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Molecular switch-like regulation in motor proteins

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Motor proteins are powered by nucleotide hydrolysis and exert mechanical work to carry out many fundamental biological tasks. To ensure their correct and efficient performance, the motors' activities are allosterically regulated by additional factors that enhance or suppress their NTPase activity. Here, we review two highly conserved mechanisms of ATP hydrolysis activation and repression operating in motor proteins—the glutamate switch and the arginine finger—and their associated regulatory factors. We examine the implications of these regulatory mechanisms in proteins that are formed by multiple ATPase subunits. We argue that the regulatory mechanisms employed by motor proteins display features similar to those described in small GTPases, which require external regulatory elements, such as dissociation inhibitors, exchange factors and activating proteins, to switch the protein's function 'on' and 'off'. Likewise, similar regulatory roles are taken on by the motor's substrate, additional binding factors, and even adjacent subunits in multimeric complexes. However, in motor proteins, more than one regulatory factor and the two mechanisms described here often underlie the machine's operation. Furthermore, ATPase regulation takes place throughout the motor's cycle, which enables a more complex function than the binary 'active' and 'inactive' states.

This article is part of a discussion meeting issue 'Allostery and molecular machines'.

1. Introduction

Motor proteins transform the energy released in adenosine triphosphate (ATP) hydrolysis into mechanical work to perform fundamental biological processes, such as intracellular transport, protein degradation and energy production [1,2]. Although motor proteins are ubiquitous, their function is necessary only at relevant locations in the cell and at distinct stages of its cycle [3]. Therefore, in order to ensure adequate spatial and temporal control of their function, motor proteins must be switched between 'active' and 'inactive' states in a regulated manner [4].

The current mechanistic picture of regulation in motor proteins has been partially drawn from the detailed understanding of function activation in small GTPases (figure 1). Small GTPases activate signalling pathways in the guanosine triphosphate-bound state—the 'on' state—and do not display such activation in the guanosine diphosphate-bound state—the 'off' state [5]. The active state of small GTPases is not required at all times and, therefore, the protein's activity is switched 'on' and 'off' throughout the cell cycle in a regulated manner (figure 1). Such regulation is performed by specialized protein factors that either prevent or promote adenosine diphosphate (ADP) release—known as guanine dissociation inhibitors or guanine exchange factors, respectively—or that stimulate GTP hydrolysis—known as GTPase-activating proteins [6]. Likewise, the activities of motor proteins are regulated by additional factors, such as the motor's substrate, associated protein factors, and subunits adjacent to each other in multimeric complexes, which take on regulatory roles analogous to those described in small GTPases (figure 1).

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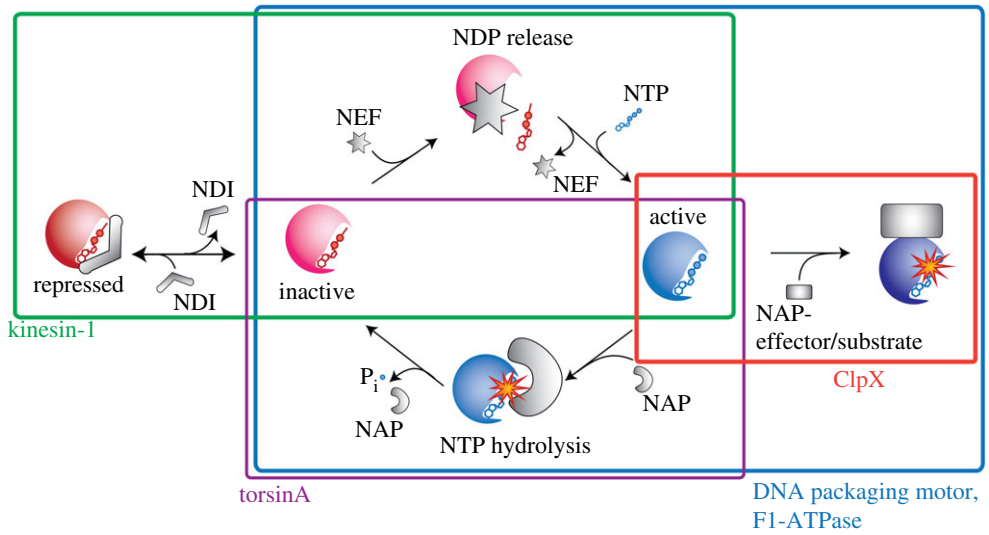

Figure 1. Small GTPase-like regulation. The activity of small GTPases is regulated by additional binding factors. Different motor proteins display distinct features of these regulatory mechanisms. The specific features exhibited by each motor are indicated in boxes with the same colour code as the name of the protein.

