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When Checkpoints Fail

Review

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Checkpoints Monitor DNA Damage and Regulate Cell Cycle Progression

A number of surveillance systems exist that interrupt cell cycle progression when damage to the genome or spindle is detected, or when cells have failed to complete an event (Weinert and Hartwell, 1988). These surveillance systems are termed checkpoints and have been given an empirical definition. When the occurrence of an event B is dependent upon the completion of a prior event A, that dependence is due to a checkpoint if a loss-of-function mutation can be found that relieves the dependence (Hartwell and Weinert, 1989). An example is the DNA damage checkpoint. Progression from G2 to M is dependent upon an intact genome (e.g. dependent upon the repair of any double strand breaks). This dependence is eliminated by deletion of the *RAD9* gene. The deletion phenotype reveals the presence of a DNA damage checkpoint, and the *RAD9* gene is a component of the checkpoint. In addition to the DNA damage checkpoint (reviewed in Elledge, 1996), mitosis is monitored by a spindle checkpoint that inhibits anaphase progression when chromosomes are not attached to the mitotic spindle (reviewed in Murray, 1995) and a spindle pole body (centrosome equivalent) duplication checkpoint (Winey et al., 1991). This review will focus on the DNA damage checkpoint. We anticipate, however, that the issues raised will apply to checkpoints in general.

The many types of DNA damage can be grouped into two categories: modification of nitrogenous bases and modification of the phosphodiester backbone (reviewed in Friedberg et al., 1995). For example, bases can be modified or even cross-linked when chemical adducts become covalently attached or when DNA undergoes spontaneous depurination or depyrimidination. These lesions are removed by excision repair pathways that excise the affected base(s), producing a temporary "excision gap," which is subsequently filled in by a repair polymerase and ligated (reviewed in Friedberg et al., 1995). Phosphodiester bonds can be broken, resulting in nicks or double-stranded breaks in DNA. Broken DNA ends are highly recombinogenic. They are repaired in S. cerevisiae primarily by a homology-based recombination reaction that prefers sister chromatids over homologs, and in mammalian cells primarily by nonhomologous end joining (reviewed in Friedberg et al., 1995) or by de novo addition of new telomeres (Wilkie et al., 1990).

Sources of damage can be extrinsic, as well as intrinsic, to the cell. Extrinsic sources include irradiation and chemical mutagens. Intrinsic damage is generated by the cell itself, either as a result of DNA metabolism or as a result of spontaneous chemical reactivity of DNA. Furthermore, different types of damage can be incurred at different stages of the cell cycle. For example, most cells rest in G1 and must accumulate most of their oxidative damage to DNA during this stage, S phase cells risk incomplete replication and nucleotide misincorporation, and cells undergoing mitosis risk chromosome breakage during the segregation of sister chromatids.

Genetic studies have identified many of the components of checkpoints in the yeasts Saccharomyces cerevisiae (reviewed in Murray, 1995) and Schizosaccharomyces pombe (reviewed in D'Urso and Nurse, 1995), and cancer prone syndromes have revealed several in human cells as well (Table 1). The use of checkpoint-defective mutants is a powerful technique for analyzing cellular processes. Pleiotropic properties of checkpoint genes, however, have limited genetic dissection of the pathways. For example, genes required for cell cycle arrest in response to DNA damage have been shown to be required for DNA repair, apoptosis, and transcriptional induction. Some checkpoint genes are required for several different stages of arrest in the cell cycle, and some are required for essential processes and must be studied either as special alleles or in the presence of suppressors.

The DNA damage checkpoint acts at three stages in the cell cycle, one at the G1/S transition, one that monitors progression through S, and one at the G2/M boundary (Table 1). Even though there are several arrest points within the cell cycle, we consider the DNA damage surveillance system to be one checkpoint because all arrests are signaled by DNA damage and because many of the genes necessary for arrest at one stage of the cell cycle are also necessary for arrest at other stages (Table 1).

We think of the DNA damage checkpoint as a signal transduction system that communicates information between a DNA lesion and components of the cell cycle. As such, it is expected to have activating signals, transduction components, and cell cycle targets. The emphasis in the field currently is on identifying these elements and defining their biochemical mode of action. In this review, we will focus on the consequences of the DNA damage checkpoint for the repair or transmission of DNA damage. We relate this discussion to an emerging field that will ultimately deal with the logic of checkpoints: that is, how and why do different checkpoint arrests vary with regard to lesion discrimination, sensitivity, recovery, adaptation, and the decision to apoptose.

Consequences of Checkpoint Failure

Cell cycle arrest mediated by a checkpoint may fail for a variety of reasons. First, like all cellular processes, checkpoints must have an intrinsic error rate. Second,

Table 1. S. cerevisiae and human genes in which loss of function mutations have eliminated or attenuated DNA damage or spindle integrity checkpoints.

Arrest points: G1/S and G2/M refer to the DNA damage checkpoint that arrests cells in G1/S and G2/M; the S phase arrest refers to the checkpoint that slows the rate of S phase progression in the presence of DNA damage, whereas the S/M arrest refers to the checkpoint that arrests cells before or during S-phase in response to inhibitors of nucleotide biosynthesis such as hydroxyurea (in yeast) or PALA (in mammalian cells); M refers to the mitotic arrest that monitors spindle integrity. "+" indicates that a given gene is required for an activity, "−" indicates that it is not, and "±" indicates a partial effect. A "+" under DNA repair indicates either that a mutation in a gene affects lesion processing or that the purified protein has been shown to possess an activity that modifies DNA. In the ninth column, a "+" indicates kinase homology and a "++" indicates that kinase activity has been shown directly. A "+" under apoptosis means that the gene product is required for apoptosis under at least some conditions.

