It’s good to unwind: how Hel308/He1Q helicases are good for health

Tabitha Jenkins, Sarah Northall, Edward Bolt and Panos Soultanas (The University of Nottingham and the University of Bristol, UK)

In every living system the information for maintaining and propagating life is contained within a genome. This ‘book of life’ is written in the language of DNA. For life to exist, access to this is essential in order to replicate and repair genetic information for the benefit of future generations. An important group of molecular motor proteins, the helicases, allows access to this book of life by opening up the DNA double helix and exposing the DNA text of individual strands for replication, transcription, translation and repair. Like all molecular motors, helicases use chemical energy derived from the binding and hydrolysis of nucleotide triphosphates, usually ATP, to carry out mechanical tasks. They come in a variety of structures and employ different mechanisms to carry out diverse mechanical tasks. One large group of helicases are required for repairing DNA that has become damaged by radiation, chemicals or other proteins stuck on the DNA ('roadblocks'). These DNA repair helicases are also diverse in form and function, having evolved in all forms of life to deal with many different types of DNA damage. However, they have the common property of being able to slide along and unwind DNA molecules to allow DNA repair enzymes access to fix the damage.

Genome maintenance and DNA repair by helicases

Closely related DNA repair helicase proteins evolved in organisms called archaea and multi-cellular eukaryotes ('metazoans', e.g. humans), to protect their cells from DNA damage. It seems, on face value, bizarre that archaea and humans share very similar helicases, given the differences in their lifestyle and cell morphologies. However, these things make sense in the context of molecular evolution. Fundamental properties of DNA repair and DNA replication in archaea and metazoans have been conserved throughout evolution, because replication is essential to cell survival and for the generation of progeny. Archaeal Hel308 and metazoan He1Q are DNA repair helicases that help to preserve the integrity of DNA, especially by mobilizing to help DNA replication that has encountered problems, such as chemical damage to DNA or an immovable protein roadblock. This type of DNA repair can be called 'replication-linked repair'. Originally also termed Hel308 in metazoans (its name was changed in metazoans from Hel308 to He1Q about a decade ago, but persists for archaea), the enzyme was first recognized during a screen to identify mutations giving hypersensitivity to DNA crosslinking agents. This screen identified an N-terminal superfamily 2 (SF2, see below) 3’-5’ helicase fused to a C-terminal DNA polymerase, Mus308, from Drosophila melanogaster which became the founding member of the Hel308 family. Exactly what He1Q and Hel308 do to support replication-linked repair is still being worked out, but some clues have emerged from genetic and biochemical analyses, atomic resolution structures of Hel308 and from possible effects of dysfunctional He1Q on human health.

An unrepaired replication catastrophe is harmful to human health

Genes encoding He1Q and Hel308, respectively helq and hel308, can be deleted in cells using CRISPR-Cas9 editing, or in more traditional ways, to observe resulting cell ‘phenotypes’ to try and understand the cellular function of the deleted gene.

helq/hel308 gene deletions cause a change in the efficacy of DNA replication and cells become hypersensitive to chemicals that are able to cause DNA inter-strand crosslinks of the type that would block DNA replication. We infer from this that in healthy cells, Hel308 and He1Q help DNA replication to overcome encounters with DNA damage, and to assist in repairing the damage. These kinds of replication problems caused by loss of helicases such as He1Q are often referred to as ‘genome instability’,
which is dependent on replication-repair processes. Defects of this kind are associated with several human diseases, including cancers, developmental and neurological abnormalities, and premature ageing syndromes. Fanconi anaemia (FA) is one prominent example of the catastrophic effects on human health caused by the inability to repair DNA damage associated with DNA replication, particularly, the repair of inter-strand crosslinks. Defects in any one of eight DNA repair proteins, though predominantly FANCA, FANCC and FANCG, leads to FA, which affects most body organs and causes increased cancer risk.

