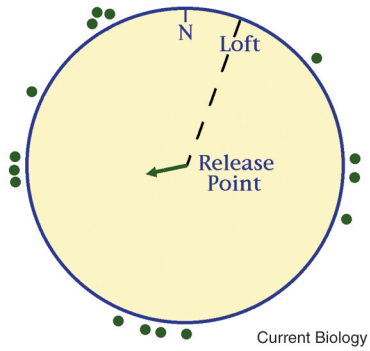

Figure 3. Pigeons are disoriented at magnetic anomalies.
In contrast to the results shown in Figure 1, pigeons released on sunny days at magnetic anomalies are disoriented in proportion to the strength of the anomaly. The minor perturbations in the otherwise-smooth gradients of magnetic-field parameters do not affect compass readings, but (over distances smaller than 50 m) can suggest disparate map positions, as the pigeon flies. The departure bearings at this site are taken at 1 km from the release site; the mean vector is not significantly orientated, and is directed about 125° counterclockwise of the loft. Data taken at other distances out to 10 km yield similar results. At greater distances, most birds become homeward oriented.

rich rock increases local field strength enough to alter map readings but not compass direction, should leave the birds reoriented or disoriented. Indeed, at smooth gradients they are reoriented, whereas at irregular ones they are ludicrously disoriented (Figure 3).

Pigeons, like most animals with maps, possess enormous numbers of magnetite grains in the ethymoid sinus. There are species in which magnetic sensitivity may be mediated in other ways, for example magnetic induction in elasmobranchs, or paramagnetic effects in the visual pigments of silvereye birds, but magnetite-based compasses seem to fit the behavioral data in nearly all cases. In species using a map for navigation, there are far too many of these grains for a mere compass, but enough to easily determine map location at least at an order of magnitude better than the 2 km estimated from returns of visually impaired pigeons.

Pigeons subjected to gradually increasing and decreasing intense magnetic fields should have their magnetite grains remagnetized. If the map sense is directional, like a typical hand compass, the birds should be disoriented. On the other hand, if the map is axial, that is, if it cannot distinguish between north and south, the orientation should be better. Birds treated with magnetic fields are indeed better oriented. However, if the birds are subjected to a sharp intense pulse, either kind of map organ should be severely impaired, which is exactly what is observed.

Whichever cues the map sense involves, it seems likely that it is this information which allows low-resolution first-time migrants to achieve high-resolution returns—and high-resolution southward trips in future years. Far and away the biggest question in long-distance navigation is the nature of the map sense. Second is the issue of recalibration, which is still puzzling. The interaction of these specific learning programs doubtlessly holds many magnificent secrets.

Further reading

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Hypothesis: A helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors

Jonathon Howard[1] and Susanne Bechstedt

Sensory reception by mechanically sensitive cells is mediated by an ion channel coupled to a molecular spring [1]. This gating spring is thought to transmit an external force (e.g. from a sound wave or a tactile stimulus) to the ion channel. The force alters the probability of the ion channel being open. This produces an electrical signal that is conveyed to the central nervous system [2]. One such channel has recently been identified as a member of the NOMP-C subfamily of TRP channels [3,4]. However, the molecular identity of the gating spring remains unknown. We propose that the gating spring is a helix (or bundle of helices) formed by the 29 ankyrin domains of this mechanoreceptive subfamily of TRP channels.

The NOMP-C proteins in the bristle sensilla of *Drosophila* [3] and in the inner ear and lateral line of zebrafish [4] contain 29 consecutive ankyrin domains (ANK repeats) at their amino termini (Figure 1A,B). A similar channel has been identified in worms [3]. Crystallographic studies of a fragment containing 12 ANK repeats revealed a highly regular structure that forms approximately 40% of one turn of a helix [5]. Extrapolation of this structure suggests that 29 ANK repeats would form almost exactly one helical turn. We call this hypothetical structure the 'ANK helix' (Figure 1C).

