Specialized cytonemes induce self-organization of stem cells

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Spatial cellular organization is fundamental for embryogenesis. Remarkably, coculturing embryonic stem cells (ESCs) and trophoblast stem cells (TSCs) recapitulates this process, forming embryonic-like structures. However, mechanisms driving ESC–TSC interaction remain elusive. We describe specialized ESC-generated cytonemes that react to TSC-secreted Wnts. Cytoneme formation and length are controlled by actin, intracellular calcium stores, and components of the Wnt pathway. ESC cytonemes select self-renewal–promoting Wnts via crosstalk between Wnt receptors, activation of ionotropic glutamate receptors (iGLURs), and localized calcium transients. This crosstalk orchestrates Wnt signaling, ESC polarization, ESC–TSC pairing, and consequently synthetic embryogenesis. Our results uncover ESC–TSC contact–mediated signaling, reminiscent of the glutamatergic neuronal synapse, inducing spatial self-organization and embryonic cell specification.

Significance

Many questions about how stem cells communicate with neighboring cells and self-organize to initiate tissue formation remain unanswered. We uncovered mechanisms employed by embryonic stem cells (ESCs) and trophoblast stem cells (TSCs) to form embryo-like structures. We describe ESC-generated cytonemes that react to self-renewal–promoting Wnt ligands secreted by TSCs. We identified glutamatergic activity upon formation of ESC–TSC interaction. This cellular connection is required for the transmission of Wnt signals to ESCs for Wnt/β-catenin pathway activation, a process that regulates morphogenesis. Given that many stem cell types express glutamate receptors and rely on niche-secreted Wnt ligands for self-renewal, we propose that Wnt and glutamatergic signaling crosstalk may prove prevalent in various mammalian tissues to regulate stem cell–niche interactions.

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pathways (e.g., Wnt3a). Therefore, we immobilized purified Wnt3a and Wnt5a onto microbeads, distributed the microbeads around single ESCs, and investigated the interaction between cytonemes and Wnt beads. Our results indicate that ESCs can distinguish between signals and selectively reinforce a connection to the self-renewal Wnt3a ligand in an LRP6-dependent process. This signal recruitment is also mediated by the activity of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) kainate glutamate receptors at the cytonemes, which produces calcium transients. We identified the roles of intracellular calcium stores, Wnt receptors, DVL2, and β-catenin in regulating the formation and length of ESC cytonemes.

In conclusion, we demonstrate that ESCs possess specialized cytonemes that react to self-renewal signals and orchestrate ESC–TSC pairing, setting the basis for spatial organization and specification of embryonic tissues.

**ESCs Extend Cytonemes to Initiate Contact with TSCs**

ESCs and TSCs possess the ability to self-sort and organize when cultured together to generate embryonic structures (2–4). By time-lapse imaging, we investigated how the initial interaction between cell types was achieved. Single TSCs, which constitutively expressed enhanced green fluorescent protein (eGFP), displayed limited movement (Fig. 1A). We used ESCs expressing the F-actin reporter Ftracm-mRFP1 (18), permitting visualization of fine membrane structures during ESC–TSC interactions. We observed single ESCS extending protrusions that transiently contacted TSCs. After the initial contact, ESCs reacted by directing a larger protrusion to establish a stable contact with TSCs (reactive interaction; RI), often followed by ESC–TSC pairing (Fig. 1B and Movie S1). We did not observe TSCs contacting ESCs in a similar manner to establish ESC–TSC pairing.

ESCs rely on activation of the Wnt/β-catenin pathway for self-renewal (19, 20). Therefore, we investigated whether TSCs secrete Wnt ligands that are received by ESCs. We profiled the transcripts of the 19 Wnt genes in TSCs, showing the expression of 16 Wnt transcripts (SI Appendix, Fig. S1A). Importantly, the interaction of ESCs and TSCs can result in activation of the Wnt/β-catenin pathway in ESCs, as indicated by the 7 oligomerized T cell factor (TCF)-binding site (TCS-TCF)-eGFP (21) ESC reporter line (Fig. 1C and SI Appendix, Fig. S1B). To verify if the TSCs were in fact the source of the Wnt ligands, we used a short-term inhibition of Wnt ligand secretion to minimize the potential impact on TSC maintenance and identity. Accordingly, ESCs incubated with TSCs pretreated with inhibitor of Wnt production-2 (IWP2), a small molecule that blocks the secretion of Wnt ligands (22), for 24 h significantly reduced the magnitude of activation, similar to that of ESCs cultured alone (Fig. 1C and SI Appendix, Fig. S1B). These results indicate that TSCs produce Wnt ligands that are received by ESCs to activate the Wnt/β-catenin pathway.

