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The Nuts and Bolts of Ring-Translocase Structure and Mechanism

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Abstract

Ring-shaped, oligomeric translocases are multisubunit enzymes that couple hydrolysis of nucleoside triphosphates (NTPs) to directed movement along extended biopolymer substrates. These motors help unwind nucleic acid duplexes, unfold protein chains, and shepherd nucleic acids between cellular and/or viral compartments. Substrates are translocated through a central pore formed by a circular array of catalytic subunits. Cycles of nucleotide binding, hydrolysis, and product release help reposition translocation loops in the pore to direct movement. How NTP turnover allosterically induces these conformational changes, and the extent of mechanistic divergence between motor families, remain outstanding problems. This review examines the current models for ring-translocase function and highlights the fundamental gaps remaining in our understanding of these molecular machines.

Introduction

The processive and forceful movement of extended biopolymers, such as nucleic acids and polypeptides, is vital for a wealth of cellular transactions. Many of these events are catalytized by multi-subunit, ring-shaped **N**ucleosidyl-**T**ri**P**hosphatases (**NTP**ases) that actively couple NTP turnover to directed substrate movement. A large number of oligomeric translocases belong to a broad grouping of proteins known as the **A**dditional **S**trand **C**atalytic glutamat**E** (**ASCE**) superfamily [1,2]. The ASCE group is itself composed of several subfamilies, including RecA-like ATPases and **AAA+** (**A**TPases **A**ssociated with various cellular **A**ctivities) enzymes (Fig 1a). Ring-translocases typically form homohexameric assemblies (Fig 1b), although heterohexamers, homopentamers and homoheptamers also exist.

The core RecA- and AAA+-type folds are related, consisting of a conserved five-stranded, parallel β-sheet sandwiched between several α-helices (Fig 2a) [1,3,4]. Each protomer contains a pair of conserved sequence motifs – termed Walker A and B – that interact with both the phosphate groups of bound nucleotide and an essential Mg^{2+} cofactor to control

catalysis (Fig 2b,c) [3,5,6]. The Walker motifs are often accompanied by a conserved glutamate residue (the namesake of the ASCE family), which resides either within the Walker-B sequence or at a spatially proximal position [7,8]. When present, this amino acid helps polarize a water molecule for nucleophilic attack on the bound NTP to elicit hydrolysis.

The NTPase active site lies at the interface between adjoining protomers, such that the catalytic motifs of each motor subunit are complemented by a conserved arginine (or lysine) from a partner subunit (Fig 1b, Fig 2b,c). In addition to contributing functionally to NTP hydrolysis, this “arginine-finger” facilitates intersubunit communication, allowing NTP binding and product release to induce conformational changes between neighboring protomers and consequently around the ring [1,8,9].

RecA and AAA+ NTPases are often described as a single motor family. However, this grouping overlooks significant differences between the two protein classes. In particular, the core ASCE folds of RecA and AAA+ enzymes are differentially oriented in higher-order oligomers such that their translocation pores run roughly perpendicular to one another (Fig. 2d) [10]. Consequently, the primary sequence positions of both the arginine finger and the substrate-interacting elements that form the pore are not conserved between the two families (Fig 2a). Other family-specific functional motifs (e.g., the Sensor-II element of AAA+ proteins) similarly are not shared. This fundamental difference in quarternary organization likely underlies some of the disparate functional properties of these evolutionarily related motors.

Coordination of NTPase status

In all ring-translocases, cycles of nucleotide binding and hydrolysis are coupled to conformational rearrangements of substrate-binding elements. To date, three major types of mechanisms – rotary, concerted and stochastic – have been proposed for the progression of nucleotide turnover within the system. In the rotary model (Fig 3a,b), hydrolysis proceeds from one subunit to the next in sequential fashion similar to the mechanism demonstrated for the F_1 ATPase [6,11], a RecA-type ACSE NTPase that forms a portion of the ATP synthesis machinery of mitochondria, chloroplasts and bacteria. By contrast, the concerted model (Fig 3c) invokes an “all-or-none” mechanism, in which all NTPase sites in the translocase bind, hydrolyze, and release nucleotide simultaneously. The stochastic or “probabilistic” model (Fig 3d) posits a mechanism with no set order to hydrolysis events.

