A TALE OF TOROIDS IN DNA METABOLISM

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A strikingly large number of the proteins involved in DNA metabolism adopt a toroidal — or ring-shaped — quaternary structure, even though they have completely unrelated functions. Given that these proteins all use DNA as a substrate, their convergence to one shape is probably not a coincidence. Ring-forming proteins may have been selected during evolution for advantages conferred by the toroidal shape on their interactions with DNA.

PROCESSIVITY
The ability of an enzyme to catalyse more than one turnover before releasing the substrate or product of the reaction.

REPLISOME
The multi-protein assembly at the junction of the DNA replication fork.

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High-resolution studies of protein structure have led to the discovery of several ring-shaped macromolecules that have essential metabolic functions. These proteins generally contain several subunits, and provide an enclosed environment for chemical reactions that may otherwise be unfavourable. Examples of such circular proteins include the F_1-ATPase[1], molecular chaperones that catalyse protein folding (for example, *Escherichia coli* GroEL)[2], the proteasomes that catalyse protein degradation[3], and bacterial light-harvesting complexes[4].

Among these diverse toroids is a subset of proteins dedicated to DNA metabolism, that physically or chemically manipulate DNA during DNA replication, repair and recombination[5]. In fact, a disproportionately large number of the proteins involved in DNA metabolism assume a circular shape. They range from sliding clamps, which passively diffuse on DNA, to the helicases that catalyse ATP-fuelled DNA unwinding, or to enzymes such as bacteriophage λ exonuclease and topoisomerases that chemically modify DNA. Many of these proteins seem to have unrelated evolutionary origins and catalyse very different reactions on DNA, although they share a quaternary ring-shaped structure.

So why have so many proteins adopted the same overall shape? Possibly because they all work on DNA — the distinctive structure and properties of the DNA polymer could have stimulated convergence to the toroidal form. The sheer number and diversity of toroidal proteins using DNA as a substrate — in contrast to protein or RNA substrates — also implies that this form has had a marked effect on the development of complex DNA metabolic processes. This review describes prominent ring-shaped proteins that work on DNA, and highlights the effect of their toroidal form on function, to try and understand why this form is so successful in DNA metabolism.

Circular sliding clamps

Sliding-clamp proteins belong to the family of PROCESSIVITY factors, whose main function is to increase the lifetime of other proteins on DNA. Clamps are an integral part of the REPLISOME, where they facilitate a stable interaction between DNA polymerase and the primer–template during DNA replication[6,7]. The speed of replication is influenced greatly by these proteins, as the polymerase alone tends to fall off the primer–template every few nucleotides, slowing down DNA synthesis. In contrast, when tethered to DNA by a sliding clamp, the polymerase becomes highly processive and extends the primer by thousands of nucleotides without falling off, resulting in efficient replication of genomic DNA[8–10].

Early biochemical studies showed that clamps move freely on DNA and fall off the ends of linearized DNA molecules, therefore predicting that these proteins interact with DNA using a sequence-independent topological linkage[11]. This hypothesis was confirmed by the first crystal structure of a clamp, *E. coli* β, which revealed a circular protein with a central hole large enough (35 Å diameter) to accommodate double-stranded/duplex DNA with no steric hindrance (FIG. 1a)[12]. Two semi-circular β monomers form a tightly sealed ring that can remain stably linked to DNA with a half-life of about

