“Geometric Clutch” Hypothesis of Axonemal Function: Key Issues and Testable Predictions

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INTRODUCTION

Cilia and flagella are both powered by the molecular motor dynein. Dynein cross-bridges induce tubulin sliding, creating a displacement between the outer doublets of the axoneme and generating a bend along the axoneme, giving rise to the ciliary/flagellar beat. A plausible explanation for dynein cross-bridge formation and termination in the working axoneme has recently been suggested [Lindemann, 1994a,b]. This proposal, dubbed the “Geometric Clutch” hypothesis, is based on the concept that force (or strain) develops transverse to the axoneme (in the plane of bending), and acts as the main regulator of dynein action. This transverse force, or “$t$-force,” is correctly oriented to alter interdoublet spacing, thereby modifying the probability of a dynein head forming an attachment between neighboring outer doublets. The $t$-force is a product of the cumulative longitudinal tension (or compression) on each doublet times the local curvature of the axoneme and can be expressed as:

$$t\text{-force} = \frac{d\theta}{ds} \times F$$

where $d\theta/ds$ is the curvature, expressed as the change in angle per unit of length, and $F$ is the total accumulated tension (or compression) on the doublet. A computer simulation utilizing the $t$-force concept to regulate engagement of force-producing bridges [Lindemann, 1994a,b] has duplicated many observed behaviors of living cilia and flagella. This simulation generated stable, repetitive beats, with bends developing at the basal (anchored) end of the axoneme and propagating distally. Key features, including the distinctive effective and recovery strokes of cilia, could be reproduced by initially assigning a greater probability of dynein cross-bridges forming on one side of the axoneme [Lindemann, 1994b].

Figure 1, taken from Lindemann [1994b], uses this hypothetical mechanism to summarize the events leading to the creation of a beat. Each numbered diagram depicts a triplet of outer doublets from one side of the axoneme (corresponding to doublets 2, 3, and 4 on one side of a real axoneme, and doublets 7, 8, and 9 on the other). The dominant side (having a higher initial probability of forming attachments) includes the bridges forming the principal bend and is called the P-bridge set. The bridges on the opposite side generate the reverse bend, and are labeled R-bridges. The figure illustrates the actions leading to flagellar oscillation in a step-wise progression. 1: With the axoneme initially in a straight position, both sets of bridges experience sporadic, random bridge attachment. 2: The P-bridge side has a higher probability of bridge attachment and undergoes a cascade of attachment. This results in a sliding episode that simultaneously inhibits formation of R-bridges due to force transfer through the interdoublet links. 3: Increased bending generates a negative $t$-force on the P-bridges that is strongest at the basal end, initiating bridge detachment. This is coupled with continued force transfer inhibition of the R-bridges due to force transfer through the interdoublet links. 4: As P-bridge detachment propagates, R-bridges experience positive $t$-force due to passive bending on that side, resulting in a cascade of R-bridge attachments. 5: R-bridge attachments reverse the curvature, inhibiting
P-Bridges
1 Random bridge attachment
2 Cascade of bridge attachment due to adhesion
3 Initiation of detachment
4 Propagation of detachment
5 Delayed initiation due to force transfer
6 New episode of attachment

R-Bridges
1 Random bridge attachment
2 Inhibition due to force transfer from P side
3 Delayed attachment due to force transfer
4 Initiation of attachment
5 Propagation of attachment
6 Initiation of detachment

Fig. 1.
the P-bridges through force transfer. 6: This reversed
curvature now produces a negative t-force on the basal
end R-bridges, initiating a chain-reaction of detachment
on that side. Meanwhile the P-bridges are experiencing
positive t-force from passive links and residual active
bridges, resulting in a new episode of attachment that
renews the cycle. The arrows labeled with a P in the
diagram represent passive force from stretching passive
interdoublet links (i.e., nexin). The arrows labeled with an
A depict active forces from dynein bridges, and the
A* labeled arrows indicate active force transferred from
the opposing side.

