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The Role of ATP-Dependent Machines in Regulating Genome Topology

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Abstract

All cells must copy and express genes in accord with internal and external cues. The proper timing and response of such events relies on the active control of higher-order genomic organization. Cells use ATP-dependent molecular machines to alter the local and global topology of DNA so as to promote and counteract the persistent effects of transcription and replication. X-ray crystallography and electron microscopy, coupled with biochemical and single molecule methods are continuing to provide a wealth of mechanistic information on how DNA remodeling factors are employed to dynamically shape and organize the genome.

Introduction

Cells employ a variety of strategies to ensure the timely, high-fidelity expression and copying of genetic information. Many of these strategies are coupled to ATP, the energy currency of cells. Energy derived from ATP binding and hydrolysis can be used by nucleic acid-dependent machines to drive non-equilibrium processes such as DNA unwinding and compaction, as well as large-scale genomic ordering. Nucleoside triphosphate (NTP) turnover can also control reaction directionality, processivity, and chemical timing, all while tying these reactions to internal energy status.

Although the long, intertwined nature of DNA poses a challenge for its compaction, the packing densities of DNA within the nucleus of a eukaryotic cell typically approaches only ~1% of its theoretical maximum (by comparison, viruses can achieve much higher packing densities, up to ~70%) [1]. The packaging of cellular DNA is therefore less concerned with surmounting the physical problem of limited space than it is with controlling chromosome superstructure as a regulatory strategy. The intricate link between three-dimensional

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genomic organization and gene expression places chromosome topology and the machines responsible for its manipulation and maintenance at the center of myriad critical processes.

Why is energy needed for topological transitions?

Extreme DNA bends, as well as duplex melting, are unfavorable transitions and thus require energy to occur. Free energies for DNA base pair steps calculated by single molecule unzipping and thermal melt experiments range from 0.8 – 2.5 kcal/mol [2,3]. DNA base stacking, hydrogen bond formation, and electrostatic repulsion of the phosphate backbone all contribute to the relative rigidity of DNA on short length scales, although short-lived, spontaneous deformations have been reported to occur [4–6]. DNA shorter than its persistence length (~150 bp) is traditionally considered relatively inflexible, and thus typically requires more energy to bend than is generally accessed by thermal fluctuations.

It is well established that the energetic compensation for costly bending and melting transitions can be readily paid for by protein-DNA contacts (e.g., see [7–9]). In addition, enzymes can use NTP binding and hydrolysis to alter DNA structure and topology. In this review, we will briefly touch on four classes of molecular “machines” – replicative DNA helicases, highly-processive DNA and RNA polymerases, motors that remodel DNA architecture proteins, and enzymes capable of altering DNA looping and topology – that channel NTP turnover into the steady state remodeling of chromosome organization. Each class of machine uses nucleotide hydrolysis for a specialized task, with binding and hydrolysis frequently employed to ensure reaction directionality and efficiency, as well as to promote energetically unfavorable transitions in DNA. Nature has evolved a variety of folds that couple NTP binding and hydrolysis to cycles of nucleic acid binding and release; this review will focus on enzymes belonging to the large Additional Strand Catalytic E (ASCE) ATPase superfamily (specifically RecA, AAA+, and ABC type proteins), GHKL ATPases, cellular RNA polymerase, and the Pol A and Pol X families of replicative polymerases.

Replicative helicases

Cellular replicative helicases are nucleic acid translocases that encircle DNA substrates (Figure 1). ATP binding and hydrolysis drive directional helicase movement, which separates template strands for use by DNA polymerases. The replicative helicases of cells form hexameric assemblies [10,11] that translocate along single-stranded DNA segments [12,13]. The encirclement of only one DNA strand allows these enzymes to unwind duplexes by a strand exclusion mechanism [14–16], segregating the leading and lagging strands away from each other. Replicative helicases are members of the broad ASCE ATPase family, but fall within three distinct clades [17]. In bacteria, the replicative helicase is formed from a homohexameric assembly of RecA ATPase subunits [18,19], whereas in archaea, eukaryotes and certain dsDNA viruses, the replicative helicase is composed of AAA+ ATPase subunits [20–22].

