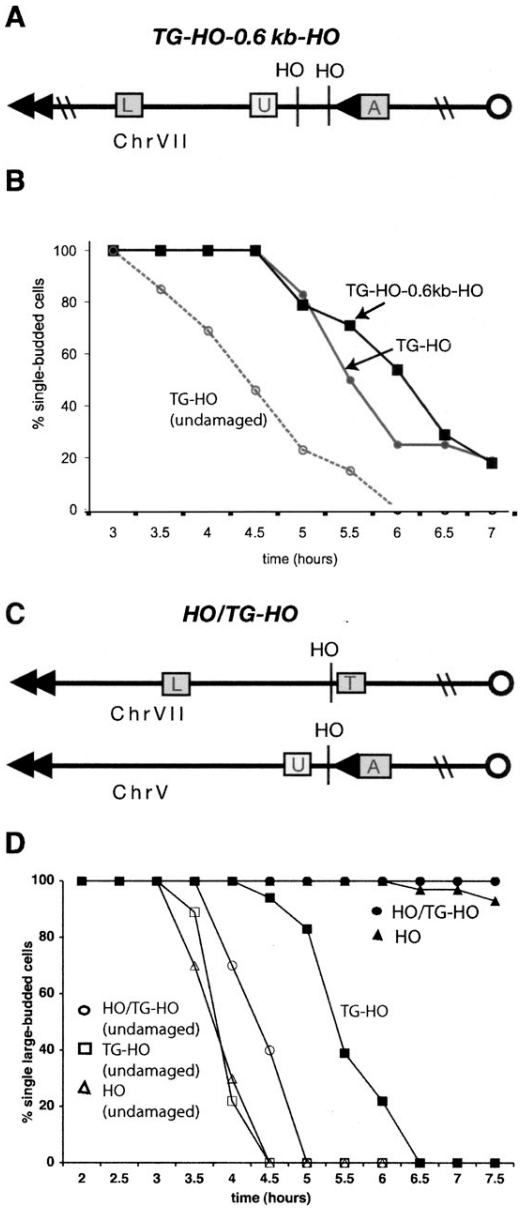
Figure 6. The anticheckpoint produces regional inhibition



additional break in *cis* has no effect on anticheckpoint function of telomere repeat

additional break in *trans* generates normal checkpoint-dependent arrest

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Figure 7. The anticheckpoint activity does not require telomerase, RIF2, or wild-type SIR2 activity