Myriad studies on a variety of ring-translocase systems have provided data supporting one model or another. Structures of nucleotide-bound or -free hexameric translocases organized into radial arrangements of discrete nucleotide-binding states (akin to those seen for F_1) have been linked to biochemical studies supporting a rotary mechanism [12–19]. The visualization of fully symmetrized-particles in which all subunits occupy similar conformations and undergo uniform, *en bloc* transitions between nucleotide binding events has at times been taken as evidence for the concerted model [20,21]. Other analyses, particularly for some protein unfoldases, have shown that individual NTPase subunits can in certain circumstances bind nucleotide and function independently of each other, suggesting a lack of enforced firing order [22,23].

To date, few hexameric motors have been imaged with a translocation substrate. The two exceptions – the papilloma virus E1 replicative helicase and the Rho transcription termination factor – act on single-stranded (ss) nucleic acid chains [12,15]. Recent structures of both proteins, bound to either ssDNA (E1) or ssRNA (Rho), reveal a radially asymmetric arrangement of NTPase states (interestingly, E1 adopts a similar conformation even in the absence of DNA and nucleotide [17]). In both cases, NTPase states are distributed around

the ring in a sequence that appears to recapitulate a full hydrolysis cycle (Fig 3b). These structures provide some of the strongest visual evidence yet supporting a rotary firing model (discussed below).

How generalizable are the observations for E1 and Rho to oligomeric ASCE NTPases as a whole? For non-processive ring or notched-ring systems (e.g., the NSF/p97 vesicle fusion regulators or the polymerase clamp loaders), concerted rounds of NTPase activity may be an equally, or even more applicable, means to power activity than rotary approaches. By contrast, the asymmetric “dimer-of-trimers” and “trimer-of-dimers” states visualized for many processive ring-translocases appear most consistent with a rotary mechanism (e.g., see [14] and [24]). Whether these symmetrized ring-forms represent a slight functional divergence from the asymmetric E1 and Rho models, or whether they convert into an asymmetric conformation upon binding substrate is not known. Insofar as the SV40 large T-antigen (LTag), computational studies indicate that concerted, NTP turnover may be incapable of inducing directional movement [16]; moreover, while there is some debate as to whether LTag translocates along one or two DNA strands [25,26], the structure of the closely related E1 helicase suggests that LTag is an ssDNA motor also likely to operate by a rotary mechanism. For the ClpX protein unfoldase, proposed initially to function by a stochastic mechanism, studies have shown that certain combinations of functional and inactive protomers can cooperatively influence the activity of the entire ring [22]. This latter finding, together with a recent dimer-of-trimers structure of ClpX [13], suggests that the peptide translocase typically employs a rotary NTPase mechanism, but retains sufficient flexibility to allow alternative subunits to hydrolyze NTP if one or more partner protomers is inactivated. Thus, it appears that the most prevalent mechanism of NTP turnover in ring-shaped translocases relies on the sequential, radial progression of hydrolysis around the translocase ring.

The peculiar properties of ClpX, mirrored to some extent by other ring-translocases, raise intriguing questions about the plasticity of the NTPase mechanism. Here, plasticity refers to the degree to which neighboring NTPase sites are obligated to turn over nucleotide in a fixed sequence. As most ring-translocases form homo-oligomers, this issue has been difficult to address due to the inherent difficulty of inactivating specific subunits. Nonetheless, titration experiments with different ratios of defective and wild-type subunits have shown that certain motors cannot tolerate even a single defective protomer without losing function [19,27,28], whereas others (including ClpX) are more permissive [22,29–31].

We propose that these differences imply the existence of a continuum with respect to the plasticity of ring NTPase firing order. In this spectrum, the behavior of many RecA-type proteins (e.g., the T7 gp4 helicase and Rho) can be considered “strictly” sequential [27,28], whereas several AAA+ motors (the MCM helicases, possibly ClpX) would fall into a “loosely” sequential category [22,31]. Given that every subunit in a ring translocase is sandwiched between two partner protomers, there are ample opportunities for binding and hydrolysis events to be allosterically sensed at a distance. What is less clear is how disparate ring-translocase systems have adapted to be more or less permissive of subunits that do not fire properly.

