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Rhes Tunnels: A Radical New Way of Communication in the Brain's Striatum?

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Abstract

Ras homolog enriched in the striatum (Rhes) is a striatal enriched protein that promotes the formation of thin membranous tubes resembling tunneling nanotubes (TNT)—“Rhes tunnels”—that connect neighboring cell and transport cargoes: vesicles and proteins between the neuronal cells. Here the literature on TNT-like structures is reviewed, and the implications of Rhes-mediated TNT, the mechanisms of its formation, and its potential in novel cell-to-cell communication in regulating striatal biology and disease are emphasized. Thought-provoking ideas regarding how Rhes-mediated TNT, if it exists, in vivo, would radically change the way neurons communicate in the brain are discussed.

Keywords

intercellular cargo; membrane scanning; mutant Huntingtin (mHTT) transport; neuronal contacts; protrusion synapse; striatal behavior; vesicle delivery

1. Introduction

1.1. Membranous Protrusions are Characteristic of Diverse Cell Types (Selected Studies between 1970 and 1980)

Most cells harbor membranous protrusions. The earliest evidence showing cells with membrane protrusions making intercellular adhesive contacts, which is influenced by temperature or nutritional status, with neighboring cells was observed in rat leukemia cells.^[1] In testicular biopsies, long membranous protrusions that were proposed to allow the flow of raw materials in or out of the seminiferous tubule^[2] were observed from the basal lamina to the germinal epithelium. During photoreceptor development, the emergence of membranous protrusions that resemble the elongating filopodial processes described for growing axons and dendrites has been reported.^[3] In an in vitro system, erythrocytes rearrange their membranes and interact with each other via long membranous protrusions.^[4] The root cells of the inner ear are linked together via membranous folding within the

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Conflict of Interest

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Supporting Information

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intercellular space and contain small protrusions that are predicted to play a role in extracellular transport.^[5] Giant-celled glioblastoma under electron microscopy (EM) show membranous undulations with occasional filopodia-like protrusions.^[6] In plerocercoid larvae cultivated in vitro, the neurosecretory vesicles release their content via protrusions extending through the axolemma into the intercellular space.^[7] Neurosecretory granule cells in insects make activity-based membrane protrusions that can make contact with cell membranes of neuronal axons.^[8] Tumor cells from the liver make cellular protrusions that interact with endothelial cells.^[9] Epithelial cells in the capillaries show membrane protrusions in response to histamine shock, a condition where histamine exerts a local dilator effect upon capillaries.^[10] Hepatocytes produce cellular protrusions after carbon tetrachloride toxicity.^[11] Oral mucosal cells are characterized by well-defined polarized external protrusions, which appear to be profoundly altered in cells that transform into cancerous cells.^[12]

1.2. Membranous Protrusions are Characteristic of Diverse Cell Types (Selected Studies between 1980 and 2010)

Bacteria can also interact with and enter animal cells via forming filamentous protrusions.^[13] Various types of cellular membranous contact each other in tooth cells, between ameloblasts and odontoblasts.^[14] In rod photoreceptor disk membranes, scattered, thin, branched short filaments appear to link disk rims with the adjacent plasma membrane, which is speculated to have a role in phototransduction via membrane dynamics during synaptic activity.^[15] In the postpartum rat uterus, many bulbous membranous protrusions and cytoplasmic blebs were found to be phagocytosed by fibroblast-like cells and macrophages.^[16] EM observation revealed that the surface of the naked eggs responded with the formation of many small cytoplasmic protrusions (viz., fertilization cones) at sperm attachment sites.^[17] Membranous intercellular bridges connecting two adjacent villi in human placenta have been reported.^[18] Membranous cytoplasmic protrusions from endometrial cells during the period of conceptus attachment were implicated in secretory processes.^[19] In birds, special cells such as Grandry corpuscles produce numerous protrusions that seem to interact with Schwann cells.^[20] Cytotoxic T-cells have shown to make finger-like membranous protrusions into virus-infected target cells.^[21] Ultrastructural analysis of osteoblastic cells found numerous membranous protrusions whose cytoplasm contained rough endoplasmic reticulum and Golgi membranes.^[22] Membranous basal protrusions, which contain microfilaments observed between epithelial and immune cells, are proposed to play a role in intercellular transport.^[23] Scanning electron microscopy of rabbit buccal epithelial cells showed small protrusions that appear to intercellularly connect between cells.^[24] At the ultrastructural level, lymph capillaries show a very thin and indented wall with protrusions toward the lumens of the vessels.^[25] Immunofluorescence and EM studies revealed that the *Shigella flexneri* spread between cells via membranous protrusions.^[26] EM studies showed a physical interaction between Langerhans cells and T-cells via membranous protrusions.^[27] Stereocilia, the inner ear hair cell, had numerous protrusions of various sizes and shapes.^[28] Moving bacteria that reach the plasma membrane were found to induce the formation of long membranous protrusions that are internalized by neighboring cells.^[29] The Poxviridae family virus, *vaccinia virus*, and *coxsaievirus* can travel between cells via actin-based membranous protrusions.^[30]

1.3. Membranous Protrusions are Characteristic of Diverse Cell Types (Selected Studies from 2010's onward)

More recent studies further report multiple roles for cellular protrusions in various cell types. In cultured leafhopper cells, the rice dwarf plant virus is intercellularly transmitted by a leafhopper vector via actin-based cellular protrusions.^[31] Stromules, the thin dynamic protrusions found between the plant plastids, can exchange viruses, molecules, or organelles.^[32] While not all the protrusions exchange materials, the protrusions induced by interleukins can promote cell-to-cell adhesion.^[33] In cultured enterocytes, the listeria bacteria spreads between cells via cellular protrusions involving membrane modeling proteins.^[34] Membrane protrusions are also known to play a role in the mating of ciliated protozoa.^[35]

Filopodia-like long protrusions containing glycoposphatidylinositol-anchored proteins and the transmembrane Wntreceptor frizzled-7 were found between distant epithelial cells during embryonic development in chick embryos.^[36] Cellular protrusions (also called tumor microtubes) connecting cancer cells in glioma cancer have been reported.^[37] In the visual system, the highly dynamic filopodial protrusions have been shown to play a critical role in synaptogenesis and circuit maturation.^[38] The finger-like protrusions appear to be necessary for myoblast cell fusion in drosophila and for leukocyte migration.^[39] Similarly, cellular protrusions appear to control the maturation of the mesoderm in Xenopus.^[40]

2. Biological Significance of Cellular Protrusions

The studies described above indicate that cellular protrusions play a major role in cell-to-cell communication. This raises fundamental questions: What are the evolutionary advantages of cellular protrusions and how are they beneficial to cells? We can logically assume that, when cells came into existence a few billion years ago, they had a better chance of survival if they could communicate with one another. That communication could be in the form of chemicals that could freely diffuse from one cell to reach another, but such communication is daunting and risky because the chemicals may lose their potency in the medium in which they travel and because there is no apparent specificity in the direction of travel to the target cell. Thus, cells may have evolved a system wherein communication could be more secure, for example, via cellular protrusions that could physically interact with neighboring cells. Such communication establishments would have far more effective ways of signaling and transporting molecular cargoes from the donor cell to a specific recipient cell as described for cytonemes and tunneling nanotube (TNT)-like protrusions below.

