REVIEW

Not just another hole in the wall: understanding intercellular protein trafficking

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Development and differentiation of multicellular organisms requires cell-to-cell communication. In plants direct signaling and exchange of macromolecules between cells is possible through plasmodesmata. Recently direct exchange of membrane-bound vesicles and organelles has been demonstrated between animal cells through formation of cytoplasmic bridges (tunneling nanotubes) in vitro. Here we review recent developments in cell-to-cell trafficking of macromolecules in plants and animals.

Cell-to-cell communication is essential to coordinate developmental events in both animals and plants. Patterning of organs and specification of cell types requires intercellular signaling to communicate positional information. The signaling molecules involved in these developmental events include small molecules, polypeptide ligands, and small RNAs. Surprisingly, in plants, transcription factors may also serve as signaling molecules by trafficking from cell to cell, presumably through intercellular channels known as plasmodesmata (Lucas et al. 1995; Perbal et al. 1996; Sessions et al. 2000; Nakajima et al. 2001; Wada et al. 2002). Recent evidence suggests the possibility that intercellular channels may also exist in animal cells, allowing the transit of macromolecules (Baluska et al. 2004a; Onfelt et al. 2004; Rustom et al. 2004).

Determining how proteins move between cells and what molecules mediate movement is a primary goal in understanding intercellular communication. Since in plants, most protein movement is assumed to occur through plasmodesmata, this involves determining how proteins reach the plasmodesmata and once there, how they move into the neighboring cell. Here we review recent progress made in understanding the intercellular trafficking of the transcription factors LEAFY (LFY) and SHORT-ROOT (SHR). We also discuss work on viral movement proteins (MPs) that suggests that the plant endomembrane system plays a role in movement to and through plasmodesmata (for reviews, see Cilia and Jackson 2004; Oparka 2004). Intriguing new data regarding protein movement between animal cells will be reviewed as well as how these insights might relate to the mechanisms regulating protein movement in plants.

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Holes in walls: plasmodesmata provide cell-to-cell connectivity

Nearly all plant cells are connected to their neighbors by plasmodesmata. Plasmodesmata are formed at high density in primary pit fields, areas where the primary walls of the contacting cells are thin. In function, plasmodesmata are similar to gap junctions in that they allow communication and exchange of ions and metabolites between neighboring cells. However, plasmodesmata differ from gap junctions in their ability to allow the passage of large molecules. In their simplest form, plasmodesmata can be thought of as two concentric cylinders that connect the plasma membranes, cytoplasm, and endoplasmic reticulum (ER) of neighboring cells (Fig. 1). The plasma membrane delimits the outer cylinder of the plasmodesma and connects the neighboring cells. Internally the ER forms the second cylinder (desmotubule) that links the ER in the two adjacent cells. The plasmodesma thus provides multiple routes for intercellular communication, one through the cytoplasmic space between the desmotubule and the plasma membrane (often referred to as the cytoplasmic sleeve or plasmodesmal pore), another via the plasma membrane itself, and a third using the ER (Blackman and Overall 2001; Roberts and Oparka 2003). When first identified, plasmodesmata were largely regarded as fixed structures only allowing intercellular movement of molecules small enough to pass through their cytoplasmic sleeve. It is now clear that plasmodesmata are not static. Instead, they are able to modify their size exclusion limit (SEL) on a continuum from open (or dilated), where relatively large molecules are able to traffic freely, to closed, where no movement is permitted.

