REVIEW

Maintenance of Genome Stability in *Saccharomyces cerevisiae*

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Most human cancer cells show signs of genome instability, ranging from elevated mutation rates to gross chromosomal rearrangements and alterations in chromosome number. Little is known about the molecular mechanisms that generate this instability or how it is suppressed in normal cells. Recent studies of the yeast *Saccharomyces cerevisiae* have begun to uncover the extensive and redundant pathways that keep the rate of genome rearrangements at very low levels. These studies, which we review here, have implicated more than 50 genes in the suppression of genome instability, including genes that function in S-phase checkpoints, recombination pathways, and telomere maintenance. Human homologs of several of these genes have well-established roles as tumor suppressors, consistent with the hypothesis that the mechanisms preserving genome stability in yeast are the same mechanisms that go awry in cancer.

Maintaining the stability of the genome is critical to cell survival and normal cell growth. Most human cancers display some form of genome instability (*1–4*). In general, cancer cells can be divided into two fundamental classes (*2*): those that show increased rates of chromosome instability and those that show increased rates of point mutations and frameshift mutations in microsatellite repeat sequences [called the MSI phenotype (*4*)]. Although the MSI and chromosome instability phenotypes were once thought to be mutually exclusive, several tumors have been described in which the two phenotypes coexist (*5*). Not all cancers exhibit ongoing genome instability. The progression of chronic myelogenous leukemia, for example, is driven by a single genomic alteration—a chromosomal translocation that fuses the *BCR* and *ABL* genes (*6*). Genome rearrangements are not restricted to cancer; stable genome rearrangements have been documented as inherited mutations that cause a number of other human diseases (*7*, *8*).

How does genome instability arise? In the case of cancers caused by mutations in mismatch repair genes, the mechanisms responsible for the elevated mutation rate are reasonably well understood (*1*, *4*). Much less is known about the molecular mechanisms that cause genome rearrangements, what pathways might suppress these rearrangements, and whether defects in such pathways underlie the ongoing genome instability seen in many cancers. Here we review new insights into these questions that have emerged from recent genetic studies of the yeast *Saccharomyces cerevisiae*.

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Assays for Detecting Genome Instability

The utility of *S. cerevisiae* systems for studying genome rearrangements was first recognized nearly 20 years ago. In these initial assays, an extra copy of a DNA sequence was inserted at a site on an unrelated chromosome (ectopic site), and this was followed by selection for recombination between the ectopic sequences (*9–11*). This strategy produced chromosomal translocations that appeared to arise by normal mitotic recombination, but whether these recombination events are accurate models of the chromosomal rearrangements seen in leukemias and other cancers is unclear.

More recently developed assays allow detection of a broader spectrum of genome rearrangements, particularly those that are not necessarily promoted by homologous recombination. One such assay involves measuring the rate of rearrangement of the left arm of chromosome V containing *CAN1* alone or *CAN1* and a *URA3* gene inserted telomeric to *CAN1*. This region of chromosome V does not contain any essential genes. Rearrangements are detected in haploid cells by measuring the rate of loss of *CAN1* or loss of both *CAN1* and *URA3* combined with mapping and sequencing the breakpoints of the selected rearrangements (*12–14*). This assay detects the formation of translocations and interstitial deletions, with little or no homology at their breakpoint junctions, chromosome fusions similar to those predicted to be formed by breakage-bridge-fusion-bridge cycles, and terminal chromosomal deletions associated with de novo telomere addition (*12–15*). Adaptations of this assay include measuring the rate of loss of *URA3* and *HIS3* markers on chromosome III (*14*) or the loss of *CAN1*, *URA3*, and *ADE2* markers on chromosome XV (*16*).

A number of assays have been developed in which a double-strand break induced by HO endonuclease has been used to direct genome rearrangements and to study the addition of telomeres, primarily at adjacent telomere "seed" sequences (*17–19*). In addition, a diploid cell system has been designed that measures the loss of hemizygous or heterozygous *URA3* markers on either chromosome III or chromosome V (*20*). These systems detect chromosomal rearrangements that are mediated by repeated sequences such as the mating-type loci and retrotransposon Ty elements (*21*). The *CAN1* gene, in conjunction with other genetic markers, has been used to measure the rate of both mitotic recombination and chromosome loss in diploid cells (*22*). Finally, numerous assays have been developed to detect rearrangements mediated by repeated sequences, including fully homologous as well as partially homologous, divergent sequences (*23–25*). The latter assays are directed at understanding how cells maintain genome stability when repeated sequences are present. This is particularly relevant to higher eukaryotic cells, which contain considerable numbers of repeated sequences (*26*, *27*).