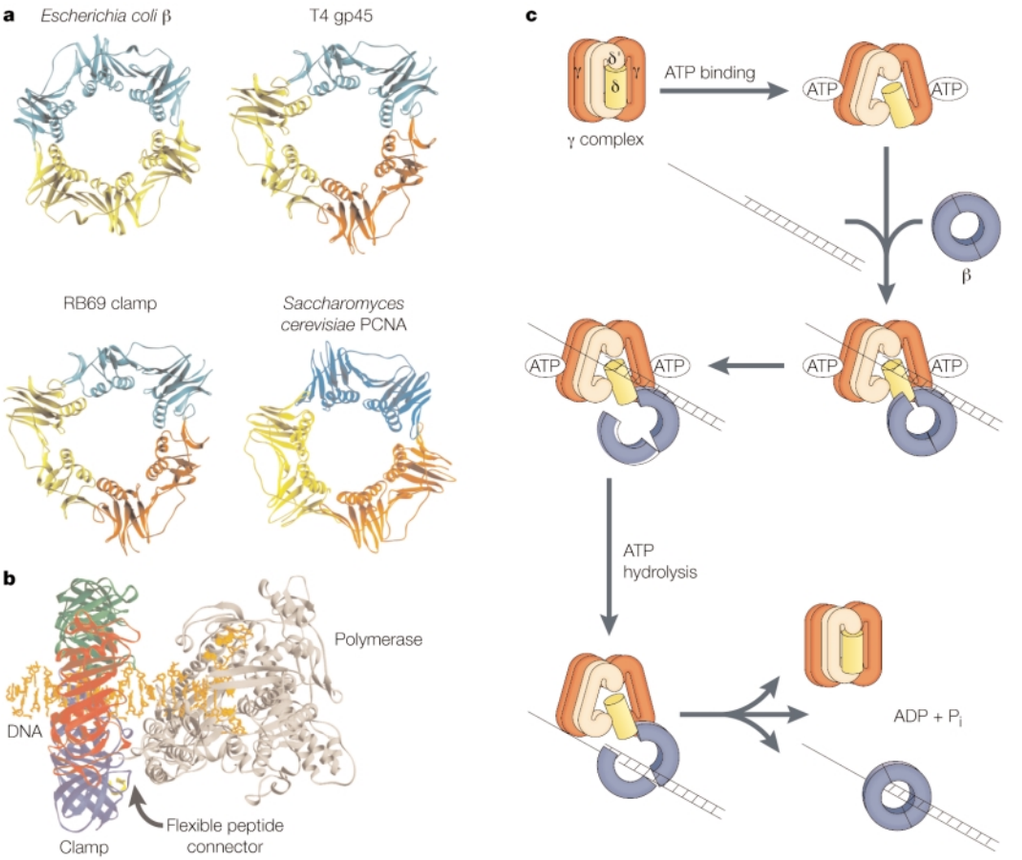

Figure 1 | **Circular sliding clamps. a** | Top views of sliding clamps from bacteriophage to humans, showing the central channel of about 35 Å that encloses duplex DNA. **b** | Model structure of the phage RB69 clamp bound behind the polymerase (grey) on DNA (orange) through a short peptide connector. **c** | Model mechanism of ATP-driven clamp-loader-catalysed assembly of the *E. coli* β clamp on DNA. Clamp assembly starts when two or more γ-subunits (red) bind ATP and change the conformation of the γ-complex , so that the δ-subunit (yellow) is exposed[85,86]. The δ-subunit binds the dimeric β-clamp (blue) and opens it at one interface[82,86]. The ATP-bound γ-complex also binds primed DNA with high affinity and brings it in close proximity to the open clamp[85]. Next, ATP hydrolysis by the γ-subunits is coupled to closure of the clamp around DNA and release of the topologically linked clamp–DNA complex for use by DNA polymerase[87].

100 minutes[13]. The inside of the ring is positively charged and probably contacts the DNA phosphate backbone through nonspecific, water-mediated interactions, which allow the ring to slide in a sequence-independent fashion on DNA. Sliding clamp proteins from bacteriophage (T4 and RB69 clamps)[14,15], *Saccharomyces cerevisiae* (the yeast proliferating cell nuclear antigeny, yPCNA)[16] and humans (hPCNA)[17] have similar toroidal structures despite minimal sequence homology, but these proteins are smaller, so three monomers are required to form a ring with about the same dimensions as β (FIG. 1a). A recent structure[15] of the RB69 clamp in complex with a carboxy-terminal peptide of the polymerase reveals the connection between the polymerase and the clamp on DNA (FIG. 1b). This has clarified further how circular sliding clamps act as mobile tethers for DNA polymerases and increase their processivity.

Hexameric helicases

Like sliding clamps, hexameric helicases are toroidal proteins that bind DNA within the central channel and move along it. But in this case, translocation is an energy-driven process fuelled by ATP, and is coupled to unwinding of dsDNA[18,19]. Most complex organisms (including bacteria) have several helicases that act in DNA replication, repair and recombination. One example highlighting the importance of helicase activity in nucleic acid metabolism is the BLM helicase, mutations in which are linked to Bloom's syndrome[20] (a human genetic disorder that leads to defects in growth and fertility as well as increased susceptibility to cancer). The *E. coli* DnaB helicase[21] and rho[22] (an RNA–DNA helicase and transcription terminator) were among the first helicases found to function as hexamers, and electron microscopy revealed that the six subunits form a ring (FIG. 2a). Hexameric ring-shaped helicases have now been found in various organisms including bacteriophage (for example, T7 gp4 (REFS 23,24), T4 gp41(REF. 25) and SPP1 gp40S (REF. 26)), viruses (for example, SV40 T antigen[27]), bacteria (for example, *E. coli* DnaB[28], rho[29] and RuvB[30]),

eukaryotes (for example, human mini-chromosome maintenance (MCM) complex[31] and BLM helicase[32]) and archaea (for example, *Methanobacterium thermoautotrophicum* MCM[33]) (FIG. 2a).

The helicase rings range from 120–140 Å in diameter with an inner diameter of 20–40 Å, which is large enough to accommodate single- or double-stranded DNA[18]. Whereas helicases from the T7 (REFS 23,34) and T4 (REF. 35) bacteriophages as well as *E. coli* DnaB[36] encircle ssDNA, the bovine papilloma virus E1 helicase[37] and SV40 T antigen[38] might encircle ssDNA or dsDNA. One model for DNA unwinding places the hexameric helicase at the replication fork with one ssDNA strand bound within the ring and one excluded from the ring[18]. FIGURE 2b depicts how the T7 helicase binds the 5′ strand inside the ring with a specific polarity[23], and can unwind DNA in the 5′–3′ direction but exclude the 3′ strand. Consistent with this 'exclusion' model, the unwinding activity of both the T7 and T4 helicases is strongly inhibited by a large block in the 5′ ssDNA, but is insensitive to a similar block in the 3′ ssDNA[39–41]. So at the fork junction, just the unidirectional translocation of the helicase ring on a single DNA strand could drive duplex DNA unwinding. Other versions of this model suggest that helicases might also manipulate the excluded ssDNA and/or dsDNA at the fork (through interactions with the outside of the ring) to destabilize the base pairs in duplex DNA. Unwinding could be aided further by interaction between the helicase and DNA polymerase. For example, both the T7 helicase[42] and DnaB[43,44] bind their respective DNA polymerases. The polymerase may assist passively by imposing unidirectionality on the helicase (into the duplex DNA) or by an active mechanism in which the forward driving force of the polymerase accelerates helicase activity.