Crystal structures of the papillomaviral E1 (AAA+) and bacterial DnaB (RecA) helicases have revealed that single-stranded DNA adopts a helical path through the central pore of a hexamer (Figure 1a [23,24]). Loops within the pore track the phosphate backbone of the single-stranded DNA. The motor subunits of each helicase do not form a symmetric ring,

but rather an asymmetric, “spiral-staircase” type of arrangement that wraps around and engages DNA in a non-sequence specific manner. During processive movement, each subunit is thought to undergo coordinated, rigid body movements, coupled to sequential cycles of ATP binding and product release (Figure 1b), that help shepherd the nucleic acid through the helicase pore in a manner somewhat akin to a bucket-belt elevator. In the E1 hexamer, each individual subunit binds to just one nucleotide unit in the DNA, whereas in DnaB, two nucleotides of DNA were found to associate with each protomer, suggesting that the hexameric motor as a whole would move only one or two bases per ATP consumed overall, respectively. Consistent with this prediction, single molecule studies on DnaB-like helicases have reported small stepping sizes on the order of ~1 bp/NTP [25–27].

Regardless of step-size, a hexameric helicase will unwind one full turn of DNA and generate one positive supercoil in front of the motor for every 10–11 nucleotide units it translocates (Figure 1c). If allowed to accumulate, this supercoiling will build up torsional “back-pressure” in the form of overwound DNA that will resist further unwinding and stall helicase advancement [28]. At present, there is relatively little information as to how much force replicative helicases can generate during movement, but preliminary single-molecule analyses of both E1 and the bacteriophage T7 gp4 protein (a bacterial-like helicase), have indicated that the isolated motors are relatively non-processive during duplex unwinding and prone to slippage [26,29]. Such weak activity might seem at odds with the need to replicate large (megabase) contiguous segments of DNA, but there is compelling recent evidence that the coupling of replicative helicases to a second motor, namely a DNA polymerase, can greatly stimulate DNA unwinding [25,30]. This issue will undoubtedly be investigated in greater detail in the coming years.

Processive synthesis motors

Highly-processive cellular DNA polymerases and RNA polymerase consist of multi-domain or multi-subunit complexes in bacteria and eukaryotes (Figure 2). Replicative DNA polymerases not only associate with a hexameric helicase, but also with priming and processivity factors to form a large macro-assembly termed the replisome. Because DNA and RNA polymerases both unwind DNA to read out base sequence information, their processive movement directly alters DNA superhelicity. As first noted by Liu and Wang in their “twin-supercoiled domain” model [31], positive supercoiling of a DNA double helix will occur ahead of a translocating unwindase and negative supercoiling behind it (Figure 2b). Direct observation of this phenomenon, from bacterial plasmids [32] to genome-wide scales in human cells [33], has been described for transcription. DNA replication similarly generates positive DNA supercoils ahead of the replication fork [34], as well as negative supercoiling on the intact leading strand [35], and can also result in the formation of intertwined “pre-catenated” DNA strands behind the replisome [36] (Figure 2d). These latter structures lead to DNA tangles that must be unlinked prior to cell division [36–38].

Although there are many different polymerase folds (Figure 2a and c) [39], both RNA polymerase and replicative DNA polymerases tend to adopt a roughly analogous shape that can be likened to a curled right hand or claw [40–43]. A single template strand is guided through the polymerase catalytic center, which is coupled to structural elements that move in

response to nucleotide binding, help sense appropriate base-pairing, and promote primer-template translocation. The energy liberated from NTP hydrolysis not only helps overcome the entropic cost of assembling an ordered polymeric chain from individual building blocks, but can also be used to generate directed movement and force. For example, *E. coli* RNA polymerase has been found to generate as much 25 pN of pulling force [44]; by comparison, classical motor proteins such as myosin or kinesin typically produce ~3–6 pN of force [45,46]. The replicative DNA polymerases of certain phage systems similarly have been reported to help “push” an associated hexameric helicase along DNA, improving DNA unwinding efficiency and processivity [30,47]. The bacteriophage phi29 DNA polymerase has been reported capable of translocating against 20pN of force without effect on its polymerization cycle [48]; however, the amount of force that can be exerted by a complete replisome has yet to be established experimentally.