Next, we determined whether the ESC–TSC interaction itself is affected by IWP2 treatment of TSCs. We observed that ESCs contact treated TSCs transiently via the protrusions; however, in 76% of cases this was not followed by ESC–TSC pairing (non-reactive interaction; Fig. 1A and B and Movie S2). We obtained similar results using a different Wnt secretion inhibitor, Wnt-C59 (ref. 23, Fig. S1C). We speculated that the ESC protrusions are cytonemes that sense TSC-derived Wnt ligands, which are essential for the establishment of stable contacts during ESC–TSC pairing. To confirm this, we generated a double knock-out (dKO) of the Wnt coreceptors LR5P and LR5P6 in ESCs (LRP5/6dKO) and observed that the transient contact between cytonemes and TSCs was unaffected. However, these ESCs had a significantly reduced ability to establish stable contacts with TSCs, similarly to the ESC interaction with IWP2-pretreated TSCs (Fig. 1B). Furthermore, both IWP2 (or Wnt-C59) treatment and LR5P5/6dKO ESCs resulted in a significant reduction in the formation of synthetic embryo structures (3) in three-dimensional (3D) culture (SI Appendix, Fig. S2). Our results suggest that specialized ESC cytonemes induce ESC–TSC pairing, an essential step in synthetic embryogenesis.

To study the specificity of these cytonemes for Wnt ligands, we covalently immobilized purified Wnts to microbeads and investigated the cytoneme-bead interactions.

**ESCs Selectively Recruit Wnt Ligands Required for Self-Renewal**

We previously described a system of a localized Wnt3a bead which recapitulates a niche signal essential for self-renewal and oriented asymmetric cell division (ACD) of single ESCs (20). Wnt5a, also produced by TSCs, cannot activate the Wnt/β-catenin pathway in ESCs (20). Importantly, Wnt5a beads do not induce ACD in ESCs (20). Using this Wnt-bead approach, we aimed to investigate the mechanisms by which ESCs interact with localized niche signals. We incubated single cells in close proximity to Wnt3a beads or Wnt5a beads (SI Appendix, Fig. S1D) and monitored initial cell-bead contact by live imaging. Primary observations revealed that ESCs utilize thin cytonemes to contact the bead and can react by directing a larger cytoneme to recruit the bead to the plasma membrane (reactive interaction [RI]) to form a stable contact (Figs. 1D and 2A and Movie S3). Although Wnt5a has high protein sequence similarity to Wnt3a, our assay indicated a significantly higher proportion of reactive interactions when cytonemes encountered Wnt3a beads (76% RI) relative to Wnt5a beads (43% RI) (Fig. 1E). This suggests that ESC cytonemes selectively react to Wnt ligands required for self-renewal.

We also tested the reactivity of cytonemes to control beads—inactive Wnt3a beads (iWnt3a beads) treated with dithiothreitol (DTT) to break the disulfide bridges in Wnt ligands, thus disrupting protein tertiary structure to render it inactive (refs. 20 and 24 and SI Appendix, Fig. S1D)—or to uncoated beads. Our results indicate that the cytonemes are unable to react to iWnt3a beads or uncoated beads efficiently (31% and 20% RI, respectively; Fig. 1E). To further confirm the selectivity of the cytonemes, we exposed ESCs to beads coated with bovine serum albumin (BSA), a nonsignaling molecule that often adheres nonspecifically to cellular membranes. Here, only 34% of interactions were reactive (Fig. 1E), reduced like the other control beads.

In summary, ESCs generate ligand-selective cytonemes to identify and recruit Wnt signals required for self-renewal. This ligand-based selectivity also governs the efficiency of stable ESC–TSC contacts and pairing.

To determine how cytonemes achieve this dual functionality, we analyzed their composition and dynamics.

