Cancer Prevention: Current Research Journal

Short Review

Cell Membrane Nanotubes in Development

Moysés Nussenzveig H[1,2]

[1]Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Brazil

[2]Institute of Physics, Federal University of Rio de Janeiro, Brazil

ARTICLE INFO

Received Date: March 09, 2020
Accepted Date: May 10, 2020
Published Date: May 15, 2020

KEYWORDS

Cell Membrane
Nanotubes
Development

Citation for this article: Moysés Nussenzveig H. Cell Membrane Nanotubes in Development. Cancer Prevention: Current Research Journal. 2020; 2(1):113

Corresponding author:
Moysés Nussenzveig H,
Institute of Physics, Federal University of Rio de Janeiro, 21941-972, Brazil,
Email: hmoyses@globo.com

ABSTRACT

Cell Membrane Nanotubes (CMN) are tubular protrusions of the cell membrane that allow it to communicate with distant cells. CMN are employed in cell invasion by practically all pathogens, suggesting that they are of ancient origin. They also play a role in cancer propagation, with possible therapeutic implications. Recent work has shown that they can establish an analogue of a synaptic connection. I have proposed that CMN are the ancestors of the nervous system. Here I review and provide additional evidence for this conjecture, by employing results of a remarkable 12-year effort by the Imachi laboratory, that has produced a stable culture of a Lokiarchaeon associated with the origin of life on Earth. Ultrastructural analysis revealed that this Lokiarchaeon produces CMN, both straight and curved. This has led to a proposed solution to one of the most controversial problems of molecular cell biology, the origin of eukaryotes, in which the properties of CMN are crucial. Intracellular tubules are also reviewed.

INTRODUCTION

CMN

Cells are complex dynamical open systems very far from equilibrium, that interact strongly with their environment. In particular, they often interact with other cells. Such interactions must have arisen very soon after the emergence of life. One way that they may be mediated is by extension of some sort of connecting "cables" between cells. Those cables exist: they have been designated by different names (cytonemes, tunneling nanotubes, tumor microtubes) [1,2]. I refer to them generically as Cell Membrane Nanotubes (CMN). Here I deal with spontaneously formed CMN. One can also produce artificial ones by pulling on cell membranes with Optical Tweezers (OT) (reviewed in [3].

I have hypothesized that CMN are the ancestors of the nervous system [4]. The problem of the evolution of the nervous system has been discussed for metazoans and ctenophores [5,6]. For Drosophila, two seminal publications of the Kornberg Lab [7,8] already hinted at analogies between cytonemes and neuronal axons. However, the earliest ancestors of the nervous system remained unidentified.

Another possible communication route between cells is through the encapsulation and transmission of messages in vesicles. It is employed in the nervous system for chemical signal transmission through axons.

TNT

The first laboratory detection of spontaneous CMN formation was made by Rustom et al [9], employing PC12 cells. They were seen forming straight connections between distant cells and creating complex networks among them. They were named tunneling nanotubes (TNT), and their observed mode of formation in [9] was by extension of filopodia-like protrusions. The protrusions probe the environment until contact with the target cell is established, forming the TNT link.

As characterized by Rustom et al [9], TNT's are straight, have lengths up to several cell diameters and radii of 25-200 nm, below the resolving limit of optical microscopes (they become visible by diffraction). They contain F-actin, but not microtubules. Unlike filopodia, they are extended above the substrate. They penetrate within the connected cells, linking the cells' cytoplasms on both sides.

They mediate selective transfer of molecules, vesicles and organelles between the connected cells. They contain Myosin Va, a motor protein known to transport cargo along actin filaments.

What are other functions of TNTs? They play a variety of roles, as will now be discussed.

Role in the immune system

Watkins and Salter [10], working with a culture of dendritic cells, the initiators ←←←of immune response, injected E. coli antigen into a dendritic cell. A few seconds later, a Ca2+signal (detected by GFP) propagated to other dendritic cells via TNTs. One cell extended a lamellipodium and moved towards adjacent dendritic cells. These results were taken to demonstrate that nonneuronal cells can transmit signals to distant cells through TNTs, an early hint at a connection CMN – nervous system.

Help and rescue

In a paper on a TNT network linking U-87 MG glioma cells [11], a blebbing (apoptotic) cell was seen receiving a vesicle sent by another cell through a TNT connection, suggesting that a TNT can intermediate help, by transmitting an apoptosis signal. This was not demonstrated in [11], but it was confirmed by later independent observations [12].