Here, we review various mechanisms of regulation in different motor proteins. We note that all these mechanisms share a general feature: the motor's function is controlled by stimulation or repression of its ATPase activity, which is regulated allosterically by different factors. We show that regulatory factors can control the motor's ATPase activity at two distinct stages of the enzymatic cycle: nucleotide exchange and ATP hydrolysis. First, we discuss specific cases in which these factors control nucleotide exchange by preventing or facilitating ADP release. Then, we describe in detail the functions of two highly conserved elements of ATP hydrolysis activation: the arginine finger and the glutamate switch. We show how the action of two repressing or two activating elements can combine resulting in a heavily inhibited or heavily activated motor activity. Throughout the review, we consider the implications of these mechanisms in multimeric ring motors.

2. Dissociation inhibitors and exchange factors in motor proteins

Kinesin-1 is an essential protein that transports vesicles and organelles towards the (+) end of microtubules [7]. For kinesin to achieve the velocity of approximately 900 nm s^{-1} observed during transport [8], its ATPase cycle—which includes ADP release and other chemical transitions—must take place at a rate of approximately 110 s^{-1} (assuming an 8 nm step size). Interestingly, unloaded kinesin-1 remains mostly in its ADP-bound state, displaying ADP release rates of about 0.00048 s^{-1} [9], whereas in the presence of its cargo and microtubules, these rates are increased to approximately 300 s^{-1} [10]. Kinesin-1 switches between these heavily repressed and transport-competent states through the combined action of two regulatory factors that control its nucleotide exchange: kinesin-1's own tail and microtubules. Indeed, in the absence of cargo, kinesin-1 remains in the heavily repressed state (figure 2*a*) by using its tail domain as a built-in inhibition factor that prevents ADP dissociation (figure 1). Deletion of the tail domain is known to activate the ATPase activity without the need of cargo binding, and inhibition is re-established by the addition of exogenous tail peptide [11]. The crystal structure shows that the tail folds and cross-links the motor domains, preventing the movement of the ATPase domains and, presumably, the allosteric signal necessary to promote ADP release [12]. However, even after binding to its cargo, kinesin-1 still binds tightly to ADP (ADP release rates of around 0.005 s^{-1}; figure 2*a*, [13]. Only in the presence of microtubules, cargo-bound kinesin-1 displays ADP release rates competent for regular transport, indicating that the polymer track functions as an exchange factor that facilitates ADP release (figure 2*a*). Indeed, crystal and biochemical studies suggest an allosteric path that promotes ADP release in kinesin-1 after binding to microtubules [14]. In particular, the interaction between kinesin-1 ATPase head and residue E415 in α-tubulin is thought to produce conformational changes that are propagated to the nucleotide-binding site to promote the release of ADP [15].

Kinesin-1 depends on its exchange factor to increase its ATPase activity. However, in multimeric ring motors, exchange factors are necessary to ensure timely release of ADP at appropriate times during the motor's cycle. The subunits and, in some cases, the subunit's subdomains can adopt the exchange factor role by promoting ADP release in the neighbouring nucleotide-binding pocket [16–18]. Next, we will briefly describe three ring motors that rely on exchange factors for their proper operation.

