TrwB: An F₁-ATPase-like molecular motor involved in DNA transport during bacterial conjugation

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Abstract

The mechanism by which TrwB acts as a DNA transporter in bacterial conjugation is analyzed. Based on a parallelism between TrwB and F₁-ATPase, TrwB would use the energy derived from ATP hydrolysis to pump DNA through its central channel, in a manner similar to that used by F₁-ATPase to produce a rotary movement of the central γ-subunit.

Keywords: TrwB; DNA transport; Bacterial conjugation; Molecular motor

1. Conjugative DNA trafficking

Bacterial conjugation is an example of macromolecular trafficking between cells. Conjugative plasmids transfer their DNA between most bacteria, thus representing one of the main causal agents of the spread of antibiotic resistance among pathogenic bacteria. Bacterial conjugation systems (including the related Vir system that Agrobacterium tumefaciens uses to transfer DNA to plants) show important similarities to both DNA replication and protein transport systems. The translocated substrate is a nucleoprotein particle that crosses the bacterial envelope into other bacterial or eukaryotic cells, crossing the kingdom boundaries. Conjugation can be visualized as a DNA rolling-circle replication (RCR) system linked to a type IV secretion system (T4SS) [16]. A two-step mechanism for DNA transport was proposed by our group [17]. The process is mediated by proteins encoded by two gene regions of conjugal plasmids. The first region encodes the DNA transfer replication (Dtr) proteins, while the second one encodes the mating pair formation (Mpf) proteins. Our model system—plasmid R388—codes for just three Dtr proteins (TrwA, TrwB and TrwC). Its Mpf region consists of eleven proteins (TrwD to TrwN) involved in the assembly and function of a T4SS, which includes the formation of an extracellular pilus.

TrwC is a member of the relaxase protein family that cleaves the DNA at the origin of transfer (oriT), helped by accessory proteins that form the so-called relaxosome. The nucleoprotein particle transported by the T4SS consists of TrwC covalently attached to a single-stranded form of the plasmid DNA. TrwC has relaxase and DNA helicase activities [11,18]. In the cytoplasm of the donor cell, the relaxase cleaves the DNA strand to be transferred (T-strand) at oriT, unwinds the cleaved strand and, once a full-length T-strand has been displaced, terminates the reaction by rejoining both ends. Conjugative relaxases belong to a superfamily of DNA strand-transferases, which includes proteins that initiate and terminate RCR, both in plasmids and phages [14]. A model for DNA processing was proposed which bears a resemblance to the replication mechanism followed by bacteriophage ΦX-174. In its original version [11], it was assumed that TrwC remained in the donor cell. Recently, Draper et al. [8] demonstrated that the relaxase protein is efficiently transported to the recipient by the T4SS, where it would presumably carry out the termination reaction. TrwC relaxase activity is enhanced by TrwA, a small tetrameric protein with a dual role in conjugation. By its binding to the oriT, TrwA acts as an activator of the oriT-cleavage reaction, probably by changing DNA topology. In addition, it is a transcriptional repressor of the trwABC operon [19,20].
TrwB is the third plasmid-encoded protein in R388 <i>Dir</i> region. It is an integral membrane protein, responsible for coupling the relaxosome with the DNA transport apparatus during cell mating [6,7]. It plays an important role by guiding the T-strand through the membrane, up to the recipient cell. The precise pathway that DNA follows to cross four lipidic membranes, plus other barriers such as lipopolysaccharides or peptidoglycan cell walls, remains unclear. Most research efforts have been addressed to finding out how DNA is first processed and then transferred to the recipient cell, but even at this stage the key mechanism used to pass the DNA through the transport complex is unknown. TrwB-like coupling proteins are key proteins involved in the process. The crystallographic structure of TrwBΔN70, the soluble fraction of TrwB, reveals a molecule with two domains: (1) an α/β twisted open-sheet domain that contains the nucleotide-binding site (NBD) and is reminiscent of RecA and DNA ring helicases, and (2) a potentially mobile “all α-domain” (see below). In the structure, six equivalent protein monomers associate to form an almost spherical quaternary structure that is strikingly similar to F1-ATPase [9,10].

