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Review

RecQ helicases and topoisomerases: components of a conserved complex for the regulation of genetic recombination

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Abstract: Maintenance of genomic stability relies on the efficient and accurate execution of DNA repair pathways, and is essential for cell viability and the prevention of cancer. Mutation of genes encoding RecQ helicases or topoisomerases gives rise to genomic instability through excessive recombination. Here, we review the recent biochemical and genetic evidence to indicate that these two classes of protein act in concert in a conserved pathway to maintain genomic stability by preventing inappropriate recombination.

Key words. RecQ helicases; topoisomerase; Bloom's syndrome; Werner's syndrome; genetic recombination; genomic instability.

Interest has been growing in recent years, in elucidating the essential role eukaryotic homologues of the *Escherichia coli* RecQ protein play in maintaining genomic stability. This has arisen primarily from the finding that mutations in three genes encoding human RecQ homologues, BLM, WRN and RECQ4, are associated with three distinct clinical disorders; Bloom's syndrome (BS), Werner's syndrome (WS) and Rothmund-Thomson syndrome (RTS), respectively [1–3]. BS is characterised by retarded pre- and post-natal growth resulting in proportional dwarfism, facial sun-sensitivity, immunodeficiency, male sterility and female sub-fertility. BS individuals also have an elevated cancer risk [4, 5]. On average, the onset of cancer in BS individuals occurs during the second decade of life and affects most organ sites that are seen in the general population. WS also predisposes individuals to the development of cancer, although in this disease, a more limited range of tumour types, mostly of non-epithelial origin, is seen. WS is classified as a progeroid disorder, since affected individuals show, prematurely, many of the features associated with normal ageing. These include the loss or greying of the hair, atherosclerosis, osteoperosis, cataracts and the development of type II diabetes [reviewed in ref. 6]. Individuals suffering from RTS are generally short in stature, have skin and skeletal abnormalities which give rise to poikiloderma and a characteristic facial shape, respectively, and are prone to developing skin neoplasms [7]. The fact that cancer predisposition is associated with BS, WS and RTS has highlighted the important role played by RecQ family helicases in the maintenance of genome stability and the prevention of tumorigenesis [for recent reviews of the RecQ family, see [refs. 8, 9].

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The RecQ family of DNA helicases

E. coli recQ gene is a component of the RecF recombination pathway and encodes a DNA helicase that unwinds DNA with a 3′–5′ directionality [10]. Homologues of RecQ are present in uni- and multicellular eukaryotes (fig. 1). Structurally, the proteins can be organised essen-

tially into three domains: a central catalytic domain that contains seven helicase motifs that are present in other DNA and RNA helicases, and amino- and carboxyl-terminal domains that flank the helicase domain (fig. 1). The sequence similarity to other helicases is also borne out enzymatically in that several RecQ homologues have been purified in recombinant form and shown to be bona fide DNA helicases, with the same 3′–5′ directionality as displayed by RecQ [10–15]. Within the RecQ family, strong sequence homology is generally restricted to the catalytic helicase domain, with the flanking N- and C-terminal domains showing limited or no homology. These flanking domains, unlike the catalytic domain, also vary considerably in size between the different RecQ homologues (fig. 1). The larger, divergent N- and C-terminal domains are found only in eukaryotic family members, and thus their presence defines a sub-group of RecQ helicases. BLM, WRN and RECQ4 belong to this sub-group of larger RecQ helicases and are encoded by three of five known human RecQ helicase genes (fig. 1). The *RECQL* and *RECQ5* genes encode the smaller human RecQ helicases. In the case of *RECQ5*, alternative splicing gives rise to three proteins, RECQ5α, -β and -γ, that all contain the helicase domain, suggesting that they are functional helicases. The presence of multiple RecQ homologues most probably reflects duplication and functional diversification of the ancestral *recQ* gene and seems to have coincided with the emergence of metazoans, since budding and fission yeast contain a single RecQ helicase, designated Sgs1 and Rqh1, respectively (fig. 1).

The divergent N- and C-terminal domains most probably serve to functionally differentiate between the various RecQ helicases. Several motifs have been identified in the C-terminal domain of certain RecQ helicases including nuclear localisation signal sequences, and a putative DNA-binding domain. WRN contains an additional recognisable motif, which is located in the N-terminal domain and forms the core of an exonuclease domain (fig. 1) [16–19]. This domain, when expressed either together or independently of the helicase domain, has nuclease activity. However, no such additional catalytic activities have been ascribed thus far to any of the other known RecQ helicases. The N- and C-terminal domains also mediate protein-protein interactions and several proteins have been identified that specifically interact with RecQ helicases. One conserved interaction, the function of which remains enigmatic, is the association of RecQ helicases with type IA topoisomerases.