3. Membranous Protrusions as Intercellular Communication Bridges

Earlier studies in sea urchin and in drosophila showed thin, actin-positive cellular protrusions playing a role in transporting signals between cells.^[41,42] In drosophila, the membranous protrusions were termed cytonemes and were found to be involved in the transportation of morphogene (Wg), and Notch signaling; dsRNA and RNAi machineries; and exosomes, and to contain glutamate synapses.^[42,43] While cytonemes in drosophila can make contact with neighboring cells, these contacts are believed to be closed ended, analogous to neuronal synapses.^[44] Cytoneme-like protrusions were also found in vertebrates: in human neutrophils, B-cells, and mast cells; in pituitary glands; in

mesenchymal cells; in pericytes in vivo; and between bacteria and neutrophils.^[45] Whether they are open- or closed-ended contacts remains unknown. Despite these studies, the exact molecular mechanisms by which cytonemes are produced, elongated, and interacted with target cells and cargo deliverers remain unclear. In *Drosophila*, genes such as *diaphanous*, *shibire*, *neuroglian*, and *capricious* are implicated in the formation of *Drosophila* cytonemes.^[46] Similarly, the subcellular localization of signaling ligands such as Hh, Wg, Delta, and Spz have been implicated in the generation of cytoneme-like protrusions.^[47] Also, the leucine-rich G-protein-coupled receptors (Lgrs), such as Lgr4 and Lgr5 promotes the formation of long actin-rich cytoneme-like membrane protrusions in HEK293 cells.^[48] These studies indicate that the cytoneme-like protrusions initially identified in *Drosophila* can also be found in mammalian cells, but the exact mechanisms or their role remains less clear. A recent study has proposed the provocative idea that cytonemes may have a role in facilitating morphogen gradient and delivery during *Drosophila* development.^[49]

4. Discovery of TNTs and TNT-Like Protrusions in Diverse Cell Types

TNTs were first described in neuronal PC12 cells. TNTs in PC12 cells are long, thin protrusions connecting two cells and have been shown to facilitate the selective transfer of membrane vesicles and organelles.^[50] Unlike filopodia-like protrusions, TNT structures can promote a direct transfer of cargoes between cells, and they are fragile and often sensitive. They can easily be destroyed by cell fixing agents such as paraformaldehyde. Also, it is believed that TNT-like protrusions can make open-ended connection, analogous to plasmodesmata in plant cells.^[51] Cryo-EM studies revealed TNTs can be bundle of multiple TNT and focused ion beam milling combined with scanning electron microscopy (FIB-SEM) studies showed a possibility of open-ended-like connections.^[52] However, it is widely believed that TNTs must be in an open-ended state at a certain point to transfer cargoes. That state may be highly dynamic and transient; due to resolution limitations, it is difficult to conclusively establish the open-ended states of TNTs using fluorescent techniques.

Two broad mechanisms have been proposed for the emergence of TNTs. The “cell dislodgement mechanism” proposes that two cells (e.g., during cell division) retain a thin thread of membrane as they move apart, forming TNTs. Alternatively, cells make active TNT-like protrusions that are most often rich in actin cytoskeleton, which makes physical contact with neighboring cells.^[53] The mechanism of TNT formation remains unclear, however, as it is expected to be quite different depending upon cell types. Nevertheless, TNT and TNT-like studies have exploded, demonstrating the existence of TNTs in diverse cell types and their role in the cell-to-cell transportation of organelles, prions, and viruses as well as their role in cancer progression, as described below.

5. Signaling and Transport Roles of TNTs and TNT-Like Protrusions in Diverse Cell Types

In myeloid-lineage dendritic cells and monocytes, TNTs can propagate calcium (Ca^{2+}) signals.^[54] HIV infection of macrophages can induce TNTs that help in HIV propagation.^[55] In SH-SY5Y cells, TNTs are shown to propagate Ca^{2+} signals.^[56] TNTs were observed between human mesenchymal multipotent stromal cells (MMSCs) and rat renal tubular cells,

and they can transport mitochondria from cell to cell.^[57] Between primary human proximal tubular epithelial cells, extensive exchange of cargo vesicles and organelles occurs via TNTs.^[58] In breast cancer cells, the multi-drug resistant regulator P-glycoprotein (P-gp) is transported between the cells via TNTs.^[59] The TNTs found between malignant cells or between normal mesothelial cells show the transfer of proteins, mitochondria, and Golgi vesicles.^[60] HeLa cells can induce TNTs and TNT-dependent transmission of electrical and calcium signaling and small molecular diffusion, and mitochondrial re-localization was found between human retinal pigment epithelial cells.^[61] TNTs formed among osteoclast precursors and were shown to transport membranous vesicles and proteins.^[62] TNTs formed between B- and T-cells known to transport H-Ras protein.^[63] Human mesothelial cells are connected by TNTs.^[64] A long TNT-like nanotube was found in an osteosarcoma tissue and formed between osteosarcoma cell lines.^[65] Human laryngeal squamous cell carcinoma cells also form TNT and transport mitochondria.^[66] Human glioblastoma cells also connect via TNTs.^[67] Prion proteins are found to be transported by TNTs in CAD (Cath.-a-differentiated) cells, a central nervous system-derived catecholaminergic neuronal cell line.^[68,69] Studies revealed the presence of TNTs in microvascular endothelial cells and transporting lysosomes and mitochondria.^[70] Primary B-cell precursor cells also use TNTs to transport cytokines and interferons.^[71] Monocyte-derived macrophages transmit HIV via TNTs.^[72] Ovarian cancer cells connected by TNTs can transport mitochondria.^[73] The bidirectional exchange of cytoplasmic components between human adipose tissue-derived mesenchymal stem cells (MSCs) and primary T lymphocytes is also found to be mediated by TNTs.^[74] B-cells are known to spontaneously form extensive TNT networks containing lysosomes and mitochondria.^[75] Hematopoietic progenitors can form TNTs and transfer stem cell markers with primary mesenchymal stromal cells.^[76] TNTs between MSC and corneal epithelial cells transport mitochondria.^[77] Lung epithelial cells are connected by TNTs and transport the influenza virus.^[78] TNTs promote intercellular mitochondrial transfer between heterogeneous cells, followed by an increase in the invasiveness of bladder cancer cells.^[79] Pancreatic cancer cells and tissues were shown to produce TNTs.^[80] TNTs were also observed between endothelial progenitor cells and osteoclast precursors cells.^[79] TNT-like protrusions were found to be involved in mRNA and protein transfer between keratinocyte and Langerhans cells in vivo.^[81] A recent study demonstrated that stromal TNTs contribute to the development of cancer drug resistance in leukemia. Correlative light-electron microscopy and electron tomography found that stromal TNTs contain vesicles, provide membrane continuity with cell bodies, and can be open ended.^[82]

A growing number of studies has demonstrated that viruses, as well as neurodegenerative disease-linked proteins, use TNTs for transmission and spreading.^[72,78,83,84] Prions, a pathogenic agent, engage TNTs in intercellular transportation in CAD cells and primary neurons.^[69,85] Likewise, tau fibrils, the biochemical hallmark of Alzheimer disease, increase TNT numbers and are transported via TNTs in HeLa and CAD cells.^[84,86] Similarly, α -synuclein, a major constituent of Lewy bodies, the pathological hallmark of Parkinson disease, has been shown to be efficiently transported via TNTs in multiple cell types.^[87] Thus, TNT-like structures were found in multiple cell types in vitro and in vivo, and in transporting, vesicles, proteins, and viruses, but the mechanisms of their role or existence, if any, in the nervous system remain less clear.