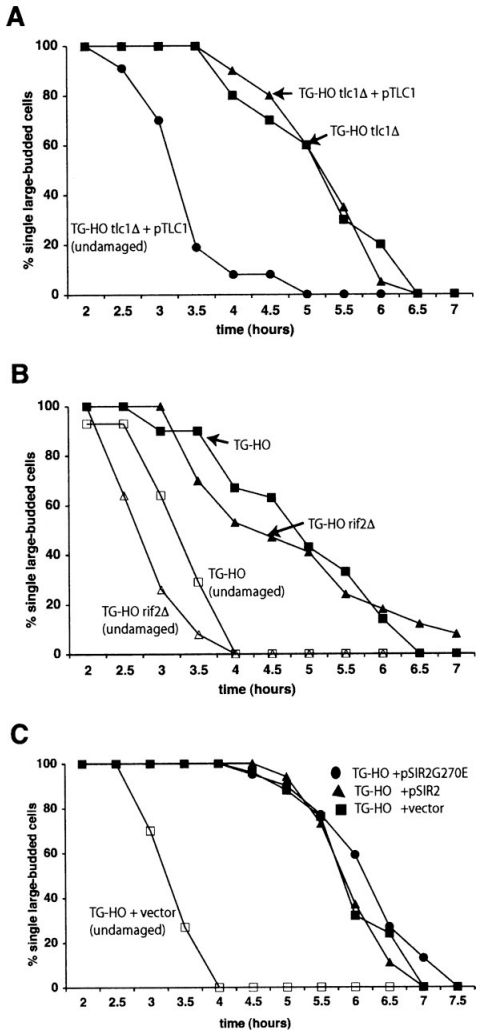

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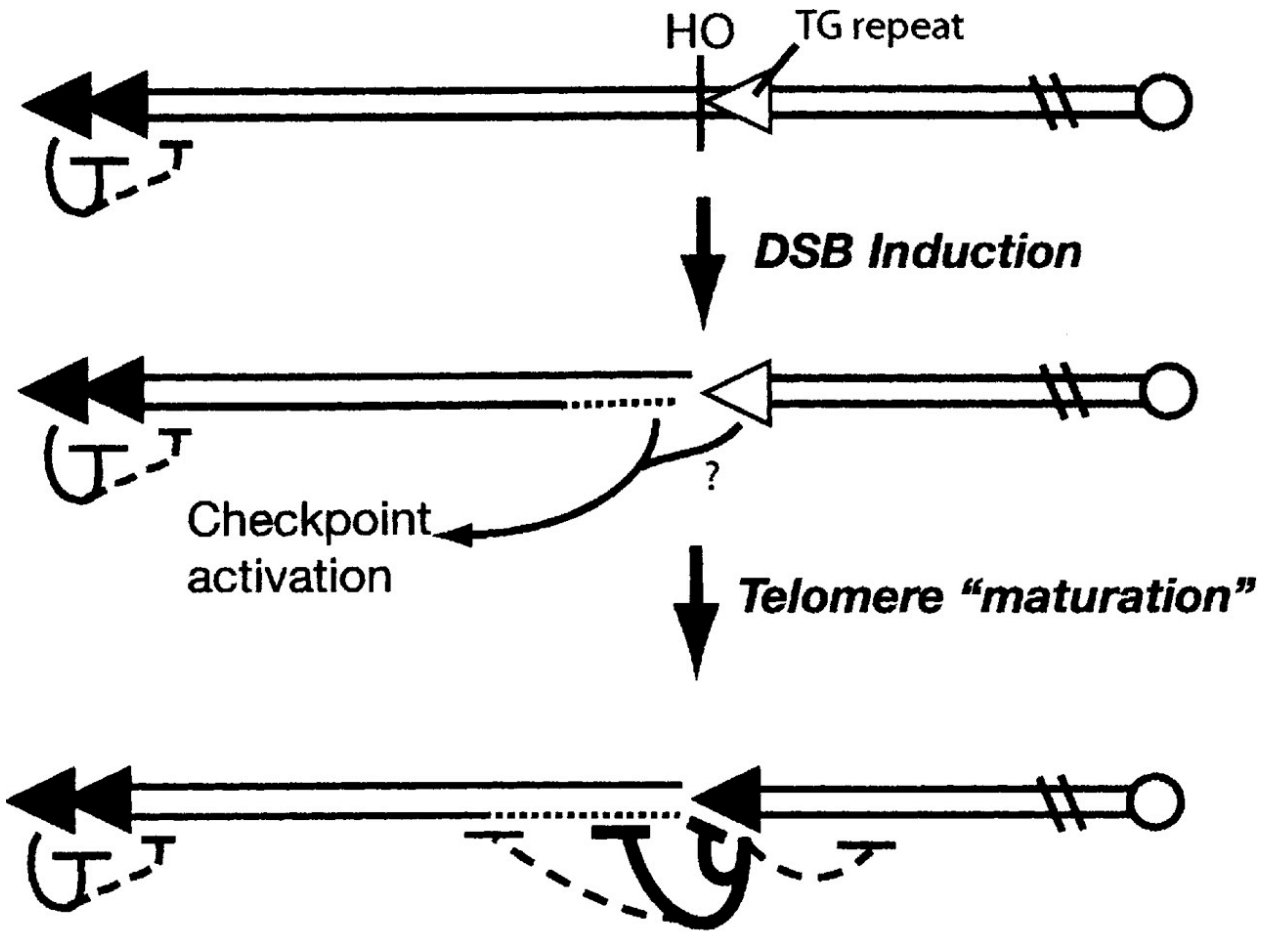
Figure 8. Model of telomere maturation and anticheckpoint activity



Local and regional anticheckpoint activity

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Conclusions

- an internal telomeric sequence can inhibit a DNA damage checkpoint response at an adjacent DSB
- DSBs adjacent to ectopic telomere sequences activate the G2/M checkpoint, but the duration of checkpoint activation is shorter (1-2h vs. 8-12h)
- checkpoint attenuation occurs upstream of Rad9 and Rad53
- mechanism does not involve repair, adaptation, gene silencing, or telomere elongation, nor does it work in *trans*

Model: telomeric repeats act as an "anticheckpoint" locus via the recruitment of unknown factors or novel topology.