**ESCs Produce Actin-Based Cytonemes that Contain Components of the Wnt Signaling Pathway**

Single ESCs appear to predominantly present bimodal, elongated cytonemes that emanate from the cell body (Fig. 2A and Movie S4), although more cytonemes can form subsequently (Figs. 2B and 4 B and C and SI Appendix, Fig. S3A). Cytonemes are dynamic and retract upon cell rounding prior to division (Fig. 2A). Cytonemes can form on different substrates and in culture media that support the self-renewal of single ESCs (SI Appendix, Fig. S3 A and B). These cytonemes are also present in ESC lines with different genetic backgrounds (SI Appendix, Fig. S3C). The time required to generate cytonemes varies between different ESC lines, but after five hours the percentage of cells with cytonemes is similar (SI Appendix, Fig. S3D). Scanning electron microscopy (SEM) revealed two major cytoneme types that both appear capable of interacting with the Wnt3a bead: thin cytonemes (~100-nm length) emanating from the main body of the cell and other nanostructures that are generated from larger cytonemes (Fig. 2B).

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To further characterize ESC cytonemes, we investigated their molecular composition. All observed cytonemes are composed mainly of actin, with tubulin restricted to the large cytonemes (Fig. 2C). Inhibition of actin polymerization via Cytochalasin D (SI Appendix, Fig. S4 A–C) prevented the formation of new cytonemes and restricted the motility of both the cell and the existing cytonemes (SI Appendix, Fig. S4C). Blockade of actin filament bundling using the Fascin inhibitor (Fascin-G2) also prevented cytoneme formation (SI Appendix, Fig. S4D). Therefore, cells without bundled actin filaments lose the capacity to recruit beads. Conversely, inhibition of tubulin polymerization by Colcemid did not impact this process (SI Appendix, Fig. S4 E–J).
Cytonemes contain components of the Wnt signaling pathway. On average, 58.9% of the observed cytonemes (n = 39 of analyzed single ESCs) contain LRP6 and all cytonemes have the downstream signaling component β-catenin (SI Appendix, Fig. S4 K and L). Contact with the Wnt source (Wnt3a bead or TSC) leads to the polarization of the Wnt coreceptor LRP6 and β-catenin in the ESC toward the area of contact (Fig. 2 D and E).

In summary, to selectively recruit the self-renewal signal Wnt3a, ESCs produce actin-based cytonemes that contain receptors and downstream effectors of the Wnt signaling pathway. Cytoneme-Wnt3a bead contact polarizes the bulk of the Wnt signaling components to the Wnt source.

ESC Cytonemes Activate Ionotropic Glutamate Receptors in Response to a Self-Renewal–Promoting Wnt Source

Contact-mediated signaling shares similarities with the neuronal synapse (25). In both systems, cellular protrusions are extended to receive a paracrine signal from the producing/presynaptic cell. In the neuronal synapse, calcium (Ca^{2+}) influx is often involved in neurotransmission and Ca^{2+} channels can be found on the pre- and postsynapse (26). We examined the generation of Ca^{2+} transients at the cytonemes of ESCs upon contact with a Wnt source. To do this, we generated a stable cell line expressing the free-cytoplasmic Ca^{2+} sensor GCaMP6s (27) and employed live imaging every six seconds (Fig. 3 A and B). A stable contact

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between an ESC and a TSC generated localized Ca\textsuperscript{2+} transients on the ESC cytoneme (Fig. 3A). Similarly, upon a stable contact between a Wnt3a bead and the ESC cytoneme, 72% of the cells generated localized Ca\textsuperscript{2+} transients at the area of contact (Fig. 3B and C). Only 24% of the cells had these transients when contacting an iWnt3a bead (Fig. 3B and C). Importantly, the Wnt3a beads induced more frequent and longer-duration Ca\textsuperscript{2+} transients than the iWnt3a beads (SI Appendix, Fig. S5A).

Chelating Ca\textsuperscript{2+} from the media by ethylene glycol tetraacetic acid (EGTA) significantly reduced Ca\textsuperscript{2+} transients (only 25% of the cells) upon a stable contact between a Wnt3a bead and the cytoneme (Fig. 3C and SI Appendix, Fig. S5A). These results suggest that Ca\textsuperscript{2+} influx is mediated by activated Ca\textsuperscript{2+} protein channels/transporters at the Wnt3a bead contact area. We sought to investigate those protein channels or transporters.