6. Rhes, a Striatal-Enriched Brain Protein, Induces TNT-Like Protrusions

Ras homolog enriched in the striatum (Rhes) is a small GTPase family member containing the GTP/GDP-binding domain (Figure 1A). Its mRNA is highly expressed in dopamine D1 receptor (D1R) and dopamine D2 receptor (D2R)-expressing neurons, in cholinergic interneurons in the striatum, and in other brain regions.^[88] Rhes is induced by thyroid hormones and can inhibit the cAMP/PKA pathway and N-type Ca^{2+} channels (Cav 2.2).^[89] We have found several new roles for Rhes in the striatum. Rhes can directly bind to and activate the mammalian target of rapamycin (mTOR) in a GTP-dependent manner, and this promotes L-DOPA-induced dyskinesia in a pre-clinical model of Parkinson disease.^[90] Rhes also contains a C-terminal small ubiquitin-like modifier (SUMO) E3-like domain (Figure 1A) and physiologically regulates SUMO modification via “cross-SUMOylation” of E1 (Aos1) and E2 (Ubc9) enzymes.^[91] Rhes affects autophagy via Beclin1, independent of mTOR signaling.^[92] Rhes also inhibits striatal motor activity through a “Rhesactome” protein network in the striatum.^[93] We also demonstrated a critical role for Rhes in Huntington disease (HD). We found that Rhes interacts with mHTT (mutant huntingtin), the genetic risk factor of HD, and makes it more soluble by adding SUMO.^[93,94] Thus, Rhes and mHTT interaction increases cellular toxicity, consistent with independent studies.^[94–96] Rhes also plays a critical role in mutant tau-mediated toxicity.^[97] In addition, a rare, highly conserved de novo mutation (R57H) in Rhes was detected in twins diagnosed on the autism spectrum.^[98] These studies illustrate significant and multifarious roles of Rhes in neuronal functions and neurological diseases. However, we know much less about how Rhes modulates various biological processes and integrates various signaling to promote diverse brain functions.

Recently, we discovered that Rhes is a potent inducer of TNT-like protrusions. As our striatal proteomics data found that Rhes-mediated motor functions have a strong link to endocytosis pathways,^[93] we sought to study its putative role in endocytosis. Surprisingly, during live-cell imaging, we found that striatal neuronal cells^[99] transfected with GFP-Rhes, but not by GFP control, showed numerous cellular protrusions positive for GFP-Rhes.^[100] Differential interference contrast (DIC) images showed numerous protrusions interconnecting cells in GFP-Rhes expressing striatal neuronal cells compared to GFP, indicating that these protrusions are induced by Rhes.^[100] Striatal neuronal cells do not express endogenous Rhes, whose expressions normally appear during postnatal day 8 or later in striatum in vivo.^[101] Therefore, the exogenous expression of Rhes in striatal neuronal cells mimics striatum and will not compete with endogenous Rhes’ function or the machineries involved in the protrusions. The robust membranous protrusions induced by Rhes that are interconnecting striatal neuronal cells has piqued our curiosity, and we launched a detailed study and found that they resemble TNTs in many aspects, such as size (<150 nm) and length (100–150 μm); hover above the substratum; are positive for actin; and interact with and transport cargoes to neighboring cells.^[100] This is the first report, to our knowledge, showing that the brain-enriched protein can induce TNT-like protrusions and transport cargoes between cultured striatal neuronal cells.

7. Rhes Transports Vesicles and Proteins through TNT-Like “Rhes Tunnels”

Rhes-positive TNT-like protrusions are membranous and transport vesicles, lysosomes, and endosomes. Endosomes travel at a speed of 0.012 mm s^{-1} , which is ≈ 150 times much slower than retrograde transport of cargo in neurons^[102] (Movie S1, Supporting Information). We did not detect markers of mitochondria, Golgi, or peroxisome in the Rhes-induced TNT-like protrusions. Intriguingly, Rhes itself as vesicular puncta is transported via TNTs to the adjacent cells. Thus, Rhes engineers TNT-like cellular protrusion, “Rhes tunnel,” which serve as a “highway” for vesicle as well as Rhes protein itself. This raised the possibility that Rhes tunnels can also transport other proteins that are known to interact with Rhes and regulate their functions, such as mHTT, the polyglutamine expanded HD protein, and mTOR kinases. Notably, mHTT itself cannot be transported, but in the presence of Rhes, mHTT is robustly transported to neighboring cells via TNT-like Rhes tunnels.^[100] The transportation is protein specific because normal HTT (<25 glutamines), mTOR, and wt-Tau are not transported in TNT-like Rhes tunnel. Based on live imaging and vesicle markers, we presume that mHTT protein is transported as vesicular cargo, although whether this is the case for all the unknown proteins that are transported in TNT-like Rhes tunnel remains to be determined. But the mechanisms by which Rhes induces TNT-like Rhes tunnels, transport vesicles, and protein cargo, also remain less clear.

8. The Promotion of TNT-Like Rhes Tunnels Lies in its C-Terminal Membrane Binding and the SUMO E3-like Ligase Domain of Rhes

We found that C-terminal farnesylation cysteine site, the C263 of Rhes (Figure 1A) is critical for TNT-like Rhes tunnel forming activities. Because Rhes C263S is totally defective in making TNTs, suggesting that membrane binding via C263 is crucial for its Rhes tunnel-inducing activity. The activity also lies to a certain extent in the SUMO E3-like ligase domain, 171–266aa region, but the amino acids responsible for this remain unknown. Cells that are deficient in SUMOs were still able to promote TNT-like Rhes tunnels, indicating Rhes can induce TNT-like protrusions independent of its SUMOylation activity. Interestingly, the 171–266aa region of Rhes is highly conserved among different species, indicating that TNT-like Rhes tunnel activity may be conserved in the animal kingdom.

9. Rhes-Induced TNTs Have Multiple Roles in Striatal Biology and Disease

9.1. Intercellular Transfer of Cargoes as a Normal Physiological Function in the Striatum

Neurons communicate by means of synapses, gap junctions, and exosomes. The presence of TNTs, which is observed in cultured neuronal cells, is not firmly established in the intact brain. This is due to fragile nature of these TNTs and lack of specific marker to distinguish them from the other types of protrusions. But protrusions such as cytonemes or membranous thin tubes were found in diverse cell types in vivo.^[41,42,103] In the striatum, which is made up of two major D1R- and D2R-positive striatal neurons, the existence of TNTs or cytonemes remains unknown. Because striatal-enriched Rhes, which is highly enriched in both D1R and D2R-expressing neurons, induces robust TNT-like Rhes tunnels, it raises a

possibility that TNT-like processes are common in the striatum. If so, what are the implications for such intercellular signaling? One possibility would be that the exchange of materials via TNT-like Rhes tunnels would be a stochastic process benefitting the co-existence of multiple cell types, in addition to other established modes of synaptic communication. In our preliminary findings, we found that Rhes, which promotes mitophagy, can travel via TNT-like Rhes tunnel to neighboring striatal neuronal cells and interact with damaged mitochondria there.^[104] Thus, TNT-like Rhes tunnels may be used for the purpose of mitochondrial surveillance. Striatum also consists of two distinct anatomical regions—matrix and striosomes—that are distinguished based on the different neurochemical markers.^[105] A thought-provoking hypothesis is whether TNT-like protrusions connect these anatomically distinct regions of the striatum and in turn regulate the striatal-dependent behaviors of the brain. For example, Rhes may transfer receptors containing vesicular cargo across these two regions via TNT-like Rhes tunnels in the striatum. Whether such possibilities exist in the striatum requires further studies.

