Self-organized networks: Darwinian evolution of dynein rings, stalks, and stalk heads

J. C. Phillips

Cytoskeletons are self-organized networks based on polymerized proteins: actin, tubulin, and driven by motor proteins, such as myosin, kinesin, and dynein. Their positive Darwinian evolution enables them to approach optimized functionality (self-organized criticality). Dynein has three distinct titled subunits, but how these units connect to function as a molecular motor is mysterious. Dynein binds to tubulin through two coiled coil stalks and a stalk head. The energy used to alter the head binding and propel cargo along tubulin is supplied by ATP at a ring 1,500 amino acids away. Here, we show how many details of this extremely distant interaction are explained by water waves quantified by thermodynamic scaling. Water waves have shaped proteins throughout positive Darwinian evolution, and many aspects of long-range water–protein interactions are universal (described by self-organized criticality). Dynein water waves resembling tsunami produce nearly optimal energy transport over 1,500 amino acids along dynein’s one-dimensional peptide backbone. More specifically, this paper identifies many similarities in the function and evolution of dynein compared to other cytoskeleton proteins such as actin, myosin, and tubulin.

The active parts of dynein motors consist of three parts, the AA1–AA6 rings, the antiparallel coiled coil CC1 and CC2 stalks extended from AA4 and connected to the stalk head, and the CC buttress extended from A5. Each ring contains about 300 aa, while CC1, CC2, and the stalk head contain about 100 aa each (4). Here, we use thermodynamic scaling to analyze the rings and the stalk and identify their evolutionary refinements. Thermodynamic scaling utilizes recently discovered critical conformational features of hydropathic interactions, which are associated with long-range strain–field interactions and, thus, is well suited to discussing subtle evolutionary changes in shape connected with such long-range interactions.

Thermodynamic scaling utilizes only one-dimensional amino acid sequences, so it appears to lack the large quantity of information contained in three-dimensional static structures. Traditional methods for analyzing sequence evolution (phylogenetics) utilize single-site comparisons (BLAST and its derivatives). Single-site methods often lead to the conclusion that evolution is merely neutral and not progressive or positive (6). One of the goals of thermodynamic scaling is to show the improvements evolution makes for many proteins, as discussed in earlier articles on cytoskeleton proteins (actin and tubulin; refs. 7 and 8). Technically such improvement is expected for self-organized networks approaching a critical point (9, 10).

Thermodynamic transitions involving short-range interactions, such as ATP hydrolysis, can be described as thermodynamically first-order, as can large changes, such as unfolding or cleaving. Long-range interactions are second-order and small, and are simplified near a thermodynamic critical point, where a recently discovered hydropathicity scale (MZ) is extremely accurate in describing long-range, water-mediated interactions (11). Altogether 127 hydropathicity scales were proposed by 2000, which utilized small databases and were never compared to each other, leaving the impression that they were all qualitative. The most popular of the early scales (KD) has turned out to be the second most accurate overall (12, 13) and is effective in describing strong interactions, such as globin metabolism. The long-range (cooperative) metabolic interactions of hemoglobin are quantified by combining MZ and KD evolutionary profiles (3).

Significance

Proteins are the prime example of self-organized networks, as they have benefited from extensive natural (Darwinian) selection. Here, we quantify the dynamical shapes of dynein as they have evolved through interactions with water films. The interactions are long-range and are easily identified, and their improvement by evolution varies with the functions of parts of this molecular motor. It appears that evolution has brought human dynein close to a dynamical critical point, indicative of intelligent design.

Author contributions: J.C.P. performed research, analyzed data, and wrote the paper.

Received December 3, 2019; revised April 1, 2020; accepted April 1, 2020.

www.pnas.org/cgi/doi/10.1073/pnas.1920840117

PNAS | April 7, 2020 | vol. 117 | no. 14 | 7799–7802
Results
Dynein sequences are very well conserved (human and chicken, 98% identity). Given a huge choice of sequences from the genomic era database, we have chosen to study evolution of dynein heavy chain (stem excluded) from worm (Q14204) to human or mouse (P34036), rather than from slime mold (A0A1S0UA63) to human. For worm BLAST reports stronger identities (68%) and positives (81%) than for slime (46%) and (65%), and only a few gaps (1% for worm, 4% for slime).

Our first step is to compare the hydropathic “roughness” or variance ratios of the hydropathic profiles $\Psi(aa, W)$ as functions of the sliding window width $W$ (3, 7–10), calculated separately for the AAA1–AAA3 rings and the predominantly coiled coil stalk. The results using the short-range KD scale are shown in Fig. 1, and those for the MZ scale in Fig. 2. As expected, the curves with the two scales are roughly similar, and the stalk is qualitatively different from the rings. However, these important qualitative differences are absent for $W = 1$, which is why phylogenetics was unable to prove positive evolution from sequences alone (6, 13).

