Redundancy, insult-specific sensors and thresholds: unlocking the S-phase checkpoint response
Jennifer A Cobb, Kenji Shimada and Susan M Gasser

DNA damage that is not properly repaired during genomic replication is a major source of gross chromosomal rearrangements and sequence loss during cell proliferation. In higher eukaryotes such mutations increase the risk of cancer. Eukaryotic cells have multiple checkpoint responses activated by DNA damage and stalled replication forks. We focus here on fork-associated events that activate and respond to S-phase checkpoint kinases.

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Abbreviations
Cdk cyclin-dependent kinase
ChIP chromatin immunoprecipitation
DSB double-strand break
FHA forkhead homology-associated
HU hydroxyurea
MMS methylmethane sulfonate
RDS damage-resistant DNA synthesis
RPA replication protein A
Sc Saccharomyces cerevisiae
Sp Schizosaccharomyces pombe
ssDNA single-stranded DNA

Introduction: DNA replication and checkpoints
The DNA damage checkpoint response can be broken down into three basic steps: first, a sensing (which often entails processing) of the DNA lesion; second, amplifying the signal to elicit a response; and third, a cellular reaction to the signal transmitted through an ‘effector’ kinase, which generally phosphorulates target proteins to slow cell-cycle progression, stabilize fork structure, and activate repair [1]. Defining the proteins involved in the S-phase checkpoint response has been particularly challenging because different insults provoke activation of the kinase cascade through different pathways, and a single insult can activate parallel pathways. The complexity of the S-phase checkpoint is further compounded by the fact that components of the replisome themselves are implicated at all three levels of the checkpoint response [2]. For example, replication forks participate in checkpoint activation: DNA polymerase ε (pol ε) and its associated protein Dpb11 (TopBP1 or Cut5 in humans or fission yeast, respectively; see Table 1) act upstream to signal kinase activation, as does the ssDNA binding protein RPA. Yet DNA polymerases and RPA are also downstream targets for phosphorylation by checkpoint kinases, which ensure proper resumption of DNA synthesis. Finally, adding a new twist to the story, recent data suggests that the S-phase checkpoint requires a threshold level of damage prior to activating the checkpoint signal, allowing the cell to ‘tolerate’ a certain level of fork-associated ssDNA without provoking cell-cycle arrest [3]. Later in the cell cycle, this ‘tolerance’ appears to be lost, and the persistence of unique strand breaks or replication forks in G2 phase prevents progression into mitosis.

In this review, we focus on new components of the S-phase checkpoint response, which help explain how fork-associated damage and stalled forks produce a checkpoint-activating signal. The signal is then amplified through fork-associated adaptors, often containing BRCT domains. This ultimately stabilizes the replisome and prevents mitosis. Results obtained with genetically compromised budding and fission yeast strains have been instrumental in sorting out redundant pathways, and have often proven to be excellent paradigms for mammalian cells.

Sensing damage in S phase
The ‘sensor’ kinases in the S-phase checkpoint signaling cascade are the ATM/ATR kinases (ataxia-telangiectasia mutated-and Rad3 related), called ScTel1/Mec1 or SpTel1/Rad3 in budding and fission yeast, respectively (see Table 1). These are necessary for the activation of the Chk2 ‘effector’ kinase (termed ScRad53 or SpCds1) in response to DNA damage during S-phase. A second effector kinase, Chk1, responds primarily to processed breaks and is important for the arrest of progression into mitosis, but not for of replication fork stability [1]. An S-phase-specific checkpoint response is activated when replication forks stall as a result of nucleotide depletion (i.e. elevated concentrations of hydroxyurea [HU]), or when they collide with lesions in DNA treated with alkylating agents, such as methylmethane sulfonate (MMS). In budding yeast, the detection of MMS-induced damage requires replication, as its damage is ‘revealed’
only when moving forks encounter modified nucleotides [4**]. This agent is therefore specific for inducing fork-localized damage, in contrast to other agents, such as phleomycin, bleomycin or γ irradiation, which engender modifications and double-strand breaks (DSBs) in non-replicating DNA, as well as at replication forks. The superposition of DSB-induced and fork-associated signaling pathways may be responsible for the complexity of the mammalian checkpoint response to ionizing radiation [5*].