S-Phase Checkpoints Suppress Spontaneous Genome Rearrangements

Checkpoints (Fig. 1) were originally identified as pathways that promote cell cycle delay or arrest in response to DNA damage or mitotic spindle damage, thereby giving the cell time to repair the damage (*28*, *29*). The hypersensitivity of checkpoint-defective mutants to killing by DNA damaging agents suggested that checkpoints might function to suppress genome instability. A survey of *S. cerevisiae* checkpoint genes revealed that mutations that disrupt the replication checkpoint (*rfc5-1*, *dpb11-1*, *mec1*Δ, *ddc2*Δ, and *dun1*Δ mutations) significantly increase the rate of genome rearrangements (*14*). In contrast, mutations in genes required for the classical G_1 and G_2 DNA damage checkpoints and the mitotic spindle checkpoints have little if any effect.

These results suggest that the DNA replication checkpoint—originally defined as the checkpoint that causes hydroxyurea-induced cell cycle arrest and inhibits firing of late-replication origins (*30*, *31*)—plays a critical

role in suppression of spontaneous genome instability. Conversely, these results imply that replication errors play a causal role in the formation of genome rearrangements (*14*). The function of the replication checkpoint in suppressing genome instability likely includes regulating cell cycle progression in response to replication errors, modulating DNA repair functions, ensuring the establishment of sister chromatid cohesion, and maintaining stalled replication forks in a state that allows them to restart DNA synthesis. All of the genome rearrangements seen when this checkpoint was inactivated involved deletion of a chromosome end, coupled with de novo addition of a new telomere. The latter result is consistent with the idea that chromosome breaks occur during S-phase and that in the absence of cell cycle arrest and repair, these broken DNAs persist into late S-phase and G_2, at which point they become substrates for telomerase, which is more active in these stages of the cell cycle (*14*, *19*, *32*).

A second S-phase checkpoint, the intra-S checkpoint, causes reduced rates of DNA replication and slower cell cycle progression in response to treatment with DNA damaging agents (*33*, *34*). There are at least two partially redundant branches of the intra-S checkpoint—one that requires *RAD17*, *RAD24*, and other genes (*35*), and one that requires *SGS1* (*36*). Both of these checkpoint branches are interfaced with many of the same downstream signal transduction and effector functions as the replication checkpoint (*30*, *31*, *33*, *34*, *36*–*38*). Inactivation of each individual intra-S checkpoint branch has a negligible effect on genome instability. However, simultaneous inactivation of both branches results in a synergistic increase in the rate of genome rearrangements to levels that are even higher than those observed when the replication checkpoint is inactivated (*14*, *38*). Consistent with the idea that the intra-S checkpoint suppresses such genome instability, treatment of *S. cerevisiae* strains containing mutations in genes required for the DNA damage checkpoint (e.g., *RAD9*, *RAD17*, *RAD24*.) with low doses of methyl methane sulfonate that only activate the intra-S checkpoint (*36*, *39*) causes a large increase in the frequency of genome rearrangements. A similar effect is seen when an *orc2-1* mutation is combined with a *rad9* mutation (*21*). These results suggest that the two branches of the intra-S checkpoint are redundant with regard to suppressing genome instability that may result from DNA replication errors.

Remarkably, simultaneous inactivation of both the intra-S checkpoint branches and the replication checkpoint by appropriate combinations of mutations (e.g., *mec1 tel1* double mutants, *mre11 mec1* double mutants, and other multiple-mutation combinations) causes a massive increase (12,000-fold and greater) in the rate of genome rearrangements (*14*, *38*). One interpretation of these results is that numerous errors occur in S-phase that can result in genome instability, but instability is rarely manifested because of extensive redundancy in cell cycle checkpoints.

S-phase checkpoints may also play a quite different role in suppressing genome instability. Recently, it was shown that checkpoint defects and recombination defects independently increase chromosome loss in diploid cells, and that combination of the two causes a synergistic increase in the rate of chromosome loss (*22*). The enhancement of chromosome loss was attributed to failure of the cells to arrest or delay in S-phase in response to double-strand breaks (or other damage) that accumulate in S-phase (due to lack of recombination). One implication of these results is that increased chromosome loss caused by checkpoint and recombination defects would exacerbate the effect of the genome rearrangements caused by checkpoint and recombination defects and result in greater genome instability.

