Chapter 3
Structure and Mechanisms of SF2 DNA Helicases

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Abstract Effective transcription, replication, and maintenance of the genome require a diverse set of molecular machines to perform the many chemical transactions that constitute these processes. Many of these machines use single-stranded nucleic acids as templates, and their actions are often regulated by the participation of nucleic acids in multimeric structures and macromolecular assemblies that restrict access to chemical information. Superfamily II (SF2) DNA helicases and translocases are a group of molecular machines that remodel nucleic acid lattices and enable essential cellular processes to use the information stored in the duplex DNA of the packaged genome. Characteristic accessory domains associated with the subgroups of the superfamily direct the activity of the common motor core and expand the repertoire of activities and substrates available to SF2 DNA helicases, translocases, and large multiprotein complexes containing SF2 motors. In recent years, single-molecule studies have contributed extensively to the characterization of this ubiquitous and essential class of enzymes.

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Superfamily II Helicases and Translocases

Helicases are a subset of nucleic acid (NA) translocases, enzymes that convert the energy of ATP hydrolysis into directional motion along polar NA substrates [1–6]. Superfamily II (SF2) helicases and translocases make up the largest and most structurally diverse group of these motor proteins [1]. Much like SF1 enzymes (see Chap. 2), SF2 proteins are so grouped owing to a common set of "helicase signature motifs" [4–8]. Further structural and functional studies have shown, however, that the two conserved domains containing the signature motifs form a general-purpose motor core, the primary biochemical function of which being the transduction of energy produced through ATP binding and hydrolysis into directional motion along a NA lattice [1, 9]. This motor core consists of two RecA-like folds termed helicase domain 1 (HD1) and helicase domain 2 (HD2); HD1 and HD2 correspond to RecA-like folds 1A and 2A in SF1 motors [1, 10]. Changing affinities of the two motor domains for DNA during the ATPase cycle bias the energy landscape and enables directional translocation. Helicase activity itself results from the actions of accessory domains unique to each enzyme that couple the biochemical activity of NA strand separation to the translocating action of the motor core [4–6]. Interestingly, SF2 enzymes exhibit a range of modular accessory domain architectures that expand the repertoire of the superfamily to include such diverse activities as replication fork reversal, chromatin remodeling, and even peptide export, all coupled to the action of the conserved motor core [11–13]. Recent sequence analysis of SF2 proteins identified six major families involved in DNA metabolism, each named after their archetypal member (RecG, RIG-I, RecQ, Rad3, Ski2, and Swi/Snf); these families within SF2 are the foci of this chapter [1].

Figure 3.1a shows the relative composition of SF2, grouped by substrate specificity [1, 4–6]. RNA helicases make up a clear majority of SF2 proteins when sorted this way, with the DEAD-box family of these enzymes being particularly numerous. *Bona fide* DNA helicases make up the next largest group, followed by ss- and dsDNA-specific translocases. Singleton et al. proposed a system for classifying helicases with respect to translocation direction, or polarity [5]. The orientation of the SF2 motor core to its NA substrate within the context of this system is shown in Fig. 3.1b: motors that operate in the 3′–5′ and 5′–3′ directions are classified as SF2A and SF2B, respectively. Although SF1 and SF2 bind NA lattices in the same relative orientation, that is, with HD1 oriented towards the 5′ end of the translocation strand, SF2 enzymes interact primarily with the phosphodiester backbone of their NA substrates while SF1 helicases intercalate the nucleobases in specific pockets of the motor core enabling a "Mexican wave"-like translocation (see Chap. 2) [4–6, 8, 14, 15]. The motor domain orientation and interaction of SF2 motors with the phosphodiester backbone is reflected in the B-form conformation of the NA lattice in first three panels of Fig. 3.1b. Many DEAD-box helicases are hypothesized to dismantle secondary structures present at sites essential to RNA quality control [16]. This highly local helicase function requires relatively limited processivity; structural and functional studies have shown that these enzymes make more extensive contacts with the heterocyclic bases of their NA substrates, a situation that would necessarily hinder the progress of an SF2 motor core optimized for back-

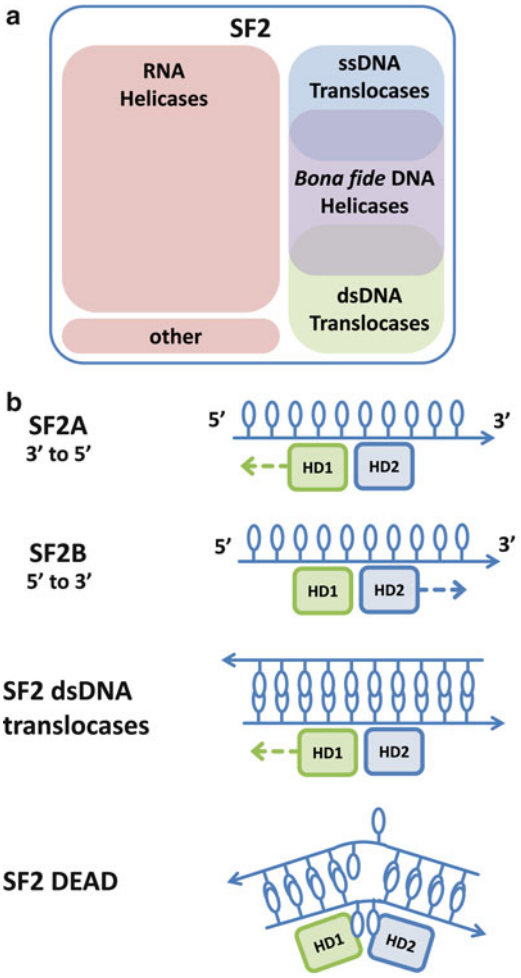

Fig. 3.1 Superfamily II, a varied class of NA motor proteins. (**a**) Shows the major groups of SF2 grouped by substrate specificity. RNA helicases make up the largest portion, with DNA helicases and translocases making up (roughly) the other half. (**b**) Displays the binding modes of SF2A and SF2B enzymes as described by Singleton et al. [5]. The *lower panels* show the orientation of the helicase domains in SF2 dsDNA translocases and DEAD-box proteins

bone-mediated translocation [4–6, 8, 16]. A diagram representing DNA binding by this group is shown in the last panel of Fig. 3.1b.