The translocation and DNA unwinding activity of helicases depends on their ATPase (or NTPase) activity[18,19]. The binding affinity of hexameric helicases to DNA is increased in the presence of NTP (nucleoside 5′-triphosphate) relative to NDP (nucleoside 5′- diphosphate), indicating that nucleotide binding and hydrolysis modulate their interaction with DNA[45,46]. Furthermore, rapid kinetic analyses of T7 helicase[47] and *E. coli* rho[48] indicate that the hexamers may use a sequential NTPase mechanism (similar to F_1-ATPase[1]), in which one active site after another binds and releases DNA — coupled to NTP binding, hydrolysis and NDP release — driving both stepwise movement of the helicase ring on DNA and subsequent DNA unwinding (FIG. 2c).

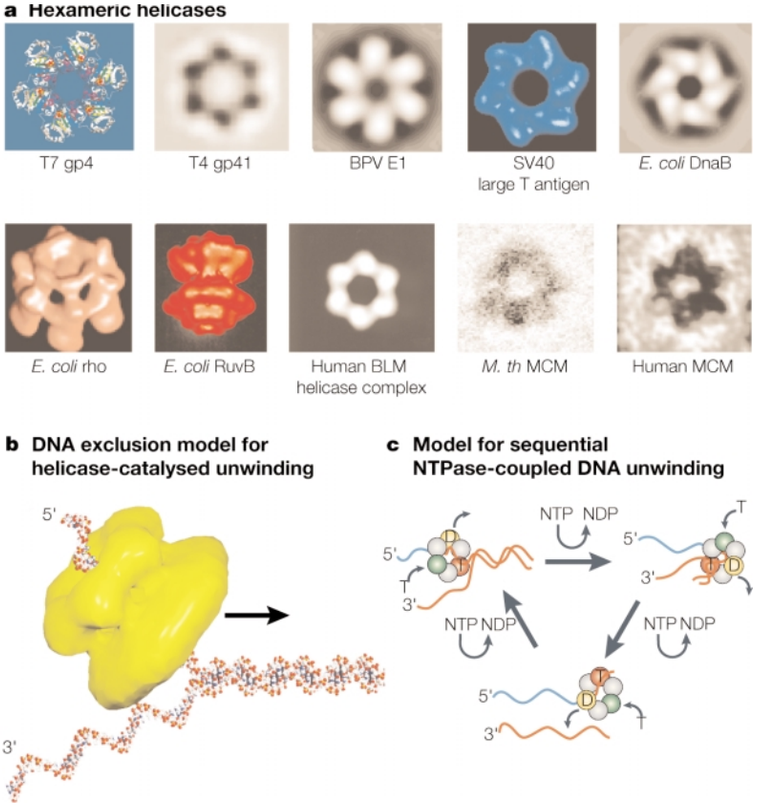

Figure 2 | **Circular helicases. a** | Top views of hexameric helicases from bacteriophage to humans, showing the central channel of about 25–35 Å that encloses single- or double-stranded DNA. **b** | Bacteriophage T7 helicase (yellow) encircling the 5′ strand and excluding the 3′ strand to unwind DNA in the 5′–3′ direction. **c** | A stepwise model mechanism for helicase translocation and DNA unwinding in which active sites on the hexamer consecutively bind NTP (T, red) and DNA, hydrolyse NTP (D, yellow), and release the hydrolysis products as well as DNA.

Recombination proteins

Many proteins in DNA recombination pathways (including DNA-repair-coupled recombination) assemble into multi-subunit toroids containing a central channel. For example, the Red system in bacteriophage λ uses at least two toroidal proteins. One of these, the λ exonuclease, is a trimer of identical subunits arranged in a ring (FIG. 3a)[49]. It binds dsDNA within the central channel, where the exonuclease active sites are located, and degrades one DNA strand in the 5′–3′ direction to generate long 3′ ssDNA overhangs. The width of the channel varies from about 30 Å at one end of the ring to roughly 15 Å at the other, such that dsDNA fits in one end and only ssDNA can fit in the other. The structure implies that the enzyme moves with a specific orientation on DNA, taking in dsDNA at one end, degrading the 5′ strand, and expelling the 3′ strand from the other. This DNA product can be processed further for recombination by the single-strand annealing pathway and the double-strand break repair pathway. Both pathways create primed DNA intermediates, which are then replicated to complete recombination or repair.