F1-ATPase, a subdomain of ATP-synthase, is formed by three dimers of α- and β-subunits ($\alpha_3\beta_3$), and one copy of the γ-subunit, a central protein that rotates as the motor's chemical cycle proceeds [19]. The catalytic site at the interface of each α−β dimer is formed by the nucleotide-binding pocket of the β-subunit and several residues contributed by the non-catalytic α-subunit, including a highly conserved *trans*-acting arginine residue, α-R373 [20] (figure 2*b*). It was shown that ATP binding to the non-catalytic α-subunit site is anti-cooperative and promotes the release of ADP from the adjacent β-subunit [21]. Interestingly, in motors containing mutants of the highly conserved arginine finger, α-R373, the β-subunits are known to remain in the

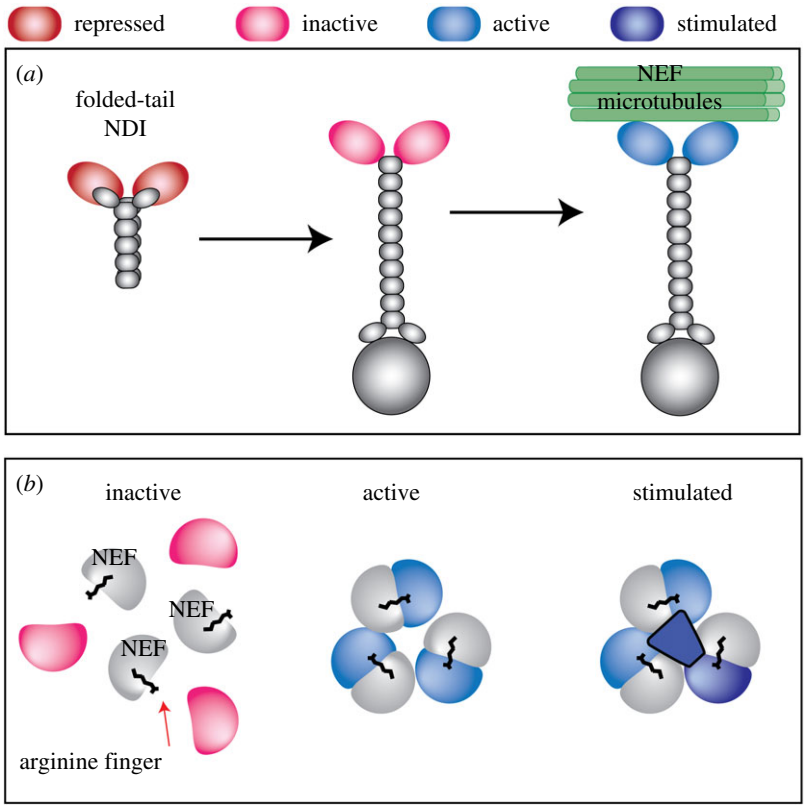

Figure 2. Multiple mechanisms of regulation operate in a single motor protein. (*a*) The tail of kinesin-1 serves as a built-in nucleotide dissociation inhibitor, NDI (left). Tail-inhibition is released upon cargo-binding (middle). The microtubules play the role of a nucleotide exchange factor (NEF) by accelerating ADP release (right). (*b*) In F1-ATPase, the α-subunits serve the role of the nucleotide exchange factor. In solution, the β-subunits do not release ADP (left). In the ring configuration, the β-subunits are released from the ADP-inhibition by the arginine finger of the α-subunit (middle). The activity of the ring motor is further enhanced by the presence of the γ-subunit.

ADP-inhibited state [16]. Additionally, replacing the arginine finger for an unnatural amino-acid analogue of lysine, Lyk—which has the same length as the arginine—prevented the β-subunit ADP-inhibited state [22], indicating that the length of the *trans*-acting side chain is sufficient for nucleotide exchange. These observations suggest that the ATP-bound α-subunit uses its arginine finger to facilitate ADP release from its neighbouring β-subunit (figure 2*b*).

A surprisingly similar mechanism was recently suggested for the φ29 DNA packaging motor. The φ29 DNA packaging motor is a pentameric ring ATPase that encapsidates one copy of the viral genome into a pre-formed protein shell. During a motor's operation, the subunits are known to undergo ADP-ATP exchange one at a time. ATP binding in one subunit induces the release of ADP in the neighbouring catalytic pocket [23]. ADP release is the slowest process during nucleotide exchange and, thus, the subunits tend to stay bound to the nucleotide unless they are induced to release it [24]. A recent single-molecule study shows that mutants of the highly conserved arginine finger, R146K, display even slower ADP release rates, demonstrating that the arginine residue is necessary for normal ADP release [17]. Based on the published structure [25], the catalytic site and the arginine finger are located at opposite sides of each subunit but are connected through a direct peptide linkage (figure 3*b*). These observations are consistent with a mechanism in which ATP binding in one subunit triggers an allosteric signal that is propagated via this linkage to its arginine finger that, in turn, facilitates the release of ADP in the adjacent catalytic pocket.

