CellPress

Special Issue: 25 Years of Trends in Cell Biology

Review

Signaling by Cellular Protrusions: Keeping the Conversation Private

Michael Buszczak,[1,*] Mayu Inaba,[1,2] and Yukiko M. Yamashita[2,*]

Information exchange between different cells makes multicellular life possible. Signaling between cells can occur over long distances, as in the case of hormone signaling, or it can take place over short distances between immediately juxtaposed neighbors, as in the case of stem cell-niche signaling. The ability of signal-sending and -receiving cells to communicate with one another in a specific manner is of paramount importance in the proper development and function of tissues. Growing evidence indicates that different cellular protrusions help to achieve specificity in signaling that occurs between distinct cell types. Here, we focus on new roles for cellular protrusions in cell-to-cell communication, drawing special attention to how stem cells use specialized extensions to promote reception of self-renewing signals emanating from the niche.

Trends

Cytonemes are specialized filopodia found in diverse tissues that promote signaling between specific cells over varying distances.

Tunneling nanotubes (TNTs) share some structural similarities with cytonemes, and can traffic diverse cargos such as mitochondria, endosomal vesicles, viruses, and Ca^{2+}.

Microtubule-based nanotubes (MT nanotubes), formed by *Drosophila* germline stem cells, provide an exclusive surface area for productive signaling between niche cells and stem cells.

Emerging Roles for Cellular Protrusions in Cell-to-Cell Signaling

The ability of cells to communicate with each other to coordinate their activity (e.g., proliferation, cell fate determination, migration) is of fundamental importance to the formation and operation of multicellular organisms. During tissue development and homeostasis, specific cells produce signaling proteins that instruct target cells to adopt particular fates and behaviors. Similar signaling specificity is also observed in stem cells niches. Niche cells must instruct stem cells to self-renew, while excluding closely positioned differentiating progeny of stem cells from receiving these same signals. Given the cellular complexity of tissues, a multitude of signal-sending and -receiving combinations exist; yet, only a handful of signaling pathways, such as Wnt, Notch, Hedgehog (Hh), Egfr (epidermal growth factor receptor), and cytokine pathways are known to regulate these processes. Accordingly, the specificity of cell–cell signaling cannot be achieved simply by choosing a single signaling pathway that is dedicated to a particular cell–cell combination. Although much has been learned about how cells communicate with one another, the mechanisms that ensure the selectively of these interactions remain poorly understood. For example, while we know how interactions between specific ligands and receptors elicit signaling cascades within the cell, we know less about how the right types of cells respond to the right types of signals in a complex environment.

In recent years, cellular protrusions have emerged as a means by which communication between cells can be conducted in a highly specific manner. Among these specialized protrusions are cytonemes [1–3], tunneling nanotubes (TNTs) [4,5], and microtubule-based nanotubes (MT nanotubes) [6]. These protrusions can be distinguished based on their diameter and

[1]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9148, USA
[2]Life Sciences Institute, Department of Cell and Developmental Biology Medical School, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109, USA

*Correspondence:
michael.buszczak@utsouthwestern.edu (M. Buszczak) and
yukikomy@umich.edu (Y.M. Yamashita).

 http://dx.doi.org/10.1016/j.tcb.2016.03.003
© 2016 Elsevier Ltd. All rights reserved.

CrossMark

Table 1. Comparison of Cellular Protrusions

Name	Cytonemes	Tunneling Nanotubes	Microtubule Nanotubes	Primary Cilia
Cytoskeletal components	Actin	Actin or microtubules	Negative for acetylated tubulin	9+0 microtubule organization, positive for acetylated tubulin
Size	0.1–0.4 μm in diameter up to 700 μm in length	<0.7 μm in diameter ~1000 μm in length Microtubule based: >0.7 μm in diameter ~1000 μm in length	~0.3 μm in diameter ~6 μm in length	~0.25 μm in diameter ~30 μm in length
Genes for formation	Diaphanous, shibire, neuroglian, and capricious SCAR/WAVE CP (cpa or cpb) Pico/Lamellipodin	No known universal mechanism. Some TNTs require M-sec, RalA, LST1, and Cdc42	IFT	IFT
Signals and cargo	Dpp, FGF, EGF, Shh	Ca^{2+}, mitochondria, endosome, lysosome, virus, prion, bacteria	Dpp	Hh, Wnt, Notch
Refs	[1–3,19]	[30,33,34,45,56–60]	[6]	[52–54]