Depending on the number of NOMP-C subunits, the channel complex may contain up to four ANK helices surrounding the intracellular face of the pore-forming region of the channel. We suggest that an arrangement of the ANK helices with their axes perpendicular to the plasma membrane (Figure 1D) has several mechanical and structural properties that would ideally suit a gating spring.

First, a full turn of a helix allows transmission of force from one end of the structure to the other without creating a torque. This geometric constraint provides an explanation for the remarkable conservation of exactly 29 ANK repeats in this otherwise highly divergent protein subfamily found in worms, flies and fish.

Second, the stiffness of an ANK helix is expected to be similar to that measured for the gating spring. The stiffness κ of a helical spring composed of a material with shear modulus G is given by $\kappa = Gr^4/4NR^3$, where r is the radius of the material, N is the number of turns and R is the radius of the helix [6]. Substituting values of G = 1 GPa (characteristic of a rigid protein, [6]) as well as r = 0.75 nm, N = 1 and R = 4.5 nm from the structural model gives κ = 0.9 mN/m (1 mN/m = 1 pN/nm). Four ANK helices in parallel would be four times as stiff. This is an upper limit to the stiffness, as structural defects would tend to increase the compliance. This stiffness is similar to the stiffness of the elastic element of fly bristle hair receptors (3 mN/m [7]), as well as to that of of the gating spring in vertebrate hair cells (~1 mN/m [8]).

Third, the ANK helix should be highly deformable and should be able to withstand compression or extension by a factor of two, even though the constituent protein cannot be deformed more than a few percent without yielding [6]. Thus, the ~20 nm long ANK helix should act as a linear spring for compressions and extensions of up to 10–20 nm. From a mechanical point of view, the importance of the arrangement postulated in Figure 1D is that the ANK helices are expected to contribute perhaps most of the compliance of the mechanosensory gating apparatus. This compliance is necessary to allow the channel to fluctuate rapidly between closed and open states such that small deformations of the cell will lead to a graded change in the opening probability.

Our model locates the gating spring on the intracellular side, directly adjacent to the structural elements that are thought to constitute the gate of channels in the potassium-channel superfamily [9]. This location accords with the ultrastructure of the arthropod sensillum, which shows no obvious extracellular compliant elements. By contrast, on the intracellular side of the plasma membrane there are 'membrane-integrated cones' [7], which connect the plasma membrane to the microtubule cytoskeleton in the tubular body, the likely site of mechanotransduction (Figure 1E). The resting dimension of a cone corresponds to that of a cluster of ANK helices. Because these cones are compressed by excitatory deflections of the bristle and stretched by inhibitory deflections [10], we predict that compression of the ANK helix in bristle receptors will open the TRP channel.

The ANK helices may be the gating springs for vertebrate hair cells, although there are several caveats. First, an intracellular location of the gating spring contradicts the prevailing view that extracellular filaments,