In the single-strand annealing pathway, the β protein of phage λ binds ssDNA overhangs and facilitates base-pairing with homologous regions in another single strand of DNA. Electron microscopy shows that the β protein forms oligomeric rings of various sizes[50]. These include small (roughly 12-subunit) rings in the absence of DNA, and larger (15- or 18-subunit) rings in the presence of ssDNA and Mg^{2+}. In the presence of dsDNA, β protein preferentially forms a long protein filament on the DNA duplex (FIG. 3b). Interestingly, when presented with a heat-denatured DNA fragment (roughly four kilobases), β protein first forms large rings on the ssDNA and then makes long helical fila-

STRAND EXCHANGE
The process by which a single DNA strand switches from one duplex DNA molecule to base pair with a complementary strand from a second, homologous duplex DNA molecule.

HOMOLOGOUS RECOMBINATION
The process by which segments of DNA are exchanged between two DNA duplexes that share high sequence similarity.

CATENATION/DECATENATION
Topoisomerases catenate (join) or decatenate (separate) two circular DNA molecules by cutting one DNA strand, passing a second strand through the break, and resealing the break in DNA.

ments under DNA annealing conditions, implying that β rings on ssDNA nucleate β–dsDNA filaments. These structural data, along with biochemical analysis of β–DNA interactions[51], indicate a possible mechanism for β-catalysed annealing of homologous ssDNAs (FIG. 3b). Initially, large β rings may form on the ssDNA overhangs (created by λ exonuclease), possibly wrapping DNA on the outside of the ring such that the bases are exposed. This may accelerate annealing by removing secondary structures, and by presenting the bases for rapid recognition and pairing with a complementary strand. Once annealing begins, the rings seem to convert into the helical β–dsDNA filament shown in FIG. 3b. It is not clear whether β protein dissociates soon after the duplex DNA is formed or if the nucleoprotein filament has a further role in recombination. Structural studies of proteins analogous to β protein, such as bacteriophage P22 Erf[52] and *E. coli* RecT[53], indicate that these proteins form rings and may form filaments (RecT), and implies that they could use similar mechanisms to catalyse ssDNA annealing.

During double-strand break repair, the *E. coli* RecA protein binds ssDNA overhangs (to form a helical RecA–DNA filament), and facilitates STRAND EXCHANGE with homologous regions in a duplex DNA strand. RecA and its bacteriophage and eukaryotic homologues (UvsX and Rad51, respectively)[54,55] catalyse ATP-dependent strand exchange between homologous ss- and ds-DNA substrates[56]. These proteins form helical filaments on ssDNA, which facilitate formation of heteroduplex DNA (between ssDNA and its dsDNA partner) during strand exchange. Recently however, RecA and Rad51 have been found to assemble into rings that could also have some function in HOMOLOGOUS RECOMBINATION[57,58]. The ring may be a vestigial form of the RecA protein, but its persistence in higher eukaryotes, including humans (hRad51), indicates that the ring form may be functional. Moreover, the human DMC1 protein, a meiosis-specific RecA homologue that can weakly catalyse strand exchange *in vitro*, has so far been detected only as an octameric ring (FIG. 3c)[59]. The central channel of the ring is wide enough to encircle ss- and ds-DNA regions of duplex DNA containing single-stranded overhangs. Although hDMC1 rings can stack on DNA, the protein does not seem to form helical filaments on DNA (unlike RecA). These structural data raise the possibility that RecA and its homologues function as rings in an as yet unknown manner during DNA recombination.

Recently, the human RAD52 protein was found to assemble into heptameric rings with a large funnel-shaped channel, 40–60 Å in diameter[60] (FIG. 3d). Eukaryotic Rad52 proteins are essential for DNA recombination and promote annealing of ssDNAs[61]. It is not clear how Rad52 uses its toroidal shape to facilitate ssDNA annealing; it might function like the bacteriophage β protein and Erf to present ssDNA appropriately for rapid annealing, although there is no evidence that Rad52 forms filaments on DNA. Rad52 seems to work with both Rad51 and RPA (replication protein A, a eukaryotic ssDNA-binding protein) in Rad51-mediated strand-exchange reactions, and with RPA for ssDNA annealing[62–64]. One way Rad52 rings could stimulate strand exchange is by binding ssDNA and nucleating or accelerating formation of the Rad51–DNA filament. Likewise Rad52 could modulate the interaction between RPA and DNA to stimulate the annealing reaction. Ongoing studies of how Rad52 binds DNA and its protein partners (Rad51, RPA) will help elucidate its mechanism of action in DNA recombination.