The consequences of topological strain induced by translocating polymerase motors has a global effect on chromosome architecture (Figure 2b and d). In addition to altering supercoiling locally, if positive DNA supercoils resulting from processive unwinding are not removed, polymerase stalling can occur [49]. Moreover, there appears to be a tight coupling between both transcription and replication and the status of DNA architectural factors. For example, the torsional stress of processive RNA polymerase movement can, in eukaryotes, destabilize nucleosomes [50] and induce the ejection of H2A/H2B histone dimers [51]. In replication, one of the MCM helicase subunits (MCM2) bears a histone H3/H4 binding domain that competes for DNA binding by wrapping around the perimeter of an H3/H4 tetramer [52], and nucleosome occupancy and spacing in turn seem to regulate the size of Okazaki fragments produced by the replisome [53]. The tight link between RNA polymerase and DNA remodeling in particular is underlined by the observation that changes in supercoiling levels can influence promoter activity [54–56], and that DNA topoisomerases have recently been found to associate with and in some instances regulate RNA polymerase directly [57–60]. A full understanding of the extent to which the cell both controls and contends with the topological consequences of replication and transcription remains to be determined.

Chromatin remodeling enzymes

ATP-dependent chromatin remodelers mobilize histone octamers bound to DNA. Alterations in chromatin structure catalyzed by ATP-dependent chromatin remodelers can result from the movement, eviction, assembly, and subunit exchange of nucleosomes (Figure 3a). ATP-dependent chromatin remodelers contain an ASCE ATPase element whose core structural fold is most closely similar to bacterial replicative helicases, but that is formed from a tandem, linked pair of RecA-type domains, rather than by assembly into a hexameric ring (Figure 3b). The motor subunits of chromatin remodeling enzymes belong to the Snf2 family of super-family 2 (SF2) helicases and are thought to act in many instances as dsDNA translocases that can generate superhelical torsion within constrained DNA substrates [61]. Snf2 motor proteins have been grouped into numerous subfamilies – such as SWI/SNF, CHD/Mi-2, ISWI/ACF, and INO80/SWR1 – based on both the sequence of the ATPase fold and on the presence of accessory domains that emanate from the motor domain polypeptide [62,63].

Although a detailed mechanistic understanding of chromatin remodeler functions is lacking, how remodelers engage the nucleosome to catalyze nucleosome mobilization and histone exchange is beginning to take shape. X-ray structures of Snf2 motor proteins have been solved for Rad54 [64,65] and Chd1 [66], and have indicated that, like other SF1 and SF2 helicases [67], the ATP-dependent opening and closing of the tandem RecA domains is coupled to cycles of tight and weak dsDNA-binding events that ratchet the duplex through the motor (Figure 3c). EM studies, coupled with crosslinking data, have revealed a global picture for the architecture of the large (> 1MDa) INO80 [68], SWR1 [69], and RSC chromatin remodeler complexes [70] bound to a nucleosome (Figure 3f–h). Compared to RSC, which fully engulfs the nucleosome [70–72], INO80 and SWR1 cradle their nucleosome targets, leaving a portion of the nucleosome open and accessible to other factors. Other remodeling complexes, such as ISWI/ACF and CHD1 are insufficiently large to either engulf or cradle the nucleosome (Figure 3d and e), but instead associate with nucleosomes at discrete positions [73–76].

Exactly how ATP-dependent remodeler conformational changes rearrange specific nucleosome contacts to promote nucleosome translocation and force generation is not understood. Single molecule measurements have shown by that DNA contacts within the nucleosome are not all energetically equivalent [77,78], that the peak force required for unzipping nucleosome bound DNA is 30–40pN (10–20pN forces are required for unwinding naked DNA [79]), and that strong contacts occur at the nucleosome dyad [80]. Single molecule experiments with ISWI have suggested that several rounds of ATP hydrolysis can build up strain within the nucleosome before DNA slides around the octamer in discrete steps [81]. How remodelers engage key positions of a client nucleosome and generate sufficient strain required to break strong histone-DNA contacts remains an area of future inquiry.