length, and by cytoskeletal elements involved in their formation, that is, actin filaments or microtubules (Table 1). Instead of 'broadcasting' signals from the source cell to a plethora of cells by simple diffusion, cellular protrusions allow signals to be transmitted from a source cell to target cells in a selective manner over a range of distances. For example, protrusions can form between immediately juxtaposed neighbors or between cells positioned far apart (across multiple cells). In both cases, the specificity excludes other cells from engaging in the conversation. Here we review recent progress in our understanding of signaling protrusions and discuss how they may promote specific cell–cell communication in multicellular organisms during development and tissue homeostasis. In particular, we focus on how signaling protrusions mediate short-range signaling as observed in stem cell-niche signaling.

Cytonemes: Specialized Filopodia Promote Long-Range Signaling

Multiple types of signaling protrusions with specific functions have been identified to date. These protrusions can be distinguished based on their diameter and length, and by the cytoskeletal elements involved in their formation, that is, actin filaments or microtubules (Table 1). Among them, cytonemes are actin-based thin thread-like (hence the name) structures that typically have a diameter of 0.2 μm and a broad range of lengths that can reach up to 700 μm [7]. Cytonemes can be labeled with both soluble- and membrane-bound GFP in living tissue and appear particularly sensitive to fixation, which may explain why they evaded detection for so long.

In general, cytonemes function to transmit signals between two cells that are positioned far away from each other. These long cellular extensions were first observed to protrude from *Drosophila* wing imaginal disc cells, where they promote signaling such as bone morphogenic protein (BMP), fibroblast growth factor (FGF), and Hh that are essential for tissue pattern formation. Since then, cells from a variety of *Drosophila* larval tissues have been shown to form cytonemes or cytoneme-like structures (Figure 1A, Key Figure), including eye imaginal discs, air sac primordium (ASP) in the tracheal system, and cells of the abdominal epidermis [2,7–12]. Early studies found that cytonemes emanating from laterally positioned cells within the wing disc oriented towards the A/P or D/V compartment boundaries [7]. The directionality of cytoneme orientation suggested that they form in response to a localized source of chemoattractant. Indeed, FGF and Decapentaplegic (Dpp), a BMP family member, both promote cytoneme formation in the wing disc, while cells within different tissues form cytoneme-like structures in

