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Discovering autoinhibition as a design principle for the control of biological mechanisms

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ABSTRACT

Autoinhibition is a design principle realized in many molecular mechanisms in biology. After explicating the notion of a design principle and showing that autoinhibition is such a principle, we focus on how researchers discovered instances of autoinhibition, using research establishing the autoinhibition of the molecular motors kinesin and dynein as our case study. Research on kinesin and dynein began in the fashion described in accounts of mechanistic explanation but, once the mechanisms had been discovered, researchers discovered that they exhibited a second phenomenon, autoinhibition. The discovery of autoinhibition not only reverses the pattern in terms of which philosophers have understood mechanism discovery but runs counter to the *one phenomenon-one mechanism* principle assumed to relate mechanisms and the phenomena they explain. The ubiquity of autoinhibition as a design principle, therefore, necessitates a philosophical understanding of mechanisms that recognizes how they can participate in more than one phenomenon. Since mechanisms with this design are released from autoinhibition only when they are acted on by control mechanisms, we advance a revised account of mechanisms that accommodates attribution of multiple phenomena to the same mechanism and distinguishes them from other processes that control them.

1. Introduction

Much scientific research on biological mechanisms focuses on how they account for phenomena—e.g., the division of a cell, the contraction of a muscle, the synthesis or degradation of a protein. From this perspective, it is surprising that many molecular mechanisms in biology are organized so that they autoinhibit—that the parts of the mechanism act on others in a manner that renders the mechanism unable to perform the phenomenon for which it is responsible. Autoinhibition involves *intra*molecular interactions between distinct domains of a molecule such that one region impedes the activity of another. For a mechanism to generate the phenomenon with which it is identified, it must be released from autoinhibition through *inter*molecular interactions between the target protein and binding partners that serve to alter the protein's conformation, activating the formerly inhibited domains (Pufall & Graves, 2002).

We argue that mechanisms operating to inhibit their ability to produce the phenomenon with which they are identified should be viewed as instantiating a design principle: a commonly implemented pattern of organization that can be described generally and realized in different molecular implementations. In section 2 we introduce recent discussions of design principles, including discussions of how they provide generalized principles that can be invoked in explanations, including mechanistic explanations. While there has been discussion of the explanatory roles of design principles in both philosophy and various areas of biology, there has been relatively little discussion of how scientists discover design principles. We focus on the discovery of autoinhibition, using the discovery of the autoinhibition of the molecular motors kinesin and cytoplasmic dynein as examples.

Although, as Pufall and Graves demonstrate, autoinhibition is a design principle widely instantiated in biological mechanisms, it is typically not discovered in the same manner as new mechanists have described the discovery of mechanisms—starting with the phenomenon, identifying the responsible mechanism, and then figuring out its components and how they are organized (Illari & Williamson, 2012). As the cases of kinesin and dynein show, that these mechanisms inhibit themselves was only recognized after they were identified as the mechanisms responsible for cellular phenomena such as axonal transport. Moreover, autoinhibition was concealed by the very experimental procedures widely used to study molecular mechanisms. Such procedures are

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designed to reliably produce the phenomenon in which researchers are interested, which requires overriding the processes resulting in autoinhibition. Only as broader inquiry was proceeding—inquiry using other experimental tools—did researchers studying both kinesin and dynein come to recognize that much of the time these mechanisms generated a different phenomenon, autoinhibition.

As background to research leading to the discovery that both kinesin and dynein inhibit themselves, in section 3, we discuss the research leading to the discovery of kinesin and cytoplasmic dynein as the motors responsible for axonal transport and the development of explanations of their ability to produce movement from the hydrolysis of ATP. This research proceeded in the manner the new mechanists have characterized: researchers identified a phenomenon of interest and associated it with a mechanism, then decomposed the mechanism into relevant parts and determined how they operate in an organized fashion so as to generate movement. In sections 4 and 5, we turn to how researchers discovered that the motors autoinhibit when not needed for transport. The paths to discovering that kinesin and dynein autoinhibit were quite different. In the case of kinesin, it occurred shortly after the discovery of the protein itself. In the case of dynein, it took considerably longer. We take advantage of the differences in the two cases to further elaborate on the reasoning that goes into piecing together accounts of how the proteins act to inhibit themselves and how other processes in the cell release them from that state and enable them to perform their activities of transporting cargo.

In section 6 we consider the implications of the case studies of kinesin and dynein for the understanding of mechanisms. As we have noted, the discovery that these mechanisms autoinhibit followed on their discovery as the mechanisms that generated the motility needed for axonal transport. This not only reverses the typical pattern of mechanism discovery but also provides a different perspective on the relation between mechanisms and phenomena, challenging the one phenomenon-one mechanism principle that most mechanists have adopted. In mechanisms that autoinhibit, the same mechanism is involved in different phenomena. Which phenomenon they engage in depends on how they are controlled. Since autoinhibition is, as we argue, a design principle widely implemented in biological mechanisms, this critical point against standard accounts of mechanism derives not only from our case study (which we use to illustrate the distinctive pattern involved in the discovery of autoinhibition) but from the whole suite of autoinhibitory mechanisms in the cell. The ubiquity of autoinhibition, in turn, motivates an alternative philosophical understanding of mechanisms that countenances how mechanisms can behave differently under different conditions of control. To understand how control processes act on mechanisms and determine what phenomenon they produce, we draw on a reconceptualization of mechanisms as systems that constrain flows of free energy. We conclude in section 7.

2. Autoinhibition as a design principle

A common theme in philosophy of biology is that the biological world is contingent and accordingly that there are no laws in biology. Smart (1963) argues that biological phenomena lacked the regularity required to be subsumed under laws. Beatty (1995) argues that biological systems, as the products of evolution, are contingent; as a result, any generalizations that are found do not qualify as laws. The lack of recognizable laws was a factor leading Bechtel and Richardson (1993/2010) to reject the D-N model of explanation and argue that many explanations in biology took the form of identifying mechanisms.[1] Resisting this tradition of denying laws in biology, Green (2015) draws upon examples in systems biology to show that what systems biologists refer to as *design principles* provide generalizations that can be invoked in biological explanations. She quotes Ma, Trusina, El-Samad, and Lim's (2009) characterization of design principles as "organizational rules that underlie what networks can achieve particular biological functions" (637).

To highlight a system's organization, a common strategy in systems biology is to represent the entities and interactions of components of a system as nodes and edges in a network. Such a representation is indifferent to the identities of particular components as these are ancillary to the pattern of organization represented in the network. In his pioneering research using network representations, Alon and his collaborators (Milo et al., 2002; Shen-Orr, Milo, Mangan, & Alon, 2002) identified numerous particular subnetworks within larger networks specifiable in this way. Each subnetwork involves two to four nodes connected in the same manner, which Alon and colleagues referred to as *motifs*. For instance, Fig. 1A illustrates a *coherent feedforward network*. It consists of three nodes, labeled X, Y, and Z (S in the input to the motif), in which node X activates node Z both directly and by activating Y which in turns activates Z. Using Boolean modeling, they showed that if node Z acted as an and-gate and if it took time for each node to respond to inputs from the previous node, such a motif would act as a persistence detector—node Z would only become active if input S was maintained sufficiently long for Y to become active and for both X and Y to send outputs to Z.[2] An important feature of motifs is that they abstract over details about the identity of X, Y, and Z; as a result, the analysis of how the motif functions explains what happens in all instantiations. Fig. 1B shows an even simpler motif explored by Tyson and Novák (2010)—a double negative feedback loop which, with appropriate parameters, enables switching between two stable regimes. In this case, the need for appropriate parameters limits the applicability of the motif, but it still generalizes over a wide domain.

Motifs illustrate fundamental features of design principles—they are ways of organizing components in which the resulting function does not depend on the specific features of the entities realizing the nodes; as a result, the motif itself can be appealed to in explanations of a diverse set of phenomena. Green, Levy, and Bechtel (2015, p. 16) capture this in their characterization of design principles as "patterns of organization that can be specified abstractly, supplying an explanation for a given behavior that occurs across a range of cases in which the organizational pattern is realized."

The word *design* is closely associated with the idea of a designer. Green et al. emphasize, however, that design principles can arise through the course of evolution without a designer. They need not even be adaptations—the product of natural selection. Nonetheless, they may be promoted by natural selection—one can view natural selection as exploring different designs. In this spirit, Lim, Lee, and Tang (2013, p. 202) characterize design principles as "archetypal classes"—"common patterns for how diverse and complex regulatory [systems] … achieve a particular function." When considering evolution, they can serve as "attractors" in the "underlying landscape within which evolution can explore." As attractors, Lim et al. characterize them as patterns that would regularly appear "if one could hypothetically replay evolution over repeatedly."

Systems biology is not the only area of biology invoking design principles. They are also employed in cell and molecular biology. One of the examples of design principles that Lim et al. present is the common organization that Steitz (1999) showed to be exhibited by different DNA

[1] Mechanisms are intended to generalize over many instances in which a phenomenon is produced, but there may be no generalizations across mechanisms responsible for different phenomena.