Machines that regulate the topological homeostasis of DNA

DNA topoisomerases are responsible for resolving topological changes imparted by processive motors and, as such, are critical for efficient transcription and replication [82–84]. Topoisomerases can be broadly divided into three classes: DNA nicking/religating swivelases (types IB and IC), single DNA-strand passage enzymes (type IA), and double DNA-strand passage machines (types IIA and IIB) [85]. All type II topoisomerases consume ATP as part of their reaction mechanism through the combined use of a GHKL-family ATPase domain [86] and an RNaseP-type “transducer” domain, the latter of which participates in ATP hydrolysis and links the GHKL fold to other parts of the enzyme. ATP binding leads to homodimerization of the GHKL fold, whereas ATP hydrolysis and product release promote dimer dissociation, allowing the system to reset. All type II topoisomerases identified to date assemble into homodimeric, heterotetrameric, or heterohexameric assemblies that coordinate the action of their ATPase regions with an associated nucleolytic region for cleaving and resealing DNA.

Despite their use of ATP, most type II topoisomerases catalyze thermodynamically favorable topological transformations such as the removal of DNA supercoils (Figure 4a). The requirement for ATP in the type II topoisomerase reaction cycle is thought to help

regulate the formation of cytotoxic double-stranded DNA breaks formed by the enzyme during strand passage [87]: by forming a tight, but reversible interface between type II topoisomerase subunits, the GHKL ATPase provides a mechanism for coordinating the timing of domain closure with DNA cleavage, and for preventing of the escape of broken DNA until religation occurs. The use of ATP also likely helps promote unidirectional strand passage. Although the hydrolysis of ATP has been found to allow the internal flexion of two domains within the ATPase [88–90], at present, it is unclear exactly how conformational changes are physically propagated from the ATPase center to the more distal nucleolytic active site to promote DNA cleavage, opening, and strand passage.

The one type II topoisomerase that is capable of using the free energy of ATP to actively push DNA into a higher energetic state is DNA gyrase. Gyrase introduces negative supercoils into relaxed DNA substrates in an ATP-dependent manner, an activity that derives from the ability of the enzyme to wrap DNA into a positive supercoil prior to strand passage [91–93]. Single molecule studies have established that the trapping of a positive supercoil for strand passage and relaxation is accelerated by ATP binding [94], indicating that there exists long-range conformational coupling between the ATPase and DNA wrapping elements, which reside on different gyrase subunits. Single particle EM reconstructions have captured a state of gyrase in which the ATPase domains are dimerized and the DNA wrap can be seen [95] (Figure 4b and c); however, single molecule efforts also have identified several enzyme substates that have yet to be understood structurally [94,96,97].

While the supercoiling and catenation state of duplex DNA is directly modulated by topoisomerases, yet another class of ATP dependent enzymes, known as structural maintenance of chromosome (SMC) proteins, can act to bring together DNA segments at a distance [98]. SMC proteins (along with their RAD50/RecF homologs) help promote long-range intermolecular or intramolecular DNA interactions, and use ATP binding to regulate chromosome condensation, cohesion, and DNA repair [99–101]. Chromatin conformation capture methods have revealed that SMC proteins help globally organize the genomes of both eukaryotes and bacteria [102–104].

SMC proteins employ an ASCE-family ATP binding cassette (ABC) ATPase, a fold structurally related to RecA-like and AAA+ATPases. However, as with the GHKL ATPases, the SMC ATPase dimerizes upon the binding of ATP (Figure 4d and e). SMC proteins are generally large (>150kDa), with a long, internal coiled-coil segment that interrupts the amino- and carboxy-terminal halves of the ABC fold. A “hinge” domain, which sits in the middle of the coiled-coil region, serves as a second dimerization element for promoting inter-SMC interactions (Figure 4d and e). SMC proteins do not act alone, but with a multitude of accessory subunits; these factors can bind to the SMC ATPase heads and coiled regions to create a third set of bridging interactions that link subunits together. Thus far, a number of structures exist for pieces of individual and multipartite SMC complexes (reviewed in [98,105]), but a complete, high-resolution view of these assemblies is still lacking.

