ELSEVIER

Binding and unwinding: SF3 viral helicases

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The SF3 helicases, distinct from the more prevalent SF1 and SF2 helicases, were originally identified in the genomes of small DNA and RNA viruses. The first crystal structures of SF3 helicases have been determined, revealing a closer structural relationship to AAA+ proteins than to RecA, consistent with their participation in replication initiation. In conjunction with origin-binding domains, SF3 helicases are responsible for distorting DNA before replication forks can be assembled. At these forks, the SF3 helicases act as replicative helicases. The simian virus 40 SF3 helicase forms a hexameric ring, anticipated to be characteristic of the entire superfamily.

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Abbreviations

AAA+	ATPases associated with a variety of cellular activities
AAV	adeno-associated virus
BPV	bovine papillomavirus
EM	electron microscopy
HPV	human papillomavirus
ITR	inverted terminal repeat
LTag	large T antigen
MCM	minichromosome maintenance
ORC	origin recognition complex
ORF	open reading frame
ori	origin of replication
SV40	simian virus 40

Introduction

Helicases are enzymes that use the energy of ATP hydrolysis to translocate along DNA or RNA and unwind double-stranded regions [1,2]. Organisms employ a surprisingly large number of distinct helicases to catalyze the nucleic acid strand separation steps of DNA replication, recombination, repair and transcription [3]. Despite their deployment in a wide range of cellular circumstances, all helicases share common structural features, including the Walker A and B ATPase motifs and, in those cases where structures are known, at least one domain that is topologically related to RecA [4]. In addition, helicases employ a common mechanism, whereby ATP hydrolysis is coupled to the movement of protein domains relative to each other; this in turn is transduced into the unwinding of duplex nucleic acids. For monomeric helicases, the relative motion is between two RecA-like domains [5,6], whereas for ring-shaped hexameric helicases, adjacent monomers shift relative to each other [7,8]. Key to this conformational switch is an arginine in one domain that senses the γ-phosphorylation state of the nucleotide bound to a neighboring domain [9].

Helicases have been classified into three main superfamilies on the basis of sequence comparisons [10]. SF1 and SF2 helicases are widely distributed and contain related sets of seven conserved sequence motifs. By contrast, the SF3 superfamily [11] consists of helicases encoded only by small DNA and RNA viruses (and their prophage remnants in cellular genomes [12•]), and only three sequence motifs were identified, contained within a limited ~100 amino acid region: Walker A and B motifs, and a novel motif C. Later, another conserved motif was noted, designated B′, sandwiched between motifs B and C [13].