9.2. Disease Implication for Rhes-Mediated Intercellular Transport of mHTT via TNT-Like Protrusions in the HD

It remains a mystery why striatum in the HD is highly vulnerable to mHTT.^[106] We linked Rhes interaction with mHTT as a principle cause for such vulnerability. Rhes promotes mHTT modification by SUMO that leads to more soluble and toxic forms of mHTT in cell and animal models of HD.^[94,95] Now we show that Rhes promotes intercellular transport of mHTT, which is partially SUMO dependent, because SUMO-defective mHTT is transported less efficiently.^[100] Thus, while the TNT-like Rhes tunnels formation by Rhes appears to be independent of SUMO, SUMO contributes to the transport of mHTT. One possibility might be that SUMO modification of mHTT by Rhes may help solubilize mHTT, which can then be transported more efficiently via TNT-like Rhes tunnels. It is unclear whether Rhes can transport aggregated forms or only soluble forms of mHTT. Because full-length mHTT, which has less tendency to form aggregates, is also present in TNT-like Rhes tunnels,^[100] we predict that Rhes may favor the soluble forms. Nevertheless, from the HD perspective, the intercellular transport of mHTT by Rhes via Rhes tunnels may have significant implications, and it raises several questions: Is mHTT intercellularly transported in vivo? Does such transport affect lesion in the striatum? If so, what are the mechanisms? Studies from human and animal models do suggest that mHTT is transported between the cells in the brain. The mHTT aggregates were found in healthy striatal cell transplants in the striatum of HD patients.^[107] Healthy human neurons were found to contain mHTT when co-cultured with HD mouse brain slices.^[108] In *Drosophila*, mHTT was found to spread from olfactory receptor neurons to various parts of the brain.^[109] Similarly, human mHTT was found in the striatum of normal mice that had received intraventricular placement of human HD neurons.^[110] Whether the cell-to-cell transport of mHTT by TNT-like Rhes tunnels is involved in such a process remains an open question. However, MRI imaging of HD patients show between-region connectivity with increasing disease progression, which may facilitate inter-neuronal mHTT transport.^[111] Going forward, it is important to design cellular models in which we first understand the implications of mHTT that is being transported to neighboring cells. Our preliminary data indicate that mHTT transported by Rhes is associated with organelles, such as lysosomes, in neighboring cells.^[100] Therefore, it raised

the question whether association of mHTT with lysosomes may induce abnormal lysosomal signaling. This notion is consistent with our finding that mHTT induces nutrient-induced activation of mTOR, which is localized on the lysosomes.^[112] Such intercellular activation of mTOR by mHTT may affect intercellular homeostasis and in turn promote tissue-specific damage. For example, high mTOR activation may diminish autophagy in neighboring cells and affect its protein and organelle turnover and thus initiate cell-type-specific lesions. These notions should be validated experimentally in vitro and in vivo in future studies.

9.3. Model Describing Mechanisms of Rhes-Mediated TNT-Like Protrusion Formation, Elongation, and Fusion

Actin polymerization inhibitor abolishes TNT-like Rhes tunnel formation, indicating a role for Rhes in regulating actin dynamics in promoting Rhes tunnels.^[100] Consistent with this notion, Rhes interacts with actin-binding proteins such as Capza and Exo-70 and activates mTOR, which is involved in actin dynamics.^[90,93,100] But what triggers Rhes to promote the induction of TNT-like Rhes tunnel is unclear. As Rhes is also associated with vesicles such as endosomes^[100] (Movie S1, Supporting Information) in the cytoplasm and TNT-like Rhes tunnels, we propose a possibility that TNT-like Rhes tunnel induction is in a continuum where the cytoplasmic Rhes-bound vesicular cargoes may set a stage for the induction. For example, upon receptor activation in the striatal neurons, Rhes may remove the receptors from the membrane and send them as vesicular cargoes via TNT-like Rhes tunnels to neighboring cells (neuron or non-neuron) for endocytosis and degradation. As a proof of this concept, Figure 1B,C, show transport of CD9–10, a tetraspanins cell surface glycoproteins with four transmembrane domains and histamine-3 receptor (H3R) being transported in TNT-like Rhes tunnels to the neighboring cells, respectively. Thus, it is possible that, when the neurons are overwhelmed with endocytic mechanisms, they can employ intercellular endocytosis via TNTs as a backup option to dispose of the cell surface proteins and receptors. But the question whether neurons or non-neuronal cells, such as glia, would be involved in such TNT-linked intercellular endocytosis mechanisms remains speculation. The cells that normally do not express endogenous receptors or intracellular signaling cascades would be an ideal source for such putative TNT-linked intercellular endocytosis and degradation, as the receptors that are being transported are technically non-functional and will not elicit additional signals that may complicate brain functions. In addition, different types of cells may be involved in TNT-mediated signaling and cargo disposal, based upon the types of cargoes being delivered. Alternatively, TNT-like Rhes tunnels involved in physiological communications are essential for normal cell-to-cell communication in the striatum. For instance, analogous to cytonemes, the Rhes-mediated TNTs may also transport secretory growth factors to stimulate neighboring neurons, or transport receptors to neighboring interneurons to potentiate behavioral output. Therefore, it is imperative to further understand the mechanisms of formation and role of TNT-like Rhes tunnels.

Our working model is that the vesicle-bound Rhes pushes the plasma membrane to initiate protrusions, which are then immediately accompanied by an increased membrane biogenesis and actin polymerization that will lead to an elongation of the protrusions, which then interact with the acceptor cell membrane (Figure 2). On the membrane, we think that TNT-like Rhes tunnels may not directly open into the lumen of the acceptor cell, but we propose

two broad delivery mechanisms: a) touch, snatch, and integrate and b) touch, deliver, scan, and enter. In the former “snatching” model, a part of TNT-like Rhes tunnels in close proximity to the acceptor cells are snatched by the acceptor cells. This model is analogous to that involved in the transfer of plasma membrane fragments from the presenting cell to the lymphocyte, termed trigocytosis.^[113] In the latter “scanning” model, the cargo, such as vesicles, detaches from the TNTs and travel across the plasma membrane (scan) of the acceptor cells and then get endocytosed (Figure 2). But many questions remain unanswered, for example, how membrane lipid layers of donor and acceptor cells fuse, and what are the physical constraints of these fusion, and how the Rhes tunnels overcome these constraints to deliver cargoes? Further validation of these models and investigation of detailed mechanisms will help in understanding the role of TNT-like Rhes tunnels in physiological and pathological contexts in the striatum.

