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Loading strategies of ring-shaped nucleic acid translocases and helicases

Valerie L O'Shea¹ and James M Berger^{1,2}

¹Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, USA

²Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94705, USA

Abstract

Ring-shaped nucleic acid translocases and helicases catalyze the directed and processive movement of nucleic acid strands to support essential transactions such as replication, transcription, and chromosome partitioning. Assembled typically as hexamers, ring helicase/translocase systems use coordinated cycles of nucleoside triphosphate (NTP) hydrolysis to translocate extended DNA or RNA substrates through a central pore. Ring formation presents a topological challenge to the engagement of substrate oligonucleotides, and is frequently overcome by distinct loading strategies for shepherding specific motors onto their respective substrates. Recent structural studies that capture different loading intermediates have begun to reveal how different helicase/translocase rings either assemble around substrates or crack open to allow DNA or RNA strand entry, and how dedicated chaperones facilitate these events in some instances. Both prevailing mechanistic models and remaining knowledge gaps are discussed.

Introduction

Nucleic acid-dependent translocases play crucial roles in essential cellular processes, coupling the energy of NTP hydrolysis to the directed movement of target nucleic-acid substrates. Ring helicases comprise a sub-class of translocase that move along single DNA or RNA strands to unwind duplex regions. Structurally, ring-translocases and helicases are constructed as an oligomeric assembly of six catalytic NTPase subunits, often coupled to accessory domains, which encircle substrate oligonucleotides within a central pore (reviewed in [1]).

Although key insights into how nucleic acids are moved through a ring helicase/translocase pore in response to nucleotide turnover are starting to emerge [2,3,4••] (Figure 1), the biophysical mechanisms that facilitate substrate binding by these toroidal motor proteins are both diverse and relatively poorly understood. By contrast to certain bacteriophage packaging motors, which can in principle thread onto the ends of linear viral chromosomes

[5], nucleic acid segments in cells frequently lack such accessible sites. In these instances, loading presents a topological problem that can be addressed by one of two general strategies that involve either the assembly of a ring around DNA or RNA, or the opening of a preformed ring to permit substrate binding [6]. However, there also exists a wealth of variations on these themes, which can be subdivided into self-directed or chaperoned loading events, whereby the translocase recognizes and engages nucleic acids on its own, or uses auxiliary ring-loading factors to assist loading (Figure 2).

All ring helicase/translocase systems identified to date belonging to either the RecA-like or AAA+ (ATPases Associated with various cellular Activities) subfamilies of P-loop NTPases [7–9]. In both systems, a bipartite ATPase active site is formed between subunits, while structurally diverse accessory domains, usually found at either the N-terminus or C-terminus of the ATPase fold, serve an array of functions, from directing the motor domains to target sites, to binding loading partners or providing additional functions. Interestingly, a number of auxiliary ring-loading factors also turn out to be AAA+ proteins (reviewed in [10]), and correspondingly oligomerize into higher-order assemblies that utilize ATP binding and/or hydrolysis in their reaction cycles. Although the mechanisms by which ring assembly or ring opening is facilitated at a molecular level have yet to be fully elucidated, new structural and mechanistic insights are beginning to reveal intriguing similarities and differences in these processes for a variety of helicase/translocase systems.

Ring assembly

FtsK — rapid, sequence-specific and self-directed ring assembly and disassembly

Members of the RecA-like FtsK/SpoIIIE family of dsDNA translocases assist with chromosome segregation during cell proliferation and sporulation (reviewed in [11]). FtsK directionally translocates chromosomal DNA with remarkable speed (5 kb/s) to bring a particular sequence, termed a *dif* site, into position at the septum for homologous recombination by the XerCD recombinase [12–14]. FtsK motors are able to independently assemble from monomers into hexamers around duplex DNA in a process that does not depend on ATP or Mg^{2+} cofactors [9,15]. Assembly of the translocase around DNA is facilitated by a specific 5'-GGG(A/C)AGGG-3' sequence (known as KOPS or FRS) that properly orients the motor onto DNA to initiate directional translocation [16–18]. The structural basis for this sequence discrimination has been established by the co-crystal structure of a KOPS/FRS duplex with the C-terminal winged-helix domain (WHD) of FtsK, termed the γ element [19], which reveals that three γ domains from distinct FtsK protomers bind cooperatively to a single KOPS/FRS duplex (Figure 3a). The FtsK disassembly/assembly reaction has been shown to be very fast and can lead to motor reversal during translocation [14,16]. How this rapid change in oligomerization state is triggered by KOPS/FRS recognition and how disassembly/assembly leads to a switch in translocation polarity is not known.