Key Figure

The Structure and Function of Signaling Protrusions

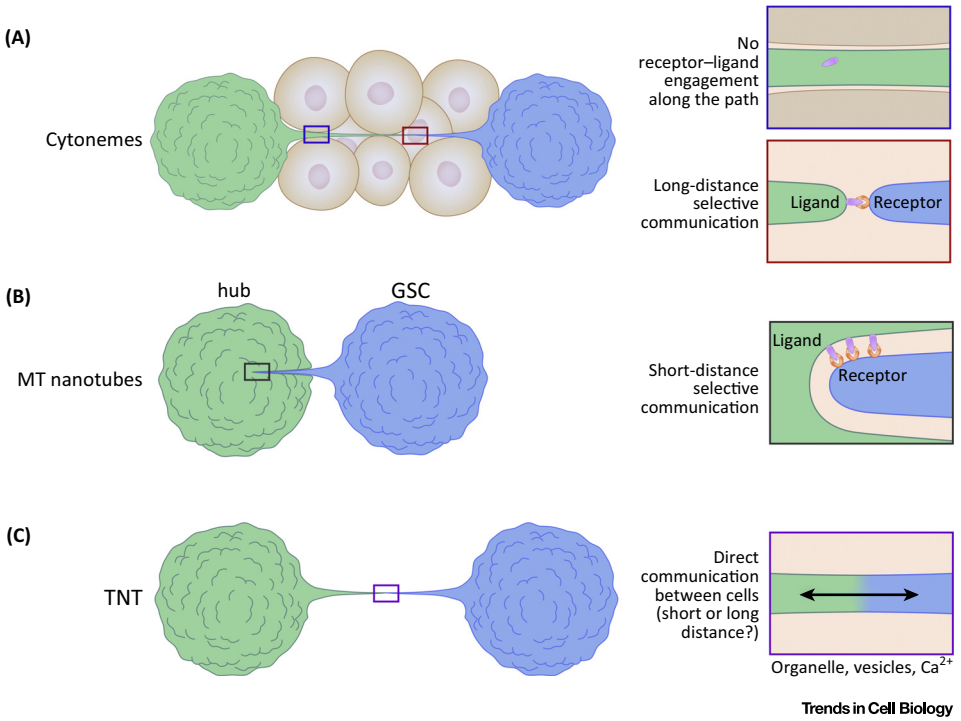

Figure 1. (A) Cytonemes were originally found in *Drosophila* imaginal discs. Cytonemes transport ligands and receptors such that cells at a distance from one another can directly and specifically communicate within the context of complex tissues. Similar 'specialized filopodia' have been discovered in vertebrate embryos. (B) Microtubule-based nanotubes (MT nanotubes) are found in *Drosophila* male germline stem cells (GSCs). MT nanotubes protrude into the hub cell niche. The bone morphogenic protein (BMP) ligand (Decapentaplegic, Dpp) produced by hub cells interacts with the receptor Tkv presented on the surface of MT nanotubes, leading to stem cell-specific activation of Dpp signaling. (C) Tunneling nanotubes (TNTs) are found in various types of cells, mainly in culture. TNTs function as cellular 'conduits' between cells to transport mitochondria, vesicles, and Ca^{2+}, among other factors.

response to Notch and Hh [13,14]. Moreover, the formation of cytonemes appears to be specific for distinct signaling pathways [10]. Co-labeling experiments performed in the eye imaginal disc revealed that an EGFR–GFP fusion protein specifically labels a subset of the total population of cytonemes within a given cell. Disruption of EGF signaling or ubiquitous expression of an activated form of EGF ligand (cSpi) led to the formation of short cytonemes extending in all directions. Similarly, overexpression of *dpp* in wing discs and overexpression of Bnl (a *Drosophila* FGF ligand) within cells of the ASP resulted in the formation of short cytonemes in all directions, suggesting that a defined and limited source of ligand promotes directional growth and/or stabilization of cytonemes. Double-labeling experiments using Tkv–GFP and BTL–Cherry fusion proteins provided compelling evidence that different signaling components traffic in and out of cytonemes in a highly regulated manner. However, the basis of this selectivity remains poorly understood.

The formation of cytonemes or cytoneme-like structures has also been observed in vertebrate tissues. For example, a recent study showed the presence of long thin filopodia in chick embryos [15]. Similar to results obtained using *Drosophila*, Sonic Hedgehog (Shh), which plays a crucial

role in pattern formation during early embryogenesis, formed distinct particles that remained associated with the signal-sending cell through these long thin cytoplasmic protrusions, which often extended across several cell diameters. Live-cell imaging showed that these particles moved along these cytoneme-like extensions in a net anterograde direction. Signal-responding cells also formed thin filopodia-like structures that contained specific subsets of Shh coreceptors. These extensions reached out to make contact with those extending from Shh-producing cells, suggesting that these protrusions assist with both sending and receiving specific signals over distances of many cell diameters. Cytoneme-like structures have also been observed in other vertebrate systems such as zebrafish and mice. In zebrafish embryos, thin projections, which were generated as a remnant of cell division, connected a significant fraction of epiblast cells, allowing for protein transfer between cells [16]. Also in zebrafish embryos, Wnt8a and its receptor Frizzled localized on specialized filopodia, which likely influenced neural plate pattern formation [17]. Furthermore, in mouse embryos, opposing non-neural ectoderm cells extended thin projections during neural tube closure, forming a bridge between them [18]. These results suggest that specialized filopodia or cytonemes form in different cell types across species.

Besides helping to establish signaling gradients within developing tissues over distances of many cell diameters [19], cellular protrusions have also been implicated in paracrine signaling between stem cells and their supportive niche cells. Work in the *Drosophila* ovary suggests that cap cells, which form the germline stem cell (GSC) niche, extend cytoneme-like extensions to communicate with their somatic cell neighbors [20]. These extensions appear to mediate transport of Hh ligands from cap cells to a second population of neighboring somatic cells called escort cells. In turn, Hh signal transduction in escort cells helps to promote the maintenance of GSCs. Disruption of actin polymerization, through transgenic expression of a constitutive form of Diaphanus or myristoylated Wasp within cap cells, results in a loss of these cellular protrusions. Together these results suggest that cap cell cytonemes likely share common features with those first described in *Drosophila* imaginal discs.

More recent work suggests that Lgr4 and Lgr5, markers and important regulators of a number of different stem cell populations in mammals, promote the formation of cytonemes in cell culture. [21]. However, the extent to which Lgr4- and Lgr5-mediated stem cell signaling relies on cytonemes in adult niche cells *in vivo* remains an open question.

