CELL BIOLOGY

# Reach Out and Touch Someone

Cellular extensions called cytonemes may allow nonneuronal cells to communicate directly with each other over long distances.

**Pernille Rørth**

How do cells in the body communicate over long distances? Neurons do so through long cellular extensions—axons and dendrites—that establish direct contacts (synapses) with target cells. Other cells are thought to receive information through secreted signaling molecules that diffuse through a tissue. For example, cells that become the fingers of the hand receive patterning information from one side of the limb primordia, with the proximal cells receiving more signal than the distal ones and, as a consequence, taking on a different digit identity. On page 852 of this issue, Roy *et al*. (*1*) provide evidence that signal-receiving cells do not passively wait, but reach out and grab the signal by making direct contact with signal-sending cells (see the figure).

Patterning a field of cells by concentration-dependent signals from a localized source makes intuitive sense. Thus, the suggestion that long, thin cellular extensions, specialized filopodia called cytonemes, might be responsible for such patterning was considered somewhat unorthodox (*2*). Cytonemes were initially described in *Drosophila* cells that respond to Decapentaplegic (Dpp), a signaling molecule that patterns imaginal discs (flat, epithelial tissue from which adult structures develop). Subsequently it was discovered that individual cytonemes differ and display receptors appropriate for the signal they perceive (*3*). This and the present work mostly involved the study of *Drosophila* air sac cells. Like vascular cells in vertebrates, they make many long filopodia.

Roy *et al*. use the reconstitution of a split fluorescent molecule, one part attached to signaling cells and the rest to distant receiving cells, to show that these cells are in physical contact. After contact, endocytosis of activated receptors returns them to the body of the receiving cell. Mutations that perturb formation of cytonemes or proper cell-cell contacts ("synapses") interfere with signaling, suggesting that these long-distance cell contacts are important. Such contact-mediated signaling could convey spatial information from a source to a field of cells if there are more short cytonemes than long ones, or if the signal is attenuated with cytoneme length.

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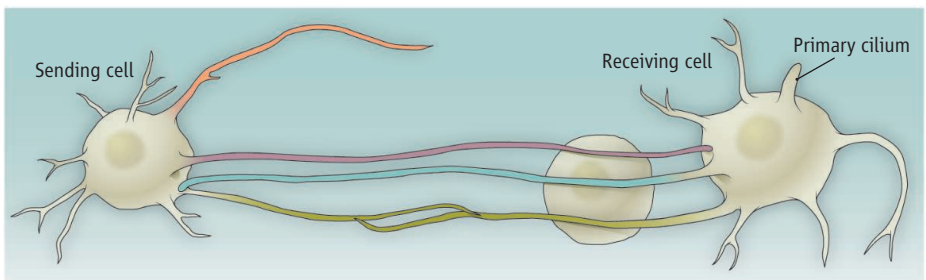


**Membrane extensions in signaling.** Filopodia-like cytonemes with different receptors or signaling molecules (different colors) are made by signal-receiving and -sending cells, and make direct cell-to-cell contacts.

Long, thin, actin-rich cellular membrane extensions classified broadly as filopodia are known from many contexts. "Tunneling nanotubes" directly connect distant cells in culture and allow mechanical and $Ca^{2+}$-based coupling directly or via gap junctions (*4*–*6*). And signal transfer decreases with nanotube length. Nanotubes transfer endocytic vesicles and F-actin, whether directly or by transcytosis. Thus, the parallel to endocytosis-dependent cytoneme signaling is striking.

Cytonemes have been implicated not just in receiving signals, but also in sending signals, as illustrated in two recent studies of the signaling molecule Hedgehog (Hh in *Drosophila* and Shh in vertebrates) (*7*, *8*). Careful genetics and quantitation in *Drosophila* (*8*) and sophisticated live imaging in chick embryos (*7*) provide evidence that the lipid-modified Hh/Shh proteins spread by traveling in, or on, cytonemes. The chick study also examined signal-receiving cells and found that filopodia from sending and receiving cells make direct contact (*7*). In light of the present study this is intriguing, but quantitation of such contacts and evidence for functional relevance are needed.

