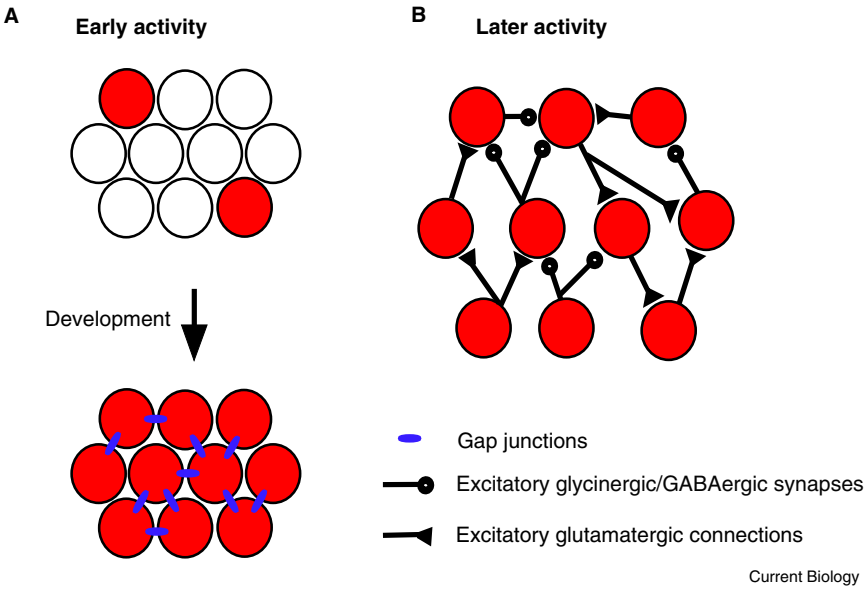

Figure 1. Two forms of activity in the developing network.

(A) Early in development, before networks are synaptically coupled, spiking activity and associated calcium transients (red circles) are experienced in individual neurons (upper network) or synchronously expressed in many neurons within the gap junction-coupled network (lower network). (B) Once the network becomes synaptically connected, spiking activity and associated calcium transients are expressed throughout the network as all neurons are recruited through recurrent excitatory synaptic connections (glutamate and GABA/glycine).

that cannot join the normal synchronized network, or if earlier-developed halorhodopsin-expressing neurons alter the circuit such that the existing synchronized network is now not competent to incorporate later-developing neurons. In addition, it will be important to determine if hyperpolarization of other subsets of spinal neurons could similarly disrupt network construction.

Further, the study [5] clearly shows that, at the inception of network formation, the contralateral synaptic connections produce alternation. It is not yet clear how this alternation would be mediated. In mature spinal networks contralateral alternation is mediated through chloride-dependent synaptic inhibition. On the other hand, it is not apparent that such synaptic inhibition exists, as more mature zebrafish spinal networks exhibit chloride-dependent synaptic depolarizations [14]. Finally, it is interesting to think about the original experiments that block sodium-dependent spiking activity, but give rise to spinal circuits that express relatively normal locomotion. It may be that sodium-dependent action potentials were blocked, but calcium transients and/or synaptic transmission remained and supported important developmental steps. Alternatively, activity may be important normally, but developing circuits are capable of compensating for the loss of this activity through novel strategies that allow for the generation of networks with seemingly normal functional behavior.

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Cell Polarity: Tension Quenches the Rear

A combination of biophysical perturbations and computer simulations shows that leading edge protrusion in crawling cells increases membrane tension, which constrains the protruding front to one side of the cell, thereby maintaining its polarity.

Alex Mogilner[1,*] and Jie Zhu[1]

Polarization — the ability to maintain distinctive front, rear and sides — is characteristic of both single cells and cells in tissues [1]. The signature of motile cells' polarization is asymmetrical establishment of sites

of actin polymerization [2], which, together with asymmetrical signaling, produces directional migration in response to chemotactic gradients [3] (Figure 1A). Mechanisms by which crawling cells confine actin assembly to a single leading edge are largely unknown. A recent paper by Houk and colleagues [4] takes a fresh look at this problem and shows that frontal protrusion increases membrane tension, which polarizes the cell by constraining the sides and rear.

Neutrophils, the immune system cells used in the recent work, are known to migrate directionally in response to external peptide gradients [5]. These cells can also polarize spontaneously in response to a sudden increase in a uniform chemoattractant concentration. One of the key characteristics of this polarization is elevated Rac GTPase activity at the cell front; Rac-dependent signaling is also necessary for polarization [5]. Fluorescent probes have revealed signaling components that localize either to the front (phosphoinositide lipids, Rac) or back (Rho) of the cell and have provided evidence for front–back mutual inhibition [6,7].

