However, the liver's anatomy indicates that the central zone might constitute a more-protected reservoir of cells, and might be a preferable location for cells involved in homeostatic self-renewal.

Hepatocytes in the pericentral region immediately adjacent to the central vein are known to replicate slightly faster than other hepatocytes in normal conditions[6], and to be the only hepatocyte population that expresses genes activated by the Wnt signalling pathway[7,8]. Wang and colleagues used genetic techniques in mice to indelibly label cells expressing a Wnt-responsive gene, such that these cells and their descendants fluoresced. They then tracked this fluorescent lineage and showed that pericentral hepatocytes self-renew — the cells remain close to the central vein and are not normally replaced by other hepatocytes. Over time, these cells give rise to descendants outside the pericentral zone that can replenish up to 40% of the liver's mass under normal conditions (Fig. 1). These findings lead to the question of how pericentral hepatocytes differ from other hepatocytes, and whether such differences depend on proximity to the central vein. Consistent with this, it is now recognized that a stem cell's identity can be dependent on the signals that it receives from its local environment[9,10].

Mammalian cells typically carry two copies of each chromosome, but most hepatocytes carry several copies of this chromosome complement and exhibit chromosomal imbalances on division, making them less than ideal candidates for the population that replenishes the liver[11]. Wang *et al.* observed that many pericentral hepatocytes have the normal chromosome complement, and so seem better suited to replicating their genomes faithfully when they divide. Finally, the authors found that Wnt signals released from the endothelial cells that make up the central vein are required to maintain the proliferation of pericentral hepatocytes and thus their function in replenishing liver cells.

The discovery that pericentral hepatocytes, along with other hepatocytes[1,3], contribute to liver homeostasis opens up many avenues for study. For instance, the relative contribution of each of these cell types to homeostatic regeneration is not known. The role of pericentral hepatocytes in regeneration following non-periportal forms of liver damage also remains to be determined. Could a better understanding of the cells enable self-renewal to be enhanced? Manipulating the Wnt pathway *in vivo* might provide insights along these lines, as has been shown for liver 'organoids' grown *in vitro*[12].

Perhaps the most important question is whether the pericentral hepatocytes behave as a niche-dependent stem-cell population[9,10]; that is, whether any hepatocyte placed in the pericentral region, under the influence of endothelial Wnt signalling, would become Wnt-responding, faster-replicating cells, functioning like the original pericentral cells. This crucial test could be carried out by ablating pericentral cells, for example through the transient induction of diphtheria toxin, and determining whether other hepatocytes take on their role. If it could be shown that any hepatocyte — or at least any hepatocyte with a normal chromosome complement — when placed in the pericentral region or exposed to the correct Wnt signalling could be 'activated' to become a more-efficient cell for liver homeostasis, this could have an impact on treatments for chronic liver disease.

But almost all hepatocytes, regardless of their position in the liver, can self-renew and contribute to liver homeostasis[1,3]. Thus, it may be that it is not appropriate to consider whether any one hepatocyte population is the true homeostatic stem cell. Instead, a more pertinent question might be whether some hepatocytes are better at self-renewing than others.

Interestingly, the authors found that pericentral hepatocytes are the only adult hepatocyte population to express Tbx3, a transcription factor that is essential for the development of hepatoblasts[13], the precursors of hepatocytes and bile-duct cells in the early embryo. Direct signalling from adjacent endothelial cells promotes embryonic hepatoblast growth[14]. Thus, the pericentral hepatocytes live in an environment that shares features with embryonic liver development. But hepatoblasts are bipotential, whereas the pericentral hepatocytes seem to give rise to hepatocytes only, indicating differences in the networks that regulate these cells types. Understanding the similarities and differences between the pericentral hepatocytes and hepatoblasts, and between pericentral hepatocytes and other hepatocytes in the liver, is sure to provide crucial insights for the liver and regeneration research fields. ■

