occurs during the acute phase of ischaemia, owing to lack of oxygen. This might explain why there is initially no extensive cell death during ischaemia, but that it instead occurs after reperfusion, when NAD^+ levels are once again replenished. There could therefore be a window of opportunity for inhibiting ischaemic injury by blocking necroptosis before reperfusion occurs.

Little is known about the composition of complex IIb and how it is regulated. The present study reveals one aspect of this complex, together with a new layer in the regulation of necroptosis. But there is still much to be learnt. For instance, given that acetylated RIP1 does not interact with RIP3, how does RIP3 bring sirtuin-2 to RIP1? It could be that RIP1 and RIP3 form a transient interaction complex that must be stabilized by RIP1 deacetylation. It is equally likely that there are other scaffold proteins that bring RIP1 and RIP3 together. Also, the kinase activity of RIP1 and RIP3 is essential for their interaction: the two proteins both phosphorylate themselves and each other. But it is unclear how phosphorylation and deacetylation are coordinated to achieve the active state of complex IIb. Finding answers to these questions will be a major goal, especially given the relevance of necroptosis to human disease. ■

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BIOCHEMISTRY

Molecular hurdles cleared with ease

Single-molecule studies reveal that a ring-like enzyme that encircles and 'slides' along one strand of duplex DNA, separating it from the other strand, overcomes molecular barriers in its path by transiently opening its ring. SEE ARTICLE P.205

MICHAEL A. TRAKSELIS & BRIAN W. GRAHAM

For all the benefits that are gained by encoding genetic information in double-stranded DNA, the long, linear, antiparallel arrangement of complementary strands in DNA duplexes provides challenges for enzymes that replicate genomes. Many proteins involved in replication have therefore adopted oligomeric, toroidal conformations that allow them to remain bound to, and to act on, DNA over long distances. One such protein complex is the DNA helicase, which regulates the initiation of replication, separates duplex strands and can traverse many obstacles in its path.

Most organisms have a circular DNA-replication helicase consisting of six subunits. The large T antigen of simian virus 40 is a well-characterized example, and serves as a model for deciphering the mechanism of DNA unwinding in eukaryotes (organisms that include plants, animals and fungi). Conflicting evidence has fuelled a debate about whether a single or a double hexamer of large T antigen encircles and acts on single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) during unwinding. On page 205 of this issue, Yardimci *et al.*[1] convincingly show that a double hexamer of large T antigen assembles at replication origins, and then separates into two single hexamers, which unwind dsDNA by encircling and translocating along each ssDNA in the 3′-to-5′ direction*.

In addition to its hexameric unwinding ability, the authors describe the surprising ability of large T antigen to bypass large objects that are covalently attached to the encircled translocating strand, highlighting the hexameric structure's remarkable plasticity. Although other hexameric helicases have been observed to have similar translocating and unwinding abilities in single-molecule studies[2–5], this is the first report of a helicase overcoming a covalent block to unwinding.

Replication helicases face three main challenges. First, the enzymes must intimately associate with the DNA, but at the same time be able to slide efficiently along it over great (molecular) distances. Second, separation of the stable duplex into single strands is required before each of the strands can be replicated. And third, genomes are huge, and the strands of duplex DNA are bound by an array of

*This article and the paper under discussion[1] were published online on 28 November 2012.

different proteins along its length, which may interfere with replication.

Previous structural studies[6–8] have indicated that a double hexamer of large T antigen pumps dsDNA into the protein's central channel (Fig. 1a), where it separates into two ssDNA sequences that are extruded out of side channels to form rabbit-ear-like loops. This double-hexamer structure was predicted to remain intact during the entire course of unwinding. Large T antigen also initiates DNA replication by binding specifically to replication origins as a double hexamer. The assembly of a double hexamer is an intriguing initiation activity, but it is inconsistent with nearly every other unwinding mechanism identified for replication helicases, in which single hexamers act through a steric exclusion mode: they encircle one strand while excluding the other.

