bound state. Taken together, these unique features of the MCAK motor domain tune it to identify and stabilize curved protofilaments at MT ends. Notably, our current knowledge on how kinesins alter MT dynamics is heavily influenced by work on kinesin-13s, largely because mechanistic details of how other kinesins work do not exist. We await further studies to see if the kinesin-13 paradigm is universal, or if other kinesins use unique biochemistries to shape the MT cytoskeleton.

Where can I find out more?

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Independent acquisition of sodium selectivity in bacterial and animal sodium channels

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Electrical signaling in animal nerves and muscles is largely carried out by proteins in the superfamily of voltage-gated ion channels [1]. These proteins are based on a single homologous domain, but different types exist as single-domain tetramers, two-domain dimers, or four-domain proteins that comprise the whole pore-forming structure [1]. Four-domain channels are hypothesized to have evolved from a single-domain ancestor by two rounds of internal duplication [2]. The role that a channel plays in a cell's physiology is largely determined by its selectivity for specific ion species and by the stimulus that opens the channel — its method of 'gating'. The voltage-gated sodium (Na_v) and calcium channels (Ca_v), which drive the upstroke of action potentials and transduce electrical signals into cellular signals, respectively, both have the four-domain architecture, whereas voltage-gated potassium channels (K_v) have only one domain. Crystallographic studies have led to important discoveries about ion permeation and gating in the single domain K_v channels, but structural studies of the four-domain Na_v and Ca_v channels have not achieved the same level of precision [3], leaving the atomic details of these important proteins in the dark. The recent discovery of and subsequent crystallographic work on a voltage-gated, sodium-selective, single-domain channel in bacteria ($BacNa_v$) was therefore greeted with excitement as a potential model of four-domain Na_v channels [4–6].

The selectivity filter of $BacNa_v$ channels is very different from that of eukaryotic Na_v channels, however, and these studies often lack clear statements of homology between the two channel types [4–6]. $BacNa_v$ channels are often referred to as 'ancestors' of Na_v channels [5], a claim whose evolutionary meaning is difficult to interpret. Basic research on the organismal function of $BacNa_v$ channels, moreover, has lagged behind the sophisticated structural studies. This situation leaves it unclear whether the molecular correlates of function are truly comparable between eukaryotic Na_v and $BacNa_v$ channels. We help address this by grounding the relationship of $BacNa_v$ channels to other major channel groups in an evolutionary framework.

The constituent domains of four-domain channels have what may be called molecular serial homology, where all four domains are equally related to the single-domain precursor [2]. We therefore followed the procedure of Strong *et al*. [2] and broke the four-domain channels into their constituent domains, making the smallest homologous unit (the domain) into the operational taxonomic units in the phylogeny. Figure 1 shows strong support for the traditional view of ion channel evolution [2], with a single origin of the four-domain structure in Na_v and Ca_v channels. DI and DIII form a clade, as do DII and DIV, in keeping with the hypothesis of two sequential rounds of internal gene duplication [2].

$BacNa_v$ channels fell outside the four-domain group with strong support, rejecting the notion that $BacNa_v$ channels can be considered Na_v channels [4] in an evolutionary sense. Instead, they grouped near CatSper channels, consistent with earlier studies showing that both $BacNa_v$ and CatSper channels are used as pH sensors in the bacterial and sperm cells in which they are respectively expressed [7,8]. We therefore propose that the $BacNa_v$, CatSper, and the novel single-domain protist types be viewed provisionally as a pH-gated group, based both on evolutionary relatedness and conservation of function.

This tree rejects the possibility of $BacNa_v$ channels being placed within Na_v channels, but it is still possible that $BacNa_v$ are functionally similar to the precursors of animal Na_v channels. There are two mutually exclusive hypotheses about the evolution of ion selectivity in voltage-gated ion channels (Figure S1 in Supplemental Information, published with this article online). In one scenario (Figure S1A), sodium selectivity is independently acquired in $BacNa_v$ and animal Na_v channels. In the other, $BacNa_v$ channels are similar in function to the common ancestor

of all non-K_v channels, and selectivity for sodium is the ancestral state for all these channels (Figure S2B).

To test these hypotheses, we used ancestral state reconstruction to estimate whether functionally characterized $BacNa_v$ channels have the same amino acids in their ion selectivity filter as the channel ancestral to extant $BacNa_v$ channels. This method uses an evolutionary model to reconstruct the most likely ancestral sequence for a clade given an alignment and a tree.