MT Nanotubes: Sipping Signals from the Niche

Adult stem cells help to maintain tissue homeostasis. These stem cells often reside in specialized microenvironments, or niches, that specify stem cell identity [22]. Niches produce a variety of signaling molecules and growth factors that keep resident stem cells in an undifferentiated state. Current models suggest that niche signaling is short-range in nature, thus limiting the self-renewal capacity and proliferation of stem cells to a physically confined space. Accordingly, cells produced by stem cell divisions that are displaced outside the niche space will undergo differentiation. Restraining niche signaling in this manner likely prevents overproliferation of stem cells, thus reducing the likelihood of tumorigenesis [23].

Despite the appeal of these models, stem cell-niche signaling often involves ligand–receptor combinations that, in other contexts, act over relatively long distances. However, little is known about how stem cell-niche signaling is spatially confined such that only stem cells receive self-renewing signals, while non-stem cells are restricted from gaining access to signals emanating from the niche.

Insights into possible mechanisms that underlie the specificity of niche-stem cell signaling have come from the study of model systems. For example, *Drosophila* male GSCs reside at the apical

tip of the testis, where they attach to a cluster of postmitotic somatic cells called the hub. Hub cells function as a major component of the stem cell niche by secreting at least two ligands, Upd, a Janus kinase (JAK)–Signal Transducer and Activator of Transcription (STAT) pathway ligand, and Dpp, a BMP ligand [24]. Although these ligands are thought to diffuse over a long-range in other contexts [25–29], they act within an extremely short-range (just one cell diameter) in the GSC niche to limit the self-renewal of stem cells to a physically confined space.

Live-cell imaging experiments revealed the presence of thin protrusions that extend from male GSCs and project into the hub cell cluster (Figure 1B) [6]. Similar structures were not readily observed in differentiating GSC progeny. Further analysis showed that these extensions were microtubule-based, and utilized intraflagellar transport (IFT) molecules for their formation [6]. However, they lacked a 9+0 triplet microtubule structure and tubulin acetylation and did not associate with the basal body, in contrast to primary cilia. Thus these structures appeared to represent a new variant of thin cellular extensions and were named MT nanotubes based on their morphology (Table 1). MT nanotubes can be marked by both GFP–α-tubulin and membrane-bound–GFP, but not by cytoplasmic GFP, suggesting the existence of a diffusion barrier that limits general access to these structures.

MT nanotubes help GSCs receive niche signals. More specifically, these extensions promote Dpp signaling within GSCs, but do not foster JAK/STAT pathway activity. Similar to cytonemes, GFP-tagged Tkv, the receptor for Dpp, localized to discrete puncta that moved within MT nanotubes. Colocalization analysis suggested that Dpp, produced by hub cells, associates with the Tkv receptor expressed by GSCs on the surface of MT nanotubes. However, this finding does not preclude the possibility that ligand–receptor interactions can take place in other areas of the GSC hub cell interface. In addition, the manipulation of the size and frequency of MT nanotubes, through the modulation of IFT–B components, impacts Dpp signal transduction within GSCs: increasing the thickness of MT nanotubes increased signaling, as marked by phospho-Mad (pMAD) staining, while decreasing the frequency of MT nanotubes led to a reduction of pMAD. Genetic manipulation of MT nanotubes within individual GSCs through clonal analysis indicated that these structures help to maintain functional stem cells. Interestingly, *dpp* overexpression throughout the testis led to ectopic formation of MT nanotubes in germ cells distant from the hub. This observation suggests that Dpp signaling component(s) may promote the formation and/or stabilization of these structures. This finding raises the question, which comes first, Dpp signaling or MT nanotube formation. Interestingly, overexpression of a dominant-negative form of the Tkv receptor, which retains the extracellular, ligand-binding domain but lacks the intracellular domain required for signal transduction, increased MT nanotube formation [6]. This finding suggests that the ligand–receptor interaction, which normally occurs only at the interface of the hub and GSCs, is sufficient to induce MT nanotube formation. Once MT nanotubes are formed, they engage in robust signaling at the surface of MT nanotubes, reinforcing Dpp signaling in GSCs. However, the field does not clearly know how much Dpp is produced by hub cells and whether this ligand is secreted uniformly across the entire cell surface of hub cells. Keeping in mind the caveats of misinterpreting localization data based on tagged transgenes, the expression of Dpp–GFP suggests that hub cells may produce a limited amount of Dpp that appears to remain inside the hub area. Thus, MT nanotubes may act like a straw that is used by GSCs to gain greater access to limited, and potentially sequestered, niche signals.