In the case of both irradiation- and MMS-induced lesions, damage-resistant DNA synthesis (RDS) occurs in checkpoint-deficient cells. Recent work in yeast suggests that rather than a reduced fork rate, the checkpoint-mediated drop in DNA synthesis reflects a delay in the firing of late origins of replication [4**,6]. This slows the completion of S phase indirectly, providing a longer period of time for repair and the completion of genomic replication. A recent extension of this study suggests that although late-origin inhibition extends S phase, it is the preservation of fork integrity that is critical for cell viability [4**]. Specifically, it was shown that the role of ScMec1 in suppressing origin firing in the presence of MMS could be genetically separated from its role in preventing fork collapse [4**]. Thus, the suppression of origin activation and fork stabilization are independent functions of the ScMec1 kinase. Moreover, the stabilization of existing forks was shown to be the more crucial event for cell survival. This separation of function was shown using the mec1-100 allele, which has a significantly delayed and reduced level of ScRad53 activation in response to both HU and MMS [7]. One can conclude, therefore, that the role of ScRad53 kinase is also distinct from that of ScMec1 in the maintenance of fork stability. These results underscore the importance of using specific assays, and not simply cell survival, to monitor the checkpoint response.

The biochemical characterization of replication forks suggests that extended ssDNA regions are produced during fork stalling and contribute to checkpoint activation. Indeed, kinase recruitment to an ssDNA–RPA complex could itself be the critical event that triggers the checkpoint signaling cascade (Figure 1). This is supported by recent microscopy and chromatin immunoprecipitation (ChIP) experiments showing a recruitment of ScMec1–Ddc2 to the replication fork shortly after treatment with genotoxic agents [4**,8**,9**].

<table>
<thead>
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<th>Table 1: Conserved damage and replication checkpoint genes.</th>
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This table summarizes the most universal S phase checkpoint factors, presented with their names in budding yeast, fission yeast and mammalian/vertebrate systems, based on work from many laboratories. In the main text, reference to species-specific proteins is designated by the prefix ‘Sc’ or ‘Sp’ for budding and fission yeasts, respectively. PCNA, proliferating cell nuclear antigen; RFC, replication factor C; PIK, phosphoinositol-3-kinase; FHA, fork-head-associated.
During unperturbed replication, RPA binds both leading and lagging strands, yet the recruitment of ScMec1–Ddc2 is not observed [8**,9**]. It is proposed that only replication forks undergoing unusual or prolonged stress, possibly accumulating abnormally long stretches of ssDNA-bound RPA, become sites of ScMec1–Ddc2 recruitment (Figure 1). Support for this comes from the study of a point mutation in the largest RPA subunit, Rfa1. The Scrfal–t11 mutant is proficient for replication but is partially defective for the checkpoint response to the stretches of ssDNA that accumulate at telomeres in a cdc13 mutant [15]. Cells carrying this allele are defective for recruiting ScDdc2 to ssDNA in response to a DSB [11**] and are partially compromised for ScDdc2 recruitment to replication forks on HU [16**]. This correlates with the reduced activation of ScRad53 in response to HU, and may reflect the inability of rfa1–t11 to load or promote the stable binding of ScMec1–Ddc2 at stalled forks. In a variant of this model based on two thermosensitive mecl alleles (Scmecl–4 and –40), the ScMec1–Ddc2 complex is proposed to stabilize forks in chromosomal zones through which the replication fork proceeds slowly, even in the absence of external insult [17].

**S-phase-specific responses**

As ssDNA and RPA are also implicated in the activation of the checkpoint response at processed DSBs [18], it remains unclear how the sensing of ssDNA at a replication fork differs from a G2-specific DSB response. Two explanations seem likely. The first involves cell-cycle-specific modifications of components in the checkpoint signaling pathway. The second invokes the physical proximity of replication fork enzymes as modifiers of the response. With respect to the former, not only damage-dependent, but cell-cycle-specific modifications are likely to influence the activity of adaptor proteins such as ScRad9, ScMrc1 or RFA itself [11**]. This most likely affects proteins involved in the amplification of the signal, and often involves proteins containing the conserved BRCT domain, such as ScRad9, TopBP1 (SpCut5 or ScDpb11), and the BRCA1 partner, BACH1 [19**,20**,21]. The BRCT domains of BRCA1 and TopBP1 were recently shown to be phospho-specific domain recognition motifs, whose specificity varies among the different BRCT-domain-containing proteins. The BRCA1 partner, BACH1, for example, undergoes a cell-cycle specific modification in G2 (probably by a Cdk), which is necessary for the interaction [19**]. In other cases, the recognition motif may be an ATM/ATR target site [20**]. These studies and the documented presence of ScDpb11 (TopBP1) at replication forks [21], suggest that the modification of BRCT domain-containing proteins contributes to the cell-cycle specificity of the checkpoint response.