Preventing Genomic Instability

type of instability	*pathway preventing it*
repeat expansion	mismatch repair
breakage-fusion-bridge	telomeric complexes
rDNA locus (repetitive)	Sir2 (silencing)
replication derived:	
fragile sites	checkpoints in S
whole chromosome timing	??

Cycles of Instability

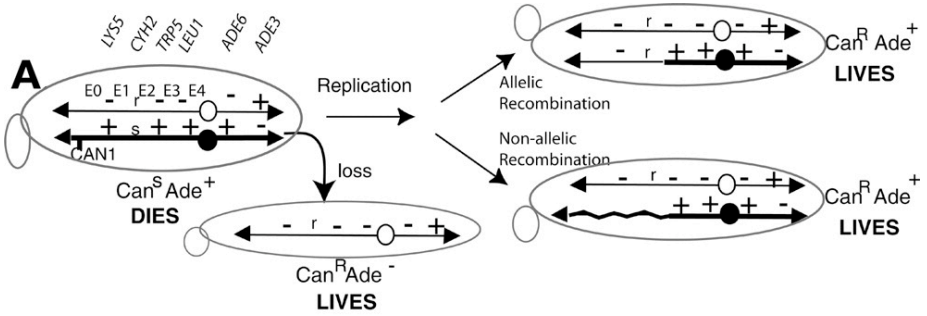
Unstable chromosomes are transient and undergo stochastic alterations (translocation, duplication, loss), making them difficult to characterize in bulk.

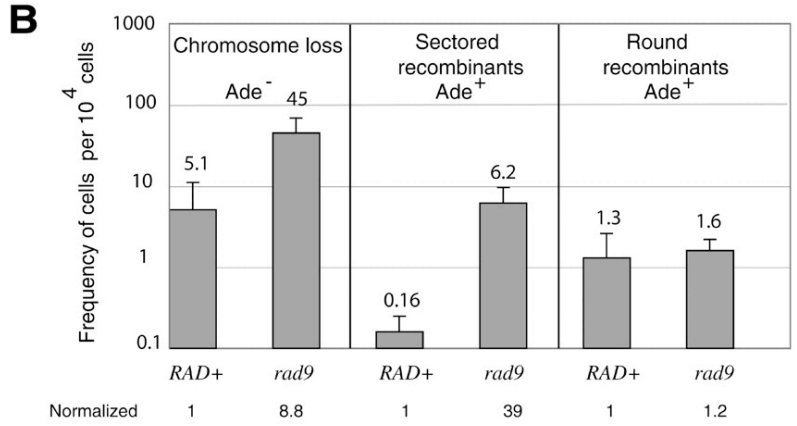
Single-cell approaches are being used in yeast (ChrVII assay) and human cell culture (somatic cell genetics) model systems to study this phenomenon.

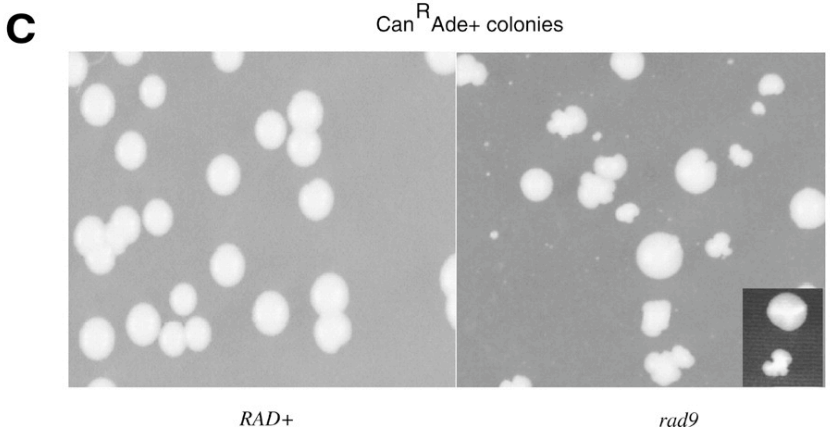
Cycles of chromosome instability are associated with a fragile site and are increased by defects in DNA replication and checkpoint controls in yeast

Anthony Admire,[1,2] Lisa Shanks,[1] Nicole Danzl,[1,3] Mei Wang,[4] Ulli Weier,[4] William Stevens,[1] Elizabeth Hunt,[1] and Ted Weinert[1,5]