[2] As Alon's use of Boolean modeling makes clear, motifs and other design principles are often analyzed in computational terms. As in the case of the double-negative feedback loop discussed below, such modeling reveals the specific conditions (reflected in parameters in the computational model) under which the design principle will realize the specific effect. In many cases, qualitative analysis, such as provided in the text, suffices to appreciate the effect. We treat design principles as patterns of organization that can be analyzed either qualitatively or quantitatively.

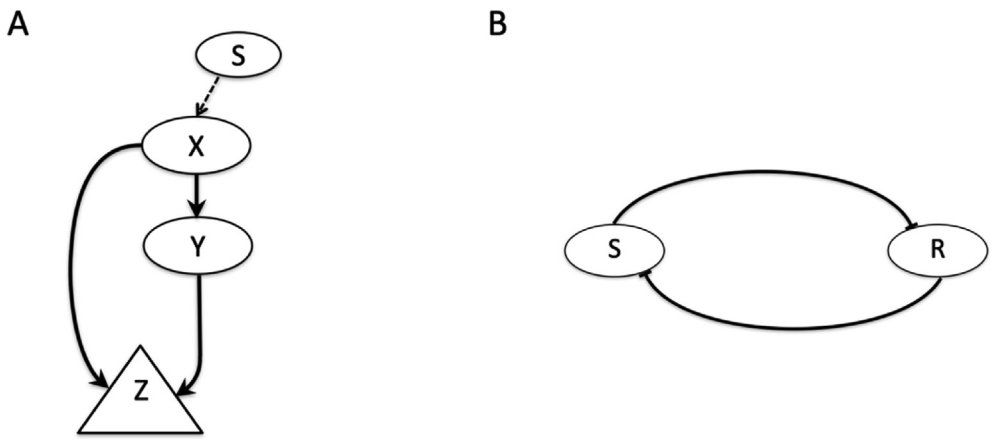

Fig. 1. A. A coherent feedforward loop motif. B. A double negative feedback loop motif.

polymerases (Fig. 2). In this case, it is the common features of the organization of the different proteins that is viewed as a design principle and is invoked to explain the functioning of the protein as a polymerase. Cell biologists Rafelski and Marshall (2008) similarly appeal to abstract features of mechanisms to propose ways in which "mechanisms pattern the architecture of the cell." They explain that they borrow the term *design principle* from engineering to designate "simple rules that, when followed in the design of a machine, ensure or at least increase the likelihood of proper assembly or function" (593). In discussing a design that could control the size of developing cellular structures, for instance, what they term *molecular rulers* have lengths corresponding to the desired length of the structures of which it controls the development. For example, the gene *H* product dictates the length of the λ-phage tail by attaching to the growing tail and preventing the action of a growth-terminating factor until the tail outgrows the "ruler" (gene *H* product).

Yet another field in which design principles are invoked is synthetic biology. Stein and Alexandrov (2015), for example, invoke actual protein switches found in cells as a basis for engineering switches to perform new functions. One of the design principles they develop is of particular relevance for our discussion below—autoinhibition. They present a cartoon (Fig. 3) to illustrate the design principle through which a ligand (L) can activate a switch, releasing the autoinhibitory domain AI,

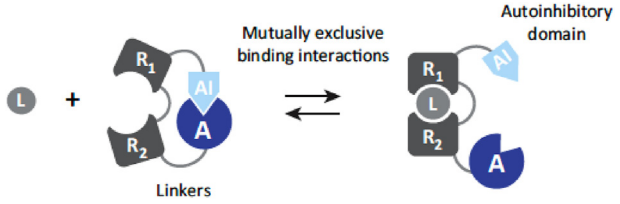

Fig. 3. A cartoon showing how a ligand binding to a switch can release a target from autoinhibition. Adapted from Stein and Alexandrov (2015) with permission from Elsevier.

rendering protein A active. Like network diagrams, by using abstract shapes a cartoon like this makes clear that the design can be instantiated by different components.

As emphasized by Green (2015), one reason design principles are philosophically important is that they make "room for generality in biology." This virtue is well captured in Salvador's (2008) discussion of the implication of Alon's identification of motifs:

> molecular biology might one day be structured around a number of simple laws or principles whose understanding hinges largely on engineering considerations similar to those applying to human

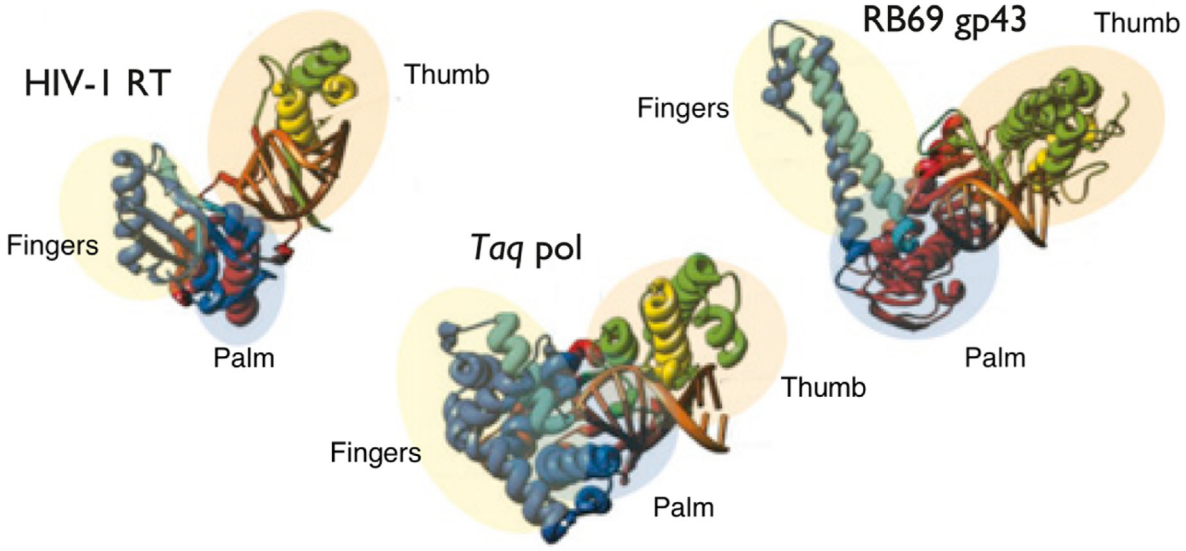

Fig. 2. A structural design principle involving regions designated palm, thumb, and fingers illustrated in three DNA polymerases. Adapted from Steitz (1999); reproduced under Creative Commons CC BY license.

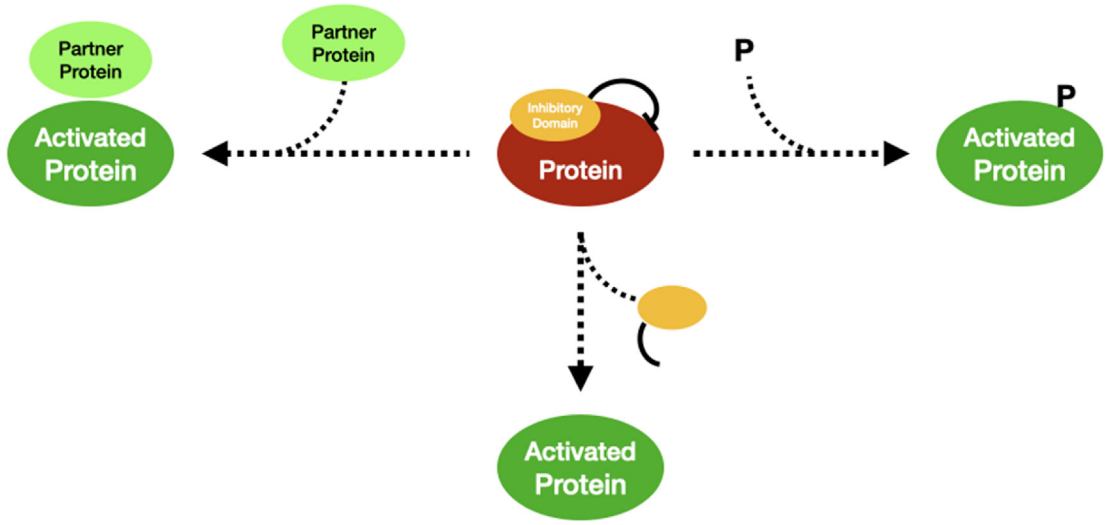

Fig. 4. The inhibited state of a protein (center of diagram) involves an intramolecular interaction which prevents it from performing its activity. Activation of a protein requires intermolecular interactions between a signal and the autoinhibiting protein. Three ways in which this can happen are illustrated. Notice that the abstract specification of the principle makes no reference to any particular proteins.