Type IA topoisomerases

Topoisomerases are ubiquitous enzymes that facilitate many processes of DNA metabolism such as transcription, replication and chromosome condensation and segregation [20]. They act by passing intact DNA strands through transient DNA nicks or breaks in the same or other DNA molecules, and thus can alter the superhelical density of DNA, or remove knots and catenanes that arise during replication [20].

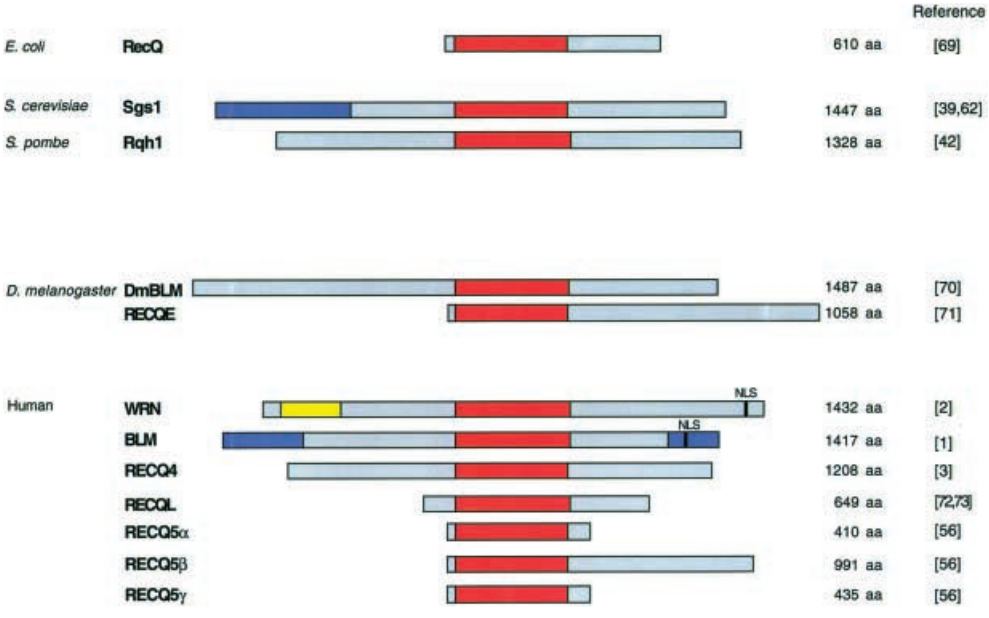

Figure 1. Alignment of RecQ family helicases. Proteins have been aligned relative to the seven helicase motifs which collectively constitute the helicase domain (red). Regions of limited or no homology are shown in grey. Domains which mediate interactions with topoisomerase III are indicated in blue. The yellow box indicates the residues required for the exonuclease activity of WRN. Also shown are the positions of nuclear localisation signal sequences (NLS).

Topoisomerases fall into one of two classes, termed type I and type II. Type I topoisomerases transfer single-stranded DNA (ssDNA) through ssDNA nicks, whereas type II topoisomerases pass double-stranded DNA (dsDNA) through dsDNA breaks. Type I topoisomerases can be divided further into two sub-classes, termed type IA and type IB, that are structurally unrelated and have distinct mechanisms of action. In a type I topoisomerase catalytic cycle, the enzyme forms a transient covalent bond, via a tyrosine residue in the active site, with either the 5′ end (type IA topoisomerases) or the 3′ end (type IB topoisomerases) of the cut DNA. Type IB topoisomerases are only present in eukaryotes, whereas type IA topoisomerases exist in both pro- and eukaryotes.