Condensin and cohesin complexes have been proposed to encircle DNA within their ring-like structures in ATPase controlled manner [106–108] (Figure 4f). How multiple DNA segments might be coordinated by SMC action is not known at a physical level; however, a recent structure involving the SMC-like protein Rad50 demonstrates that at least one duplex can be bound by the ATPase domain directly (Figure 4d [109]). Single molecule work has recapitulated the SMC-mediated compaction of DNA [110–112], and AFM studies suggest that multiple SMCs may also interact with one another to promote long-range DNA compaction [113,114]. Recent structural and crosslinking studies have indicated that the coiled-coiled domains of SMC complexes can undergo a conformational rearrangement upon binding of ATP and DNA, which may foster communication between the hinge and ATPase domains [115], or possibly promote hinge opening [116,117]; for cohesin, proteolytic cleavage of one of the accessory subunits also regulates subunit opening and DNA release [118,119]. Coordination between DNA loading and exit of chromosome segments within SMC complexes may occur through an ATP-coordinated gating mechanism, reminiscent of the type II topoisomerases, but full structural and mechanistic characterization of DNA binding, loading, and exit from SMC complexes awaits further study.

Conclusion

While there is great structural diversity in the enzymes that use ATP in order to effect structural transitions in DNA, these machines all use ATP binding and hydrolysis to drive intra- and inter-subunit motions that can generate force and/or capture and reshape nucleic acid segments. The detailed mechanisms of how the chemical energy of ATP is harnessed by these enzymes are varied and are in many cases incomplete, in part due to a lack of structural information for complete molecular assemblies bound to appropriate substrates. Structures of larger and larger assemblies are starting to emerge, however, aided by recent advances in EM and X-ray crystallographic capabilities. In addition, single molecule studies continue to fill in key gaps in our knowledge of the dynamics and nucleotide coupling of these systems. Nanomanipulation techniques, coupled with traditional biochemistry, will undoubtedly continue to reveal catalytic substeps and conformational intermediates that may be targeted for understanding by future structural efforts.

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Highlights

- ▶ Unwinding a DNA duplex poses topological challenges to replication and transcription.
- ▶ Cells have many energy-dependent strategies for maintaining topological homeostasis.
- ▶ NTP-fueled molecular machines shape the topological landscape of the genome.

Figure 1. Replicative Helicases form hexameric assemblies and translocate along single stranded DNA

(a) Comparison of the structures of the homohexameric papillomavirus E1 replicative helicase (left, PDB ID: 2GXA [23]), a homohexameric bacterial replicative helicase (center, PDB ID: 4ESV [24]); and the eukaryotic MCM2-7 helicase as part of the CMG complex (right, EMD ID: 2772 [120]). In all three structures, DNA (magenta) binds to the central pore of the hexamer. Different ATPase subunits are colored (or marked by colored labels) for clarity.

(b) ATP hydrolysis scheme for E1 (left) and DnaB (right). Cartoon representations are colored as in (a) with nucleotide depicted as red stars. The black arrows indicate the direction of sequential nucleotide hydrolysis around the hexameric ring relative to the view in which 3' DNA faces up, out of the pore.

(c) Cartoon representation of the supercoiling transitions in DNA imparted by replicative helicase movement, with positively supercoiled DNA accumulating ahead of the replicative helicase and melted DNA segregated for replication by polymerases behind. CMG (left) and DnaB (right) have opposite polarities on DNA, but both orient their ATPase subunits toward the duplex end of the fork.

Figure 2. Processive synthesis motors impart distinct supercoiling transitions in DNA
(a) RNA polymerases *Escherichia coli* (left, PDB ID: 4YLN [121]) and *Sacharomyces cerevisiae* (right, PDB ID: 5C3E [122]) bound to transcription bubbles, colored as indicated. RNA synthesis results in a bubble of un-base-paired DNA that is contained within the RNA polymerase core folds.
(b) Diagram of supercoiling transitions resulting from RNA synthesis. Positive supercoils accumulate ahead of RNA polymerase and negative supercoils behind, with RNA synthesis from rNTP monomers occurring within the RNA polymerase core.