> designed circuits. The major breakthroughs in the exact sciences occurred when the main regularities (laws) were discovered and then explained. From this process ensued the predictive power that earned these sciences the qualifier "exact," which still sets them apart from biology. If a similar process is nowadays taking place in molecular biology this is largely through the discovery and explanation of design principles (193).

Our brief discussion illustrates a wide range of designs that biologists in different domains of biology refer to as design principles. We turn now to the specific example on which we focus, autoinhibition. Treating it as a widespread phenomenon, Pufall and Graves (2002) characterize autoinhibition abstractly: "intramolecular interactions between separable elements within a single polypeptide provide a common regulatory strategy [in which] one region of a protein interacts with another to negatively regulate its activity" (422). The intramolecular interactions are illustrated in a cartoon fashion in Fig. 4 in which a domain of a protein inhibiting its activity is indicated by an edge-ended line between an oval representing the inhibitory domain and another representing the activity of the protein. Since there are conditions in which the activity of the protein is required, the figure also identifies three ways in which an inhibited protein can be released from autoinhibition through intermolecular activities.

Without labeling it a design principle, Pufall and Graves argue that autoinhibition is a "common regulatory strategy to modulate protein function." In support of this claim, they provide a detailed account of seven examples and list over thirty other instances. This frequent occurrence suggests that it is what Lim et al. characterized as an evolutionary attractor and appropriately characterized as a design principle.

Autoinhibition is useful in explaining the contribution of proteins to cell activities. The activities performed by proteins are invoked in explaining how cells generate different phenomena. But most phenomena (e.g., cell division, synthesis of proteins, autophagy), are only useful to the cell on some occasions and at other times are detrimental. For instance, cell division is useful to construct a multicellular organism, but unconstrained cell division is a feature of cancer. Employing a design in which an intramolecular interaction inhibits the ability of the protein to perform its activity ensures that it will not act except when a signal specifically releases it from autoinhibition.

As we discussed, both philosophers and biologists have articulated the significance of design principles for understanding explanation in biology. In the spirit of mechanists concerned with discovery (Bechtel & Richardson, 1993/2010; Craver & Darden, 2013), we take up the question of how they are discovered. The example of autoinhibition is useful for this purpose since experiments are designed to enable mechanisms to operate and hence involve procedures that effectively release proteins from autoinhibition whether researchers understand this explicitly or not. As Pufall and Graves note, the assays used to study protein function frequently "bias" researchers to focus on the active state of the protein and not notice that regions of it may serve an autoinhibitory function.[3] This poses the question: how do researchers come to notice instances of autoinhibition? To address this question, we turn to research on two molecular mechanisms—the motor proteins kinesin and dynein. The paths to discovering that kinesin and dynein autoinhibit were quite different. The discovery occurred relatively quickly in the case of kinesin—shortly after the discovery of the protein itself—while, in the case of dynein, discovering that it had this design took considerably longer. This difference across cases works in our favor, philosophically, as it helps more fully characterize the process and significance of the discovery of autoinhibition. In both cases, the discovery that the mechanism autoinhibits followed on the discovery of the mechanism itself. Accordingly, we turn to the discovery of kinesin and dynein in the next section, reserving to sections 4 and 5 the analysis of how each was found to autoinhibit.

3. Discovering the motors responsible for axonal transport

In this section we describe how researchers, starting from observations of fast axonal transport, discovered the responsible mechanisms. This involved identifying two mechanisms they took to be loci of control (Bechtel & Richardson, 1993/2010) for that phenomenon—the molecular motors kinesin and dynein, and characterizing how, in each case, their movement patterns drive axonal transport. The movement pattern of the motors now became the phenomenon to be explained. To do so, researchers decomposed them into their organized parts and operations and demonstrated that the proposed mechanism can generate the phenomenon of motility. This pattern of discovery is familiar to mechanist philosophy of science according to which researchers identify a phenomenon of interest and, decomposing it into parts and localizing

[3] Pufall and Graves offer this as a reason to "predict that there are undoubtedly many more examples of autoinhibition to be discovered" (453).

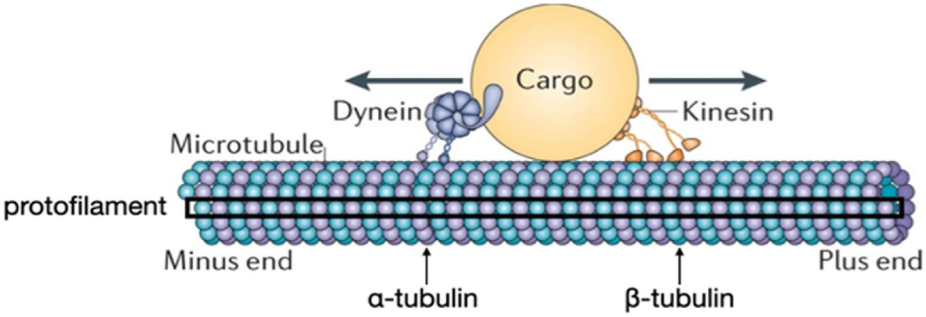

Fig. 5. Cartoon of microtubule with dynein moving cargo toward the minus end and kinesin toward the plus end of a microtubule. Adapted from Hancock (2014) with permission from Springer Nature.

functions to those parts, specify the mechanism responsible for it (Illari & Williamson, 2012).

Fast axonal transport was first identified through research on nerve regeneration that began during World War II. When researchers found that constricted axons swelled to two or three times their normal diameter at the point of constriction, they attributed the swelling to material moving through the axoplasm and accumulating at the point of constriction. Using radioactive isotopes that became available after the war, investigators followed the movement of labeled material through the axon. By cutting out segments of the axons at different times, they were able to determine when various molecules reached each section. Such experiments revealed that transport occurs in two directions, toward and away from the center of the cell. These were dubbed *anterograde* and *retrograde* axonal transport respectively (Grafstein & Forman, 1980). Attaching video cameras to microscopes, Allen, Allen, and Travis (1981) directly visualized the movement of radioactively tagged organelles and proteins and distinguished different rates at which cargo was transported. Even the slow transport they observed was faster than could be explained by diffusion. Accordingly, researchers began searching for the responsible mechanism. By extracting axoplasm out of axons and observing that particle transport still occurred, Brady, Lasek, and Allen (1982) concluded the mechanism resided in the cytoplasm and did not involve the plasma membrane.

Research during the same period had identified a cytoarchitecture within cells that consisted of microtubules, microfilaments and intermediate filaments. Microtubules are long (sometimes as long as 50 μm), hollow cylinders (approximately 25 nm in diameter), typically consisting of 13 protofilaments. Each protofilament is made of heterodimers of α- and β-tubulin proteins (Fig. 5). Typically, microtubules are arranged in the cell a bit like the spokes of a wheel, extending from what is designated the "minus-ends" near the nucleus or centrosome of the cell to the "plus-ends" at the cell periphery. Schnapp, Vale, Scheetz, and Reese (1985) showed that axonal transport occurred along microtubules by correlating images of vesicles moving along filaments under a video microscope with electron micrographs of the same material. Under EM, these researchers were able to identify the filaments along which vesicle movement occurred as single microtubules. While electron microscopy enabled these researchers to identify microtubules as the tracks, it did not identify the motor driving movement along those tracks.

Recognizing that movement faster than that achieved through diffusion required a source of energy and that this would most likely be provided by hydrolysis of ATP, Vale and his colleagues initiated a search among proteins associated with microtubules for those that hydrolyze ATP (ATPases). Initially expecting the ATPase to be attached to transported vesicles, they used centrifugation to purify microtubules and vesicles from axons and, combining them with the soluble fraction from the centrifugation process on glass coverslips, observed the vesicles to move, like transported cargo, along the glass coverslips. Running a control experiment to ensure that they were observing vesicles and not aggregated proteins, these researchers combined only the soluble fraction and microtubules on a glass coverslip. Since they believed the motor would be bound to vesicles, they anticipated no movement. To their surprise, the microtubules began to slide over the coverslip. Now knowing that the motor was in the soluble fraction, they were able to isolate and characterize it, naming it *kinesin* (from the Greek word *kinein*, to move).[4] Kinesins turned out to constitute a large superfamily of proteins, more than 40 of which occur in mammals. Most kinesins transport cargo to the plus end of microtubules. We focus primarily on kinesin-1, the founding member of the superfamily, referring to it simply as *kinesin*.

In a preparation in which researchers had immobilized microtubules on glass coverslips and observed kinesin-bound beads moving along them, Vale et al. (1985) inhibited the activity of the kinesin and observed that minus-end directed transport still occurred along microtubules. They concluded that kinesin only drives anterograde movement and that another motor is responsible for retrograde movement. Vallee, Wall, Paschal, and Shpetner (1988) identified the second ATPase and, employing electron microscopy, demonstrated that it was "structurally equivalent" to axonemal dynein, a motor that had been identified 20 years earlier as responsible for movement of cilia (Gibbons & Rowe, 1965). The new dynein came to be known as *cytoplasmic dynein*.