The SF2 Motor Core

Translocation of SF2 motor cores along NA lattices is accomplished by the concerted action of the HD1 and HD2 moieties [2, 4–6, 8]. Current data suggest this action mirrors the "inchworm" model of translocation proposed for other helicase superfamilies (Chaps. 1 and 2) [2, 4–6, 9, 16]. Containing 11 conserved motifs

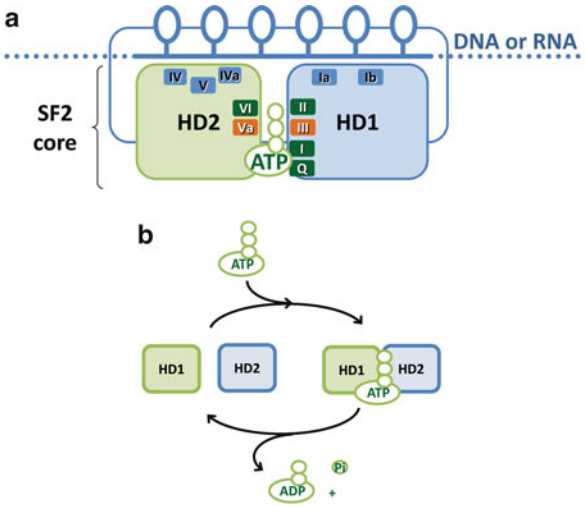

Fig. 3.2 The SF2 motor: its structure and behavior during ATP binding and hydrolysis. (**a**) Shows the orientation of the helicase signature motifs within the motor core and their relationship to the NA lattice when bound to ATP. (**b**) Shows the relative positions of HD1 and HD2 through the course of the ATP hydrolysis cycle

(Q, I, Ia, Ib, II, III, IV, IVa, V, Va, and VI), the SF2 helicase domains are diagrammed in Fig. 3.2a [1]. A subset of the P-loop NTPases, SF2 enzymes use the sterically optimized Walker A and B motifs to hydrolyze NTPs [2]. The SF2 DNA translocases and processive helicases possess a Q motif that confers specificity for ATP [17]. Helicase motifs I and II, containing the Walker A and B boxes, are among the most conserved motifs across the superfamily [1]. Motif VI is also involved in the coordination and hydrolysis of NTPs [2, 18, 19]. Located at the interface between the RecA-like folds, motifs I and II—along with motifs Q, III, Va, and VI—compose a pocket formed when the helicase domains are brought into close proximity upon ATP binding [2, 5, 6, 14]. This process is depicted in Fig. 3.2b. The subsequent hydrolysis of ATP to ADP and inorganic phosphate collapses this pocket and allows the separation of the helicase domains. Motifs III and Va contact both DNA-binding and NTP hydrolysis moieties within HD1 and HD2 (respectively) and are believed to play an essential role in transmitting the energy of ATP hydrolysis into motor function [2, 18, 19].

Motifs Ia, Ib, IV, IVa, and V make extensive contacts with the phosphodiester backbone of the DNA lattice [4, 14]. The tuned affinities of these motifs for NA substrates are believed to be the antecedents of helicase polarity [5, 6]. Furthermore, the inchworm model of translocation predicts that these affinities are dynamic through the course of the ATP hydrolysis cycle; however, the details of how this variation is tied to specific conformational transitions of the helicase domains remain poorly understood, mainly due to the dearth of structural information from SF2 enzymes [2, 9, 16].

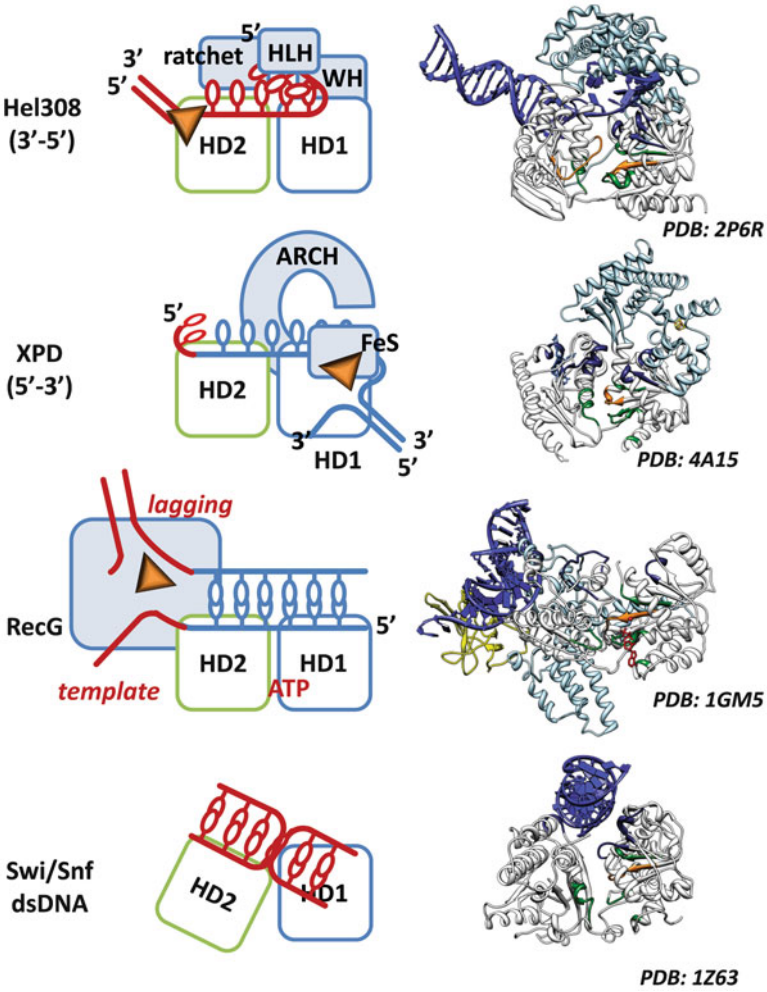

Fig. 3.3 Structures of several representative SF2 motors. The high-resolution crystal structures (from indicated PDB entries) of several SF2 motors are shown alongside diagrammatic representations. Substrate nucleic acids are shown in *violet* in the crystal structures and *red*/*blue* in the diagrams. Motifs in the crystal structures are color coded as follows: *green*—ATP binding; *dark blue*—DNA binding; *orange*—involved in the communication between ATP- and DNA-binding sites. HD1 and HD2 are *white* and are aligned the same as on the cartoon. In the diagrams, the auxiliary domains are in *light blue* (pin/wedge structures are shown in *yellow*)
ATP adenosine triphosphate; *SF1* superfamily I; *NTP* nucleoside triphosphate; *FRET* Förster resonance energy transfer; *GC* guanine-cytosine; *TIRFM* total internal reflection fluorescence microscopy; *OB* oligonucleotide binding fold; *bp* base pair

Accessory Domains Dictate Biological Function

The helicase domains of SF2 motors often contain long insertions that are believed to function in coupling ATP hydrolysis and translocation activities to accessory biochemical function [1]. These regions act in concert with accessory domains separate from the motor core to enable the in vivo functions of SF2 proteins. An example of this phenomenon is the presence of a protruding region of β-hairpin secondary structure between motifs Va and VI in the Ski2-like helicase Hel308 [20]. Structural data for this enzyme indicate that this structure may function as a "pin" that separates substrate NA strands and thus plays a crucial role in coupling translocation to helicase activity. Similarly, accessory NA-binding domains facilitate Hel308's unwinding of duplex DNA [20]. Figure 3.3 shows the domain orientation of several SF2 proteins for which structural data are available. These examples—Hel308 and members of the Rad3, RecG, and Swi/Snf families—show explicitly the degree to which domains outside of the motor core influence the nucleoprotein interactions within the SF2 core and beyond to yield the intermediates required for essential processes in NA metabolism including DNA cross-link repair, replication fork rescue, and chromatin remodeling.