2. Role of TrwB in DNA transport

The crystallographic structure of the soluble fraction of TrwB reveals important similarities to F1-ATPase and hexameric helicases [10]. Although the function of these proteins is different, they share a common feature: they all operate as molecular motors. In view of this structural analogy, it was reasonable to think that TrwB could operate as a DNA transporter, an idea backed up by several lines of evidence.

The TrwB amino acid sequence includes conserved Walker motifs, which are present in proteins that hydrolyze ATP, but until recently, ATPase activity had not been demonstrated for this protein. Interestingly, TrwBΔN70 is an ATPase that requires DNA for activation and displays positive cooperativity for ATP hydrolysis in the presence of DNA, with at least three catalytic sites involved, underscoring an oligomeric state [29]. Moreover, in the same work it was also shown that DNA is the key factor that promotes TrwBΔN70 oligomerization. The protein could recognize the conjugative T-DNA through the central channel, similarly to the mechanism described for T7 gene 4 helicase [2]. In general, 5′ → 3′ ring helicases have a preference for forked DNA substrates with two single-stranded tails. The 5′ tail passes through the center of the ring [33], whereas the 3′ tail contacts the outside of the ring [2]. In a similar way, we propose that the T-DNA would have its 5′ tail transferred through the internal cavity of TrwB. TrwBΔN70 structure reveals a ring of six tryptophan residues in the central channel (Fig. 1a, left panel), suggesting a possible role in DNA binding and translocation. In fact, mutation of that residue (W216A) was deleterious for DNA conjugation [29]. The mutation did not affect protein oligomerization, but abolished DNA-dependent ATP hydrolysis, suggesting a key role of this residue in DNA translocation through the central pore of the hexamer. Interestingly, a single mutant monomer seemed to inactivate a whole hexamer, as judged by in vitro ATPase experiments with mixed hexamers [29].

The cytoplasmic channel entrance in the TrwB hexamer is plugged by a ring of asparagine residues (Asn271 of loop αI–αJ from each subunit) and restricted to ~8 Å in diameter [10]. Such a small diameter at the entrance of the channel might be considered a priori a drawback of the proposed model, since it is too narrow for DNA to pass through. However, the Asn271 plug is the narrowest part of the channel, which is otherwise ~20 Å wide along its entire length. By analogy to F1-ATPase (Fig. 1, right panels), we think that the solved TrwB structure could represent an inactive conformation of the enzyme, the aperture of the channel being coupled to ATP hydrolysis. Therefore, we propose an alternating opening of the subunits, similar to the so-called “binding change mechanism” described for the F1-ATPase.

F1-ATPase is the globular domain of the F1F0-ATPase, or ATP synthase. When separated from the intact enzyme, the F1 component hydrolyzes ATP. F1-ATPase consists mainly of a α3β3 heterohexamer, where α and β subunits are arranged alternately around a central γ-subunit (Fig. 1, right panels). Each α- and β-subunit contains one nucleotide binding site, but only nucleotides bound to the β-subunits participate directly in catalysis. The structure of bovine mitochondrial F1-ATPase shows a marked asymmetry in the conformation of the β-subunits. This asymmetry explains the “binding change mechanism” since it allows the binding of substrate (ATP) and release of products (ADP and Pi) to occur simultaneously at separate catalytic sites on the enzyme. In the crystal structure, one β-subunit (β6γ) binds ADP, the second (β3γ) binds ATP, and the third (β1γ) is in a conformation with low nucleotide affinity (corresponding to the “empty” or “open” conformation) [5]. Interconversion between the different states is achieved by rotation of the central γ-subunit relative to the α- and β-subunits [1] (Fig. 1, right panels).

However, there are some differences between TrwB or ring helicases and F1-ATPase. F1-ATPase has only three catalytic subunits, whereas in TrwB or hexameric helicases there are potentially six equal catalytic sites. A modified version of the binding change mechanism was proposed for hexameric helicases [27] and we propose here that a similar mechanism could also work for TrwB. DNA helicases are molecular motors that move along DNA to separate the strands of dsDNA, coupling NTPase activity to movement. In contrast, TrwB is anchored to the inner membrane through two transmembrane α-helices per monomer (in their N-terminal regions) and, therefore, the protein cannot move along DNA. Instead, once the DNA is bound, it is pushed directly through the internal cavity by using energy derived from ATP hydrolysis in a way analogous to F1-ATPase. The latter consists of six subunits arranged around a central coiled-coil γ-subunit. Analogously, the central channel in TrwB could be occupied by a single DNA strand, which might be transferred through the internal cavity in a 5′ → 3′ direction (Fig. 2).