In agreement with these observations, the general theoretical hypothesis has been that cell polarization is a self-organizing process, resulting from feedback loops between various signaling components (Figure 1A). Starting from the famous Turing work (reviewed in [8]), mathematical models showed that *both* local activation and global inhibition are required to generate stable polarization. Specifically, two recent quantitative models suggested that slowly diffusing autocatalytic activator molecules and rapidly diffusing inhibitor molecules stably separate in space [9] (Figure 1A), or a limited protrusion activator is sequestered to the cell front and depleted from the rear [10]. Testing these models is challenging because, while various local activation mechanisms are known, neither the molecules responsible for inhibition are known, nor has it been determined whether inhibition is diffusion-based.

Houk *et al.* [4] cleverly circumvented this difficulty by forcing the cell into a peculiar growth-cone-like morphology (Figure 1B) that neutrophils adopt in the presence of uniform chemoattractant following brief heat shock. They simulated a few basic reaction–diffusion models [8] in this geometry, where diffusion through the narrow tether connecting cell front and rear is extremely slow. They found that all examined models make the same prediction: the pseudopod (top in Figure 1B) is activated and protrusive, while the cell body (bottom in Figure 1B) is inhibited and quiescent. Furthermore, the models predicted that if the cell body is suddenly disconnected from the pseudopod, it will remain quiescent for a long time because the information that the inhibitory influence has been removed is passed down by slow diffusion.

Surprisingly, when the passive rear was disconnected from the pseudopod with a focused laser beam, the cell body reanimated rapidly and started to protrude. This is inconsistent with the

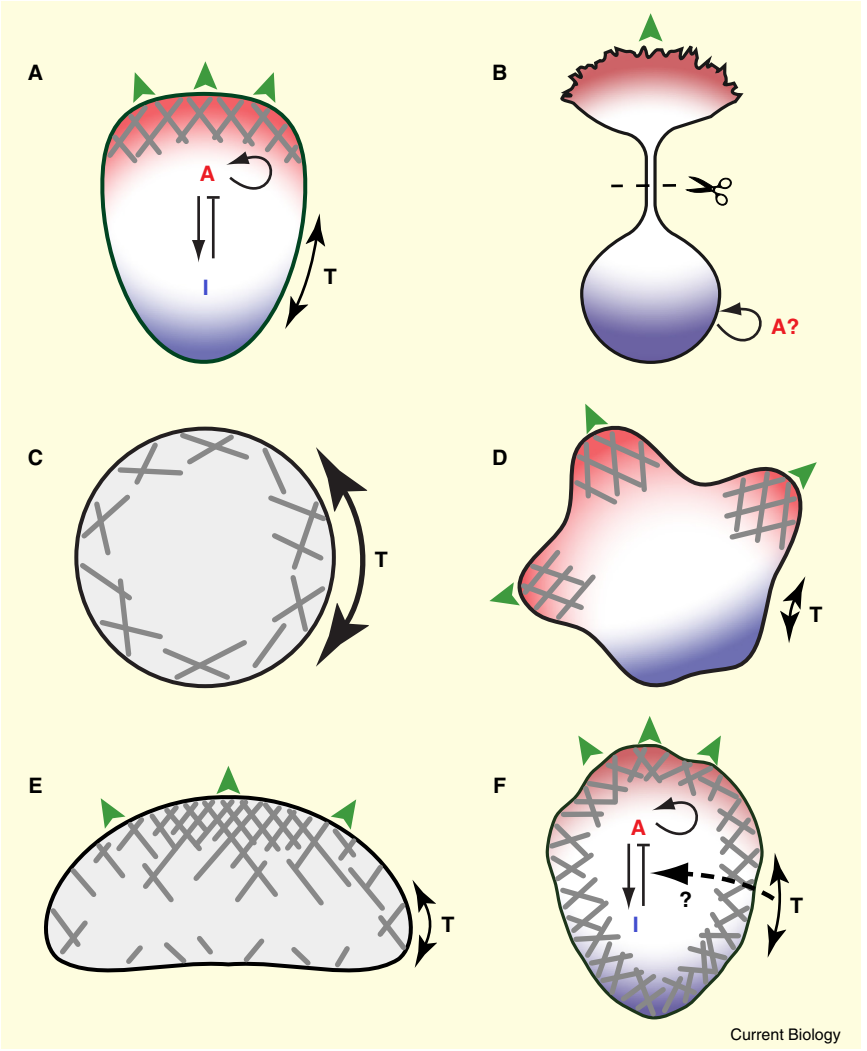

Figure 1. Membrane tension is the inhibitory signal determining cell polarity.

