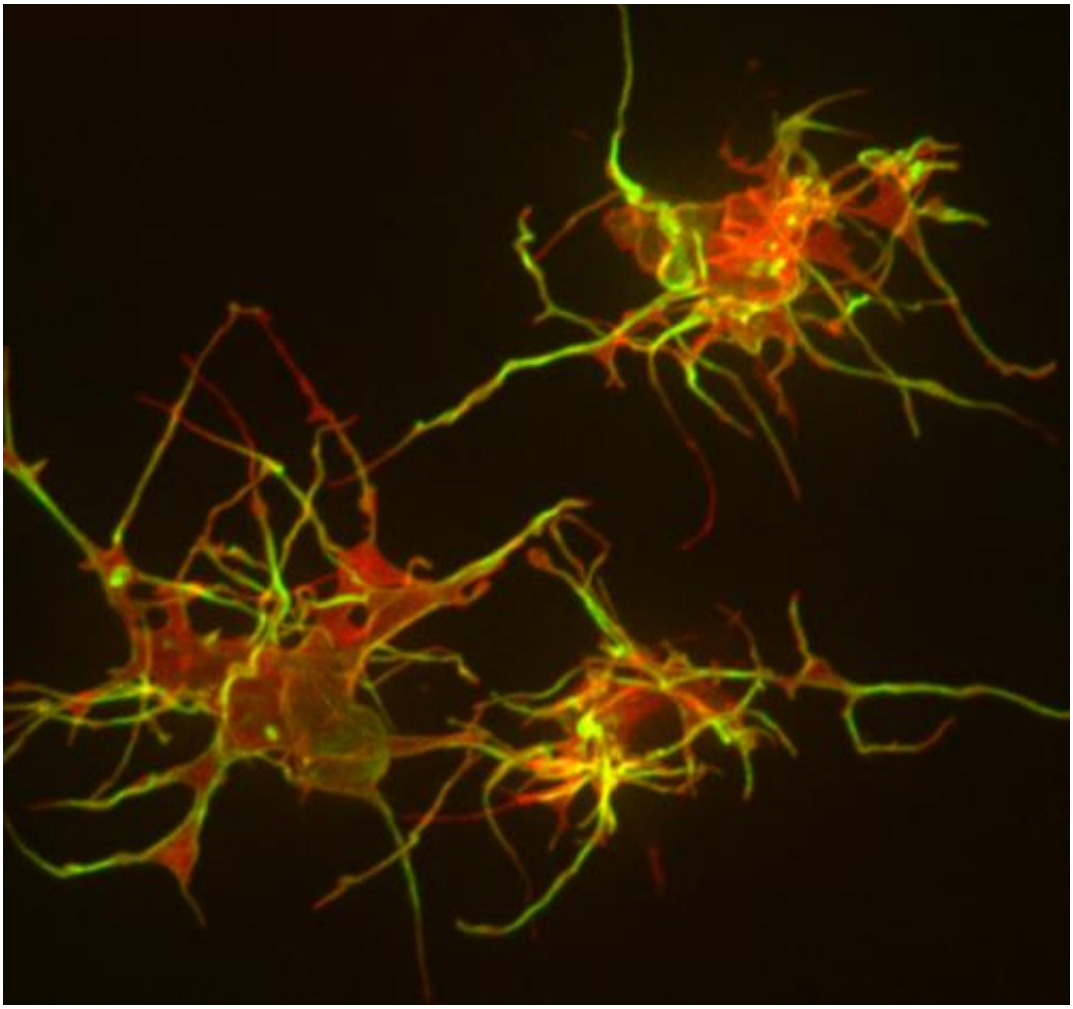
Discovery alters understanding of long-distance intercellular communication

April 29 2013, by Krista Conger



These mesenchymal cells from a developing chick embryo have developed long, finger-like projections that reach out across relatively long distances to touch those of other cells. This membrane-to-membrane contact allows the direct exchange of signaling molecules between cells separated by several neighboring cells. Credit: Esther Llagostera

(Phys.org) —In a finding likely to fundamentally reshape biologists' understanding of how vertebrate cells communicate, researchers at the Stanford University School of Medicine and the UC-San Francisco have discovered a new type of cellular structure that directly delivers and receives payloads of signaling molecules between distant neighbors in a developing embryo.

The seeming specificity of the interaction contrasts starkly with the commonly held notion that signaling molecules are released from one cell and float, or diffuse, through the intercellular space to their targets. While this finding does not preclude the use of diffusion as a signaling method, it identifies another new, surprising avenue of long-distance cellular communication.