In general there are two modes by which molecules may traffic through plasmodesmata. Cytoplasmically localized proteins whose molecular weight is below the

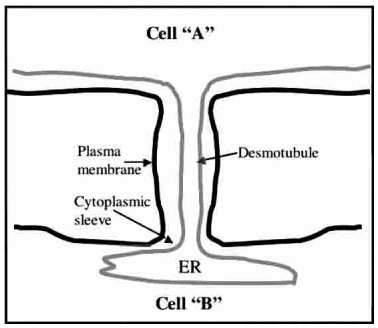

Figure 1. Diagram of plasmodesma connecting cells "A" and "B."

plasmodesmatal SEL can move between cells by nontargeted (or passive) movement. These proteins do not require an interaction with plasmodesmata for movement and likely diffuse through the cytoplasmic sleeve. Examples of nontargeted movement are the cell-to-cell trafficking of green fluorescent protein (GFP; Crawford and Zambryski 2001) and the LFY; (Wu et al. 2003) transcription factor. In contrast, some proteins must interact with plasmodesmata or associated proteins to increase the SEL and allow for their own movement. These proteins show targeted (or selective) movement. Targeted movement has been shown for viral MPs, which facilitate the spread of new viruses between cells, and the KNOTTED1 (KN1; Lucas et al. 1995) and SHR (Gallagher et al. 2004) transcription factors (Zambryski 2004).

Movement of GFP and LEAFY is nontargeted

Proteins that are sufficiently small to move between cells without an increase in the SEL are likely to move by simple diffusion. This has been shown for cytoplasmically localized (free) GFP and LFY (Crawford and Zambryski 2000; Wu et al. 2003). Free GFP has been shown to move extensively between cells in the *Arabidopsis* root meristem (Imlau et al. 1999; Sena et al. 2004) as well as in the leaf blade. Consistent with movement by diffusion, multimeric GFP (2XGFP) does not move as extensively as monomeric GFP (Crawford and Zambryski 2000, 2001).

The subcellular localization of GFP also affects the extent of protein movement. In *Nicotiana* leaf epidermis, addition of a nuclear localization signal to GFP reduced both the number of cells from which the protein could traffic and the distance traveled. Significantly, movement of 2XGFP was nearly blocked by the addition of a nuclear localization signal. This was presumably because at ~54 kDa the dimeric GFP was too large to diffuse freely from the nucleus back into the cytoplasm. Alternatively the kinetics of nuclear import might outpace entry back into the cytoplasm by diffusion. Movement of GFP was completely blocked by addition of signals that target the protein either to actin or into the lumen of the ER, although actin and ER are both components of plasmodesmata. These experiments tell us that for nontargeted movement the protein must be available in the cytoplasm, presumably because the protein is diffusing through the cytoplasmic sleeve.

More recently, the plant-specific transcription factor LFY has also been shown to move by a nontargeted mechanism (Wu et al. 2003). LFY is required for the transition from vegetative to reproductive development. Both the *LFY* mRNA and protein are normally expressed in all of the three layers that make up the floral primordia (L1, L2, and L3); however, when *LFY* is expressed in just the L1 layer (epidermis), the protein is able to move into the L2 and L3 layers and to rescue the *lfy* phenotype. Wu et al. (2003) showed that movement of LFY (47 kDa) in the shoot apical meristem of *Arabidopsis* is limited in a similar fashion to that of movement of 2XGFP, with the extent of protein movement correlating with the amount of protein present in the cytoplasm.

The correlation between the extent of LFY movement and availability in the cytoplasm strongly suggests that LFY movement is nontargeted. To strengthen this argument, Wu et al. (2003) made deletions spanning the LFY protein to determine what effect these mutations would have on movement, the idea being that if LFY movement were targeted, specific domains within LFY would be required for interaction with plasmodesmatal associated proteins to promote its movement. Mutation or elimination of these movement domains should eliminate LFY trafficking. If, however, LFY simply diffuses through plasmodesmata, a specific domain would not be required for movement. The latter scenario was found; none of the mutations eliminated LFY movement. It is possible that there are redundant movement domains in LFY; however, the dependence of LFY movement upon cytoplasmic localization and the lack of any mutations that abolish movement are consistent with the movement of LFY being nontargeted.