The ChrVII Assay for chromosome loss and rearrangement



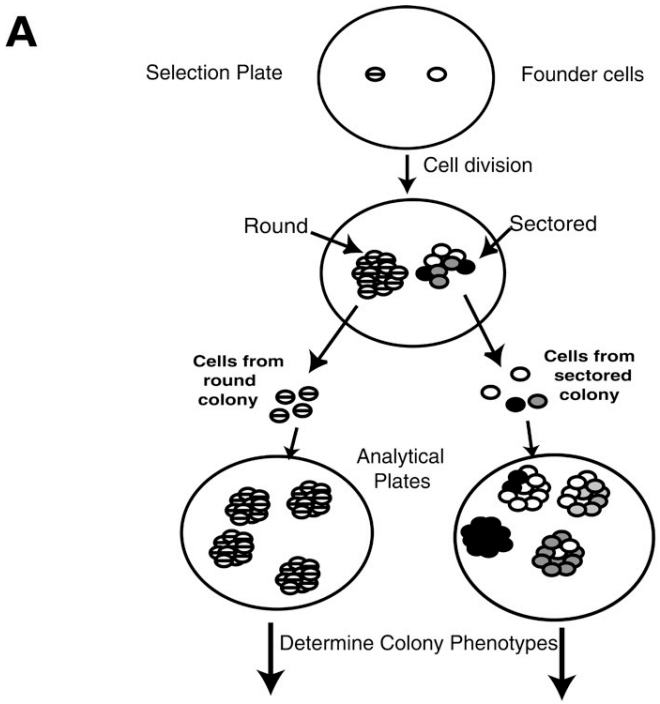




two classes of mitotic recombinants: round vs. sectored

Anthony Admire et al. Genes Dev. 2006; 20: 159-173

Sectored colonies contain genetically unstable recombinants

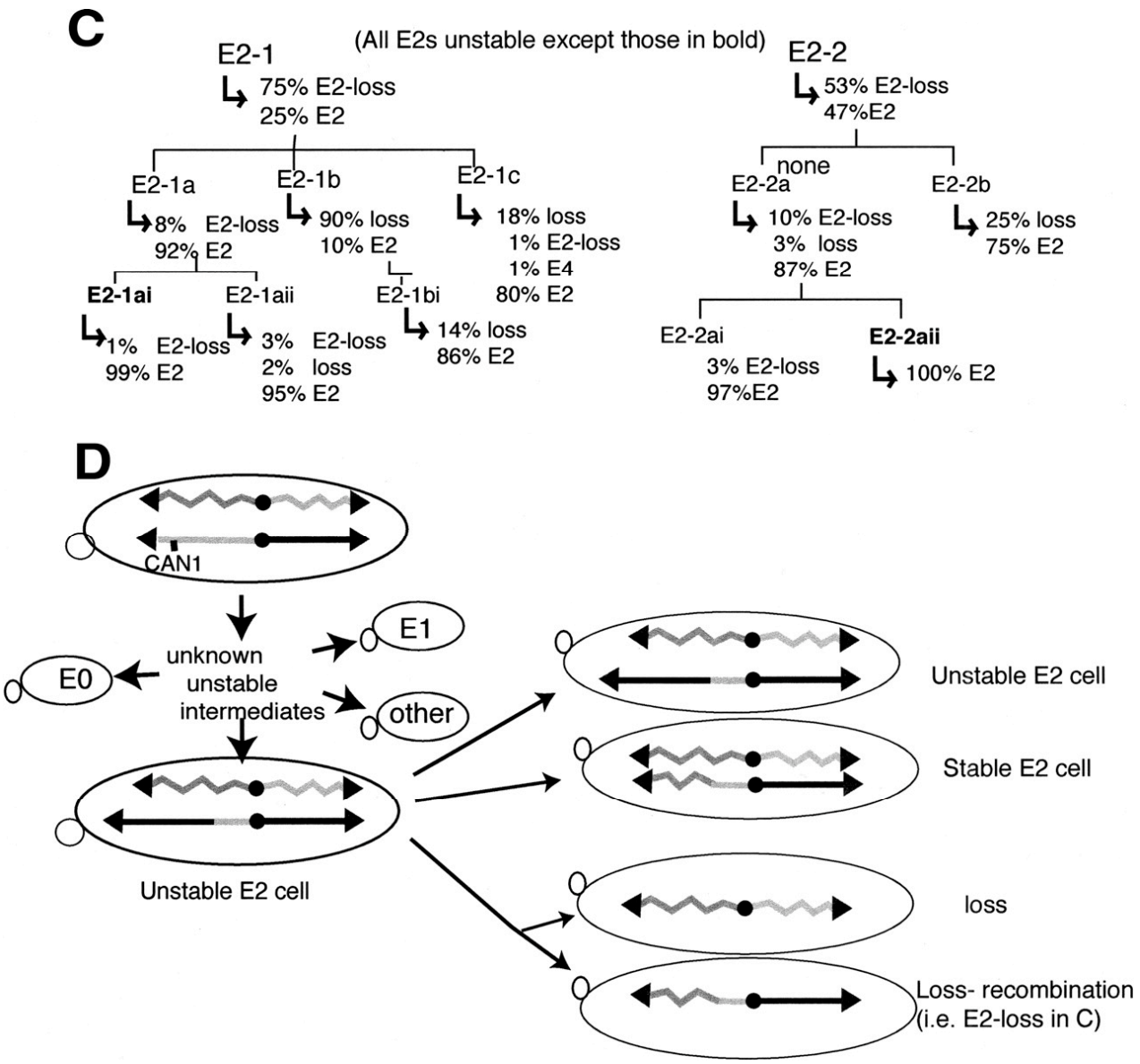


	Colony #						Loss	fragmented
rad9 round	1	0	0	132	0	0	2	0
	3	106	0	0	0	0	0	0
rad9 sectored	4	1	27	15	2	0	3	3