The previous examples suggest a conserved allosteric process connecting ATP binding in one subunit to ADP release in the adjacent binding pocket via the arginine finger. Although experimental evidence about the details of this allosteric pathway is still needed, communication between adjacent binding pockets is also thought to underlie nucleotide exchange in other ring ATPases. An interesting variation of this mechanism is seen in the disagregase Hsp104, a hexameric ring ATPase from yeast that targets protein aggregates to resolve them [18]. The subunits of Hsp104 contain two binding domains: NBD1 and NBD2. A mutagenesis study shows that ATP binding to each protomer, NBD2 or NBD1, increases ATP turnover by almost 10-fold in the other. ADP release is known to be the rate-limiting step for the hydrolysis reaction, and thus, the observed increase in ATPase rate is thought to result from facilitating ADP release in one binding domain upon binding of ATP by the other [18]. Although, the allosteric regulation has been shown to occur in both directions, NBD2 is thought to be the main engine of Hsp104, whereas NBD1 is thought to play more of a regulatory role.

The examples presented above show that nucleotide factors operate in many motor proteins to regulate their ATPase activity. Mechanisms for nucleotide exchange often operate in combination with factors that modulate ATP hydrolysis rates. In what follows, we will review some of these activating factors and their associated mechanisms to stimulate or suppress ATP hydrolysis.

3. ATPase modulation via the arginine finger

Most ASCE (additional strand catalytic E) proteins reach high steady-state ATPase activity only in their oligomeric form

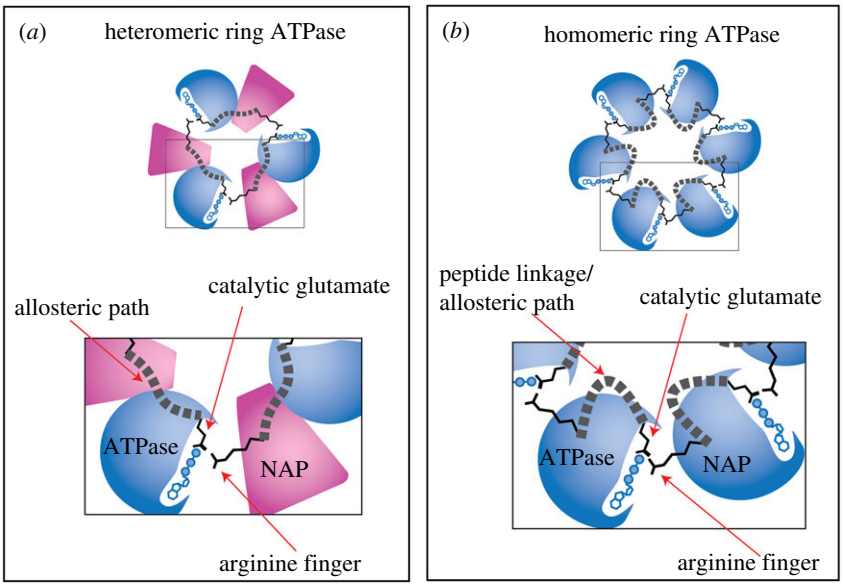

Figure 3. Catalytic pockets in heteromeric and homomeric ring motors with NTPase-activating proteins (NAP). (*a*) In heteromeric ring motors, two different proteins form the catalytic pocket: the ATPase subunit (blue) and an activating protein with no intrinsic ATPase activity (pink). Signals are transmitted by the activating protein to the adjacent catalytic pocket through allosteric pathways (grey dashed line). (*b*) In homomeric ring motors, the catalytic pocket is formed by two identical subunits. Signals are transmitted to the next catalytic pocket through peptide linkages connecting the nucleotide-binding site and the arginine finger (grey dashed line).