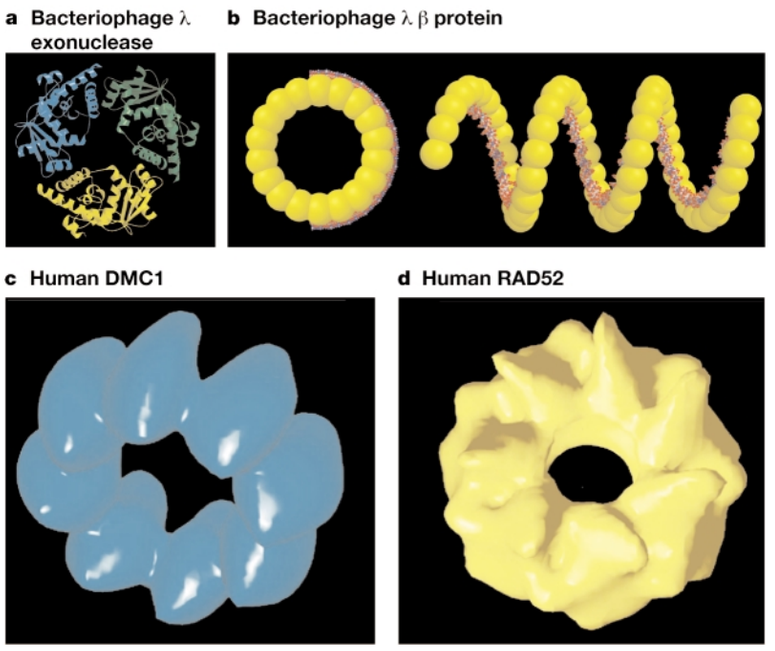

Figure 3 | **Circular recombination proteins. a** | Top view of the bacteriophage λ exonuclease, showing the central channel that binds DNA. **b** | The β protein of bacteriophage λ, as a ring with single-stranded DNA wound around it (left) and as a nucleoprotein filament with duplex DNA (right). **c** | Octameric human DMC1, a meiosis-specific homologue of *E. coli* RecA. **d** | Heptameric human RAD52.

Topoisomerases

DNA topoisomerases break one or both strands of the double helix, pass a single strand or duplex DNA, respectively, through the break and re-ligate the strand(s) to catalyse changes in the topological state of DNA. These enzymes change the 'superhelicity' of DNA and CATENATE or DECATENATE DNA molecules, and are therefore essential for solving topological problems that entangle DNA during replication, recombination, repair or transcription, as well as chromosome condensation and segregation[65]. Topoisomerases belong to two main families: type I enzymes that break only one DNA strand transiently (and are divided further into type IA and type IB topoisomerases), and type II enzymes that break both strands of the duplex. High-resolution structures of topoisomerases from each family have been solved, and all have a toroidal shape with a large central cavity that can accommodate DNA.

Escherichia coli topoisomerases I and III (FIG. 4a) are type IA enzymes in which four domains of a single polypeptide form a toroid with a hole about 25 Å in diameter[66,67]. The proposed mechanism of type IA topoisomerases involves initial DNA binding to an

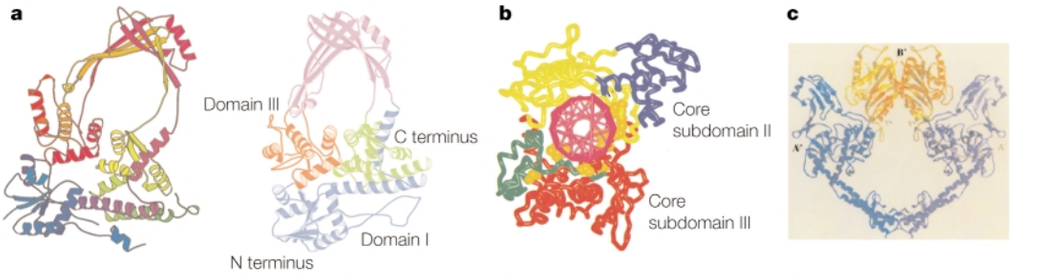

Figure 4 | **Circular topoisomerases. a** | Type IA enzymes, *E. coli* topoisomerase I and III. Domains I and III are thought to move to expose the active site (where one DNA strand is cleaved) and open a gate for entry of the other strand into the central cavity. This is followed by re-ligation and release of both DNA strands to change the topology of DNA. **b** | A type IB enzyme, human topoisomerase I. The core subdomains II and III are thought to part to allow entry of dsDNA (pink) into the central cavity, where one DNA strand is cleaved. The other strand is passed through the break, followed by re-ligation and release of the DNA. **c** | A type II enzyme, *Saccharomyces cerevisiae* topoisomerase II DNA-binding fragment. The proposed ATP-driven mechanism involves binding and cleavage of one dsDNA at the upper domain, parting of the broken strand to allow passage of another duplex into the cavity, then re-ligation and release of both DNAs.

outer site on the ring. This induces ring opening, cleavage of one strand and passage of the other strand through the break into the central cavity, re-ligation of the broken strand and release of DNA, to yield a product with changed topology.

The human topoisomerase I shown in FIG. 4b is an example of a type IB enzyme, the structure and mechanisms of which are unrelated to those of type IA enzymes. In this case, a bi-lobed monomer forms a closed clamp-like structure that encircles the DNA duplex[68,69]. The active site is on the inside of the toroid, and strand cleavage, strand passage and re-ligation all occur in the central cavity.

The third family of topoisomerases, the type II enzymes, catalyse the transport of one dsDNA strand through another in a reaction coupled to ATP[70,71]. FIGURE 4c shows the carboxy-terminal DNA binding or cleavage fragment of *S. cerevisiae* topoisomerase II, in which two monomers are arranged around a central cavity about 50–55 Å in diameter[72]. The enzyme uses energy from ATP to bind one dsDNA strand, cleave it, part the ends for a second DNA strand to pass through into the central cavity, and then re-ligate the first strand. The second strand is then released from the cavity to complete the reaction.

As topoisomerases nick or break DNA as part of their reaction, it is critical that they hold on to the reaction intermediates until the breaks in DNA are resealed, to avoid DNA damage. The toroidal design of topoisomerase addresses this problem, with the central cavity serving as a trap for the DNA intermediates until the reaction is complete.