Another aspect of the firing-order models that has not been (and indeed cannot be) addressed by structure is whether hydrolysis proceeds at constant monotonic rate between subunits. Interestingly, single-molecule analyses of the bacteriophage φ29 packaging protein – a pentameric, RecA-type ring NTPase that translocates along double-stranded DNA – provide compelling evidence for a rotary firing mechanism [32,33]; however, these efforts also indicate that packaging occurs in well-defined 10-base pair “bursts” composed of four rapid and successive substeps. Here, each packaging cycle appears to consist of a loading

phase in which four (or five) ATPase sites engage ATP, followed by a second phase in which four consecutive hydrolysis events propel the motor along DNA. Similarly, presteady-state studies on RNA substrates containing single, phased deoxyribonucleotides indicate that Rho requires a 2'-OH on every seventh base for efficient translocation, an observation difficult to reconcile with a uniform stepping mechanism [34]. Available structural data are not necessarily inconsistent with these biochemical findings. For instance, the sequential order of ATPase states seen in Rho or E1 could reflect an intermediate in which a hydrolysis burst has only partly occurred. Alternatively, it may prove that different motor systems have significantly distinct properties with respect to the relative timing of hydrolysis between subunits. At present, no one system exists in which complementary structural and solution data are available to resolve this issue.

Substrate interactions and NTPase/translocation coupling

A particularly fundamental question in ring-shaped motors is how substrate movement through the translocation pore is effected by catalytic events at distal NTPase sites. Although the polymer-binding regions of different motor families are divergent in their primary sequence, they typically adopt either loop or β-hairpin structures. In all cases, except where a six-fold symmetric ring is observed, these elements wrap around the central axis of the translocation pore in a helical or semi-helical array [12–15,21]. When nucleotide-bound and -free states are compared for a given translocase (including fully-symmetrized forms), the vertical position of a given loop or hairpin in the pore appears coupled to the nucleotide status of its associated NTPase center. Thus, subunit "rocking" events that frequently accompany NTP binding, hydrolysis, and release also help lever translocation loops up and down the ring axis [12–15,20,21,35], walking the motor along substrate.

Whether substrates are passed from one subunit to the next in a "bucket-brigade" fashion, or whether each subunit escorts a single portion of the substrate through the entirely of the central channel, has been addressed by substrate-bound structures of E1 and Rho [12,15]. Both enzymes manifest a right-handed, "spiral staircase" of pore loops, upon which the translocation substrate sits curled into a matching helical configuration (Fig 4a). Both proteins also overwind the substrate, compressing it in the pore, a finding corroborated by recent single-molecule studies of the RecA-type helicase, DnaB [36]. As the progression of NTPase states is followed around the ring (from E → T → DP), the loops wrap around the ring axis in a smoothly graded manner (Fig 1b, 4a), with the transition between states at the beginning or end of the cycle marked by a single subunit positioned midway between the top and bottom of the ring. Interestingly, the most extensive loop/substrate contacts are manifest by ADP-bound subunits in the case of E1, and ATP-bound in Rho.

Given their divergent sequences and positions (Fig 2a), several aspects of the Rho and E1 translocation loops are remarkably similar. In both instances, the loops curl around either the 5' or 3' face of the phosphodiester backbone, appearing to "tug" on substrate in a direction consistent with their differing translocation polarities (Fig 4a). Both hexamers also contain one protomer whose loop has disengaged from bound substrate to allow it transit from one end of the staircase to the other. DNA or RNA contacts occur primarily between the loops and the sugar/phosphate backbone, and although lysines are present in both enzymes to neutralize the negative charge on the nucleic acid, most interactions are not electrostatic.

Together, these observations are most consistent with an escort model in which a pore loop or hairpin contacts a nucleobase at the start of an NTPase cycle, and then chaperones it continuously through the translocation pore. The intermediate steps in the migration of each hairpin correlate with NTPase status, thereby enforcing a gradual, directed movement of substrate as successive subunits take their turn releasing spent hydrolysis products and

binding fresh NTP molecules. Modeling of a full NTPase cycle, by morphing the NTPase state of one subunit to the next, reveals a smooth, conveyor belt-like translocation of substrate through the central channel by a circular, but vertical, wave of pore loop movements [12,15]. Both Rho and E1 appear to take a one-base step along its substrate for each ATP consumed; however, because of the escort mechanism employed, each subunit of the translocase actually traverses a distance of six bases over the course of a single NTPase cycle.