Much work remains to be done with regard to characterizing the form and function of MT nanotubes. Like cytonemes, it remains unclear how MT nanotubes are made specific for different signaling pathways and how trafficking in and out of these extensions is regulated. Whether other stem cell populations use similar extensions to gain greater access to niche signals remains an open question. Nonetheless, the discovery of MT nanotubes opens a new

avenue for further understanding how communication between niche cells and stem cells is regulated in an *in vivo* setting.

Tunneling Nanotubes (TNTs) in Cell–Cell Communication

First discovered in cultured cells [30], TNTs have been mostly investigated in *in vitro* settings, but the use of mosaic/chimeric or transgenic conditions in which only a subpopulation of cells express markers for TNTs has recently allowed for the visualization of TNTs *in vivo* [16,18,31,32]. The relationship between TNTs and other thin protrusions such as cytonemes and MT nanotubes are not well understood. TNTs with various dimensions and molecular components have been reported (Table 1) [33], and they are roughly classified into two categories: 'thin TNTs' are less than 0.7 μm in diameter and composed primarily of F-actin, whereas 'thick TNTs' are greater than 0.7 μm in diameter and contain both F-actin and microtubules [33]. Interestingly, a recent study demonstrated that these two types of TNTs may be interconvertible, depending on stimulation [34]. Morphological and cytoskeletal characteristics of some TNTs resemble that of cytonemes, whereas other TNTs resemble MT nanotubes. As our understanding deepens, some of these structures may become united under the same label.

Studies on TNTs clearly demonstrate that they mediate local communication between cells by functioning as a conduit (Figure 1C). TNTs can transfer organelles (e.g., mitochondria, lysosomes) [31,33–40], endosome vesicles [30,41,42], and pathogens (e.g., HIV virus [43], prions [44], and bacteria [33]). TNTs can also mediate the propagation of cell death signals, including the transfer of active caspases [45,46], and influence membrane potential and calcium signaling [47–49]. Thus, TNTs regulate a broad spectrum of intercellular communication in different contexts.

With regard to stem cell-niche signaling, TNTs appear to mediate transfer of SARA (Smad anchor for receptor activation) endosomes between osteoblasts and hematopoietic progenitors and regulate Smad signaling within osteoblasts [42]. Although a number of studies have shown that osteoblasts do not serve as the niche for hematopoietic stem cells [50], osteoblasts are known to regulate restricted progenitors. Therefore, osteoblast regulation of hematopoietic progenitor cells may still present an attractive model to study niche-stem cell-like interactions via TNTs in an *in vivo* setting. Furthermore, considering the observation that these structures can extend several cells diameters in length, TNTs may allow certain cells to directly influence stem cell identity and activity at a distance.

Recent studies have provided evidence for the presence of TNTs in mammalian tissues. Visualization of thin protrusions in complex tissues requires mosaic or chimeric labeling, in which only a subset of cells express a marker (such as GFP-tagged protein) that localizes to TNTs in a background of non-expressing cells. By using chimeric mice that have received GFP-marked bone marrow-derived cells, the formation of TNTs was observed in MHC class II+ cells in the corneal stroma [32]. TNT formation increased upon injury, suggesting a role in cell–cell communication during inflammation. In addition, *in vitro* co-culture of cardiomyocytes with human multipotent adipose-derived stem cells (hMADS) promoted TNT formation, which correlates with an enhanced ability of hMADS to promote angiogenesis and repair of damaged cardiomyocyte tissue [51]. These studies predict further *in vivo* roles for TNTs.

Primary Cilia in Stem Cells

Primary cilia represent another type of signaling protrusion (Table 1) [52], mostly studied in the context of a 'fluid environment'. Primary cilia extend from cells and either receive humoral factors that have been secreted into the extracellular fluid or sense the flow of the fluid itself (mechanosensing). In this context, primary cilia do not appear to function in local or contact-dependent signaling. However, primary cilia are found in tissues that contain tightly packed cells, such as

epidermal cells. Since epidermal cells are attached to their neighbors, their primary cilia likely come in contact with adjacent cells. In this context, primary cilia appear to participate in both Shh signaling [53] and Notch signaling [54]. Removal of primary cilia in mouse postnatal epidermal tissues leads to tissue hyperplasia due to expansion of follicular cells, caused by activated Shh signaling [53]. In embryonic epidermal tissue, primary cilia regulate Notch signaling to promote differentiation [54]. Notch3 receptor localizes to the primary cilia in suprabasal cells, where Notch signaling is active. Although the source of Notch ligand(s) has not been determined in this context, the juxtacrine mechanism of Notch signaling indicates that the ligand(s) must be presented by the neighboring cells, and the ligand–receptor interaction likely occurs on the surface of the primary cilia.

