JCB Comment

A Src-astic response to mounting tension

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The nerve growth cone binds to a complex array of guidance cues in its local environment that influence cytoskeletal interactions to control the direction of subsequent axon outgrowth. How this occurs is a critical question and must certainly involve signal transduction pathways. The paper by Suter and Forscher (2001, this issue) begins to address how one such pathway, an Src family tyrosine kinase, enhances cytoskeletal linkage to apCAM, a permissive extracellular cue for Aplysia growth cones. Interestingly, they show that applied tension increases this kinase's localized phosphorylation that in turn further strengthens linkage. This suggests a potential positive feedback mechanism for amplifying and discriminating guidance information to guide growth cone motility.

The nerve growth cone is the sensory motile tip of growing axons (Ramon y Cajal, 1890), and its cellular behavior has been well studied in culture (Jay, 1999). It guides axon growth by moving in response to chemical cues present in the developing embryo (for review see Muller, 1999). Addressing the mechanisms by which this occurs is of fundamental importance in understanding how the vast numbers of neurons that make up the nervous system are correctly wired together. Within recent years there has been a convergence of research on the cellular mechanisms of growth cone motility and on guidance cues and their downstream signals, but how these mechanisms are integrated is not well understood.

Growth cones move by resolving the local imbalance of forces applied across their cytoskeletal connections to membrane receptors and their underlying ligands. Indeed, it has long been known that locally applied tension (via a microneedle) can steer growth cones, and this steering involves F-actin and microtubules (Heidemann et al., 1991). The clutch hypothesis models the action of the forces provided by actin polymerization, retrograde flow, actomyosin-based tension and adhesion at focal contacts (Mitchison and Kirschner, 1988). Protrusion occurs by actin assembly when an actin filament is fixed with respect to the substrate at a contact point (i.e., a clutch is engaged). The clutch is composed of a complex of actin-associated proteins that simultaneously bind actin filaments and receptors for cell or substrate cues (Jay, 1999).

The connection of this clutch to membrane receptors that in turn differentially bind to environmental cues was once thought sufficient to guide growth cone movement. More recently, it has become clear that many of these cues also elicit signal transduction changes (Schuch et al., 1989) that in turn may affect how force is generated or coupled in the growth cone. How guidance cues direct motility via signaling is an important and unresolved question.

Suter and Forscher (2001) address this question using a well-characterized system, the growth cones of Aplysia bag neurons grown on poly-L-lysine in culture. Although this system does not lend itself well to in vivo studies, it is a particularly useful cell biology preparation. The growth cones are relatively stationary (which may accentuate cytoskeletal movement) and are unusually large and flat, permitting beautiful imaging of both actin and microtubules, both by immunocytochemistry and by dynamic imaging. The authors have previously shown, using a restrained bead interaction assay, that apCAM antibody-coated bead can generate cytoskeletal-mediated traction force (Suter et al., 1998). When the bead was pulled by a microneedle, a trail of F-actin and microtubules moved in response, suggesting a linkage between apCAM and the cytoskeleton in the growth cone.

Suter et al. (1998) tested the hypothesis that tyrosine phosphorylation is involved in this linkage. They observed an increase in tyrosine phosphorylation localized around the bead when the apCAM-antibody–coated bead is restrained by a microneedle, but not when the bead is unrestrained. This increase can be inhibited by 2,3-butanedione-2-monoxime, a general myosin inhibitor that can inhibit retrograde flow. Furthermore, Genestein (a general tyrosine kinase inhibitor) and PP1 (specific for Src family kinases) inhibit this increase as well as apCAM-mediated traction force but do not affect retrograde flow. Finally, Suter et al. (1998) showed that steering events generated by pulling on an apCAM-coated bead caused an increase of a phosphorylated Src family kinase around the contact point of the bead and this increase is reduced by PP1. Together, these results argue strongly for an Src family kinase that localizes and activates at an apCAM–clutch linkage in response to applied traction force (Fig. 1 a). This paper ties the cellular mechanical basis of how growth cones move to how guidance cues trigger signal transduction events, two fields of interest that are primed to converge. Although previous work