SV40 large T antigen (LTag) — sequence-specific and self-directed ring assembly—Small dsDNA viruses such as polyomavirus and papillomavirus use ring-shaped superfamily III (SFIII) helicases to co-opt the replication machineries of host eukaryotic cells and aid viral proliferation. Like FtsK, SFIII enzymes also form by the

assembly of monomers into productive hexamers around DNA (reviewed in [20]). The polyomavirus Large T-antigen (LTag) is a multi-domain, multifunctional machine that fuses an AAA+ motor domain to an N-terminal domain responsible for localizing the protein to viral replication origins [21,22]. During initiation, the N-terminal domains from four LTag protomers initially recognize inverted repeat sequences in the SV40 origin [23,24], forming a complex that recruits eight additional LTag protomers in an ATP-dependent manner to generate a head-to-head double hexamer that encircles DNA (reviewed in [20]). Following assembly, the LTag double hexamer splits into two single hexamers that each translocate along a complementary single DNA strand [25••]. Recent work has established how two LTag subunits initially engage an asymmetric origin DNA during the first stages of assembly, showing that the ATPase domains of LTag play a direct role in origin discrimination (Figure 3b) [26•]. At present, it is not known how a pair of origin-associated LTag dimers nucleates assembly of the LTag double hexamer, or how the double hexamer intermediate is resolved into two functional single hexamers concomitant with the melting of origin DNA.

E1 — chaperoned sequence-specific ring assembly—Like its LTag relative, the papillomavirus E1 protein is another SFIII helicase that loads onto DNA by assembling directly onto a viral replication origin through a double-hexamer intermediate (reviewed in [20,27]). However, while E1 uses an N-terminal DNA binding domain similar to that of LTag for origin recognition [24,28], its motor elements do not initially bind DNA directly. Instead, the AAA+ domains of two E1 protomers first form a complex with a homodimer of the papillomavirus E2 transcription factor, which binds to sequences adjacent to E1 recognition elements and helps to chaperone the formation of productive E1 origin interactions (reviewed in [27]). X-ray crystallography has shown that E2 binding blocks the canonical AAA+ oligomerization surface of E1, ensuring that E1 protomers do not associate prematurely [29] (Figure 3c); ATP binding, which is necessary for E1 assembly, is thought to remodel the E2 binding site on E1, promoting dissociation of the chaperone to allow E1/E1 dimers to form. During formation of the E1 hexamer, the N-terminal and AAA+ domains of E1 cooperate to form a double trimer intermediate that uses ATP turnover to locally melt origin DNA [30,31••]. The structure of this intermediate, and how it assembles with additional E1 subunits to form two functional hexameric helicases, has yet to be resolved.

Ring opening systems

Rho — formation of an open-ring ground state

In bacteria, the termination of certain transcripts is dependent upon Rho, a homohexameric RNA translocase capable of unwinding RNA/DNA heteroduplexes and displacing stalled RNA polymerases from template (reviewed in [32]). In bacterial cells, where transcription and translation are coupled, the 5' end of mRNAs is occluded by the ribosome, preventing Rho from threading its way onto substrate. Each Rho subunit is composed of an N-terminal, oligonucleotide/oligosaccharide binding (OB) fold that binds pyrimidine-rich RNA, along with a C-terminal RecA-like ATPase domain (reviewed in [33]). Similar to FtsK, Rho is targeted to nucleic acid substrates by virtue of its accessory nucleic-acid binding domains

independent of ATP binding or hydrolysis [34]; however, Rho differs in that it forms stable hexamers in the absence of RNA or nucleotide cofactors.