(c) Leading strand DNA polymerases from *Thermus aquaticus* (left, PDB ID: 3E0D [123]) and *Sacharomyces cerevisiae* (right, PDB ID: 4M8O [43]) contain a core DNA polymerase domain composed of “thumb,” “palm” and “fingers” structural elements, colored as indicated. These structural elements form the catalytic site for DNA strand synthesis.

(d) Cartoon representation of the supercoiling transitions in DNA created by replisome movement. dNTPs are used to synthesize daughter strands complementary to leading and lagging strand templates. Replisome movement results in positive supercoiling ahead of the replisome and DNA crossings that can result in DNA catenanes if not resolved. The arrow indicates the direction of replisome movement.

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Figure 3. Chromatin remodeling enzymes

(a) Cartoon representation of the structural transitions in nucleosomes that can be catalyzed by ATP-dependent chromatin remodelers.

(b) Structure of the Snf2 ATPase from *Danio Rerio* Rad54 (PDB ID: 1Z3I [65]). The tandem RecA ATPase domains are colored orange and blue, with inserts specific to the Snf2 family in gray.

(c) Cartoon representation of the structural transitions undergone by tandem RecA fold. The RecA-like subunits contain residues important for ATP binding and hydrolysis, and for nucleic acid binding, that are brought together upon binding nucleotide and DNA. Repetitive cycles of this open/closed transition with ATP turnover is proposed to generate the DNA movements and/or forces necessary for nucleosome remodeling events.

(d–f) EM reconstructions (top) and cartoon representations (bottom) of nucleosome remodelers with proposed nucleosome binding sites and Snf2 ATPase domain positions indicated. (d) ACF dimer bound to a mononucleosome [75]. (e) ISW1a ATPase bound to a mononucleosome containing linker DNA (EMD ID: 1877 [76]). (f) RSC [71]. (g) SWR1 bound to a mononucleosome (EMD ID: 5638 [69]). (h) INO80 (EMD ID: 2385 [68]).

Figure 4. Type II DNA topoisomerases and SMC complexes

(a) DNA unlinking (left) and supercoil altering events (right) catalyzed by type II topoisomerases. Duplex DNA is represented as black and magenta lines. Removal of DNA catenanes and knots can be catalyzed by type II DNA topoisomerases (left), as can supercoil relaxation (right). The asterisk denotes that supercoiling of relaxed DNA is uniquely carried out by DNA gyrase.

(b) Cartoon representation of DNA gyrase, showing the wrapping of DNA and the location of ATP binding within the GHKL ATPase subdomains.

(c) 3D cryo-EM map of full-length DNA gyrase bound to DNA (EMD ID: 2361 [95]) with the ATPase-DNA cleavage core of yeast Top2 bound to DNA (PDB ID: 4GFH [124]) and the gyrase carboxy-terminal domains (PDB ID: 1SUU [125]) docked into the density. Linker regions are indicated with dotted lines. Topoisomerase subunits are in blue and gray, DNA bound to the cleavage core of Top2 is represented as magenta spheres.

(d) Structures of an SMC hinge dimer with associated, extended coil-coil elements from *Pyrococcus furiosus* (top, PDB ID: 4RSJ [115]), juxtaposed next to the dimerized ABC ATPase subdomains of Rad50 bound to double stranded DNA and nucleotide (bottom, PDB ID: 4W9M [109]). SMC monomers are colored in blue and gray, DNA is shown as magenta spheres, and fragments of associated accessory subunits are depicted in yellow. Extended coiled-coil linkers are indicated as dotted lines (not to scale), that would connect the two regions in a full-length Smc/Rad50 complex.

(e) Cartoon representation of structural transitions that SMC proteins thought to undergo during DNA trapping. SMC monomers dimerize through their hinge domains, with their ABC ATPase subdomains linked by an accessory protein bridge. ATP binding can cause the ABC ATPase subdomains to dimerize and transient openings in the accessory protein bridge or possibly in the hinge domain may allow DNA to access the interior of the cavity formed by the SMC coiled-coil domains.

(f) Proposed trapping of two DNA duplexes has the potential to bring distal segments in close proximity (left) to each other or link two segments from distinct chromosomes (right) within the SMC ring interior.