With the identification of these two motor proteins, research proceeded on two fronts. First, researchers shifted their attention away from fast axonal transport and toward the detailed movement of the motors themselves, characterizing the stepping patterns of kinesin and dynein as they moved along the microtubules. Second, they developed mechanistic explanations for these stepping patterns, seeking to understand the means by which the motors step in the characteristic ways they do. The development of an innovative tool—the single-molecule motility assay—was crucial in studying kinesin movement in greater detail. Using purified kinesin and microtubules, Howard, Hudspeth, and Vale (1989) reconstituted kinesin-driven motion by immobilizing single kinesin molecules "heads-up" on glass coverslips. This enabled them to observe, under a video microscope, single kinesin molecules pushing microtubules around. An alternative version flipped this geometry, immobilizing microtubules on glass coverslips and coating tiny plastic beads with kinesin. The movement of the beads was then visible as they were carried along the microtubule track by the kinesin motors. By analyzing the motion of these beads or microtubules, researchers were able to draw inferences to the stepping activities of the kinesin motor driving it. They determined, for instance, that it walked "processively," taking steps in which one of two "heads" remained attached to the microtubule while the other head

[4] In successfully isolating kinesin, these researchers developed a novel technique that built on an earlier discovery that AMP-PNP, a non-hydrolyzable ATP analog, stopped transport along microtubules (Lasek & Brady, 1985). This led Vale and his team to use AMP-PNP to bind the as-yet-unidentified motors to microtubules and then purify the microtubules along with presumably, the attached motors. When the purified microtubules were treated with ATP to counter the effects of AMP-PNP, the material released was examined and found to contain a novel protein that, when combined with ATP and microtubules in a motility assay, caused microtubules to slide.

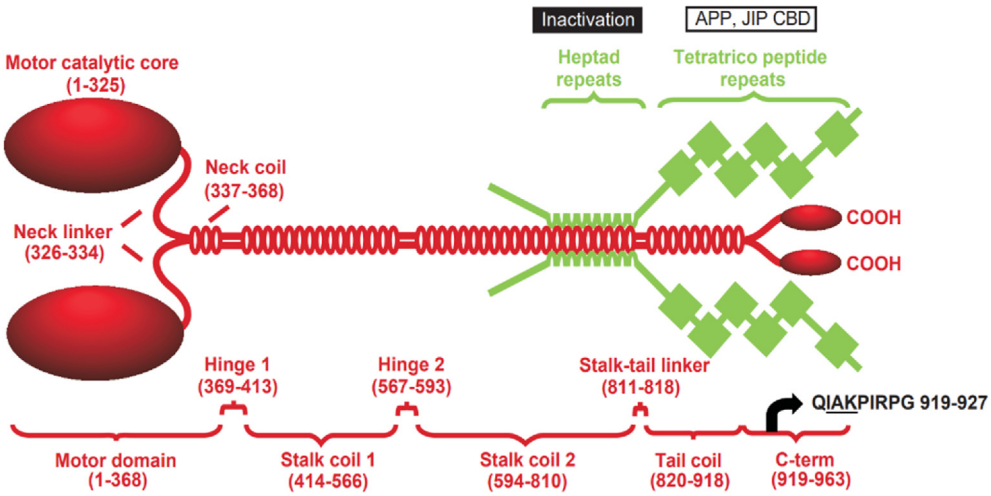

Fig. 6. Structure of kinesin 1. The parts in red represent the heavy chains which dimerize into the motor heads at the N-terminal end, while those in green constitute the light chains. Adapted from Schanpp (2003) with permission from Journal of Cell Science. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

moved, that it could take many steps before totally detaching, and that the two heads are asymmetric in their movement (Bollhagen, 2021). A similar scenario played out in the case of dynein. Single molecule studies of dynein revealed that they also move processively, but that their walking was more erratic than that of kinesin, with occasional backwards and sidewise steps (Qiu et al., 2012; Reck-Peterson et al., 2006).

Even as research aimed at characterizing the motor's stepping patterns proceeded, investigations directed at explaining these activities was initiated. A first step was to determine the parts of the motor molecules. Kinesin was found to consist of two N-terminal heavy chains and two C-terminal light chains which bind to cargo (Bloom, Wagner, Pfister, & Brady, 1988; Scholey, Heuser, Yang, & Goldstein, 1989). From electron micrographs, Hirokawa et al. (1989) revealed that the heavy chains form an elongated coiled-coil which dimerizes at a "neck linker" into globular heads at one end and binds the two light chain tails at the other (Fig. 6).

Higher resolution EM studies revealed that the globular heads contained the loci of ATP binding and hydrolysis and of microtubule binding. These heads became the foci in attempts to explain how the energy released in ATP hydrolysis generated motion. By crystalizing kinesin in different states of ATP hydrolysis, researchers demonstrated that the heads adopted different conformations before and after hydrolyzing ATP. Rice et al. (1999) developed a scenario according to which the conformation of the overall molecule changes as it binds ATP, hydrolyzes it, and then expels the resulting ADP and Pi. Among the consequences of these changes is that kinesin binds to and subsequently detaches from the microtubule. The conformation change also affects the linker that connects the two heads so that when one head is detached from the microtubule, it is forced forward to where it binds to the next binding site towards the plus-end of the microtubule (Fig. 7).[5]

Similar findings revealed how dynein produces movement. Like kinesin, dynein is a dimer of two proteins, each of which contains a heavy chain that forms a globular head. However, studies of its structure revealed important differences (Neuwald, Aravind, Spouge, & Koonin, 1999). The motor domain in the globular head consists of a ring of six AAA+ (ATPases associated with cellular activities) modules, four of which are capable of hydrolyzing ATP (only the first produces the force used to move the motor). The microtubule-binding site is separated from the motor domain at the end of a coiled-coil stalk. Researchers have

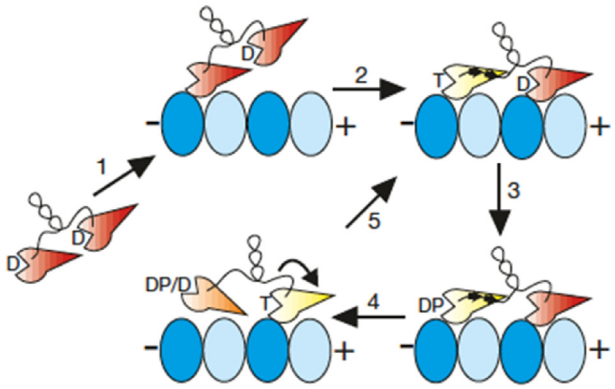

Fig. 7. Mechanistic account of kinesin walking. Depending on whether the kinesin bound ATP (T), hydrolyzed it to ADP (DP if phosphate is still at the site, D once it has been jettisoned), each head is bound or released from the microtubule. The force exerted on the linker moves the trailing head, once free, ahead of the previously forward head, where it binds the microtubule again. Reproduced with permission from Springer Nature. Rice et al. (1999).

developed detailed models of how the conformation changes induced by ATP hydrolysis alter the configuration of the head, which in turn alters the stalk so as to change whether the microtubule binding site can bind the microtubule (Fig. 8). These models further describe how force generated by ATP hydrolysis is communicated to the linker that joins the two heads and propels movement towards the minus-end of the microtubule.

The research described in this section fits the accounts of discovery by the new mechanists according to which, a mechanism is sought to explain how a phenomenon is produced (Machamer, Darden, & Craver, 2000) or how some task is carried out (Bechtel & Richardson, 1993/2010). As Darden (2008) states, "identifying a puzzling phenomenon is the first step in an investigation of a mechanism." The phenomenon or task to be explained provides a "perspective" from which researchers can study the mechanisms underpinning them, deploying the heuristics and strategies mechanist philosophers have identified and characterized—e.g. decomposition and localization (Bechtel & Richardson, 1993/2010), schema instantiation and forward and backward chaining (Craver, 2007; Darden, 2008). The mechanism, once discovered, is understood as the mechanism *for* the phenomenon or task the identification of which initiated the inquiry. In our case, the phenomenon of interest was, initially, axonal transport. Upon discovery of kinesin and dynein, their stepping patterns

[5] For a detailed account of this research as well as research on a similar motor, myosin, and a discussion of how the mechanisms arrived at differ from standard new mechanist accounts of mechanisms, see Bechtel and Bollhagen (2021).