	5	18	3	19	2	0	3	1
	6	0	25	19	4	0	1	5
rad9 sectored % (443 cells from 9 colonies)		**8**	**28**	**48**	**3**	**0**	**9**	**4**
RAD+ sectored	7	0	0	20	1	0	10	1
	8	0	0	4	2	1	25	1
RAD+ sectored % (241 cells from 8 colonies)		**18**	**1**	**24**	**2**	**1**	**51**	**5**

E2 region of ChVII is particularly destabilized in *rad9* cells

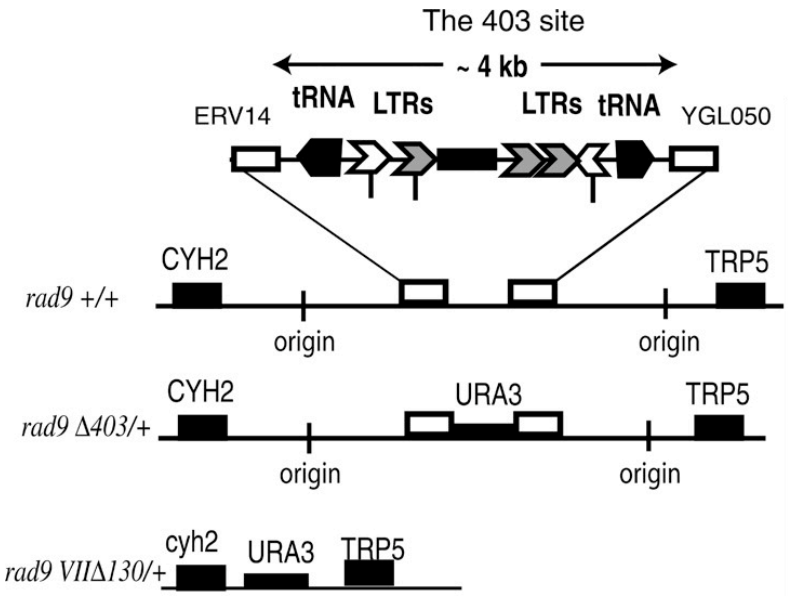
Anthony Admire et al. Genes Dev. 2006; 20: 159-173