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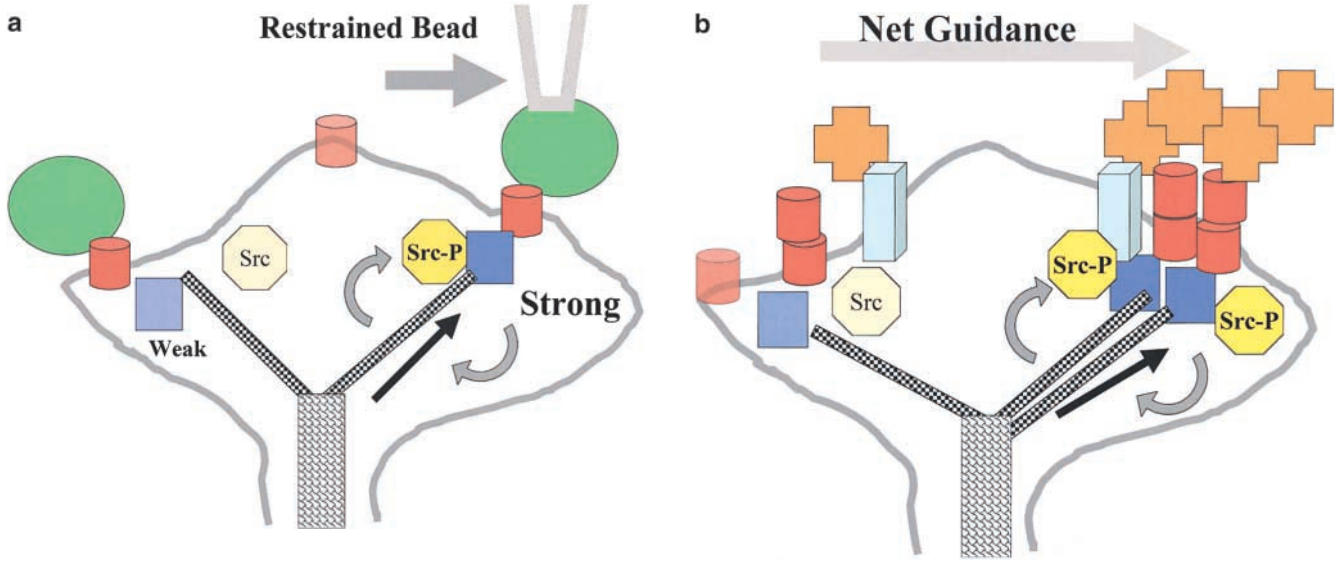

Figure 1. **Tension strengthens cytoskeletal linkage to apCAM via an Src family kinase, a positive feedback mechanotransduction.** (a) In response to tension applied to a restrained AntiapCAM-coated bead (large green circle) attached to apCAM (red cylinders), an Src family kinase (yellow hexagon) is recruited and activated to further increase linkage between apCAM and actin filaments (checked rectangle) via a molecular clutch (blue square). Actin filaments are in turn connected to microtubules (cobbled rectangle). This mechanotransduction establishes a positive feedback that increases cytoskeletal coupling in response to applied tension (gray curved arrows) and may guide subsequent axon outgrowth. (b) The positive feedback observed in Fig. 1 a may have a role in amplifying differential responses to concentration or interconnectivity of guidance cues. Such mechanisms may allow integration of different cues such as CAMs (red cylinders) and extracellular matrix (orange crosses). Differences in tension could locally enhance the clutch at either CAMs or integrins (light blue rectangles) via an Src family kinase (yellow hexagons). The strength of binding of cues or the compliance of their underlying substrate could increase tension to further enhance coupling at that site. This positive feedback mechanotransduction (gray curved arrows) would act both as a signal amplifier and more importantly a discriminator. This would enhance cytoskeletal movement toward regions of higher effective binding or compliance resulting in a net guidance decision.

has implicated tyrosine phosphorylation (Wu and Goldberg, 1993; Worley and Holt, 1996) and Src family kinases (Ignelzi et al., 1994) in neurite outgrowth, none until now has provided evidence for a potential mechanism of action.