In [11], it was also observed, by OT pulling on a TNT, that beyond a threshold pulling force the extracted nanotube splits into two branches. This effect was termed I–Y bifurcation. The converse function, rescuing threatened cells, is exemplified by the transfer of mitochondria. Mitochondria, the powerhouses of eukaryotic cells, are essential for cell respiration. Mitochondrial transfer through CMNs has been verified in a variety of situations (e.g., [2]).

Roles of CMN in infections

Practically all known types of pathogens opportunistically employ CMN for cell invasion. Önfelt et al [1] showed that BCG bacteria surf along TNT between macrophages, and then get phagocytosed, transmitting infection.

Bacterial communication through CMN was detected by Dubey and Ben-Yehuda [13], allowing transient acquisition of antibiotic resistance. This happened in an interspecies manner, between B. subtilis and Staphylococcus aureus, and even between B. subtilis and the evolutionarily distant bacterium Escherichia coli.

Flu virus employs TNT for transmission between cells. Retrovirus build CMN bridges for cell-to-cell transmission [14]. HIV propagates among T cells through CMN, increasing infectivity by orders of magnitude [15].

Prions hijack TNT for intercellular spread [16]. The TNT network in this connection has been compared with an Internet of Cells. TNTs also spread tau and other prion-like diseases [17].

CMN and Cancer

←←The important role of CMN in cancer propagation was stressed by Osswald et al [2], who studied the development of glioblastomas (regarded as incurable tumors) in mouse brains, over a period of up to 1 year. They detected the growth of CMN, up to 500 μm long and with ≅ 1μm diameter. They named such CMNs tumor microtubes. The CMNs contained mitochondria and microvesicles. The microtube tips were compared to neuronal growth cones during development, showing frequent dendritic arborization. They invade the normal brain, and nuclei from cell division travel along them.

The paper explains why, in contrast with other brain tumors, glioblastomas are resistant to radiotherapy and chemotherapy. Indeed, after a tumor cell is killed, a new nucleus travels through a CMN to replace it. This suggests that blocking CMN formation might be a possible chemotherapeutic target [18].

Osswald et al [2] emphasize the differences between tumor microtubes and TNTs. However, Pontes et al [11] had seen the

formation of a network connecting glioblastoma cells that showed typical features of TNTs. This apparent contradiction could arise from the difference between in vitro and in vivo experiments. It is also possible that CMNs assume different forms depending on the environment.

Various structures of CMN

Ultrastructural study of CMNs connecting T cells [14] revealed that, in contrast with previously found TNTs, they did not connect the cells' cytoplasms on both sides, as had been noticed in Rustom et al [9].

In Figure 6 C1 of [14], where the scale bar is 0.5 μm, it can be estimated that the gap between the CMN tip and the membrane of the cell to which it connects is of the order of 0.1 μm, a typical order of magnitude for a synaptic cleft in the central nervous system. Curved and tortuous TNT were seen in vivo, within an inflamed transparent mouse cornea, connecting neighboring dendritic cells [19]. The curvature becomes visible in this three-dimensional environment.

CMN and neurons

I now sum up the above results, comparing the above features of CMN with those of neuronal axons.

(i) Both CMN and neurons transmit signals between distant cells.←

(ii) Calcium signaling ←←As Watkins and Salter [10] demonstrated for dendritic ←←←cells, calcium ion signals are transmitted between cells through TNTs. As reported by Northcut [6], voltage-gated Na channels, employed in the nervous system, evolved from Ca channels, and they predated neurons.

(iii) Electrical and chemical signaling – In the nervous system, signal transmission is both chemical, through vesicles containing neurotransmitters, and electrical, through electrical synapses. Transmission of molecules and vesicles through TNTs was already found in Rustom et al [9]. The analogue of electrical synapses for CMN was demonstrated by Wang et al [20]. It allows for bidirectional exchange through a gap junction.

(iv) Analogues of axons and dendritic networks-As was mentioned in Section 1.5, Osswald et al [2], in their study of glioblastoma invasion, compared cancer microtube tips with neuronal growth cone tips during development, with frequent dendritic arborization.

(v) Role of TNTs in neural development-It was proposed by Wang et al [20], based on hippocampal growth studies, that developing neurons establish electrical coupling and exchange of calcium signals with astrocytes via TNTs.