Extended lineage analysis of unstable cells: persistent heterogeneity



Anthony Admire et al. Genes Dev. 2006; 20: 159-173

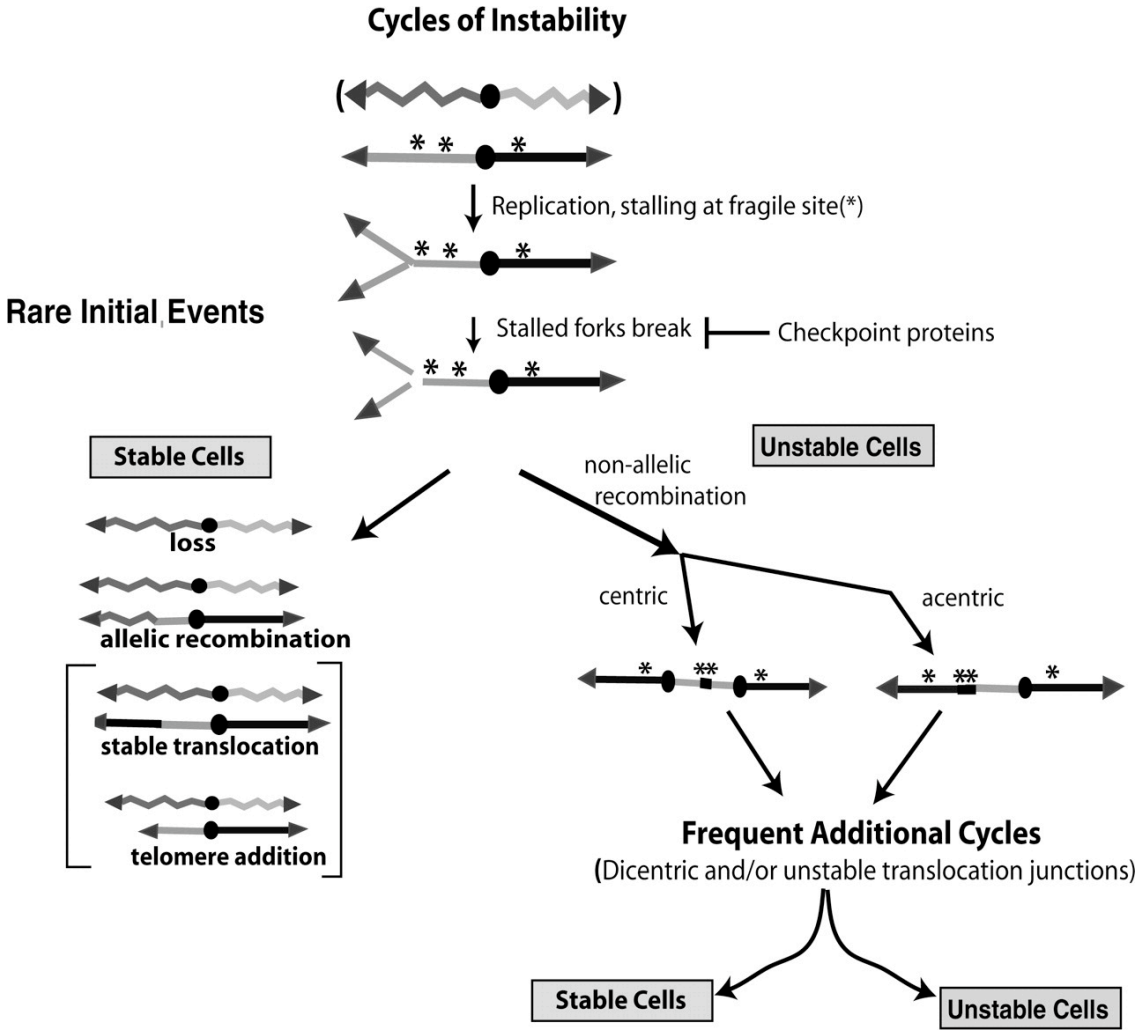
Figure 6. Deletions of the ChrVII 403 E2 site decrease chromosome instability



Chromosome Instability frequency x10 $^{-4}$			
loss	sectored	430-535	round
29 +/-11	8.3 +/- 5.6	13/14	1.5 +/- 0.4
32 +/-25	3.3 +/- 0.6	2/13	0.8 +/- 0.2
96 +/- 10	1.7 +/- 0.5	n.d.	0.4 +/- 0.2

