Molecular biology of Hel308 helicase in archaea

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Abstract
Hel308 is an SF2 (superfamily 2) helicase with clear homologues in metazoans and archaea, but not in fungi or bacteria. Evidence from biochemistry and genetics implicates Hel308 in remodelling compromised replication forks. In the last 4 years, significant advances have been made in understanding the biochemistry of archaeal Hel308, most recently through atomic structures from cren- and eury-archaea. These are good templates for SF2 helicase function more generally, highlighting co-ordinated actions of accessory domains around RecA folds. We review the emerging molecular biology of Hel308, drawing together ideas of how it may contribute to genome stability through the control of recombination, with reference to paradigms developed in bacteria.

DNA helicases in replication, repair and recombination
Since many stages of replication, repair and recombination require ssDNA (single-stranded DNA) substrates, helicases can oblige by separating strands of base-paired dsDNA (double-stranded DNA). Unwinding parental duplex for genome duplication at replication forks requires protein complexes containing helicases gp41 (bacteriophage), DnaB (bacteria) or MCM (minichromosome maintenance) (eukaryotes and archaea). Accessory helicases, e.g. PriA, Rep, UvrD, Dda, PcrA and Rrm3, may aid the processivity of replicative helicases during strife [1–4]. Chemical damage to nucleotides, both genome-wide and at replication forks, is accessed for excision repair by helicases of Uvr (bacteria) [5], XP and Fanc protein complexes (both eukaryotes and archaea) [6]. Defects in XP and Fanc proteins in humans manifest as the disorders Fanconi’s anaemia and xeroderma pigmentosum, which are characterized by a variety of clinical symptoms caused by genome instability.

Recombination events are linked to excision repair and replication to deal with lesions using an intact homologous DNA molecule as a repair template. The onset and early stages of recombination may be controlled by helicases as directional motors that depose proteins or DNA strands to expose ssDNA that stimulates recombination. Alternatively, such helicase clearance roles may prevent recombination by disassembling recombination intermediates. These kinds of event, summarized in Figure 1, have been linked to bacterial helicases RecQ [7], UvrD [8] and Rep [9], although the exact roles of these proteins have been elusive. Late in recombination, branch migration and dissolution reactions are catalysed by Holliday junction helicases (e.g. RecQ [10,11], bacterial RuvAB [12] or RecG [13]) to disentangle synthesized DNA molecules.

Where Hel308 sits in this broad church of genome biology is discussed below. The absence from archaea of sequence homologues of bacterial recombination helicases made it tempting to seek analogous roles for Hel308. Evidence currently points to involvement of Hel308 early on in recombination or other gene-conversion events that are linked to blocked replication, perhaps in a role similar to that of Escherichia coli RecQ or UvrD protein.

Hel308 in archaea
Designation of Hel308 arose from isolation in Drosophila of mutations that cause hypersensitivity to DNA-cross-linking reagents [14]. One group of Drosophila mutants (musa308) mapped to what is now known as PolQ [15], a translesion polymerase that is active in excision repair [16]. PolQ has proposed helicase domains (but no reported helicase activity) that were used to identify novel human helicases implicated in DNA repair, unearthing hel308 [17]. The rapid accumulation of genome sequence data identified Hel308 homologues in archaea, including in the minimalist genome of the hyperthermophile Nanoarchaeum equitans, but not in bacteria or yeast. Conservation of DNA replication and repair protein machinery in eukaryotes and archaea, but not in bacteria, is not unusual and has been commented on several times, e.g. reviewed by Barry and Bell [18].