The conclusion that LFY movement is likely nontargeted was somewhat surprising since earlier work by Lee et al. (2003) suggested that LFY movement was affected by NON-CELL-AUTONOMOUS PATHWAY PROTEIN 1(NtNCAPP1) from *Nicotiana tabacum*. NtNCAPP1 was identified as a protein that interacted with *Cucurbita maxima* PHLOEM PROTEIN 16 (CmPP16). Plants expressing a dominant negative form of NtNCAPP1 restricted movement of CmPP16 and tobacco mosaic virus (TMV) MP but not KN1. Interestingly, these plants had a phenotype similar to overexpression of LFY, suggesting that NtCAPP1 normally interacts with NtLFY and inhibits its movement. This is in contrast to CmPP16 and TMV MP whose movement appears to be facilitated by NtNCAPP1. It seems possible that NtLFY may move by a different mechanism than *Arabidopsis* LFY or that the NtNCAPP1 dominant negative indirectly affects diffusion through plasmodesmata. Testing directly whether NtLFY or LFY interact with NtNCAPP1 or testing whether the NtNCAPP dominant negative affects movement of other nontargeted proteins like GFP would help to answer these questions.

SHORT-ROOT movement cannot be explained by diffusion

SHR, a member of the GRAS family of transcriptional regulators, is required both for correct specification of

the endodermal cell layer and normal patterning of the root (Helariutta et al. 2000). Recently, SHR movement was shown to be dependent upon its presence in the cytoplasm (Gallagher et al. 2004). SHR is expressed in stele cells in the *Arabidopsis* root meristem. In these cells the SHR protein localizes both to the nucleus and the cytoplasm. The SHR protein moves from cells in the stele into the adjacent endodermal cell layer where the protein is detected only in nuclei. This has been shown both by immunolocalization and visualization of a fully functional fusion of GFP to SHR (Nakajima et al. 2001). When a strong nuclear localization signal is added to SHR–GFP (SHR–NLS–GFP), the protein no longer moves (Gallagher et al. 2004). Sena et al. (2004) showed that when SHR–GFP is expressed in epidermal cells where the protein is also efficiently nuclear localized, there is no movement into adjacent layers. Inhibition of SHR movement by nuclear localization represents a potential mechanism by which SHR trafficking is limited to the endodermis. Interestingly, Sena et al. (2004) also showed that when SHR was expressed in the root epidermis in a *scarecrow* (*scr*) mutant background (SCR is a GRAS family protein that functions downstream of SHR), the SHR protein did not efficiently nuclear localize and was able to move into the adjacent cell layer. These data provide direct evidence that SHR must localize to the cytoplasm in order to move and indicate a role for SCR in limiting SHR movement, potentially by regulating the subcellular localization of SHR.

Unlike LFY whose movement is also regulated by the degree to which the protein is nuclear localized, SHR movement appears targeted. Isolation of an informative new allele of *shr*, *shr-5*, showed that while cytoplasmic localization of SHR is required for movement, it is not sufficient. The *shr-5* allele contains a point mutation in T289 that converts this residue to an isoleucine. Expression in plants of a SHR–GFP construct with T289 mutated to isoleucine showed that this mutation causes loss of specific nuclear localization in stele cells and complete loss of movement. Loss of movement could not be attributed to protein degradation, aggregation, or loss of stability, indicating that T289 plays a role in SHR movement. Since T289 by sequence analysis is a potential phospho-acceptor, a phosphomimetic allele of SHR–GFP (T289 was converted to glutamate) was made. However, it was also unable to localize to nuclei or move, suggesting that the role of T289 in movement may not be as a phospho-acceptor (Gallagher et al. 2004). Since at least one GRAS protein appears to be glycosylated (Swain et al. 2001) it remains possible that T289 is modified in this way.