Suppression of Genome Rearrangements by Recombination Functions

Homologous recombination promotes genetic exchanges and the repair of DNA damage (*40*). Studies of bacteriophage and bacteria have demonstrated that recombination can occur between one end of a double-strand break and an intact DNA, resulting in the formation of a replication fork (Fig. 2A, and discussed further below). This type of recombination has been implicated in initiation of phage replication, repair of stalled or collapsed replication forks, and certain types of genetic exchanges (*41*, *42*). A similar form of recombination, termed break-induced replication (BIR) (Fig. 2A), occurs in *S. cerevisiae* (*43*, *44*). A survey of yeast recombination genes (*15*) revealed that some mutations [*rad51*, *rad59*, *rad54*, *rdh4*, *rad55* (at 30°C

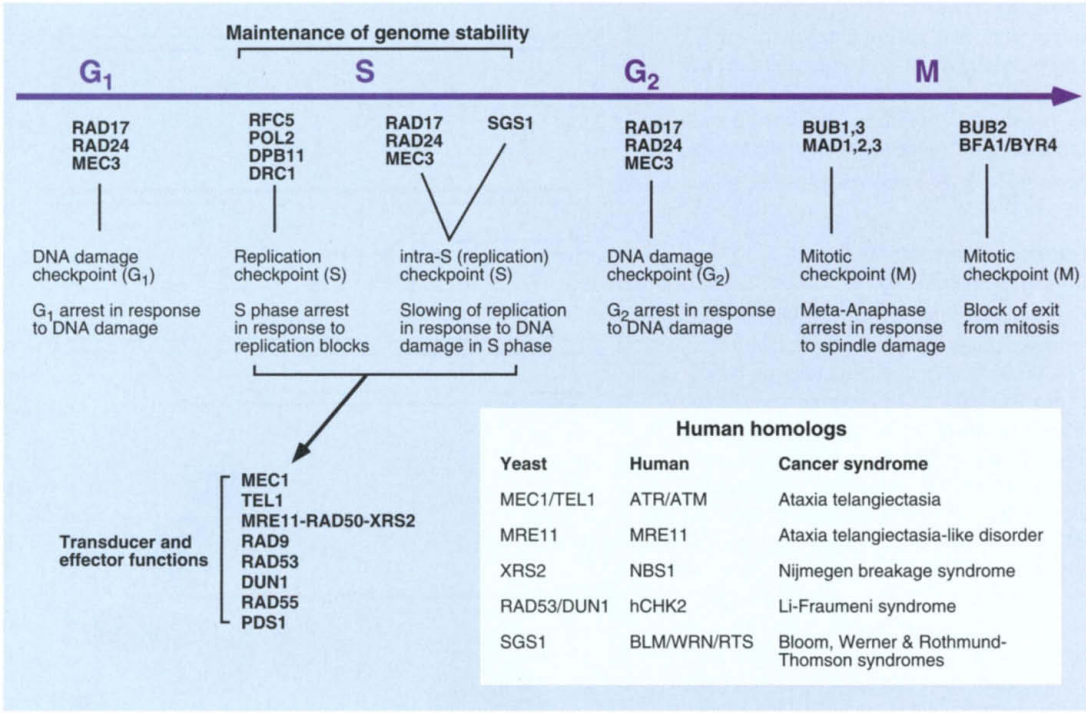

Fig. 1. Summary of *S. cerevisiae* DNA damage, replication, and mitotic checkpoints. The different stages of the cell cycle are indicated above the horizontal line. Below the line are listed a subset of the proteins that function in the indicated checkpoint branches. These proteins are thought to detect the "damage" that triggers each checkpoint. The primary effect of activating each checkpoint is shown below the proteins. Listed below the three checkpoint branches that function in S-phase are many of the known proteins that function in the downstream signal transduction cascade or are targeted by this cascade. The box at the right lists human homologs of yeast checkpoint genes that are mutated in human cancer susceptibility syndromes.

only), and *rad57* (at 30°C only)] caused only small increases in genome instability, whereas other mutations and combinations of mutations [*rad52*, *rad55* (at 23°C only), *rad57* (at 23°C only), *rad51* + *rad59*, and *rad54* + *rdh54*] elicited much more dramatic genomic derangements. Mutations in *mre11*, *rad50*, and *xrs2* also promote genome rearrangements, although these same mutations simultaneously disrupt cell cycle checkpoints, end-joining reactions, telomere maintenance, and recombination (*12*, *15*, *45*, *46*). Overall, these results parallel the genetic requirements for BIR and are distinct from the genetic requirements for classical double-strand break repair (*44*, *47*). This raises the possibility that BIR may be an important pathway for the suppression of genome instability in *S. cerevisiae*. The types of genome rearrangements that accumulate in recombination-defective cells are a mixture of terminal deletions with associated de novo telomere addition, translocations, and deletions, some of which appear to be formed by nonhomologous end joining (NHEJ) (*12*, *15*).