The innate stability of the Rho hexamer initially suggested that Rho might load onto mRNAs by a self-directed ring opening mechanism. Electron microscopy studies have supported this model, showing that Rho forms notched hexameric particles that homogeneously shift into closed rings when long RNAs and nucleotide are present [35,36]. X-ray crystallography has provided additional corroborative data, with one structure capturing the Rho hexamer in a split-ring, or 'lockwasher'-shaped intermediate that orients the free 3' end of RNA bound by the helicase's OB fold domains toward the central translocation channel of the motor (Figure 4a) [37]. Comparison of this structure with a closed-ring state that contains RNA bound within the translocation pore (Figure 1b) indicates that Rho utilizes inter-subunit hinging motions to convert between open and closed forms [3]. Biochemical studies have suggested that RNA binding to the OB folds of Rho may allosterically influence ring opening and closure [38]; how these signals propagate between subunit interfaces and with ATP binding events in real time is presently not understood.

T7 gp4 — self-directed ring opening

The T7 gene 4 protein (gp4) is a RecA-family, bi-functional helicase-primase responsible for replicating the bacteriophage's genome (reviewed in [39]). Upon binding nucleotide, gp4 forms oligomeric rings that can independently load onto closed-circular, single-stranded DNA [40,41]. Unlike Rho, both six-membered and seven-membered closed rings of gp4 have been visualized by EM and X-ray crystallography [42–45]. In higher-resolution structures, each N-terminal primase domain within a gp4 ring can be seen to sit atop not its own subunit, but on the adjacent RecA domain of a partner subunit; a linker region between the primase and helicase domains, which is required for oligomerization, further connects neighboring protomers together (Figure 4b) [44–46].

The prevalence and stability of closed gp4 oligomeric rings has suggested that the helicase uses a self-directed ring breaking strategy for loading onto DNA. However, unlike Rho, which has been observed to spontaneously form open lockwashers, ring opening in gp4 appears to require DNA. Kinetic studies of loading using changes in protein fluorescence have been interpreted to suggest that DNA binding by one of the primase domains in a ring may help unlatch two protomers, allowing the helicase ring to open and engage substrate [47]. Consistent with this idea, a construct of gp4 lacking the primase domain has been crystallized as a spiral filament with six protomers per turn [48]. However, biochemical and EM analyses of crosslinked gp4 oligomers also have suggested a different model, in which DNA binding by a pre-formed heptamer may help trigger the loss of one subunit, leaving a gap through which single-stranded DNA can enter into the pore [43]. Although the subunit ejection model does not require the formation of a spiral intermediate to support loading, it also does not preclude such an event; additional studies are needed to resolve or reconcile these schemes to further clarify the molecular mechanism by which gp4 loads itself onto substrates.

DnaB — chaperoned ring-breaking...at least some of the time

DnaB-family proteins serve as central replicative helicases of bacteria (reviewed in [49]). Structural studies, including both EM and X-ray crystallography, have shown that DnaB orthologs readily form stable, closed hexameric rings [50–58]. In higher-resolution crystal structures, DnaB-family helicases have been found to adopt a two-tiered architecture, whereby a set of N-terminal primase interaction domains form a trimer of dimers that sits atop a pseudo-six-fold symmetric ring of RecA-type ATPase domains [53–55].

In *Escherichia coli*, DnaB forms a stable 6:6 complex with the DnaC protein [59,60], a factor needed for loading of the helicase both onto closed-circular, single-stranded DNA and onto nascent replication bubbles formed by the DnaA initiator protein [61–63]. A recent EM reconstruction of a dodecameric DnaB/DnaC complex (DnaBC) has established that DnaC aids in the loading process by enforcing an open, lockwasher-shaped configuration on the helicase (Figure 4c) [64••]. The action of DnaC, which is itself an AAA+ ATPase [65], is reminiscent of the clamp-loader complex, a distantly related AAA+ relative that opens ring-shaped, DNA polymerase processivity clamps and deposits them onto DNA (reviewed in [66]). However, DnaC does not require either ATP or its associated AAA+ domain for loading DnaB onto DNA [61,64••]. Instead, the AAA+ domains of DnaC form a nucleotide-dependent spiral that appears to stabilize an open lockwasher state for DnaB [64••,65], while ATP hydrolysis by DnaC is thought to promote release of the loader from the helicase to allow ring closure [61]. Interestingly, in opening DnaB, DnaC also remodels multiple oligomeric contacts within the helicase's N-terminal collar, an event that appears to stimulate DNA unwinding by the motor [64••]. How DnaB opening is coordinated with deposition of the helicase onto a newly-melted replication origin is not understood, but is known to require not just DnaC, but also the bacterial initiator, DnaA [65,67,68].