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DNA REPLICATION

# Strand separation unravelled

**The DNA double helix must be separated into single strands to be duplicated. A structure of the Mcm2–7 helicase enzyme responsible for this activity yields unprecedented insight into how the process is initiated.** SEE ARTICLE P. 186

MATTHEW L. BOCHMAN & ANTHONY SCHWACHA

The successful replication of double-stranded DNA, an essential part of cell division, depends on a helicase enzyme that separates the two component strands. Although simple helicases have been extensively studied[1], much less is known about the complex replicative helicases found in eukaryotes (the group of organisms that includes animals, plants and fungi). But that is about to change. On page 186 of this issue, Li *et al.*[2] capitalize on advances in cryo-electron microscopy[3] to resolve the structure of a eukaryotic helicase, Mcm2–7, to a near-atomic resolution of 3.8 ångstroms — around five times higher than the best Mcm2–7 structure reported so far[4]. Combined with previous studies, this structure indicates how a key step in DNA replication occurs: the initial 'melting' of double-stranded DNA into single strands.

Mcm2–7 has a central role in eukaryotic DNA replication. Like similar helicases from bacteria, archaea and viruses, it unwinds double-stranded DNA (dsDNA) by binding one strand in its central channel, excluding the other. Energy, provided by the enzyme's ability to hydrolyse ATP molecules, enables the complex to translocate along the bound DNA,

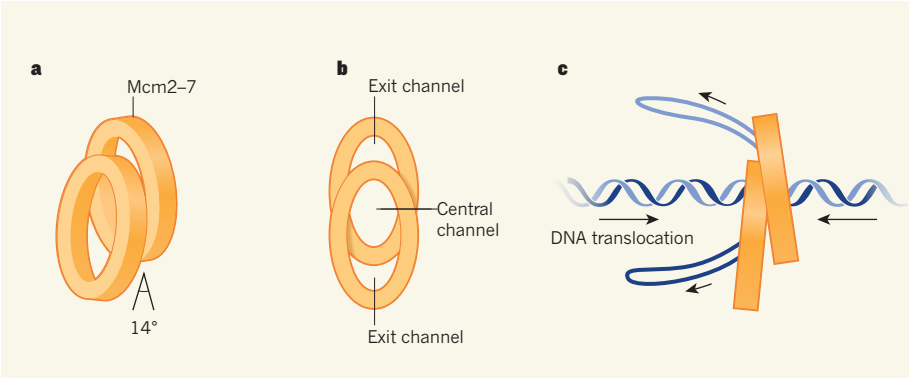


**Figure 1 | Structure of a strand separator.** **a**, The Mcm2–7 helicase is a doughnut-shaped enzyme composed of six different subunits (individual subunits not shown) that is vital for DNA replication[5]. Li *et al.*[2] resolve a structure that contains two copies of this hexamer, which they suggest is involved in the DNA melting process that converts double-stranded DNA (dsDNA) into single strands to initiate DNA replication. In the authors' structure, the two hexamers are tilted at a 14° angle relative to one another. **b**, This conformation forms a narrow central channel between the two hexamers through which dsDNA can pass, together with two exit channels. **c**, dsDNA pumped through the central channel might be extruded through the exit channels as single-stranded DNA 'rabbit ears', which can then be replicated.

resulting in unwinding of the complementary strand[1]. But the doughnut-shaped Mcm2–7 is structurally and functionally different from other helicases, because it is the only known hexameric helicase to be derived from six different subunits (Mcm2 to Mcm7) instead of from six copies of the same subunit. This feature has allowed portions of the complex to evolve extra, specialized functions that are thought[5] to be crucial to the enzyme's ability to load onto DNA and to activate its unwinding activity — two landmark regulatory events during DNA replication.