The second argument is based on the fact that the Mre11 complex — MRX or MRN, for ScMre11–Rad50–Xrs2, or Mre11–Rad50–Nbs1 in mammalian cells — is implicated...
in DSB processing in both S and G2 phases, although it leads to the activation of ScTel1 and ScChk1 in G2 phase [22*,23**,24] and not ScRad53 (CHK2 or SpCds1), which is stimulated during the S-phase response. The MRX–Tel1 signal is activated in parallel to the ScMec1 (ATR) response to ssDNA [24] (Figure 2). Similar parallel pathways appear to function in *Xenopus* extracts [14**]. In fission yeast, an unbranched checkpoint response to MMS-induced fork-associated damage was shown to require SpRad3 and SpCds1, but not MRX or SpTel1 [25**]. This is consistent with an MRX-independent checkpoint pathway being stimulated by fork-associated damage in *Saccharomyces cerevisiae*. It is striking that SpMre11 is not required for the response to fork arrest by HU, and that MMS does not provoke the Tel1-activated modification of either Mre11 or Xrs2 in budding yeast [23**,24,25**]. This allows us to differentiate fork-associated damage that activates through the ScMec1–Mrc1–Dpb11–Rad53 pathway from non-fork-associated DSBs or damage that cannot be repaired by events at the replication fork. These latter appear to be processed by MRN (MRX) and to activate the checkpoint through Tel1 (ATM; see Figure 2). In mammalian cells the MRN complex acts through a BRCT domain-containing protein called Mdc1 to suppress RDS in response to irradiation in S phase [26*,27*]. The MRN–Mdc1 interaction is apparently needed for the recruitment of Mre11 to repair foci. We propose that the close proximity of checkpoint adaptors such as ScMrc1 and ScTof1 at the replication fork, or the rapid and efficient repair by either translesion synthesis or nonreciprocal recombination pathways, may disfavor use of the MRN/MRX pathway for fork-associated damage.

**Novel ‘adaptors’ are fork-associated in S phase**

An increasing number of S-phase-specific checkpoint proteins have been shown to be integral components of the replisome, being present both at stalled and normal replication forks [8**,9**,10*,16]. Two proteins, ScTof1 or SpSwi1 [28,29] and Sc/SpMrc1 [8**,30,31] have recently been shown to be fork-associated and are contenders for the role of the fork specific ‘adaptor’ or ‘amplifier’ in the checkpoint signaling cascade. In both budding and fission yeasts, Mrc1 is required for the activation of ScRad53 and ScCds1 during replication stress [30,31], and SpMrc1 physically interacts with SpCds1 in yeast two-hybrid studies [31]. A model has been proposed in which phosphorylation of ScMrc1 by ScMec1–Ddc2, which is recruited to abnormal stalled

![Figure 2](image)

**Figure 2**

A model for checkpoint activation in response to a DSB. (a) The induction of a DSB is followed by the recruitment of the MRX (*S. cerevisiae*) or (b) MRN (mammalian) complex that processes the break. This may be a transient or reversible step, and it is not known whether MDC1 is required here or only at a later step in the formation of mammalian repair foci. At this point, the full checkpoint response is silent. Extended ssDNA (dashed line), which is generated by MRX/N nuclease activity, may be required to trigger a global checkpoint response. (c) In *S. cerevisiae*, the binding of two sensor kinases Tel1 and Mec1–Ddc2 and subsequent MRX phosphorylation by Tel1 leads to the activation of effector kinases Rad53 and Chk1 through the Rad9 mediator. In mammalian cells, ssDNA generation and/or chromatin modification appears to convert ATM from an inactive to an active form (monomer). The active ATM phosphorylates H2AX (*H2AX*, or histone H2A in yeast) early in the response. The formation of repair foci requires MDC1 and is followed by the recruitment of other mediators 53BP1 and BRCA1. Although the molecular nature of focus formation is not known, the stable assembly of these factors near a DSB coincides with checkpoint activation and recruitment of repair proteins.
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forks, in turn recruits and activates ScRad53 [8**] (Figure 1). Indeed, in fission yeast, SpMrc1 is phosphorylated by SpRad3 in response to replicational stress, and mutation of its potential SpRad3/Tei1 target sites strongly impairs both SpMrc1 modification and its interaction with SpCds1 [32**]. In budding yeast, the mutation of the potential ScMec1 S/TQ phosphorylation sites compromises its ability to activate ScRad53 in response to replicational stress [8**]. Thus, mrc1 mutants have checkpoint defects similar to ScRad53 and SpCds1 mutants, resulting in increased sensitivity to HU [8**,32**].