In clinics, HelQ defects have been associated with breast and ovarian cancers, oesophageal squamous cell carcinoma and reproductive problems, although the precise mechanistic links between HelQ function and such human pathologies is far from clear. A 2013 study into statistical links between human DNA repair pathways and risk of oesophageal squamous cell cancer and gastric cancer, two major causes of cancer death worldwide, identified several genes of significance. These included HelQ and its homologue PolQ, alongside much more established players in preventing genome instability such as BRCA1. Although this study used a limited sample set over 10 years in North Central China, it begs the question, what are the roles of HelQ in relation to preventing disease?

One line of enquiry for answering this is from observations that HelQ interacts with a group of proteins called RAD51 and RAD51 paralogues. These are crucial for replication-linked repair because they control a set of complex DNA manipulations called homologous recombination. This process is most ‘famous’ for making crossovers in meiotic cell division, but it is also an important DNA repair process in all cells, from bacteria to metazoans. It is especially notable for being a relatively accurate mode of DNA repair, compared with other repair processes such as DNA end-joining. HelQ makes direct physical interaction with the RAD51 paralogue complex BCDX2, and this somehow promotes accurate and reliable repair by homologous recombination. Components of the complex, RAD51C and RAD51D, have been associated with ovarian cancer risk while RAD51B and XRCC2 have been associated with breast cancer risk. Does this suggest that HelQ defects could also potentially increase ovarian and breast cancer risk? Some research has indeed highlighted that defects specifically in amino sequences of HelQ are causative for ovarian cancer. Studies using a viable HelQ-deficient mouse model, where HelQ was no longer detectable, identified fertility defects and a higher proportion of mice developing two or more primary tumours compared with wild-type controls. As the mystery of HelQ is unwound, it is likely to highlight new understanding of its roles in preventing disease and in the biology of human cells more generally.

**Structure begets function in Hel308 and HelQ**

Being able to see the three-dimensional structure of protein at atomic resolution is key to understanding function, and is important for delineating evolutionary relationships between hundreds of different helicases, and understanding their relative roles.

Phylogenetic classification of helicases was initially based on seven highly distinct conserved amino acid sequence motifs, two of which make up the Walker A and Walker B sites of nucleotide triphosphate (NTP) binding. The explosion of protein sequence information over the last generation has seen a concomitantly large increase in the number of proteins classified as helicases. No one ‘core’ helicase domain is found in all, although RecA-like ATPase domains are common. There are six helicase superfamilies (SF1–6), of which SF1 and SF2 are the largest and show major structural and functional differences to SF3–6. SF2 is the largest and most diverse of the families, comprising helicases that contribute to transcription, DNA repair, chromatin rearrangement and RNA metabolism. The sub-division of SF2 into 10 further clades is also based on sequence homology.
Interestingly, Hel308 and HelQ group into the Ski2-like helicase clade, proteins more noted for single-stranded RNA translocation and double-stranded RNA melting rather than DNA repair.

The Ski2-like features of Hel308 are readily apparent in the structure of the enzyme from *Archaeoglobus fulgidus*, a sulphur-metabolizing organism, which includes binding to a tailed duplex DNA molecule (Figure 2). An outstanding feature of this is the positioning of the winged-helix domain, which is apparently dislocated from the DNA, but packed tightly against a RecA-like ATPase domain. Recent published and unpublished work from the authors’ labs suggests how this winged-helix domain may in fact be required for ssDNA translocation by Hel308 and, by extension, human HelQ, which is predicted to have the same domain. Continued work to understand the precise translocation mechanism of Hel308 and HelQ is underway and unwinding ability will improve our understanding of the role of Hel308 and HelQ, which shares 36.9% sequence similarity to Hel308.

Common structural features between Hel308, Mtr4 and Brr2, other Ski2-like helicases, include a ratchet domain (Figure 2), a winged-helix domain packed tightly against an ATPase RecA-like domain and a C-terminal helix-hairpin-helix (HhH). One might speculate that these domains are essential for Hel308 to unwind DNA and recruit specific repair proteins, features common in regulating RNA metabolism and degradation, therefore explaining the similarity. There may also be mechanistic details to Hel308/HelQ functions that we have yet to uncover.