A recent study reported that primary cilia also form strong adhesive connections with neighboring cells within a Madin–Darby canine kidney (MDCK) cell monolayer [55]. This cilium–cilium adhesion is glycoprotein-dependent, but does not appear to involve typical cell adhesion, as it is independent of Ca^{2+}. Unlike TNTs, the transfer of material between two cells was not observed along the adhered primary cilia. However, ligand–receptor interactions can occur at the interface of the primary cilia where they adhere to each other, opening the possibility that these structures likely influence local signaling events.

Outstanding Questions

How many types of cellular protrusions exist across diverse tissues and species?

To what extent do cells rely on specialized protrusions to foster specific signaling events over both short and long distances?

Are similar mechanisms involved in the formation of different cellular protrusions?

Are different types of protrusions specific for a certain spectrum of signaling pathways, or do they serve as a general platform for all communication between different cells?

What mechanisms direct components of specific pathways to specific cellular protrusions?

Concluding Remarks

The spectrum of signaling protrusions has expanded in recent years. While models of simple diffusion have dominated the field of cell signaling, the discovery of cytonemes, TNTs, and MT nanotubes suggest that different cells use a number of mechanisms to communicate with one another. Signaling protrusions function over a variety of distances. Long protrusions extending over many cell diameters allow for the specific delivery of ligands from a source to a target without influencing cells in between. Protrusions formed over a shorter distance provide an exclusive surface area on which two adjacent cells can send and receive signals, while preventing their neighbors from listening in on the conversation. Both types of protrusions appear to enhance the specificity of cell–cell communication.

Future studies are required to deepen our understanding on how specialized filopodia and protrusions form and how they regulate signaling between cells (see Outstanding Questions). Indeed, morphological and cytoskeletal characteristics between protrusions raises the possibility that different cellular protrusions described to date may be interconvertible depending on physiological context and may not represent fundamentally distinct structures. Some TNTs resemble cytonemes, whereas other TNTs resemble MT nanotubes. Cytonemes and TNTs are both dependent on F-actin and both participate in the trafficking of vesicles. Furthermore, the underlying structure of TNTs can change. For example, a recent study showed that stimulation of PC12 cells by UV-induced damage converts TNTs to MT–TNTs that contain microtubules in addition to actin filaments [34]. These findings suggest the possibility of interconversion, but one must also consider the possibility that variations in particular attributes may simply reflect how protrusions respond to specific stimuli, rather than fundamental differences in their structures and functions. Future studies that comprehensively compare various cellular protrusions under different conditions may necessitate some renaming and recategorization of these specialized cellular extensions. As our understanding deepens, some of these structures may become united under the same label. Lastly, it will be important to comprehensively characterize signaling protrusions *in vivo* and *in vitro* to facilitate the understanding of the biological significance of signaling protrusions in a more cohesive manner.

Acknowledgments

We are grateful to the Buszczak and Yamashita lab members, Sunny Wong for helpful information, and Jose Cabrera for figure illustration. We apologize for not being able to cite all relevant studies owing to space limitation. The work in the

Buszczak lab is supported by the National Institute on Aging (NIA; AG047318). The work in the Yamashita lab is supported by the Howard Hughes Medical Institute.