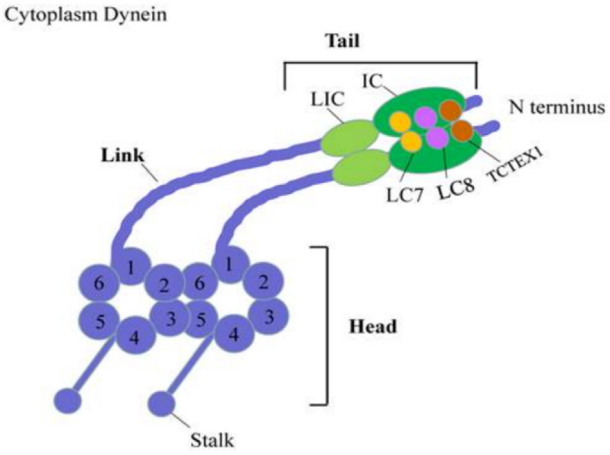

Fig. 8. The structure of dynein. Reproduced under Creative Commons CC BY license from Xiao, Hu, Wei, and Tam (2016).

became the phenomena to be explained. These phenomena provided a perspective which was embodied in the single-molecule motility assay that enabled researchers to investigate the stepping patterns of the individual motor proteins. Researchers then decomposed the motors and pieced together accounts of the mechanisms *for* motor movement.

There is no question that the single-molecule motility assay was extremely productive in advancing mechanistic explanations of how kinesins and dyneins generate anterograde and retrograde movement respectively. The assay, however, is designed to make the motors generate movement, concealing the fact that much of the time the motors are unable to move as a result of inhibiting themselves. Recognizing that they instantiated the design principle of autoinhibition involved a shift away from the perspective in which movement is *the* phenomenon for which the motors are responsible to a perspective in which researchers could recognize that these motors autoinhibit and only produce motility when released from autoinhibition. From this new perspective, these motors are controlled by processes in the cell. Researchers arrived at this different perspective by different trajectories in the cases of kinesin and dynein; accordingly, we discuss them separately in the next two sections.

4. Discovering autoinhibition in kinesin

In the previous section we described how Howard et al.'s single-molecule motility assay enabled researchers to establish that kinesins walk processively. We did not note that the researchers first attempt to show that a single kinesin was capable of moving a microtubule failed to generate motion. The researchers offered two explanations for this failure: 1) either single kinesin molecules cannot move microtubules or 2) kinesin "denatures"—breaks, essentially—when it binds to the glass. Assuming the latter, these researchers pre-treated their coverslips with other proteins (tubulin and cytochrome *c*) to prevent the hypothesized denaturation. With the pre-treatment, they observed what they inferred to be microtubules sliding across single kinesins.

Another study published the same year advanced a different understanding of the pretreatment. Hisanaga et al. (1989) showed that most kinesins in cells are unattached to microtubules and exist in a "folded" conformation with their cargo-binding "tails" in close proximity to their MT-binding hydrolytic heads. The researchers found that when suspended in a buffer with high salt concentration, kinesins unfolded, assuming an extended conformation. Hackney, Levitt, and Suhan (1992) confirmed these findings and used them to account for a prior biochemical finding that purified kinesin motor domains with their tails removed hydrolyzed ATP faster than full length kinesin. Initially this was puzzling since it was not clear why the presence of the tail region would reduce the activity of the hydrolytic heads. Hackney et al. offered an explanation: the folded conformation, available only to the full-length kinesin, represents an autoinhibited state: by bringing the tail and head regions together both the ATPase and MT-binding sites become inaccessible (Fig. 9). They concluded that the "folded conformation is enzymatically inhibited and may represent a soluble pool of the enzyme" (p. 8700). This explained why Howard et al. had to pretreat their coverslips. Rather than preventing denaturation, as Howard et al. had put it, the pre-treatment released kinesin from inhibiting itself, enabling MT-binding and uninhibited ATP hydrolysis. Hackney et al. hypothesized that a similar inhibition-releasing mechanism could operate *in vivo*.

The shift from the language of "denaturing" to that of "inhibiting"[6] marks an important shift in perspective. From the perspective embodied in the single-molecule motility assay, the target phenomenon is the movement of the motor. In order to study motor movement, techniques must generate movement reliably. From this perspective, a motor that is not generating movement is simply not producing its phenomenon. In short, it is "broken" or, "denatured." To think of a motor as inhibiting itself is to adopt a new perspective from which a motor that is not generating motion is, nonetheless, seen as functioning properly. The phenomenon it is generating is merely different from motility. Once a perspective on the motors is adopted which attributes to them a distinctive function—autoinhibition—inquiry can move in new directions.

First, once researchers adopted the perspective that, in addition to motility, kinesins engage in autoinhibition, they can make that a focus of inquiry. Coy et al. (1999), for instance, theorized about its physiological significance: if kinesins did not inhibit themselves, they would take futile, non-cargo carrying trips down microtubules, over-accumulate on microtubule tracks causing traffic jams, and wastefully hydrolyze ATP (back-of-the-envelope calculations suggested they would do so at a rate comparable to the total metabolic rate of humans).[7] Other investigations filled in details of kinesin's autoinhibited conformation. A productive line of research drew upon Verhey et al.'s (1998) determination that the heptad repeats shown in Fig. 6 are responsible for the binding of the kinesin heavy chains (KHCs) to the kinesin light chains (KLCs). The researchers further determined that the heptad repeats are necessary, but not sufficient, for inhibition of microtubule binding as the 64 KHC residues closest to the C-terminal are also required. They advanced a model in which the heptad repeats of KLC induce an interaction between the C-terminal tail and hydrolytic heads of KHC that prevents microtubule-binding. Once crystallographic analysis was possible, Kaan et al. (2011) could identify the

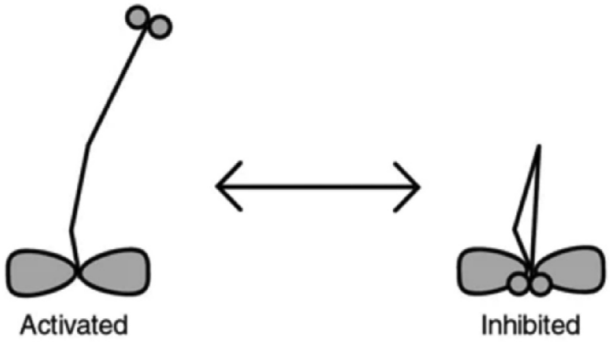

Fig. 9. Basic "tail-inhibition" model. Reproduced with permission from Springer Nature. Coy, Hancock, Wagenbach, and Howard (1999).

[6] Hackney et al. described part of kinesin inhibiting other parts, but did not use the term *autoinhibition*. Later publications that did use the term (Kaan, Hackney, & Kozielski, 2011; Verhey & Hammond, 2009) cite Hackney et al. as establishing that kinesins autoinhibited.

[7] More recently Kelliher et al. (2018) have drawn on findings that kinesins bind to receptors on the Golgi apparatus to advance a new hypothesis that a function of kinesin autoinhibition is to maintain Golgi outposts in dendrites.

components involved in autoinhibition and advanced a "double lockdown model" according to which the tail region of folded kinesin cross-links its ATP hydrolyzing heads resulting in a non-motile structure that inhibits ADP release.

Recognizing that kinesins inhibit themselves also pointed kinesin researchers to another new line of inquiry: determining what releases kinesin from autoinhibition. This led to the discovery of the first molecule that couples kinesin to cargo, Sunday Driver (SYD). Bowman et al. (2000) found it in the course of investigating the *Drosophila syd* mutant that exhibited the same defective transport phenotype as was produced by deletion of a subunit of kinesin itself. To explain its role, the researchers drew on contemporaneous research by Ito et al. (1999) and Kelkar, Gupta, Dickens, and Davis (2000) that revealed that SYD acts as a scaffolding protein in the MAPK/JNK signaling pathway that regulates cell functions such as autophagy. A scaffolding protein provides a structure along which proteins can be spatially organized so that they can easily interact with other proteins involved in the same process. Drawing on this framing, Bowman et al. proposed that SYD provides a scaffold that forces kinesin out of its autoinhibitory state and so enables it to bind cargo (in this case a vesicle) and begin to traverse a microtubule (Fig. 10).

The findings about SYD were soon generalized. SYD is one of three JNK interacting proteins (JIPs). Research on the other two (JIP1 and JIP2) provided compelling evidence that they facilitate binding to other cargo when bound to a further membrane-associated protein, ApoER2 (Verhey & Rapoport, 2001). Drawing on their own and other research (Byrd et al., 2001), Verhey and Rapoport advanced a schema on which kinesin figures in the JNK-pathway. First, the cargo binding protein binds to the motor which releases it from autoinhibition, binds the motor to cargo and provides a scaffold for intracellular signaling kinases in the pathway. Next, the complex is transported to the nerve terminal where the cargo fuses with the plasma membrane (step 1 in Fig. 11), binds its extra-cellular ligand (step 2), phosphorylates a signaling kinase (step 3), and releases the kinesin (step 4) which resumes its autoinhibited configuration and diffuses (or is itself transported) through the cell.

Situating kinesin in this larger activity of transporting signaling molecules provides a different perspective on kinesin. It is not just a motor that generates movement from ATP but an entity whose operation is controlled by other entities in its environment. This is facilitated by cargo binding proteins. They determine which phenomenon a kinesin is to exhibit—autoinhibition or active transport. This perspective motivated much additional research during the past decade that has resulted in identifying additional cargo-binding proteins and additional means by which kinesin is released from autoinhibition (Lin & Sheng, 2015) and its transport activities regulated (Sirajuddin, Rice, & Vale, 2014).