Hel308 [then called ORF (open reading frame) mth810] from the archaeon Methanothermobacter thermautotrophicus was first proposed to be part of a predicted exosome, through sequence similarity to Ski2 helicases involved in releasing RNA molecules for degradation [19]. No more was heard until 2005, when Mth810 (molecular mass of 75 kDa) was described for its actions at forks and Holliday junctions from M. thermautotrophicus and Pyrococcus furiosus (ORF PF0677, 82 kDa) [20,21]. Mth810 was called Hel308a (‘a’ for archaea) on the basis of sequence conservation with human Hel308, and identical properties of human Hel308 and Mth810 in ssDNA-stimulated ATPase activity and 3′→5′ polarity of unwinding. The P. furiosus enzyme was called Hjm (Holliday junction migration) for its ability to unwind...
Figure 1 | Hel308 in recombination-dependent DNA replication at blocked replication forks

Hel308 (blue shape) loads on to a blocked lagging strand template as a motor that clears DNA and proteins (e.g. RPA (replication protein A), light blue ovals). Although this may provoke recombination from ssDNA, it could also remove stalled protein complexes that interfere with replication or other events. Hel308 may load RadA recombinase (grey hexagons) on to ssDNA, triggering recombination by strand exchange. Alternatively, a clearance role of Hel308 may extend to removing RadA from ssDNA, preventing recombination in favour of an alternative repair strategy. In this anti-recombinogenic role, Hel308 may stabilize a replication fork, promoting repair by translesion polymerases.

Isolated archaeal Hel308 protein is a ssDNA-stimulated ATPase that unwinds duplex DNA from a 3’ (but not a 5’) ssDNA overhang as its minimal substrate [20]. No activity is detectable on fully base-paired duplex DNA. It unwinds a variety of branched DNA structures, including an RNA–DNA hybrid [25], but is most active on forked molecules, removing the lagging strand of a fully base-paired model fork [20,26]. This substrate versatility is reminiscent of bacterial RecQ helicases [7]. The genetic screen identifying M. thermautotrophicus Hel308 showed recQ-like activity in replication-impaired E. coli strains [20]. Similar genetic analysis showed that Hel308 did not interact with Holliday junctions, and is therefore more likely to be involved in processing DNA at early stages of events that remodel blocked replication forks [20]. Ideas of how Hel308 may be involved in these events are described in Figure 1 and are expanded on below.

Reactions of archaeal Hel308: remodelling replication forks?

It is not yet possible to superimpose biochemical data from archaeal Hel308 on to less comprehensive genetic data from mostly eukaryotic studies. Drosophila and Caenorhabditis elegans hel308 promote repair of DNA cross-links [22,23], and its inactivation in Drosophila provokes homologous recombination [24]. Genetic analysis in the archaean Haloferax volcanii showed hel308 to be an essential gene (T. Allers, personal communication). However, analysis of archaeal hel308 in bacteria, alongside biochemical of the isolated protein, has developed a working hypothesis that archaeal Hel308 may have very similar functions to the bacterial SF2 (superfamily 2) helicase RecQ, or the SF1 helicase UvrD. RecQ and UvrD are not widespread in archaea, and RQC and HRDC motifs common to RecQ proteins cannot be detected in Hel308 sequences.

Is Hel308 in archaea analogous to E. coli RecQ or UvrD?

RecQ helicases are a family of proteins that contribute to genome stability by controlling homologous recombination at replication forks. E. coli RecQ can remodel replication forks to initiate recombination, by generating ssDNA for RecA-catalysed strand exchange [27,28], and it can also
Figure 2 | Hel308 from *M. thermautotrophicus* efficiently unwinds DNA at 50°C

*M. thermautotrophicus* Hel308 efficiently unwinds >100 bp of DNA in 5 mM ATP and 5 mM MgCl₂ at 50°C. Helicase substrate was prepared from polymerization of [32P]-end-labelled M13 primer annealed to M13 ssDNA. Lane 1, 95 nt marker DNA; lane 2, boiled substrate; lane 3, control (no Hel308); lane 4, 100 nM Hel308; lane 5, 100 nM K51L Hel308, walker A mutant.

Help from its friends?