By step 6, it becomes obvious that the mechanism
necessary for stable oscillations has been set in motion.
The new episode of attachments on the P-bridge side will
ultimately result in a repeat of all the earlier events. It is
apparent, at this point, that certain axonemal properties
are crucial to the validity of this hypothesis. Firstly, there
must be a small (but finite) probability for random dyne-

in cross-bridge attachment even without the influence of
the t-force, otherwise initiation of the cycle from a
straight standstill would not be possible. There must also
be elastic linkages that contribute to the initial engage-
ment of bridges once oscillations have begun (illustrated
in step 4 of the R-bridge side). These linkages must also
act to restrict the splaying of the axoneme during the
bridge detachment phase of the cycle (depicted in step 4
of the P-bridge or step 6 of the R-bridge sets). Finally,
there must be some transfer of longitudinal force from
one side of the axoneme to the other. Without this trans-
fer, the probability of bridge attachment on the opposing
side would increase as soon as the curvature increased
(as in R-bridge step 2). This would prompt simultaneous
bridge engagement on both sides of the axoneme, pre-
vailing the growing bend from ever reaching the thresh-
hold necessary for bridge detachment. The importance of
this side-to-side transfer of force in the axoneme is
brought into focus by the t-force concept. The goal of
this review is to correlate the events and processes of the
Geometric Clutch mechanism with recent experimental
observations on cilia and flagella. Where possible, test-
able predictions of the hypothesis will also be explored.

THE T-FORCE

The t-force itself is not a mysterious or arbitrary
concept. When a flexible structure is curved and under
tension, it experiences force transverse to the axis of
tension. This is equally true for cables of a suspension
bridge or doublets of a flagellum. In either case, exter-
nally applied forces must also be active or the structure
would snap taut or buckle. In a suspension bridge, the
t-force is holding the highway up through support cables
that drop from the main cable. In the axoneme, the best
candidates for application of external force on the outer
doublets include interdoublet nexin links and radial
spokes. High quality micrographs [Avolio et al., 1986;
Goodenough and Heuser, 1982; Sugrue et al., 1991] sug-
gest that there is another set of interdoublet connectors
(other than nexin links) synchronous with the outer dyne-

in arms (at 24 nm intervals). In a passive section of a
flagellum (where dynein heads are not forming attach-
ments), all of the t-force needed to prevent the axoneme
from splaying apart must come from one or more of these
linkage types. Most of the force driving the axonemal
beat comes from the dynein arms between doublets 2, 3,
and 4 on one side and doublets 7, 8, and 9 on the oppo-
site side [Lindemann, 1994a]. These doublets can be
squeezed closer together or pulled farther apart by the
t-force. The orientation of the radial spokes (mainly per-
pendicular to the plane of bending at these doublets) does
not dispose them to act as a resistant force. Therefore,
it is reasonable to assume that most of the force counter-
balancing the t-force at these doublets must come from
the nexin and/or the other interdoublet connectors.

INTERDOUBLET SPACING

A necessary postulate of the Geometric Clutch hy-
pothesis is the assumption that the t-force is large enough
to significantly alter interdoublet spacing. On one hand,
high quality micrographs depicting changes in interdou-
blet spacing during oscillation have not been attained.
However, Gibbons and Gibbons [1973] reported that
"the average spacing between tubules appears to be
rather less when a bridge is present than when it is not,
suggesting that formation of a cross bridge is associated
with a moving together of the affected doublet tubules."
In addition, a decrease in interdoublet spacing has been
demonstrated during rigor [Gibbons, 1975] and with the
addition of certain cations [Warner, 1978; Zanetti et al.,
1979], enforcing the idea that interdoublet spacing does
change when dynein bridges are attached.