	Arrest Point					Transcription Induction	DNA Repair	Kinase Homology (+) or Activity (++)	Apoptosis
	G1/S	S	G2/M	M	S/M				
S. cerevisiae									
RAD9	+[1]	+/−[2,3]	+[4]	−[4,5]	−[6]	+/−[7]	+[8]		
RAD17	+[38]	+/−[3]	+[6]	−[6]	−[6]	+/−[7]	+[8]		
RAD24	+[9]	+/−[3]	+[10]		−[10]	+/−[7]	+[8]		
MEC3	+[2]	+/−[2,3]	+[10]	−[2]	−[10]	+/−[7]	+[8]		
MEC1		+[11]	+[10]		+[10]	+[7]		+[12]	
RAD53	+[13]	+[11]	+[10,13]	−[13]	+[10,13]	+[13,7]		++[14]	
POL2	−[15]		−[15]		+[15]	+[15]			
DBP11					+[16]				
RFC5					+[17]				
PDS1		−[3]	+[18]	+[18]	−[18]				
MPS1			−[19]	+[19]	−[19]			++[20]	
MAD1				+[5]					
MAD2				+[5]					
MAD3		−[3]		+[5]					
BUB1				+[21]	−[21]			++[22]	
BUB2				+[21]	−[21]				
BUB3				+[21]	−[21]				
Human									
ATM	+[23]	+[24]	+[25]			+[23]		+[26]	−[27]
p53	+[23]	−[28]	+/−[29,30]	+[31]	+[32,33]	+[23]	+[34]		+[35,36]
p21[CIP1]	+[37]			−[37]					−[37]

[1]Siede et al., 1993; [2]Longhese et al., 1996; [3]Paulovich et al., 1997; [4]Weinert and Hartwell, 1988; [5]Li and Murray, 1991; [6]Weinert and Hartwell, 1993; [7]Kiser and Weinert, 1996; [8]Lydall and Weinert, 1995; [9]Siede et al., 1994; [10]Weinert et al., 1994; [11]Paulovich and Hartwell, 1995; [12]Kato and Ogawa, 1994; [13]Allen et al., 1994; [14]Zheng et al., 1993; [15]Navas et al., 1995; [16]Araki et al., 1995; [17]Sugimoto et al., 1996; [18]Yamamoto et al., 1996; [19]Weiss and Winey, 1996; [20]Lauzé et al., 1995; [21]Hoyt et al., 1991; [22]Roberts et al., 1994; [23]Kastan et al., 1992; [24]Painter and Young, 1980; [25]Zampetti-Bosseler and Scott, 1981; [26]Savitsky et al., 1995; [27]Meyn et al., 1994; [28]Wyllie et al., 1996; [29]Aloni-Grinstein et al., 1995; [30]Stewart et al., 1995; [31]Cross et al., 1995; [32]Livingstone et al., 1992; [33]Yin et al., 1992; [34]Mummenbrauer et al., 1996; [35]Lowe et al., 1993; [36]Clarke et al., 1993; [37]Deng et al., 1995; and [38]Siede et al., 1996.

like many signal transduction systems, they exhibit adaptation. That is, even though damage remains unrepaired, after an interval of arrest the cell may resume progress through the cell cycle (Sandall and Zakian, 1993). Third, cells with defective checkpoints may be at an advantage when selection favors multiple genetic changes. Cancer cells are usually missing some checkpoints, probably because this loss permits a greater rate of genomic evolution (reviewed in Hartwell and Kastan, 1994). The same selective pressure might occur during the evolution of organisms when rapid change is advantageous. The conditions under which checkpoints fail can be exploited to ask what are the consequences of checkpoint failure. Such studies will provide light into what checkpoints are good for. Hopefully, a consideration of the consequences of checkpoint failure will ultimately help reveal why they have their particular error rates, adaptation characteristics, and selective pressures. The consequences of checkpoint failure for the DNA damage checkpoint can depend both on the type of damage and on the stage of the cell cycle.

Therefore, to appreciate the consequences of checkpoint failure, we need to consider not only the type of damage and the stage of the cell cycle in which it has occurred, but also what happens as the cell progresses to the next stage of the cell cycle. If damage fails to be repaired within the stage of its origin, the nature of the damage can be changed as the cell passes to the next stage, resulting in the formation of secondary lesions. For example, if a G1 cell that has single-stranded breaks in its DNA progresses through S phase, the single strand lesions will be converted to secondary lesions, i.e., double strand breaks. Moreover, some options for repair may be lost if the cell cycle progresses to the next stage prior to repair. Segregation of broken chromosomes may lead to loss of the acentric fragment, precluding the possibility of end-to-end joining. We will consider both of these types of consequences, namely formation of secondary lesions and loss of repair options, following loss of checkpoint control within S, or at the G1/S or G2/M transitions.