(A) In polarized motile cells, protrusion (green arrowheads) driven by the polymerizing actin network (gray sticks) is localized to the leading edge. The protrusion generates a membrane tension T. The polarity can arise if a slow activator molecule (A, red) has autocatalytic dynamics and enhances production of a fast inhibitor (I, blue), which in turn inhibits the activator. (B) In the experiment with the stretched morphology, the reaction–diffusion models predict that the rear remains inactive when it is separated from the front, unless signaling molecules have fast kinetics (loopy arrow). The experiment shows that the rear reactivates rapidly after the separation, while both modeling and data indicate that fast kinetics is unlikely. (C) Increasing tension inhibits all protrusion and relevant signaling. (D) Decreased tension allows multiple protrusions to emerge. (E) In motile fish keratocytes, the polymerizing actin network generates membrane tension that pushes the leading edge forward, contains the sides and crushes the actin network at the rear. (F) In the polarized neutrophil, membrane tension is likely to feedback on the signaling pathways.

prevalent view that reaction–diffusion mechanisms underlie communication between cell parts, so how does the front inhibit the rear? One possible answer lays in a classical experiment [11] showing that lamellipodial fragments of fish keratocyte cells can switch from a symmetric stationary state to a polarized motile morphology if simply prodded mechanically. A theoretical model [12] suggested the explanation: if actin network polymerization focuses at one end of the cell, actin filaments pushing at the membrane at that end generate membrane tension. This tension mechanically quenches protrusions elsewhere. Importantly, the tension spreads across the whole cell surface virtually instantly, on a sub-second scale [12], so mechanical inhibition is fast.

To test whether tension affects protrusion, Houk *et al.* [4] applied suction causing the cell to bulge into a micropipette, thereby increasing cell tension. Within seconds after this mechanical perturbation, protrusion ceased, the pseudopod retracted into the cell body, and the activity of Rac and the recruitment of the SCAR/WAVE complex (a driver of actin assembly) were inhibited (Figure 1C). Notably, these effects of tension were rapidly reversible. Increasing tension by applying hypotonic buffer caused similar effects. To see whether the tension is necessary to restrict the protrusion to one side of the cell, Houk *et al.* [4] used hypertonic buffers. The resulting drop in tension was followed by multiple protrusions and SCAR/WAVE recruitment around the cell (Figure 1D). Thus, the tension is both sufficient and necessary to polarize the cell.

Next, Houk *et al.* [4] used optical traps to pull tethers from the membrane and measure their tension. They found that, indeed, membrane tension nearly doubled during leading edge protrusion. Thus, the inhibitory mechanical signal was generated by the protrusion at the front, but one subtle question remained: which force was responsible for the cell polarity — the in-plane tension in the plasma membrane lipid bilayer, or the myosin-generated tension in the cytoskeleton that underlies the membrane [13]? This question was answered by relaxing the cytoskeleton tension with blebbistatin, a myosin inhibitor, which in fact caused the membrane tension to go up slightly and led to just one protruding lamellipodial extension. Therefore, membrane tension was responsible for the polarization.

Thus, Houk *et al.* [4] demonstrated that the rapid mechanical inhibition is the essential element of the polarization machinery: leading edge protrusion increases the membrane tension, which propagates rapidly and inhibits protrusions at the sides and rear. This exciting discovery raises a number of questions that no doubt will occupy cell biologists for years to come: how does leading edge protrusion create the membrane tension? How does the tension inhibit protrusion at the sides and rear and why doesn't the tension squash the very protrusion at the front that created this tension? Are biochemical pathways coupled to the mechanical one and, if so, how?

A very recent study of fish keratocyte lamellipodial fragments [14] hints at answers to the first two questions (Figure 1E): in the fragments, the actin network density is graded around the boundary, so fewer actin filaments at the sides push at the membrane, create tension and get stalled by it. Denser filaments at the front are able to grow against this tension, while at the rear the tension crushes the actin network, which has been weakened by depolymerization, and hauls it forward. Notably, this mechanism in the fragment works as well without myosin action. Another possibility is suggested by nematode sperm cell locomotion, in which the membrane tension away from the front reorients cytoskeletal fibers parallel to the cell boundary, so that the growth of fibers does not result in protrusion [15].