To address this inconsistency, Yardimci *et al.* performed single-molecule studies of large T antigen in the process of unwinding origin-containing dsDNA. They tethered dsDNA to a surface in a microfluidic flow chamber and added large T antigen so that it bound to the DNA. They then added a cell extract containing replication proteins, to initiate DNA replication, followed by a type of nucleotide that, when incorporated into replicated dsDNA, could be tagged with a fluorescent label. By monitoring the growth of newly replicated, fluorescently labelled DNA, Yardimci *et al.* calculated the fork rate (the rate of bidirectional replication). They observed that when both ends of the original DNA were tethered to the surface such that it was stretched to prevent rabbit-ear ssDNA from forming, the fork rate was the same as that of DNA tethered at just one end. This is a telling result — if large T antigen unwinds DNA as a double hexamer, then its ability to unwind the doubly tethered DNA should be reduced, because it cannot pump such dsDNA into its central channel. The authors' results therefore show that the large T antigen double hexamer separates into single hexamers, which each unwind DNA in opposite directions from the origin (Fig. 1b).

To further distinguish whether ssDNA or dsDNA passes through large T antigen's central channel, Yardimci *et al.* tested the helicase's ability to unwind dsDNA in which the protein streptavidin was non-covalently attached to the leading and/or lagging strands. If dsDNA is encircled by large T antigen, streptavidin would be removed if it is on either of the single strands, or on both strands; but if ssDNA alone is encircled by the helicase, streptavidin would be removed only from the translocating strand. Consistent with a single hexamer encircling only one strand, the authors observed streptavidin displacement from the leading strand but not from the lagging strand. The researchers also performed single-molecule experiments in which dsDNA was non-covalently tagged with large fluorescent labels called quantum dots, instead of streptavidin. These experiments confirmed that unwinding occurs in the 3′-to-5′ direction, with the helicase travelling along the leading strand.

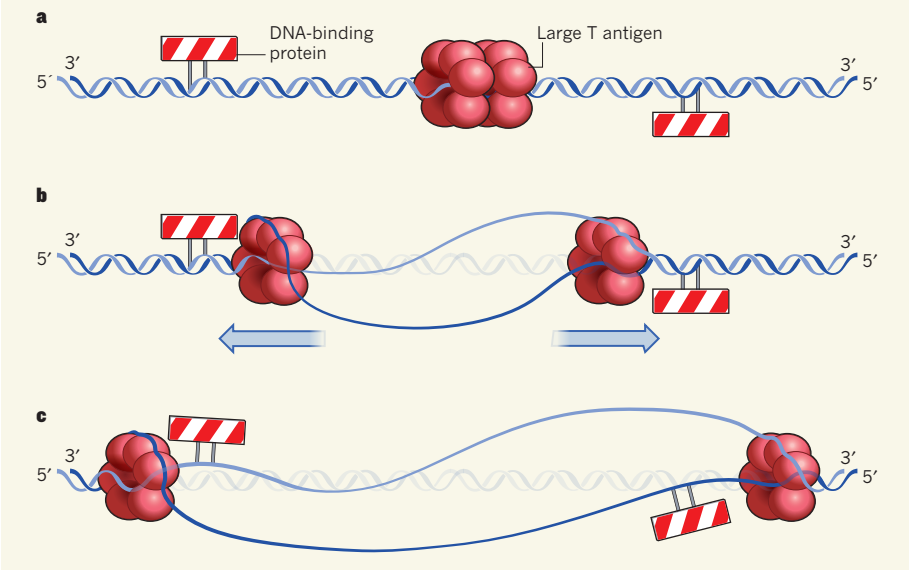

Figure 1 | Mechanism of DNA unwinding by the large T antigen of simian virus 40. Hexameric DNA-replication helicase enzymes, such as large T antigen, bind to double-stranded DNA (dsDNA) at sites known as replication origins before separating the duplex into single strands. **a**, Yardimci *et al.*[1] report that large T antigen binds dsDNA as a double hexamer. **b**, The double hexamer then splits into two single hexamers that each track along a 'leading' strand in the 3′-to-5′ direction while excluding the other, 'lagging', strand, unwinding dsDNA as they go. **c**, If the hexamers encounter a 'roadblock', such as a protein covalently attached to the leading strand, they can bypass it efficiently without dissociating from the DNA.