In addition, mrc1 cells exhibit a slow and damage-inducing S-phase [30] that can not be suppressed by upregulating RNR1. This suggests a role for ScMrc1 in replication that might be independent of ScRad53 activation [8**]. Consistently, ScMrc1 is loaded in a Cdc45-dependent manner at normal replication forks, shortly after origin firing [8**,9**]. The reduced level of checkpoint activation in strains with reduced origin activity (e.g. orc2-1) may thus reflect a reduced presence of the ScMrc1-mediated signal [3*,4**]. Importantly, when all ScMec1-phosphorylation consensus sites are mutated in ScMrc1, cells progress normally through an unperturbed S phase despite an inability to mount a checkpoint response [8**]. This confirms that ScMrc1 has two distinct functions: one that ensures fork progression and a second that ensures the checkpoint response.

The second S-phase-specific ‘adaptor’ candidate for checkpoint signalling is ScTof1 or SpSwi1. ScTof1 was identified in a screen for genes that have a synergistic effect with ScRad9, showing increased sensitivity to DNA-damaging drugs [28]. The contribution of ScTof1 to ScRad53 activation appears to be restricted to S-phase and does not contribute to UV-induced transcription of RNR3 during G1 or to the cdc13-1 induced block to anaphase in G2/M [28]. ScTof1, like ScMrc1, is present at replication forks during normal S-phase progression, and remains associated with the fork through HU challenge [9**]. Both ScMrc1 and ScTof1 correlate with replication fork movement, a process that is Mec1/Tei1-independent [9**]. In fission yeast, SpSwi1 is essential for the proficient activation of SpCds1, and is necessary to prevent fork collapse in the rDNA and the newly identified programmed fork pausing site that is necessary for mating type switching [29**]. Limiting levels of SpSwi1 or ScTof1 may also be responsible for the threshold needed for Rad53 activation in S-phase [37**].

During a checkpoint response, only extremely low levels of the ‘effector’ kinase ScRad53 have been detected at replication sites [9**]. Clearly, however, ScRad53 activation does protect stalled forks from pathological rearrangements that produce extensive single-stranded gaps, hemi-replicated intermediates, and the accumulation of Holliday junctions through fork reversal [33]. Moreover, irreparable structures appear to form in rad53 mutants that result in fork collapse [4**,**6,34]. It is not known if ScRad53 is a ‘hit and run’ enzyme or is simply insensitive to formaldehyde fixation. In a strain bearing a mutation that eliminates kinase activity (Scrad53-K227A) the association of DNA polymerases α and ε with stalled forks was reduced, yet RFA1 and ScDdc2 accumulate at these same sites [16*]. This suggests that the absence of ScRad53 kinase activity contributes to a progressive degeneration of stalled fork structures. It is possible, on the other hand, that polymerase destabilization under these conditions reflects the low dNTP levels in this strain. Indeed, in a rad53 null allele bearing an sml1 deletion — which leads to ribonucleotide reductase upregulation — did not reduce polymerase recovery at stalled replication forks [10*]. Intriguingly, recent data suggests that the two FHA domains of ScRad53 are involved differentially in the DNA damage and DNA replication checkpoint responses [35**]. Whereas FHA2 is required in vivo for ScRad9-dependent Rad53 phosphorylation and activation in G2 phase, mutation of FHA1 specifically compromised the response to HU arrest [35**]. It is of major interest to identify the relevant ligand for the ScRad53 FHA1 domain, and to determine whether this interaction helps recruit ScRad53 to forks, or may be responsible for the high threshold of damage required for checkpoint activation in S-phase cells [37**].