Anthony Admire et al. Genes Dev. 2006; 20: 159-173

Figure 7. Cycles of chromosome instability arising from fragile sites



Anthony Admire et al. Genes Dev. 2006; 20: 159-173

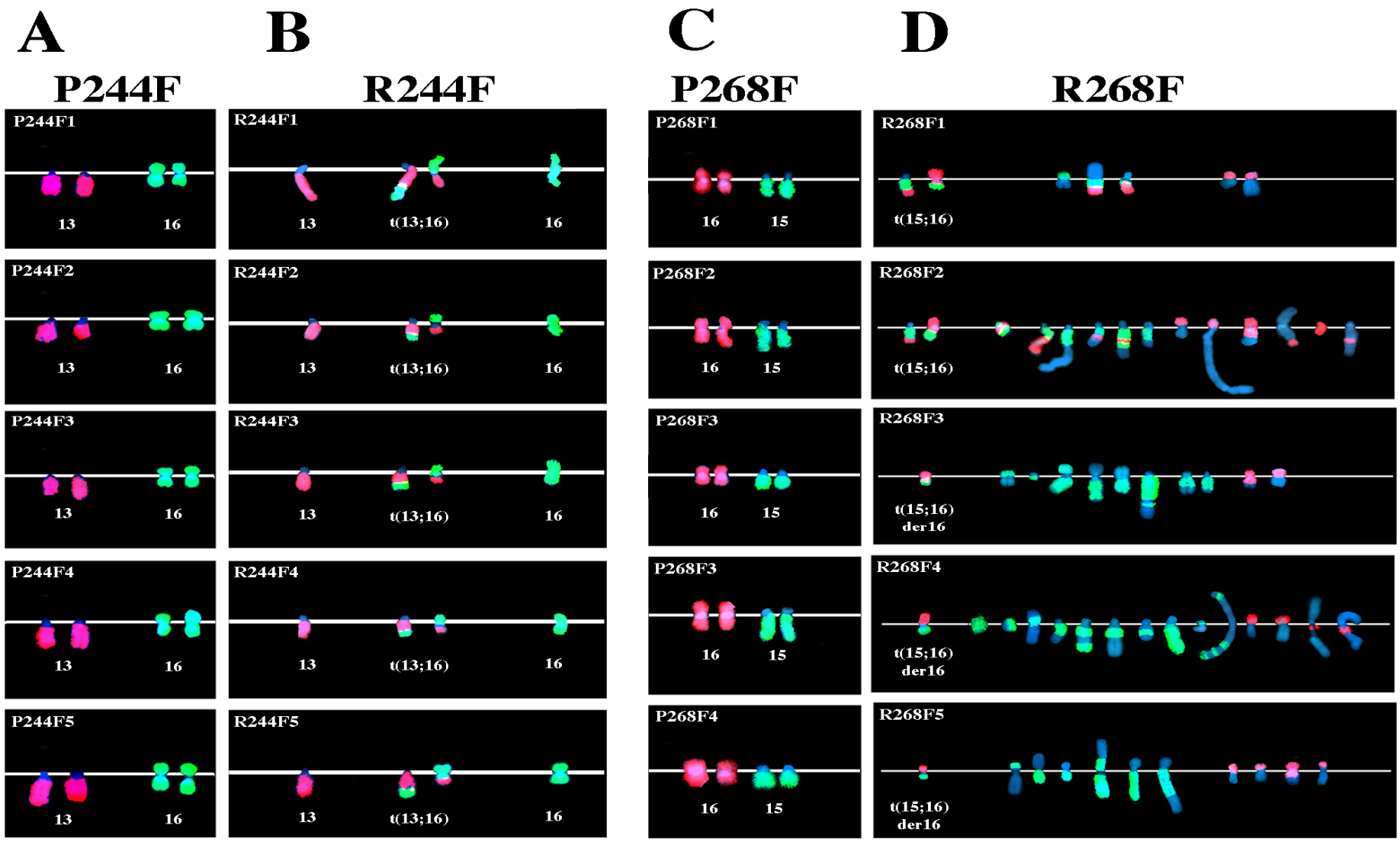
Conclusions

• Chromosome instability (sectoring) is persistent in a subpopulation of cells selected for loss of a genetic marker.

• Cycles of chromosome instability may involve altered replication at specific loci (E2, tRNA loci), resulting in mitotic recombinants (dicentrics, translocations).

• Loss of such loci can reduce the frequency of instability, loss of replication checkpoint proteins increases it.

Unstable Chromosomes in Mammalian Cells



Leslie Smith and Mathew Thayer

BMB, OHSU