Of particular interest is the demonstration by Suter et al. (1998) that applied tension can differentially increase the clutch (an internal mechanism) such that traction force generation can be more efficient. This positive feedback mechanism is not dependent solely on adhesion but also on the generation of tension internally. Such a positive feedback mechanism could enhance small local differences in binding or compliance to increase the resulting statistical bias such that a guidance decision occurs.

Links between applied tension and receptor–cytoskeleton interactions and signaling have been observed before. Using laser-trapped fibronectin beads, Choquet et al. (1997) showed a localized proportional strengthening of the cytoskeletal linkages to occupied integrin in response to applied tension, and pharmacological inhibition suggested a role for dephosphorylation. Additionally, applied mechanical stress was shown to modulate cyclic AMP signaling (and subsequent transcriptional regulation) when integrin receptors were occupied (Meyer et al., 2000). Recently, Balaban et al. (2001) have correlated force compliance between cell and substrate with the assembly of focal adhesions. Together, these studies and the current paper suggest that applied tension can alter signaling pathways to modulate cell behavior, and this may be a general mechanism.

There may be a physiological role for the mechanotransduction observed in this paper and for that previously observed (Choquet et al., 1997). An unresolved issue is how growth cones make distinct guidance decisions in a smeared and complex spatial distribution of guidance cues. How a growth cone reads gradients is not clear but it is thought that these gradients bias the direction growth cone movement over time. The growth cone may use increased cytoskeletal linkage upon localized binding to a favorable substrate to enhance the statistical bias when selecting its direction of subsequent movement (Fig. 1 b). The strength of binding of cues or the compliance of their underlying substrate could increase tension to further enhance coupling at that site. This positive feedback mechanotransduction would act both as a signal amplifier and more importantly a discriminator. We have seen marked advances in methods for measuring local traction forces (Dembo et al., 1996) and observing the dynamic localization of signaling molecules and binding interactions (Balaban et al., 2001), and it will be interesting to correlate localized changes in signal transduction events with local traction forces to test this idea.

Although this work is compelling, many questions remain. How might tension activate or localize a tyrosine kinase? It is easy to envision that tension applied across a protein complex may cause conformation changes that reveal or enhance docking or phosphorylation sites. This has not yet been demonstrated and would require visualizing changes in a single molecular complex held under tension. How does a single growth cone integrate a diverse array of

extracellular information? Is there an interplay between CAM-mediated systems studied here and those mediated by integrin (Choquet et al., 1997)? This interplay will be complex. Some studies suggest that tyrosine kinases affect CAMs and integrins differentially (Williams et al., 1994), whereas others suggest that Src may have similar effects at least on certain substrates (Felsenfeld et al., 1999). Also, it is not known if similar signaling mechanisms act in the transmission of repulsive cues, and it will be important to address how growth cones read positive and negative cues in the same environment.

How do different neurons contacting the same guidance cues respond differentially? With regard to tyrosine kinases, there is a remarkable complexity and context dependence for growth cones. For example, neurite outgrowth from neurons from different Src family knockout mice is different, but only on specific substrates (Ignelzi et al., 1994). This observation is further confounded by compensatory increases in other Src family members in response to knockout (Grant et al., 1992). Worley and Holt (1996) observed either increase or decease in neurite outgrowth in response to tyrosine kinase inhibition depending on the cell type. Other signal transduction pathways must also impinge on these growth cone decisions. Growth cone response to a wide array of guidance cues can be switched from attraction to repulsion by altering intracellular cyclic nucleotides (Song et al., 1998).

These complexities illustrate the need to segregate which kinase does what and whether other signaling pathways converge on coupling or other cellular events during guidance. Furthermore, an in-depth functional analysis of the molecular clutch is required to establish how an Src family kinase strengthens the clutch. In the field of signal transduction, we have been better at assessing what can occur than what does occur during cellular processes. We need to assess how growth cones integrate their complex in vivo cues to address if the mechanisms of mechanotransduction seen in culture are physiologically important.

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