10. Conclusion and Outlook

Cell-to-cell communications via membranous protrusions, making physical cell-to-cell contact and delivering cargoes, continue to be discovered in the numerous cell types. But the idea of whether the neuronal cells in the brain are individual units (neuron theory) or physically connected (reticular theory) is a controversial, decades-old topic, even among Nobel Prize winning scientists.^[114] For example, while Cajal believed that brain cells are not physically connected and function as individual units, Golgi believed that brain cells are indeed physically connected and functions as a collective team.^[115] Consistent with this idea, as indicated above, TNT-like protrusions form physical conduits between neuronal cells. Therefore, it is fair to assume that TNT-like physical contacts are an example that supports the reticular theory and Golgi’s view. In the nervous system, even if they are not permanently connected between the cells, they form a transient physical bridge to transport signals and cargo between the cells. Moreover, it is also possible that these transient and highly fragile structures are vulnerable to Golgi’s staining and hence may have been disrupted and consequently unavailable for drawing by Cajal.^[116] In any case, TNT-like Rhes tunnels represent a unique mode of cellular communication, which—if their existence is proven unequivocally in vivo—will radically change the ways we understand how brain cells in the nervous system interact and function. Brain cells may not be isolated islands but rather interconnected islands exchanging materials via TNT-like membranous protrusions, which are considered the ancestors of the nervous system.^[117]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Yip DK, Auersperg N, J. Cell Biol. 1974, 63, 109. [PubMed: 4278552]
- [2]. Chakraborty J, Nelson L, Jhunjhunwala J, Young M, Kropp K, Cell Tissue Res. 1976, 174, 261. [PubMed: 1000575]
- [3]. McLaughlin BJ, J. Comp. Neurol. 1976, 170, 347. [PubMed: 993372]
- [4]. Lutz HU, Lomant AJ, McMillan P, Wehrli E, J. Cell Biol. 1977, 74, 389. [PubMed: 560378]
- [5]. Galic M, Giebel W, Arch. Oto-Rhino-Laryngol. 1977, 214, 319.
- [6]. Tani E, Nakano M, Itagaki T, Fukumori T, Acta Neuropathol. 1978, 41, 61. [PubMed: 205080]
- [7]. Gustafsson MK, Wikgren MC, Cell Tissue Res. 1981, 220, 473. [PubMed: 7296642]
- [8]. Scharrer B, Wurzelmann S, Cell Tissue Res. 1977, 184, 79. [PubMed: 922864]
- [9]. Kawaguchi T, Endo M, Tobai S, Nakamura K, Gan 1979, 70, 277. [PubMed: 467893]
- [10]. Nikulin A, Hegewald G, Gmaz-Nikulin E, Plamenac P, Barenwald G, Zentralbl. Allg. Pathol. Pathol. Anat. 1980, 124, 99.
- [11]. Stacey NH, Fanning JC, Toxicology 1981, 22, 69. [PubMed: 7336438]
- [12]. Chomette G, Leclerc JP, Szpirglas H, Auriol M, Vaillant JM, Pathol., Res. Pract. 1981, 171, 345. [PubMed: 7279786]
- [13]. Ryc M, Grabovskaya KB, Zentralbl. Bakteriologie A 1981, 249, 302. [PubMed: 7023130]
- [14]. Burgess AM, Katchburian E, Anat J 1982, 135, 577.
- [15]. a)Roof DJ, Heuser JE, J. Cell Biol. 1982, 95, 487; [PubMed: 6815210] b)Brandon C, Lam DM, J. Comp. Neurol. 1983, 217, 167. [PubMed: 6886050]
- [16]. Inouye S, Iyama K, Araki C, Usuku G, Virchow's Arch. B 1983, 42, 235. [PubMed: 6134388]
- [17]. Ohta T, J. Exp. Zool. 1985, 234, 273. [PubMed: 3998685]
- [18]. Burton GJ, J. Anat. 1986, 147, 245. [PubMed: 3693075]
- [19]. Guillomot M, Betteridge KJ, Harvey D, Goff AK, Reproduction 1986, 78, 27.
- [20]. Watanabe I, Usukura J, Yamada E, Arch. Histol. Cytol. 1985, 48, 89.
- [21]. Patterson S, Byrne JA, Lampert PW, Oldstone MB, Lab. Invest. 1987, 57, 29. [PubMed: 3496491]
- [22]. Bhargava U, Bar-Lev M, Bellows CG, Aubin JE, Bone 1988, 9, 155. [PubMed: 3166832]
- [23]. Hashimoto Y, Komuro T, Cell Tissue Res. 1988, 254, 41. [PubMed: 3197084]
- [24]. Saito H, Sato K, Ikenoya T, Shika Kiso Igakkai Zasshi 1989, 31, 44. [PubMed: 2519252]
- [25]. Marchetti C, Piacentini C, Bull. Group. Eur. Rech. Sci. Stomatol. Odontol. 1990, 33, 19.
- [26]. Kadurugamuwa JL, Rohde M, Wehland J, Timmis KN, Infect. Immun. 1991, 59, 3463. [PubMed: 1910001]
- [27]. Concha M, Vidal A, Garces G, Figueroa CD, Caorsi I, J. Invest. Dermatol. 1993, 100, 429. [PubMed: 8384233]
- [28]. Arima T, Uemura T, Yamamoto T, Hear. Res. 1987, 25, 61. [PubMed: 3492483]
- [29]. Cossart P, Kocks C, Mol. Microbiol. 1994, 13, 395. [PubMed: 7997157]
- [30]. a)Reeves PM, Bommarius B, Lebeis S, McNulty S, Christensen J, Swimm A, Chahroudi A, Chavan R, Feinberg MB, Veach D, Bornmann W, Sherman M, Kalman D, Nat. Med. 2005, 11, 731; [PubMed: 15980865] b) Reeves PM, Smith SK, Olson VA, Thorne SH, Bornmann W, Damon IK, Kalman D, J. Virol. 2011, 85, 21; [PubMed: 20962097] c) Paloheimo O, Ihalainen TO, Tauriainen S, Valilehto O, Kirjavainen S, Niskanen EA, Laakkonen JP, Hyoty H, Vihinen-Ranta M, J. Virol. 2011, 85, 6714. [PubMed: 21525342]
- [31]. Chen Q, Chen H, Mao Q, Liu Q, Shimizu T, Uehara-Ichiki T, Wu Z, Xie L, Omura T, Wei T, PLoS Pathog. 2012, 8, e1003032. [PubMed: 23166500]
- [32]. Krenz B, Jeske H, Kleinow T, Front. Plant Sci. 2012, 3, 291. [PubMed: 23293643]