It may help to judge the function of a helicase if we know the proteins with which it interacts. Evidence was recently presented for Hel308 remodelling a fork into a Holliday junction, and for interaction of Hel308 with the resolvase protein, Hjc [32]. The experiments imply that a blocked replication fork could be regressed into a Holliday junction by Hel308, provoking recombination by recruitment of Hjc to catalyse Holliday junction resolution, generating DNA ends. This is reminiscent of a model of replication and recombination interplay first proposed in eukaryotes [33] and later demonstrated for bacterial RecG [13]. No details are available for the nature of Hel308–Hjc interaction, the mechanics of how this results in junction resolution or whether it can be supported with other non-archaeal resolvases. Under some conditions, Hjc can cleave replication forks as well as Holliday junctions in *vitro* (I.L. Woodman and E.L. Bolt, unpublished work).

A *Pyrococcus* Hel308 also reportedly interacts with the sliding-clamp protein PCNA (proliferating-cell nuclear antigen) [26]. Little is known about Hel308–PCNA interaction: no mediating amino acid residues have been confirmed, and there is no idea of how the interaction affects protein function. A putative PIP (PCNA-interacting protein) box sequence motif for PCNA interaction at the Hel308 C-terminus was reported in *Pyrococcus* Hel308 proteins [26], but this is not present in any other archaea. Hel308 atomic structures [25,31], and unwinding data from isolated enzyme (Figure 2), make it unlikely that PCNA is required to improve the processivity of helicase unwinding, but a role for PCNA somehow recruiting Hel308 to forks cannot be ruled out. There is mention, although no data, of Hel308 interacting with RadA recombinase [26]. This is intriguing as a potential mechanism for Hel308 and RadA together promoting recombination after remodelling of a blocked fork. Overall, more robust studies are needed to determine physical and functional interactions of Hel308 and their biological significance.

Atomic structures of Hel308

Atomic structures of Hel308 from the euryarchaeon *Archaeoglobus fulgidus* [25] and the crenarchaeon *Sulfolobus solfataricus* [31] revealed a five-domain protein monomer that surrounds DNA and drives a β-hairpin plough through duplex to separate strands (Figure 3). The structures represent a significant step forward in closing the gap in knowledge between mechanisms of superfamily 1 and superfamily 2 helicases.

Apparent processivity of Hel308 on simple *in vitro* substrates is readily explained by Hel308 clamping DNA using all five domains. DNA is bent into a ‘dog-leg’ shape that can be interpreted, with caution, as being equivalent to a fork structure that is lacking part of a leading strand, but with an intact lagging strand. DNA is unwound from an initial 2 bp ATP-independent DNA melting by a β-hairpin loop in domain 2. Further unwinding is fuelled by ATP binding and hydrolysis in RecA folds shared between domains 1 and 2.

process branched recombination intermediates when acting in concert with topoisomerase III [11,29]. The recQ-like phenotype of introducing archaeal hel308 into *E. coli* replication-deficient strains [20,26] may arise because Hel308 gains access to blocked forks, clearing away obstacles to generate ssDNA that provokes recombination. Alternatively, this ‘fork clearance’ activity may also act counter to recombination, by removing D-loops and/or recombinase proteins in favour of alternative events to overcome blocked forks. This would be more like the bacterial superfamily 1 helicase UvrD, which is required for *E. coli* to survive DNA-crosslinking reagents [30]. UvrD has been proposed to have a fork-clearing role [8], and may displace stalled protein complexes in other contexts away from replication forks. Hel308 is apparently quite processive (Figure 2), and can displace other proteins and streptavidin beads from ssDNA molecules [31], the basis of which is explained by atomic structures of Hel308–DNA complexes (see below) (Figure 3). Archaeal Hel308 does not complement the UV-sensitivity of an *E. coli* uvrD strain (E.L. Bolt, unpublished work), but the recent development of more refined *E. coli* recQ, uvrD, rep, recA and ruv strain combinations may make it possible to distinguish between recombinogenic and anti-recombinogenic roles of archaeal Hel308, albeit in a heterologous system.
Figure 3 | Atomic structure of Hel308-DNA complex, and the position of domain 5