E. coli has two type IA topoisomerases, Top I and Top III, which are encoded by the *topA* and *topB* genes, respectively (fig. 2). The *topB* gene encodes a polypeptide of 653 amino acids, in which a central stretch of 530 residues, that contains the catalytic active site, is 22% identical and 40% similar to Top I. Outside this region, the two proteins diverge, particularly in the carboxy-terminal portions of the proteins, which have a role in substrate binding [21, 22]. In the case of Top III, the C-terminal 50 residues are rich in charged amino acids. Loss of this region abolishes nearly all DNA binding and, consequently, DNA relaxation activity [21]. An additional property of Top III, absent from the Top I protein, is an ability to decatenate newly replicated plasmid molecules in an in vitro replication system [23, 24]. This activity is also dependent on the C-terminal 50 residues of Top III [22]. The C-terminal domain of Top I is considerably larger than that of Top III, comprising approximately 300 amino acids. It too contains regions of charged residues, but in addition contains three tetra-cysteine Zn(II) atom-binding motifs, which are found in many DNA-binding proteins. Fusion of the C-terminal 312 residues of Top I to the first 604 amino acids of Top III results in a chimeric protein that exhibits relaxation activity comparable to the full-length Top III protein [22]. However, the fusion protein is inefficient at decatenating end-stage replication products and thus reveals distinct functional roles of the C-terminal domains of Top I and Top III [22].

In budding and fission yeast, the *TOP3* and *top3*$^+$ genes, respectively, encode the sole type IA topoisomerase found in each of these organisms (fig. 2) [25–27]. In each case, these proteins bear greatest sequence similarity to bacterial Top III. To date, at least two type IA topoisomerases have been found in higher eukaryotes, termed TOPO IIIα and TOPO IIIβ (fig. 2) [28–30]. At the sequence level, these enzymes are more similar to Top III than Top I, although both isozymes contain an extended C-terminal domain containing a Zn^{2+}-finger motif, which shows limited homology to the C-terminal region of Top I.

Where tested, the type IA topoisomerases only relax negatively supercoiled DNA, positively supercoiled DNA being refractory to this class of topoisomerase [28, 31]. However, with the exception of bacterial Top I, which maintains DNA supercoiling homeostasis in vivo in conjunction with two type II topoisomereases, DNA gyrase and Top IV [32], the other type IA topoisomerases only weakly relax negatively supercoiled DNA [31, 33–35]. Thus, these other type IA topoisomerases are unlikely to contribute to the removal of DNA supercoils. Indeed, genetic studies indicate that *E. coli* Top III and *Saccharomyces cerevisiae* Top3 contribute little to the supercoiling status of the bacterial and yeast genomes, respectively [31, 32]. Consistent with this is the finding that type IA topoisomerase mutants in several organisms have severe phenotypes: loss of Top3 in *Schizosaccharomyces pombe* is lethal, and murine TOPO IIIα is required for embryonic development [25, 26, 36]. This indicates a lack of functional redundancy between type IA topoisomerases and other classes of topoisomerase.

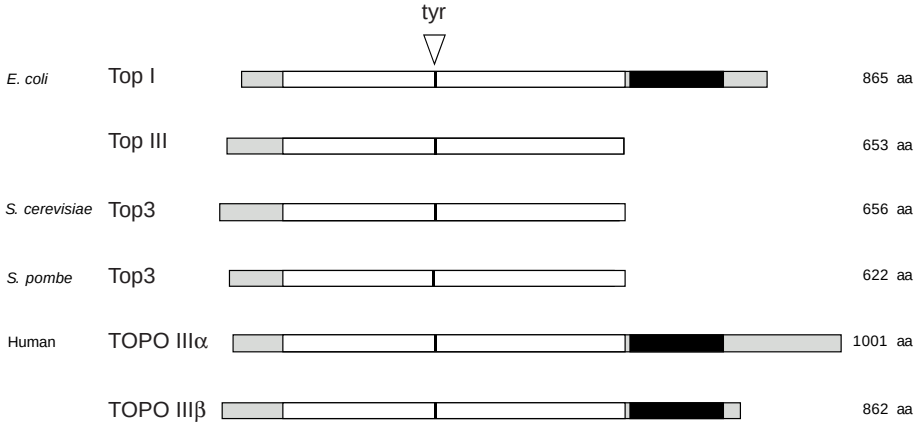

Figure 2. Alignment of type IA topoisomerases. Proteins have been aligned relative to the conserved tyrosine residue in the active site, as indicated by the black vertical bars. Areas in white represent a region of homology present in all members. In eukaryotes, the region of homology between various members extends to the amino terminus. Black boxes indicate the location of putative (in the case of eukaryotic homologues) or demonstrated (for *E. coli* Top I) Zn(II) binding tetracysteine motifs.