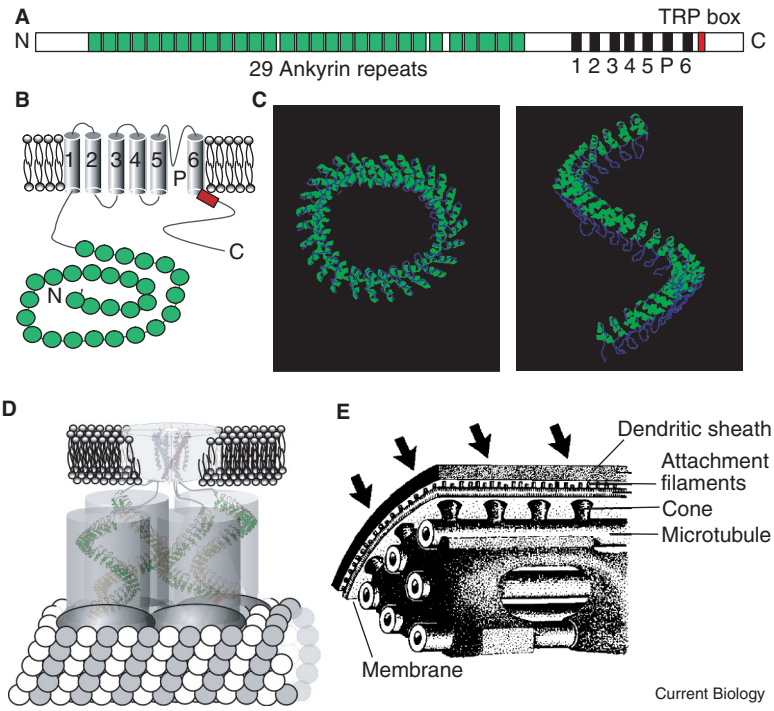

Figure 1
(A) Primary structure of the NOMP-C class of TRP channels showing the 29 ankyrin repeats (green), the 6 putative transmembrane helices (black), the pore-forming region (P) and the TRP box (red). (B) Postulated secondary structure of NOMP-C. (C) Two views of the proposed structure of 29 ANK repeats built by extrapolation of the structure of a 12-ANK fragment of ankyrin [5]. The left image is looking along the axis of the helix. The right image is looking perpendicular to the axis. (D) Hypothetical structure of the mechanotransduction apparatus in arthropod bristle receptors shows the ion channel in the membrane and the four ANK helices that connect it to the microtubule. In this figure the connection is indirect. (E) Ultrastructure of the insect mechanoreceptor showing the membrane-integrated cones that connect the plasma membrane to microtubules in fly bristle receptors (from [18]). In the fly, a deflection of the bristle causes compression (arrows) and opens the channel. An approximate scale is provided by the 25 nm diameter of the microtubules (in E), whose constituent tubulin subunits (represented as circles in D) have a diameter of 4 nm.

termed tip links, are the gating springs of hair cells. However, this view has recently been challenged by electron microscopy of the tip link that showed a structure that is probably too rigid and inextensible to serve as the gating spring [11]. On the other hand, the 'insertional plaques', located where the tip links connect to the plasma membranes and the underlying actin cytoskeleton, appear quite compliant and are large enough to accommodate a bundle of ANK helices [12]. Thus, the hypothesis that the ANK helix is the gating spring is not inconsistent with the ultrastructure of the hair cell. A second potential problem is that because the vertebrate channels open in response to tension in the tip link (caused by shear between the stereocilia [8]), our hypothesis predicts that it is extension, rather than compression, of the ANK helix that gates the channel open. How structural differences between the vertebrate and invertebrate channels could reverse the sign of gating is not clear. The third caveat is that the vertebrate gating spring is highly extensible: it has constant stiffness up to ~50 nm [8] and can extend up to 100 nm [13]. While these large extensions could be achieved if the ANK repeats were to unfold like the Immunoglobulin repeats of titin [14], thereby protecting the channel from damage due to large deformations of the hair bundle, it is not clear that the dynamics and nonlinearity of protein folding and unfolding are compatible with the gating process. A final caveat is that genes encoding TRP channels with 29 ANK repeats have not been found in the human or mouse databases.

The promiscuity of the ANK protein–protein interaction domain [15] could allow connection to the microtubule cytoskeleton in invertebrate mechanoreceptors and to the actin cytoskeleton in hair cells. In the case of the hair cell, the channel may connect to the actin filaments via the myosin motors that mediate adaptation [13]. The large number of ANK repeats in a tetramer of ANK helices could provide the scaffold necessary for assembling the large ensemble of myosins hypothesized to form the adaptation apparatus in hair cells [16], though the binding of the motors will decrease the compliance of the spring.

In summary, we have postulated that the ANK helical bundle forms the gating spring, a compliant element that transmits an externally derived force to the molecular gate of a mechanoreceptive ion channel. In this view, the ANK helix is functionally homologous to the ligand-binding domain of calcium-gated potassium channels [9]. It is possible that the ANK repeats found in other TRP channels [17] are not simply anchoring domains, but play an active role by transmitting mechanical force to the channel's gate. Even ankyrin itself, which contains 24 ANK repeats that likely form a nearly complete helix [5], may function to transmit tension from the cytoskeleton to ion transporters so that ion flux across the plasma membrane can be regulated by the mechanical state of the cell.

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