Huang et al. (28) recently demonstrated the generation of Ca\textsuperscript{2+} transients at the cytonemes of Drosophila air sac primordium that are essential for Decapentaplegic (Dpp) signaling. These Ca\textsuperscript{2+} transients require glutamatergic activity mediated by the glutamate receptor GluRII. Crosstalk between ionotropic glutamate receptors, Ca\textsuperscript{2+} influx, and Wnt signaling has also been described in the neuronal synapse (29). We aimed to investigate whether this feature also exists at the ESC cytoneme. Transcript profiling (SI Appendix, Fig. S5B) and proteomic analysis indicate that ESCs express ionotropic glutamate receptors (iGLuRs) (30, 31). Initially, we incubated single ESCs with Wnt3a beads in the presence of inhibitors of iGLuRs: MK801 (a noncompetitive antagonist of the N-methyl-D-aspartate [NMDA] receptor) or cyanoquinoxaline (CNQX), a competitive antagonist for the AMPA and kainate receptors. Only CNQX treatment significantly reduced the proportion of cells that produce Ca\textsuperscript{2+} transients at the

Fig. 3. ESC cytonemes exhibit glutamate receptor activity upon contact with a Wnt source. (A) Representative frames from time-lapse imaging of an ESC expressing the Ca\textsuperscript{2+} reporter GCaMP6s and Ftractin-mRuby3, where a cytoneme contacts an eGFP-expressing TSC (green, dashed black line). (Top) interaction between ESC and TSCs, (Scale bar, 10 μm) (middle) generation of Ca\textsuperscript{2+} transients upon cytoneme-TSC contact; with fluorescence signal represented by heat-map colors using the Fire look-up table in Fiji; (Bottom) position of the cytoneme in the interaction (yellow arrowhead points to the tip of the cytoneme). Time is expressed in minutes and seconds. (Scale bar, 5 μm) (B) Representative frames from time-lapse imaging of an ESC expressing GCaMP6s, where a cytoneme contacts a Wnt3a bead. (Top) generation of Ca\textsuperscript{2+} transients upon cytoneme-Wnt3a bead contact; (Bottom) absence of Ca\textsuperscript{2+} transients, with the bead shown as a black sphere in the DIC panels, outlined by a yellow dashed circle. Time is expressed in minutes and seconds. (Scale bars, 10 μm.) DIC, differential interference contrast. (C) Quantification of the percentage of cells with Ca\textsuperscript{2+} transients in the cytonemes, in different ESC cell lines, in the presence of Wnt3a or iWnt3a beads or cells treated with CNQX (10 μM), MK801 (20 μM), or EGTA (2 mM). (D) Quantification of the percentage of reactive (green) or nonreactive (red) interactions between ESCs and Wnt3a beads in the presence of 5-μM TG, 2-mM EGTA, 10-μM CNQX, 20-μM MK801, or control treatment water or dimethyl sulfoxide (DMSO; CNTRL). Water or DMSO treatment yielded similar percentages of interactions. n ≥ 30 cells from three independent experiments. (E) Quantification of the percentage of reactive (green) or nonreactive (red) interactions between ESCs and TSCs in the presence of 10-μM CNQX. n = 55 cells from three independent experiments. Asterisks indicate statistical significance calculated by Fisher’s exact test for C, D, and E; ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
cytonemes upon a stable contact with a Wnt3a bead (19%) (Fig. 3C). Here, the transient frequency was low and each lasted only 21.8 s, on average, relative to 78.2 s without CNQX (SI Appendix, Fig. S5A). Consequently, CNQX treatment significantly reduced the proportion of reactive interactions with Wnt3a beads, thus impacting Wnt3a-bead recruitment to the plasma membrane (Fig. 3D). The agonist kainate can reverse the effects of CNQX on Ca^{2+} transients and the reactivity of the cytonemes to Wnt3a beads.
Importantly, the proportion of ESC cytonemes that establish a stable contact with TSCs and mediate ESC–TSC pairing, as well as the proportion of synthetic embryo structures (3) that can form in 3D, was significantly reduced in the presence of CNQX (Fig. 3E and SI Appendix, Fig. S2).

Next, we asked if the activity of iGluRs, intracellular Ca\(^{2+}\), and Ca\(^{2+}\) influx are required for cytoneme formation. CNQX and MK801 had no significant effect on cytoneme formation (SI Appendix, Fig. S5 E and F). Depleting intracellular Ca\(^{2+}\) stores by Thapsigargin (TG, a SERCA pump blocker) (32, 33) significantly compromised cytoneme formation (SI Appendix, Fig. S5E). However, Thapsigargin treatment did not affect the ability of the existing cytonemes to react to Wnt3a beads (Fig. 3D). On the other hand, EGTA in the media significantly reduced the proportion of reactive cytonemes (Fig. 3D). This supports the role of iGluR-mediated signaling, which includes Ca\(^{2+}\) influx to the cell, in the recruitment of localized Wnt3a to cells.