Telomere Damage–Induced Genome Instability

Ever since McClintock described the breakage-bridge-fusion-bridge cycle (*48*), it has been known that telomeres are needed for the stability of chromosomes (*49*). When telomere maintenance functions (Fig. 2B) are absent from growing *S. cerevisiae* cells, the telomeres continually shorten and the cells ultimately senesce (*50*–*52*). From the senescing cell population, survivors can arise in which new telomeres have been added to the chromosome ends by recombination (*47*, *53*). This recombination appears to occur between the chromosome end and telomeric or subtelomeric sequences that may be located on another chromosome. This recombination-dependent telomere maintenance has been suggested to occur by either of two pathways of BIR (*47*, *54*, *55*).

Two different types of genome instability have been observed in telomerase-defective yeast. The first arises when a chromosome end is degraded to an internal sequence that is homologous to sequences on other chromosomes (*16*). This end then invades homologous sequences on other intact chromosomes and primes BIR, resulting in a nonreciprocal translocation. [This type of rearrangement is virtually the same as that observed when a HO break was induced near the left end of chromosome III adjacent to a sequence that was homologous to a sequence at the HMR mating type locus; the sequence at the HO-break site primed BIR at the HMR sequence, resulting in a nonreciprocal translocation (*18*)]. A second form of genome instability is seen when mutations in either *TLC1* or *EST2* (which inactivate telomerase) are coupled with a mutation in *TEL1*. This combination results in a 2400-fold increase in the rate of genome rearrangements. About 60% of the rearrangements are nonreciprocal translocations, and 40% are chromosome translocations in which the broken end of the centromere-containing chromosome V fragment is fused to the shortened telomere of another chromosome (*15*). A similar effect is seen when mutations in either *TLC1* or *EST2* were combined with a mutation in *MEC1*, although the rates of genome instability observed are much lower (*15*).

These results suggest several possible mechanisms by which telomere dysfunction can produce genome instability. Shortening of the telomere is ultimately detected as DNA damage that activates a TEL1-dependent checkpoint and to a lesser extent a MEC1-dependent checkpoint, possibly leading to activation of repair functions (*15*, *56*). The MRE11-RAD50-XRS2 complex and the RAD55 subunit of the RAD55-RAD57 complex, two components of BIR pathways, are phosphorylated in a TEL1-dependent and a MEC1-dependent manner, respectively, in response to checkpoint activation (*57*, *58*). Under these circumstances, BIR would either restore functional telomeres or lead to translocations if degradation of the chromosome from the telomere had exposed an appropriate region of homology (*16*, *18*, *47*). In the absence of the checkpoint, BIR may be suppressed, and in the absence of BIR, other pathways such as NHEJ reactions could result in high rates of chromosome fusions (*15*). Chromosome fusions might also occur in the presence of the checkpoint if chromosome fragmentation or degradation did not expose regions of homology that are substrates for BIR; this has been observed to occur at very low rates (*16*). An alternative, but not mutually exclusive, possibility is that telomeres and/or a functional TEL1-dependent checkpoint might suppress NHEJ reactions that lead to chromosome fusions.