In addition to SHR, mutations in rice thioredoxin h protein, (RPP13-1), KN1, and Heat Shock Protein 70 chaperone homologs from pumpkin (CmHsc70-1 and CmHsc70-2) have all been shown to affect their trafficking (Ishiwatari et al. 1995; Aoki et al. 2002), suggesting the presence of a specific movement domain that gates plasmodesmata. Indeed transfer of the movement domain from CmHsc70-1 to the nontrafficking CmHsc70-3 is able to confer the ability to move (Aoki et al. 2002). However, an autonomous movement domain that can confer movement onto nonrelated proteins independent of the original context has not yet been identified. This is in contrast to the HIV TAT transduction domain (Nagahara et al. 1998) and other transduction peptides that can confer the ability to traffic from cell to cell to nonrelated proteins in mammalian systems (for review, see Joliot and Prochiantz 2004). This suggests that movement domains in plants are context specific.

Role of the endomembrane system and cytoskeleton in protein movement

Learning whether a protein moves by targeted or nontargeted movement is one step in understanding cell-to-cell trafficking. One must also determine how the protein reaches the plasmodesmata in the first place. It is possible that some proteins may randomly contact plasmodesmata by diffusion through the cytoplasm, while others may require transport along cytoskeletal elements. Recent work, however, suggests that at least some proteins are carried to plasmodesmata by interaction with proteins associated with the endomembrane (for review, see Oparka 2004).

In plant cells, the endomembrane system includes the ER, Golgi bodies, vacuole, and vesicles. The endomembrane is required for cell division and delivery of proteins to the cell surface. As in animal cells, the plant endomembrane system is able to localize various proteins to different subdomains in the plasma membrane. In plants this is well illustrated (as noted by Jurgens and Geldner 2002) by the apical localization of the auxin influx carrier AUX1, the basal localization of the efflux carrier PIN1, and the lateral localization of COBRA all within the same cell. In animal cells, targeted secretion in epithelial cells is achieved by the differential localization of syntaxins to plasma membrane subdomains; in plant cells the mechanisms are not yet known.

Evidence is accumulating that the endomembrane system also participates in targeting non-cell-autonomous proteins to plasmodesmata. TMV MP is known to localize to the ER (Mas and Beachy 1999). Additionally, NtNCAPP1, which participates in the movement of MP and CmPP16 in tobacco leaves, also localizes to the outer surface of the ER membrane (Lee et al. 2003). Recently Laporte et al. (2003) showed that grape vine fan leaf virus MP interacts with KNOLLE, a t-SNARE, syntaxin family protein. In a screen for interactors with cauliflower mosaic virus (CMV) MP, Huang et al. (2001) identified an *Arabidopsis* protein with similarity to a prenylated rab acceptor (PRA1) from rat. PRA1 interacts with components of the vesicle transport machinery, rabs (small GTPases from the Ras super-family) in particular, and a component of the v-SNARE (vesicle SNAP receptor) complex. Interestingly Escobar et al. (2003) found that the N-terminal portion of Rab11 associates with plasmodesmata when expressed in *Nicotiana benthamiana* leaves as a GFP fusion.

The specific interaction of MPs with vesicles or vesicle-localized proteins prompted Oparka (2004) to propose the "grab-a-rab" hypothesis in which non-cell-

autonomous proteins traffic to the cell membrane by hitchhiking on vesicles destined for particular plasma membrane subdomains associated with plasmodesmata. In support of this hypothesis, expression of antisense of *LeRab11a* in tomato led to plants with reduced apical dominance, determinate growth, branched inflorescences, abnormal floral structure, and ectopic shoot growth on leaves. These pleiotropic effects of loss of LeRab11a on plant growth prompted the investigators to suggest that the protein must somehow participate in endo- or exocytosis of hormone carriers/receptors and/or homeodomain proteins (Lu et al. 2001). Based upon the results of Escobar et al. (2003), it may be that loss of LeRab11a disrupts movement through plasmodesmata.