Checkpoint Control of the G1/S Transition and of S Phase Progression Rate

Although cells do not require an undamaged template for replication, eukaryotic cells have mechanisms to

avoid replicating damaged DNA (reviewed in Naegeli, 1994). For example, DNA repair proteins remove or reverse DNA lesions to restore the integrity of the template. Given enough time for repair, the cell might avoid replicating damaged DNA altogether. To increase the time available for repair prior to replication, the DNA damage checkpoint arrests cells with a G1 DNA content in response to some types of DNA damage. During this G1/S delay, cells are able to repair much of the damage, thereby restoring the template before replication. Cells also utilize the DNA damage checkpoint within S phase. Replicating bacterial (Cairnes and Davern, 1966), yeast (Siede et al., 1994; Paulovich and Hartwell, 1995; Paulovich et al., 1997), or mammalian cells (Painter and Young, 1980; Larner et al., 1994) decrease the rate of ongoing DNA synthesis in response to DNA damage; this inhibition may reflect control at the level of origin initiation and/or at the level of fork progression (Painter and Young, 1980; Larner et al., 1994). The value of the G1/S and S phase arrest in response to DNA damage may be best understood by considering the consequences of unrestrained replication in the presence of DNA damage. Since it is likely that failure of control over the entry into S from G1 and failure of control over replication within S phase have the same consequence for lesion processing (the lesion is replicated rather than repaired), we consider loss of these two controls together.

Failure to Regulate Progression into or through S Phase

If the DNA damage checkpoint fails, DNA repair will be compromised and cells will experience consequences of replicating the damaged template. These consequences will be determined at least in part by the type of damage being replicated. First, when a replication fork encounters a covalently modified base (e.g. thymine dimer) in the template strand, the fork may stop. In a mechanism that is not well understood, replication resumes downstream of the damage, resulting in the formation of a secondary lesion, a daughter strand gap that encompasses the damage. Second, replication of single strand nicks results in replication fork breakage and the formation of double strand breaks (reviewed in Kuzminov, 1995b). Replication of gapped DNA would also result in the formation of a double-stranded break and concomitant breakage of the replication fork; in addition, the broken sister chromatid would suffer a deletion. Unlike one-strand lesions, double strand breaks confer high risk for loss of heterozygosity and gross chromosomal instability manifest as DNA amplification, chromosome rearrangement or truncation, and chromosome loss or gain (discussed below). Third, in some instances the replication machinery is able to replicate across lesions in the template DNA, so-called translesion synthesis (reviewed in Friedberg et al., 1995). Replication across adducts results in misincorporation of noncognate bases in the nascent strand and the potential to fix a mutation during either subsequent replicative or repair synthesis. Fourth, base mismatches arise by occasional incorporation of the wrong base during DNA replication and are removed by the mismatch repair system (reviewed in Friedberg et al., 1995). Replication of DNA containing a mismatch produces two new duplexes, neither of which contains a mismatch, but one of which contains a mutation. Hence, replication of a mismatch results in the fixation of a mutation in one of the daughter duplexes, since the option for mismatch repair is lost.

Why Does Premature Entry into S Phase Result in Genetic Instability?

In the previous examples, the origins of damage, the mechanism of its repair, and the consequences of progression past a cell cycle arrest point are at least clear in outline. However, the nature of some forms of DNA damage, such as that associated with unregulated progression into S phase, is unknown. Even in the absence of extrinsic damage, unregulated entry into S phase can result in genomic instability and/or cell death. Progression through the G1 phase can be accelerated in either yeast or mammalian cells by the overproduction of G1 cyclins (Nash et al., 1988; Ohtsubo and Roberts, 1993; Quelle et al., 1993; Resnitzky et al., 1994; Vallen and Cross, 1995). Such cells enter S phase prematurely and exhibit genetic instability and an enhanced dependence on checkpoint functions for survival (Vallen and Cross, 1995; Zhou et al., 1996).

One possibility for why inappropriate entry into S phase results in genetic instability is that cells may commonly have DNA damage that cannot be repaired during S phase and so must be repaired prior to entry into S phase. This could be due to cell cycle–associated differences in DNA repair or in the DNA itself (such as its chromatin structure), or simply due to the fact that replication forks encounter lesions faster than DNA repair systems can clear them. Another possibility is that inappropriate entry into S phase may actually cause DNA damage. For example, cells might fail to activate enough replication origins to permit completion of replication before they enter mitosis, or shortening the G1 phase may result in commencement of S phase with abnormal nucleotide pools. Ribonucleotide reductase (RNR) facilitates the conversion of ribonucleoside diphosphates to deoxynucleoside triphosphates, which are precursors to DNA replication. RNR activity is cell cycle regulated, and *RNR* gene message levels are induced in late G1 (reviewed in Elledge et al., 1992). Premature entry into S phase could result in inadequate RNR activity (Yarbro, 1992; Weinert et al., 1994), which could cause depletion of nucleotide pools and stalling of replication forks (Petes and Newlon, 1974), both of which have been shown to lead to genetic instability; in bacteria (reviewed in Kuzminov, 1995a), and probably also in yeast (Keil and Roeder, 1984; Voelkel-Meiman et al., 1987), stalled replication forks are unstable and are prone to breakage and restoration by recombinational repair, resulting in increased recombination rates in a variety of cell types. Instability of stalled replication forks could explain the high recombination and chromosome loss rates in yeast cells overproducing CLN1, as well as the elevated rates of gene amplification in human $p53^{-}$ cells (which are defective in the G1/S DNA damage checkpoint) treated with PALA (an inhibitor of nucleotide biosynthesis) (Livingstone et al., 1992) and in rodent cells overproducing Cyclin D (Zhou et al., 1996). Additionally, alterations of dNTP concentrations induce mutations due to deleterious effects on DNA polymerase fidelity (reviewed in Kunz et al., 1994). The importance

of having sufficient nucleotide pools before entering S phase is reflected in the fact that human cells are thought to monitor nucleotide pools directly and arrest at a p53-dependent G1 block when these pools are low (Linke et al., 1996).