(A) Image of *A. fulgidus* Hel308 bound to 3'-tailed ssDNA, produced from PDB code 2P6R (see also [25,34]). Domain 5 (salmon pink) engages with ssDNA 10–12 nt distal from the point of strand separation at the β-hairpin. (B) Arg^644^ (Arg^649^ in *M. thermautotrophicus* Hel308) engages ssDNA in addition to several other DNA contacts arising from the peptide backbone. Mutation of Arg^649^ in *M. thermautotrophicus* Hel308 severely curtails ssDNA-stimulated ATPase activity. Arg^642^ (Arg^647^ in *M. thermautotrophicus*) orients away from DNA as a salt bridge with the helicase ratchet domain 4. (C) Conserved RAR (Arg-Ala-Arg) motif at the C-terminal domain 5 of Hel308 proteins. Afu, *A. fulgidus*; Mth, *M. thermautotrophicus*.

causing co-ordinated movements of a helicase ratchet generated by domains 2 and 4. A proposed helicase mechanism is described in detail, and goes some way to unifying observed actions of SF2 helicases more generally, by comparison with hepatitis C virus helicase NS3 and RNA helicases Ski2 and Vasa [25]. RecQ helicase structures do not share these unwinding characteristics, although similar cellular functions of Hel308 and RecQ could have arisen by convergent evolution of function from different ancestral genes.

An appealing observation from the *A. fulgidus* structure is a fingertip-grip on 3'-tailed ssDNA by Hel308 domain 5 (Figures 3A and 3B). Biochemistry on this domain showed the interaction to be functionally important as a ‘molecular brake’ coupling ssDNA binding to ATPase and unwinding activities [31,34]. We mutated a triad of arginine residues in *Mth* Hel308 domain 5 (R^647^XRAR^651^) that is conserved in archaeal and metazoan Hel308 (Figure 3C) [34]. R^649^A protein had dramatically reduced ssDNA-stimulated ATPase activity, but was unchanged in its basal ATPase activity. ATPase activity of R^647^A mutant even in the absence of ssDNA was comparable with the ssDNA-stimulated activity of wild-type protein. There was no difference in DNA binding by each mutant protein compared with wild-type, but fork unwinding was reduced in each case. The *A. fulgidus* Hel308 atomic structure revealed that Arg^649^ (and Arg^651^) contact ssDNA distal to the RecA core, but Arg^647^ and other domain 5 residues contact the helicase ratchet domain 4 (Figure 3B). We interpreted this as the engagement of Hel308 domain 5 on ssDNA triggering productive ATPase activity, leading to helicase unwinding. A similar proposal arose from removal of domain 5 from crenarchaeal Hel308, in this case with a resulting increase in ATPase activity and helicase unwinding [31]. In this way, Hel308 could be controlled to respond to regions of ssDNA arising at compromised replication forks, or elsewhere in the genome. It may be important that there is a significant difference between the positioning of domain 5 in structures of *A. fulgidus* Hel308 with and without DNA, and we await an ATP-bound structure for more insight. Armed with structural insights into Hel308 DNA binding by domain 5, 10–12 nt away from a dsDNA–ssDNA junction, we are using a variety of fork substrates to test how they are engaged by the helicase and how the disposition of the fork affects Hel308 function.

Closing remarks

The cellular role of Hel308 in archaea, and metazoans, is not clear, but a link to controlling potential recombination events arising during DNA replication seems likely. Atomic structures give useful insight into the biochemical function of archaeal Hel308, but genetic analysis in archaea is required to flesh out ideas from biochemistry and heterologous genetics of Hel308. This would be useful to establish this helicase into epistasis groups, and to screen for recombination defects or hyper-recombination phenotypes.
We are grateful for funding from the Biotechnology and Biological Sciences Research Council for a studentship to I.L.W. [grant number BB/DS26602/1/W].

References
1 Heller, R.C. and Marians, K.J. (2007) Non-replicative helicases at the replication fork. DNA Repair 6, 945–952
15 Boyd, J.B., Sakaguchi, K. and Harris, P.V. (1990) mus308 mutants of Drosophila exhibit hypersensitivity to DNA cross-linking agents and are defective in a deoxyribonuclease. Genetics 125, 813–819

Received 4 August 2008
doi:10.1042/BST0570074