Other toroids

Several other toroids function in nucleic-acid metabolism, including NAD^+-dependent DNA ligase (*Thermus filiformis*)[73], the *trp* RNA-binding attenuation protein (*Bacillus subtilis* TRAP)[74], the bacteriophage head–tail connector (for example, ϕ29 connector)[75] and translin[76] (FIG. 5). The NAD^+-dependent DNA ligase is a monomeric protein with four domains arranged in a ring (FIG. 5a). Suh and colleagues have proposed[73] that the ligase may bind DNA within the central cavity, in a manner analogous to human topoisomerase I, as it seals the nick in dsDNA. They also speculate that its clamp-like shape may allow the ligase to slide on DNA until it encounters a nicked site.

The TRAP protein forms an 11-subunit ring that helps to terminate transcription of the *trp* operon. The crystal structure of TRAP in complex with RNA shows how each subunit interacts with a GAG triplet repeat (FIG. 5b)[74]. When tryptophan levels in the cell are high, TRAP binds these repeats to facilitate formation of a transcription-terminator hairpin structure in the messenger RNA, which blocks further mRNA synthesis.

The head–tail connector protein has several functions in the bacteriophage life cycle. It is a circular assembly of 12 identical subunits that bind DNA[77,78] and also interact with an accessory ATPase factor. Together, these proteins pump DNA into the phage head[75], possibly by an ATP-dependent mechanism analogous to that of RuvB (a ring-shaped DNA pump/helicase involved in homologous recombination) (FIG. 2)[79].

Translin is an octameric toroid that binds specific sequences at ssDNA ends of broken DNA[76]. Translin is highly conserved among vertebrates[80], implying that it serves essential functions in these organisms. Possibly, it recognizes chromosomal DNA breakpoints, and could also form a scaffold for assembly of other proteins that repair or otherwise process broken DNA.

Ring assembly on DNA

The closed ring structure of the proteins described above, and the fact that they generally bind DNA in the central channel, raises the question of how DNA gains access to the inside of the ring. The answers are as varied as the protein rings themselves. Topoisomerases (IA and IB) and DNA ligase seem intrinsically capable of large conformational changes that open and close the ring — much like a clam shell — around DNA[67,73,81]. Other pro-

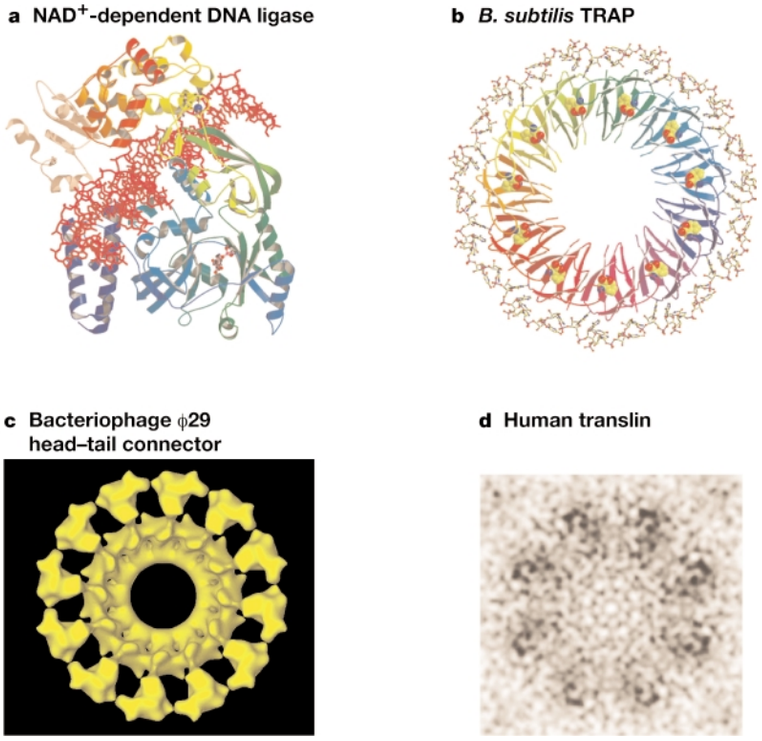

Figure 5 | **More circular proteins. a** | *Thermus filiformis* NAD⁺-dependent DNA ligase with dsDNA modelled within the central channel. **b** | *B. subtilis trp* RNA binding attenuation protein (TRAP) bound to 11 tryptophan molecules and 11 repeating GAG triplets; similar TRAP binding to mRNA *in vivo* facilitates formation of a transcription-terminator hairpin structure in the RNA, effectively blocking tryptophan biosynthesis. **c** | The bacteriophage ϕ29 head–tail connector that promotes packaging of DNA into the phage prohead. **d** | Human translin, an octameric protein ring that recognizes chromosomal DNA breakpoints.

teins require assistance to form a topological link with DNA. For example, the circular sliding-clamp proteins are assembled around DNA by MOLECULAR MATCHMAKER proteins, known as 'clamp loaders', in a reaction fuelled by ATP (FIG. 1c). Clamp loaders from *E. coli* (γ complex)[82], bacteriophage T4 (gp44/62)[83] and eukaryotes (replication factor C or RFC)[84], are multi-protein complexes in which the ATPase activity of one or more subunits drives the other subunits to bind the circular clamp and DNA, open the clamp and close it around the DNA[82–87].