Failure of the G2/M Arrest

Double-stranded DNA breaks activate the DNA damage checkpoint, preventing progression through mitosis. Mammalian cells have a nonhomologous end-joining activity that fuses together broken DNA, and direct end-to-end joining of the centromeric and the acentric fragments may be the primary mechanism by which breaks are repaired in mammalian cells (reviewed in Friedberg et al., 1995). This process may result in a deletion of DNA near the break, presumably due to exonucleolytic degradation. The DNA damage checkpoint facilitates repair both by increasing the time for repair and by transcriptionally inducing gene expression. If the G2/M arrest fails, the broken chromosome may be subjected to mitosis, and the centromere-containing and acentric fragments may be partitioned into separate nuclei, precluding the possibility of their undergoing end-to-end fusion. This situation can lead to a variety of outcomes. For example, the broken chromosome may be degraded and lost altogether. Chromosomes are lost at elevated rates in yeast checkpoint mutants (Weinert and Hartwell, 1990) in response to both spontaneous damage and targeted double-stranded DNA breaks (Sandell and Zakian, 1993). Even if both chromosome fragments end up in the same nucleus following mitosis, and can undergo end-to-end fusion at a later time, there may be consequences. For example, the region deleted at the fusion junction may be larger if the rate of DNA degradation varies in the cell cycle. It has been shown in yeast that a broken plasmid is degraded much more quickly in S phase than in G1 (Raghuraman et al., 1994). Alternatively, rather than undergoing degradation, the centromere-containing fragment may be "healed." A telomere can be added (Matsumoto et al., 1987; Wilkie et al., 1990), thus generating a truncated chromosome and uncovering recessive mutations on the homolog. Finally, the broken chromosome may be replicated, producing truncated sister chromatids and restoring the option for an end-to-end fusion event, this time between sisters. This fused molecule will be dicentric and will therefore be broken in the next mitosis, after which it can fuse again. This is commonly referred to as the bridge-breakage-fusion cycle, and has been documented in plants and mammals (McClintock, 1941; Ma et al., 1993). One result of the bridge-breakage-fusion cycle is the loss of telomere-proximal sequences on the chromosome. Bridge-breakage-fusion cycles can also lead to chromosomal rearrangement and gene amplification (Ma et al., 1993). Since the point of rebreakage is likely to be different from the original point of fusion, one chromatid will have an inverted duplication of the region near the breakage point. Because this process occurs iteratively, this region may become amplified.

Failure of the G2/M delay precludes repair from the sister chromatid template. Whereas recombinational repair may be only a minor repair pathway in mammalian cells, it is the major pathway for double strand break repair in yeast. On passing through mitosis, sister chromatids are separated and are no longer available as templates for repair. Sister chromatid exchange is also important for lesions other than double-stranded DNA breaks. Bypass replication of some DNA adducts leaves a DNA gap in the nascent strand. This gap is repaired using the sister chromatid as a recombinational template. Failure of the G2/M arrest precludes the use of a sister as template.

While sister chromatids have been shown to be the preferred template for recombinational repair (Kadyk and Hartwell, 1992), homologs can also serve as recombination substrates. The use of a homolog may be disadvantageous, however, because it may lead to loss of heterozygosity. Moreover, even homolog recombination may be less efficient if not completed at the checkpoint arrest; it has been demonstrated that a double-stranded DNA break is recombinationally repaired off of a homolog less efficiently in a checkpoint-deficient (*rad9*) strain than in a wild-type strain (Sandell and Zakian, 1993). While this may reflect the loss of some aspect of RAD9p function other than its role in the G2/M arrest, it is also possible that a cell is better able to perform recombination at this arrest. Alternatively, the broken DNA may be less stable in S phase, and therefore be degraded more quickly in the ensuing cycle.

Failure to Regulate DNA Replication May Result in an Increased Dependence on G2 Arrest

If failure to control replication of nicks were to result in the formation of DNA double strand breaks, the G2/M delay regulating progression through mitosis would be activated. During this downstream arrest, cells would have an opportunity to repair strand breaks recombinationally and potentially avoid losing a chromosome in mitosis or initiating a round of bridge-breakage-fusion cycles. Therefore, one would predict that loss of the G2/M delay might be more consequential to a cell carrying a defect in G1/S or S phase checkpoints than to an otherwise wild-type cell. This prediction is as yet untestable in yeast, since no mutation identified so far eliminates the G1/S delay without also eliminating the G2/M delay. Work in mammalian cells, however, has shown that pentoxyphylline and caffeine (which override the G2/M delay) enhance the sensitivity of cells that are defective in the G1/S delay to DNA damaging agents (Fan et al., 1995; Powell et al., 1995; Russell et al., 1995).