Equation 1 makes it possible to calculate the t-force
acting during the beat cycle by using an estimate of force
per dynein, commensurate with independent measure-
ments [Kamimura and Takahashi, 1981; Oiwa and Ta-
kahashi, 1988; Gittes et al., 1993]. In the case of a 10
μm cilium, the greatest t-force acts to pull doublets
apart, and has a peak value of ≈ 7.5 × 10⁻⁵ dynes/μm
[Lindemann, 1994b]. This dynein-produced force must
be countered by the elastic force of interdoublet linkages,
or the axoneme would break apart. The computer simu-
lation was able to calculate an upper limit estimate of
elasticity (0.6 dyne/cm) by using a value just large
enough to "arrest" the microtubule sliding and produc-
ring the characteristic configuration observed in cilia. The
nexin link periodicity of ≈ 100 nm [Stephens, 1970]
provides 10 nexin per \( \mu \text{m} \), requiring each linkage to bear 7.5 \( \times 10^{-9} \) dyne during the period of maximum t-force. This would equate to stretching each link 1.25 \( \times 10^{-4} \) cm, or 1.25 \( \mu \text{m} \), a value five times the diameter of the axoneme!

Using a more conservative estimate of dynein force (1 \( \times 10^{-8} \) dyne/dynein), and engaging only half of the dynein arms over only 8 \( \mu \text{m} \) of the axonemal length, would reduce the tension on the doublet to 6.6 \( \times 10^{-6} \) dyne, reducing the required elastic force to 4.2 \( \times 10^{-7} \) dyne/nexin at a curvature of 0.063 radians/nexin. This still requires an added displacement of 70 nm between doublets 2 and 4, or doublets 7 and 9. This is the lowest possible approximation, yet it is still an extreme distortion which is more than sufficient to regulate cross-bridge formation. In actuality, this low estimate is probably not feasible because the resultant total dynein force would not drive a cilium at the frequencies and velocities observed in nature. It now becomes clear that the axoneme experiences a tremendous amount of t-force. The challenge will be to identify the system holding the axoneme together under such phenomenal stress.

Currently, only two other structures, the interdoublet connectors and the radial spokes, are recognized as possibly responsible for keeping the axoneme intact when subjected to maximal t-force. The existence of motile, spokeless flagella [Schrevel and Besse, 1975; Brokaw et al., 1982; Gibbons et al., 1985] strongly implicates the interdoublet connectors. The specific periodicity and attachment sites of these linkages is somewhat controversial (as they are generally obscured by the dynein arms in electron micrographs). Goodenough and Heuser [1982] find linkages in register with the dynein and identify them as dynein “stalks.” Sugrue et al. [1991] hypothesize that these stalks may actually be interdoublet connectors superimposed by the dynein. Under freeze etch electron microscopy, it was determined that these connectors do not maintain fixed attachments and are capable of detaching and reattaching as the doublets slide past each other [Goodenough and Heuser, 1982]. They are also more closely spaced than the nexin links, making them a good prospect for providing supplemental mechanical support in maintaining axonemal integrity. In fact, first reports localized them only to the basal region of the flagellum [Ringo, 1967], where t-forces reach their maximum amplitude.

The radial spokes in normal cilia/flagella may also play a role in stabilizing the axoneme (spoke-free axonemes are reportedly more fragile) [Gibbons et al., 1985; Goldstein and Schrével, 1982]. The radial spokes could serve to squeeze together outer doublets 9, 1, and 2, and also doublets 4, 5–6, and 7 when t-force is at its peak. This mechanical crunching could act to limit doublet excursion in response to increasing t-force.