(vi) Glutamate signaling-Glutamate is the principal excitatory neurotransmitter in the brain [21]. Nerve impulses trigger its release from vesicles across the synapse to activate receptors in the post-synaptic cell. It also is important in the regulation of growth cones and synaptogenesis during brain development. Very recently, Huang et al [7] found that it plays an essential role in cytoneme-mediated signaling (cytonemes are CMN) in Drosophila development.

(vii) Synaptic connections – Huang et al [7] were able to determine the structure of cytoneme-mediated connections by employing the GRASP technique [22]. In this method, two complementary fragments of GFP are attached to different cells. When a synaptic connection is established between them, GFP fluorescence is seen. The result they found agrees with the synaptic cleft structure seen in [7].

(viii) Plants - Ca channels and glutamate receptors are also employed for communication in plants [23]. They appear to have existed before plants and animals had diverged [24].

The eight features listed above support the conjecture [4] that CMN are the ancestors of the nervous system.

ORIGIN OF LIFE AND OF THE EUKARYOTES

Origin of life

The Earth's primordial atmosphere lacked oxygen. With no ozone layer, penetrating ultraviolet radiation bathed land surfaces, rendering them hostile to life. Thus, plausible hypotheses for life's origin situate it deep in the oceans. For a recent detailed discussion of the origin of life, see [25].

It was proposed by Martin and Russell [26] that life began at deep-ocean hydrothermal vents, where up to the present are found tens-of-meters-tall carbonate mounds, inhabited by microorganisms believed to be representative of the earliest life forms.

The deep ocean Lost City Vent Field (depth about 800 m) was discovered in 2000 [27], in an expedition employing a remotely operated imaging vehicle and the submersible Alvin. It is currently rich in microorganisms that were the only life forms during the first two billion years after life's origin. They are of two types, Bacteria and Archaea, with very different

features, both of them Prokaryotes, i.e., microorganisms without nuclei.

The mounds have a microscopic sponge-like cellular structure with metallic catalytic linings. Through the channels flows circulating sea water. Present in this water are thermal, electrical and pH gradients. This system behaves like an electrochemical reactor, an ideal hatchery for the origin of cells. Acetyl thioesters, related to Acetyl CoA, are continually generated. They are surmised to be precursors of RNA in the RNA world model [28]. For arguments in support of this model, see [29]. One of them is that modern cells have a cytoplasm reminiscent of sea water and employ Acetyl CoA in the Krebs cycle. A recent argument is the discovery [30] by the Cassini–Huygens NASA mission of a plume of icy particles and vapor emanating in hydrothermal jets from the Saturn's moon Enceladus, containing molecular oxygen, nitrogen and aromatic compounds. In [31] it is reported that an archaeon can be grown in a laboratory under Enceladus-like conditions.

Origin of eukaryotes

Given the universality of the genetic code, bacteria and archaea are surmised to have had a common ancestor, known as LUCA (Last Universal Common Ancestor).

Eukaryotes have nuclei, but their most important difference from prokaryotes is that they have internal organelles, the mitochondria, with their own very small genomes, that provide them with an independent energy source. The large energy per gene ratio of mitochondria allowed eukaryotes to increase their complexity (nuclear genome) by many orders of magnitude, in contrast with prokaryotes [32].

The origin of eukaryotes is one of the most controversial problems in biology. In the model of Martin and Müller [32], the endosymbiosis between an anaerobic and chemosynthetic methanogenic archaeon (host) and an alpha-proteobacterium (the symbiont) gave rise to mitochondria.

The host utilized hydrogen (H2) and carbon dioxide (CO2) to produce methane, while the symbiont, capable of aerobic respiration, expelled H2 and CO2 as byproducts of anaerobic fermentation processes. A second endosymbiosis with a cyanobacterium would have given rise to a photosynthetic vegetable cell. The bacterium would have been engulfed by the archaeon, an extremely unlikely event, that may have occurred only once.

Evidence for this hydrogen hypothesis is that mitochondria, like bacteria, have double membranes; the mitochondrial genome is very similar to the bacterial one; mitochondria, like bacteria, multiply by binary fission. The eukaryotic cell nucleus, to which most of the engulfed bacterium genes migrated, would have been formed at a later step. A very serious difficulty with this model is in the engulfment mechanism. It could not have been analogous to phagocytosis, because archaea have neither the required proteins nor enough energetic resources.