There is no available structure for any HelQ protein as yet, although interesting atomic resolution structures from two of the most closely related proteins of HelQ, Hel308 and PolQ, offer some ideas as to how HelQ may function, and also highlight some tantalizing possibilities that HelQ may have unusual functions. One of particular interest is the involvement of PolQ in alternative end joining, being able to mediate the rejoining of two 3’ resected DNA strands. The high sequence similarity to HelQ could suggest new roles of HelQ. A HelQ homologue in humans is called PolQ, which comprises helicase-like domains fused to a family A DNA polymerase. Although helicase activity from PolQ has not been detected, this part of the protein has been structurally determined as a dimer of dimers, forming a tetramer arrangement of PolQ helicase domains in solution. It is proposed that each PolQ dimer operates on either side of a DNA molecule, working together to process DNA, possibly by melting and re-annealing it, processes that might contribute to specialized pathways of DNA repair. This structure of PolQ helicase domains has also allowed some structural modelling of its homologue HelQ (Figure 3). Data from the authors’ labs suggests that HelQ may form different multimers, including tetramers and it has been interesting to note that in these models the proposed helicase ratchet of HelQ is orientated inwards toward the ring of the HelQ, where it could (speculatively) interact with DNA that is encircled.

### Hel308 helicases as tools in medicine and genome editing

Hel308 helicases have been studied as part of developing DNA nanopore technology. Nanopores are membrane proteins that have been designed to control a flux of ions through the membrane pore, where a change in current can be used to
analyze movement of specific molecules, such as translocating DNA. More recently, nanopore technology has been applied for the analysis of enzyme kinetics at a single molecule level, in real-time. One type of analysis, single-molecule picometre-resolution nanopore tweezers (SPRNT), was used to look at individual Hel308 molecules. The *Mycobacterium smegmatis* porin A (MspA) nanopore was fused to Hel308. Single-stranded DNA bridges the physical interaction between Hel308 and MspA. The DNA, bound to Hel308, is attracted through the MspA membrane pore via an electric field, resulting in Hel308 positioned onto the MspA. Once Hel308 is attached, movement of DNA through the pore is dependent on motor activity of the enzyme, rather than the change in electrical current. A change in the ion current is then measured to determine the DNA position and sequence. Data from this study has been used to detail how Hel308, and by extension, other SF2 helicases, move along the DNA.

HelQ could be used as a new diagnostic marker for ovarian cancer, a disease that is currently challenging to diagnose and has poor prognosis. Understanding the structure and function of HelQ could be used to help diagnosis and treatment of this and other types of cancers. With this potential of HelQ in cancer detection, there are possibilities of HelQ being used as a target in personalized cancer therapy. This technology could be used to inhibit cancer cells by inhibiting replication of damaged cells, inhibiting HelQ could knock out an essential protein in the pathway. There is already a large existing market of anti-cancer drugs that target DNA replication, for example, cisplatin, which binds to DNA causing strand crosslinks that block replication. However, most patients relapse after treatment due to cisplatin-resistant disease caused by up-regulation of DNA repair, and therefore alternative therapies are constantly needed.

As noted throughout this article, the importance of human HelQ helicase for promoting human health is based on its involvement in DNA repair linked to replication and recombination. Readers may be aware of advances made in the editing of genomes, using biotechnologies based on discoveries from fundamental research into CRISPR-Cas9 genome editing, particularly editing that inserts a new desired DNA sequence at or near the site of Cas9 DNA cleavage DNA. As CRISPR-Cas9 genome editing progresses, we will need a more thorough knowledge of fundamental DNA repair processes in these cells, in order to optimize editing, and to understand the advantages and hazards of the technology when introduced into biological systems. Understanding functions of HelQ, and several other DNA repair proteins that are tied with CRISPR-Cas editing, is needed for this.

Further reading