THE NEXIN LINKS

Nexin links play a key role in the Geometric Clutch mechanism. They provide elasticity to resist the action of the dynein arms, while contributing passive elastic force that results in the development of positive (bridge engaging) t-force. A bull sperm flagella that has lost its native ability to beat will often respond actively to an externally imposed flagellar bend [Lindemann and Rikmenspoel, 1972; Lindemann et al., 1980; Lindemann et al., 1995]. Passive bending stretches the nexin links and creates a positive t-force that assists in dynein bridge engagement. Thus, micromanipulation studies appear to confirm that element of the hypothesis. Nexin links have been observed to stretch up to ten times their resting length [Olson and Linck, 1977; Warner, 1976]. This extreme nexin elasticity is a necessary property for a workable hypothesis. The model predicts that a dynein bridge force of 1.2 \( \times 10^{-7} \) dyne/dynein would require the nexin link elasticity to be less than 0.06 dyne/cm per nexin link. If the nexin were any stiffer, even complete activation of all dynein arms could not bend a 10 \( \mu \text{m} \) cilium into the extreme curve observed at the end of the effective stroke. Good evidence indicates that only half of the dynein arms are likely to be attached at any one time [Sugrue et al., 1991], making the actual value more likely 0.03 dyne/cm per nexin link. Additionally, if the dynein bridge force were lower, the nexin value must also be lower, making the likeliest figure somewhere between 0.01 and 0.03 dyne/cm per nexin link. The computer simulation yields life-like beating with an elasticity constant within this range. Future experimental verification of this estimate is important.

THE CRITICAL SWITCHING LIMIT

The Geometric Clutch mechanism predicts that episodes of dynein-tubulin attachment are terminated by the t-force. This t-force is defined by the product of local curvature and longitudinal strain on the doublets. Thus, if the product of dynein force and local curvature does not reach the critical t-force magnitude necessary to initiate bridge detachment, dynein disengagement fails, and switching arrests. In hindsight, when vanadate and Ni\(^{2+}\) were used to produce “arrested” cilia and flagella [Lindemann et al., 1980, 1995; Satir et al., 1991], they may actually have been reducing the dynein force contribution to such a level that the critical t-force limit could not be reached.

Subjecting non-inhibited cilia or flagella to mechanical or viscous restraint may identify the pivotal degree of curvature necessary to permit a beat to reverse direction. If this critical limit is not reached, the beat should “stall.” Similarly, a requisite number of dyneins
must pull together to reach the switching limit. This limit may be detectable on short cilia or flagellar fragments following microdissection. In the highly stiffened macroflagella of bull or rat sperm, the critical length (and number of dynein arms) may be much greater due to the increased resistance to bending. This might make such a threshold easier to detect in large mammalian sperm.

If “arrested” cilia and flagella are the result of switching failure, increasing the t-force by manipulating the arrested axoneme to increase its existing curvature (increasing the product of curvature and bridge force) may result in production of a single beat. Induction of a “single beat” of this nature would support the existence of a critical t-force requirement.

Additionally, if paralyzed mutants have functional axonemal dynein, in spite of a very low probability of cross bridge formation, micromanipulation of a severe flagellar bend should result in bend propagation. This would substantiate the assumption that the defect is in the cascade mechanism and that the dynein arms are capable of responding if subjected to sufficient positive t-force.

There are naturally occurring stationary cilia that exhibit a reactive beat in response to tactile stimuli [Moss, 1993]. This mechanical sensitivity can be explained if these specialized cilia maintain a sub-critical t-force for bridge detachment. Analyzing the effect of applied stimulus on the t-force might resolve the mechanism of mechanical sensitivity.

THE BASAL ANCHOR

The basal body, or anchoring structure (i.e., the connecting piece in mammalian sperm) plays an important role in the t-force mechanism. In the Geometric Clutch model, bend formation and propagation from the base arise as a consequence of the summation of active and elastic forces toward the basal anchor. Without the basal anchor, initiation of bridge attachment and detachment episodes becomes more difficult and less well organized. Isolated flagellar fragments with no basal body typically cannot maintain a coordinated beat [Goldstein, 1969; Lindemann and Rikmenspoel, 1972; Okuno and Hiramoto, 1976]. However, a positive t-force is produced in the middle of a mechanically bent segment even without a basal anchor. This is due to the production of sliding and the stretching of elastic links at both ends of the axoneme. The stretched links contribute maximal tension in the center of the flagellar fragment, as a positive t-force develops. The dynein arms should then engage, propagating the induced bend. This experiment was actually conducted over twenty years ago on bull sperm [Lindemann and Rikmenspoel, 1972], with the predicted result. It would be interesting to see if this is a universal property of cilia and flagella.