A striking observation in favor of our hypothesis is the fact that the “all-α domain” in TrwB is oriented towards the cytoplasm, whereas in ATP synthase it is in the opposite orientation to interact with the γ-subunit, and indirectly, with the membrane. Therefore, the TrwB “all-α domain” is inverted relative
Fig. 1. Structural comparison between TrwB and F1-ATPase. Structural features of TrwB, the α3β3 subcomplex from *Bacillus* PS3 and F1-ATPase from bovine mitochondria are shown on the left, central and right panels, respectively. Bottom and lateral views of the structures are represented in A and C, respectively, whereas B is a schematic representation of the complexes. For added clarity, only α-, β- and γ-subunits have been represented in mitochondrial F1-ATPase (magenta, blue and green colors, respectively). In the TrwB hexamer, the ring formed by six tryptophan residues in the central channel has been depicted in red (A, left panel). The figure underscores TrwB sixfold symmetry, a threefold symmetry in the α3β3 subcomplex, which relates the three α- and the three β-subunits, and a pseudo-threefold symmetry in mitochondrial F1-ATPase (B, panels left, central and right, respectively). The latter is ultimately caused by the asymmetric positioning of the γ-subunit relative to the α3β3 subassembly. F1-structure supports a catalytic mechanism in which the three β-catalytic subunits are in three different states of the catalytic cycle at any instant (βTP, βDP and βE). βTP has ATP bound, βDP has ADP bound and βE is empty. Interconversion of the states is achieved by rotation of the central γ-subunit relative to the α3β3 subassembly (right panel), while the non-catalytic α-subunits retain a similar conformation. Bacterial α3β3 subcomplex was crystallized without the γ-subunit and no bound nucleotides. As a consequence, its three β-subunits adopt a similar conformation, equivalent to the βE-subunit of mitochondrial enzyme (central panel). The red arrow points out the movement that converts the βTP closed conformation into the βE open conformation. The figure was produced by using Pymol (http://pymol.sourceforge.net) (Protein Data Bank coordinates 1BMF, 1SKY and 1E1Q for mitochondrial F1-ATPase, bacterial α3β3 subcomplex and TrwB, respectively).

In F1-ATPase, protein structural asymmetry supports the binding change catalytic mechanism explained in Fig. 1. Until now, no structural asymmetry of the ring subunits has been found in TrwB that can give us a clue to possible intermediates in the catalytic pathway. Interestingly, in the atomic structure of the thermophilic *Bacillus* PS3 α3β3 subcomplex—without the γ-subunit and no bound nucleotide [26]—the β-subunits adopt an identical conformation, which results in a hexamer with an exact threefold symmetry. The threefold symmetry axis relates the three α- to the three β-subunits as shown in Fig. 1 central panels. Thus, the α3β3 heterohexamer can be considered a structural equivalent of the TrwB symmetric homohexamer (compare central and left panels in Fig. 1). It is tempting to propose that the asymmetry caused by the γ-subunit in F1-ATPase
Fig. 2. A modified binding-change mechanism for TrwB-mediated DNA translocation. F₁-ATPase and TrwB structures are shown in A and B, respectively, represented in opposite orientations relative to the inner membrane. The “all-α-domain” in TrwBΔN70 is shown in red (residues 184–297). The equivalent domain in F₁-ATPase corresponds to a C-terminal bundle of helices (residues 364–474), which constitute a mobile domain that acquires a different conformation in the three catalytic subunits and contains the residues involved in the interaction with the γ-subunit (black double arrow). TrwB lacks a central coiled-coil subunit, showed in green in the F₁-ATPase structure. Instead, one single DNA strand (light green in the figure) might be translocated through the internal cavity in TrwB. DNA translocation could be driven by the movement of the “all-α-domain” as a consequence of ATPase activity (by analogy to F₁-ATPase). The tryptophan ring (shown in black) might interact with the DNA via base stacking and thus, push the DNA through the channel. Protein data bank codes are the same as used in Fig. 1. The DNA drawing was created as a separate image and superposed on TrwB structure.