Although the action of *E. coli* DnaC on DnaB would appear to resolve the immediate question of how a ring-shaped helicase can be physically opened by an exogenous loading factor, it is unclear whether all DnaB orthologs rely on a similar loading mechanism. For example, the helicase and helicase loader of Gram-positive organisms such as *Bacillus subtilis* (whose DnaB and DnaC counterparts are known as DnaC and DnaI, respectively) has been shown to form a stable 6:6 complex [69,70]; however, the *B. subtilis* loader has been reported to act in a different manner, chaperoning the assembly of the helicase around single-stranded DNA as opposed to opening a preformed ring directly [71]. In this regard, the recent imaging of *Geobacillus stearothermophilus* DnaB in complex with the *B. subtilis* DnaI loader (and a fragment of the DnaG primase protein) is notable [72•], in that it exhibits a closed-ring structure that may correspond to a 'post-assembly' state (Figure 4d). Moreover, the DnaB protein of many bacteria and bacteriophage do not have identifiable DnaC/DnaI-family loaders. For example, the DnaB-family helicases of bacteriophages T4 and SPP1 (gp41 and G40P, respectively) both have non-ATP dependent and unrelated chaperones that are thought to directly facilitate ring opening to permit loading onto single-stranded DNA [73,74]. The bacterium *Helicobacter pylori* likewise has no discernable DnaC/DnaI loader, and studies of its cognate DnaB helicase have revealed many distinctive properties — including the ability to form double hexamers that have not been seen in other DnaB homologs [75•] and the ability to support replication of an otherwise unviable *E. coli*

strain that bears a defective copy of DnaC [76] — which suggest that the helicase either uses an as-yet-unidentified loader or is capable of undergoing self-directed loading. Additional studies are clearly needed to delineate how replicative helicase loading strategies agree and differ across bacterial and bacteriophage systems.

Eukaryotic Minichromosome Maintenance (Mcm2–7) — chaperone-controlled ring-closing and reopening?

The Minichromosome Maintenance complexes (MCMs) are AAA+-type ATPases that serve as the principal replicative helicases of eukaryotes and archaea (reviewed in [77,78]). As with most DnaB-family proteins, MCMs spontaneously form homohexameric (archaea) or heterohexameric (eukaryotes) rings on their own that can encircle DNA ([79•,80•] and reviewed in [81]). However, MCMs also have been shown to exhibit substantial organizational plasticity, having been imaged in heptameric, double-hexamer, and even spiral states (reviewed in [81]).

Consistent with their ability to form stable rings, the loading of MCMs onto DNA is thought not to proceed through a subunit assembly mechanism, but rather through the controlled opening of preformed hexamers by specific chaperone factors. In eukaryotes, several proteins — including the Origin Recognition Complex (ORC), Cdc6, and Cdt1 — collaborate in an ATP-dependent manner to deposit MCM2–7 (the eukaryotic form of the complex) onto DNA in an inactive state during the G1 phase of the cell cycle, forming a stable pre-replicative complex (pre-RC) (reviewed in [82]). Like viral SFIII enzymes, MCM2–7 is initially loaded as a double hexamer intermediate (Figure 4e) [83,84]. However, as cells progress into S phase, multiple processing events — including post-translational modifications and the recruitment and choreographed association of the accessory factors Cdc45 and GINS (reviewed in [85]) — are required to subsequently dissociate the MCM2–7 double hexamer into two, active single hexamers that ultimately serve as the motors for advancing a bidirectional replication fork [86,87•,88].

How MCM2–7 is processed during loading has garnered much attention. Although archaeal MCM relatives have been imaged as closed rings on their own (reviewed in [81]), EM studies have found that MCM2–7 predominantly forms open, left-handed lockwashers in either the presence or absence of ATP (Figure 4f) [79•,80•]. These structural findings accord with biochemical analyses, which first reported the ability of MCM2–7 to spontaneously open and load onto closed-circular, single-stranded DNA substrates, and which showed that the interface between the MCM2 and MCM5 subunits is particularly prone to separation [89]. By contrast, cryo-EM reconstructions of MCM2–7 in an ATP-bound context of the CMG (i.e. bound to Cdc45 and GINS) or a pre-RC (bound to ORC, Cdc6, Cdt1, and short DNA duplex) have captured conformational states for the helicase in which the MCM2/MCM5 breach is occluded, thus blocking access into the ring (Figure 4g) [79•,90•]. Interestingly, in the case of the pre-RC complex, ORC and Cdc6 (which likewise are AAA+ ATPases) form a spiral structure reminiscent of that seen for *E. coli* DnaC and for clamp loaders, suggesting that the pre-RC directly remodels the conformation of MCM2–7 to facilitate loading [90•]. In this context, the pre-RC complex imaged by EM has been proposed to correspond to a ‘post-loading’ state, in which helicase deposition has just been