Intriguingly, Shh signaling is associated with another membrane-bound cellular extension in signal-receiving cells. In vertebrates, but not *Drosophila*, Shh signal transduction appears to occur at the primary cilium (*9*–*11*). The primary cilium is quite different from filopodia. It is a single, stable, microtubule-based structure. It is not known whether Shh-carrying filopodia contact the cilium to transfer signal; perhaps they are functionally independent specializations.

Knowing that both sending and receiving cells use cytonemes prompts consideration of what this mode of signaling contributes. Roy *et al*. argue, but do not strictly prove, that cytoneme-mediated contact is required for signaling. Cytonemes might find their target cells by chemotaxis, sensing the secreted molecules. Alternatively, cytonemes might initially grow in an unguided manner and selectively stabilize contacts with the appropriate signaling cell. Only quantitative live imaging can discriminate between these alternatives.

Long-range filopodia also play a role in membrane-tethered signaling interactions between Delta and Notch (*12*). Here, modeling and quantitative live imaging show that the dynamics of filopodia are required to refine patterning (*13*). Compartmentalization by cytonemes with only one type of signaling receptor might also allow highly sensitive signal detection, unaffected by other pathways.

Signaling by touch has several features not afforded by signaling at a distance using secreted molecules. Signaling events can include gap junctions and $Ca^{2+}$-based signals, as seen with nanotubes. The local membrane context can be sampled for the presence of the appropriate mechanical characteristics or adhesion molecules, for example, and this may direct signals only to specific recipient cells. And localized signaling may minimize signal loss. However, restricting signaling through a limited number of cytonemes also makes signaling more stochastic. The "private conversations" thus set up may not reach all intended recipients. Making the cytoneme contacts dynamic allows broader target sampling and, possibly, signal averaging over time. The stochastic differences between signals received may thus be reduced. But the one-on-one restricted signaling interactions may also be used actively, to make each cell-signaling contact unique.

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Direct communication between adjacent cells underlies tissue organization. Communicating with a dispersed community of cells presents quite different challenges: Decisions must be made about which cells to communicate with, and how. Cytonemes, nanotubes, and other filopodia-like structures can be used for long-distance communication, but there is still limited information about their biological importance. The most informative experiments, like selective disruption of connections and detailed analysis of the consequences, are also the hardest to carry out. Direct communication allows private cell-to-cell conversations: Freely transmitting the signal makes it simpler to reach many cells. Employing a combination of these two signaling mechanisms may optimize strategies for decision-making in development. Even the nervous system, with its elaborate and sophisticated use of long-distance cell-cell connections, also uses dispersed signals to modulate general outputs such as mood and other emotions.


**References**

1. S. Roy, H. Huang, S. Liu, T. B. Kornberg, *Science* **343**, 124462 (2014); 10.1126/science.1244624.
2. F. A. Ramírez-Weber, T. B. Kornberg, *Cell* **97**, 599 (1999).
3. S. Roy, F. Hsiung, T. B. Kornberg, *Science* **332**, 354 (2011).
4. A. Rustom, R. Saffrich, I. Markovic, P. Walther, H. H. Gerdes, *Science* **303**, 1007 (2004).
5. S. C. Watkins, R. D. Salter, *Immunity* **23**, 309 (2005).
6. X. Wang, M. L. Veruki, N. V. Bukoreshtliev, E. Hartveit, H. H. Gerdes, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17194 (2010).
7. T. A. Sanders, E. Llagostera, M. Barna, *Nature* **497**, 628 (2013).
8. M. Bischoff *et al.*, *Nat. Cell Biol.* **15**, 1269 (2013).
9. D. Huangfu *et al.*, *Nature* **426**, 83 (2003).
10. K. C. Corbit *et al.*, *Nature* **437**, 1018 (2005).
11. R. Rohatgi, L. Milenkovic, M. P. Scott, *Science* **317**, 372 (2007).
12. C. de Joussineau *et al.*, *Nature* **426**, 555 (2003).
13. M. Cohen, M. Georgiou, N. L. Stevenson, M. Miodownik, B. Baum, *Dev. Cell* **19**, 78 (2010).


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STRUCTURAL BIOLOGY

# Unraveling a Flavivirus Enigma

The structure of a flavirirus nonstructural protein provides mechanistic understanding for many of its functions.