In conclusion, the formation of ESC cytonemes is regulated by intracellular calcium stores. Activated AMPA/kainate receptors enable localized influxes of Ca\(^{2+}\) on cytonemes that facilitate the recruitment of the self-renewal signal Wnt and mediate ESC–TSC pairing and the formation of synthetic embryos.

**Components of the Wnt Pathway Regulate the Formation and Selectivity of Cytonemes**

The distribution patterns of Wnt receptors and downstream effectors such as β-catenin within cytonemes prior to cell–signal contact suggests that ESCs may utilize these proteins to regulate reactivity to an instructive signal. To examine this, we generated knock-out (KO) cell lines and studied the effect of the loss of each component on cytoneme formation and the recruitment of Wnt3a beads. Specifically, we generated LRPS5KO, LRP6KO, LRPS/6dKO, and DVL2KO and utilized the published conditional β-catenin KO cell line (βKO) (35).

We initially characterized these cell lines including the expression of pluripotency markers, the protein levels of components of the Wnt/β-catenin pathway, and the ability of the different KO cell lines to activate the Wnt/β-catenin pathway. Please refer to SI Appendix, Supplementary Text and Figs. S6 and S7 for a full description of the generation and characterization of the KO cell lines.

Briefly, when cultured in 2i media that maintain ESC self-renewal (36), KO cell lines of the receptors and DVL2 maintain levels of pluripotency markers comparable to those of wild-type (WT) cells (SI Appendix, Fig. S6). Importantly, in this media CHIR 99021 (a glycogen synthase kinase 3 β [GSK-β] inhibitor) can activate the Wnt/β-catenin signaling pathway by inhibiting the destruction complex, overriding the need for Wnt proteins, the receptors, or DVL2 (19). The freshly knocked-out β-catenin cell line has reduced levels of pluripotency markers compared to the WT cell line (SI Appendix, Fig. S7).

In the presence of purified Wnt3a ligands, leukemia inhibitory factor and serum-containing media LRPS5KO has a significant reduction in activation of the Wnt/β-catenin pathway in comparison to WT, and LRP6KO is significantly further compromised. LRPS/6dKO cannot activate the Wnt/β-catenin pathway in this media. Furthermore, DVL2KO, which has elevated levels of DVL1 and DVL3, can activate the Wnt/β-catenin pathway even more than the WT cells in the presence of Wnt3a ligands (Fig. 4D).

We next addressed whether the aforementioned differences between WT and mutant cells impact cytoneme formation and reactivity to niche signals. WT cells elongate and mainly produce bimodal cytonemes, but they can also have additional smaller cytonemes. Combined, these cytonemes, averaging five per single WT cell with an average maximum length of 29.9 μm, facilitate interaction with the surrounding Wnt3a beads (Fig. 4 B–D and Movie S4). Similarly, the cell lines LRPS5KO, LRPS6KO, and LRPS/6dKO have, on average, four cytonemes. LRPS5KO cytonemes are marginally shorter when compared to WT cytonemes (Fig. 4 B–D, SI Appendix, Fig. S8 A–C, and Movie S5). On the other hand, LRPS6KO has significantly shorter cytonemes (21.1 μm) than WT and the cytonemes are even shorter in the LRPS/6dKO cells (16.3 μm) (Fig. 4 B–D, SI Appendix, Fig. S8 A–C, and Movie S6). DVL2KO cells are rounder than WT cells and contain significantly fewer cytonemes (an average of three cytonemes) that are very short (10 μm) in comparison to all other studied cell lines (Fig. 4 B–D, SI Appendix, Fig. S8 A–C, and Movie S7). The round morphology is more prominent in βKO (Fig. 4B), although βKO has more (an average of seven) and shorter cytonemes than the WT cells (Fig. 4 B–D, SI Appendix, Fig. S8 A–C, and Movie S8). The aforementioned statistically significant changes in the number and length of the protrusions of each KO cell line (Fig. 4 C and D) are reflected in the increased time required by these cells to detect Wnt3a beads in their local environment (Fig. 4E and SI Appendix, Fig. S8E). Importantly, in these measurements the initial distance between the cell and the bead was similar for all cell lines (SI Appendix, Fig. S8E).

