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HYPOTHESIS

RecQ-like helicases: the DNA replication checkpoint connection

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SUMMARY

The eukaryotic homologues of the *Escherichia coli* RecQ DNA helicase play conserved roles in the maintenance of genome stability. Results obtained in yeast and mammalian systems are beginning to form a coherent picture about what these helicases do to ensure normal cell division and why humans who lack these enzymes are cancer prone. Recent data suggest that the yeast enzyme Sgs1p, as well as two human homologues, which are encoded by the Bloom's and Werner's syndrome genes, function during DNA replication and possibly in a replication checkpoint specifi to S phase.

Key words: Sgs1p, Helicase, Checkpoint, S-phase, DNA replication, Bloom's syndrome, Werner's syndrome

INTRODUCTION

The RecQ family of helicases includes the *Saccharomyces cerevisiae* gene *SGS1* (Gangloff et al., 1994; Watt et al., 1995), the *Schizosaccharomyces pombe* gene *rqh1*$^+$ (Stewart et al., 1997), and at least fi e human genes, of which three are implicated in heritable diseases: *BLM* is mutated in Bloom's syndrome (BS; Ellis et al., 1995), *WRN* in Werner's syndrome (WS; Yu et al., 1996; Imbert et al., 1996) and *RecQL4* in the Rothmund-Thomson syndrome (RTS; Kitao et al., 1999a,b). These disease-related genes encode DNA helicases with extensive homology; yet each syndrome has distinct, characteristic features. Patients who are homozygous for mutations in *BLM* have increased rates of chromosomal rearrangement, chromosome breakage, sister chromatid exchange and recombination, which together result in a pronounced predisposition to cancer (reviewed by German, 1993). Disruption of the murine gene causes embryonic lethality (Chester et al., 1998), and fibroblasts from these embryos have defects in chromosome stability like those described for cultured cells from BS patients, which suggests a conservation of function among mammals. Mutations in the closely related *WRN* helicase correlate with phenotypes of premature aging – such as greying hair, scleroderma-like skin, osteoporosis, diabetes mellitus and cancer – before the age of 40 (reviewed by Epstein et al., 1966). Consistently, cultured fibroblasts from *wrn*–/– mice also show premature cellular senescence (Lebel and Leder, 1998). Finally, RTS patients are characterized by abnormalities in skin and skeleton, as well as both premature aging and a predisposition to neoplasia (Houwing et al., 1991; Kitao et al., 1999a,b).

Although yeast cells lacking either *SGS1* (*S. cerevisiae*) or *rqh1*$^+$ (*S. pombe*) are viable, the mutant strains have phenotypes resembling those described for the human syndromes. Inactivation of Sgs1p results in an increase in DNA recombination, enhanced chromosome missegregation and a shortened life-span (i.e. the mean number of cell divisions of the yeast cell; Watt et al., 1996; Sinclair et al., 1997), while rqh1p deficien y correlates with a hypersensitivity to DNA damage (Stewart et al., 1997). Many authors have speculated on the events that underlie these phenotypes, and yet the precise cellular function of the eukaryotic RecQ-like helicases has remained obscure. Below we summarize results from a number of studies that support the hypothesis that these helicases function in genomic DNA replication and perhaps, more specificall , in an S-phase-specific checkpoint that responds to perturbations in replication fork movement.

FUNCTIONS DURING S-PHASE OF THE CELL CYCLE

It has long been noted that cultured cells from BS and WS patients manifest defects in S phase of the cell cycle. Cells lacking *BLM* have an abnormal profile of DNA replication intermediates (Lonn et al., 1990) and retarded DNA-chain growth (Hand and German, 1975), while WS cells were impaired in S-phase progression (Poot et al., 1992). In yeast, Cho et al. (1998) recently showed that the levels of *SGS1* transcripts peak in S phase, *SGS1* expression apparently being controlled by two SCB (Swi4p-Swi6p cell cycle box) elements that are typically found in the promoters of genes whose products are required for late G_1 or S phase progression. More striking, however, is the sharp fluctuation in the levels of Sgs1 protein: Sgs1p is abundant in S phase, but is barely detectable as cells traverse metaphase and early G_1 (Frei and Gasser,

2000). In S-phase yeast nuclei, the protein has a focal distribution that overlaps significantly with sites of de novo DNA synthesis and with ORC, a six-protein complex essential for initiation of DNA replication (Frei and Gasser, 2000). Consistent with this is Lebel and colleagues' demonstration that the Werner's helicase co-fractionates on sucrose gradients with replication proteins and co-precipitates with PCNA, a processivity factor for polymerase δ in DNA replication and repair events (Lebel et al., 1999). Other workers find that WRN protein interacts with replication protein A (RPA; Brosh et al., 1999) and immunolocalizes in discrete foci throughout the nucleoplasm of mammalian cells (Shiratori et al., 1999). Recently WRN was shown to stimulate DNA polymerase δ activity in vitro, albeit in a manner independent of the WRN helicase function (Kamath-Loeb et al., 2000).