In the kinesin case, soon after it was identified as the motor driving anterograde transport, investigation of key features of the assay used to demonstrate kinesin motility compelled researchers to associate a second phenomenon—autoinhibition—to the mechanism. This led researchers not just to focus on what was required to release kinesin from autoinhibition so that it would produce motility but to situate kinesin in a larger context in which its activity is controlled by cargo binding proteins. These controlling cargo-binding proteins determined when kinesin was released from its autoinhibited state and transported cargo. In this case, the recognition that kinesins instantiated the design principle of autoinhibition initiated research into the entities that released it from autoinhibition and thereby regulated its activity. This is not the only trajectory research can take, however. Research on dynein reveals a different trajectory.

5. Discovering how cytoplasmic dynein is controlled

While developing procedures to reconstitute dynein motility *in vitro*, researchers came to recognize that other molecules had to be added to their preparation in order for dynein to generate movement. Researchers immediately conceptualized these additional molecules as controlling or regulating dynein's behavior. It took twenty-five years, however, for researchers to recognize that the molecules were, specifically, releasing the motor from autoinhibition. We examine how this research proceeded and reflect on why these additional components were considered control elements rather than simply parts of the mechanism for transport. We conclude this section by considering how this research culminated in the understanding that dynein produces a second phenomenon, autoinhibition, when these regulatory components are not present.

After developing an assay in which they could demonstrate retrograde movement along microtubules, Schroer, Steuer, and Sheetz (1989) tried to reconstitute dynein-driven motility using purified dynein. They found that purifying dynein and adding it back to a preparation of microtubules did generate movement, but much slower movement than in their initial preparation. The researchers concluded that some factor or factors other than dynein was required to generate normal movement. Gill et al. (1991) showed that normal dynein movement could be restored by adding a large protein complex that they isolated from the original preparation and named *dynactin* (*dynein activator*). Moreover, Gill et al. demonstrated that when they removed it completely from a dynein preparation (the initial purification of dynein was only partial), motility was totally suppressed. Dynactin, they concluded, was required for dynein to generate retrograde transport.

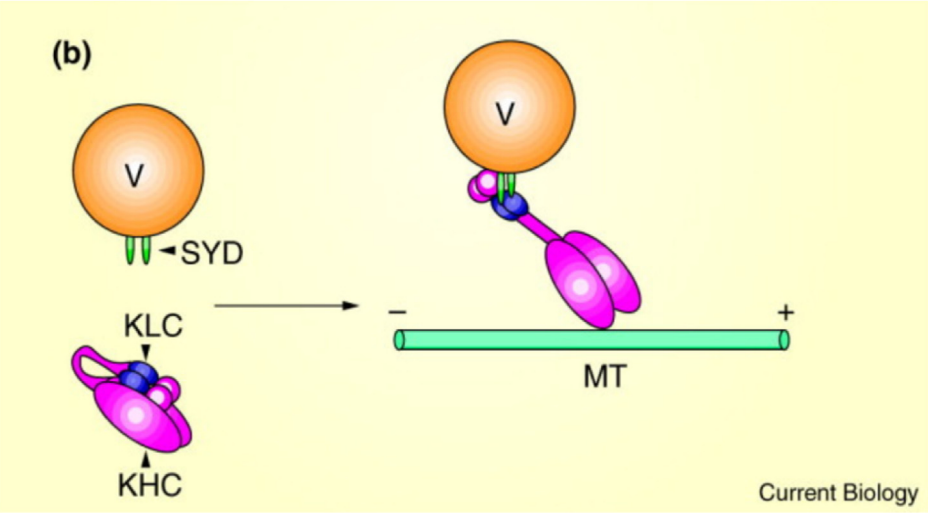

Fig. 10. SYD linking kinesin to vesicular cargo. Reproduced with permission from Elsevier. Hays and Li (2001).

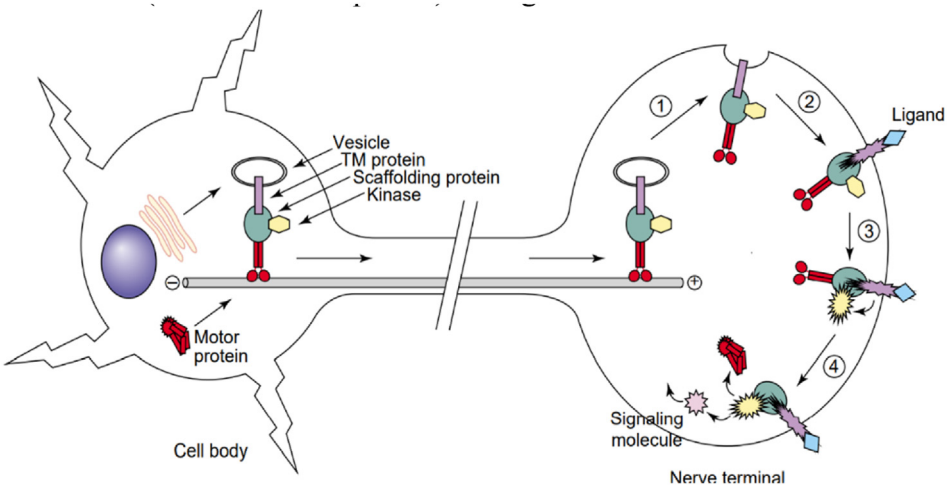

Fig. 11. Cargo releases kinesin from autoinhibition in the cell body. The kinesin transports the signaling components to the nerve terminal, where the transmembrane protein is inserted into the cell membrane and eventually releases kinesin, which returns to its autoinhibited state. Reproduced with permission from Elsevier. Verhey and Rapoport (2001).

The discovery of dynactin initiated an inquiry into how it interacts with dynein. One hypothesis stemmed from Gill et al.'s determination that the gene *dynactin* exhibited 50% sequence identity to the Drosophila *Glued* gene. Subsequent electron microcopy studies showed that the shared sequence corresponded to a $p150^{Glued}$ dimer that forms an arm (shown in Fig. 12) that binds to both dynein's intermediate chain and the microtubule (Waterman-Storer, Karki, & Holzbaur, 1995). The significance of dynactin binding to the microtubule proved controversial. Since without dynactin, dynein could not maintain motility over long distances, King and Schroer (2000) proposed that the arm provided an additional contact that could keep dynein on the microtubule. However, Kardon, Reck-Peterson, and Vale (2009) demonstrated that if, in yeast, they rendered dynactin's arm unable to bind the microtubule, processivity still increased over preparations without dynactin. More recently Ayloo et al. (2014) have argued for important differences between yeast and mammalian dynein. They advance evidence that, in mammals, dynactin often binds to the microtubule before dynein, recruits dynein to it, and keeps dynein tethered to the microtubule (sometimes braking dynein's movement). They argue that these activities are essential for dynein to transport small cargoes, which employ only a few dyneins, and in regions of the cell in which there are few microtubules.

In spite of finding dynactin to be necessary to produce movement *in vitro*, the investigators did not simply treat it as an additional part of the mechanism for retrograde transport. Rather, they construed dynactin as *regulating* or *controlling* dynein which they continued to view as having a distinctive status, namely, the motor that drives the motion by transforming ATP into mechanical motion. In other words, they viewed the motor as the mechanism for transport and dynactin as regulating this mechanism. However, unlike in the kinesin case in which researchers already understood the motor as capable of inhibiting itself and, thus, understood the binding partners as releasing kinesin from its autoinhibited state, dynein researchers did not yet understand dynein to be capable of autoinhibition. Thus, prior to the discovery of dynein autoinhibition, dynactin was viewed as regulating dynein but not specifically by releasing it from autoinhibition.

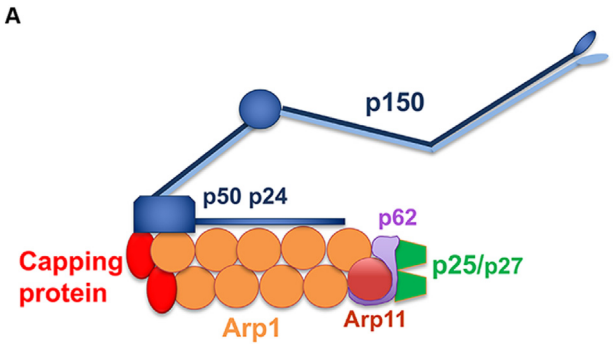

Fig. 12. A schematic representation of the structure of dynactin. Reproduced under Creative Commons License CC BY from Zhang, Qiu, and Xiang (2018).