The research was published online April 28 in *Nature*.

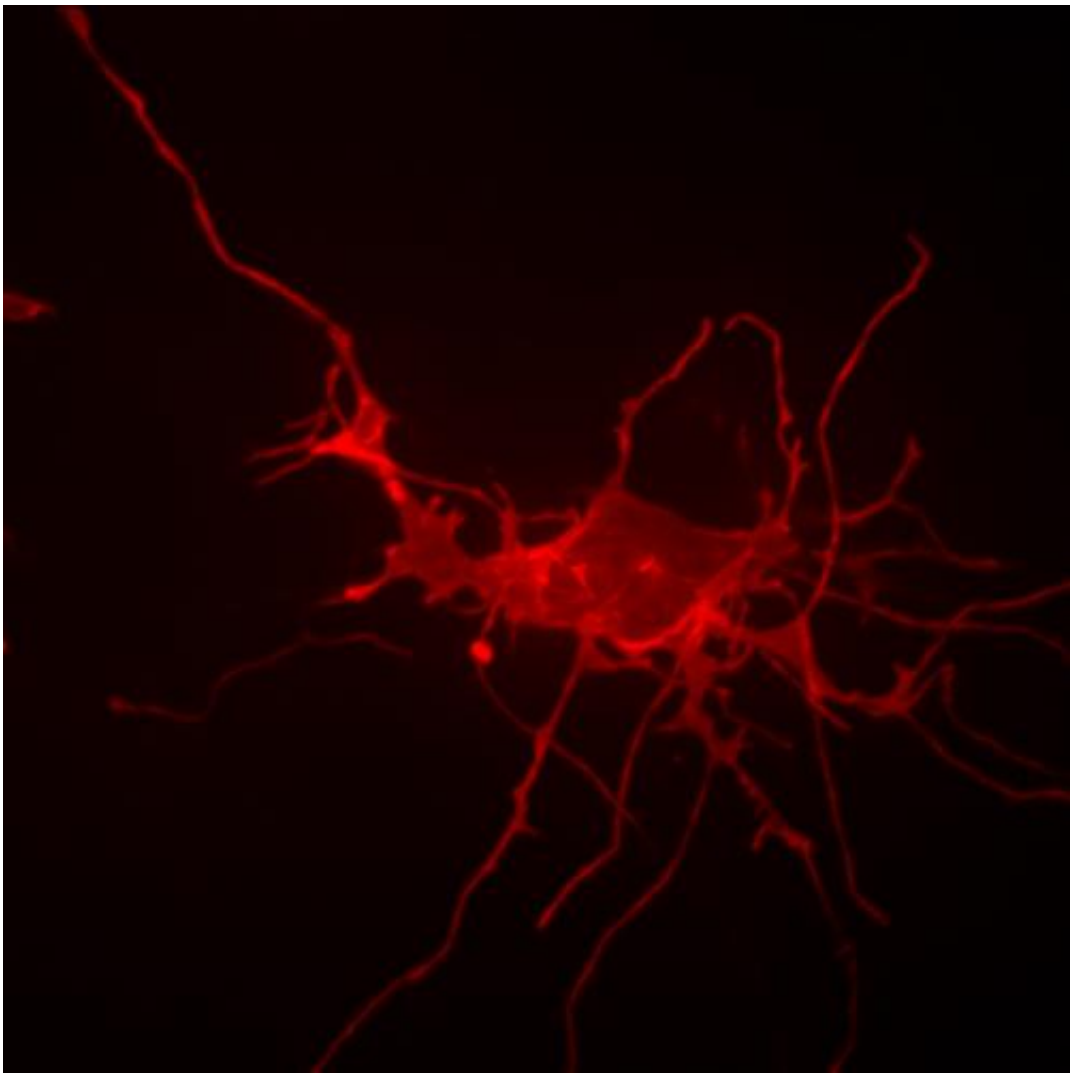
"The presence of these structures, which we call cytoplasmic extensions or a type of specialized filopodia, was really unexpected," said Maria Barna, PhD, an assistant professor of developmental biology and of genetics at Stanford. She and her colleagues used high-resolution, real-time imaging techniques to see the previously invisible extensions that form finger-like projections. These projections are destroyed by conventional techniques that are used to preserve tissues.