Hexameric DNA helicases also form stable closed rings in the presence of Mg^{2+} and/or NTP, so they need a mechanism for assembly around DNA[18] (FIG. 2). Studies of the bacteriophage T7 helicase[88] and *E. coli* rho (D. E. Kim and S. S. Patel, unpublished observations) indicate that the DNA may first bind outside the ring, inducing conformational changes that open the ring and allow DNA to slip inside. Several helicases also interact with accessory protein factors that may facilitate their assembly on DNA. In bacteriophage T4, for example, the gp59 protein stimulates interaction between the gp41 helicase and DNA[89]. In *E. coli*, the DnaC protein facilitates assembly of the DnaB helicase on DNA[90]. Likewise, the Cdc6 protein may be required for assembly of the MCM helicase complex onto DNA[91,92]. Both gp59 (REF. 93) and DnaC[94] bind their respective helicases as well as DNA with high affinity, and may work by simply increasing local concentration of the helicase near DNA (where it can self-assemble around DNA). Alternatively, these proteins could be molecular matchmakers that load the helicase ring onto DNA, possibly by ATP-driven mechanisms analogous to those used by clamp-loader proteins.

The requirement for a mechanism of assembly around DNA introduces a measure of specificity to the interaction between DNA and toroidal proteins, and presents a method of regulating their activity on DNA. This point is highlighted by the λ exonuclease ring, which does not self-assemble around DNA and does not use an accessory exonuclease-loader. Consequently, λ exonuclease can initiate DNA degradation only at free dsDNA ends (which can thread into the ring), and its nuclease activity is limited to specific loci such as breaks in dsDNA[95]. Regulation of the accessory proteins that catalyse ring assembly on DNA further influences the activity of toroidal proteins, and can have far-reaching consequences on DNA metabolism. For example, Cdc6, the MCM helicase loader, is subject to cell-cycle regulatory pathways[97], and helicase-catalysed unwinding of the replication origin is required for assembly of replication-initiation proteins on DNA[7,96,97]. So modulation of Cdc6 activity affects MCM activity on DNA, which in turn can affect initiation of replication. Similarly, individual components of the eukaryotic RFC complex, which is responsible for loading (and unloading) PCNA clamps onto DNA, have been implicated in cell-cycle checkpoint pathways[84]. Therefore modulation of clamp assembly (through the clamp loader) and consequent modulation of DNA polymerase activity may be one method by which cell-cycle regulatory pathways influence DNA metabolism and vice versa.

Why rings?

Within the classes of toroids discussed above, there are a few proteins that have similar functions but do not assume a toroidal shape. Most notable among these are the monomeric and dimeric DNA helicases as well as the monomeric processivity factors of DNA polymerases. *E. coli* Rep helicase and UvrD (helicase II) have two subunits that appear to alternately bind and release DNA (in an ATPase-coupled mechanism), for unidirectional translocation and DNA unwinding[19], and a recent study indicates that the UvrD helicase is also active as a monomer[98]. The PcrA helicase seems to function as a monomer, with DNA binding and release alternating between two sites on the same protein, allowing the helicase to move like an 'inch-worm' on DNA[99].

So why do so many DNA helicases function as rings? The most prominent benefit of a circular shape is the ability to form a topological link with DNA. Hexameric helicases bind DNA within the central channel, ensuring that when DNA is released from one site during translocation it does not dissociate completely but remains trapped within the ring until bound by another site (FIG. 2c)[18]. Non-toroidal helicases have the core DNA-unwinding activity, but a topological link with DNA may confer the advantage of high processivity on toroidal helicases. Indeed, most of the hexameric helicases examined so far

MOLECULAR MATCHMAKER
A macromolecule that increases the affinity of two or more other molecules for each other, usually through a reaction using energy from ATP binding and hydrolysis.

Table 1 | Structure and function of protein rings involved in DNA metabolism

Protein rings	Examples	Activity on DNA	Mode of action
Sliding clamps	*E. coli* β, *S. cerevisiae* and human PCNA, bacteriophage T4 gp45.	Increase the processivity of replicative DNA polymerases to several thousand nucleotides synthesized per DNA binding event.	The protein ring encircles DNA, binds DNA polymerase and diffuses passively along the duplex, acting as a mobile tether for the polymerase as it duplicates DNA.
Hexameric helicases	SV40 T antigen, bacteriophage T7 gp4, *E. coli* DnaB, human MCM complex.	Unwind dsDNA to generate ssDNA substrates for DNA metabolic processes such as replication, repair and recombination.	The protein ring encircles ss- (in some cases ds-) DNA, and separates the two strands of the duplex fuelled by energy from NTP binding and hydrolysis.
DNA recombination proteins	Human RAD51, RAD52, DMC1, bacteriophage λ β protein.	Facilitate homologous DNA recombination.	Present ss- or dsDNA in conformations that favour ss-DNA annealing or strand exchange with dsDNA, alone or in complex with other recombination proteins.
Topoisomerases	*E. coli* topoisomerase I, human topoisomerase I, *S. cerevisiae* topoisomerase II.	Change the superhelicity of DNA and catenate/decatenate duplex DNA molecules.	Cleave one (ss or ds) DNA strand, pass a second strand through the break, and re-ligate the broken DNA. Activity of type II enzymes is fuelled by ATP.
Others	Bacteriophage λ exonuclease. Bacteriophage ϕ29 head–tail connector. Human translin.	3′–5′ DNA exonuclease. DNA transport protein. Breakpoint recognition protein.	Encircles DNA and processively degrades one strand. Pumps DNA into the phage head using ATP. Binds (encircles?) duplex DNA ends.