Fig. 3. A two-step model for the “pumping mechanism” in conjugative DNA transport. Both steps are explained in detail in the text. Horizontal black lines represent bacterial membranes, traversed by dark green cylinders that represent the T4SS. TrwC (in blue) is represented as a two-domain circle + oval (relaxase + helicase) shape. TrwB (green) is anchored to the inner membrane and is represented as a monomer (A) or a hexamer (B) with an orange-like shape. Pre-existing DNA is represented by a thin continuous black line, and newly replicated DNA by a dashed arrow. The red arrow indicates the nic site. The curved arrow on TrwB indicates postulated motion forces required for DNA movement.

can also be induced in TrwB after DNA binding. Recent biochemical studies support this idea, since TrwB oligomerizes in the presence of DNA, the same substrate that stimulates its ATPase activity [29]. The above results and observations led us to propose a rather more detailed model to explain the mechanism of conjugative DNA transport, which is a combination of the mechanism followed by F₁-ATPase and hexameric helicases.

3. The “pumping mechanism” for DNA transport

The model is based on the initial transport of TrwC (linked to the DNA) through the T4S channel, followed by active translocation of the T-DNA by means of the TrwB pump. The process can be summarized in two main steps (Fig. 3).

3.1. Relaxase shooting

Upon reaction triggering, TrwC cleaves the DNA and becomes covalently bound to the 5‘ end of the cleaved T-DNA strand. TrwC bound to the T-DNA is transported to the recipient cell piloting the trailing DNA, probably by using the energy provided by VirB11 and/or VirB4 ATPases from the T4SS. Donor DNA synthesis begins from the 3’ end of the cleaved strand and a RCR system provides the T-DNA that will be transferred. The newly synthesized DNA strand dis-
places the old strand, which is transferred to the recipient cell through the T4SS. Transfer of the nucleoprotein implies that the T-DNA:TrwC complex must be directed towards the inner membrane. TrwB plays an essential role in this process by coupling the relaxosome to the T4SS [7]. TrwB is a transmembrane protein in equilibrium between monomeric and hexameric forms [12,13]. Recent data have shown that the protein oligomerizes upon binding a DNA substrate [29]. Therefore, we postulate that the protein plays a coupling role as a monomer. Once the relaxase, covalently bound to the DNA, is shot through the channel, TrwB wraps onto the DNA as a hexamer (see next step).

3.2. DNA pumping

TrwB assembles into a hexamer in the inner membrane, wrapping the T-DNA in its internal cavity. Through an ATPase activity, the protein is able to pump the DNA through the membrane. Fig. 3 underlines the expected asymmetry of TrwB during catalysis. It is difficult to imagine that DNA simultaneously interacts with six subunits within the channel of a TrwB ring. DNA might bind to a positively charged loop in the cytoplasmic domain of a subunit in an “open” conformation. Following the binding-change mechanism, the subunit changes to a “closed” conformation and by doing so, the DNA is sent into the internal cavity. At the same time, and with the same “closing” movement, the DNA is also rotated within the channel towards another catalytic subunit, so the “pumping mechanism” takes place iteratively. At the end of a replication cycle, when the translocated TrwC reaches the nic site for a second time, it carries out a second cleavage reaction, which rejoins the two ends of the original T-strand, thus liberating it.

4. Is the RecA-like domain an essential fold for a molecular motor with a rotary device?

ATP synthase is probably one of the best characterized molecular motors. The enzyme shares with all helicases a common feature: they are made of the same structural building block, the RecA-like domain. F1-ATPase, TrwB and hexameric helicases, among others, assemble six of these domains to form a ring, so each NTP-binding site is formed by structural elements contributed by two adjacent RecA-like domains. In all these cases, the motor device consists of six subunits arranged in a ring, in which the energy derived from NTP hydrolysis is coupled to a rotary device. By an alternating mechanism of aperture and closure, the catalytic subunits work as the “pistons” in a motor device. In some cases, as in ATP synthase, such a movement produces the rotation of the γ-subunit in the internal cavity. In others, such as helicases or DNA pumps, interaction of the DNA with the catalytic subunit in an “open” conformation allows, by means of ATP hydrolysis, a translation movement of the DNA into the internal cavity, coupled to rotation towards the next catalytic subunit. Therefore, a general rotary pumping model for DNA movement can be proposed that applies to a wide variety of hexameric enzymes working as molecular motors. It is expected that key aspects in the proposed mechanism can be tested experimentally in the near future, such as DNA passage through the central channel or a possible rotation of DNA during transport. Single molecule experiments have proved to be an excellent tool to analyze these aspects [22–24].