[26,27]. By contrast, as monomers, the subunits bind nucleotides with low affinity and support low rates of ATPase hydrolysis, preventing futile energy consumption. The high ATPase activity observed after oligomerization results from catalytic pocket sites at the interface of each pair of adjacent subunits [28] (figure 3). ATP hydrolysis rates in the catalytic sites are enhanced by *trans*-acting arginine residues that stabilize the transition state (figure 4*a*). In heteromeric ring motors, ATP hydrolysis in the catalytic subunits—containing both the nucleotide-binding pocket and catalytic residues—are activated by the non-catalytic subunits—contributing the arginine residues (figure 3*a*). The catalytic and non-catalytic subunits have different sequences and often differ significantly in their tertiary structures. In homomeric ring motors, ATP hydrolysis in the catalytic sites is activated by arginine residues provided by identical neighbouring subunits (figure 3*b*). In each subunit, the nucleotide-binding pocket and catalytic residues are located at the opposite side of the arginine finger. Below we review several heteromeric and homomeric ring ATPases where subunits take on the role of activating factors to stimulate ATP hydrolysis in the neighbouring catalytic pocket. We will also discuss allosteric processes that enable sequential subunit stimulation.

4. The arginine finger in heteromeric ring motors

The $\alpha_3\beta_3$ ring complex of F1-ATPase exhibits its maximum ATPase activity in the presence of the γ-subunit (figure 2*b*), but also displays significant ATPase activity alone (approx. 25% of the full F1-ATPase) [29]. The $\alpha_3\beta_3$ intrinsic ATPase activity is supported by three catalytic sites formed at the interface of each α−β dimer (figure 2*b*). Early biochemical studies showed that catalytic β-subunits alone do not display significant ATPase activity and require the presence of the non-catalytic α-subunits to attain detectable enzymatic activity [30]. Moreover, mutants of the highly conserved arginine finger were shown to decrease the ATPase activity by a factor of 10^3 [16]. Although some of this reduction derives from larger ADP inhibition of the β-subunit (see previous section), single-molecule studies have shown that the transition most affected by the substitution is ATP hydrolysis [16]. These studies, together with molecular dynamic simulations, show that the α-subunit plays an activating role by contributing its *trans*-acting arginine to the catalytic site (figures 3*a* and 4*a*). Interestingly, recent studies based on high-speed atomic force microscopy show that rotor-less $\alpha_3\beta_3$ complexes exhibit ATP-induced dynamic conformational changes and binding asymmetry similar to those observed during the operation of the full F1-ATPase complex [31]. This observation indicates that the α- and β-subunits are fully competent to propagate signals between catalytic sites without the concourse of the γ-subunit (figure 3*a*). The fact that mutants of the arginine finger abolish multi-site catalysis [32] suggested that the α-subunit, via its arginine finger, plays an important role in transmitting signals between catalytic sites. Isolated β-subunits are known to undergo large conformational changes upon nucleotide binding [33] that closely resemble the open-to-closed conformation transition. By contrast, isolated α-subunits exhibit much more limited conformational changes upon ligand binding in bulk studies [34]. However, molecular dynamic simulations show that the open-to-closed conformational transition in α-subunits is essentially barrierless [35], implying that the non-catalytic subunit could easily mirror the β-subunit's conformational changes upon induction. Moreover, mutagenesis studies have identified a set of residues in the vicinity of the arginine finger in the α-subunit that are necessary for nucleotide-binding cooperativity and multisite hydrolysis but that have no effect on unisite catalysis—the hydrolysis rate when only one out of the three catalytic sites is occupied with nucleotide [32]. Based on the crystal structure of the full F1-ATPase complex, this set of residues is thought to amplify conformational changes occurring in the arginine finger during the ATP hydrolysis transition state [36]. Although more experimental evidence is required, the inherent flexibility of the α-subunit suggests an allosteric path that allows long-range communication between β-subunit catalytic pockets (figure 3*a*).

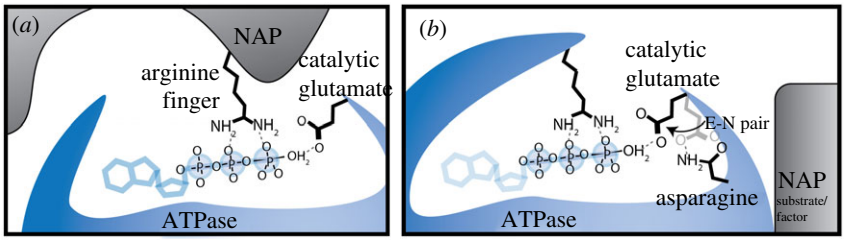

Figure 4. Molecular mechanisms for ATP hydrolysis activation. (*a*) An arginine finger interacts with the γ and β phosphates to stabilize the transition state during the water nucleophilic attack. (*b*) The catalytic glutamate pairs with an asparagine when the protein is in the inactive state. The residue is released from the pair by external activating factors to activate ATP hydrolysis. NAP, NTPase-activating protein.