are active in genomic DNA replication, which requires exceptionally processive helicase and DNA polymerase activity for efficient synthesis of long chromosomal DNA. In contrast, the Rep (and UvrD) and PcrA helicases function mainly in DNA repair and replication of small phage or plasmids in bacteria[100], which require unwinding of relatively short lengths of DNA.

Similarly, multimeric circular sliding clamps may confer higher processivity on replicative DNA polymerases than monomeric processivity factors that do not encircle DNA, such as UL42 (an accessory protein for the herpes simplex virus DNA polymerase[101]), the small subunit of mitochondrial polymerase γ[102] or thioredoxin (an accessory protein for T7 polymerase)[103]. Interestingly, the polymerase γ accessory subunit has almost no effect on stability of the interaction between polymerase γ and DNA. Instead it increases polymerase processivity by increasing the rate of DNA polymerization[104]. So the monomeric processivity factors may differ from circular sliding-clamp proteins in their mechanism of action, which could explain the observed structural differences.

The λ exonuclease, which can hydrolyse about 3,000 nucleotides per DNA-binding event, is another example of how the toroidal shape allows a protein to act with high processivity (and speed) on DNA[95]. Other rings, such as the β-recombination protein of bacteriophage λ[50], and TRAP[74], highlight how the ordered assembly of repeating nucleic-acid-binding sites (as in a ring) can be used to arrange DNA (or RNA) into specific conformations required for activity. In other proteins, such as DNA topoisomerases, the ring provides a secluded chamber where intermediates can be trapped until the reaction is complete. This property seems particularly important for type II topoisomerases, which must manipulate two dsDNA strands without letting go of either until strand transfer is complete (FIG. 4c)[65].

A ringing endorsement

The long list of ring-shaped proteins illustrates the usefulness of this shape in a variety of DNA-metabolic processes (TABLE 1). Indeed, a single toroidal protein can have different functions simply by virtue of its shape. For example, the bacteriophage T4 clamp gp45 (FIG. 1), originally discovered as a polymerase processivity factor, is also a mobile scaffold on DNA for the transcriptional co-activator T4 gp33 and the sigma factor T4 gp55, facilitating their interaction with RNA polymerase at late-gene promoters[105]. Similarly, the eukaryotic PCNA clamp is an accessory protein not only for replicative DNA polymerases, but also for DNA ligase I, DNA (cytosine-5) methyl transferase, Fen1 and XPG endonucleases, and several other proteins that work on DNA[6]. These proteins seem to have acquired (and retained during evolution) the ability to bind circular clamps, whose DNA-tracking ability may enhance their activity by allowing them to rapidly target their sites of action on DNA.

The widespread use of the toroidal shape is highlighted further in a phylogenetic study by Koonin and colleagues[106] describing the common roots between the RecA/Rad51/DMC1 family of ATP-dependent recombinases (FIG. 3) and the DnaB family of hexameric helicases (FIG. 2). It has been known for some time now that proteins such as RecA, F_1-ATPase, the rho transcription terminator, and the PcrA and T7 helicases have a common core domain responsible for NTP binding/hydrolysis, and related conformational changes that drive their activity (REF. 107 and references therein). There are further similarities in the oligomerization domains of RecA and hexameric helicases[24], and Koonin and colleagues propose that an ancestral RecA protein was recruited for helicase function as the DNA replication system evolved in bacteria. Given the advantages of forming a topological link with DNA, it is not surprising that a ring-form-

ing, DNA-binding, RecA-like protein was a useful progenitor for hexameric helicases. These proteins have retained their core NTPase domain and the ability to form oligomeric rings, despite having diverged in sequence and secondary structure.

Links

DATABASE LINKS Bloom's syndrome | BLM helicase | DMC1 | RAD52 | translin | Cdc6 | PCNA | Fen1 | DNA ligase 1 | DNA methyltransferase | XPG | RAD51 | RFC complex | human topisomerases | F_1-ATPase | DnaB helicase | DnaC | Rho | RecA | *E. coli* topisomerase | *S. cerevisiae* topisomerase II

ENCYCLOPEDIA OF LIFE SCIENCES DNA helicases | Polymerase processivity

We have described a variety of proteins that work on DNA and share an overall toroidal shape. The fact that many of these proteins are evolutionarily unrelated, and have highly specialized structures and functions, makes their convergence to a common shape quite striking. Moreover, persistence of this shape through evolution in proteins with common roots, but divergent functions, highlights its advantages in protein transactions with DNA, and its effect on nucleic-acid metabolism. As more protein structures come under increasingly high-resolution scrutiny, we expect the list of toroidal proteins to expand and diversify even further.

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