Dynactin was just the first additional component that researchers discovered was required for dynein to produce retrograde motion. Researchers soon discovered that dynactin on its own does not tend to bind to dynein and when it does, the resulting dimer is unstable. Swan, Nguyen, and Suter (1999) found that, in *Drosophila*, Bicaudal D[8] (BicD; in mammals BicD has two homologues, BicD1 and BicD2) promoted their binding. Hoogenraad et al. (2001) showed that BicD proteins form a complex between dynein and dynactin and Rab6, a small GTPase situated on membranes of vesicles synthesized in the Golgi apparatus. McKenney, Huynh, Tanenbaum, Bhabha, and Vale (2014) revealed that BicD2 provides a rigid structure to which both dynein and dynactin bind (Fig. 13). Researchers responded to these findings as they had to dynactin—they did not treat BicD as a component of the mechanism for retrograde transport but as acting to regulate its activity.

The fact that Rab6, a protein on the Golgi apparatus, is part of the complex that forms with dynactin, dynein, and BicD, pointed to a more specific role for BicD—recruiting dynein to an organelle requiring transport. Since vesicles produced in the Golgi apparatus are just one type of cargo transported by dynein, researchers searched for other agents that enable other cargos to bind to dynein. To date, they have identified several and the cargos to which they bind: Rab11-FIP3 binds recycling endosomes, Hook3 binds secretory vesicles, and Spindly binds kinetochore (Canty & Yildiz, 2020).

From this, researchers concluded that dynactin and BicD (or another cargo-binding protein) are required to generate retrograde transport and act by controlling dynein's operation. This raised the question of what dynein does when it is not generating retrograde transport. An early micrograph by Amos (1989) had shown dynein in a conformation in which its "two heads fused together, forming a dimeric globular particle with two separate tails" (a conformation Amos named *phi* for its shape). This finding, however, was largely neglected until Torisawa et al. (2014)

[8] The protein was so named as it was first identified in a *Drosophila* mutant in which the anterior segments of the embryo become a set of second posterior segments.

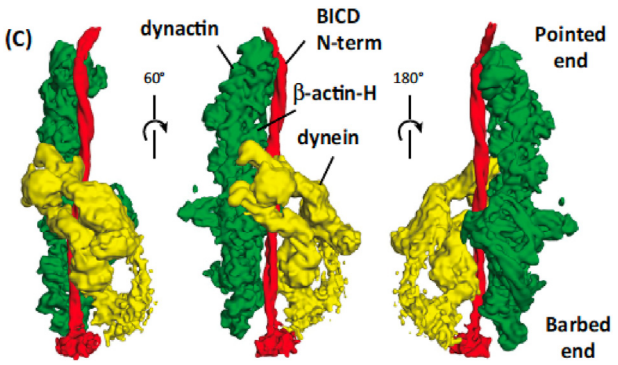

Fig. 13. Role of BicD (red) in generating a bond between dynein (gold) and dynactin (green) based on cryo-EM. Reproduced with permission from Elsevier. Hoogenraad and Akhmanova (2016). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

drew attention to it and identified the phi-conformation as an auto-inhibited state in which dynein's two heads are stacked with their C-terminal sides facing each other and their stalks crossed. In this configuration the microtubule binding domains are facing in opposite directions, enabling only one of them to bind a microtubule. This makes processive movement impossible (Fig. 14). When ATP is available, dynein in the phi conformation can bind and release from the microtubule, but this merely leads to dynein diffusing along the microtubule with a slight bias towards the minus end. Torisawa et al. also found that if they forced the two heads apart by inserting a rigid rod (emulating the effect of BicD), dynein movement became directed and processive. Given the role of cargo-binding proteins in recruiting BicD to dynein, they proposed this control process ensured that dynein only assumed a structure in which it could act as a motor when cargo was in need of transport.

Recognition that dynein instantiates the design principle of auto-inhibition has led to an explosion of proposals as to how dynactin and BicD figure in autoinhibition release. The use of a rod by Torisawa et al., for example, suggested that this is the role played by the Arp1 component

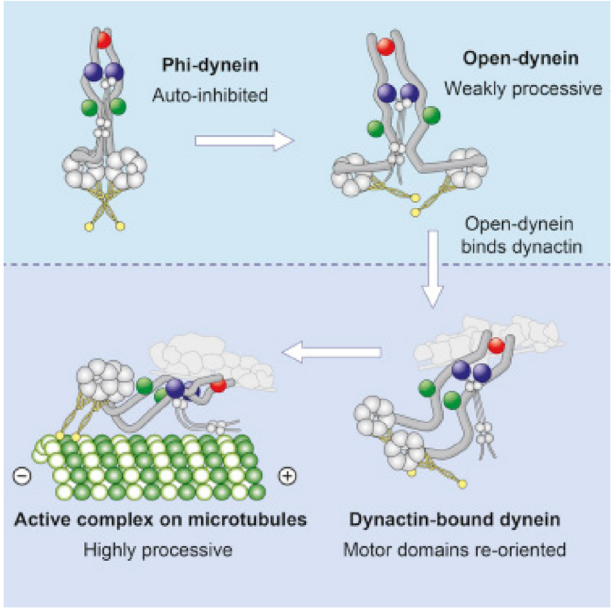

Fig. 14. Transformation of dynein from phi-particle conformation to binding with dynactin and becoming an active motor. Reproduced under Creative Commons License (CC BY) from Zhang et al. (2017).

of dynactin (this proposal received further support from an EM study by Zhang et al., 2017). As we noted, dynein and dynactin on their own do not bind and BicD has been viewed as playing an activating role by providing a rigid structure along which both dynactin and dynein can bind. McKenney (2018) proposes that binding to dynactin and BicD breaks the symmetry of the autoinhibited dynein. Researchers have developed similar accounts of the mechanical action of other cargo adaptors (Olenick & Holzbaur, 2019; Reck-Peterson, Redwine, Vale, & Carter, 2018).

The research on dynein followed a different trajectory as that on kinesin, but both resulted in a major change in perspective from one in which the motors were just understood as engaging in motility to one in which they were normally autoinhibited and only produced motility when cargo needed to be transported. In the case of dynein, the shift started with the recognition that additional elements were needed for dynein to generate motility and the treatment of these as control elements, necessary for dynein to generate processive movement. It was not until 2014 that researchers came to see dynein as capable of adopting a functionally distinctive state and autoinhibiting when these control elements were not active. Once its capacity to autoinhibit was recognized, it was further recognized that the specific roles of dynactin and BicD were to release the motor from its autoinhibited state. Thus, in the case of dynein as well as kinesin, researchers came to adopt a perspective on the motor from which the motors were seen as performing two distinctive functions—movement and autoinhibition—under different conditions of control.

6. Implications of discovery of autoinhibition for philosophical accounts of mechanisms

Our account of the discovery that kinesin and dynein instantiate the design principle of autoinhibition—molecular mechanisms inhibiting themselves and only producing the phenomenon of motility when released by a control process—has significant implications for standard accounts of mechanistic explanation (Bechtel & Abrahamsen, 2005; Machamer et al., 2000). We begin with implications for the characterization of the discovery process as beginning with characterizing a phenomenon and then discovering the mechanism responsible for it. The discovery of kinesin and dynein autoinhibition followed the reverse path—starting with the mechanisms and determining that, in addition to motility, they exhibit autoinhibition. This also brings into question the common assumption of a one-to-one mapping of a phenomenon unto a mechanism. We then turn to implications of the fact that mechanisms are subject to control for standard accounts of the constituency of mechanisms. We argue that an alternative conception of mechanism as consisting of constraints that direct the flow of free energy provides a better understanding of how mechanisms are subject to control.

Standard accounts of mechanism discovery embrace a *phenomenon-first* approach to inquiry, as described by Illari and Williamson (2012, p. 123):

> All mechanistic explanations begin with (a) the identification of a phenomenon or some phenomena to be explained, (b) proceed by decomposition into the entities and activities relevant to the phenomenon, and (c) give the organization of the entities and activities by which they produce the phenomenon.

On this view, the characterization of the phenomenon is the reference point for identifying the mechanism. The initial research on both kinesin and dynein adhered to this strategy, seeking mechanisms *for* active transport and, subsequently, *for* the stepping patterns of the motor mechanisms. But the research leading to the discovery of autoinhibition departed from this approach, instead starting with the mechanisms and developing from investigations of the mechanisms a characterization of a second phenomenon for which it was responsible: autoinhibition. In this process, the mechanisms served as the reference points for discovering the phenomena.

There is precedent in the mechanist literature for identifying phenomena based on an account of a mechanism. In her discussion of "phenomenon reconstitution," Kronfeldner (2015) describes how researchers can pick out a particular "causal factor," experiment and collect data on it, and then treat it as explanatory with respect to a different phenomenon than that which researchers were initially investigating. Bechtel and Richardson (1993/2010) tell a similar story in their discussion of the "Mendelian trait" which was initially understood as a macroscopic phenotypic trait (e.g., eye color). Finding that patterns of phenotypic inheritance cannot be explained in terms of single genes, scientists re-identified the phenotypic trait with something that could be explained in terms of single genes—enzyme activity. Thus, the phenomenon to be explained in terms of single genes was "reconstituted" from the phenotypic trait to enzyme activity.