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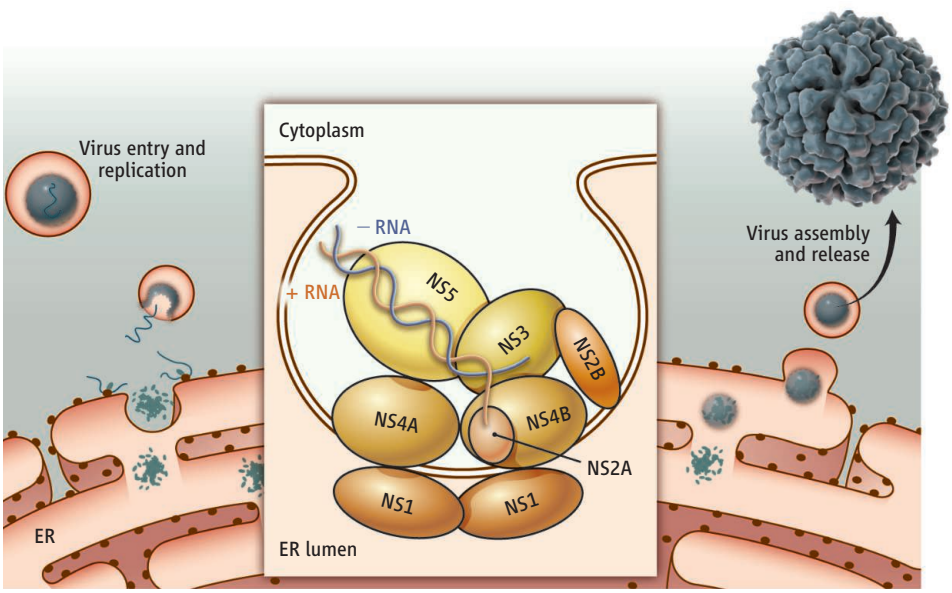
There is growing concern about the spread of flaviviruses, such as dengue virus and West Nile virus, to new geographic areas as they can cause major epidemics and represent global public health threats. Controlling these viruses requires a better molecular understanding of how they infect cells. Nonstructural protein 1 (NS1) is perhaps the most enigmatic flavivirus protein. During infection, NS1 exists in two distinct forms, travels to various compartments, decorates itself with different molecular disguises, and plays numerous roles in its infectious cycle and disease pathogenesis (*1*). How this protein manages all of this has been a puzzle since its discovery in 1970 (*2*). Crystallizing NS1 has daunted many researchers because of the heterogeneity of its glycosylation and association with lipids, but as reported on page 881 of this issue, Akey *et al.* (*3*) have accomplished this task. The unusual structural details revealed about NS1 may guide the design of compounds that inhibit viral replication and provide clues as to how it contributes to different stages of the virus life cycle and disease.

Flavivirus NS1 is a glycoprotein with a molecular mass of 46 to 55 kD, depending on its glycosylation status. The crystal structures of dengue virus NS1 (3 Å resolution) and West Nile virus NS1 (2.8 Å resolution) exhibit a similar hexameric arrangement of three dimers, confirming the hexameric structure (30 Å resolution) indicated by a cryoelectron-microscopy analysis (*4*). Each monomer displays an unusual fold consisting of three regions: a "β-roll" domain that dimerizes with that of another monomer; a "wing" domain that resembles a helicase domain; and a "β-ladder" domain that aligns with that of another NS1 molecule to form an extended β-sheet ladder. The ladder forms the plane of the NS1 dimer, with a hydrophobic side (exemplified by a "greasy finger" loop) that can associate with the membrane. The hydrophobic side of each dimer faces the interior of the hexamer. Remarkably, recombinant NS1, which does not possess any transmembrane domain, can convert large liposomes into smaller lipid-protein nanoparticles. This demonstrates that NS1 can directly modulate the lipid membrane without additional cellular proteins. Such lipid-modulation activity and its underlying structure could account for the myriad functions of NS1.

After flavivirus entry into a cell by endocytosis, the virus particle is released into the cytoplasm. Viral genomic RNA is translated into proteins and replicated, and virus assem-




**Assist before leaving.** Flavivurus replicates at the ER surface in the infected cell. Viral NS1 protein forms dimers in the ER lumen, yet assist the replication complex on the opposite side of the membrane. Seven nonstructural proteins, together with host proteins (not shown), form the replication complex. Once immature viral particles bud into the secretory pathway, NS1 protein forms hexamers that are secreted as lipoproteins.

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