DNA replication and the S/M checkpoint

When DNA replication is incomplete, an S/M checkpoint also prevents the onset of mitosis by maintaining sister chromatid cohesion (SCC) and preventing spindle formation. Budding yeast has the unique characteristic that intranuclear microtubules begin elongating throughout G2 phase in preparation for mitosis. This explains why budding yeast cells, and not other species, downregulate spindle elongation in response to the S-phase checkpoint.

Recent work suggests that checkpoint control over spindle formation consists of dual pathways: an ScMec1–ScPds1 branch that is necessary in early S-phase, and an ScMec1–ScPds1 branch that is essential in late S phase [36] (Figure 1c). ScPds1 is also called Securin or Cut2 in humans and fission yeast, respectively.

The ScMec1p–ScRad53-dependent pathway suppresses the premature accumulation of the anaphase promoting complex (APC) specificity factor, ScCdc20 [37**]. This study shows that ScCdc20 can act independently of the APC and that one of its downstream targets may be ScAsk1, a protein originally identified in a genetic screen aimed at finding new S-phase checkpoint genes that have phenotypes similar to rad53 mutants [38]. ScAsk1 is both a target of the cyclin-dependent kinase Cdc28 and is a component of the DASH complex. DASH resides at kinetochores and is necessary for the interaction between the kinetochore and spindle microtubules [39]. The
Coordinating replication and mitosis

As the checkpoint functions of ScMec1 and ScRad53 are not essential, and because ScPds1 is dispensable for normal cell growth, we can assume that these factors do not regulate entry into mitosis during an unchallenged cell cycle. To prevent mitotic entry, a eukaryotic cell may not regulate entry into mitosis during an unchallenged cell cycle. To prevent mitotic entry, a eukaryotic cell may.

It is not clear whether this role for ScPds1 is specific to budding yeast. In cells ranging from humans to fission yeast, the S/M checkpoint response appears to activate Chk1 through ATR to prevent entry into mitosis [1**]. This is achieved at least in part by inhibiting cyclin/Cdk1 activation. A role for the Securin (Pds1) has not yet been demonstrated. Nonetheless, after ionizing irradiation, human ATM phosphorylates Smc1, another essential subunit of the cohesin complex, and site-directed mutation of Smc1 acceptor serines (aa957 and aa966) compromises the intra-S-phase checkpoint [44]. Interestingly, these same sites become modified in an ATM-independent manner after UV irradiation or HU treatment. The other PI3-like kinase, ATR, seems to be an attractive candidate effector for this modification.

Conclusions

In summary, research in various systems has begun to clarify sensors and signaling partners that mediate the checkpoint response in S phase of the cell cycle. One can distinguish pathways that respond to fork-associated damage induced by MMS, by HU-arrested forks, and by DSBs. Cyclin and Orc2 mutants have been used to show that a higher threshold level of damage is tolerated in S phase for checkpoint activation. Studies reviewed here have implicated ssDNA and RPA in signaling activation of the checkpoint, however, it remains unclear how the sensing of ssDNA at a replication fork during S phase differs from the RPA–ssDNA complex formed at a processed DSB in G2. Moreover, it is unclear what distinguishes an aberrant fork from normal replication structures. Progress has been made on the identification of components that propagate the signal. The recent characterization of two ‘adapter’ proteins, Sc/SpMrc1 and ScTof1 or SpSwi1, which are components of the replisome, has been instrumental in defining a distinctive S-phase signaling pathway to checkpoint activation. Future studies that would help clarify details of the S-phase checkpoint will include a further characterization of separation of function mutants in these proteins. This should provide insight into their molecular mechanisms, discriminating essential roles in cell viability from nonessential roles that preserve genomic integrity. Potential sensors and signals for completed replication, which may or may not involve correct sister chromatid alignment, provide still-fertile grounds for research.
Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


The best summary of DNA damage checkpoints and checkpoint-associated factors to date, with a strong focus on yeast.


Mutations in Orc2 lowers initiation efficiency and compromises the S phase checkpoint response to HU, MMS, and bleomycin. This can be reversed by restoring efficient pre-replication complex formation, and suggests the existence of a threshold for S phase checkpoint activation. Orc2 is not otherwise needed for the S-phase checkpoint response.


Authors examine the relative importance of downstream checkpoint effectors on cell viability and suggest that preventing the breakdown of stalled replication forks is essential for cell viability. The MMS-induced checkpoint response shown to depend on fork movement.