Thus, while the simple narrative of mechanistic inquiry takes it to start with the identification of a particular phenomenon and to proceed by seeking the underlying mechanism, it is recognized that, in the iterative process of mechanistic investigation, mechanisms themselves can take the lead with researchers holding them fixed to scaffold inquiry while the phenomena to be explained undergo renovation. Accordingly, after presenting the phenomenon-first account cited above, Illari and Williamson go on to characterize a more nuanced process:

> Mechanisms are individuated by their phenomena, and phenomena are also individuated by their mechanisms. This is not circular, because it happens iteratively over time. At the beginning, a mechanism is not needed to individuate a phenomenon, but the characterisation of the phenomenon may be further refined when a mechanism or mechanisms are discovered" (124).

Even on this more nuanced view, however, the process ends with a single phenomenon explained in terms of a single mechanism (or "causal factor"). In the research we described, however, the conclusion was not a single reconstituted phenomenon but the recognition that, by design, the same mechanism was responsible for two different phenomena. This is not a trivial modification of standard mechanistic accounts according to which mechanisms are individuated by the phenomena they explain. Following Glennan's (1996) assertion, "One cannot even identify a mechanism without saying what it is that the mechanism does," the principle that the identity of a mechanism is tied to the phenomenon it explains has been called Glennan's Law.[9] The identification of autoinhibition as a second phenomenon associated with molecular motors would be a violation of this principle. In light of the fact that research often does proceed from characterization of a phenomenon to the identification of a mechanism, we suggest that the one phenomenon-one mechanism principle might better be treated as a heuristic that can productively guide research but can also be expected to fail, especially as research proceeds.

Recognizing that the same mechanism can produce two incompatible phenomena, such as motility and autoinhibition, raises a further question: what determines which phenomenon it produces on a given occasion? In the cases of kinesin and dynein, it was cargo binding proteins that, by binding to the motors, induce a change in conformation that releases them from autoinhibition and enables them to adopt a conformation in which they can bind microtubules, bind ATP, and walk along the microtubule. This presents another challenge. Even before dynein researchers identified it as inhibiting itself, researchers had identified the need for dynactin and an agent like BicD in order for it to generate motility. We noted that researchers did not treat these agents as parts of the mechanism but as ones that controlled the mechanism. But on a common view about the identity of mechanisms, these agents would be identified as components of the mechanism. Craver (2007; see also Craver & Kaplan, 2020), for example, advances a constitutive relevance account for identifying the components of a mechanism. He employs the criterion of mutual manipulability—any factor whose manipulation can alter the phenomenon in terms of which the mechanism is identified and that is altered when the phenomenon is altered counts as part of the mechanism. On such a criterion, dynactin, BicD, etc., all count as constituents of the mechanism responsible for retrograde motility. Again, though, this is not how researchers understood these additional required elements.

If one adopts the mutual manipulability criterion of constitutive relevance, one can maintain Glennan's law and avoid attributing more than one phenomenon to a mechanism—from such a perspective, different mechanisms are responsible for motility and autoinhibition. The history that we analyzed in sections 4 and 5 would be the history of discovering a new phenomenon for which a separate mechanism was responsible. We resist this proposal. First, this is not how the scientists characterized their accomplishment. They understood themselves to have determined that the mechanism responsible for motility inhibited itself when appropriate control processes did not operate on it. Second, there is an important distinction to be made between mechanisms responsible for specific phenomena and control processes (mechanisms) that operate on them. Control is important not just for mechanisms that autoinhibit. Under such rubrics as *cell signaling*, biologists are increasingly focusing on how mechanisms within living organisms are controlled.

If one rejects mutual manipulability as the criterion for identifying constituents of mechanisms, one needs an alternative criterion. Such an alternative is found in the proposal by Winning and Bechtel (2018) to characterize mechanisms not as collections of entities and activities responsible for a phenomenon, but as entities that constrain flows of free energy so as to perform the work needed to produce the phenomenon to be explained. Free energy and work have not featured in new mechanist accounts. To account for the active nature of mechanisms, Machamer et al. (2000) treat activities as constituting a primitive category that does not require explanation. An alternative is to follow physics and treat free energy as required for activity. Without free energy, mechanisms are inert. The biologists investigating motility recognized this—they assumed the source of free energy for motility was provided by ATP and accordingly looked for an ATPase that interacted with microtubules. Kinesins and dyneins are both ATPases—by hydrolyzing ATP they release free energy which is then constrained to produce the movements within these proteins (Bechtel & Bollhagen, 2021).

Adopting the conception of mechanisms as sets of constraints that direct the flow of free energy, one can differentiate mechanisms from other processes that control them. The mechanism consists of the constraints that determine the work that is done from a given source of free energy. Controlling the mechanism also requires the performance of work—the mechanism is controlled by altering constraints within it. In the cases of kinesin and dynein, the cargo binding proteins perform the work of releasing the motors from autoinhibition. To perform this work, the control processes draw on their own sources of free energy and must constrain it appropriately. We cannot develop a full characterization of control processes here (for further development, see Bich & Bechtel, 2022a, 2022b); what is important for our purposes is that by attending to how mechanisms constrain free energy in the performance of work, one can distinguish mechanisms from other processes that exercise control over them. This revised account of mechanisms enables us to make sense of the researchers' distinction between kinesins and dyneins and the processes that exercised control over them. Specifically, it enables us to understand why dynein researchers did not count dynactin, BicD, etc. as merely further parts of the mechanism for motility but, rather, as parts of mechanisms controlling dynein. Moreover, one can also understand how the same mechanism can be responsible for different phenomena—different phenomena are produced when the constraints within the mechanism direct free energy differently. In the case of kinesin and dynein, they autoinhibit rather than producing motility when constraints

[9] Not all mechanists have ascribed to it. In their definition of a mechanism, Bechtel and Abrahamsen (2005, p. 423) allow that a mechanism may be "responsible for one or more phenomena."

within them prevent hydrolysis of ATP. When control processes operate on them, these constraints are altered and the motors hydrolyze ATP and generate motility.

7. Conclusion

Biologists are finding that many molecular mechanisms inhibit themselves. We have argued that autoinhibition constitutes a design principle—an abstractly characterized pattern of organization that explains phenomena across a wide range of cases—and that accounts of mechanisms that maintain that mechanisms are to be individuated by the (single) phenomenon they explain struggle to accommodate mechanisms that instantiate this design. We suggest that the account of biological mechanisms developed in Winning and Bechtel (2018) provides a positive alternative.

Mechanisms exhibiting the design principle of autoinhibition do so through *intra*molecular interactions which prevent them from generating the phenomenon characteristic of their active states. *Inter*molecular interactions between the mechanism and binding partners release the mechanisms from autoinhibition, enabling them to produce that phenomenon. Focusing on two molecular mechanisms, kinesin and dynein, we analyzed how researchers discovered that they autoinhibit. We showed that different paths were followed in the two cases. In the case of kinesin, researchers quickly recognized that the experimental protocol they deployed to investigate kinesin motility acted to release kinesin from a conformation in which it inhibited itself. Research then turned to what processes act on kinesin in living cells to control it by releasing it from autoinhibition. In the case of dynein, researchers early on recognized a that a variety of other entities were needed for dynein to produce motion *in vitro* but, contrary to what the mutual manipulability criterion would imply, researchers did not consider them part of the mechanism for retrograde axonal transport. Rather, they construed them as controlling the mechanism. Only much later did researchers recognize that such control was needed to release dynein from, specifically, autoinhibition. In both cases, the shift in perspective involved in the discovery—the shift from understanding the proteins as "denatured" or otherwise simply not producing motion in *in vitro* assays to understanding them as implementing an autoinhibitory design—prompted researchers to discover broader processes in the cell which functioned to release the motors from their autoinhibited state. In the end, researchers arrived at a framework in which molecular motors exhibit the design principle of autoinhibition and only produce motility when acted on by control processes.

Autoinhibition is widely implemented in biology. In fact, as Pufall and Graves point out, there are likely numerous undiscovered instantiations of this design principle.[10] Thus, a philosophical account of biological mechanisms needs to be able to accommodate this important organizational pattern. That autoinhibitory mechanisms exhibit two phenomena—e.g., autoinhibition and motility—with control processes determining which they exhibit on a given occasion does not fit well with the standard philosophical accounts of mechanisms. Accordingly, we provide a revised philosophical account of mechanisms that distinguishes control processes from the operations of the controlled mechanism and recognizes that one mechanism can exhibit multiple phenomena. This revised account of mechanisms is well-suited to understanding both the various biological mechanisms that implement autoinhibition as a design principle and the process involved in their discovery.

Credit statement

The authors declare that they contributed equally to all aspects of the research involved in the development of this project.

[10] See fn. 3.

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