Questions About the Logic of the DNA Damage Checkpoint

Our consideration of the consequences of checkpoint failure raises many questions about the logic of checkpoints. Although we have limited knowledge about the signals that elicit checkpoints, the DNA damage checkpoint seems to respond to different types of primary damage at different stages of the cell cycle. What is the logic of these different responses? The signals that activate the DNA damage checkpoint at different stages of the cell cycle appear to favor lesions that would cause the most serious damage if passed unrepaired to the

next stage of the cycle. For example, arrest at G1/S is sensitive to gaps remaining after excision repair because failure to arrest would permit their conversion to double strand breaks. In contrast, this checkpoint does not respond to unexcised dimers (yeast and mammalian cells: Nelson and Kastan, 1994; Siede et al., 1994) or a double strand break (yeast: Raghuraman et al., 1994). It is also sensitive to nucleotide pool depletion, probably because entry into S with inadequate nucleotide pools results in damage that can produce gene amplification (Livingstone et al., 1992; Yin et al., 1992). The arrest at G2/M is dramatically sensitive to even one double strand break because failure to arrest would lead to the irreversible loss of chromosome fragments. Another example may be mismatched DNA bases generated during a replication error. The mismatch repair system is believed to be active for a period after replication when discrimination of mother and daughter strands is still possible. The presence of a functional mismatch repair system imposes a "G2" (or possibly late S phase) delay in the presence of alkylation damage (Hawn et al., 1995), while mutational loss of mismatch repair relieves this arrest.

What are the signals that activate checkpoints? Clearly primary damage to DNA such as double strand breaks and excision gaps (or something derived directly from them) are signals for the DNA damage checkpoint. When damage is induced by radiation or chemicals, however, many cellular components in addition to DNA are modified. Any chemical changes (e.g. changes to RNA, protein, or lipid) that are well correlated with an important type of DNA damage would be reasonable signals to activate checkpoints. Indeed, in mammalian cells ceramide is released from the membrane in response to irradiation (reviewed in Hannun, 1996).

Although the G1/S, S, and G2/M cell cycle arrests respond to different types of damage and arrest the cell at different stages, many of the same components are involved at all three arrests. What is common to the three arrest stages? One commonality is that many types of DNA damage are processed to single-stranded DNA (double strand breaks, excision of damaged bases, stalled replication forks, and mismatches), and single-stranded DNA appears to be the direct signal in bacteria (reviewed in Witkin, 1991), Xenopus (Kornbluth et al., 1992), human cells (Huang et al., 1996), and possibly S. cerevisiae (Garvik et al., 1995). Indeed, the checkpoint components may be directly involved in processing damaged DNA to its single-stranded form (Lydall and Weinert, 1995). Under this model, the distinct sensitivities of the cell to different types of damage at different stages would be related to the ability of the cell to process a particular type of lesion to single-stranded DNA at each stage. Indeed, the lack of sensitivity of S. cerevisiae cells in G1 to even one double strand break is correlated with their lack of processing the break until they enter S phase (Raghuraman et al., 1994).

How are DNA lesions allocated to different repair pathways? Epstasis experiments with radiation-sensitive mutants of S. cerevisiae indicate that different repair pathways can act on the same lesion. If this conclusion is true, is the allocation of lesion processing to different repair pathways simply an invariant outcome of their relative rates and efficiencies? Results with mutants of Cdc7p, a protein involved in the initiation of S phase in S. cerevisiae, suggests that the relative allocation can be reset by events occurring at initiation of replication. Some alleles of *CDC7* are hypomutable while other alleles are hypermutable in response to UV-irradiation (Hollingsworth et al., 1992). Presumably, these differences reflect differences in the allocation of lesions to a mutagenic repair pathway instead of a nonmutagenic pathway or a lethal event. Moreover, since these repair pathways may act at different stages in the cell cycle or replication process, the adaptation characteristics of the DNA damage checkpoint at different stages of the cycle should influence the allocation as well.

Checkpoint components are involved in processes other than signal transduction. Although the existence of a checkpoint is defined by loss-of-function mutations that alleviate arrest in response to damage, some components of the DNA damage checkpoint are essential. Some checkpoint genes are necessary for repair, transcription, and replication. Since the target of the DNA damage checkpoint is likely to be an essential component of the cell cycle machinery, the existence of some essential components is not surprising. For two essential components in S. cerevisiae, however, the deletion of a third gene that is itself nonessential and has little phenotypic consequence renders the two essential components nonessential (Paulovich et al., 1997; X. Zhao and R. Rothstein, personal communication), making this explanation unlikely to be general. Their roles in transcription, repair, and replication could be related to the need to scan the DNA for lesions, recognize lesions and process them to signal, and alter the replication machinery to replicate a damaged template, respectively.

What is the logic behind the efficiencies of arrest and repair, the rates of recovery from cell cycle arrest after DNA repair, and the rate of adaptation to cell cycle arrest or of apoptosis in the presence of unrepaired DNA? Although it is clear that there are vast differences in the behavior of the DNA damage response checkpoint to different types of damage, to different stages of cell cycle arrest, and in different tissues of metazoans, our current catalog of these differences is so meager, and our understanding of their consequences so incomplete, as to preclude speculation about these important issues at this time. Nevertheless, they deserve serious experimental investigation.

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References

Allen, J.B., Zhou, Z., Siede, W., Friedberg, E.C., and Elledge, S.J. (1994). The *SAD1/RAD53* protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev. *8*, 2401–2428.