RecQ helicases and type IA topoisomerases act together in a common pathway to maintain genomic integrity

Many, if not all, RecQ helicase mutants display a loss of genomic stability [reviewed in ref. 37]. In particular, genetic recombination appears to occur aberrantly and at an excessive frequency. In BS cells, reciprocal exchanges between sister chromatids occur approximately tenfold more frequently than they do in normal cells [38]. Quadriradial chromosomes, which appear to be homologous chromosomes engaged in recombination, also occur at an elevated frequency in BS cells. In *S. cerevisiae*, deletion of the *SGS1* gene results in an increased rate of spontaneous mitotic recombination at numerous loci throughout the genome [39, 40]. Interestingly, ectopic expression of either BLM or WRN can suppress the excessive recombination phenotype of *sgs1* mutants indicating a conservation of function between eukaryotic RecQ helicases [41]. Hyper-recombination is also a feature of *S. pombe rqh1* mutants, which is particularly apparent following treatment with either the DNA replication inhibitor hydroxyurea or UV irradiation [42]. *recQ* mutants of *E. coli* show elevated levels of illegitimate recombination, which occurs between sequences that lack extensive sequence homology [43].

Type IA topoisomerases are also required for genomic integrity and, similarly, seem to function in suppressing recombination. Excessive recombination in *E. coli topB* mutants results in genomic deletions [44, 45]. Inactivation of *TOP3* in *S. cerevisiae* results in a more severe hyper-recombination phenotype than is seen in *sgs1* mutants, and particularly affects repeat sequences that occur throughout the genome [27, 39, 46]. Budding yeast *top3* mutants also grow slowly, which is partly due to a delay in the cell cycle in the S/G2 phases [27].

An apparent common mechanism of action of RecQ helicases is that they function in association with one or more type IA topoisomerase. Mutation of *SGS1* can suppress the hyper-recombination and slow growth of *top3* mutants, indicating that these two enzymes function in the same biochemical pathway [39]. The Sgs1 and Top3 proteins also physically associate, suggesting that they act together within a complex [39]. This interaction is conserved, since inactivation of $rqh1^+$ in the distantly related yeast, *S. pombe*, also suppresses the loss of $top3^+$, which is lethal due to a chromosome segregation defect [25, 26].

In humans, at least one RecQ helicase, BLM, is likely to function with a type IA topoisomerase [47, 48]. Human TOPO IIIα directly interacts with residues in the N-terminal domain of BLM (fig. 1) [48]. The interaction between *S. cerevisiae* Top3 and Sgs1 is also mediated through residues in the N-terminal domain of Sgs1 [39, 49] and therefore indicates a degree of conservation in the organisation of functional domains in these two helicases (fig. 1). A second hTOPO IIIα interaction domain also resides in the extreme C-terminal portion of BLM (fig. 1) [48]. Interestingly, although both hTOPO IIIα interaction domains map to the non-conserved regions of BLM, the presence of at least one of these interaction domains is required for *BLM* to genetically interact with *S. cerevisiae TOP3* and implies that BLM can form an active complex with yeast Top3 [48]. In contrast, the WRN protein is unable to genetically interact with yeast *TOP3* [41].

In vivo, BLM and hTOPO IIIα exist as a complex that is associated with promyelocytic leukaemia oncoprotein (PML) nuclear bodies, which appear as discrete nuclear foci and number about 10–20 per cell [47, 48, 50–52]. These nuclear structures comprise multiple proteins and have been implicated in a diverse range of cellular processes such as anti-viral defence, growth suppression, apoptosis, differentiation, transcription, cell cycle control and DNA replication [53–55]. An essential structural component of PML nuclear bodies is the PML protein. The *PML* gene was originally identified at the translocational breakpoint of the reciprocal chromosomal translocation t(15;17) in patients with acute promyelocytic leukaemia. As a result of this translocation, the *PML* gene is fused in-frame with the gene encoding the retinoic acid receptor α (RARα). Unlike PML, the PML-RARα fusion protein is seen in numerous nuclear microspeckles and results in a similar mislocalisation of BLM [52]. The localisation of BLM to PML nuclear bodies appears essential for BLM to perform its role, since the mislocalisation of BLM in murine $PML^{-/-}$ cells correlates with an elevated level of sister chromatid exchanges, the hallmark feature of BS cells [52]. While PML is required for the formation of PML bodies and the correct localisation of BLM, PML bodies are still present in BS cells [50, 52]. However, in BS cells, hTOPO IIIα is mislocalised, indicating that hTOPO IIIα is likely recruited to PML bodies via an interaction with BLM [47, 48].