More direct evidence suggesting a role for Sgs1p in DNA replication is provided by budding yeast genetics: the combination of an *sgs1* mutation with loss of another helicase, *srs2*, is lethal, although each single mutant grows with wild-type kinetics (Lee et al., 1999). Importantly, when a temperature-sensitive *sgs1* mutation is present in an *srs2* background, the cells are unable to replicate DNA at restrictive temperatures, whereas cells bearing either single mutation can. This indicates that the two helicases provide a redundant, but essential, activity for DNA replication. The efficiency of rDNA transcription also drops significantly in the double mutant (Lee et al., 1999), which suggests that Sgs1p and Srs2p serve double duty, aiding RNA polymerase I as well as one or more DNA polymerases, in a redundant fashion. We consider it likely that the defects in replication account for most of the chromosome missegregation phenotypes that characterize cells lacking Sgs1p. As described for *E. coli* (Cox et al., 2000), the failure to resolve aberrant DNA structures that form during replication fork arrest can influence chromosome segregation and/or induce unusual recombination events. In summary, these genetic studies, coupled with immunolocalization and fractionation results, suggest that Sgs1p and other RecQ-like helicases are either integral components of the DNA replication machinery or are recruited to replication forks that stall when damaged DNA or other impediments block elongation (Rothstein et al., 2000).

Could the redundant S-phase function of Sgs1p and Srs2p be that of a replication fork helicase? The best candidate for this function is the minichromosome maintenance (MCM) complex, a hexamer that has a weak but detectable helicase activity in vitro (Yu et al., 1999). MCM proteins associate with the pre-replication complex in late G_1 (Tanaka et al., 1997) and appear to move with the replication fork (Aparicio et al., 1997; reviewed by Donaldson and Blow, 1999). Elimination of any one of the six genes encoding MCM subunits is lethal in yeast, and the temperature-sensitive *mcm* mutations generally lead to arrest in G_2. This could be due either to redundancy with another helicase activity or to the nature of the temperature-sensitive defects. To check the former possibility, we combined an *sgs1* deletion with temperature-sensitive alleles of MCM components, but observed no synthetic lethality (Frei and Gasser, 2000). Similarly, the potential redundancy with helicases involved in lagging strand synthesis was examined in a strain in which *sgs1* was disrupted in a *dna2* temperature-sensitive background (Budd and Campbell, 1997). The double mutant showed only a slight drop in viability at intermediate temperatures (C. Frei and S. M. Gasser, unpublished observations), making it unlikely that Sgs1p is redundant with a lagging-strand helicase. Because replication helicases might be multiply redundant, direct crosslinking data will probably be necessary if we are to determine whether or not Sgs1p and/or Srs2p function at the replication fork.

Another possibility is that Sgs1p helps resolve the strand invasion events that occur at replication forks when template strands break or when obstacles impede polymerase progression. Data from *E. coli* show that stalled replication forks are unstable and that strand breakage often occurs, presumably because of the fragility of unpaired DNA stretches (Cox et al., 2000). A strand invasion event involving a single-stranded DNA end at a broken replication fork could allow reinitiation and permit replication to continue (Chakraverty and Hickson, 1999). Interestingly, *E. coli* RecQ contains the enzymatic activities required for this process (D-loop formation and binding to crossover structures; Harmon and Kowalczykowski, 1998). Moreover, the association of Sgs1p with type I and type II topoisomerases could provide enzymatic activity required for resolving these strand invasion events or even for separating double helical catenanes (Wu et al., 1999). Consistent with this model is the observation that both extrachromosomal rDNA circles and broken replication intermediates in replicating plasmids, accumulate dramatically in *sgs1*-deficient yeast strains (Sinclair et al., 1997; C. Frei and S. M. Gasser, unpublished observations). Whether Sgs1p serves as a normal replication helicase or mediates polymerase reinitiation in the case of strand breakage, the data summarized above strongly suggest that it functions at replication forks, either constitutively or in response to polymerase pausing.