Aloni-Grinstein, R., Schwartz, D., and Rotter, V. (1995). Accumulation of wild-type p53 protein upon γ-irradiation induces a G_2 arrest-dependent immunoglobulin κ light chain gene expression. EMBO J. *14*, 1392–1401.

Araki, H., Leem, S.-H., Phongdara, A., and Sugino, A. (1995). Dpb11, which interacts with DNA polymerase II(ϵ) in *Saccharomyces cerevisiae*, has a dual role in S-phase progression and at a cell cycle checkpoint. Proc. Natl. Acad. Sci. USA *92*, 11791–11795.

Cairns, J., and Davern, C.I. (1966). Effect of 32P decay upon DNA synthesis by a radiation-sensitive strain of *Escherichia coli*. J. Mol. Biol. *17*, 418–427.

Clarke, A.R., Purdie, C.A., Harrison, D.J., Morris, R.G., Bird, C.C., Hooper, M.L., and Wyllie, A.H. (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature *362*, 849–852.

Cross, S.M., Sanchez, C.A., Morgan, C.A., Schimke, M.K., Ramel, S., Idzerda, R.L., Raskind, W.H., and Reid, B.J. (1995). A p53-dependent mouse spindle checkpoint. Science *267*, 1353–1356.

Deng, C., Zhang, P., Harper, J.W., Elledge, S.J., and Leder, P. (1995). Mice lacking $p21^{CIP1/WAF1}$ undergo normal development, but are defective in G1 checkpoint control. Cell *82*, 675–684.

D'Urso, G., and Nurse, P. (1995). Checkpoints in the cell cycle of fission yeast. Curr. Opin. Genet. Dev. *5*, 12–16.

Elledge, S.J. (1996). Cell cycle checkpoints: preventing an identity crisis. Science *274*, 1664–1672.

Elledge, S.J., Zhou, Z., and Allen, J.B. (1992). Ribonucleotide reductase: regulation, regulation, regulation. Trends Biochem. Sci. *17*, 119–123.

Fan, S., Smith, M.L., Rivet, D.J., Duba, D., Zhan, Q., Kohn, K.W., Fornace, A.J., and O'Connor, P.M. (1995). Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res. *55*, 1649–1654.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995). DNA Repair and Mutagenesis (Washington, D.C.: ASM Press).

Garvik, B., Carson, M., and Hartwell, L. (1995). Single-stranded DNA arising at telomeres in *cdc13* mutants may constitute a specific signal for the *RAD9* checkpoint. Mol. Cell. Biol. *15*, 6128–6138.

Hannun, Y.A. (1996). Functions of ceramide in coordinating cellular responses to stress. Science *274*, 1855–1859.

Hartwell, L.H., and Kastan, M.B. (1994). Cell cycle control and cancer. Science *266*, 1821–1828.

Hartwell, L.H., and Weinert, T.A. (1989). Checkpoints: controls that ensure the order of cell cycle events. Science *246*, 629–634.

Hawn, M.T., Umar, A., Carethers, J.M., Marra, G., Kunkel, T.A., Boland, C.R., and Koi, M. (1995). Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. Cancer Res. *55*, 3721–3725

Hollingsworth, R.E., Ostroff, R.M., Klein, M.B., Niswander, L.A., and Sclafani, R.A. (1992). Molecular genetic studies of the Cdc7 protein kinase and induced mutagenesis in yeast. Genetics *132*, 53–62.

Hoyt, M.A., Totis, L., and Roberts, B.T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. Cell *66*, 507–517.

Huang, L.-C., Clarkin, K.C., and Wahl, G.M. (1996). Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G_1 arrest. Proc. Natl. Acad. Sci. USA *93*, 4827–4832.

Kadyk, L.C., and Hartwell, L.H. (1992). Sister Chromatids are preferred over homologs as substrates for recombinational repair in Saccharomyces cerevisiae. Genetics *132*, 387–402.

Kastan, M.B., Zhan, Q., el-Deiry, W.S., Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, A.J., Jr. (1992). A mammalian cell cycle checkpoint pathway utilizing p53 and *GADD45* is defective in ataxia-telangiectasia. Cell *71*, 587–597.

Kato, R., and Ogawa, H. (1994). An essential gene, *ESR1*, is required for mitotic cell growth, DNA repair and meiotic recombination in Saccharomyces cerevisiae. Nucleic Acids Res. *22*, 3104–3112.

Keil, R.L., and Roeder, G.S. (1984). *Cis*-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of S. cerevisiae. Cell *39*, 377–386.

Kiser, G.L., and Weinert, T.A. (1996). Distinct roles of yeast *MEC* and *RAD* checkpoint genes in transcriptional induction after DNA damage and implications for function. Mol. Biol. Cell *7*, 703–718.

Kornbluth, S., Smythe, C., and Newport, J.W. (1992). In vitro cell cycle arrest induced by using artificial DNA templates. Mol. Cell. Biol. *12*, 3216–3223.

Kunz, B.A., Kohalmi, S.E., Kunkel, T.A., Mathews, C.K., McIntosh, E.M., and Reidy, J.A. (1994). International Commission for Protection Against Environmental Mutagens and Carcinogens. Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. Mutation Res. *318*, 1–64.

Kuzminov, A. (1995a). Instability of inhibited replication forks in E. coli. BioEssays *17*, 733–741.