completed. Taken together, the present data suggest that MCM2–7 has a natural proclivity for opening, and that the role of ORC, Cdc6 and Cdt1 is to control this inherent flexibility to properly coordinate helicase loading with the eukaryotic cell cycle. Future studies will be needed to establish how and when the MCM2–7 ring is opened during the loading cycle, and how the MCM2–7 double hexamer intermediate is generated.

Conclusions

All ring helicase/translocase systems that do not thread onto the free ends of a target nucleic acid segment must instead overcome a common topological challenge that prevents substrates from simply diffusing into the pore of the ring. Two means for overcoming this challenge are to either assemble a helicase/translocase from monomers into a ring around DNA or RNA, or to crack open a pre-assembled ring. Different approaches for coordinating ring assembly or ring opening also are known, with helicase/translocase systems either self-directing their own loading or relying on accessory chaperones. Interestingly, nature has evolved a number of diverse variations on these themes, which can differ even between closely related helicase/translocase systems. Although many extant structures likely correspond to specific loading intermediates, the full scope of states accessed during loading, as well as the time-dependent dynamics by which these states interconvert with one another, has yet to be established for any one system. Future investigations will undoubtedly continue to uncover many surprising insights into the loading mechanisms of these essential molecular machines.

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Figure 1.

Ring helicase/translocases bind substrate nucleic acids in their central pore. **(a)** The papilloma virus E1 helicase bound to DNA (PDB ID: 2GXA [2]). **(b)** The *E. coli* Rho transcription termination factor bound to RNA (PDB ID: 3ICE [3]). **(c)** The *G. stearothermophilus* DnaB helicase bound to DNA (PDB ID: 4ESV [4••]).

Figure 2.
Summary of ring helicase/translocase loading mechanisms. See text for details.

Figure 3.

Intermediates formed by self-directed or chaperone-directed hexameric helicase/translocase systems. **(a)** Assembled (α/β) motor domains of an FtsK hexamer (left) (PDB ID: 2IUU [9]) juxtaposed next to a trimer of DNA bound γ domains (right) (PDB ID: 2VE9 [19]). Flexible linkers tether the motor and γ domains and likely help to accommodate the large-scale movements that accompany FtsK assembly/disassembly events. **(b)** Structure of an LTag dimer bound to one half of the SV40 replication origin (PDB ID: 4GDF [26]). **(c)** Structure

of the E1-binding domain of E2 in complex with the AAA+ domain of E1 (PDB ID: 1TUE [29]).

Figure 4.

Structural intermediates formed by ring-opening helicase/translocase systems. **(a)** Structure of an open-ring Rho hexamer (PDB ID: 1PVO [37]). **(b)** Structure of a gp4 heptamer highlighting the domain swapping of primase and linker elements (PDB ID: 1Q57 [45]). **(c)** 3D EM reconstruction of an ATP-bound *E. coli* DnaBC complex; docking of homologous structures into the EM density reveals a gap in the open-ring particle (EMD ID: 2321 [64••]). **(d)** Structure of a closed-ring complex of *G. stearothermophilus* DnaB helicase and DnaG helicase-binding domain (HBD) with *B. subtilis* DnaI (PDB ID: 4M4W [72•]). **(e)** 3D

EM reconstruction of the *Methanothermobacter thermautotrophicus* MCM [81] showing a crystallographic model for an N-terminal domain (NTD) dodecamer (PDB ID: ILTL) docked into the central region. **(f)** 3D EM reconstruction of an open ring *D. melanogaster* MCM2–7 complex (EMD ID: 1835 [79•]), with the homology models on the basis of archaeal MCM structure docked into the EM density. **(g)** 3D EM reconstruction of ORC, Cdc6, and Cdt1 bound to MCM2–7 and dsDNA (EMD ID: 5625 [90••]).