References

1. Kornberg, T.B. (2014) The contrasting roles of primary cilia and cytonemes in Hh signaling. *Dev. Biol.* 394, 1–5
2. Kornberg, T.B. and Roy, S. (2014) Cytonemes as specialized signaling filopodia. *Development* 141, 729–736
3. Fairchild, C.L. and Barna, M. (2014) Specialized filopodia: at the 'tip' of morphogen transport and vertebrate tissue patterning. *Curr. Opin. Genet. Dev.* 27, 67–73
4. Gerdes, H.H. and Carvalho, R.N. (2008) Intercellular transfer mediated by tunneling nanotubes. *Curr. Opin. Cell Biol.* 20, 470–475
5. Sherer, N.M. and Mothes, W. (2008) Cytonemes and tunneling nanotubules in cell–cell communication and viral pathogenesis. *Trends Cell Biol.* 18, 414–420
6. Inaba, M. *et al.* (2015) Nanotubes mediate niche-stem-cell signalling in the *Drosophila* testis. *Nature* 523, 329–332
7. Ramirez-Weber, F.A. and Kornberg, T.B. (1999) Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* 97, 599–607
8. Sato, M. and Kornberg, T.B. (2002) FGF is an essential mitogen and chemoattractant for the air sacs of the *Drosophila* tracheal system. *Dev. Cell* 3, 195–207
9. Hsiung, F. *et al.* (2005) Dependence of *Drosophila* wing imaginal disc cytonemes on Decapentaplegic. *Nature* 437, 560–563
10. Roy, S. *et al.* (2011) Specificity of *Drosophila* cytonemes for distinct signaling pathways. *Science* 332, 354–358
11. Roy, S. *et al.* (2014) Cytoneme-mediated contact-dependent transport of the *Drosophila* decapentaplegic signaling protein. *Science* 343, 1244624
12. Huang, H. and Kornberg, T.B. (2015) Myoblast cytonemes mediate Wg signaling from the wing imaginal disc and Delta–Notch signaling to the air sac primordium. *Elife* 4, e06114
13. Gradilla, A.C. *et al.* (2014) Exosomes as Hedgehog carriers in cytoneme-mediated transport and secretion. *Nat. Commun.* 5, 5649
14. Cohen, M. *et al.* (2010) Dynamic filopodia transmit intermittent Delta–Notch signaling to drive pattern refinement during lateral inhibition. *Dev. Cell* 19, 78–89
15. Sanders, T.A. *et al.* (2013) Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* 497, 628–632
16. Caneparo, L. *et al.* (2011) Intercellular bridges in vertebrate gastrulation. *PLoS ONE* 6, e20230
17. Luz, M. *et al.* (2014) Dynamic association with donor cell filopodia and lipid-modification are essential features of Wnt8a during patterning of the zebrafish neuroectoderm. *PLoS ONE* 9, e84922
18. Pyrgaki, C. *et al.* (2010) Dynamic imaging of mammalian neural tube closure. *Dev. Biol.* 344, 941–947
19. Bischoff, M. *et al.* (2013) Cytonemes are required for the establishment of a normal Hedgehog morphogen gradient in *Drosophila* epithelia. *Nat. Cell Biol.* 15, 1269–1281
20. Rojas-Rios, P. *et al.* (2012) Cytoneme-mediated delivery of hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in *Drosophila*. *PLoS Biol.* 10, e1001298
21. Snyder, J.C. *et al.* (2015) Lgr4 and Lgr5 drive the formation of long actin-rich cytoneme-like membrane protrusions. *J. Cell Sci.* 128, 1230–1240
22. Ohlstein, B. *et al.* (2004) The stem cell niche: theme and variations. *Curr. Opin. Cell Biol.* 16, 693–699
23. Morrison, S.J. and Spradling, A.C. (2008) Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* 132, 598–611
24. de Cuevas, M. and Matunis, E.L. (2011) The stem cell niche: lessons from the *Drosophila* testis. *Development* 138, 2861–2869
25. Tulina, N. and Matunis, E. (2001) Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK–STAT signaling. *Science* 294, 2546–2549
26. Kiger, A.A. *et al.* (2001) Stem cell self-renewal specified by JAK–STAT activation in response to a support cell cue. *Science* 294, 2542–2545
27. Schulz, C. *et al.* (2004) A misexpression screen reveals effects of bag-of-marbles and TGFβ class signaling on the *Drosophila* male germ-line stem cell lineage. *Genetics* 167, 707–723
28. Kawase, E. *et al.* (2004) Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the *Drosophila* testis. *Development* 131, 1365–1375
29. Shivdasani, A.A. and Ingham, P.W. (2003) Regulation of stem cell maintenance and transit amplifying cell proliferation by TGF-β signaling in *Drosophila* spermatogenesis. *Curr. Biol.* 13, 2065–2072
30. Rustom, A. *et al.* (2004) Nanotubular highways for intercellular organelle transport. *Science* 303, 1007–1010
31. Naphade, S. *et al.* (2015) Brief reports: Lysosomal cross-correction by hematopoietic stem cell-derived macrophages via tunneling nanotubes. *Stem Cells* 33, 301–309
32. Chinnery, H.R. *et al.* (2008) Cutting edge: Membrane nanotubes in vivo: a feature of MHC class II+ cells in the mouse cornea. *J. Immunol.* 180, 5779–5783
33. Onfelt, B. *et al.* (2006) Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria. *J. Immunol.* 177, 8476–8483
34. Wang, X. and Gerdes, H.H. (2015) Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. *Cell Death Differ.* 22, 1181–1191
35. Islam, M.N. *et al.* (2012) Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat. Med.* 18, 759–765
36. Vallabhaneni, K.C. *et al.* (2012) Vascular smooth muscle cells initiate proliferation of mesenchymal stem cells by mitochondrial transfer via tunneling nanotubes. *Stem Cells Dev.* 21, 3104–3113
37. Liu, K. *et al.* (2014) Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia–reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. *Microvasc. Res.* 92, 10–18
38. Pasquier, J. *et al.* (2013) Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *J. Transl. Med.* 11, 94
39. Plotnikov, E.Y. *et al.* (2010) Cytoplasm and organelle transfer between mesenchymal multipotent stromal cells and renal tubular cells in co-culture. *Exp. Cell Res.* 316, 2447–2455
40. Ahmad, T. *et al.* (2014) Miro1 regulates intercellular mitochondrial transport and enhances mesenchymal stem cell rescue efficacy. *EMBO J.* 33, 994–1010
41. Burtey, A. *et al.* (2015) Intercellular transfer of transferrin receptor by a contact-Rab8-dependent mechanism involving tunneling nanotubes. *FASEB J.* 29, 4695–4712
42. Gillette, J.M. *et al.* (2009) Intercellular transfer to signalling endosomes regulates an ex vivo bone marrow niche. *Nat. Cell Biol.* 11, 303–311
43. Sowinski, S. *et al.* (2008) Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. *Nat. Cell Biol.* 10, 211–219
44. Gousset, K. *et al.* (2009) Prions hijack tunnelling nanotubes for intercellular spread. *Nat. Cell Biol.* 11, 328–336
45. Arkwright, P.D. *et al.* (2010) Fas stimulation of T lymphocytes promotes rapid intercellular exchange of death signals via membrane nanotubes. *Cell Res.* 20, 72–88
46. Luchetti, F. *et al.* (2012) Fas signalling promotes intercellular communication in T cells. *PLoS ONE* 7, e35766
47. Watkins, S.C. and Salter, R.D. (2005) Functional connectivity between immune cells mediated by tunneling nanotubules. *Immunity* 23, 309–318