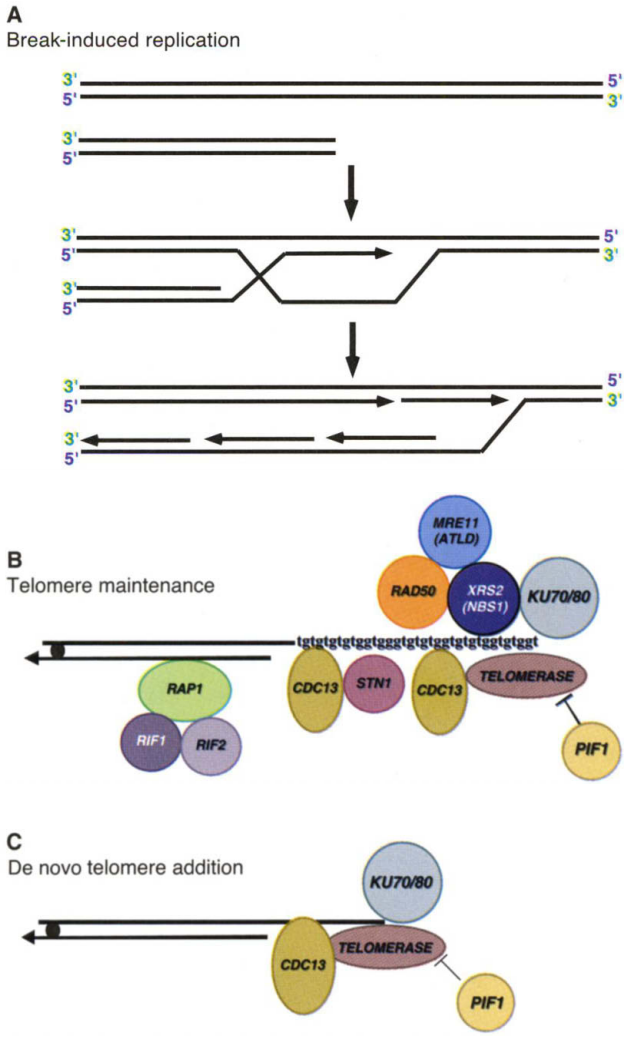

Fig. 2. Illustration of break-induced replication, telomere maintenance, and de novo telomere addition reactions. **(A)** Break-induced replication. In this example, the 3′ end of a broken DNA invades the intact DNA to form a D loop. The resulting heteroduplex region is then extended and a Holliday junction is formed. By appropriate resolution of the Holliday junction, a structure is formed that is equivalent to a replication fork. **(B)** Telomere maintenance functions. The diagram shows the end of a chromosome containing a single-stranded TG repeat end and the known *S. cerevisiae* proteins that bind to telomeres and extend the TG repeat sequences. **(C)** De novo telomere addition reactions. The diagram shows the end of a broken DNA molecule and the proteins that have been implicated in de novo telomere addition.

De Novo Telomere Additions Cause Genome Instability

Telomeres are normally maintained by the ribonucleoprotein enzyme telomerase (Fig. 2B), which extends the simple repeat sequences found at the ends of chromosomes (*59*). Telomerase is targeted to telomeres through the action of a number of proteins that bind chromosome ends, telomere repeat sequences themselves, and proteins that bind specific sequences located near telomeres. The specificity of telomerase for telomeres is evidenced by the fact that telomeres do not appear to be added to induced double-strand breaks unless these breaks are located adjacent to telomere-re-

lated repeat sequences that act as substrates for telomerase (*17*–*19*).

Given this specificity, it is remarkable that virtually every mutation that scores positive in the chromosome V marker loss assay increases the rate of rearrangements in which the end of a chromosome is deleted and a new telomere is added (*12*, *14*, *15*, *38*). These rearrangements are the result of de novo telomere addition because their formation requires functional telomerase and some, but not all, of the other telomere maintenance functions (Fig. 2C) (*15*). The high proportion of this type of rearrangement contrasts with the very low level of true de novo telomere additions at HO endonuclease–induced double-strand breaks (as compared with those that occur at break-associated telomere "seed" sequences) (*18*). This may indicate that the two processes involve distinct DNA structures.