β-subunits of F1-ATPase pair exclusively with α-subunits to form complete catalytic pockets. By contrast, other ATPase proteins can pair with various activating proteins, a strategy that possibly evolved to regulate the function of the motor by different factors at distinct locations in the cell. TorsinA, a member of the AAA+ branch of NTPases, related to proteases and Hsp proteins, illustrates this behaviour [37]. TorsinA is found in the endoplasmic reticulum and nuclear envelope of higher eukaryotes, and does not display ATPase activity in isolation. However, ATP hydrolysis by torsinA subunits is induced upon association with LAP1 at the nuclear envelope, or with LULL1 at the endoplasmic reticulum. Both activating proteins lack intrinsic ATPase activity. EM studies show that torsinA and LAP1 or LULL1 subunits form alternating hetero-hexameric rings in which the activators donate an arginine finger to complete torsinA's ATPase active site (figure 3*a*) [38]. Many questions remain to be answered about this system, including whether LAP1 and LULL1 can mediate long-range communication between torsinA subunits in a manner similar to α-subunits in F1-ATPase. Nonetheless, the ATPase activation observed in torsinA raises the possibility that other proteins are also activated by multiple activating factors, each forming a different heteromeric ring depending on their location in the cell.

5. The arginine finger in homomeric ring motors

The mechanism for ATP hydrolysis activation in homomeric ring motors (figure 3*b*) has been well characterized in a model ring ATPase, the φ29 DNA packaging motor. The operation of this motor is known to be segregated into two phases, while the subunits display a high degree of coordination [24]; in the first phase, all five subunits in the ring sequentially exchange ADP for ATP. Then, saturation of the ring with ATP is a signal that activates the first hydrolysis event. This event, in turn, initiates a cascade of hydrolysis by the remaining subunits [24]. A recent single-molecule study shows that the observed high degree of coordination results from the ability of the subunits to switch between spontaneous (poor) and stimulated (efficient) ATPase activity during the motor's operation [17]. Throughout the nucleotide exchange stage, the subunits display their basal low ATPase activity, allowing all subunits to bind nucleotide. During the catalytic phase, ATP hydrolysis at the catalytic sites is activated by the neighbouring subunit via the *trans*-acting arginine finger, R146 (figure 4*a*). According to the recently published crystal structure of the monomer [25], the conserved catalytic glutamate, E119, is connected with the putative DNA-binding loop which extends to the arginine finger, R146 (figure 3*b*). Thus, an allosteric mechanism can be envisioned for the activation of the first ATP hydrolysis by the last ATP-binding event and for the following sequential ATP hydrolysis; the last ATP-binding event is sensed by residues in the catalytic pocket. These residues propagate a conformational change that repositions the arginine finger in the next catalytic pocket to stimulate ATP hydrolysis. ATP hydrolysis in that subunit induces the repositioning of its arginine finger to stimulate ATP hydrolysis in the next catalytic pocket. By repetition of this process, ATP hydrolysis stimulation is propagated sequentially around the ring. Many ring ATPases are known to display similar sequential coordination of ATP hydrolysis, such as the Rho transcription factor and the chaperonin CCT/TRiC [39,40]. The mechanism described here for the φ29 DNA packaging motor possibly underlies the operation of similar molecular machines.

As seen above, ATP hydrolysis stimulation via the arginine finger is a highly conserved mechanism employed by many ring ATPases (figure 4*a*). Additionally, the ATPase activities of most ring motors are repressed or further stimulated upon binding other elements, such as the motor's substrate or additional protein factors. In the next section, we will describe a possible mechanism underlying the operation of these activating/repressing factors.