48. Wang, X. *et al.* (2010) Animal cells connected by nanotubes can be electrically coupled through interposed gap-junction channels. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17194–17199
49. Wang, X. *et al.* (2012) Developing neurons form transient nanotubes facilitating electrical coupling and calcium signaling with distant astrocytes. *PLoS ONE* 7, e47429
50. Morrison, S.J. and Scadden, D.T. (2014) The bone marrow niche for haematopoietic stem cells. *Nature* 505, 327–334
51. Figeac, F. *et al.* (2014) Nanotubular crosstalk with distressed cardiomyocytes stimulates the paracrine repair function of mesenchymal stem cells. *Stem Cells* 32, 216–230
52. Singla, V. and Reiter, J.F. (2006) The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* 313, 629–633
53. Croyle, M.J. *et al.* (2011) Role of epidermal primary cilia in the homeostasis of skin and hair follicles. *Development* 138, 1675–1685
54. Ezratty, E.J. *et al.* (2011) A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. *Cell* 145, 1129–1141
55. Ott, C. *et al.* (2012) Primary cilia utilize glycoprotein-dependent adhesion mechanisms to stabilize long-lasting cilia–cilia contacts. *Cilia* 1, 3
56. McCoy-Simandle, K. *et al.* (2016) Exosomes and nanotubes: control of immune cell communication. *Int. J. Biochem. Cell Biol.* 71, 44–54
57. Gerdes, H.H. *et al.* (2013) Tunneling nanotubes, an emerging intercellular communication route in development. *Mech. Dev.* 130, 381–387
58. Antanaviciute, I. *et al.* (2014) Long-distance communication between laryngeal carcinoma cells. *PLoS ONE* 9, e99196
59. Hase, K. *et al.* (2009) M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex. *Nat. Cell Biol.* 11, 1427–1432
60. Schiller, C. *et al.* (2013) LST1 promotes the assembly of a molecular machinery responsible for tunneling nanotube formation. *J. Cell Sci.* 126, 767–777