In neutrophils, the situation is likely to be more complicated than that in lamellipodial fragments: polarization and migration require phosphoinositide lipids and Rac [5], and Rho and myosin are intimately involved [7]. So, the mechanical effect of tension is probably coupled to the biochemical pathways (Figure 1E). Furthermore, a recent study of spreading fibroblasts [16] demonstrated that an increase in plasma membrane tension stopped protrusion and activated an exocytotic burst and myosin contraction. Thus, the membrane tension is coupled to membrane transport and plasma membrane area change, as well as complex physical [17] and chemical [18] plasma membrane organization, details of which will have to be elucidated.

Then, there are further questions: how is the tension regulated to allow exactly one region of protrusion, not more and not less, which is especially surprising given huge fluctuations in the tension? How does tension keep the cell polarized, yet sensitive to external signals? Does the tension work in three dimensions where the membrane is abundant and its tension likely lower? Can we rule out other directed transport mechanisms such as molecular motors driving signaling molecules from the front to the rear directly? (Microtubule-based motor transport is unlikely, as disrupting a neutrophil's microtubule network causes polarization [19], but in principle other directed transport processes are possible.)

Various cells adapted a set of evolutionarily conserved core mechanisms of polarity, including coupled reactions and transport of signaling molecules, graded cytoskeleton dynamics, and biased membrane transport. Houk *et al.* [4] add the mechanical effect of membrane tension to this repertoire; future research on mechanochemical coupling will show how the tension is integrated with other polarity pathways.

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Animal Cognition: Chimpanzee Alarm Calls Depend On What Others Know

After a wild chimpanzee encounters a model of a dangerous snake, whether or not he gives an alarm call depends on his perception of another individual's knowledge.

Robert M. Seyfarth and Dorothy L. Cheney

Birds and mammals do not always give alarm calls when they see a predator. Instead, alarm call production is affected by the presence and composition of an audience. Animals are more likely to give alarm calls when they are near other conspecifics than when they are alone, and more likely to give alarm calls in the presence of kin and preferred companions than in the presence of non-kin or rivals. To date, however, there has been no evidence that calling also depends on the signaler's perception of whether recipients are ignorant or already informed about the presence of danger. Indeed, whether any animal is even capable of making this distinction — whether any animal has a 'theory of mind' — has been the subject of considerable debate. In this issue of *Current Biology*, Catherine Crockford, Roman Wittig, and colleagues [1] describe the results of a field experiment suggesting that chimpanzees recognize when others are ignorant about the presence of danger and adjust their alarm calls accordingly.

Many field experiments have shown that, when producing vocalizations, non-human primates take into account subtle contingencies, including the context, the recipient's identity, the identity of others nearby, and the nature of their own recent interactions with their recipient and the recipient's kin [2–5]. Despite this broad sensitivity, monkeys and apes generally seem inattentive to the one feature that underlies much human communication: the perception of another individual's knowledge and beliefs [6]. The issue, however, is fraught with methodological complications [7].

Searching for a theory of mind in animals is difficult because intentions and beliefs are usually correlated with behavior, making it hard to determine whether one animal is attending to another's behavior or her mental state. Research on children, moreover, has shown that having a theory of mind is not an all-or-nothing phenomenon: before the age of two, children recognize that others have likes, dislikes, and motives, but the same children cannot distinguish between what they believe and what others believe. Like young children, animals may have a partially developed theory of mind. Several studies, for example, have shown that animals are sensitive to other individuals' direction of gaze and behavioral intentions. However, there is little convincing evidence that any animals — including in particular chimpanzees — can attribute knowledge states to others. Experiments that attempt to address this question in primates [8–10] are difficult to evaluate because of the artificial settings in which they are conducted, the involvement of humans, and repeated testing of the same individuals.

In the wild, chimpanzees form temporary parties that fluctuate in size and composition throughout the day [11,12]. This 'fission–fusion' society would seem to provide an ideal setting for the evolution of a theory of mind. Some individuals can acquire knowledge that others do not have and 'decide' whether to share it or not; others, meanwhile, must determine who knows what.

As they followed a lone chimpanzee in the Budongo Forest of Uganda, Crockford and colleagues [1] guessed where it was about to go and placed in its path a stuffed model of either a Gaboon viper or a rhinoceros viper, two highly poisonous snakes. They then waited until — with luck — the subject discovered the snake and then recorded its vocal behavior as — with more luck — other chimpanzees (termed 'receivers') approached the area.

Subjects were classified as having no prior knowledge about the snake or having some prior knowledge, either because they had already seen the snake or they had been within 50 m when an earlier discoverer had produced an '*alert hoo*' in response