Irradiated mammalian cells are analyzed for RDS in various compromised backgrounds. The pathways that prevent RDS are bifurcated, one requiring MRN processing while the other acts through the Chk2-Cdc25A-Cdc2pathway. To mimic loss of ATM, one must eliminate both pathways. This may reflect activation by both DSB and by fork-related events.


This paper shows that Mrc1p binds to early replicating sequences and moves with the replication fork. During an HU block, Mrc1p remains stably bound at sites of stalled replication. Mutations of 18 putative Mec1p/ATR acceptor sites on Mrc1 prevents its modification in response to HU, and precludes Rad53p activation, suggesting that Mec1 target sites activate Mrc1 mediator function. This function is genetically separable from the role of Mrc1 in normal fork progression.


The authors perform a chromatin immunoprecipitation on microarray of budding yeast Chr VI with 300 bp resolution (ChIP on Chip) on cells in S-phase. They observe that the checkpoint proteins Mrc1p and Top1p move together with Cdc45p suggesting direct interaction with the replication machinery. They also show that these proteins are important for maintaining a tight coupling between the replication machinery and sites of DNA synthesis during HU block.


Budding yeast DNA pol α and ε are shown to be stably bound for 40–60 min at HU-arrested replication forks in a manner dependent on Mec1p and the RecQ helicase Sgs1p. Deletion of RAD53 in an sml1 background does not destabilize polymerases like the lack of Mec1 activity.


The authors of this paper show that replication protein A (RPA) is required for the recruitment of ATR to sites of DNA damage, a process mediated through ATRIP. They also show in S. cerevisiae that Ddc2p, a homologue of ATRIP, is recruited to the DSB in an RPA-dependent manner.


Single-strand DNA gaps are shown to activate an S phase DNA-damage checkpoint that blocks the initiation of DNA replication. The pathway requires RPA and ATR, but not ATM, and leads to an inhibition of Cdc7/Dbf4 protein kinase activity.


ChiP data suggest that RFA and Ddc2/Dcc1 checkpointing increases at stalled replication forks in a strain lacking rad53. Polymerases are partially destabilized under the same conditions, possibly because of lowered dNTP concentrations.


These authors identify tandem BRCT domains (in BRCA1 and PTIP) as substrates phosphorylated by ATM or ATR kinases, in response to DNA damage. The BRCT domain directly interacts with phosphorylated BRCA1-associated carboxyl-terminal helicase (BACH1), a modification which is cell cycle regulated and is required for DNA-damage-induced G2 to M phase checkpoint arrest. Other BRCT domains also bind phosphorylated peptides.


See annotation [23-24].
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   and Cds1, and depends on Cut5 (Dbp11/TopBP1) and two helicases Srs2
   and Rq1 (Srs2 and Sgs1 or BLM). However, the pathway does not
   require Tel1/ATM, Rhp5/Rad9, Rad50, Rad32 (Mre11) or any other protein
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demonstrate that cells with reduced levels of MDC1 accumulate
   S-phase DSBs and have increased levels of H2AX. They go on to
   show that the Rad53-dependent checkpoint activity, only one is needed to activate Rad53 in response to HU. The
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The authors perform a synthetic lethal screen on microarrays with a ctf4 deletion strain to identify additional cohesion components. Results from their screen show that a subset of genes important for sister chromatid cohesion are involved in the S-phase checkpoint and DNA replication and repair. Specific genes include subunits of the MRX complex, SRS2, SGS1, MRC1, TOF1, RAD53, and RAD52.


This paper reports on a synthetic genetic array analysis using a ctf8 deletion strain to identify non-essential genes required for SCC. They biochemically analyze some of the products of their candidate genes using immunoprecipitation followed by mass spectrometry and show that complexes are formed in vivo with proteins involved both in mitotic spindle positioning and sister chromatid cohesion. The protein Tof1 was detected in their screen and suggests a functional link between checkpoint pathways, DNA replication, and cohesion.


Here it is shown that the Dbf4-dependent kinase (fission yeast Hsk1/Dfp1) interacts and phosphorylates SpSwi6 (HP1 in higher organisms) to promote heterochromatin formation and sister chromatid cohesion specifically at centromeres. This is a novel function for this kinase.


See annotations [26,27].