Kuzminov, A. (1995b). Collapse and repair of replication forks in Escherichia coli. Mol. Microbiol. *16*, 373–384.

Larner, J.M., Lee, H., and Hamlin, J.L. (1994). Radiation effects on DNA synthesis in a defined chromosomal replicon. Mol. Cell. Biol. *14*, 1901–1908.

Lauzé, E., Stoelcker, B., Luca, F.C., Weiss, E., Schutz, A.R., and Winey, M. (1995). Yeast spindle pole body duplication gene *MPS1* encodes an essential dual specificity protein kinase. EMBO J. *14*, 1655–1663.

Li, R., and Murray, A.W. (1991). Feedback control of mitosis in budding yeast. Cell *66*, 519–531.

Linke, S.P., Clarkin, K.C., Di-Leonardo, A., Tsou, A., and Wahl, G.M. (1996). A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev. *10*, 934–947.

Livingstone, L.R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T.D. (1992). Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell *70*, 923–935.

Longhese, M.P., Fraschini, R., Plevani, P., and Lucchini, G. (1996). Yeast pip3/mec3 mutants fail to delay entry into S phase and to slow DNA replication in response to DNA damage, and they define a functional link between Mec3 and DNA primase. Mol. Cell. Biol. *16*, 3235–3244.

Lowe, S.W., Schmitt, E.M., Smith, S.W., Osborne, B.A., and Jacks, T. (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature *362*, 847–849.

Lydall, D., and Weinert, T. (1995). Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. Science *270*, 1488–1491.

Ma, C., Martin, S., Trask, B., and Hamlin, J.L. (1993). Sister chromatid fusion initiates amplification of the dihydrofolate reductase gene in Chinese hamster cells. Genes Dev. *7*, 605–620.

Matsumoto, T., Fukui, K., Niwa, O., Sugawara, N., Szostak, J.W., and Yanagida, M. (1987). Identification of healed terminal DNA fragments in linear minichromosomes of Schizosaccharomyces pombe. Mol. Cell. Biol. *7*, 4424–4430.

McClintock, B. (1941). The stability of broken ends of chromosomes in *Zea mays*. Genetics *26*, 234–282.

Meyn, M.S., Strasfeld, L., and Allen, C. (1994). Testing the role of p53 in the expression of genetic instability and apoptosis in ataxia-telangiectasia. Int. J. Radiat. Biol. *66*, S141-S149.

Mummenbrauer, T., Janus, F., Müller, B., Wiesmüller, L., Deppert, W., and Grosse, F. (1996). p53 protein exhibits 3′-to-5′ exonuclease activity. Cell *85*, 1089–1099.

Murray, A.W. (1995). The genetics of cell cycle checkpoints. Curr. Opin. Genet. Dev. *5*, 5–11.

Naegeli, H. (1994). Roadblocks and detours during DNA replication: mechanisms of mutagenesis in mammalian cells. BioEssays *16*, 557–564.

Nash, R., Tokiwa, G., Anand, S., Erickson, K., and Futcher, A.B. (1988). The $WHI1^{+}$ gene of Saccharomyces cerevisiae tethers cell division to cell size and is a cyclin homolog. EMBO J. *7*, 4335–4346.

Navas, T.A., Zhou, Z., and Elledge, S.J. (1995). DNA polymerase ϵ links the DNA replication machinery to the S phase checkpoint. Cell *80*, 29–39.

Nelson, W.G., and Kastan, M.B. (1994). DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol. *14*, 1815–1823.

Ohtsubo, M., and Roberts, J.M. (1993). Cyclin-dependent regulation of G_1 in mammalian fibroblasts. Science *259*, 1908–1912.

Painter, R.B., and Young, B.R. (1980). Radiosensitivity in ataxia-telangiectasia: a new explanation. Proc. Natl. Acad. Sci. USA *77*, 7315–7317.

Paulovich, A.G., and Hartwell, L.H. (1995). A checkpoint regulates the rate of progression through S phase in S. cerevisiae in response to DNA damage. Cell *82*, 841–847.

Paulovich, A.G., Margulies, R.U., Garvik, B.M., and Hartwell, L.H. (1997). *RAD9*, *RAD17*, and *RAD24* are required for S phase regulation in S. cerevisiae in response to DNA damage. Genetics, in press.

Petes, T.D., and Newlon, C.S. (1974). Structure of DNA in DNA replication mutants of yeast. Nature *251*, 637–639.

Powell, S.N., DeFrank, J.S., Connell, P., Eogan, M., Preffer, F., Dombkowski, D., Tang, W., and Friend, S. (1995). Differential sensitivity of p53(−) and p53(+) cells to caffeine-induced radiosensitization and override of G2 delay. Cancer Res. *55*, 1643–1648.

Quelle, D.E., Ashmun, R.A., Shurtleff, S.A., Kato, J.Y., Bar-Sagi, D., Roussel, M.F., and Sherr, C.J. (1993). Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. Genes Dev. *7*, 1559–1571.

Raghuraman, M.K., Brewer, B.J., and Fangman, W.L. (1994). Activation of a yeast replication origin near a double-stranded DNA break. Genes Dev. *8*, 554–562.

Resnitzky, D., Gossen, M., Bujard, H., and Reed, S.I. (1994). Acceleration of the G1/S phase transition by expression of cyclins D1 and E with an inducible system. Mol. Cell. Biol. *14*, 1669–1679.

