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Non-conventional protrusions: the diversity of cell interactions at short and long distance

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Cells use different means to communicate within and between tissues and thereby coordinate their behaviours. Following the initial observations of enigmatic long filopodia unrelated to cell movement, it became clear that the roles of cellular protrusions are not restricted to sensing functions or motility and are much more diverse than previously appreciated. Advances in *live*-imaging and genetic tools revealed several types of non-conventional cell protrusions and their functions, ranging from tissue patterning, proliferation and differentiation control, tissue matching and cell spacing to more unexpected roles such as priming of cell adhesion as well as bidirectional coordination of tissue movements. Here, we will highlight exciting new insights into highly diverse cell behaviours elicited by protrusions and contact-dependent cell communication, essential for embryonic development across species.

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Introduction

Conventional cell protrusions such as filopodia, lamellipodia, blebs and invadopodia have been classically viewed as the driving force underlying the movement of individual or groups of cells. Filopodia, actin-based 'cell antennae', integrate soluble and membrane-bound signals encountered in the extracellular environment. These protrusions mediate both directional guidance and mechanical attachment to the substrate (ECM or other tissues) to steer cell or growth cone migration to the target location (reviewed in [1]). However, reports showing that migration processes such as axonal growth, angiogenic sprouting or neural crest collective path finding can be correctly guided without filopodia, suggest more diverse roles than previously anticipated [2–4]. High-resolution *live* imaging techniques combined with membrane tethered and cytoplasmic reporters revealed the unexpected presence of 'non-conventional' types of cellular protrusions in many different cell and tissue types. Long and directional protrusions, which did not correlate with cell motility, were described already in the 1990s in invertebrate models, suggesting that such structures may deliver signalling molecules between non-juxtaposed cells during morphogenesis [5,6]. Since then, the reported number of novel protrusion types, showing unusual morphologies and non-stereotypic behaviors and functions, increased exponentially. Their nomenclature and characterisation is loosely based on cytoskeletal composition (actin-based or microtubule-based), tissue of origin, transported molecules, stability, resistance to fixation and other structural features. However, there is currently no consensus whether they may represent specialised variations of prototypical structures, such as filopodia and cilia, or if their molecular composition is fundamentally different [7]. Based on *in vivo* function and structural similarities we report here several types of non-conventional protrusions, whose number is expected to increase and with many still requiring thorough characterization (Table 1).

On one end of the spectrum are cytonemes or signalling filopodia, actin-based protrusions up to 700 μm long, described in both *Drosophila* and vertebrates to mediate direct morphogen relay from producing to target cells (reviewed in [[13••],9]; Figure 1). On the other end are specialised protrusions that mediate mutual communication between migrating cells. The ascribed functions of this more heterogeneous group range from guidance of loosely connected cell streams [10], to topological 'matching' of zippering tissues [11,12] and mutual guidance between neighbouring tissues migrating in different directions (Figure 2). Uniquely among those, Eph/Ephrin long-range protrusions emanate from liver precursors and mesodermal cells and guide bidirectional organ placement, providing a novel mechanism of protrusion function and reciprocal tissue communication [13••].

In pathological conditions and cultured cells, it was shown that virus, proteins and organelles are exchanged by yet another type of long actin protrusion, the tunneling nanotubes (TNT), which physically connect the cytoplasm of contacting cells (reviewed in [14]). Cytoplasm exchange was also shown between cancer cells and tumor-associated macrophages, triggering a more migratory phenotype of the tumor [15]. So far, only few reports indicate

Table 1

Comparision of protrusion types based on their tissue context and proposed function.