- [33]. Meran S, Martin J, Luo DD, Steadman R, Phillips A, Am. J. Pathol. 2013, 182, 2223. [PubMed: 23583650]
- [34]. a) Rigano LA, Dowd GC, Wang Y, Ireton K, Cell. Microbiol. 2014, 16, 1068; [PubMed: 24405483] b) Sanderlin AG, Vondrak C, Scricco AJ, Fedrigo I, Ahyong V, Lamason RL, Mol. Biol. Cell 2019, 30, 2124. [PubMed: 31242077]
- [35]. Cole ES, Giddings TH Jr., Ozzello C, Winey M, O'Toole E, Orias J, Hamilton E, Guerrier S, Ballard A, Aronstein T, Eukaryotic Cell 2015, 14, 116. [PubMed: 25107923]
- [36]. Sagar, Prols F, Wiegrefte C, Scaal M, Development 2015, 142, 665. [PubMed: 25617437]
- [37]. Jung E, Osswald M, Blaes J, Wiestler B, Sahm F, Schmenger T, Solecki G, Deumelandt K, Kurz FT, Xie R, Weil S, Heil O, Thome C, Gommel M, Syed M, Haring P, Huber PE, Heiland S, Platten M, von Deimling A, Wick W, Winkler F, J. Neurosci. 2017, 37, 6837. [PubMed: 28607172]
- [38]. Sild M, Van Horn MR, Schohl A, Jia D, Ruthazer ES, J. Neurosci. 2016, 36, 5279. [PubMed: 27170125] a) Hamp J, Lower A, Dottermusch-Heidel C, Beck L, Moussian B, Flotenmeyer M, Onel SF, J. Cell Sci. 2016, 129, 3426; [PubMed: 27521427] b) Kroon J, Schaefer A, van Rijssel J, Hoogenboezem M, van Alphen F, Hordijk P, Stroes ESG, Stromblad S, van Rheenen J, van Buul JD, J. Immunol. 2018, 200, 1790. [PubMed: 29386254]
- [39]. a) Hamp J, Lower A, Dottermusch-Heidel C, Beck L, Moussian B, Flotenmeyer M, Onel SF, J. Cell Sci. 2016, 129, 3426; [PubMed: 27521427] b) Kroon J, Schaefer A, van Rijssel J, Hoogenboezem M, van Alphen F, Hordijk P, Stroes ESG, Stromblad S, van Rheenen J, van Buul JD, J. Immunol. 2018, 200, 1790. [PubMed: 29386254]
- [40]. Sonavane PR, Wang C, Dzamba B, Weber GF, Periasamy A, DeSimone DW, Development 2017, 144, 4363. [PubMed: 28982683]
- [41]. Miller J, Fraser SE, McClay D, Development 1995, 121, 2501. [PubMed: 7671814]
- [42]. Ramirez-Weber FA, Kornberg TB, Cell 1999, 97, 599. [PubMed: 10367889]
- [43]. a) Hsiung F, Ramirez-Weber FA, Iwaki DD, Kornberg TB, Nature 2005, 437, 560; [PubMed: 16177792] b) Biloni A, Sanchez-Hernandez D, Callejo A, Gradilla AC, Ibanez C, Mollica E, Rodriguez-Navas MC, Simon E, Guerrero I, Dev. Biol. 2013, 376, 198; [PubMed: 23276604] c) Rojas-Rios P, Guerrero I, Gonzalez-Reyes A, PLoS Biol. 2012, 10, e1001298; [PubMed: 22509132] d) Bischoff M, Gradilla AC, Seijo I, Andres G, Rodriguez-Navas C, Gonzalez-Mendez L, Guerrero I, Nat. Cell Biol. 2013, 15, 1269; [PubMed: 24121526] e) Huang H, Kornberg TB, eLife 2015, 4, e06114; [PubMed: 25951303] f) Gonzalez-Mendez L, Seijo-Barandiaran I, Guerrero I, eLife 2017, 6, e24045; [PubMed: 28825565] g) Huang H, Liu S, Kornberg TB, Science 2019, 363, 948; [PubMed: 30819957] h) Gradilla AC, Gonzalez E, Seijo I, Andres G, Bischoff M, Gonzalez-Mendez L, Sanchez V, Callejo A, Ibanez C, Guerra M, Ortigao-Farias JR, Sutherland JD, Gonzalez M, Barrio R, Falcon-Perez JM, Guerrero I, Nat. Commun. 2014, 5, 5649. [PubMed: 25472772]
- [44]. Yamashita YM, Inaba M, Buszczak M, Annu. Rev. Cell Dev. Biol. 2018, 34, 59. [PubMed: 30074816]
- [45]. a) Gupta N, DeFranco AL, Mol. Biol. Cell 2003, 14, 432; [PubMed: 12589045] b) Galkina SI, Sud'ina GF, Ullrich V, Exp. Cell Res. 2001, 266, 222; [PubMed: 11399050] c) Fifiadara NH, Beer F, Ono S, Ono SJ, Int. Immunol. 2010, 22, 113; [PubMed: 20173038] d) Galkina SI, Romanova JM, Bragina EE, Tiganova IG, Stadnichuk VI, Alekseeva NV, Polyakov VY, Klein T, FEMS Immunol. Med. Microbiol. 2011, 61, 114; [PubMed: 21054581] e) Budry L, Lafont C, El Yandouzi T, Chauvet N, Conejero G, Drouin J, Mollard P, Proc. Natl. Acad. Sci. U. S. A. 2011, 108, 12515; [PubMed: 21746936] f) Koizumi K, Takano K, Kaneyasu A, Watanabe-Takano H, Tokuda E, Abe T, Watanabe N, Takenawa T, Endo T, Mol. Biol. Cell 2012, 23, 4647; [PubMed: 23034183] g) Farahani RM, Sarrafpour B, Simonian M, Li Q, Hunter N, J. Comp. Neurol. 2012, 520, 3803; [PubMed: 22678627] h) Corriden R, Self T, Akong-Moore K, Nizet V, Kellam B, Briddon SJ, Hill SJ, EMBO Rep. 2013, 14, 726; [PubMed: 23817552] i) Mattes B, Dang Y, Greicius G, Kaufmann LT, Prunsche B, Rosenbauer J, Stegmaier J, Mikut R, Ozbek S, Nienhaus GU, Schug A, Virshup DM, Scholpp S, eLife 2018, 7, e36953. [PubMed: 30060804]
- [46]. Roy S, Huang H, Liu S, Kornberg TB, Science 2014, 343, 1244624. [PubMed: 24385607]
- [47]. Gonzalez-Mendez L, Gradilla AC, Guerrero I, Development 2019, 146, dev174607. [PubMed: 31068374]

- [48]. Snyder JC, Rochelle LK, Marion S, Lyerly HK, Barak LS, Caron MG, J. Cell Sci. 2015, 128, 1230. [PubMed: 25653388]
- [49]. Du L, Sohr A, Yan G, Roy S, eLife 2018, 7, e38137. [PubMed: 30328809]
- [50]. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH, Science 2004, 303, 1007. [PubMed: 14963329]
- [51]. a)Scherer NM, Mothes W, Trends Cell Biol. 2008, 18, 414; [PubMed: 18703335] b)Rustom A, Ann. N. Y. Acad. Sci. 2009, 1178, 129. [PubMed: 19845633]
- [52]. Sartori-Rupp A, Cordero Cervantes D, Pepe A, Gousset K, Delage E, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, Zurzolo C, Nat. Commun. 2019, 10, 342. [PubMed: 30664666]
- [53]. Delage E, Cervantes DC, Penard E, Schmitt C, Syan S, Disanza A, Scita G, Zurzolo C, Sci. Rep. 2016, 6, 39632. [PubMed: 28008977]
- [54]. Watkins SC, Salter RD, Immunity 2005, 23, 309. [PubMed: 16169503]
- [55]. Eugenin EA, Gaskill PJ, Berman JW, Cell. Immunol. 2009, 254, 142. [PubMed: 18835599]
- [56]. Smith IF, Shuai J, Parker I, Biophys. J. 2011, 100, L37. [PubMed: 21504718]
- [57]. a)Plotnikov EY, Khryapenkova TG, Galkina SI, Sukhikh GT, Zorov DB, Exp. Cell Res. 2010, 316, 2447; [PubMed: 20599955] b)Vallabhaneni KC, Haller H, Dumlér I, Stem Cells Dev. 2012, 21, 3104; [PubMed: 22676452] c)Pasquier J, Guerrouahen BS, Al Thawadi H, Ghiabi P, Maleki M, Abu-Kaoud N, Jacob A, Mirshahi M, Galas L, Rafii S, Le Foll F, Rafii A, J. Transl. Med. 2013, 11, 94. [PubMed: 23574623]
- [58]. Domhan S, Ma L, Tai A, Anaya Z, Beheshti A, Zeier M, Hlatky L, Abdollahi A, PLoS One 2011, 6, e21283. [PubMed: 21738629]
- [59]. Pasquier J, Galas L, Boulange-Lecomte C, Rioult D, Bultelle F, Magal P, Webb G, Le Foll F, J. Biol. Chem. 2012, 287, 7374. [PubMed: 22228759]
- [60]. Lou E, Fujisawa S, Morozov A, Barlas A, Romin Y, Dogan Y, Gholami S, Moreira AL, Manova-Todorova K, Moore MA, PLoS One 2012, 7, e33093. [PubMed: 22427958]
- [61]. Wittig D, Wang X, Walter C, Gerdes HH, Funk RH, Roehlecke C, PLoS One 2012, 7, e33195. [PubMed: 22457742]
- [62]. Takahashi A, Kukita A, Li YJ, Zhang JQ, Nomiyama H, Yamaza T, Ayukawa Y, Koyano K, Kukita T, J. Cell. Biochem. 2013, 114, 1238. [PubMed: 23129562]
- [63]. Rainy N, Chetrit D, Rouger V, Vernitsky H, Rechavi O, Marguet D, Goldstein I, Ehrlich M, Kloog Y, Cell Death Dis. 2013, 4, e726. [PubMed: 23868059]
- [64]. Thayanithy V, Babatunde V, Dickson EL, Wong P, Oh S, Ke X, Barlas A, Fujisawa S, Romin Y, Moreira AL, Downey RJ, Steer CJ, Subramanian S, Manova-Todorova K, Moore MA, Lou E, Exp. Cell Res. 2014, 323, 178. [PubMed: 24468420]
- [65]. Thayanithy V, Dickson EL, Steer C, Subramanian S, Lou E, Transl. Res. 2014, 164, 359. [PubMed: 24929208]
- [66]. Antanaviciute I, Rysevaite K, Liutkevicius V, Marandykina A, Rimkute L, Sveikatiene R, Uloza V, Skeberdis VA, PLoS One 2014, 9, e99196. [PubMed: 24945745]
- [67]. Carone C, Genedani S, Leo G, Filafferro M, Fuxe K, Agnati LF, J. Mol. Neurosci. 2015, 55, 42. [PubMed: 24996625]
- [68]. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, Olivo-Marin JC, Mannel D, Zurzolo C, Nat. Cell Biol. 2009, 11, 328. [PubMed: 19198598]
- [69]. Zhu S, Victoria GS, Marzo L, Ghosh R, Zurzolo C, Prions 2015, 9, 125.
- [70]. Astanina K, Koch M, Jungst C, Zumbusch A, Kierner AK, Sci. Rep. 2015, 5, 11453. [PubMed: 26095213]
- [71]. Polak R, de Rooij B, Pieters R, den Boer ML, Blood 2015, 126, 2404. [PubMed: 26297738]
- [72]. Hashimoto M, Bhuyan F, Hiyoshi M, Noyori O, Nasser H, Miyazaki M, Saito T, Kondoh Y, Osada H, Kimura S, Hase K, Ohno H, Suzu S, J. Immunol. 2016, 196, 1832. [PubMed: 26773158]
- [73]. Desir S, Dickson EL, Vogel RI, Thayanithy V, Wong P, Teoh D, Geller MA, Steer CJ, Subramanian S, Lou E, Oncotarget 2016, 7, 43150. [PubMed: 27223082]