6. ATPase modulation via the 'glutamate-switch' mechanism

In multimeric ring motors, ATPase activation and repression upon binding additional elements—such as the motor's substrate or additional protein factors—have been widely reported [41–43]. While these additional elements are often thought as ligands that regulate the motors' activities, the molecular mechanism that couples ligand binding to the change in a protein's ATPase activity is still not well understood. A comparative structural study of multiple motor proteins, belonging to the AAA+ superfamily of the ASCE division [44], provides important insights into this ligand-mediated regulation. This study includes the structural maps of 50 different proteins (including F1-ATPase, PspF, ORC1, HslU, RFC and SV40) found in different states, such as ADP-bound, ATP-bound, and in the presence or absence of their regulatory ligand. In the absence of the regulatory ligand, the catalytic glutamate side chain was seen to be well positioned to interact with the missing γ phosphate in the ADP-bound state, but systematically rotated away from this position by approximately 100° in the ATP-bound state. In the rotated configuration, the glutamate forms a hydrogen bond with another residue located in the vicinity, typically an asparagine (figure 4*b*). The systematic formation of the glutamate-asparagine (E-N) pair suggests an

explanation for the weak ATPase activity exhibited by many proteins in the absence of their regulatory ligand: the state of the protein corresponds to the orientation of the catalytic glutamate. In other words, the protein is inactive when the E-N pair is formed and active when the glutamate is released from it. This study also shows that, in most cases, there is a direct peptide linkage between the E-N pair and the regulatory ligand-binding region. This linkage suggests an allosteric mechanism that couples the binding event to changes in the catalytic glutamate's orientation. In the proposed mechanism, the regulatory ligand binding induces the release of the catalytic glutamate from the E-N pair to stimulate—or promotes the formation of the E-N pair to repress—the protein's ATPase activity (figure 4*b*). Additional structural maps of proteins in the presence of their regulatory ligands are still required. However, mutagenesis studies show that the substitution of the asparagine required to form the E-N pair renders the protein irresponsive to the presence of the regulatory ligand [45]. Although further experimental evidence is needed, the glutamate-switch model suggests a compelling mechanism for the activation and repression of the motors' ATPase activities by additional binding factors. In the following section, we will consider the implications of this activating/repressing mechanism in multimeric ring motors.

7. Asymmetric ATPase activity in ring motors

In multimeric ring motors, the individual subunits must coordinate their operation to perform a single biological task [46]. However, during a motor's operation, the subunits might not bind simultaneously, all at once, to the substrate or associated factors. Thus, only those subunits that bind to the motor's regulatory ligand will be stimulated or repressed, resulting in symmetry breaking of the ATPase activity around the ring complex. Moreover, as the motor's cycle proceeds, the subunits–regulatory ligand interactions change, and the ATPase activity of the individual subunits is expected to change accordingly. Here, we discuss two ring motors that display asymmetric ATPase activity: the φ29 DNA packaging motor and the protease ClpXP.