Surprisingly, Yardimci *et al.* found that many of the bulky quantum dots on the leading strand were bypassed by large T antigen, without affecting the unwinding rate. To provide a more natural block to unwinding, the authors covalently attached a protein called HpaII methyltransferase (M.HpaII) to either the leading or the lagging strand. Amazingly, in both cases, unwinding occurred with equal efficiency in spite of the covalent block.

To test whether the helicase remains attached to DNA on encountering a covalently attached blockage, or whether a new helicase is recruited instead, the authors assembled large T antigen on DNA, washed away any unbound protein and then mixed the large T antigen–DNA assembly with replication protein A to initiate unwinding. Once again, they observed that the unwinding ability of large T antigen was not significantly different when M.HpaII was covalently attached to either the leading or the lagging strands, which suggests that a preassembled helicase is able to bypass the 'roadblock' (Fig. 1c). When the researchers attached two M.HpaII proteins to DNA 60 bases apart on the leading strand, the unwinding rate of the DNA by large T antigen was again similar to that of unadulterated DNA. However, when Yardimci *et al.* tested DNA labelled with proteins attached just 14 bases apart, unwinding was strongly inhibited. It will be interesting to determine whether the second block limits large T antigen's ability to remain bound to the DNA in this case, or whether the proximity of the blocks prevents large T antigen from reassembling around ssDNA.

The authors suggest that plasticity within the hexamer ring is responsible for the transient opening of translocating large T antigen, and that this opening overcomes natural barriers to unwinding. They also argue that multiple blocks located close together prevent the reclosing of large T antigen, or stimulate the protein's dissociation from DNA. It is known that the asymmetrical hexameric arrangement of subunits in the eukaryotic replication helicase MCM2–7 — which is thought to have an analogous structure and unwinding mode to large T antigen — results in a labile interface between this helicase's MCM2 and MCM5 subunits[9]. This is thought to facilitate the opening and loading of MCM2–7 at replication origins, but might also allow the helicase to open transiently, to bypass protein roadblocks as it unwinds DNA.

It is now important to reconcile exactly how large T antigen bypasses impediments. For example, how are the molecular interactions with DNA in the central channel of the antigen disrupted by a roadblock? If the hexamer opens transiently, how does it remain associated with the DNA? Does the degree of opening depend on the size of the roadblock, and what is the mechanism for reclosure around ssDNA afterwards? Perhaps, after opening of a hexamer, large T antigen jumps or rotates over a roadblock before reassociating with the DNA, akin to a molecular hurdler. Or perhaps interactions with the lagging strand on

the external surface of the large T antigen are required to keep it from fully dissociating from DNA, similarly to what has been observed for an archaeal hexameric helicase[10].

Large T antigen, despite having served as a model for studies of the unwinding mechanisms of hexameric helicases for years, continues to surprise scientists with its abilities. It will be interesting to see whether other hexameric helicases share these abilities, or if there are specific differences between them. In the meantime, Yardimci *et al.* have greatly advanced our understanding of how helicases unwind long stretches of DNA in the presence of competing cellular processes. ■

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APPLIED PHYSICS

Molecules that convert heat into light

A class of fluorescent organic molecule has been designed that enables highly efficient light-emitting diodes to be made. The devices may turn out to be competitors to their conventional analogues. SEE LETTER P.234

BRIAN D'ANDRADE

Electronic displays are almost ubiquitous, not least because they are the means by which people interact with the Internet. The mobile display market has seen tremendous growth[1], for example, with more than 1 billion mobile phones being sold in 2006. Liquid-crystal displays are a dominant technology for display applications, but organic light-emitting diodes (OLEDs) have steadily been incorporated into mobile devices and are increasingly being used in televisions. On page 234 of this issue, Uoyama *et al.*[2] report a type of OLED that may offer an alternative to its conventional equivalents.