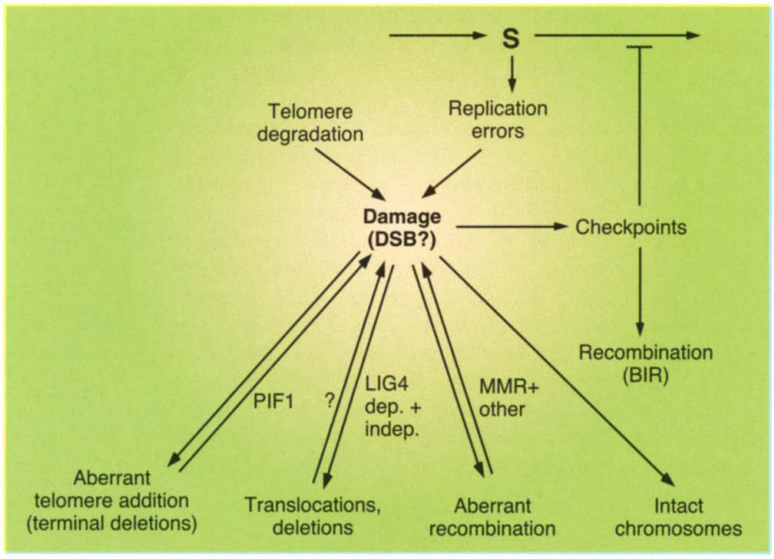

Fig. 3. Multiple pathways function to maintain genome stability in *S. cerevisiae*. DNA replication errors activate S-phase checkpoint sensors, whereas telomere damage likely activates DNA damage checkpoint sensors. These sensors then activate downstream responses, including DNA repair, that are required for suppression of genome instability and possibly cell cycle arrest or delay. Multiple pathways work to correct the damage; the primary nonmutagenic repair pathway appears to be break-induced replication (BIR), although double-strand break (DSB) repair and nonhomologous end joining (NHEJ) may play minor roles. The major mutagenic pathways are the translocation pathway(s), the de novo telomere addition pathway suppressed by PIF1, and aberrant recombination reactions that are suppressed by DNA mismatch repair (MMR) and other functions. The number of translocation pathways and whether there are pathways that specifically suppress translocations remain to be determined.

Perhaps not surprisingly, de novo telomere additions appear to be actively suppressed. Mutations in the *PIF1* gene, which encodes a DNA helicase, were identified in a screen to detect telomere maintenance functions (*17*, *60*). *pif1* mutations resulted in increased addition of telomeres at telomere seed sequences placed at a subtelomeric site, increased the length of normal telomeres, and modestly increased (~14-fold) telomere additions at an HO-break site (*17*). Strikingly, these mutations caused a 240- to 1000-fold increase in the rate of spontaneous genome rearrangements, depending on the allele tested (*15*), and they acted synergistically with other mutations that promote genome instability. The genome rearrangements induced by *PIF1* defects were all rearrangements in which the end of a chromosome was deleted and a new telomere was added, and their formation required telomere maintenance functions. Thus, PIF1 defines an enzymatic pathway that suppresses genome instability driven by de novo telomere additions; other components of this pathway have not yet been identified (*15*).

This form of genome instability may also be passively suppressed. Telomere maintenance functions apparently act on normal telomeres starting in late S-phase (*32*). However, the activity of telomere maintenance functions on telomere "seed" sequences located near an HO-induced double-strand break (i.e., de novo telomere addition activity?) is up-regulated in G_2 (*19*). Because spontaneous genome instability appears to result from errors or damage that occur during S-phase (*14*, *38*), the collaboration of checkpoints that delay progression through S-phase with cell cycle regulation of telomerase maintenance functions may suppress genome instability driven by de novo telomere addition.

At-Risk DNA Sequences

The sequence and structure of DNA can influence the rate of genome rearrangements. Simple repeat sequences such as microsatellite repeats, minisatellite repeats, and short repeated sequences located within a short distance of each other are prone to replication errors. Such sequence elements show high rates of frameshift mutations and small deletion and duplication mutations in strains containing mutations in DNA replication and repair genes (*61*–*66*). Inverted repeats, or palindromes, are cleaved to produce double-strand breaks (*67*), and in *S. cerevisiae*, aberrant processing of these structures can produce certain types of genome rearrangements (*14*, *26*). Whether palindromes play a major role in genome instability is unclear, however, as these structures are rare in the *S. cerevisiae* and human genomes (*27*, *68*).

Of perhaps greater significance to the genesis of genome rearrangements are dispersed repeated sequences such as retrotransposon Ty elements, subtelomeric elements, and the regions of homology between different chromosomes, many of which have been extensively documented by the yeast genome project. Such repeated sequences often show some level of sequence divergence. A number of investigators have shown that spontaneous and damage-induced genome rearrangements can be mediated by such dispersed repeated sequences (*16*, *69*, *70*). Such events likely occur by aberrant recombination, and in several instances BIR has been implicated as the cause (*16*, *41*). Mismatch repair functions and the SGS1 helicase are known to suppress recombination between such repeats if they show sequence divergence (*71*, *72*). Mismatch repair functions also suppress translocations mediated by short divergent repeat sequence and appear to suppress BIR events that maintain telomeres in the absence of telomerase (*72*, *73*). A number of gene products, including the RRM3 helicase, have also been shown to suppress deletion and/or gene conversion events within direct repeat arrays like ribosomal DNA sequences and artificially created repeats (*24*, *25*, *74*); however, it is unclear if these gene products suppress genome rearrangements mediated by dispersed repeated sequences.