RecG Family Helicases: Bacterial First Responders

Packed tightly inside the bacterial envelope, even the simplest DNA lattices form highly heterogeneous manifolds. Replete with stable nucleoprotein complexes and persistent NA structures, even these relatively simple genomes can prove resistant to the efficient progress of the replisome. In the case of a replication fork stall caused by a difficult element of the lattice or a collision with another DNA-translocating machine or otherwise tightly bound protein, there exist robust mechanisms for the origin-independent rescue of replication. Regression of the replication fork from the site of replisome dissociation is often the first step in these processes [21, 22]. Stalled replication fork regression is performed in prokaryotes by SF2 helicases of the RecG family [23]. Belonging to SF2A, RecG proteins translocate along dsDNA in the 3′–5′ direction while primarily contacting the DNA along the phosphodiester backbone of the template for the nascent lagging strand (see Fig. 3.3) [4]. Topologically, these proteins consist of an accessory "wedge" domain at the N-terminus linked to the conserved SF2 motor core, with this assembly capped by a short accessory domain at the C-terminus [1].

Structural and Mechanistic Insights from Thermotoga maritima

A crystal structure of RecG from *Thermotoga maritima* represents the only published structural information for this helicase family [4]. Solved in a complex with ADP and a three-way DNA junction, this structure provides valuable insight into the detailed

mechanism of SF2 motor-coupled replication fork reversal. It is hypothesized from the structural data that RecG translocation is facilitated by protein–DNA contacts at the branch point of the parent strands [24]. The contacts at the parent strand branch point occur in a dsDNA-binding conduit separating the motor core and the wedge domain, a substantial gap bridged by a 41-residue α-helical linker [4]. Several residues of the C-terminal accessory domain form a pin structure that inserts prominently into this channel. The pin structure makes contact with the DNA backbone and is linked to motif VI of the motor core by a network of hydrogen bonds. Alteration of this network as a consequence of the ATP hydrolysis cycle is believed to drive RecG translocation. Directional movement of the motor core along the dsDNA lattice brings the nascent strands of the stalled fork structure into contact with the wedge domain; it is in this way that translocation is coupled to helicase activity. The wedge domain is believed to separate the nascent DNA strands from the parental components of the stalled fork during translocation by steric exclusion of the nascent strands from a set of diverging ssDNA channels. Interestingly, the wedge domain's contacts with the liberated DNA strands facilitate the simultaneous reannealing of the fork's parental strands in its wake and the formation of a "nascent strand duplex" at its prow. Thus, the concerted action of the accessory domains during dsDNA translocation forms the Holliday junction structures typical of RecG processing of stalled replication forks [4, 20].

The In Vivo Role of RecG

A variety of in vitro studies have observed a strong preference among RecG proteins to bind dsDNA substrates possessing 3′ ssDNA branches (as shown in Fig. 3.3) [25–27]. This class of substrates includes Holliday junctions, D-loops, and R-loops—the usual suspects for recombination-mediated repair processes. The affinities of RecG proteins for these structures and the fork-like junctions that result from replisome collapse place the RecG family at the interface of DNA replication, recombination, and repair. dsDNA translocation is coupled to helicase activity through the accessory domains to yield a Holliday junction from the stalled fork [28]. The formation of these junctions may permit origin-independent replication fork restoration by the bacterial recombination machinery [29, 30]. Conversely, these Holliday junctions may also be substrates for direct resolution pathways [23]. Though it is clear that RecG proteins act as first responders at stalled forks, at this time the precise methods by which these structures are ultimately resolved are poorly understood [27, 28].

Although RecG is an exclusively bacterial helicase, regression of stalled replication forks into a "chicken foot" structure is important in all organisms [21]. Evidence of fork regression activity exists for human BLM and FANCM helicases [31, 32]. Much like RecG, FANCM is an SF2A dsDNA translocase that uses specific accessory domains to perform branch migration on a variety of three- and four-way DNA junctions [31].

RIG-I Translocases: Guides of Branched DNAs in Archaea and Eukarya

SF2 translocases often perform the initial steps in processing branched DNA structures [30]. Three- and four-way junction structures are frequent intermediates of DNA damage repair. If not processed in a timely manner, these structures can cause genome instability [33]. In most cases, the intermediates produced by SF2 translocases are subsequently modified via biochemical activities unrelated to NA translocation. The RIG-I family translocases have been linked to the resolution of many of the three- and four-way branched DNA structures observed in Archaea and Eukarya including D-loops, R-loops, Holliday junctions, and aberrant fork structures [30, 34]. Named for a human RNA translocase implicated in viral recognition, the RIG-I SF2 enzymes exhibit a wide range of biochemical activities united by common domain architecture [1]. Archaeal members of the RIG-I family possess terminal accessory domains that cleave the branched intermediates produced by the coupled action of the motor core and helicase domains [31]. These enzymes physically link the primary and secondary responses to stalled replication forks and other events yielding branched DNAs in vivo. In eukaryotes, the downstream processing of three- and four-way DNA junctions frequently occurs within macromolecular assemblies recruited by RIG-I proteins [29, 33, 35, 36]. A discrete set of mutations to the terminal accessory regions of eukaryotic RIG-I translocases abolishes their nuclease activity [31]. Interestingly, these same mutations facilitate the participation of eukaryotic RIG-I translocases in a range of macromolecular assemblies. Severing the physical link between branch point migration and downstream processing establishes a modular paradigm in eukaryotes where RIG-I translocases tune their recruitment of protein partners to their substrates, allowing them to facilitate a variety of DNA transactions essential for genome maintenance and concomitant regulation of the cell cycle [30, 31, 33].

Hef: An Archaeal Model for RIG-I Action

Isolated from *Pyrococcus furiosus*, *h*elicase-associated *e*ndonuclease for *f*ork-structured DNA (Hef) is the only available structural model for RIG-I DNA translocases [37]. Though Hef functions quite differently from its eukaryotic brethren, RIG-I translocases share a highly conserved domain architecture that permits a certain degree of generalization [1, 31]. The helicase activity that drives branch point migration by RIG-I enzymes is believed to be coupled to the action of the SF2 core by an Mph1-like accessory domain present at the N-terminus, though the detailed mechanism of this coupling is unknown [36, 37]. RIG-I proteins are SF2A enzymes that perform dsDNA translocation [1, 5]. A “family-specific” accessory domain bisects the RecA-like folds of the motor core. Containing a network of positively charged residues, this accessory domain shares marked characteristics with the DNA-binding

"thumb" domains of A-family DNA polymerases [1, 31]. The details of how this domain aligns branched DNA substrates are unclear from the available *apo*-structure, but this region is believed to recruit Hef to specific DNA junctions. Hef's C-terminal ERCC4 and helix-turn-helix domains are responsible for the cleavage of forked DNAs in a manner similar to nuclease ERCC4 (MUS81) [31, 36, 37]. In vitro observations suggest that RIG-I helicase activity stimulates nuclease action, suggesting that the activities act in concert in vivo. Human RIG-I DNA translocases retain these nuclease domains, but the deletion of a single lysine in the ERCC4 domain abrogates their nuclease activity [31].