A potential functional relationship between other human RecQ helicases and type IA topoisomerases remains to be determined. Reports that RECQ5 interacts with hTOPO IIIα and hTOPO IIIβ, when overexpressed in cells [56], and that hTOPO IIIβ interacts with Sgs1 when expressed in *S. cerevisiae* [57], suggests that a common mode of action of human RecQ helicases is to function in association with one or more type IA topoisomerases.

A role for RecQ helicases and type IA topoisomerases in the processing of recombination intermediates

How RecQ helicases act in conjunction with type IA topoisomerases to suppress recombination in the cell is unclear. In bacteria, a central step in homologous recombination, the pairing of a portion of DNA containing a

double-strand break with homologous sequences to facilitate the exchange of DNA strands, is catalysed by the RecA protein. RecQ can have dual, but opposing, effects on RecA-mediated strand exchange in vitro, suggesting that RecQ may function in the processing of recombination intermediates [58]. Similarly, RAD51, the structural and functional eukaryotic homologue of RecA, directly interacts in vitro and exists as a complex in vivo with BLM (our unpublished data). Moreover, a conserved feature of RecQ helicases is their ability to bind and disrupt synthetic four-armed DNA molecules, a structural mimic of the Holliday junction recombination intermediate [58–61] . At least in the case of BLM and WRN, these proteins can also promote branch migration of Holliday junctions over relatively large distances [60, 61].

Genetic evidence suggests that *S. cerevisiae* Sgs1 and Top3 also function in the processing of recombination intermediates during meiosis. *sgs1* and *top3* mutants show sporulation defects [40, 62, 63]. Nevertheless, both mutants are able to carry out the initial steps of meiotic recombination, that is, generate double-strand breaks and form recombinant molecules. In the case of *top3* mutants, however, the sporulation defect can be overcome if meiotic recombination is prevented by a second mutation that inactivates Spo11, which is required to initiate meiotic recombination by generating double-strand breaks [63]. Deletion of *SGS1* also partially suppresses the meiotic defects of *top3* mutants. A similar genetic relationship therefore exists between *SGS1* and *TOP3* for both mitotic and meiotic recombination, suggesting that the Sgs1/Top3 complex likely catalyses a similar step in both processes [39, 63].

Little is known about the *in vivo* substrate for eukaryotic type IA topoisomerases. A peculiar feature of several of these enzymes compared with other topoisomerases is their preference for binding ssDNA [28, 31, 33–35]. This preference has also been demonstrated for *E. coli* Top III and may partially explain the low activity shown by Top III and its eukaryotic homologues in relaxing negatively supercoiled DNA. Indeed, hyper-negatively supercoiled DNA, in which the DNA is under-wound to a greater extent than is seen in plasmids routinely purified from bacteria, is a preferred substrate for these enzymes [35]. Similarly, a negatively supercoiled heteroduplex DNA molecule that contains a short single-stranded loop is more efficiently relaxed by *S. cerevisiae* Top3 protein than is a fully dsDNA molecule [31]. Consistent with this, the activity of type IA topoisomereases can also be stimulated using conditions where the DNA helix is destabilised, such as high temperatures or low salt concentrations [28, 31, 33–35].

The genetic relationship between *TOP3* and *SGS1* or *rqh1*$^{+}$, in budding and fission yeast, respectively, suggests that RecQ helicases generate a structure that is acted upon by topoisomerase III enzymes. The ability of *E. coli* Top III to decatenate replication intermediates in vitro requires the presence of a region of ssDNA in one of the DNA molecules. This probably reflects the ssDNA preference of the enzyme and also the fact that type I topoisomerases only make ssDNA nicks which would not generally be sufficient to effect decatenation of interlinked duplex molecules. However, in the presence of active RecQ, Top III can catalyse the catenation and decatenation of covalently closed plasmid DNA [64]. This functional interaction is conserved, since RecQ can also stimulate yeast Top3 to catalyse the same-strand passage reaction [64]. The physiological role of such an activity is unknown. Indeed, while Top I cannot catalyse the same linking and unlinking reactions as Top III, ectopic expression of this bacterial enzyme can suppress the slow growth of *S. cerevisiae top3* mutants [27, 64]. However, if Top I can complement other aspects of the phenotype associated with loss of *TOP3*, such as hyper-recombination, remains to be determined. Separation of the different phenotypes associated with mutation of *TOP3* is demonstrable, at least in meiosis, where Top I expression can suppress the sporulation deficiency of *top3* cells [63]. However, the resultant spores display low viability [63].