THE CENTRAL PAIR AND RADIAL SPOKES

Enough recent evidence has been presented supporting the contention that the flagellar/ciliary central pair rotates during beating in some species [Omoto and Kung, 1979; Omoto and Witman, 1981; Kamiya et al., 1982] that the concept has been incorporated in a current instructional text [Bray, 1992]. A kinesin-like protein is believed to be associated with the central pair microtubules, and is suspected to be the motor responsible for their rotation [Bernstein et al., 1994]. Though central pair rotation is not required in the Geometric Clutch mechanism, the two concepts are not incompatible. In organisms exhibiting a more three dimensional flagellar beat, rotation of the central pair may be responsible for the change in beating plane by more uniformly utilizing all of the outer doublets. However, in the more planar beat of sea urchin and mammalian sperm flagella it is much less likely that the central pair rotate.

Modification of the beat plane in sea urchin sperm using a vibrating probe resulted in an equal recoil of the beat plane [Takahashi et al., 1991]. The most cogent interpretation of these results asserts that rotating the central pair wound up the spokes, and the beat plane followed the recoil of the central pair back to their original position. This suggests a permanent axonemal structure with a well-defined central pair orientation in relation to the beat plane. In fact, the mammalian sperm axoneme appears to contain a stable central partition that often remains intact after all other doublets have been extricated following axonemal disintegration by free microtubular sliding [Lindemann et al., 1992; Kanous et al., 1993]. This partition consists of the central pair connected to doublets 3 and 8.

The importance of radial spoke and central pair involvement in dynein bridge attachment is further diminished in view of the evidence for spoke detachment/reattachment [Warner and Satir, 1974], and the existence of motile central pair deficient mutants [Schrevel and Besse, 1975; Witman et al., 1978; Brokaw et al., 1982; Gibbons et al., 1985].

THE DYNEIN REGULATORY COMPLEX

A protein cluster on outer doublet A microtubules, called the dynein regulatory complex (drc) [Piperno et al., 1992, 1994], seems to repress dynein activity. The radial spokes appear to attach near this complex, mediating a dynein activation or derepression [Smith and Sale, 1992]. Additionally, spokeless mutants exhibit motility if they also lack the drc proteins [Piperno et al., 1994].

The currently available evidence is consistent with two different hypothetical views for the function of the
drc. An as yet undetermined mechanism may be selectively derepressing certain dynein arms during rotation of the central pair, thereby controlling sliding activation. This concept, likened to a distributor activating cylinders in a gasoline engine, has been proposed [Bray, 1992]. However, this theory does not concur with other evidence that not all species experience central pair rotation [Takahashi et al., 1991; Lindemann et al., 1992; Kanous et al., 1993], while some spokeless and/or central pair deficient mutants exhibit motility [Schrevel and Besse, 1975; Brokaw et al., 1982; Gibbons et al., 1985].

The Geometric Clutch hypothesis suggests an alternative interpretation of the drc's capacity. The base level probability of dynein arm attachment to the adjacent doublet modifies the axoneme's ability to initiate the sliding necessary for a flagellar beat cycle (see Fig. 1). The inner dynein arms are most advantageously positioned to initiate the attachment cascade necessary for creation of the flagellar beat. Alterations in inner arm orientation or length would directly impact bridge attachment probability.