Human RIG-I Translocase FANCM

The terminal helix-turn-helix motif of Fanconi Anemia Complementation Group M protein (FANCM) facilitates its interaction with the Fanconi Anemia core complex (see Chap. 9) [1, 31, 33]. FA-associated protein FAAP24 possesses inactive ERCC4 and helix-turn-helix domains that dimerize with identical domains in FANCM and link the RIG-I translocase to the FA core complex. The dimerization of these proteins is believed to activate FANCM's branch point migration activity and recruit the FA core to branch point sites made labile by RIG-I helicase activity. Branched DNA structures that stimulate FANCM helicase activity often indicate the necessity for initiating repair processes, many of which are regulated by the FA core [30, 31]. Recruitment of the core by FANCM has been further linked to the S-phase DNA damage checkpoint. FANCM's affinity for a variety of three- and four-way junctions makes it an excellent indicator of the repair status of the genome. The participation of the human FANCM in a number of FA complexes allows it to tune the cellular response to branched DNA structures and guide their processing in a way that responsively maintains genomic stability and ensures the proper progress of the cell cycle [29, 31].

The RecQ Family

Originally identified almost 30 years ago, RecQ translocases have since been identified in all three kingdoms of life and are among the best-studied SF2 proteins (see Chap. 8) [38, 39]. In vivo, RecQ helicases participate in replication fork rescue, telomere maintenance, homologous recombination, and DNA damage checkpoint signaling (see Chaps. 6, 8 and 9) [40]. There are five human RecQ helicases, and mutations in three of their genes are associated with cancer predisposition and/or premature aging as expressed in Bloom syndrome, Werner syndrome, and Rothmun-Thomson syndrome [32, 41, 42]. In contrast, bacteria and lower eukaryotes have a single RecQ family member. RecQ helicases unwind DNA with a 3′–5′ polarity and

are capable of unwinding or remodeling a variety of DNA structures in vitro. These structures include forked duplexes, D-loops, triple helices, four-way junctions, and G-quadruplex DNA. The activities of RecQ family members are often tuned by integration into macromolecular complexes catalyzing multiple enzymatic activities (discussed in detail in Chap. 8).

In addition to a common SF2A motor core, RecQ helicases contain several characteristic accessory domains. Immediately downstream of the RecA-like regions, these enzymes carry a highly conserved RecQ-C-terminal (RQC) domain [39, 43–45]. This region contains a zinc-binding domain and a winged-helix (WH) domain and is present in all RecQ helicases, save for RecQ4. Mutagenesis studies on some members of this helicase family have shown that the zinc-binding domain is likely involved in maintaining the structural stability of the protein [46, 47]. The WH domain is important for dsDNA recognition and may also be involved in protein–protein interactions [39]. At their C-termini, RecQ helicases possess a relatively varied accessory region termed the helicase-and-RNaseD-like-C-terminal (HRDC) domain. This region has been implicated in substrate recognition. These modular accessory domains (RQC and HRDC) position the SF2 core and tune its action to give rise to the varied substrate specificities and enzymatic activities of the RecQ family.

BLM: A Human Model for RecQ Action

Among human RecQ helicases, BLM (Bloom Syndrome protein) is probably the most extensively studied. Not surprisingly, it is discussed extensively in several chapters of this book (Chaps. 6, 8 and 9). Bloom Syndrome is the disease caused by a BLM deficiency [32]. This disease is associated with chromosomal abnormalities and defective response to replication stress, both due to extraordinarily high levels of homologous recombination (HR).

BLM's ability to regulate HR results primarily from the augmentation of basic SF2A translocation activity by its accessory domains. The RQC's zinc-binding region has been shown to stabilize BLM and facilitate DNA binding [32]. Similarly, the WH region of the RQC confers BLM's ability to bind G-quadruplex DNA [39]. While not essential for forked-duplex unwinding or ATP hydrolysis, the HRDC domain plays an important role in Holliday junction unwinding and, more importantly, double Holliday junction dissolution, an activity exclusive to BLM among human RecQ helicases. Sato et al. recently demonstrated that isolated HRDC alone could not bind substrate DNA [48]. Both RQC and HDRC domains together are necessary for interaction with telomere-associated protein TRF2, a factor that stimulates the BLM-mediated unwinding of telomeres. BLM's unique N-terminal regions are likely involved in macromolecular interactions of still unknown significance. Full-length BLM was found to form hexameric ring structures, while the isolated BLM helicase core was found to be active in monomeric form even under conditions that strongly favor oligomerization [49]. When studied in isolation, the helicase core domain showed low processivity, a moderate unwinding rate, and, surprisingly, an ability to

"measure" the length of the duplex it unwound (see single-molecule section below) [50]. Although numerous biochemical studies of BLM exist, the mechanochemistry and detailed coupling mechanisms between ATP hydrolysis, translocation, and other activities of BLM remain unresolved. What is clear, however, is that the protein's accessory domains dictate its substrate specificity and biochemical activity.

Rad3 Family Helicases

The Rad3 family (also known as XPD-like) is the only family of SF2 helicases shown thus far to translocate on ssDNA in the 5′–3′ direction. It is named after yeast Rad3, the first identified member of this family [51]. Members of the Rad3 family are found in all domains of life and are involved in several genome maintenance pathways [52–54]. There are typically two Rad3 helicases in bacteria, one in archaea and yeast, and four have been identified in humans (XPD, FANCJ, RTEL, and CHLR1) [55]. A distinct structural feature of this family is the presence of a large insertion in HD1's Walker A motif split into two accessory domains, the iron-sulfur (FeS) cluster-containing domain and the arch domain [52, 56, 57]. Because of the important, but still debated, roles Rad3 helicases play in genome maintenance, many members of this family are discussed in detail in later chapters (Chaps. 6, 9 and 10). Here, we will use XPD, a model Rad3 helicase, to discuss how the structural features of Rad3 enzymes enable 5′–3′ translocation on crowded lattices and concomitant duplex unwinding.

XPD: A Model SF2B Enzyme

In humans, XPD (Xeroderma pigmentosum complementation group D) helicase is one of the most important subunits of TFIIH (Transcription Factor II-H complex), an essential element of the nucleotide excision repair (NER) machinery [58–60]. Four published crystal structures of XPD helicases from three different archaeal organisms make up the body of Rad3 structural data [60–63]. Three structures are without DNA or ATP and share a common topology. The most recent structure was obtained from a complex of XPD and a short DNA fragment [60]. XPD contains two modular domains incorporated into the motor core, an FeS-cluster containing domain and an arch domain [57]. The 4Fe-4S cluster is coordinated to four cysteine residues, three of which are important for the integrity of the cluster, folding of the FeS domain, and XPD activity [64]. Situated on the other side of the Walker A box, the arch domain is composed of two sets of alpha-helices arrayed with 60° between them, their relative orientation giving rise to an arch-like structure (Fig. 3.3). All published models predict that ssDNA passes first through the groove between the HD2 and arch domains, moving then through a hole encircled by the arch, FeS-cluster, and HD1 domains. Two recent studies identified the polarity of the translocation strand as

bound by XPD and provided insights into how the enzyme achieves its directionality on the NA lattice [60, 65]. These studies also identified the FeS-cluster domain as a wedge structure involved in duplex separation.