Figure 1 shows the ancestral pore reconstruction for all sampled $BacNa_v$ channels (the full tree used for reconstruction can be found in Figure S2). Functionally characterized $BacNa_v$ channels have the selectivity filter sequence LESWAS or LESWSM [9,10]. Aspartate residues (D) were more common in the ancestral pore than in characterized $BacNa_v$ sequences. An aspartate in the sixth position, which occurs in the ancestral channel, is enough to nearly equalize the permeability to calcium and sodium in mutated channels [9]. An aspartate at both the third position, which was nearly as probable as a serine in our reconstruction, and the sixth position would strongly suggest calcium selectivity in the ancestor of $BacNa_v$ channels [9]. We therefore find it most likely that the ancestor of $BacNa_v$ channels was a non-selective, or even calcium-selective, pH-sensitive channel resembling CatSper channels in structure and function [7,8]. This result awaits functional verification, but we find strong support for the idea that selectivity for sodium is a derived trait in the channels that have been expressed and characterized.

In this study we asked whether selectivity for sodium is directly comparable in Na_v and $BacNa_v$ channels by exploring the evolutionary history of the latter group. We found that sodium selectivity almost certainly arose independently in $BacNa_v$ and Na_v channels, and that $BacNa_v$ should not therefore be thought of as evolutionary precursors of animal Na_v channels. This finding does not preclude the use of $BacNa_v$ channels as models for animal channel function, but it does highlight the importance of evolutionary considerations in such studies. Our analysis begins the work of placing $BacNa_v$ channels in an integrative framework that will allow more fruitful comparisons to animal channels in the future.

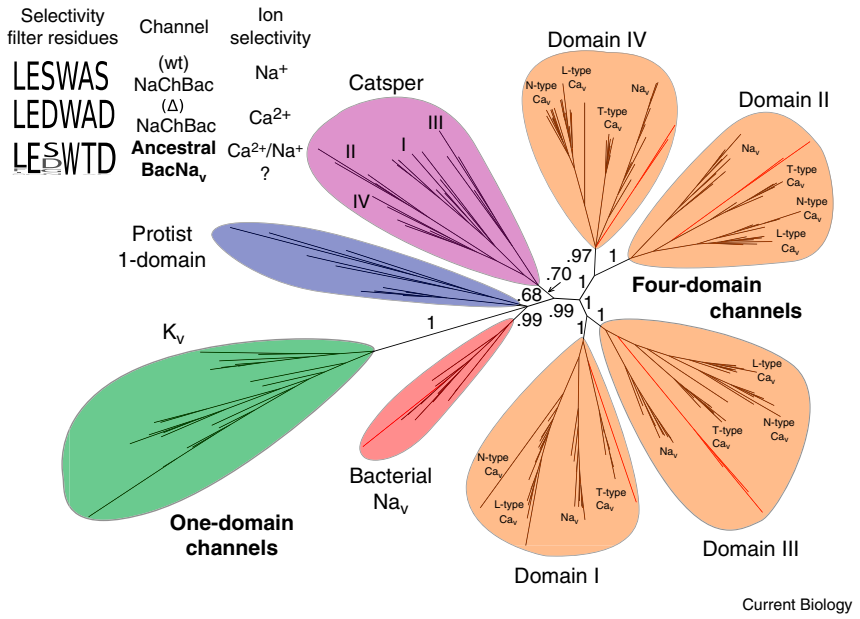

Figure 1. Unrooted tree of major ion channel types and ancestral state reconstruction of $BaNa_v$ selectivity filter.
The four homologous domains of Ca_v and Na_v channels have a single, well-supported origin to the exclusion of all the single-domain channels. The branching order of CatSper, $BacNa_v$, and eukaryotic single-domain channels is not well supported, but we do not find $BacNa_v$ near eukaryotic Na_v channels in any scenario. Novel sequences include a clade of one-domain channels in protists, and channels from early-branching zoosporic fungi (red lineages), including a horizontally transferred $BacNa_v$ channel and the first described Cav channels in fungi (Supplemental Information). Bayesian posterior probabilities are provided for interior branches. Ancestral states for the $BacNa_v$ family's selectivity filter are displayed in proportion to their *a posteriori* likelihood. The wild-type (wt) selectivity filter for the founding member of the $BacNa_v$ family, NaChBac, and a mutant channel with Ca^{2+} selectivity [9] are displayed for comparison. The ancestral pore is more similar to the calcium-selective mutant.

Supplemental Information

Supplemental Information includes two figures and experimental procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.09.025.

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