The criteria that guide us in choosing a value of $W = W^*$ to display a profile $\Psi(aa, W^*)$ are that ideally it should be the smallest value (hence strongest resolution) which shows a peak in evolutionary variance ratios. In Fig. 2, we see such a peak for the stalk at $W^* = 13$; it has a special significance, discussed below. With the KD scale, a broader peak occurs for $W^* = 21$, which also appears to be significant. The ring variance ratios do not show such a peak, but they do level off near $W^* = 29$.

Dynein has two major ATPase sites in the AAA1 and AAA3 rings. ATP binding to AAA1 triggers a cascade of conformational changes that propagate to all six AAA domains, while nucleotide transitions in AAA3 gate the transmission of conformational changes between AAA1 and the stalk (14, 15). In Fig. 3, the worm and human profiles $\Psi(aa,29)$ show a large qualitative difference in the hydrophilic minima separating AAA2 from AAA3. It seems likely that these hydrophilic extrema define the edges of these rings. In worm the three rings have similar sizes, and this is generally assumed for animals (for instance, on Uniprot “by sequence similarity”), but there are no structural data for animal species. Dynein fish sequences are incomplete, but frog dynein (F6KXW0) is similar to worm near the AAA2-AAA3 interface.

The deep human minimum in Fig. 3 arises from the strongly hydrophilic sequence 2396 RRRKGKEDEGEE 2407. This 12-aa sequence is conserved for almost all animals and birds. A possible explanation for the appearance of this extremely hydrophilic sequence is that it enhances the gating of AAA1 by AAA3. Note that most of the early (pre-2000) hydropathicity tables also list R, K, D, and E as the most hydrophilic amino acids (11). This is the case for the KD scale shown in Fig. 4; it also exhibits a strong hydrophilic minimum for the human, but not the worm, interface. The secondary minimum (near 650 in Fig. 3), which gives a clear-cut AAA2-AAA3 interface for worm with the 2007 MZ scale (Fig. 3), is absent for the 1982 KD scale (Fig. 4), a feature that favors the modern MZ scale.

Both full-length human (16) and slime (17) cytoplasmic dynein-1 have been studied by cryo-electron microscopy (cryo-EM), with emphasis on the shaft and its complex motor activity. While planar rings are observable, individual AAA units are not, so that differences between AAA2 and AAA3 in human and worm dynein have not been resolved (17, 18). Given steady improvements in cryo-EM techniques, it may be possible to test this evolutionary prediction in the future.

According to Figs. 1 and 2, the best description for the evolution of the stalk is obtained with the MZ scale and $W = 13$. The resulting profile is shown in Fig. 5. The CCI and CC2 coiled coils are labeled from the hydrophobic heptad repeats discussed below. Between them is the stalk head that binds to tubulin. Hydropathic profiling shows that the three regions are qualitatively different across all three species. CCI is overall hydrophilic, while CC2 has stabilizing and two deep hydrophilic hinges near 250 and 315, hydrophobic peaks at both ends, separated by a

![Fig. 1](image1.png)  
**Fig. 1.** The human/worm variance ratios using the KD scale show a stronger peak near $W = 21$ for the stalk than for the AAA1–AAA3 rings.

![Fig. 2](image2.png)  
**Fig. 2.** We are again looking at the human/worm variance ratios With the MZ scale, there is an unambiguous peak at $W = 13$ for the stalk, while the rings merely show a flattening near $W = 29$. The large differences from Fig. 1 (KD scale) reflect changes in short-range vs. long-range forces.

![Fig. 3](image3.png)  
**Fig. 3.** Profiles of the human and worm rings. Here, human site 1 corresponds to Uniprot Q14204 site 1868 Tyr. Worm is aligned to human by BLAST. A 9-aa gap in worm is barely visible near 110. The largest change is the deep human hydrophilic minimum near 530, which improves decoupling of AAA2 from AAA3 (see text). It is absent from worm, which suggests that worm AAA2 and AAA3 may be decoupled near 635.
stabilizing hydrophobic peak. The structure of the stalk head is discussed further below.

What is the meaning of these hydrophobic coiled coil oscillations? Here, the coiled coils are functioning as mechanical springs, tilting the stalk head to drive cytoskeleton cargo. Compressed helical pitches exclude water (hydrophobic extrema), while expanded helical pitches increase solvent accessible area, and increase water density (hydrophilic extrema). Hydropathic waves can be excited with lower energy, and linear water film surface waves can travel along the dynein surface great distances, much as linear seismic water waves travel across oceans (19–21). These linear shallow surface waves explain the remarkable distance linear tsunami wave approaching shore (21). Note that the role of gravity in shallow water waves is played in protein hydropathic waves by the van der Waals dispersion attraction between water and amino acids (22).