In isolation, XPD functions quite poorly as a helicase; on the other hand, in distributive processes like transcription and NER, this could be an asset. *Ferroplasma acidarmanus* XPD can unwind forked DNA as a monomer when assisted by RPA2, one of the cognate ssDNA-binding proteins. RPA2 stimulates DNA unwinding by XPD through a novel mechanism by providing a helix-destabilizing function [66]. Honda et al. demonstrated that XPD moves along ssDNA coated with RPA2 without displacing or stepping over it [67, 68]. Taken together, these data suggest a model whereby XPD helicase recognizes forked DNA substrates decorated with RPA2 (common structures in transcription and NER) and uses its FeS-containing domain to orient itself at the fork. RPA2 then melts the duplex ahead of the helicase, allowing XPD to advance forward and to trap the newly open bases. Retaining contact with the translocation strand, RPA2 then plays an additional supporting role by stabilizing the product(s) of helicase activity.

As yet, the other human Rad3 helicases (FANCJ, RTEL, CHLR1) and their homologs have proved resistant to high-resolution structural studies. However, based on amino acid sequence alignment, all of these helicases should have an SF2B motor core similar to that of XPD, an FeS-containing domain, and an arch domain. Additional domains integrated into or found at the termini of the motor core are involved in the interactions of each family member with its specific protein partners in the cell [53, 54]. Forthcoming structural data may elucidate how these domains work together to render the biological functions carried out by these enzymes.

The Hel308 Helicases of the Ski2 Family

Though named for an RNA-specific enzyme, the Ski2 family of SF2 helicases contains the Hel308 subfamily [1]. Exhibiting primary domain architecture nearly superimposable with both the RecQ and DEAH/RHA families, the Hel308 helicases have been identified in many archaeal and eukaryotic organisms. Implicated in several DNA repair pathways, these enzymes are believed to act primarily through processes involving recombinase nucleoprotein filaments at sites of DNA damage [33, 69, 70]. In metazoans, Hel308 enzymes are believed to function in the Fanconi Anemia (FA) pathway of recombination-mediated repair and in the regulation of RAD51 foci generated during meiosis [33].

Structural Lessons from Archaeal Hel308 Helicases

Both high-resolution structures available for the Hel308 subfamily are archaeal in origin, derived from crystallized complexes of *Sulfolobus solfataricus* and *Archaeoglobus fulgidus* helicases with partially unwound DNA structures [69, 70].

The crystallographic data suggest that Hel308 helicases bind specifically to fork-like structures possessing a 3′ overhang, a specificity enforced by their accessory domains. In addition to the conserved SF2A motor core, the Hel308 helicases possess three C-terminal accessory domains (as shown in Fig. 3.3) [1]. A β-hairpin motif inserted between motifs V and VI of HD2 is believed to act as a pin structure, coupling helicase activity to 3′–5′ ssDNA translocation by direct insertion into the duplex interface [1, 69, 70]. Interactions with the "ratchet" accessory domain are believed to facilitate strand separation by the pin structure. This domain is situated directly opposite the motor core-duplex interface and may stabilize released ssDNA, easing strand separation. Nearest to the motor core, the winged helix (WH) domain is also observed in the RecQ-like and DEAH/RHA translocases, and the folded orientation of the WH domain relative to the motor core is conserved across the Ski2, RecQ, and DEAH/RHA families [1]. The WH domain makes extensive orienting contacts with branched structures and may function as a "hinge," allowing Hel308 helicases to bind and then close tightly around substrate DNA [69, 70]. Capping the Hel308 helicases at the C-terminal is a helix-loop-helix (HLH) domain that exhibits typical DNA-binding activity and a strong affinity for ssDNA. The HLH domain is believed to function in vivo as a "brake," grasping the freed translocation strand and limiting the processivity of Hel308 helicases on branched substrates [70]. Taken together, the actions of accessory domains appear to orient Hel308 helicases to specific branched substrates and to couple their translocation along these substrates to self-limiting helicase activity.

The Role of Hel308 Helicases In Vivo

The in vivo role of Hel308 helicases has been extensively investigated [33]. Studies of *Caenorhabditis elegans* show that its Hel308 helicase, HELQ, is recruited to replication forks in response to crosslinking DNA damage. Real-time studies of U2OS cultures under crosslinking conditions support this result in human cells and agree with worm-based results placing Hel308 helicases in the FA repair pathway. Further genetic studies in *Drosophila* have shown Hel308 enzymes to play an essential role in the modulation of meiotic recombination. Interestingly, biochemical studies have shown Hel308 helicases to specifically disrupt dsDNA-RAD51 filaments, implicating these enzymes along with Rad54 translocase as postsynaptic regulators of recombination, a finding further supported by genetic results in *C. elegans*. Furthermore, Hel308 helicases have been shown in vitro to displace DNA-binding proteins via direct, though helicase-activity independent, interactions. Surprisingly, the in vitro ability of HELQ to remove RAD51 was observed to be ATPase independent. Taken together, the genetic and biochemical data suggest that Hel308 helicases act to assist the recombination-mediated repair of DNA damage encountered during replication and meiosis by resolving post-synaptic Rad51 complexes. The detailed pathway of RAD51 locus regulation, a pathway proceeding through Hel308 enzymes that provides resistance to cross-linking DNA damage and ensures proper levels of meiotic recombination, however remains unclear [29, 33].

Future work will resolve the relationship between the observed architecture of Hel308 enzymes, their observed in vitro activities, and the growing body of genetic studies placing these enzymes in essential genome maintenance pathways.

SF2 Chromatin Remodelers

Eukaryotic chromosomal DNA is packaged into a condensed structure known as chromatin [71, 72]. The basic unit of chromatin is the nucleosome, which consists of 146 bp of dsDNA wrapped around a histone protein octamer [73, 74]. Highly condensed chromatin packs and organizes dsDNA within the nucleus. On the other hand, however, nucleosomes restrict access to the templating information required by the molecular machines tasked with DNA replication, repair, recombination, and transcription [72, 75]. Chromatin remodeling complexes are multisubunit enzymes that use the energy of ATP hydrolysis to reposition, destabilize, eject, or restructure nucleosomes, thereby rendering nucleosomal DNA accessible. The importance of chromatin remodelers can be illustrated by their absolute requirement for embryonic development and their governing role in cell cycle progression [76].

The essential unit of chromatin remodeling is a single nucleosome bound by a single remodeler driven by an SF2 motor [77, 78]. While a unified model of chromosome immobilization remains elusive (see Chap. 13), many recent models support the notion that the remodeler binds to the nucleosome particle via accessory moieties, and then the motor core binds DNA at a location inside the nucleosome [75]. The translocase domain, tightly anchored to the nucleosome core, then induces directional DNA translocation, generating a remodeling strain within the nucleosome particle. This process may occur by sequential or concerted action of two domains: a DND (DNA-binding domain) that pushes DNA into the nucleosome, creating small DNA loop, and a Tr (translocation) domain that pumps the DNA loop outward.

There are four families of chromatin remodelers: Swi/Snf, ISWI, CHD, and INO80 [72, 75, 79]. Remodelers in all these families possess a Swi2/Snf2 family ATPase core (or subunit), a version of the SF2 motor core containing DExx and HELICc domains. What differentiates these families from each other is the presence of accessory domains incorporated into, or adjacent to, the SF2 translocase core. The Swi/Snf, ISWI, and CHD families each have a short insertion within the SF2 motor, while remodelers of INO80 family have a long insertion. These insertions form the accessory domains that regulate the enzymatic activity of the complex, facilitate interactions with transcription factors or other chromatin-associated enzymes, or target the complex to DNA and histones.