Russell, K.J., Wiens, L.W., Demers, G.W., Galloway, D.A., Plon, S.E., and Groudine, M. (1995). Abrogation of the G2 checkpoint results in differential radiosensitization of G1 checkpoint-deficient and G1 checkpoint-competent cells. Cancer Res. *55*, 1639–1642.

Roberts, B.T., Farr, K.A., and Hoyt, M.A. (1994). The Saccharomyces cerevisiae checkpoint gene *BUB1* encodes a novel protein kinase. Mol. Cell. Biol. *14*, 8282–8291.

Sandell, L.L., and Zakian, V.A. (1993). Loss of a yeast telomere: arrest, recovery, and chromosome loss. Cell *75*, 729–739.

Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D.A., Smith, S., Uziel, T., Sfez, S., et al. (1995). A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science *268*, 1749–1753.

Siede, W., Friedberg, A.S., and Friedberg, E.C. (1993). *RAD9*-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *90*, 7985–7989.

Siede, W., Friedberg, A.S., Dianova, I., and Friedberg, E.C. (1994). Characterization of G1 checkpoint control in the yeast Saccharomyces cerevisiae following exposure to DNA-damaging agents. Genetics *138*, 271–281.

Siede, W., Nusspaumer, G., Portillo, V., Rodriguez, R., and Friedberg, E.C. (1996). Cloning and characterization of *RAD17*, a gene controlling cell cycle responses to DNA damage in Saccharomyces cerevisiae. Nucleic Acids Res. *24*, 1669–1675.

Stewart, N., Hicks, G.G., Paraskevas, F., and Mowat, M. (1995). Evidence for a second cell cycle block at G2/M by p53. Oncogene *10*, 109–115.

Sugimoto, K., Shimomura, T., Hashimoto, K., Araki, H., Sugino, A., and Matsumoto, K. (1996). Rfc5, a small subunit of replication factor C complex, couples DNA replication and mitosis in budding yeast. Proc. Natl. Acad. Sci. USA *93*, 7048–7052.

Vallen, E.A., and Cross, F. (1995). Mutations in *RAD27* define a potential link between G1 cyclins and DNA replication. Mol. Cell. Biol. *15*, 4291–4302.

Voelkel-Meiman, K., Keil, R.L., and Roeder, G.S. (1987). Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell *48*, 1071–1079.

Weinert, T.A., and Hartwell, L.H. (1988). The *RAD9* gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. Science *241*, 317–322.

Weinert, T.A., and Hartwell, L.H. (1990). Characterization of *RAD9* of Saccharomyces cerevisiae and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. Mol. Cell. Biol. *10*, 6554–6564.

Weinert, T.A., and Hartwell, L.H. (1993). Cell cycle arrest of *cdc* mutants and specificity of the *RAD9* checkpoint. Genetics *134*, 63–80.

Weinert, T.A., Kiser, G.L., and Hartwell, L.H. (1994). Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. *8*, 652–655.

Weiss, E., and Winey, M. (1996). The Saccharomyces cerevisiae spindle pole body duplication gene *MPS1* is part of a mitotic checkpoint. J. Cell Biol. *132*, 111–123.

Wilkie, A.O.M., Lamb, J., Harris, P.C., Finney, R.D., and Higgs, D.R. (1990). A truncated human chromosome 16 associated with α thalassaemia is stabilized by addition of telomeric repeat $(TTAGGG)_n$. Nature *346*, 868–871.

Winey, M., Goetsch, L., Baum, P., and Byers, B. (1991). *MPS1* and *MPS2*: novel yeast genes defining distinct steps of spindle pole body duplication. J. Cell Biol. *114*, 745–754.

Witkin, E.M. (1991). RecA protein in the SOS response: milestones and mysteries. Biochimie *73*, 133–141.

Wyllie, F.S., Haughton, M.F., Bond, J.A., Rowson, J.M., Jones, C.J., and Wynford-Thomas, D. (1996). S phase cell-cycle arrest following DNA damage is independent of the p53/p21(WAF1) signaling pathway. Oncogene *12*, 1077–1082.

Yamamoto, A., Guacci, V., and Koshland, D. (1996). Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. J. Cell Biol. *133*, 85–97.

Yarbro, J.W. (1992). Mechanism of action of hydroxyurea. Semin. Oncol. *19(3)*, 1–10.

Yin, Y., Tainsky, M.A., Bischoff, F.Z., Strong, L.C., and Wahl, G.M. (1992). Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell *70*, 937–948.

Zampetti-Bosseler, F., and Scott, D. (1981) Cell death, chromosome damage and mitotic delay in normal human, ataxia telangiectasia and retinoblastoma fibroblasts after x-irradiation. Int. J. Radiat. Biol. *39*, 547–558.

Zheng, P., Fay, D.S., Burton, J., Xiao, H., Pinkham, J.L., and Stern, D.F. (1993). *SPK1* is an essential S-phase-specific gene of Saccharomyces cerevisiae that encodes a nuclear serine/threonine/tyrosine kinase. Mol. Cell. Biol. *13*, 5829–5842.

Zhou, P., Jiang, W., Weghorst, C.M., and Weinstein, I.B. (1996). Overexpression of cyclin D1 enhances gene amplification. Cancer Res. *56*, 36–39.