- [74]. Matula Z, Nemeth A, Lorincz P, Szepesi A, Brozik A, Buzas EI, Low P, Nemet K, Uher F, Urban VS, Stem Cells Dev. 2016, 25, 1818. [PubMed: 27596268]
- [75]. Osteikoetxea-Molnar A, Szabo-Meleg E, Toth EA, Oszvald A, Izsepi E, Kremlitzka M, Biri B, Nyitray L, Bozo T, Nemeth P, Kellermayer M, Nyitrai M, Matko J, Cell. Mol. Life Sci. 2016, 73, 4531. [PubMed: 27125884]
- [76]. Reichert D, Scheinplflug J, Karbanova J, Freund D, Bornhauser M, Corbeil D, Exp. Hematol. 2016, 44, 1092. [PubMed: 27473566]
- [77]. Jiang D, Gao F, Zhang Y, Wong DS, Li Q, Tse HF, Xu G, Yu Z, Lian Q, Cell Death Dis. 2016, 7, e2467. [PubMed: 27831562]
- [78]. Kumar A, Kim JH, Ranjan P, Metcalfe MG, Cao W, Mishina M, Gangappa S, Guo Z, Boyden ES, Zaki S, York I, Garcia-Sastre A, Shaw M, Sambhara S, Sci. Rep. 2017, 7, 40360. [PubMed: 28059146]
- [79]. Lu J, Zheng X, Li F, Yu Y, Chen Z, Liu Z, Wang Z, Xu H, Yang W, Oncotarget 2017, 8, 15539. [PubMed: 28107184]
- [80]. Desir S, O'Hare P, Vogel RI, Sperduto W, Sarkari A, Dickson EL, Wong P, Nelson AC, Fong Y, Steer CJ, Subramanian S, Lou E, Sci. Rep. 2018, 8, 9484. [PubMed: 29930346]
- [81]. a)Su Q, Igyarto BZ, J. Invest. Dermatol. 2019, 139, 2313; [PubMed: 31129057] b)De La Cruz Diaz JS, Kaplan DH, J. Invest. Dermatol. 2019, 139, 2260. [PubMed: 31648687]
- [82]. Kolba MD, Dudka W, Zareba-Kozioł M, Kominek A, Ronchi P, Turos L, Chrosicki P, Włodarczyk J, Schwab Y, Klejman A, Cysewski D, Srpan K, Davis DM, Piwocka K, Cell Death Dis. 2019, 10, 817. [PubMed: 31659149]
- [83]. a)Sowinski S, Jolly C, Berninghausen O, Purbhoo MA, Chauveau A, Kohler K, Oddos S, Eissmann P, Brodsky FM, Hopkins C, Onfelt B, Sattentau Q, Davis DM, Nat. Cell Biol. 2008, 10, 211; [PubMed: 18193035] b)Jansens RJJ, Van den Broeck W, De Pelsmaeker S, Lamote JAS, Van Waesberghe C, Couck L, Favoreel HW, J. Virol. 2017, 91, e00749; [PubMed: 28747498] c)Guo R, Davis D, Fang Y, Virology 2018, 517, 122; [PubMed: 29307597] d)Panasiuk M, Rychlowski M, Derewonko N, Bienkowska-Szewczyk K, J. Virol. 2018, 92, e00090. [PubMed: 29491165]
- [84]. Abounit S, Wu JW, Duff K, Victoria GS, Zurzolo C, Prions 2016, 10, 344.
- [85]. Gousset K, Zurzolo C, Prions 2009, 3, 94.
- [86]. Tardivel M, Begard S, Bousset L, Dujardin S, Coens A, Melki R, Buee L, Colin M, Acta Neuropathol. Commun. 2016, 4, 117. [PubMed: 27809932]
- [87]. a)Abounit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R, Zurzolo C, EMBO J. 2016, 35, 2120; [PubMed: 27550960] b)Rostami J, Holmqvist S, Lindstrom V, Sigvardson J, Westermarck GT, Ingelsson M, Bergstrom J, Roybon L, Erlandsson A, J. Neurosci. 2017, 37, 11835. [PubMed: 29089438]
- [88]. a)Harrison LM, Cell. Mol. Neurobiol. 2012, 32, 907; [PubMed: 22450871] b)Usui H, Falk JD, Dopazo A, de Lecea L, Erlander MG, Sutcliffe JG, J. Neurosci. 1994, 14, 4915; [PubMed: 8046460] c)Sciamanna G, Napolitano F, Pelosi B, Bonsi P, Vitucci D, Nuzzo T, Punzo D, Ghiglieri V, Ponterio G, Pasqualetti M, Pisani A, Usiello A, Neurobiol. Dis. 2015, 78, 146. [PubMed: 25818655]
- [89]. a)Vargiu P, De Abajo R, Garcia-Ranea JA, Valencia A, Santisteban P, Crespo P, Bernal J, Oncogene 2004, 23, 559; [PubMed: 14724584] b)Errico F, Santini E, Migliarini S, Borgkvist A, Centonze D, Nasti V, Carta M, De Chiara V, Prosperetti C, Spano D, Herve D, Pasqualetti M, Di Lauro R, Fisone G, Usiello A, Mol. Cell. Neurosci. 2008, 37, 335; [PubMed: 18035555] c)Ghiglieri V, Napolitano F, Pelosi B, Schepisi C, Migliarini S, Di Maio A, Pendolino V, Mancini M, Sciamanna G, Vitucci D, Maddaloni G, Giampa C, Errico F, Nistico R, Pasqualetti M, Picconi B, Usiello A, Sci. Rep. 2015, 5, 10933; [PubMed: 26190541] d)Harrison LM, He Y, J. Neurosci. Res. 2011, 89, 874. [PubMed: 21374700]
- [90]. Subramaniam S, Napolitano F, Mealer RG, Kim S, Errico F, Barrow R, Shahani N, Tyagi R, Snyder SH, Usiello A, Nat. Neurosci. 2012, 15, 191.
- [91]. Subramaniam S, Mealer RG, Sixt KM, Barrow RK, Usiello A, Snyder SH, J. Biol. Chem. 2010, 285, 20428. [PubMed: 20424159]