Is the translocation substrate a passive participant during motor movement? For Rho at least, each change in nucleotide state is accompanied by the rearrangement of a conserved salt bridge network that runs from the substrate-binding pore to the NTPase active site. The degree to which all available salt bridges in the network are fully formed is coupled to both RNA interactions and NTPase status. Interestingly, the network becomes fully interconnected at precisely the point where the Rho subunit making the greatest number of contacts to RNA also adopts a hydrolysis-competent (T*) state. Correct formation of the network appears necessary to properly position the catalytic glutamate and its associated nucleophilic water next to the γ-PO_4 moiety of the bound nucleotide. Thus, RNA seems to act as a “timing belt” for Rho, whereby its relative position in the translocation pore controls which catalytic center is competent to fire. The critical nature of this network is supported by its invariance among Rho homologs across all bacteria, as well as by mutagenesis studies, which have shown that altering participatory amino acids deleteriously affects both RNA binding and ATP hydrolysis, even though they do not contact either substrate or ATP directly [15,37–40].

Whether ring-shaped translocases employ a similar network to Rho is unclear. Studies in the related RecA-motor, T7 gp4, have shown that at least one intersubunit interaction is important for function [41]. The mitochondrial m-AAA unfoldase also appears to retain several amino acids that sense the nucleotide state of each ATPase site at a distance, and couple this status to substrate translocation by controlling intersubunit conformational changes [18]. By contrast, it is less clear that conserved, cross-protomer interactions outside the active site play a role in E1. To date, there has been relatively little study of residues that do not directly contact substrate molecules or participate in NTPase chemistry.

Translocation polarity

A rotary firing order, coupled with the spiral staircase arrangement of substrate-binding elements in the central channel of the ring, helps ensure unidirectional translocation of ring NTPases along a linear polymer. However, single-stranded nucleic acid translocases are known to move along their substrate in either 5’ → 3’ (RecA-like motors [42–44]) or 3’ → 5’ (AAA+ motors [45,46]). Certain dsDNA translocases similarly can show a bias for preferentially tracking along only one of the two strands [47]. The molecular logic that dictates these family-specific preferences has not been delineated.

Based on our present understanding of rotary-powered ring NTPases, there appear to be three means of altering translocation polarity. One way to picture these approaches is to consider a self-propelled nut (the motor) capable of climbing along a bolt (the substrate) (Fig 4b). The motor’s NTPase cycle dictates the spinning direction of the nut, which contains a mobile, but chiral, series of pore loops that act as “threads” to grip the substrate in a defined orientation. For two motors that move in opposing directions, they could: a) share the same firing order and threading pattern, but bind substrate in different directions, b) possess the same firing order and substrate binding orientation, but contain an inverted helical arrangement of loops (akin to inverting the handedness of the threads), or c) bind

substrate in the same manner using a similar pore-loop configuration, but utilize an inverted NTPase firing order (i.e., “spin” in opposite directions).

A comparison of the substrate-bound structures of Rho and E1 reveals that both enzymes share a common substrate binding orientation [12,15]. This property can be seen from a top-down view of the motor in which the 5’-DNA or -RNA end projects out toward the viewer (Fig 4c): in both instances each NTPase site is positioned to receive an arginine finger projecting from a *clockwise*-related partner subunit. Likewise, the arrangement of the substrate-binding loops within the central pore follows the same right-handed, spiral configuration for both helicases (Fig 4a). By contrast, the organization of ATPase states is reversed between the two proteins, running in a clockwise arrangement in E1 and counterclockwise in Rho. Thus, the opposing translocation polarities of these two motors appear to result from reversing their respective firing orders [15].

Whether the difference in translocation polarity between Rho and E1 is generalizable to RecA and AAA+ motors as a whole seems unlikely: at least one RecA-protein (Phi12 P4) and one AAA+-class enzyme (Mcm) appear to bind substrate in a manner opposite that seen for either Rho or E1, respectively [48,49]. Furthermore, AAA+ protein unfoldases appear capable of bidirectional translocation [50], while an inspection of the ClpX dimer of trimers shows that the pore loops of this protein wrap around the ring axis in a left-handed, rather than right-handed, configuration [13]. Whether this property helps accommodate the bidirectional tracking of the motor along protein chains is unclear. Further studies will be needed to resolve these outstanding questions.

Concluding remarks

A detailed picture of ring-shaped translocase mechanism has been slowly coming into focus. Progress on these systems has been hindered in part by their conformational dynamism, multistep reaction cycle, and complex organization. Although diverse models have been proposed to explain how NTP turnover is transduced into physical movement, a growing number of findings implicates a rotary firing order in coordinating hydrolysis among subunits. Substrate-bound structures of two ring translocases (E1 and Rho), which belong to two distinct branches of the ASCE NTPase family (AAA+ and RecA, respectively), reveal a highly analogous means for gripping and moving along biopolymer substrates, despite fundamental structural differences.