The number of protein motors described at a molecular level is increasing and it is tempting to think that additional proteins sharing the mentioned structural features will also operate as the F1-ATPase motor. For instance, RuvB was also described as a motor protein involved in homologous recombination. RuvB hexameric rings drive ATP-dependent branch migration by rotating DNA duplexes [32]. RuvA forms a tight tetramer that facilitates the binding of RuvB protein and maintains DNA structure in a square planar conformation, which promotes branch migration [21]. The specific dimeric endonuclease RuvC introduces nicks into two symmetrical sites near the junction center, producing two separate recombinant DNA duplexes [15]. The three proteins form the so-called RuvABC resolvosome [30]. In our system—plasmid R388—TrwC is a dimeric protein that cleaves the DNA, like RuvC. TrwA is a tetrameric protein that binds to DNA and changes its topology, like RuvA. The third protein—TrwB—is a hexameric molecular motor that, like RuvB, couples ATP hydrolysis to DNA pumping. Moreover, it was shown that RuvA modulates the ATPase activity of RuvB [25] and a similar effect has recently been observed for TrwA-TrwB (I. Tato, I. Arechaga, F. de la Cruz and E. Cabezon, unpublished results). It is tempting to speculate that the two systems operate with a similar mechanism.

Two additional F1-ATPase-like motors might be represented by FtsK, involved in chromosomal DNA separation during cell division [4] and SpoIIIE, which packages DNA in spores during Bacillus sporulation [31]. Both proteins bear only remote sequence similarity to TrwB-like proteins, mainly around the NTP binding Walker motifs, while biochemical data indicate that they catalyze similar DNA-driven motor reactions [4].

5. Conclusions and further directions

Atomic structures of proteins often reveal evolutionary relationships that cannot be clearly seen when simply looking at amino acid sequences. Such is the case for TrwB, a protein involved in single-stranded DNA transfer during bacterial conjugation. TrwB belongs to the family of coupling proteins which are present in all conjugative systems, connecting the relaxosome with the secretion machinery. The crystallographic structure of TrwBΔN70, the soluble fragment of TrwB, reveals important similarities to a disparate group of proteins involved in either DNA processing (helicases) or transforming energy (F1F0-ATPase). Although the function of these proteins is clearly different, they all operate as molecular motors. Such a relationship provided a new insight into how TrwB might function as a DNA pump. Thus, it was not surprising that DNA-dependent ATPase activity was finally reported for TrwB. In this review, we have developed a conjugative DNA transport model by incorporating a new hypothesis for the molecular mechanism by which TrwB acts as a DNA transporter. Based on a parallelism between TrwB and F1-ATPase, TrwB would
use the energy derived from the hydrolysis of ATP to pump the DNA through its central channel, in a similar way to that used by F$_1$-ATPase to produce a rotary movement of the central γ-subunit. F$_1$-ATPase and TrwB have a number of structural neighbors that constitute the RecA protein-like family in the SCOP database [3]. Thus, rotary motors composed of hexameric proteins (that may be called ROT-HEX engines for a catchy name) are clearly not confined to the use of DNA substrates but extend, in addition to ATP synthase, to transport devices for a variety of substrates, perhaps including polypeptides (such as HP0525).

TrwB can be considered as a DNA transporter and, together with its remote homologs SpoIIIE and FtsK (involved in chromosome segregation), belong to a novel group of molecular motors involved in DNA transport across membranes. As an inner membrane protein, it is able to transport DNA through lipidic membranes. Such a characteristic converts TrwB into a mechanical device with important applications, such as transport of DNA substrates from synthetic liposomes towards selected target cells. A more thorough knowledge of the biochemistry of this group of molecular motors might help us to engineer new devices of biotechnological interest for DNA delivery.

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References