Once the membrane-associated cargo reaches the plasma membrane and is released at the plasmodesma, how does the protein then pass through the plasmodesmal pore? Both actin and myosin localize to plasmodesmata and there is evidence that both play a role in gating plasmodesmata (Heinlein 2002). Volkmann et al. (2003) showed that microinjection into *Arabidopsis* roots of antibodies to myosin VIII results in enhanced movement of FITC-labeled dextrans and Lucifer Yellow between cells. The increase in cell-to-cell movement was presumably due to an increase in the SEL of plasmodesmata, suggesting that intact myosin VIII is required to limit transit through the plasmodesmal pore. In addition to gating plasmodesmata, myosin and actin may also play a role in transit through the pore. Many models of plasmodesmal structure have actin filaments traversing the cytoplasmic sleeve, providing a mechanism by which non-cell-autonomous proteins could travel through the pore. It was previously thought that microtubules played a direct role in movement of TMV MP through plasmodesmata (Boyko et al. 2000). There is now evidence, however, that microtubules are not required for passage of TMV MP through plasmodesmata. Instead, it appears that microtubules play a role in sequestering the MP in the cytoplasm and inhibit, rather than facilitate, movement (Kragler et al. 2003).

Work by Kawakami et al. (2004) with TMV MP suggests that in some cases release of the non-cell-autonomous protein from the transport vesicle may not be necessary for movement through plasmodesmata. Based on the localization of TMV MP and the kinetics of infection, Kawakami et al. (2004) suggest that TMV MP traffics through plasmodesmata as an ER membrane-associated complex that contains the MP, viral RNA, and replicase. These complexes are highly motile and traffic extensively within the cell before they reach the plasma membrane and plasmodesmata. Once at the plasma membrane, the complex is able to gate the plasmodesma and surprisingly the entire membrane-bound unit passes through the plasmodesmal pore into the neighboring cell. Both actin and myosin are required for movement of the membrane-associated complex.

Recently, Baluska et al. (2004a) showed that in maize root apices, endocytosis occurs preferentially at plasmodesmata. Using Lucifer Yellow as a fluorescent tracer, they found invagination of the plasma membrane specifically in pit fields (where primary cell walls are thin) and at plasmodesmata. Endocytosis was blocked by depolymerization of actin, but not by actin stabilization, suggesting that actin plays a role in this process. In contrast intact microtubules were not required for uptake of the fluorescent tracer.

Collectively these data indicate a role for the endomembrane in movement through plasmodesmata. The localization of MPs to the ER and in some cases their interaction with vesicle-localized proteins suggests a mechanism that brings non-cell-autonomous proteins to plasmodesmata. In addition the suggestion that TMV MP passes through plasmodesmata as an intact membrane-associated complex and the identification of endocytic vesicles specifically at plasmodesmata raise the possibility that non-cell-autonomous proteins pass through plasmodesmata as vesicle-associated complexes. Of course, the participation of the plant endomembrane system in movement through plasmodesmata is still speculative. Much work remains to determine precisely what roles (if any) vesicle-associated proteins and secretion play in cell-to-cell trafficking.

Nonclassical protein export in animals

Clues to how protein movement occurs between plant cells can be gained by examining nonclassical protein export and import in animal cells. Transfer of homeodomain proteins, which lack traditional signal sequences for secretion, has been demonstrated between metazoan cells ex vivo. These proteins include Engrailed, Hoxa5, Hoxb4, Hoxc8 Emx1, Emx2, Otx2, and Pax6 and the homeodomain from KN1 (Prochiantz and Joliot 2003). Both Engrailed 1 and Engrailed 2 are found within vesicles in vivo (Joliot et al. 1997). Analysis of chicken Engrailed 2 homeoprotein (cEN2) showed that movement involved secretion followed by internalization mediated by two regions within the conserved homeodomain referred to as the secretion and penetratin domains respectively. Prochiantz and Joliot (2003) suggest that secretion occurs by incorporation of the homeoprotein into nascent vesicles as they form. Deletion of 11 amino acids within the secretion domain prevents secretion and incorporation into vesicles, indicating a link between the two (Joliot et al. 1998). In addition, secretion is also blocked by phosphorylation of cEN2 by CK2 (Maizel et al. 2002). This is interesting because TMV MP is also blocked by phosphorylation (Waigmann et al. 2000) In mammalian cells, internalization is thought to occur by charge interactions at the membrane and membrane destabilization initiated by insertion of W48 (which is required for internalization) into the bilayer (for review, see Prochiantz and Joliot 2003).