Nonetheless, functional distinctions between various classes of ring-shaped translocases imply that likely mechanistic divergences also exist. For Rho and E1, one such difference (translocation direction) appears to arise from an inverted progression of NTP hydrolysis events around their respective rings. The degree to which this approach is extensible to other motors is uncertain: as already seen with distantly-related Superfamily-1 and -2 helicases, there exists a myriad of divergent chemo-mechanical means for altering directional polarity (see [51] and references therein). Other significant issues, such as how hydrolysis is timed, whether NTP turnover events are uniform or clustered, how NTPase cycles and translocation elements have adapted to different (and sometime non-uniform) substrates, which NTPase step constitutes the “power-stroke” for translocation, or why certain motors are more or less tolerant of subunit “misfiring”, also remain to be determined. Many more surprises undoubtedly await investigators as they continue to dig deeper into this essential and complex class of molecular machines.

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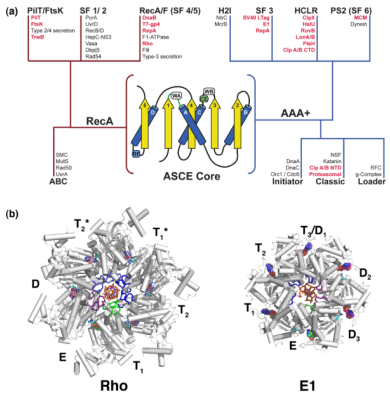

Figure 1.

RecA and AAA+ ring translocases [1]. **(a)** Phylogenetic tree showing several RecA and AAA+ clades of the ASCE superfamily. Representative family members are shown, with polymer-translocases highlighted red. At center is a diagram of the conserved ASCE fold highlighting key elements of the NTPase active site. Abbreviations: WA – Walker A, WB – Walker B, CE – catalytic glutamate, RF – arginine finger, SF1-6 – helicase superfamilies 1 through 6, PS2 – pre-sensor II insert, H2I – helix 2 insert, HCLR – (HslU, ClpABC-CTD, LonAB, RuvB), ABC – ATP Binding Cassette, AAA+ - ATPases Associated with diverse cellular Activities, ASCE – Additional Strand, Catalytic glutamate ([1]). **(b)** Substrate-bound Rho (PDB ID: 3ICE) and E1 (PDB ID: 2GXA) hexamers shown as RecA and AAA+ exemplars, respectively. The ATPase status of each subunit is labeled; loops in the translocation pore and nucleotide molecules are differentially colored accordingly (ATP-type – blue, ADP-type – magenta, nucleotide exchange – green); arginine fingers are shown as cyan sticks.

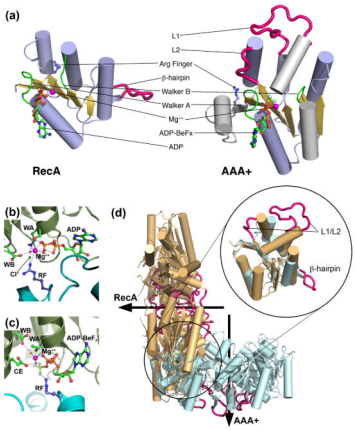

Figure 2.

Structural comparisons of RecA and AAA+ ring translocases. **(a)** Cartoon representation of AAA+ (E1) and RecA-type (Rho) ASCE domains. Conserved α-helices and β-strands are colored pale blue and yellow, respectively. Nucleotide-binding elements are green, substrate-binding loops magenta, and non-conserved insertions grey. Nucleotides and the Arg-finger are shown as ball-and-stick. **(b,c)** Close-up of the E1 (panel b) and Rho (panel c) active sites. The NTP-binding subunit is green, and the subunit donating the Arg-finger is cyan. Conserved active site elements, nucleotide, active site water molecules and ions are shown (a Cl^- ion (E1) or BeF_3 species (Rho) occupies the position of the γ-PO_4 moiety). **(d)** Superposition of a single ASCE domain within the Rho (beige) and E1 (cyan) hexamers (inset) highlights the orientational offset between the RecA and AAA+ translocation pores (magenta).

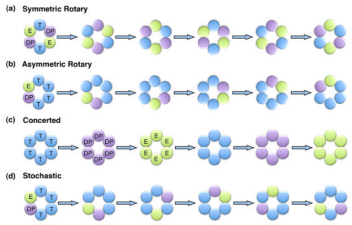

Figure 3.