One of the unique features of an OLED display is that every pixel has an OLED, which is essentially a micrometre-sized light source. The millions of red, green and blue OLEDs in a display all have to be efficient, manufacturable with good yields and capable of providing a highly consistent light output. These requirements have been achieved by improving the organic materials used in OLEDs.

One of the key drivers of OLED technology is novel chemistry. New molecules constantly offer surprises that challenge conventional wisdom and offer alternative solutions. Tang and colleagues[3] introduced the world to modern OLED designs 25 years ago. However, the devices were poor at converting input electric current to light because they incorporated inefficient fluorescent organic materials. Then, in 1998, a team led by Forrest (a physicist) and Thompson (a chemist) introduced[4] devices with phosphorescent molecules that enabled almost 100% efficient conversion. Phosphorescent molecules differ from their fluorescent analogues in that they contain a heavy metal atom such as iridium.

This work led to an explosion of phosphorescent materials that enabled many desirable characteristics, such as a variety of colours, long operational lifetimes and high efficiencies. However, it remains difficult to produce a single display that uses blue, green and red phosphorescent devices, because the operational lifetimes and colour characteristics of some phosphorescent devices do not meet the strict performance requirements for display applications. It is important to solve these problems because phosphorescent materials are the most promising materials for displays, with the potential to deliver nearly 100% efficiency. Now, Uoyama *et al.* describe how they have made efficient OLEDs that use a special type of fluorescent material.

When an organic molecule absorbs electrical energy, it moves from its original state to a higher-energy (excited) state. For high-efficiency devices, the excited molecule preferably sheds the extra energy by producing a photon of light when it returns to its original, unexcited state. The molecular transition from excited to unexcited states has a few strict rules related to quantum-mechanical conditions that must be satisfied. These rules prevent every excited molecule from emitting a photon and returning to an unexcited state. As a result, fluorescent materials used in OLEDs often generate heat instead of light. Phosphorescent materials break those rules by using a heavy metal atom, such as iridium, bonded to the organic materials (typically comprising carbon, nitrogen and hydrogen) in an individual molecule. This makes it significantly more likely that after absorbing electrical energy, a phosphorescent molecule can efficiently transition from a high energy state to a low one by emitting a photon.

Uoyama and colleagues developed fluorescent molecules that can achieve high efficiency by using heat instead of a heavy metal atom. They convincingly demonstrated the molecules' potential by making highly efficient fluorescent OLEDs with a performance comparable to that of phosphorescent OLEDs. Their fluorescent molecules are based on carbazolyl dicyanobenzene and as such have the potential to be synthesized at low cost, Uoyama *et al.* believe, because the synthetic process does not require rare metal catalysts or rare heavy metal atoms. Furthermore, the OLEDs can emit multiple colours, from sky-blue (with a peak wavelength at 473 nanometres) to orange (577 nm).

There are many challenges ahead before these molecules can be fully accepted into production. The emissive colours have to be developed for display applications; the operational lifetime of devices that incorporate the molecules needs to be comparable to, or preferably better than, state-of-the-art devices (possibly the hardest challenge); and the ability to manufacture products based on the new OLEDs must be proven. The molecules are not certain to replace existing ones, but they may be shaped into contenders in terms of the cost and quality of desirable performance characteristics. Indeed, perfecting the new molecules will take time and effort, but Chihaya Adachi, who leads the work and is a co-author of the study[2], has the experience needed to help his team to overcome these obstacles. ■

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