Another reported property of the inner dynein arms may be pertinent in facilitating dynein bridge engagement. Avolio et al. [1986] demonstrated that inner and outer dynein arms do not move symmetrically in attaching to adjacent B microtubules. This causes the doublets to skew in relationship to one another when changing from an unattached to an attached configuration. Additionally, certain subpopulations of inner arm dyneins have been reported to rotate microtubules in in vitro assays [Kagami and Kamiya, 1992]. This may significantly affect bridge attachment, since even minor rotation of B tubules alters the spatial relationship of the outer arms to the adjacent doublet [Kagami and Kamiya, 1992]. If this rotation were directed outward, it would greatly increase the probability of outer arm engagement. The inner arms, ideally positioned to initiate their own cross-bridge cascade, may be facilitating outer arm attachment to trigger interdoublet sliding episodes. This scheme is consistent with evidence that drc suppression of inner arm function is sufficient to incapacitate the beating mechanism in some flagella [Piperno et al., 1992, 1994].

The Geometric Clutch simulation of a cilium can only duplicate a natural looking effective and recovery stroke beating pattern if the bridge attachment base probability is different on opposite sides of the axoneme (the 2, 3, 4 and the 7, 8, 9 side). Cilia and flagella have the demonstrated ability to change beat cycle symmetry when the free Ca\(^{2+}\) concentration within the axoneme varies [Brokaw et al., 1974; Bessen et al., 1980; Lindemann and Goltz, 1988]. Perhaps the drc (or the spokes derepressing the drc) include a protein capable of changing configuration in response to free Ca\(^{2+}\), thereby creating a change in the switching thresholds of the affected dyneins. The existence of a caltractin/centrin linkage between the drc and inner dynein arms has been reported [Piperno et al., 1992]. However, the necessary chemical or structural difference between the opposite sides of the axoneme still requires experimental substantiation.

THE CALCIUM RESPONSE

Previous studies on cilia and flagella have demonstrated a Ca\(^{2+}\)-induced change in beat symmetry [Naitoh and Kaneko, 1972; Brokaw et al., 1974; Bessen et al., 1980; Lindemann and Goltz, 1988], even to the extent of oscillatory "arrest" at one extreme configuration of the cycle [Gibbons and Gibbons, 1980; Lindemann and Goltz, 1988; Satir et al., 1991]. This behavior can be mimicked in the Geometric Clutch computer simulation by altering the base level bridge attachment probabilities on opposite sides of the axoneme. Increasing or decreasing these probabilities could be analogous to:

1. Modifying the distance between adjacent doublets.
2. Modifying the length of the dynein arms.
3. Modifying the dynein arm projection angle relative to the adjacent doublet.

Option 1 could be accommodated by a change in spoke or interdoublet link length in response to Ca\(^{2+}\). Options 2 and 3 could result from Ca\(^{2+}\)-induced mechanical changes in the drc or dynein.

For bridge attachment probability to differ on opposite sides of the axoneme, biochemical or steric differences must be ascertained. Some such evidence has already been documented. Ni\(^{2+}\), previously shown to block flagellar Ca\(^{2+}\) response [Naitoh and Kaneko, 1972; Lindemann and Goltz, 1988; Larsen and Satir, 1992], recently demonstrated stronger inhibition of the dynein-tubulin interaction on one side of the axoneme [Kanous et al., 1993]. In an even more recent study, micromanipulation of bovine sperm models poisoned with Ni\(^{2+}\) revealed that the axoneme maintained the capability to respond, but only in one bend direction [Lindemann et al., 1995].

Additionally, good candidates for Ca\(^{2+}\)-sensitive sites have been discovered. Centrin, a Ca\(^{2+}\)-responsive contractile protein, has been localized to the axoneme [Salisbury et al., 1986; Salisbury, 1989] and, along with actin, is reported to be a component of the drc [Piperno et al., 1992].

SUMMARY

The Geometric Clutch hypothesis integrates a large body of seemingly disconnected analytical measure-
mments and observations into one conceptual framework. It remains to be established whether certain key requirements of the hypothesis are actual attributes of real axonemes. The hypothesis is rich in predictive value, as its fundamental working elements are developed directly from physical properties and structures of the axoneme. Exploration of these predictions will serve to confirm or reject the hypothesis itself, but even more importantly, may contribute to elucidation of the principles underlying ciliary and flagellar functioning.

REFERENCES


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