The φ29 DNA packaging motor is known to translocate 10 bp of DNA per cycle in a burst of four power strokes, each 2.5 bp in size, interspersed by dwell times of about 80 ms on average at saturating [ATP] [23]. Because the φ29 packaging motor is a homo-pentamer, the four power strokes reveal a crucial symmetry breaking in its operation: only 4 out of the 5 subunits perform DNA translocation. It has been proposed that the remaining subunit performs a regulatory function in the ring. In fact, temporary inactivation of the fifth subunit with ATPγS results in greatly lengthened dwell times [24] followed by a 10 base pair burst, suggesting that proper turnover of ATP by the fifth subunit is necessary to initiate the translocation cascade by the other four subunits in a timely manner. Because the motor is known to contact two DNA phosphates every 10 bp, it was proposed that the observed functional difference between otherwise identical subunits results from the periodic contact of the fifth subunit with the motor's substrate [47]. A recent single-molecule study that combines targeted mutagenesis and cryo-EM reconstruction, provides further insights into the mechanism that enables division of labour among identical subunits [17]. In this study, a substitution that abolishes the subunit's ATPase activity is shown to be tolerated in the ring only if it inactivates the regulatory subunit. Such mutant motors also display greatly lengthened dwell times separated by exactly 10 bp bursts. From these experiments, it was possible to extract the spontaneous ATP hydrolysis rates of the translocating subunits and that of the regulatory subunit. The data showed that the regulatory subunit has a threefold increase in ATP hydrolysis rate relative to its translocating counterparts. The asymmetric cryo-EM reconstruction shows that, indeed, only one of the five subunits establishes extensive contacts with the DNA prior to translocation. These observations are consistent with a mechanism in which the periodic DNA contact enhances the regulatory subunits' ATP hydrolysis rates. In agreement with this interpretation, previous bulk measurements have shown that DNA stimulates the subunits' ATPase activities in solution [48]. Moreover, the pseudo-atomic structure of the motor's subunit [25] shows that the putative DNA-binding loop is adjacent to the catalytic glutamate, E119, which provides a direct peptide linkage that connects DNA contact to the stimulation of the subunit's ATPase activity. As discussed in the previous section, in the φ29 DNA packaging motor, the first ATP hydrolysis (by the regulatory subunit) takes place quickly after saturation of the ring with ATP. Thus, the data suggest that the DNA-bound regulatory subunit is *primed* to be *further* activated by the last ATP-binding event. Moreover, the different hydrolysis rates suggest that other rates could be different in the subunit contacting the DNA. In particular, it has been speculated that ADP release takes place much faster at the regulatory subunit than in any other subunit to initiate nucleotide exchange, again activated by the binding of that subunit to the regulatory ligand. Thus, functional symmetry breaking between the subunits of the φ29 DNA packaging motor provides a mechanism by which one of the subunits triggers, in turn, first the beginning of the nucleotide exchange and then the beginning of the hydrolysis cascade.

Similar mechanisms possibly operate in the hexameric protease ClpX. ClpX recognizes ssrA-tagged proteins, unfolds them and feeds the polypeptide through its central pore into the proteolytic chamber, ClpP, for degradation [49]. The ATPase activities of ClpX subunits are known to be highly asymmetrical and consist of two classes defined by the orientation between the proteins' subdomains: ATP-unloadable (U) and ATP-loadable (L) [50]. Among the loadable type, some sites release ATP rapidly, whereas others release ATP slowly. The mechanism that produces such asymmetry is not well understood. However, the ATPase activities of ClpX subunits are known to be repressed in the presence of the proteolytic chamber, ClpP, and stimulated by ssrA-tagged substrates [49]. Two types of ClpX luminal loops are important for this ATPase regulation [51]. The first type, known as pore-1 or GYVG loops, are located in the middle of the pore and propel substrates forward along the ring channel through hydrophobic interactions. Pore-1 loops are known to influence the subunits' ATP hydrolysis rates, although the mechanism of ATPase modulation is not yet clear. The second type, known as pore-2 loops, are heavily populated with charged residues and are located at the interface between the ring ATPase and the proteolytic chamber, ClpP. Pore-2 loops extend directly from the Walker B motif, which harbours the catalytic glutamate, E185, involved in ATP hydrolysis [52], and thus, are thought to translate the motor's interactions into enhancement or suppression of ATP hydrolysis by controlling the configuration of the

glutamate residue at the catalytic site. Indeed, pore-2 loops are known to be necessary for repression of the ATPase activity by ClpP [53]. Furthermore, pore-1 and pore-2 loops form a helical inner surface running parallel to each other [52] and, thus, it is conceivable that the motor's substrate serves as an allosteric platform that supports communication between the two types of loops. Given the chemical and conformational heterogeneity of the unfolded polypeptide chain, it is possible that the asymmetric ATPase activity observed in ClpX stems from the highly irregular and continuously changing substrate-subunit interactions.

8. Concluding remarks

As shown throughout this review, different motors have adopted distinct allosteric processes to regulate specific stages of their enzymatic cycle, specifically nucleotide exchange and ATP hydrolysis. This regulation takes place either through selective interactions with activators, repressors and nucleotide exchange factors or, as observed in ring ATPases, through interactions with adjacent partners. Such regulatory interactions recall the control of activity seen in small GTPases. The mechanisms reviewed above employ highly conserved structural elements and, thus, likely underlie the operation of many other motor proteins. It is fascinating to verify how, through discrete intermolecular interactions a global, deterministic, machine-like behaviour emerges from purely stochastic molecular events.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

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