In 2015, at a hydrothermal vent in the depths of the Arctic ocean, remains of an unknown type of archaeon named Lokiarchaeon were found [29]. It seemed to be a missing link between Prokaryotes and Eukaryotes, because it already had some eukaryotic features. Its genome was reconstructed from a minute sample of sediment (about 10 grams). However, in 2017, this proposal was contested and attributed to contamination [13].

THE IMACHI EXPERIMENT

In a recently published paper, Imachi et al [33] report that they have isolated and grown living Lokiarchaea cultures. The researchers collected mud from the 2006 dive of a submersible into the 2500-meter-deep Omine Ridge off the coast of Japan. To cultivate microbes from these sediments, they built a methane-fed bioreactor that mimicked the conditions of a deep-sea methane vent.

Small samples of the collected mud were then inserted into glass tubes that contained nutrients, as well as antibiotics to eliminate possible contaminating bacteria. After one year, the authors found, by DNA analysis, that one of the tubes contained an archaeon of the Asgard superphylum, to which Lokiarchaea belong. The archaeon took between 14 and 25 days to undergo cell division (as compared to typical one hour doubling time for bacteria). This about 500 times slower growth rate is one of the reasons why it took about 12 years to complete the work.

Repeated subcultures and purification led to gradual enrichment of the archaeon. The final result was a stable (thus far quite small) lab culture, containing only this new Lokiarchaeon and a different methane-producing archaeon. Together, the two microbes formed a symbiotic relationship . The scientists named the cultured strain Lokiarchaeon Prometheoarchaeum syntrophicum after the Greek god

Prometheus, who created humans out of mud. They verified that it does contain numerous eukaryote-like genes.

What does the new Lokiarchaeon look like?

Microscopic observations showed that the Lokiarchaeon cells are small cocci, ca. 300-750 nm in diameter (average 550 nm), and generally form aggregates surrounded with extracellular polysaccharide (EPS)-like materials (Figures 2 a,b). Dividing cells had a ring-like structure around the middle (Figure 2c). The cells produce membrane vesicles (MVs; 50–280 nm in diameter (Figure 2b) and chains of blebs (Figure 2c). Thus, membrane vesicles are also of very ancient origin (cf. Sect. 1.1).

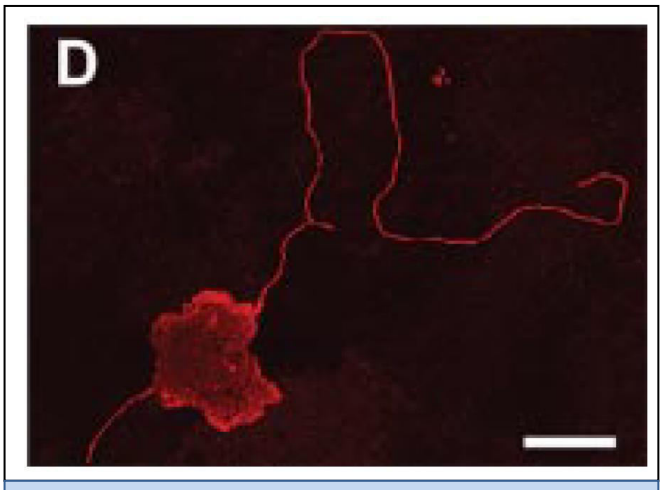

Figure 1: TNT in vivo in inflamed mouse cornea connecting neighboring (not identified) dendritic cells. Scale bar: 20 µm, After [19].

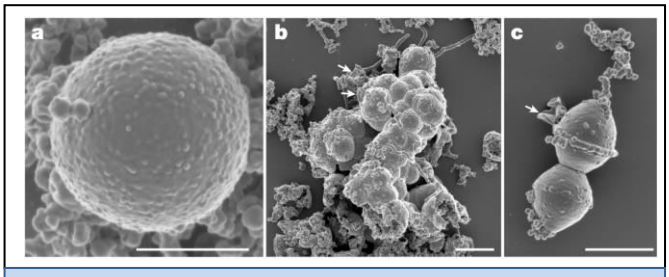

Figure 2: SEM (Scanning Electron Microscopy) of new *Lokiarchaeon*. a- single cell; b- aggregated cells covered with extracellular polysaccharide-like materials; c- dividing cell with polar chains of blebs (after [33]). Scale bars, 1 µm (b, c), 500 nm (a).