RecQ-LIKE HELICASES AND CHECKPOINT CONTROL

Perhaps more revealing with respect to human diseases are recent data suggesting a role for RecQ-like DNA helicases in an S-phase checkpoint that monitors DNA integrity. One of the characteristics associated with cancer is cellular immortalization – the potential to undergo an indefinite number of cell divisions. Often a prerequisite for immortalization is the loss of a mechanism that prevents cell cycle progression, or induces apoptosis, in the presence of damaged DNA. It is well documented that the tumor suppressor p53 plays important roles in both cell cycle arrest and in apoptosis, in response to damaged DNA. The ATM kinase, which is mutated in the disease ataxia telangiectasia (AT), functions upstream of p53 in both G_1 and G_2 phases of the cell cycle, and the loss of either p53 or ATM results in a failure to respond properly to damaged DNA (Dasika et al., 1999; Lavin and Khanna, 1999). Interestingly, the WRN helicase interacts biochemically in vitro and in vivo with the C terminus of p53 (Blander et al., 1999; Spillare et al., 1999; see Fig. 1). This interaction requires a C-terminal domain of WRN, which is highly conserved, yet poorly characterized (Morozov et al., 1997). The WRN-p53 contact might be physiologically significant, since p53-mediated apoptosis is attenuated in cultured WS cells (Spillare et al., 1999). Finally Kim et al. (1999) have shown that the ATM protein kinase phosphorylates WRN in vitro and colocalizes with the helicase and RPA in meiotic cells (Plug et