The Swi/Snf Family

Swi/Snf (Switching defective/Sucrose nonfermenting) chromatin remodelers are multiprotein complexes composed of 8–14 subunits [80]. Many of the activities carried out by this family serve to enable genome maintenance and

transcription machines' access to genetic information. Different Swi/Snf remodelers slide, unwrap, and eject nucleosomes at many loci and mediate dimer exchange and/or dimer swapping [75]. The Swi/Snf family can be subdivided into two groups, and both play distinct roles in DNA repair [77, 79]. Powered by ATP hydrolysis, Swi/Snf protein complexes carry out directional translocation on dsDNA [81]. This activity destroys histone–DNA contacts, and, according to the loop capture model, creates a transient DNA loop that propagates around the nucleosome and resolves when it reaches the other side of the nucleosome, a process that leads to nucleosome repositioning. It is not yet known whether the loop is large (>100 bp) or small (1–12 bp), but it is clear that during remodeling the contacts between histones and DNA are broken and reformed along the length of the nucleosome.

The ISWI and CHD Families

ISWI (Imitation Switch) family remodeling complexes are typically smaller than Swi/Snf assemblies, containing two to four subunits. Some members of this family optimize the spacing between nucleosomes to promote chromatin assembly and the repression of transcription, while other members randomize nucleosome spacing [82]. Much like ISWI proteins, the CHD (Chromodomain, Helicase, DNA binding) family participates in chromatin organization. Some members of this family slide or eject nucleosomes to promote transcription, while others have repressive roles. CHD remodelers may consist of a helicase subunit in lower eukaryotes but are large complexes in vertebrates [75, 83].

INO80 Remodeling Factors

Finally, the INO80 (Inositol-requiring 80) family complexes contain more than ten subunits, often with a long insertion present in the middle of the SF2 helicase domain [84]. Functionally, this family is diverse; INO80 enzymes perform a variety of transactions involved in transcriptional activation and DNA repair. SWR1 proteins are often classified as INO80 remodelers, but their functions are different from most INO80 family members in that they are able to remodel the nucleosome [72, 75].

Despite the diverse set of activities attributed to the four families of chromatin remodelers, they all contain an SF2 helicase motor essential for the remodeling activity. The varied accessory domains that interact with nucleosome, directing SF2 motor action and carrying additional enzymatic activities, bring about the diverse functions of this family.

Insights into SF2 Biochemistry from Single-Molecule Studies

In recent years, the emergence of single-molecule (SM) techniques has contributed powerfully to the study of SF2 [9, 16, 85, 86]. The ability to address the behaviors of individual molecular motors provides unprecedented insights into their function [87]. This holds particularly true in the cases where the same enzyme may perform different functions in different contexts. For example, human RecQ family helicase BLM participates in the macromolecular RTR complex to dissolve double Holliday junctions (see Chap. 8). Proper function of the RTR complex depends on the integrity of BLM HRDC domain and thereby on its oligomerization. BLM helicase has several additional roles in controlling and promoting recombination which may have different oligomeric forms or binding partners [88].

Single-Molecule Studies of RecQ Helicases

In the absence of a unified mechanism for DNA unwinding, the accurate assignment of activities to a distinct physiologically relevant form of a helicase is of paramount importance. A single-molecule FRET study on BLM helicase showed that this enzyme undertakes repetitive unwinding attempts on forked DNA substrates [50]. This behavior was observed for wild-type BLM and a mutant that lacks an oligomerization domain. In addition, unwinding time was found to be protein concentration independent, which suggested that repetitive low-processivity unwinding events on the same DNA molecule can be attributed to a single BLM monomer. Reannealing time was found to be ATP dependent, a behavior that was interpreted as strand switching by the helicase motor and translocation on the opposite (complementary) arm of the fork in the 3′–5′ direction allowing the strands to reanneal. From the FRET change histogram analysis, Yodh and colleagues concluded that BLM unwinds a well-defined length of DNA, leading to the interpretation that BLM helicase is able to "measure" the number of base-pairs unwound, and once it reaches a specific length, it rapidly reverses unwinding by switching the strand. Another interesting finding of this SM-FRET study was that the repetitive unwinding pattern persisted in the presence of human replication factor A (RPA) with only a limited increase in unwinding processivity per cycle and no effect on reannealing time. This was unexpected since RPA is known to enhance the processivity of BLM helicase on long duplex regions [89]. Furthermore, RPA significantly increased the waiting time before repeating unwinding for catalytic core BLM (lacking the RPA-binding domain). These results indicated that RPA facilitates BLM transfer back to the tracking strand and reinitiation of unwinding via direct physical interaction between the two proteins.

Another important feature of the helicase mechanism that can be definitively addressed in the single-molecule experiments is whether the enzyme is an active or a passive helicase [7, 8, 90]. This again is particularly important for helicases whose motor cores translocate along the phosphodiester backbone of ssDNA or dsDNA and therefore may be prone to slipping and back stepping [91].

Escherichia coli RecQ was one of the model helicases that were compared in a magnetic tweezer study targeted at establishing a physical reference value/parameter to judge whether a specific helicase unwinds dsDNA in an active or passive manner [92]. The theoretical approach adopted to find this reference parameter was based on comparing translocation velocity (V_{trans}) on ssDNA vs. unwinding rate (V_{un}) of dsDNA using DNA substrates of different GC contents, and with different tension forces. An active helicase destabilizes the fork, resulting in similar unwinding and translocation rates. In contrast, a passive helicase is slowed down by the presence of the dsDNA fork, which leads to a much slower unwinding rate compared to the translocation rate. Furthermore, because a passive helicase advances by trapping the spontaneously melted base pairs, its unwinding rate is expected to be sensitive to dsDNA sequence and tension applied to the two ssDNA tails of a fork. Manosas and colleagues proposed that for a helicase taking 1 bp steps and no slippage or back steps, the value of $V_{un}/V_{trans} = 0.25$ can be a good reference to judge whether a helicase is passive (<0.25) or active (>0.25). Surprisingly, RecQ was found to have V_{un}/V_{trans} values ranging from 0.9 to 0.7 under all GC ratios and tension forces tested in this study, suggesting that RecQ is an extremely active helicase (compared to the passive T4 gp41 helicase, which has values of 0.1 or less, and showed strong GC content and tension force dependence). It is important to note that all experiments on RecQ were done under monomeric conditions (picomolar concentration range), supporting previous studies suggesting that RecQ can unwind dsDNA as a monomer.

In a different FRET-based single-molecule study, the Dou and Xi groups noticed a lag in the initiation of unwinding by RecQ for longer ssDNA tails of an overhang DNA structure at a low ATP concentration [93]. At a high ATP concentration, the unwinding initiation rate was similar for longer and shorter tails of overhang DNA structures. The authors suggested that this behavior is an indicator of mutual inhibition by RecQ molecules. To prove that this is indeed the case, Pan and colleagues carried out experiments at low and high enzyme concentrations and observed that delayed initiation disappeared at low helicase concentration in the presence of a long tail overhang structure. They proposed that the mutual inhibition is due to a forced closure of the cleft between RecA-like domains HD1 and HD2 of a leading monomer by a trailing monomer before binding of ATP. Notably, preincubation of RecQ and DNA with a non-hydrolysable ATP analog before initiating the unwinding reaction prevented the unnatural closure of the cleft and alleviated the mutual inhibition effect by higher concentrations of RecQ. This phenomenon was observed in a stopped-flow experiment conducted under single-turnover conditions.