Type	Cytoskeletal composition	Involved signalling cascades	Tissue of origin	Proposed function
Cytonemes/signalling filopodia	Actin (Microtubules; only in [27,30])	Dpp/BMP, Hh, EGF, FGF, Shh, Wnt, Delta/Notch	Wing disc, thorax, muscles, ovaries and tracheae (*Drosophila*) [17–23,24••,25,26••,36,42–44]; limb bud mesenchyme and somites (chicken and quail) [27,30,31]; neural plate (zebrafish) [28,29•]	Morphogen spreading and tissue patterning
Bidirectional protrusions	Actin	Eph/Ephrin	Liver progenitors and lateral plate mesoderm (zebrafish) [13••]	Reciprocal guidance of migrating tissues (repulsion); epithelial organisation
Stem cell enveloping processes	Actin	Dpp, Hh and Notch	Intestinal and hematopoietic stem cell niches (*Drosophila*) [32,33]; germline stem cell niche (*C. elegans*) [34,35•]	Stem cell state maintenance
Microtubule-nanotubes	MT	Dpp	Testis/germline stem cell niche *(Drosophila)* [37]	Stem cell state maintenance
Embryonic cavity spanning protrusions	Actin	EGF, FGF and unknown [5,40]	Trophectoderm (mouse) [39], blastomeres (frog) [40], ectodermal and mesenchyme cells (sea urchin) [5]	Morphogen spreading and cell proliferation
(Inter)cellular bridges	Actin	unknown	Epiblast cells (incomplete cytokinesis; zebrafish) [41]; neural crest cells (chicken) [51]	Mechanical function and positional information communication
Tunnelling nanotubes	Actin	unknown	Immune cells, cancer cells, CNS cells (induced by pathological conditions) [14,15]	Exchange of organelles, vesicles and small molecules
Airinemes	Actin, Microtubules	Delta/Notch	Pigment cells (macrophage-mediated delivery; zebrafish) [45,46•,47••]	Cell spacing and migration
Myotube filopodia	Actin	Dumbfounded/Kin-of-Irre (Duf) and Sticks-n-stones (Sns)	Myoblast-myofiber fusion (*Drosophila*) [48••]	Priming of stable heterotypic cell adhesion
Cadherin fingers	Actin	Cadherin/Catenin	Endothelial cells [52•]	Directionality communication in collective migration
Zipping protrusions	Microtubules, actin	unknown	Epidermis (dorsal closure; *Drosophila* [11,53]; neural tube closure; mouse) [12,54]	Spatial matching during tissue gap closure

functional links between TNTs and *in vivo* developmental processes.

In many instances, direct 'communication-by-contact' may represent a more robust way to deliver signals rather than secretion and diffusion of molecules, considering the variability and complexity of the extracellular environment, which ranges from fluid-filled spaces to densely packed cells.

Specialised protrusions bridge the gap between cell signalling and cell guidance, becoming a means by which static and moving cells communicate to each other at short or long distances in order to coordinate their behaviours and fate decisions. However, in many cases it is still not known which molecular players are involved in such cellular interactions and what triggers protrusion formation.

In light of recently emerging functional data, we are going to highlight the diversity of signalling contexts governed by 'non-conventional' cell protrusions during development, likely representing a snapshot of what we are expecting to see in the future.

Morphogen distribution and cell fate determination

Morphogens, signalling molecules able to elicit concentration dependent responses in target cells, control patterning of tissues by inducing differential cell fate decisions in their field of action. Their ability to form diffusive gradients (Figure 1a) has long been debated due to the hydrophobicity of members such as Hedgehog (Hh) and Wingless (Wg)/Wnts [8,9]. Moreover, membrane-tethered Wg maintains the majority of its patterning functions [16]. Signalling filopodia/cytonemes have been shown to transport morphogens and their receptors between senders and receivers in numerous developmental processes (Figure 1b). These include imaginal disc and tracheal air sac patterning by Decapentaplegic/BMP (Dpp), Hh, EGF and FGF in *Drosophila* [17–23,24••,25,26••], chicken limb bud regionalization by Sonic hedgehog (Shh) [27], zebrafish neural plate patterning and chicken somite epithelialization by Wnts [28,29•,30]. They are currently the best characterised type of non-conventional protrusion, with interesting features and common motives starting to emerge.