NTP hydrolysis models for ring-translocases. NTPase states are labeled as follows: T (blue) – NTP state, DP (purple) – NDP + P_i state, E (green) – nucleotide exchange state. **(a)** Symmetric rotary mechanism. **(b)** Asymmetric rotary mechanism. **(c)** Concerted mechanism. **(d)** Stochastic mechanism. See text for details.

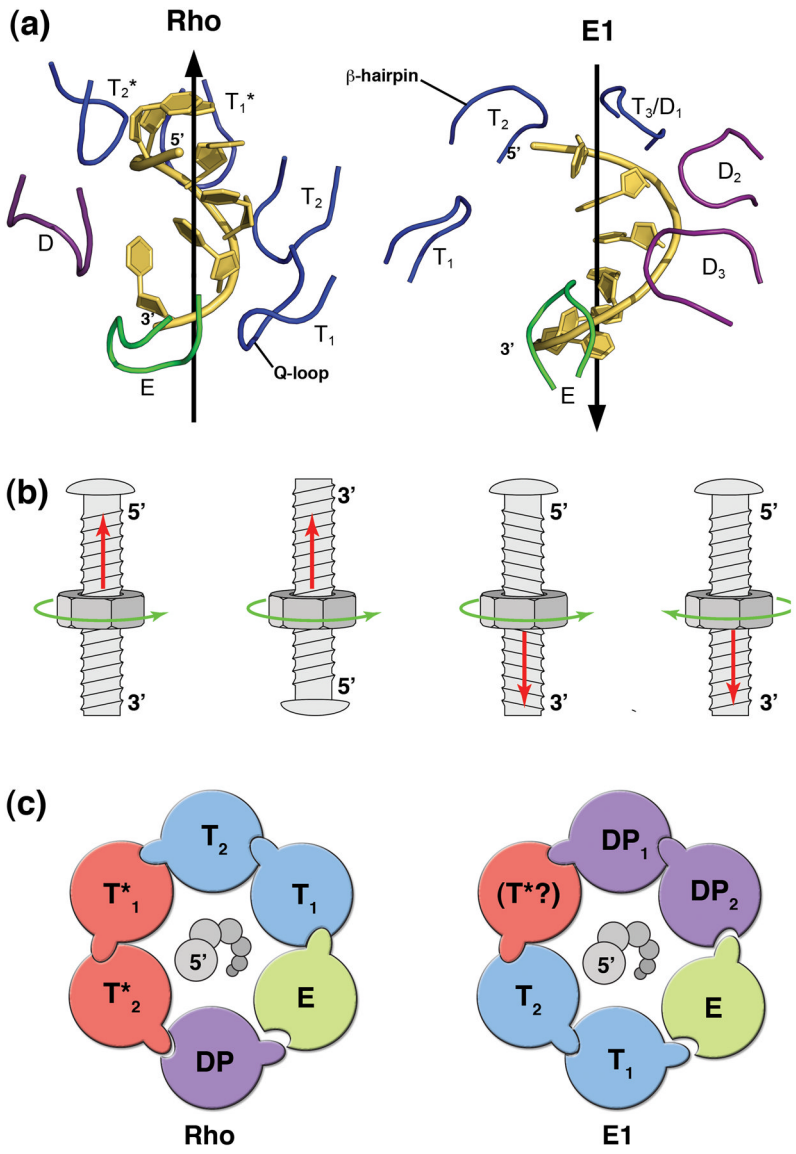

Figure 4.

Molecular determinants of translocation polarity. **(a)** Rho and E1 pore loops bound to ssRNA and ssDNA, respectively (5’ end at top). Loops are labeled colored by subunit nucleotide state in accord with Fig 1b. **(b)** A “nut-and-bolt” schema for considering translocation polarity. The direction of movement of the nut on the leftmost bolt can be reversed by: 1) reversing the substrate binding orientation (flipping the bolt, middle left panel), 2) reversing the chirality of the pore loop “staircase” (inverting the threads on the nut, middle right panel), or 3) reversing the NTPase cycle progression (turning the nut in the opposite direction, rightmost panel). **(c)** Schematic of Rho and E1 (hexamer 1) as viewed from the 5’ end of their substrates, illustrating their respective NTP hydrolysis polarities. The subunit labeled (T*?) in E1 reflects the adoption of ADP or ATP-like states by this protomer in different hexamers. Subunits are colored according to nucleotide state as in Fig 1b. Nucleic acid is shown as a spiral of grey circles.