"We now know that the morphology of a typical cell in a vertebrate embryo looks very different than what we would have imagined," said Barna. "These extremely fine, very long extensions can reach several cell diameters away. We have visualized these fascinating structures in mesenchymal cells but they may be a more generalized feature of many additional cell types." (A mesenchymal cell arises from the mesoderm —one of three main tissue types in the developing vertebrate

embryo—to become connective tissue, cartilage, bone and some blood cells.)

The researchers believe that cells use cytoplasmic extensions as a means of targeted delivery of key signaling molecules either alone or in concert with free diffusion of molecules.

"This work suggests that the cells within the embryonic limb tissue are interconnected by a dense network of dynamic extensions that mediate communication across a broad terrain," said Deborah Yelon, PhD, professor and vice chair of cell and developmental biology at UC-San Diego. "This 'network' view of long-distance signaling is a striking contrast to previous 'signal diffusion' or 'bucket brigade' models and has the potential to revamp the textbook view of the mechanisms of tissue patterning." Yelon was not involved with the study.



The finger-like projections on the cells were previously invisible to researchers using conventional imaging techniques. The scientists have shown that the extensions, which they call specialized filopodia, deliver and receive signaling molecules over relatively long cellular distances. Credit: Esther Llagostera

Barna and her colleagues found that the newly identified, nearly invisible finger-like projections burrow through tightly packed tissue in developing chick and mouse embryos for several cell-lengths. Once contact between cells through these structures has been made, signaling molecules can be delivered directly from one cell to another.

These cytoplasmic extensions, which repeatedly stretch and retract until contact is made, allow cells separated by five or more intervening cells to communicate directly though their respective membranes, and may introduce an unprecedented degree of specificity in signal delivery in vertebrates.

"It's long been known that, in many tissues and organs, there is a very small subset of progenitor cells that instruct neighboring cells how to differentiate into various cell types" said Barna. "To do so, they secrete signaling molecules that have to move through very densely packed cells to reach their targets. The cellular mechanisms for controlling the dissemination of this information are critical, but not well understood."

The researchers believe the extensions are similar to small, rod-like cellular structures called filopodia that protrude beyond the membrane of some cells to participate in cell migration and cellular interactions. However, the newly discovered filopodia are longer, thinner and more dynamic than any previously described and have unique cellular features. "In fact, they may more closely resemble signal-delivering tubular structures called cytonemes seen in insect embryos," said Barna.

Barna and her colleagues began the study by wondering how signaling molecules important in development, such as one called sonic hedgehog, or SHH, move from cell to cell in a developing chick embryo. To track the process, they needed to see whether they could visualize individual cells in real time at high resolution. They used fluorescent, membrane-bound proteins to outline individual mesenchymal cells in the developing limb of a living chick embryo—essentially tracing its boundaries against a backdrop of unlabeled neighbors. When they used their custom-made, high-power microscopes to visualize the cells, they were startled to see long, finger-like projections burrowing through the surrounding tissue.

"As we watched, the structures would rapidly extend and retract until

they found one another and formed a synapse-like connection between two distant cells," said Barna. "We realized that they could serve as conduits for cellular interactions." Forming the connection seemed to stabilize the structures, which maintained the contact for several minutes or longer.

They next labeled the sonic hedgehog molecule itself, which, surprisingly, appeared as a relatively large particle. They watched as the particle moved away from the originating cell along the length of the projection to the outstretched, finger-like projection of a recipient cell. Closer investigation showed that the recipient cell expressed SHH receptors at discrete points along the length of its filopodia. What's more, the speed and efficiency of SHH delivery along the length of the extension suggests the involvement of an actin-based motor—somewhat like a conveyer belt.

"These filopodia serve as conduits for highly organized, directional movement within tissues and three-dimensional space," said Barna. "Although one cell can have many—for example, eight to 10 of these structures—they may be divided into subsets dedicated to specific signaling molecules. For example, SHH and its receptors are found on one of the cell's eight or 10 filopodia. So there's a lot of sorting that's happening."

Barna and her colleagues are now exploring whether other types of cells also have these specialized filopodia that may have not been previously visualized, and how the structures may contribute to pathological processes like cancer and metastasis.

"This finding opens up a new frontier in cell and developmental biology ," said Barna. "How are these types of filopodia formed? What types of cellular machinery are needed to allow them to extend and retract so quickly? What type of cargo can move along these structures? We are

really just scratching the surface of what we can learn."

More information: Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning, DOI: 10.1038/nature12157

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