The KN1 homeodomain is quite diverged from cEN2 and is thought to move via plasmodesmata in plant tissues, although other modes of trafficking have not been definitively ruled out. Recently the KNI homeodomain was shown to traffic between animal cells in culture (Prochiantz and Joliot 2003). The only mutation within the homeodomain that is able to inhibit KN1 trafficking between plant cells also inhibits movement between

animal cells, suggesting that there are conserved mechanisms for movement. Interestingly the mutated sequence in KN1 is part of the nuclear import signal whereas the sequence within the cEN2 homeodomain that is required for secretion is also required for nuclear export, indicating that transport through the nucleus may be essential for entry to the secretory pathway. The LFY protein, which lacks a homeodomain, does not traffic between mammalian cells (A. Joliot, pers. comm.). It will be interesting to see if other non-cell-autonomous plant proteins like SHR can move in this system. In addition there are at least 80 homeodomain proteins in *Arabidopsis* by sequence analysis. These potentially represent a large group of non-cell-autonomous proteins in plants, of which only the KNOTTED-related homeodomain proteins (Kim et al. 2003) have been shown to move.

Transport tubules, *Listeria*, and nanotubes

While many strains of virus are able to move between plant cells by increasing plasmodesmatal SELs (through the action of MPs), some strains of virus move between cells by formation of specialized tubular structures (Huang et al. 2000). The infection of plant cell protoplasts (that lack cell walls) with any of these viruses leads to the formation of striking projections from the cell surface called transport tubules (Fig. 2). These membrane-enclosed tubules can extend at least 20 µm outward from the cell surface (van Lent et al. 1991). Transport tubules are also formed in vivo by infection with these same viruses or expression of their MPs. In planta the tubules extend between cells through plasmodesmata, apparently displacing the desmotubule, and provide a channel through which virions may pass. Since even in planta the tubules are completely membrane enclosed, the tubule must break down for the virus to exit (Pouwels et al. 2003). This mode of cell-to-cell movement may be particular to plant viruses; no endogenous plant proteins have been shown to traffic in this way.

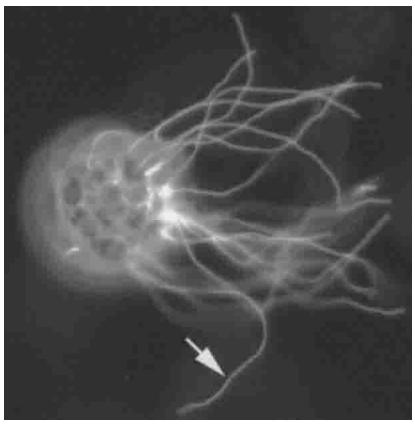

Figure 2. Tubule formation in *Arabidopsis* protoplasts expressing a cauliflower mosaic virus (CaMV) MP–GFP fusion. The arrow indicates a surface tubule. This image was generously provided by S.H. Powell.

Interestingly the ability of cells to form transport tubules is not specific to plants. Kasteel et al. (1996) showed that similar structures are formed in cultured insect cells expressing MPs from either cowpea mosaic virus (CPMV) or CMV, revealing that plants and animals share the molecular components required for tubule formation. Thomas and Maule (1999) showed that the ability of the mutated MPs to form tubules in insect cells was directly related to their ability to propagate infection in planta, suggesting that these structures are required for viral movement.