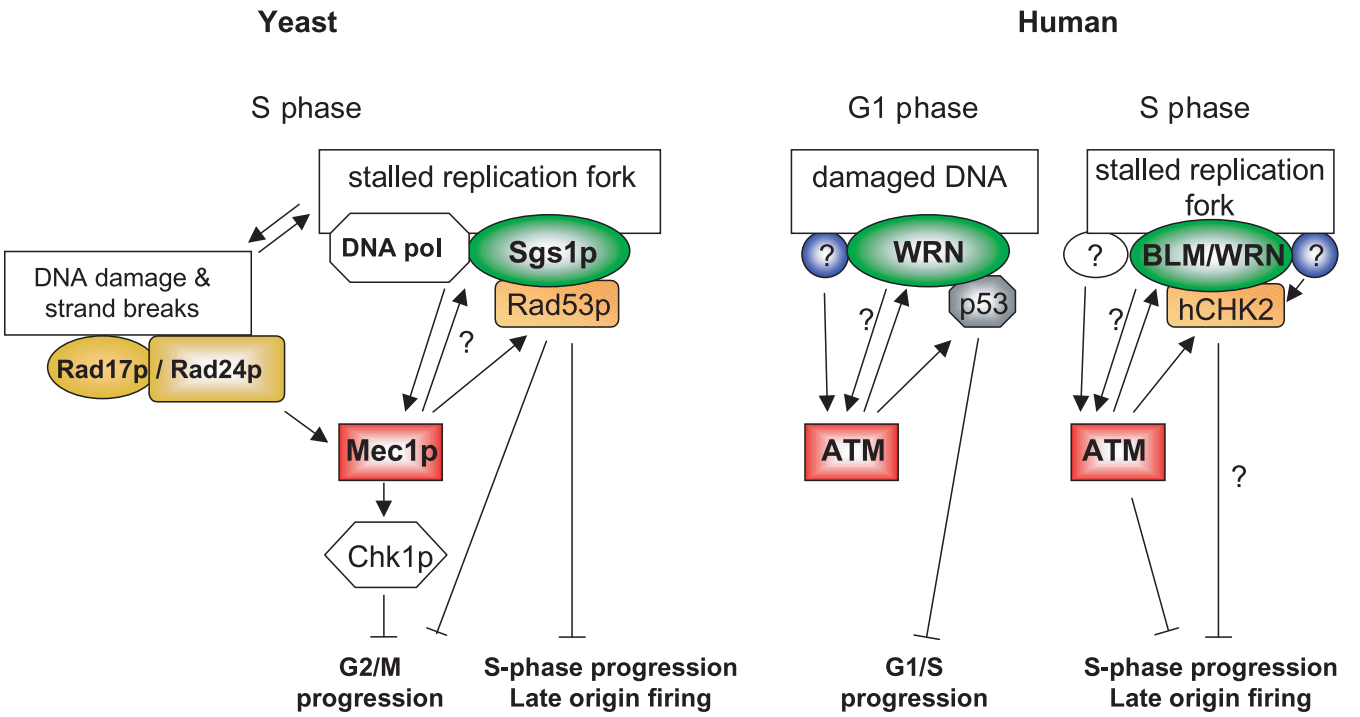

Fig. 1. A model for the putative checkpoint function of RecQ-like helicases. We propose that in S phase the *S. cerevisiae* Sgs1p and the *H. sapiens* Werner's and Bloom's helicases (WRN and BLM) are closely associated with the replication machinery. In our model, after a block in replication-fork progression due to either its encountering damaged DNA or exogenously added hydroxyurea, RecQ-like helicases recruit the yeast checkpoint protein Rad53p or its human homologue hCHK2/hCDS1 to stalled replication forks. This recruitment probably involves the activation of the helicase and/or the checkpoint protein by Mec1p or its human homologue ATM, respectively. Mec1p in yeast can also be activated by the Rad17/Rad24p pathway, which responds to damaged DNA, which can either result from or cause stalled replication forks. Activated Rad53p/hCHK2/hCDS1 then functions to slow down S-phase progression by blocking origin firing and elongation of DNA replication. The Werner's helicase might also have a G_1- or G_2-specific function as a recruitment factor for p53 to sites of damaged DNA, although evidence for this is still lacking. The role of p53 is not equivalent to that of the signal-transducing kinases Rad53p or hCHK2, although *WRN* expression is also controlled by p53 (Yamabe et al., 1998). Blue and white circles indicate potential signal-transducing proteins that remain to be identified, and question marks represent unknown but predicted interactions.

al., 1997, 1998; Walpita et al., 1999). Again, these studies provide data linking *WRN* to a mammalian checkpoint that monitors genomic integrity.

In view of their S-phase phenotypes, mammalian RecQ helicases might also function in an S-phase specifi checkpoint. Although the mechanism is still incompletely understood, early reports suggested that cultured mammalian cells delay S-phase progression both by decreasing initiation rates and by slowing fork progression at existing replication sites, in response to an activated ATM kinase (Larner et al., 1994; Painter and Young, 1980). In G_2 phase, the protein kinase hCHK2/hCDS1 is modified by ATM and appears both to upregulate p53 and to promote the arrest of the cell cycle prior to mitosis (Matsuoka et al., 1998; Bell et al., 1999; Hirao et al., 2000). In addition, hCHK2/CDS1 is activated by stalled replication forks, in a process that is independent of ATM (Chaturvedi et al., 1999; Matsuoka et al., 1998). Thus, both ATM and hCHK2/hCDS1 appear to slow down S-phase progression in response to damage, possibly through different pathways.

In *S. cerevisiae,* the ATM kinase homologue Mec1p is involved in all known DNA-damage and replication checkpoints, and is required for the activation of Rad53p and Chk1p, the homologues of human hCHK2/CDS1 and hCHK1 (Weinert, 1998; Sanchez et al., 1999). Whereas Chk1p is required to block anaphase entry in a response to DNA damage (Sanchez et al., 1999), Rad53p and Mec1p are also required to block S-phase progression in the presence of damaged DNA, and to delay mitosis in the presence of stalled replication forks (Paulovich and Hartwell, 1995). Recent results suggest that in budding yeast, Sgs1p participates in the checkpoint that responds to stalled replication forks in S phase (Frei and Gasser, 2000; see Fig. 1). Genetic analyses indicate that *sgs1* alleles form an epistasis group with a mutation in DNA polymerase ε (*pol2*-11), which prevents cell cycle arrest in response to an unscheduled block in DNA replication (Navas et al., 1995). The pol-ε/Sgs1p-dependent checkpoint activates Mec1p and Rad53p through a pathway parallel to Rad17p and Rad24p, two components of a signalling cascade that activates Mec1p and Rad53p in response to DNA damage (Fig. 1). Because stalled replication forks often result in strand breakage, provoking the DNA damage response, either of the two parallel pathways appears to be sufficient to ensure a phosphorylation of Rad53p in response to hydroxyurea. Importantly, however, loss of both Sgs1p and Rad24p fully compromises Rad53p phosphorylation, and allows passage through the cell cycle prior to completing DNA replication (Frei and Gasser, 2000). Together with the immunostaining

studies that colocalize Sgs1p and Rad53p in S-phase-specifi foci, these data suggest that in yeast Sgs1p recruits the signal transducing kinase to stalled replication forks, which prevents cell cycle progression and additional DNA damage. Reinforcing this model are recent biochemical studies, which confirm a direct interaction between Sgs1p and the FHA domain of Rad53p (C. Frei et al., unpublished results).