Do Replication Errors Underlie Spontaneous Genome Rearrangements?

Three general observations suggest that DNA replication errors may underlie spontaneous genome rearrangements. First, mutations in genes encoding DNA replication proteins cause increased rates of genome rearrangements as well as the accumulation of recombination intermediates (*12*, *14*, *64*, *75*, *76*). Second, S-phase checkpoints play a critical role in suppressing genome instability (*14*, *38*). This is consistent with the idea that checkpoint activation is required for correct repair of errors that occur during normal replication. It is also consistent with the observation that checkpoints maintain stalled replication forks in a state that allows them to resume DNA synthesis (*77*). Third, dysregulation of replication origins results in increased genome instability (*70*, *78*). It is not yet clear which DNA structures formed during DNA replication are processed to yield genome rearrangements.

Studies of bacterial systems have suggested that stalled or collapsed replication forks occur during normal DNA replication, and that these structures are converted back into functional replication forks by recombination (*42*, *79*). When bacterial DNA replication is stalled, for example, through the use of temperature-sensitive DNA helicase mutants, the stalled structures are converted to double-strand breaks by specific pathways that resolve the stalled fork structures (*79*, *80*). The broken DNA can then recombine with an intact chromosome to produce a new replication fork, and replication proteins are then reloaded onto the new fork (*42*, *81*). This process resembles BIR in *S. cerevisiae* (*41*, *43*, *44*). The similarity between the proposed role of BIR in suppressing genome instability (*15*) and the role of recombination in replication restart (*42*, *80*) suggests that processing of stalled replication forks is important in suppressing genome instability in *S. cerevisiae*. Consequently, aberrant processing of stalled replication forks may give rise to genome rearrangements. Recent studies on the interaction between replication and recombination, and on the processing of branched DNAs like those associated with stalled replication forks, appear to have set the stage to gain more insights into this critical question (*82*–*85*).

An Integrated Model for the Control of Genome Instability

Figure 3 presents a model for the pathways that produce and suppress genome rearrangements in yeast, which is based in part on the genetic data reviewed here. During DNA replication, errors occur that result in broken, stalled, or collapsed replication forks, and these may ultimately be processed to double-strand breaks or other types of structures that promote genome rearrangements. The precise nature of these "mutagenic" DNA structures is presently unknown. Degradation of chromosome ends also results in structures that promote genome instability. The replication errors trigger S-phase checkpoint sensors, whereas telomere damage likely activates the DNA damage checkpoint sensors at any stage of the cell cycle. This results in phosphorylation of recombination and repair proteins, a transcriptional response, and possibly cell cycle arrest.

These broken or damaged DNAs are normally repaired by BIR rather than by homologous recombination or NHEJ, and this prevents genome rearrangements (*15*). When BIR is inactivated, these substrates yield genome rearrangements including terminal deletions with associated de novo telomere addition, interstitial deletions, and translocations. Inactivation of pathways suppressing recombination mediated by repeat sequences results in many aberrant recombination events. Inactivation of the S-phase checkpoints can generate terminal deletions with associated de novo telomere addition, presumably because damaged DNA persists in late S-phase and G_2, where it serves as a substrate for telomerase. S-phase checkpoint defects may also increase the frequency of replication errors that ultimately produce substrates for aberrant addition of telomeres. Normally, these types of rearrangements are suppressed by PIF1 (*15*, *60*), which inhibits de novo telomere addition and channels substrates into nonmutagenic recombination. Some of the translocations and deletions observed are formed by NHEJ, but other mechanisms for their formation clearly exist (*15*). Thus, it is the extensive redundancy among pathways that suppress genome instability that appears to reduce genome instability to extremely low levels in normal cells.

Parallels in Higher Eukaryotes

As noted above, genome instability is a characteristic feature of most human cancers (*2*, *3*, *86*). Because the types of genome rearrangements in the *S. cerevisiae* mutants described here are also found in cancer cells, it is tempting to speculate that they arise through similar mechanisms. At least seven human homologs of *S. cerevisiae* genome instability genes are mutated in inherited cancer susceptibility syndromes (Fig. 1, inset) (*87*–*94*). In addition, the proteins encoded by the breast cancer susceptibility genes *BRCA1* and *BRCA2* either interact directly with proteins that function in the genome instability suppression pathways or are phosphorylated by proteins that function in suppression of genome instability described in Fig. 1 (*95*). Similarly, the Fanconi anemia gene products may also interact with these pathways (*96*, *97*). Finally, some of the proteins encoded by mismatch repair genes, including several that are prevalent cancer susceptibility genes (*1*, *2*, *4*), also function to suppress certain genome rearrangements (*72*, *73*).