- [92]. Mealer RG, Murray AJ, Shahani N, Subramaniam S, Snyder SH, J. Biol. Chem. 2014, 289, 3547. [PubMed: 24324270]
- [93]. Shahani N, Swarnkar S, Giovino V, Morgenweck J, Bohn LM, Scharager-Tapia C, Pascal B, Martinez-Acedo P, Khare K, Subramaniam S, Sci. Signaling 2016, 9, ra111.
- [94]. Subramaniam S, Sixt KM, Barrow R, Snyder SH, Science 2009, 324, 1327. [PubMed: 19498170]
- [95]. Swarnkar S, Chen Y, Pryor WM, Shahani N, Page DT, Subramaniam S, Neurobiol. Dis. 2015, 82, 66. [PubMed: 26048156]
- [96]. a)Okamoto S, Pouladi MA, Talantova M, Yao D, Xia P, Ehrnhoefer DE, Zaidi R, Clemente A, Kaul M, Graham RK, Zhang D, Vincent Chen HS, Tong G, Hayden MR, Lipton SA, Nat. Med. 2009, 15, 1407; [PubMed: 19915593] b)Seredenina T, Gokce O, Luthi-Carter R, PLoS One 2011, 6, e22231; [PubMed: 21779398] c)Baiaomonte BA, Lee FA, Brewer ST, Spano D, LaHoste GJ, PLoS One 2013, 8, e53606; [PubMed: 23349722] d)Sbodio JJ, Paul BD, Machamer CE, Snyder SH, Cell Rep. 2013, 4, 890; [PubMed: 24012756] e)Lu B, Palacino J, FASEB J 2013, 27, 1820; [PubMed: 23325320] f)Argenti M, Ph.D Thesis, Università degli Studi di Padova (Padova PD, Italy) 2014.
- [97]. Hernandez I, Luna G, Rauch JN, Reis SA, Giroux M, Karch CM, Boctor D, Sibih YE, Storm NJ, Diaz A, Kaushik S, Zekanowski C, Kang AA, Hinman CR, Cerovac V, Guzman E, Zhou H, Haggarty SJ, Goate AM, Fisher SK, Cuervo AM, Kosik KS, Sci. Transl. Med. 2019, 11, eaat3005. [PubMed: 30918111]
- [98]. Vadgama N, Pittman A, Simpson M, Nirmalanathan N, Murray R, Yoshikawa T, De Rijk P, Rees E, Kirov G, Hughes D, Fitzgerald T, Kristiansen M, Pearce K, Cerveira E, Zhu Q, Zhang C, Lee C, Hardy J, Nasir J, Eur. J. Hum. Genet. 2019, 27, 1121. [PubMed: 30886340]
- [99]. Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, Cattaneo E, MacDonald ME, Hum. Mol. Genet. 2000, 9, 2799. [PubMed: 11092756]
- [100]. Sharma M, Subramaniam S, J. Cell Biol. 2019, 218, 1972. [PubMed: 31076452]
- [101]. Harrison LM, Lahoste GJ, Ruskin DN, Brain Res. 2008, 1245, 16. [PubMed: 18929545]
- [102]. Gibbs KL, Kalmar B, Sleigh JN, Greensmith L, Schiavo G, Neurosci J Methods 2016, 257, 26.
- [103]. a)Chinnery HR, Pearlman E, McMenamin PG, J. Immunol. 2008, 180, 5779; [PubMed: 18424694] b)Salas-Vidal E, Lomeli H, Dev. Biol. 2004, 265, 75; [PubMed: 14697354] c)Caneparo L, Pantazis P, Dempsey W, Fraser SE, PLoS One 2011, 6, e20230; [PubMed: 21647454] d)Pyrgaki C, Trainor P, Hadjantonakis AK, Niswander L, Dev. Biol. 2010, 344, 941; [PubMed: 20558153] e)Teddy JM, Kulesa PM, Development 2004, 131, 6141. [PubMed: 15548586]
- [104]. Sharma M, Jarquin UNR, Rivera O, Kazantzis M, Eshraghi M, Shahani N, Sharma V, Tapia R, Subramaniam S, Proc. Natl. Acad. Sci. U. S. A. 2019, 116, 23760. [PubMed: 31676548]
- [105]. Brimblecombe KR, Cragg SJ, ACS Chem. Neurosci. 2017, 8, 235. [PubMed: 27977131]
- [106]. a)Subramaniam S, Snyder SH, Neuropharmacology 2011, 60, 1187; [PubMed: 21044641] b)Rikani AA, Choudhry Z, Choudhry AM, Rizvi N, Ikram H, Mobassarah NJ, Tulli S, Ann. Neurosci. 2014, 21, 112. [PubMed: 25206077]
- [107]. Cicchetti F, Lacroix S, Cisbani G, Vallieres N, Saint-Pierre M, St-Amour I, Tolouei R, Skepper JN, Hauser RA, Mantovani D, Barker RA, Freeman TB, Ann. Neurol. 2014, 76, 31. [PubMed: 24798518]
- [108]. Pecho-Vrieseling E, Rieker C, Fuchs S, Bleckmann D, Esposito MS, Botta P, Goldstein C, Bernhard M, Galimberti I, Muller M, Luthi A, Arber S, Bouwmeester T, van der Putten H, Di Giorgio FP, Nat. Neurosci. 2014, 17, 1064. [PubMed: 25017010]
- [109]. Babcock DT, Ganetzky B, Proc. Natl. Acad. Sci. U. S. A. 2015, 112, E5427. [PubMed: 26351672]
- [110]. Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, Bae J, Lee N, Li L, Im W, Kim M, Kim HS, Oh SH, Kim TA, Ko JJ, Aube B, Oueslati A, Kim YJ, Song J, Acta Neuropathol. 2016, 132, 577. [PubMed: 27221146]
- [111]. Johnson EB, Gregory S, Prog. Mol. Biol. Transl. Sci. 2019, 165, 321. [PubMed: 31481169]
- [112]. Pryor WM, Biagioli M, Shahani N, Swarnkar S, Huang WC, Page DT, MacDonald ME, Subramaniam S, Sci. Signaling 2014, 7, ra103.
- [113]. Joly E, Hudrisier D, Nat. Immunol. 2003, 4, 815. [PubMed: 12942076]

- [114]. a)Barbara JG, Morphologie 2010, 94, 4; [PubMed: 19850504] b)Bock O, Endeavour 2013, 37, 228. [PubMed: 23870749]
- [115]. Glickstein M, Curr. Biol. 2006, 16, R147. [PubMed: 16527727]
- [116]. DeFelipe J, Prog. Brain Res. 2013, 203, 201. [PubMed: 24041282]
- [117]. Nussenzveig HM, Eur. Biophys. J. 2019, 48, 593. [PubMed: 31280337]

Figure 1.

A) Rhes domains and GTP/GDP and farnesylation (lipid)-binding sites. B,C) Representative confocal image of striatal neuronal cells transfected with GFP-Rhes and mCherry CD9–10, a cell surface glycoprotein (B) or GFP-Rhes and H3R, a histamine-3 receptor (C). Differential interference contrast (DIC) microscopy image of TNT connecting cells (1 and 2) are shown. Arrow indicates Rhes-induced TNT-like protrusions “Rhes tunnels.” Confocal images were acquired on a Zeiss LSM 880 microscope. Image Credit: Dr. Manish Sharma.

Figure 2.

A) The pencil sketch of Rhesus-induced TNT-like protrusion from donor cell to acceptor cell. B) Vesicle-based initiation of protrusion formation. C) Elongation of protrusion accompanied by a leading vesicle. D) Delivery models: 1) touch, snatch, and integrate model and 2) touch, deliver, scan, and enter model.