Studies of the fission yeast gene *rqh1*$^+$ (also called *hus2*$^+$ or *rad12*$^+$) have suggested a slightly different S-phase function, since *rqh1*$^-$ mutations decrease the viability of cells as they recover from an arrest provoked by hydroxyurea (Davey et al., 1998; Murray et al., 1997; Stewart et al., 1997). Rather than directly implicating *rqh1*$^+$ in a checkpoint response, Murray et al. (1997) propose a unique role in the resumption of growth following genomic insult, perhaps in a recombination pathway that allows the replication fork to bypass DNA damage (Murray et al., 1997). Nonetheless, even in this scenario, the notion that RecQ homologues are specialized replication-fork helicases is relevant: it remains possible that in fission yeast the checkpoint signaling function is redundant with that of another protein.

YEAST AS A MODEL SYSTEM FOR HUMAN DISEASES

Many of the genes mutated in human diseases are structurally conserved throughout the eukaryotic kingdom. Nonetheless, examples in which yeast mutant phenotypes resemble those of the human disease, and can be complemented by the human gene, are relatively rare. Notably, expression of the human *BLM* or *WRN* gene in an *sgs1*-deficient yeast cell suppresses some, but not all, of the Sgs1-related phenotypes (Yamagata et al., 1998; Neff et al., 1999). Either gene can suppress the hyper-recombination phenotype, whereas only *BLM* behaves like *SGS1* itself, restoring the slow growth phenotype of a DNA topoisomerase III mutant (in a *top3 sgs1* double mutant, see below) and suppressing sensitivity to HU (Yamagata et al., 1998). This may argue that the function of *SGS1* resembles that of *BLM* more closely than that of *WRN*. Nevertheless, there is an intriguing correlation between phenotypes of yeast cells lacking functional Sgs1p and WS cells in culture, in that both show an enhanced degree of cellular senescence (Sinclair et al., 1998). Moreover, *BLM* but not *WRN* suppresses the short-lived phenotype of the *sgs1*-null allele, although it did not repress the accumulation of extrachromosomal circles of rDNA, the putative 'cause' of yeast aging (Heo et al., 1999).

Such observations demonstrate clearly how important it is to identify the defects relevant to a given disease, particularly when yeast and human homologues share only a subset of overlapping functions. Intriguingly, both yeast and human RecQ-like DNA helicases interact with DNA topoisomerases II and III. Genetic studies have indicated that an *sgs1 top1* double mutant has a slower growth rate than either single mutant, whereas the *sgs1* mutation suppresses the reduced growth rate provoked by loss of topoIII, a single-strand, type I topoisomerase (Gangloff et al., 1994; Lu et al., 1996; Watt et al., 1995). Recently it was shown that the BLM helicase interacts biochemically with DNA topoisomerase IIIα (Wu et al., 2000), and that the two proteins colocalize by immunofluorescence. Thus, as proposed by Chakraverty and Hickson (1999), the study of topoIII might provide further insights as to the physiological role of the RecQ family of DNA helicases in mammalian cells. In this context, it is interesting that yeast Top3p is also implicated in an S-phase DNA damage checkpoint (Wu et al., 1999; cited in Chakraverty and Hickson, 1999).

Although novel functions for RecQ-like helicases may remain to be discovered, the proposed role for Sgs1p in the DNA replication checkpoint in yeast provides a convenient working model for the function of BLM, WRN and RTS helicases in human cells. Loss of a DNA replication checkpoint can result in aneuploidy owing to premature mitotic division and can thus be responsible for the cancer-prone character of BS and RTS patients. The hypothesis portrayed in Fig. 1 summarizes what is known about the function of Sgs1p in the S-phase checkpoint pathway in yeast, which is thought to respond to stalled replication forks directly and to DNA damage indirectly. In brief, RecQ-like helicases appear to associate with the replication machinery and to recruit signal-transducing kinases, such as Rad53p, to the site of lesion. Most of the interactions proposed in the mammalian pathway remain speculative (see question marks in Fig. 1); yet, in view of the conserved nature of checkpoint pathways in general, we can safely predict that many of the players will be the same. Mutations in the ATM kinase already indicate that the replication checkpoint will play a role in mammalian genomic stability. Future studies should reveal the exact function of the large and ubiquitous RecQ-like helicases through a systematic analysis of the proteins they recruit to the replication fork.

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Note added in proof

During revision of this text, a paper appeared by Wang et al. (*Genes Dev*. (2000), **14**, 927-939) showing that mammalian BLM is associated with a complex that contains BRCA1, MSW2, MSW6, MLW1, ATM, RAD50, MRE11 and NGS1 proteins. These proteins colocalize in foci in response to hydroxyurea and ionizing radiation in S-phase cells, which strongly supports the model that RecQ-like helicases are part of a sensor for DNA damage in S phase.

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