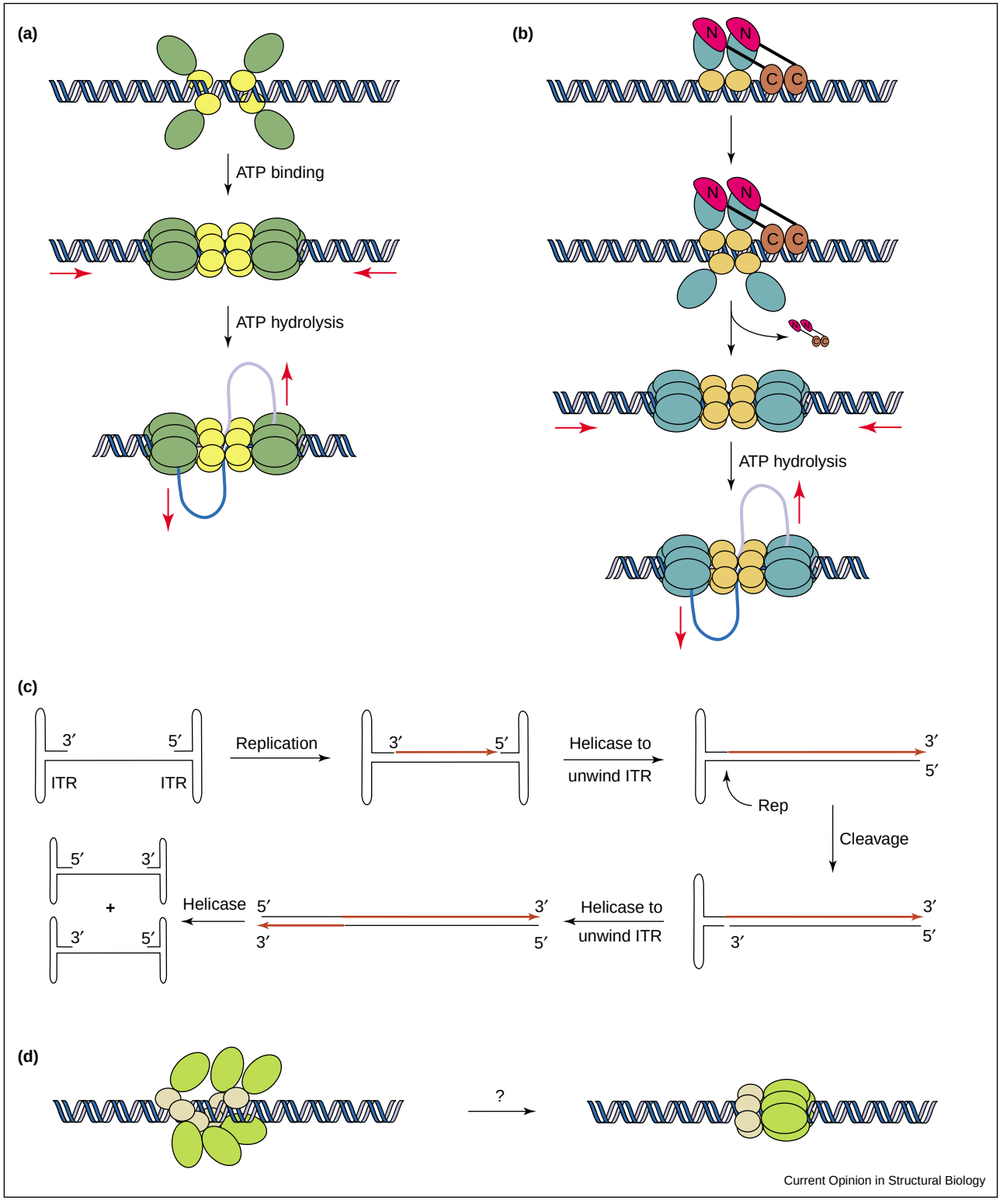
The structures of several SF1 and SF2 helicases have been determined in various substrate-bound states [3,9,14]. However, only within the past two years have the first structures of SF3 helicases been solved. These include the helicases of simian virus 40 (SV40) [15••] (PDB code 1N25), adeno-associated virus (AAV) type 2 [16••,17] (PDB codes 1S9H and 1U0J) and human papillomavirus serotype 18 (HPV18) in complex with part of the viral E2 protein [18••] (PDB code 1TUE). In all three cases, the SF3 helicases are domains of larger proteins known as origin-binding or initiator proteins, although in AAV the helicase is also expressed alone through the use of an alternative promoter.

In this review, we will discuss the specialized roles that SF3 helicases play during viral genome replication and how structural features of this distinct helicase superfamily are translated into virus-specific functions.

Initiating genome replication

Viruses cannot replicate in the absence of host cells and certain proteins that these cells provide. Nevertheless, they are decidedly idiosyncratic about the extent to which they depend on host cell functions. Large viruses travel

Figure 1



Schematic showing current models of the binding and assembly of viral origin-binding proteins on viral origins of replication. The structurally related DNA-binding domains are in shades of yellow and the SF3 helicase regions are in green/blue. **(a)** Assembly of head-to-head double hexamers of SV40 LTag. In the first step, DNA-binding domains recognize four closely spaced inverted pentanucleotide sequences, which are arranged as two pairs. In the presence of ATP, double hexamers assemble. It has been proposed that DNA is unwound within the central chambers of the hexamers, exiting through gaps between the two domains in the helicase and where the DNA-binding domains meet, as shown