All KO cell lines establish interactions with Wnt3a beads via cytonemes (Fig. 4F). Knocking out LRPS6 significantly reduces the ability of a cell to subsequently recruit the Wnt3a bead to the plasma membrane, whereas knocking out LRPS5 does not (Fig. 4G). LRPS/6dKO also exhibits compromised ability to recruit Wnt3a beads to the cell and to pair with TSCs (Figs. 4G and 1A and B). We found that LRPS/6dKO also impaired the generation of Ca\(^{2+}\) transients upon cytoneme–Wnt3a bead contact (only 36% of the cells; Fig. 3C). The frequency and duration of the Ca\(^{2+}\) transients observed at LRPS/6dKO cytonemes were reduced in comparison to WT (SI Appendix, Fig. S5A). These results emphasize the essential crosstalk between Wnt coreceptors and Ca\(^{2+}\) transients at the cytonemes for the recruitment of localized Wnt signals and ESC–TSC pairing.

The interactions of DVL2KO cytonemes are less reactive than those of the WT, whereas the reactivity of the βKO cytoneme interactions is unaffected (Fig. 4G).

Taken together, the evidence shows that LRPS6 plays an important role in the initial recognition and subsequent recruitment of the Wnt3a bead to the plasma membrane after contact, while LRPS5 is not required for this process. DVL2 and β-catenin regulate the length and number of cytonemes and the resulting efficiency of detecting the self-renewal signal without compromising its recruitment to the plasma membrane.

In summary, we identified specialized ESC-produced cytonemes and the mechanism they use to detect and selectively react to self-renewal Wnts. This process requires crosstalk between Wnts and the family of glutamate receptors that involves an influx of Ca\(^{2+}\) and promotes ESC–TSC pairing required for synthetic embryogenesis.

**Discussion**

Cytoneme-mediated signaling is a means of highly specific paracrine signal transduction, allowing both signal amplitude and duration to be controlled with exquisite precision. Yamashita and colleagues identified, in male germ line stem cells, microtubule-based protrusions that contain bone morphogenetic protein (BMP) receptors and extend to the hub cells in Drosophila testis, thereby recruiting the ligand that is essential for their maintenance (37). Identification of cytonemes and understanding of their function in mammalian stem cells remains limited.

Here, we show that ESCs use specialized cytonemes to distinguish between niche signals and preferentially select Wnt ligands promoting their self-renewal, such as those secreted by TSCs. After the recognition of the Wnt source, ESCs generate larger cytonemes to facilitate robust signaling and pairing with TSCs to promote synthetic embryogenesis.
The multiplicity of secreted ligands and the difficulty of visualizing Wnt proteins in situ make it challenging to study how Wnt reception occurs in ESCs. To circumvent these issues, we utilized a reductionist approach of immobilizing purified Wnt ligands on microbeads, distributing them near single ESCs, and observing their interaction by time-lapse imaging.

We found that ESCs generate actin-based cytonemes that contain Wnt receptors, which represent, on average, 60% of the total protrusions that ESCs form. After a Wnt source is detected, the bulk of the receptors and β-catenin polarizes toward the Wnt. A larger cytoneme enriched with Wnt receptors and AMPA/kainate receptor subunits form a stable contact with the Wnt source. Consequently, LRP6-AMPA/kainate receptor crosstalk is initiated and generates localized Ca²⁺ transients. This crosstalk is required to allow the reactive interaction, Wnt signaling, and ESC–TSC pairing that form the basis of cellular communication and spatial self-organization.

The finding that ESCs utilize iGluR-containing cytonemes indicates a striking similarity to aspects of the neuronal synapse. Both systems show selective, directed cellular protrusions for the purpose of reinforcing a niche. The multiplicity of secreted ligands and the difficulty of visualizing Wnt proteins in situ. Wnt signaling, and ESC–TSC pairing that form the basis of cellular communication and spatial self-organization.

The findings, and our own data, may suggest that the neuronal signaling in the Drosophila investigations have demonstrated that components of the pre-order spatial signaling and organization. Recent comprehensive purposes of reinforcing a niche. Both systems show selective, directed cellular protrusions for the purpose of reinforcing a niche. The multiplicity of secreted ligands and the difficulty of visualizing Wnt proteins in situ. Wnt signaling, and ESC–TSC pairing that form the basis of cellular communication and spatial self-organization.

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