The CC1 and CC2 coiled coils are stabilized by hydrophobic heptad repeats based on similar amino acids with one or two (1 or 2) CH$_3$ side groups and no benzenoid rings (23). These amino acids are (KD hydropathicities): hydroneutral Gly is 157 (1), Ile (254) (2), Leu (240) (2), Val (248) (2), Ala (214) (1), and Met (202) (1). The heptad repeats span 109 aa for both CC1 and CC2. The heptad sequence analysis (23) establishes KD as the scale better suited to describing short-range CC1-CC2 contact binding, because small Ala appears as hydroneutral on the MZ scale, which instead emphasizes long-range curvatures.

The KD heptad repeats appear to be similar for CC1 and CC2 (23), although the large-scale MZ profiles with W = 13 are qualitatively different (Fig. 5). Looking more closely, we see that the hydrophilic minimum in CC1 near 70 corresponds to the hydrophobic maximum in antiparallel CC2 near 280. Binding CC1 to CC2 tends to compensate the variations in water density and, thus, supports hydrophobic heptad-mediated binding by reducing variations in helical pitches.

The most dramatic effects of dynein evolution occur in the stalk head, whose profile is shown in Fig. 6, enlarged from the central region of Fig. 5. Binding to tubulin occurs through the three hydrophobic peaks A–C. In slime mold, peak A is the strongest and should provide most of the binding. In animals, all three peaks can contribute to increasing the binding strength. This is in good agreement with the experiment, which finds that animal stalk heads bind to tubulin in vitro, while slime and other primitive stalk heads do not (17, 23).

More generally, evolution has leveled sets of (more often) hydrophobic and (less often) hydrophilic extrema in many proteins (24). Such leveling optimizes protein hydrodynamics, in accordance with Sethian’s level set hydrodynamic theory (25–27). Looking more closely at Fig. 6, we see that cargos in tubulin could be moved by rocking the shaft head between the two stronger peaks A and B, with peak C serving as a pivot. This corresponds to the prestroke and poststroke conformations observed with optical tweezers (28, 29). Note that some of the motor features are merely mechanical. For example, the CC hinges near the shaft head are probably mechanical and not associated with the water film (17). The CC in AAA5 may act primarily as a buttress supporting the shaft, as CC2 has already hydrophobically reflected the ATP AA1 water wave signal back to the shaft head. A model showing small tilts of flexible stalks observed by polarized total internal reflection fluorescence microscopy is also consistent with this rocking model (30).

Discussion

The complexity of the architecture of dynein and the difficulties in carrying out atomically detailed simulations, both due to the long time scales and inaccuracies in the force fields (especially water), have led to the creation of coarse grained (CG) models. Such models with multiple parameters based on crystal structures...
from several species have been used to analyze AAA and linker interactions separately (31). The methods used here on dynein have previously been tested on other cytoskeleton proteins (7–9), as well as many other proteins (3, 10, 24), and have consistently yielded new insights into positive Darwinian evolution of protein functions from sequences alone. Because the genomic sequence database is so much larger than all other protein databases, in retrospect these multiple successes appear inevitable, once started. The starting problem here is associated with the “first-cut” nature of BLAST, which itself effortlessly yields many positive results. This has led many researchers to limit themselves to point (W = 1 or perhaps 2) comparisons of protein sequences, structure, and function (6) without exploring the possibilities of optimizing wavelengths of hydrophatic profiles by selecting larger values of W from evolutionary trends.

Here, we have found that the positive effects of evolution are especially dramatic in dynein, where the linear waves in monolayer water films propagate along the protein chain 1,500 aa from their source in AAA1 to the dynein stalk hubulin binding sites. Within a W = 1 perspective, such “action at a distance” looks impossible in the presence of thermal fluctuations, while it is natural enough in terms of shallow water waves with W ∼ 10–30 bound to an aequously and critically self-organized amino acid backbone. Note that close to a critical point large-density fluctuations may involve only small energy differences incorporated over long wavelengths.

Can the present results be extended using molecular dynamics simulations? Perhaps—the details of ATP hydrolysis have already been discussed for actin (32, 7). At least the rocking balance in the presence of thermal fluctuations between the A, B, and C hydrophobic peaks in the dynein shaft head might be accessible. Comparisons of slime and human shaft head dynamics may be possible and would test our picture of self-organized criticality.

An historical note: The underlying mechanism of molecular motors has been discussed for decades, for instance by Hudhey (1957) and Feynman (1963), usually in terms of harnessing thermal fluctuations, a task apparently involving Maxwell demons for “thermal rectification” (33–35). We have shown here that Darwinian selection has shaped motor proteins so that even linear water waves can transmit chemical energy over very long distances. Our statistical mechanical approach was anticipated by Schrodinger in 1943 (36, 37). Self-organized criticality was used in a schematic model to derive modular (or “punctuated”) evolution in 1995 (38), and fractals (39) emerged explicitly in 2007 (11). The concept of self-organized criticality, with potential applications to living matter, has been widely discussed for decades (40).

Data Availability. All data associated with the manuscript are available at Uniprot under accession no. Q14204.