The process of viral spread by transport tubules in plants is reminiscent of *Listeria* infection. *Listeria monocytogenes* is a facultative intracellular parasite that infects animal cells, causing Listeriosis in humans. Spread of Listeria occurs by formation of tubular extensions on the cell surface that extend deep into neighboring cells. The neighboring cell then takes up the bacteria by a poorly understood process that is sometimes referred to as paracytophagy (Robbins et al. 1999). The tubular structures are required for infectivity and are formed by the bacteria's ability to nucleate actin from their surface, which provides the force required to deform and extend the plasma membrane (for review, see Cameron et al. 2000). There is no evidence that plant viruses are capable of nucleating actin. There is, however, data suggesting that MPs may themselves serve this function. MPs from CPMV, which induce tubules, form multimeric chains at the cell surface. They have also been shown to bind GTP. GTP binding of CPMV MP is involved in tubule formation, at least in insect cells. Carvalho et al. (2004) speculate that assembly of MP bound at the plasma membrane into microtubule-like structures may provide the force necessary to generate the transport tubules. In addition, Laporte et al. (2003) showed that individually neither actin nor microtubules are required for tubule formation. Disruption of both actin and microtubules, however, caused tubules to form intracellularly, usually near the nucleus (as opposed to near the cell wall) and often in aster-like structures.

Recently, structures with striking similarity to transport tubules from plant cells have been reported in animal cells. These structures, referred to as tunneling nanotubes (Fig. 3), can actually form between many different cell types in tissue culture (Baluska et al. 2004b; Onfelt et al. 2004; Rustom et al. 2004). Remarkably, when tunneling nanotubes from neighboring cells meet, they appear to coalesce and to form a cytoplasmic bridge between the cells. Both Rustom et al. (2004) and Onfelt et al. (2004) show that proteins can be selectively transported between cells through these tubules. Interestingly Rustom et al. (2004) showed that synaptophysin (a marker for early endosome and endosome-derived vesicles) and myosin Va were both present as discrete units within the tubule and showed some degree of colocalization. The discovery of tunneling nanotubes in animal cells raises the intriguing possibility that the cytoplasmic continuity between cells is not specific to plants and certain stages of insect development. This is also suggested by the similarity of the tunneling nano-

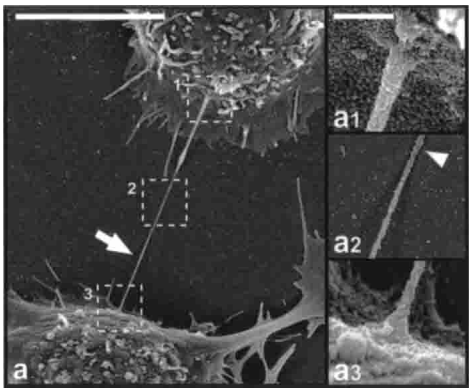

Figure 3. Tunneling nanotubes. (*a*) Tunneling nanotubes have formed between PC12 cells in culture. Images in *a1–a3* are magnifications of the corresponding regions in *a*. Bars, 10 μM. This image is reprinted with permission from Rustom et al. (2004) (© 2004 American Association for the Advancement of Science. http://www.sciencemag.org).

tubes to cytonemes found in vivo in *Drosophila* wing imaginal discs. Like transport tubules or tunneling nanotubes, cytonemes are thin actin-based extensions of the plasma membrane. Cytonemes were shown to extend from imaginal disc cells on the lateral flanks of the wing disc toward signaling centers associated with the anterior/posterior border; however, they have not been shown to form cytoplasmic bridges between cells (Ramirez-Weber and Kornberg 1999).

Summary and conclusion

Until recently, direct communication by intercellular trafficking of macromolecules through cytoplasmic bridges was thought to occur primarily in plants. The discovery of tunneling nanotubes between mammalian cells in tissue culture raises the possibility that cytoplastic continuity may exist in animals as well. In addition, although plants and animals independently evolved multicellularity, it appears that both groups share at least some of the same factors for cell-to-cell signaling. The regulated movement of the KN1 homeodomain between animal cells and the formation of transport tubules in insect cells illustrate this point. As the factors and mechanisms that regulate intercellular protein movement in plants and animals are discovered and understood, it will be interesting to see which are universal and which are specific.

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