More than 50 yeast genes have now been implicated in the suppression of genome instability. Whether human homologs of additional genes in this group function as tumor suppressors—particularly in the more common solid tumors—is an important question for future investigation.

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VIEWPOINT

Checking That Replication Breakdown Is Not Terminal

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Two new studies help to clarify the relationship between checkpoint proteins, recombination, and replication fork integrity.

DNA double-strand breaks (DSBs) are major pathological DNA structures in mitotic cells. Left unrepaired, these breaks can result in chromosome translocations and missegregation of genetic information, destabilizing the genome and potentially contributing to carcinogenesis. Spontaneous DSBs are caused by endogenous DNA damaging agents, but they also occur at stalled DNA replication forks in particular mutant backgrounds.

Replication-associated DSBs were first demonstrated in helicase-defective *Escherichia coli* (*1*). Surprisingly, DSB formation during replication requires enzymes involved in homologous recombination (HR). In *E. coli*, stalled replication forks are restarted by HR-mediated repair (*2*) (Fig. 1). Perhaps DSBs arise because processing of DNA structures at damaged forks by HR enzymes generates DNA intermediates that are susceptible to cleavage. This interpretation is consistent with a recent study showing that rescue of stalled replication forks in *E. coli* by the RecG helicase involves unwinding of both nascent strands at the fork and their subsequent annealing to form a four-stranded Holliday junction (HJ) (*3*). Inappropriate resolution of such a HJ would result in a DSB at a stalled fork.

HR proteins in yeast and other eukaryotes also play a role in maintaining replication fidelity and preventing the accumulation of DNA damage, including DSBs [e.g., (*4*, *5*)]. Eukaryotic replication checkpoint proteins were originally identified for their ability to prevent cell entry into mitosis during replication, but they also prevent genome instability through regulation of DNA repair within S phase (*7*). For example, in the fission yeast *Schizosaccharomyes pombe*, both recombination and replication checkpoint proteins are required for promotion of cell survival when DNA damage in S phase cannot be removed (*6*).

In this issue of *Science*, a new study by Sogo *et al.* (*8*) helps to clarify the relationship between checkpoint proteins, recombination, and replication fork integrity. The authors used electron microscopy to visualize stalled replication intermediates in the budding yeast *Saccharomyes cerevisiae* in the presence or absence of the replication checkpoint. In wild-type cells, these intermediates were largely bifurcating and double-stranded, with only limited regions of single-stranded (ss) DNA. In contrast, the replication intermediates isolated from checkpoint-defective (*rad53* mutant) cells showed extensive ssDNA regions and large numbers of reversed forks. This suggests that the replication checkpoint suppresses the formation of HJ-like replication intermediates. By implication, the absence of the checkpoint function may allow DSBs to occur through inappropriate processing of HJs.

In related work, also in this issue, Cha and Kleckner (*9*) demonstrate that DSBs occur in replication checkpoint–deficient *S. cerevisiae* cells during replicative stress. Specifically, these authors find that in the absence of Mec1 (a chromosome-bound signal transduction protein involved in DNA replication, repair, and recombination), DSBs occur late in S phase at specific genomic loci that correspond to slowly replicating regions in unstressed cells. These "replication slow zones" (RSZs) map between active replication origins, but deletion of the origins does not affect DSB formation, suggesting that RSZs are intrinsically susceptible to breakage during replication. The simplest interpretation is that RSZs exhibit slow replication because replication is more difficult in these regions, perhaps because DNA binding proteins need to be removed. Thus, RSZs are likely to experience additional difficulties when the supply of deoxynucleoside triphosphates (dNTPs) is depleted by the experimental conditions.

In both *E. coli* and *S. cerevisiae*, DSBs are induced when helicase activity is perturbed (*1*, *10*). Thus, checkpoint proteins may coordinate replication and recombination during replicative stress caused by global dNTP inhibition, localized DNA damage, or refractory chromatin architecture resulting from repetitive sequences or the binding of proteins that must be removed by specialized helicases. It is interesting that the replication checkpoint is not essential for viability in *S. pombe*, as it is in *S. cerevisiae*. This is because the two yeasts regulate ribonucleotide reductase (RNR) in different ways: Induction of *S. cerevisiae* RNR activity in S phase

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