Single-Molecule Studies of Rad3 Helicases

Due to the lack of structural information from Rad3 helicases, SM studies have been particularly useful in addressing the mechanisms of their activity [67]. The first SM study of XPD helicase combined the well-known SM-TIRFM technique with distance-dependent quenching of fluorescent dyes by the iron-sulfur cluster observed in all members of the Rad3 helicase family [94]. The ability to follow helicase movement

by monitoring the FeS-mediated fluorescence quenching allowed Honda and colleagues to address an intriguing question: what happens when a translocating helicase encounters a roadblock [68]. The main finding of this multiparameter SM study was that distinct ssDNA binding proteins had a differential effect on the translocation activity of XPD helicase. RPA1 (a homodimeric ssDNA-binding protein) competed with XPD for ssDNA access, while RPA2 (an ssDNA-binding protein containing a single OB fold) targeted XPD to the RPA2-ssDNA complex. In a pseudo-tricolor experiment, Honda and colleagues directly observed that XPD was able to share the lattice with RPA2 and to bypass this ssDNA-binding protein without displacing it from ssDNA. RPA2 was found, however, to significantly slow down XPD translocation. The authors proposed that the ability of XPD to translocate on protein-coated ssDNA depends on the high flexibility of the relative position of the arch domain with respect to iron-sulfur domain, which may permit an opening motion to accommodate the protein–DNA complex into the hole made by the arch and iron-sulfur domains and HD1 (Fig. 3.3). Whether this is indeed the case remains to be determined and will likely require further development of novel SM techniques.

A recent atomic force microscopy (AFM) study by the Tainer and Barton groups showed that XPD helicase may cooperate with other proteins that are efficient in DNA charge transfer (CT) in order to localize to the vicinity of damage in DNA [95]. XPD helicase was found to redistribute on kilobase DNA substrates in the vicinity of a lesion which prevents CT, while a CT interaction-deficient mutant of XPD did not have this redistribution ability. The authors proposed that the ability of redox sensitive proteins like XPD to redistribute in the vicinity of lesions might be a good strategy to reduce the search process required to find lesions across the genome.

Insights into SF2 Chromatin Remodeling Activities

Single-molecule analyses have been particularly useful in discriminating between a number of models proposed to explain the activities of the Swi/Snf family of DNA translocases and nucleosome remodeling motors. One member of this family, Rad54 (see Chap. 9), is an important player in homologous recombination and in the recombination-mediated repair of broken chromosomes [96, 97]. The enzyme remodels DNA structures (including nucleosomes), assists Rad51-mediated strand invasion into nucleosome bound DNA, and facilitates disassembly of Rad51 nucleoprotein filaments from dsDNA. The first indirect indication that hRad54 (human Rad54) moves on dsDNA came from the observation that it generates supercoils on closed circular DNA [98]. It was a much later SM study, however, which unambiguously revealed the motion of Rad54 molecules, decorated with fluorescently labeled antibodies, on lambda dsDNA (approximately 50 kb long) stretched by hydrodynamic flow [99]. Rad54 was observed to bind DNA randomly and with no particular preference for substrate orientation. The observed velocity of translocation (~300 bp/s) was much faster than the movements usually detected during remodeling processes and was ATP dependent. Translocation rates were varied widely, with a tenfold difference between the slowest and fastest molecules. Rad54

processivity was remarkably high (~11 kb) and, unexpectedly, it was independent of ATP concentration, a behavior opposite to that observed in many other DNA motor proteins. A number of peculiar features of the Rad54 translocation were observed. These included pausing, direction reversal, and velocity change. The small sample size, however, prevented firm conclusions. In summation, the authors proposed that there is a change in the molecular species undertaking the translocation after pausing or direction reversal, which could reflect a multimeric Rad54 complex with a different monomer being used after the translocation restart since there was no observed correlation between velocity before and after a pause or directional reversal. Another interesting observation reported in this study was that at least half of the molecules exhibited a "lag" phase before starting translocation. Furthermore, the duration of this lag was reduced with increasing ATP concentration. This behavior may originate from a rate-limiting ATP-induced structural transition necessary for starting translocation.

In a following study, the same group used a very similar experimental approach to monitor the activity of yeast Tid1, a Rad54 homolog, which interacts with the meiotic recombinase Dmc1 [100]. Tid1 displayed more complex translocation behavior than Rad54 with most of the molecules showing the characteristic velocity change. In addition, the average translocation rate was two- to fourfold slower than Rad54; however, their processivities were similar. Similar to Rad54, Tid1 showed uncorrelated translocation rates before and after pauses and reversals, a finding indicative of a multimeric active form of this motor protein. Finally, Tid1 showed a wide distribution of translocation rates with a tenfold difference between the fastest and the slowest molecules.

The observed ATP dependence of the lag phase can be at least in part explained by the observations from another single-molecule study. Lewis et al. investigated the conformational transitions of an archaeal Rad54 catalytic core (Rad54-cc) [101]. Previous crystallographic studies showed that, in the absence of ATP, the Rad54 catalytic core adopts an open conformation with or without substrate DNA [102]. Ensemble FRET results showed that the catalytic core closes upon binding to DNA, while subsequent ATP binding did not change FRET [101]. Interestingly, a detectable conformational transition occurred after ATP hydrolysis but before ADP release (as inferred from FRET measurements in the presence of ATP, ADP, and an analog of the ATP hydrolysis transition state). In the same study, SM-FRET measurements performed on immobilized Rad54-cc showed that the protein is free to open and close in the presence of DNA. It was unclear, however, whether these conformational transitions corresponded to DNA molecule binding and dissociation. FRET histogram analysis revealed that the equilibrium between open and closed states was shifted towards a lower FRET state (open) in the absence of DNA, and towards a higher FRET state (closed) in the presence of DNA, though the FRET distributions in both cases were remarkably broad. Repeated SM-FRET measurements with freely diffusing protein molecules led to the same FRET distributions. Additionally, no discernible differences in conformational states were detected in the presence of DNA and in the presence and absence of ATP. Notably, the FRET-based study, which utilized truncated Rad54cc, did not produce any evidence of the multimeric

forms of active Rad54 complex, which contrasted with the observations made in the optical tweezer study of full length yeast Rad54 [99]. This discrepancy may be due to the omission of modular auxiliary domains responsible for protein oligomerization in the second study.

Recently, a sub-second AFM study was performed on full-length human RAD54 [103]. Based on volume measurements, the authors concluded that RAD54 functions as a monomer. Diffusion of the protein molecules in the absence of DNA was found to be six orders of magnitude slower than theoretically expected for a monomer, which could either indicate multimerization or surface interaction effects. Consistent with the SM-FRET studies, adding ATP or a non-hydrolyzable analog led to an increase in diffusion coefficient. RAD54 molecules were one order of magnitude faster in the presence of DNA than proteins alone on the imaging surface. Surprisingly, adding ATP in the presence of DNA did not lead to any detectable enhancement of Rad54 mobility on the NA chain.