Two structures containing Mcm2–7 have been described previously. One represents the CMG complex[6,7], which is active during the phase of DNA replication known as elongation, when complementary DNA is synthesized for each existing strand. The complex contains one Mcm2–7 hexamer and two other essential replication factors that activate the enzyme's DNA unwinding ability. By contrast, the second structure[8,9] is an inactive form of the enzyme, which has been isolated from cells before they replicate. This structure contains two Mcm2–7 hexamers in a head-to-head orientation, enclosing dsDNA in the central channel. Helicase structures such as this Mcm2–7 double hexamer (Mcm2–7 DH) are rare, and so its purpose has been a cause for debate.

One reasonable conjecture is that the Mcm2–7 DH participates in DNA melting. Whereas DNA unwinding enlarges a pre-existing single-stranded DNA (ssDNA) region during elongation, DNA melting, which is an earlier process, initiates replication by locally transforming dsDNA into ssDNA. Local melting provides a site for the subsequent assembly of a DNA replication fork — the full complement of proteins that enable duplication of the genetic material[10]. Although melting has been well studied in bacteria, little is known about how it occurs in eukaryotes[10]. Li and colleagues' structure, when combined with other data, is highly consistent with a role for the Mcm2–7 DH in DNA melting, for several reasons.

First, both the current study and a previous one[4] demonstrate that the two Mcm2–7 hexamers in the Mcm2–7 DH are offset along the long vertical axis of the hexamer, at a 14° tilt relative to one another (Fig. 1a). This offset restricts the dimensions of the central channel (Fig. 1b). Although DNA is not visible in the authors' structure, these data suggest that dsDNA will be kinked at the interface between the two hexamers. Sharp DNA bending is known to cause local DNA melting[11], and may contribute to the unwinding of dsDNA during transcription[12]. Thus, a DNA kink between the two Mcm2–7 hexamers could serve to initiate DNA melting.

Second, although helicases normally interact productively only with ssDNA, a specific form of the Mcm complex (Mcm467) has been shown to bind to and translocate along dsDNA[13]. This is consistent with a potential role for Mcm2–7 in manipulating dsDNA during melting. Finally, unlike bacterial hexameric helicases, some viral replicative helicases, such as papillomavirus E1 and simian virus-40 (SV40) large T-antigen, initially form dsDNA-containing DHs that resemble the Mcm2–7 DH (refs 14, 15). These structures locally melt DNA and then uncouple into single hexamers to unwind DNA during elongation.

How might dsDNA melting occur? Electron microscopy indicates that the SV40 large T-antigen DH can act as a pump[13], in which dsDNA enters each hexamer from flanking regions and ssDNA is extruded in 'rabbit ear' structures at the interface between them[14]. Consistent with such a mechanism in eukaryotes, the misalignment of the two hexamers in the Mcm2–7 DH creates two exit channels at the hexamer interface through which rabbit ears might be extruded (Fig. 1c). Thus, the Mcm2–7 DH might melt DNA in a manner analogous to melting on SV40 large T-antigen, with local unwinding of the bent DNA forming a highly flexible hinge to facilitate ssDNA extrusion. Such a model had been proposed to explain Mcm2–7 DNA unwinding during elongation[13]. Because the Mcm2–7 DH seems to be enzymatically inactive, further research will be needed to identify the factors required to activate the DH for melting, as well as to determine how the individual Mcm2–7 hexamers physically uncouple and are remodelled into the ssDNA-bound form needed for elongation.

Given the technical advances in cryo-electronmicroscopy, a flood of high-resolution structures should become available in the near future. However, such structures provide only a static glimpse of the target protein, a particularly limiting problem for the study of dynamic processes such as DNA replication. Because Mcm2–7 is only one of many molecular motors involved in DNA replication, understanding the dynamic nature of their interactions is essential for a complete understanding of DNA replication. To this end, single-molecule studies using reconstituted eukaryotic replication systems[7,16] have begun to shed much-needed light on the dynamics of this process. Together, these varied experimental approaches should yield a holistic understanding of the vital process of DNA replication. ■

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