The ability of some type IA topoisomerases to decatenate linked DNA molecules may function in separating newly replicated chromosomes. However, it has been hypothesised that it could act to disrupt early recombination intermediates [65, 66]. Such an event would be desirable following, for example, inadvertent pairing of DNA strands over an extensive region that might occur between homeologous (similar but non-identical) sequences or repeat sequences at different genomic locations (fig. 3). Indeed, locating and identifying the ‘correct’ homologous sequence to initiate recombination poses a considerable logistical problem, particularly in organisms with large and partially repetitive genomes. Both *sgs1* and *top3* mutants have phenotypes that suggest they may have defects in establishing productive recombination intermediates. Recent evidence suggests that *S. cerevisiae sgs1* mutants appear able to use only the sister chromatid, and not the homologous chromosome, in the recombinational repair of dsDNA breaks [67]. In *top3* mutants, gene conversion events, arising as a result of recombination between genes on heterologous chromosomes, can extend considerable distances into regions of non-homologous sequences [46]. The hyper-recombination phenotypes of RecQ helicase and type IA topoisomerase mutants may therefore not result from uncontrolled recombination. Rather, inappropriate recombination events, which are normally disrupted by a RecQ helicase/type IA topoisomerase complex, are converted into recombinant molecules or alternatively persist, with the result that chromosomes are covalently interlinked. It may be significant, therefore, that chromosome segregation defects are also a feature of *sgs1* and *rqh1* and *S. pombe top3* mutants (fig. 3) [25, 42,

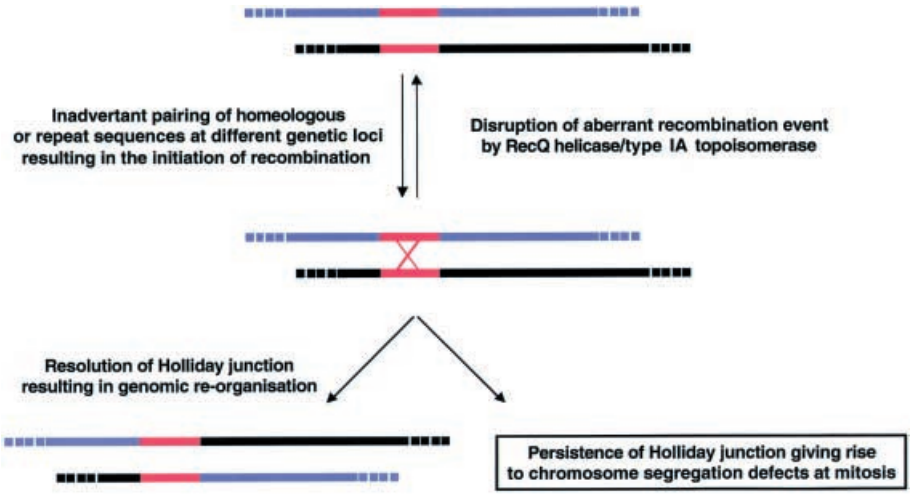

Figure 3. Potential role for RecQ helicase/type IA topoisomerase complexes in the reversal of inappropriate recombination events. See text for details.

62]. Furthermore, demonstration that expression of a recombinant Holliday junction resolvase can partially suppress some of the phenotypic effects of deleting *rqh1*$^+$ is consistent with the persistence of unresolved recombination intermediates in these mutants [68].

Conclusions and future perspectives

RecQ helicases and type IA topoisomerases have been highly conserved through evolution and appear to act in concert to maintain genomic stability. Disruption of this complex can have devastating effects, such as cell growth defects or even death, and in multicellular organisms can result in an increased susceptibility to the development of cancer. While analysis of mutant phenotypes and biochemical studies with purified enzymes are consistent with a role for RecQ helicases and type IA topoisomerases in the processing of recombination intermediates, in precisely which in vivo processes these proteins function remains to be determined. Many RecQ helicase mutants have defects in some aspect of replication [reviewed in refs. 8, 37]. Given that RecQ helicase mutants are viable, they are unlikely to play a critical catalytic role as part of the 'core' replication machinery. However, a subtle defect in replication could be the underlying cause for the hyper-recombination phenotype in RecQ helicase mutants. A key area of research will therefore be to further characterise the functional interactions between RecQ helicases and type IA topoisomerase and the roles these enzymes play in the co-ordination of recombinational DNA repair pathways and the process of DNA replication.

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