Presence of CMN

The Lokiarchaeon cells also form unique membrane-based, straight as well as curved and tortuous protrusions, with a diameter of about 80-100 nm and various lengths (Figures 3g-h). Some protrusions, remarkably, display complex branching, unlike known archaeal protrusions, but similar to the I-V bifurcations of [11].

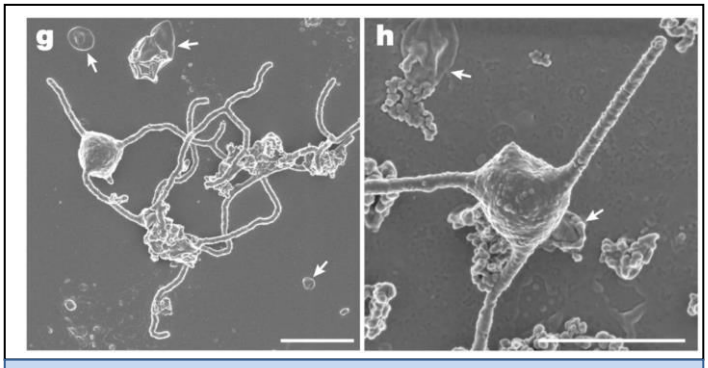

Figure 3: Production of long straight and curved protrusions (after [33]). Scale bars, 1 µm (g,h).

In this symbiosis, PAPLA and PA mutually benefit - PAPLA can allot energy metabolism to PA and indirectly obtain energy from organotrophy via AAC while PA is fed 2-oxoacids for energy production (Figure 4f). Here, PAPLA endogenize PA and we arrive at LECA possessing symbiosis congruent with that of extant eukaryotes and their mitochondria.

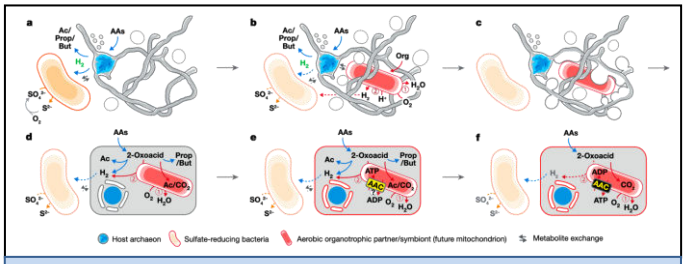

Figure 4: Steps in the proposed *Entangle-Engulf-Endogenize* model for eukaryogenesis. a-c entangle d-e engulf f endogenize (after [33]).

Origin of eukaryotes

Prior to endosymbiosis, the Last Eukaryotic Common Ancestor (LECA) archaeon likely interacted with Sulfate-Reducing Bacteria (SRB) and O2-utilizing organotrophs. The O2-utilizing partner was likely a facultative aerobe capable of aerobic and anaerobic H2-generating organotrophy. In this three-member interaction, the SRB could syntrophically scavenge H2 from both the pre-LECA archaeon and facultatively aerobic partner.

One of the facultatively aerobic partners was likely the pre-mitochondrial alphaproteobacterium (PA; i.e., future

mitochondrion) as it has been proposed that PA would be capable of both aerobic and anaerobic H2-generating organotrophy. Evolution of the symbiosis likely led to PA endosymbiosis into the pre-LECA archaeon, resulting in a transitional PA-containing pre-LECA archaeon (PAPLA) using PA as an O2-scavenging and building-block-providing symbiont essential for growth under microaerobic conditions, even without SRB.

Given the structure of extant eukaryotic cells, it is logical to presume that the pre-LECA archaeon engulfed their metabolic partner. The archaeon may have produced protrusions and/or microvesicles. For an archaeon syntrophically growing in a narrow space (e.g., sediment pore), it may have been possible for the protrusions/MVs, helped by microvesicles (Figure 3b), to fuse and inadvertently surround its partner, resulting in phagocytosis-independent engulfment. Note that vesicles, the alternative communication route mentioned in Sect. 1.1, also appear to have existed since the origin of life.