ACF is another member of Swi/Snf family that is involved in chromatin remodeling and gene silencing (see Chap. 13) [104]. It repositions nucleosomes to create evenly spaced assemblies. These evenly spaced assemblies are critical for higher order chromatin folding, a process associated with long-term transcriptional repression. Blosser et al. studied the dynamics of nucleosome remodeling by human ACF using SM-FRET [105]. The authors adopted a three-color assay in which the nucleosome was labeled with a FRET donor–acceptor pair, while ACF was labeled with another, orthogonal dye. This experimental strategy allowed the authors to monitor simultaneously the events of nucleosome remodeling by FRET and the binding events of ACF and to correlate the dynamics of both entities. It was found that the gradual translocation of nucleosomes by ACF is interrupted by well-defined pauses. Surprisingly, several aspects of the remodeling process were found to be ATP-dependent: the binding of ACF, translocation of the nucleosome, and pauses. When the nucleosome was positioned at the end of DNA, the first pause occurred after 7 bp of translocation. Subsequent pauses were separated by 3–4 bp. The same behavior was observed when the nucleosome was positioned centrally, which means that this behavior may be a fundamental characteristic of remodeling by ACF. The authors suggested that the origin of these pauses is an ATP-dependent conformational transition of the enzyme that prepares the nucleosome for the next round of DNA translocation. Experiments with centrally positioned nucleosomes showed that ACF complex can translocate the histone octamer back and forth a total distance of more than 200 bp and switch directions more than 20 times. Statistical analysis of the fluorescence intensity and photobleaching behavior of the labeled ACF showed that when ACF binding events lead to bidirectional remodeling, there is always a dimer of ACF molecules. Conversely, binding events associated with unidirectional remodeling were observed to be due to ACF monomers. Interestingly, another study published at the same time used biochemical data and electron microscopy to show that ACF acts as a dimeric motor to separate nucleosomes [104].

SM tools can directly observe (and often in real time) details of the chromatin remodeling process including the structure of the nucleosome after remodeling and the displacement range per remodeling event. Yeast Swi/Snf is a transcriptional

activator that uses the energy of ATP hydrolysis to regulate the accessibility of the genetic code by changing chromatin structure [106]. By unzipping single DNA molecules using an optical trapping setup, Shundrovsky et al. probed some of the finer details of yeast Swi/Snf complex action [107]. Remodeling by Swi/Snf occurred in both directions on dsDNA. In addition, remodeled nucleosomes showed a continuous distribution of relocation sites around their original positions on DNA. The novel aspect of this SM approach is that it can directly detect the precise locations of the DNA-histone contacts, while the ordinary end-to-end stretching approach detects only the relative locations of the interactions. Unfortunately, the shapes of the response curves modeling the relationship of applied force to the number of unzipped DNA bases (or "disruption signature") in the presence of nucleosomes were similar with and without Swi/Snf (i.e. any structural changes to the nucleosomes were not stable enough to be detected). This result was used as evidence that the nucleosome resumes its classical structure after remodeling under the selected experimental conditions and spatial resolution of the technique used in this study. This study, however, did not show in any way that nucleosome structure is unchanged during the remodeling process [71]. Applications of this method to other systems may lead to more informative results.

RSC, or Remodels the Structure of Chromatin, is about tenfold more abundant in the cell than Swi/Snf [108]. It has been implicated in transcriptional regulation, sister chromatid cohesion, chromosome stability, and DNA repair. Two SM studies of RSC (and Swi/Snf) were published in 2006, both showing very interesting aspects of the remodeling process [81, 109]. The two studies were complementary to each other: one of them done on bare DNA without nucleosomes [109], while the other was done with nucleosomal DNA [81]. The experimental design of both studies was based on detecting changes in the length of single DNA molecules fixed at both ends.

In the former study [109], RSC was found to induce transient DNA length shortening (which was confirmed by AFM). This was interpreted as translocation of RSC on DNA resulting in a loop generation. Both ATP concentration and tension force affected the size of the translocated loop. The extent of underwinding during loop formation differed between (−) and (+) supercoiled DNA (scDNA); this was explained as a consequence of either the difference in the size of the DNA loop formed on (+/−) scDNA or the difference in the number of generated negative supercoils. The authors noted that loop formation was associated more closely with the underwinding of DNA, though a combination of both supercoiling processes is also possible. Notably, the generation of supercoils on a topologically closed DNA by a translocating SF2 motor has also been observed for hRad54. Based on the Worm-like Chain (WLC) model, the authors estimated that RSC generates much less than one turn of loop per every 10.5 bp of (−) scDNA translocated. The amount of negative rotation generated was between −0.15 and −0.04 rotations per 10.5 bp translocated, perhaps because the motion of RSC along DNA might be broken down into steps of ~12 bp in length. Interestingly, although the length of the translocated loop assumed a Gaussian distribution, the time taken to form the loop could be modeled by an exponential distribution. This difference in statistical behavior may reflect a change in RSC remodeling activity in response to tensile load during translocation.

The authors thus proposed a “thermal ratchet model” to describe RSC translocation. In this model, the RSC–DNA complex proceeds upon ATP binding into active translocation mode, with at least two contacts between DNA and RSC. Most of the loops formed due to translocation of DNA by RSC are removed in an ATP-dependent manner, probably due to translocation in the opposite direction. Sometimes, the loop collapses rapidly, either due to translocase disengagement or due to losing another contact that constrains the DNA.

Zhang et al. found that both RSC and yeast Swi/Snf switch translocation direction on DNA that has nucleosomes, but with a lower probability compared to their behavior on bare DNA [81]. Loop formation on bare DNA occurs only at very low DNA tension (<1 pN), while on DNA with a nucleosome present it could be observed at much higher tensions (1–6 pN). The average loop size on bare DNA was significantly larger than the average loop size observed on nucleosomal DNA (~100 bp). Following the same trend, the translocation rate on nucleosomal DNA substrates was much slower than on bare DNA (12 bp/s). Another interesting observation in the experiments done with nucleosomal DNA was the occurrence of a burst of loop formation and dissipation activity, a behavior that was not observed on bare DNA.

The differences between the studies of RSC were ultimately suggested to reflect a specific recognition of the nucleosome by the remodeler and a strong coupling between nucleosome association and remodeler translocation. It is important to note that the SM approach used in both studies cannot directly discriminate between nucleosome mobilization due to loop formation and that achieved by another mobilization mechanism; however, there were clear similarities observed between the translocation activities of Swi/Snf and RSC complexes that may represent general features of chromatin remodeling by Swi/Snf-like enzymes.

Concluding Remarks

Clearly, the emergence of SM methods has contributed extensively to the characterization of SF2 helicases and translocases. Without a doubt, the maturation of these techniques will provide many more useful findings. Future work with SF2 enzymes must place particular emphasis on building a more detailed mechanism of duplex separation and filling the great need for high-resolution structural data. Progress in these areas will advance the larger goal of a deepened understanding of translocation and helicase activities in biological contexts and the regulation of these activities within the macromolecular complexes in which they so often play central roles. A deeper understanding of SF2 enzymes will no doubt shed light on many essential cellular processes and enrich the larger picture of NA metabolism.

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