Such an engulfment process would assimilate the partner and simultaneously form a chromosome-bounding membrane structure (Figure 4d-f) topologically similar to that of the eukaryotic nuclear membrane. In this symbiosis, PAPLA and PA mutually benefit – PAPLA can allot energy metabolism to PA and indirectly obtain energy from organotrophy via AAC (ADP/ATP Carrier), while PA is fed oxoacids for energy production. Here, PAPLA endogenizes PA and one finally arrives Figure 4f) at LECA possessing symbiosis congruent with that of extant eukaryotes and their mitochondria.

This sequence of steps, referred to as the Entangle-Engulf-Endogenize (E3) model for the origin of eukaryotes, is illustrated in Figure 4. The verification that the most primitive micro-organisms already produced CMNs also reinforces the proposal that they are the ancestors of the nervous system.

CONCLUSION

CMN have existed since the origin of life and they have likely played a crucial role in the origin of eukaryotic cells.

INTRACELLULAR TUBULES

Besides the intercellular nanotubes considered so far, there exist also, within eukaryotic cells, Intra Cellular Tubules (ICT). Well-known examples are the tubular networks in the endoplasmic reticulum and Golgi apparatus, which display CMN features such as the V-Y bifurcations reported in [11].

Other ICT play important roles in intracellular communication, such as lysosome reformation [34] and mitochondrial fission and fusion [35,36]. The scale of those ICT can be two to three orders of magnitude smaller that of CMN, so that correlated scaling of properties is to be expected.

Lysosomes used to be regarded as a waste disposal system for degradation of biomolecules by hydrolytic enzymes. However, it was discovered that they are involved in a variety of other cell processes, including reforming of the cell membrane, apoptosis and energy metabolism. A recycling process known as autophagy maintains lysosome homeostasis. Its terminal step is autophagic lysosome reformation (ALR) [34]. The steps in this process are illustrated in Figure 5.

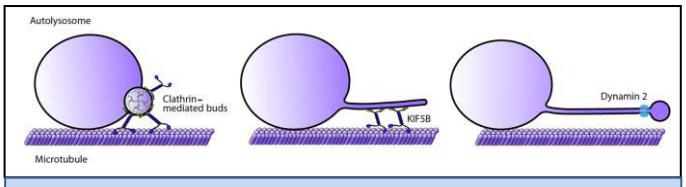

Figure 5: Formation of bud at the autolysosome surface is followed by elongation of membrane tubules, transportation along a microtubule by motor protein KIF5B, and proto-lysosome scission by dynamin 2 (From [34]).

The proto-lysosomes formed by this process mature into new lysosomes, completing the lysosome reformation process. Since the size of autolysosomes ranges from a few hundred nanometers to several micrometers, their membrane tubules are consistent with (relatively thin) CMN.

Mitochondria (MT) are highly dynamic cell organelles. They can form constantly changing tubular networks. Their shapes may vary from elongated tubules to spherical. Their chief role is in ATP production by oxidative phosphorylation.

They also contribute to metabolic regulation and to apoptosis, among other functions. They divide by binary fission, similarly to the bacteria, from which they descend. Two MT can combine by fusion, integrating their contents. These processes are regulated by GTPases of the dynamin family. Studies by electron cryo-tomography [37] indicate that Mfn proteins of this family produce tethering of opposing MTs, followed by docking by GTP hydrolysis and local fusing of the two MTs.

MT fission [38] starts by the dynamin-related protein Drp1 binding to an integral protein situated in the outer mitochondrial membrane at the fission site. An ER microtubule

wraps around the MT, forming a ring-shaped collar. Other proteins promote actin assembly at the ER–mitochondria interface. Myosin may then exert contractile force to constrict the microtubule. GTP hydrolysis-narrowing finally results in fission. This process is pictured in Figure 7.

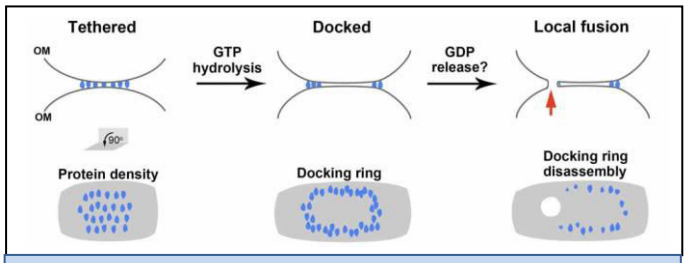

Figure 6: Steps sequence in MT fusion (from [37]). The upper a finally and lower figures are longitudinal and transverse views of this process, with Mfn proteins represented in blue.

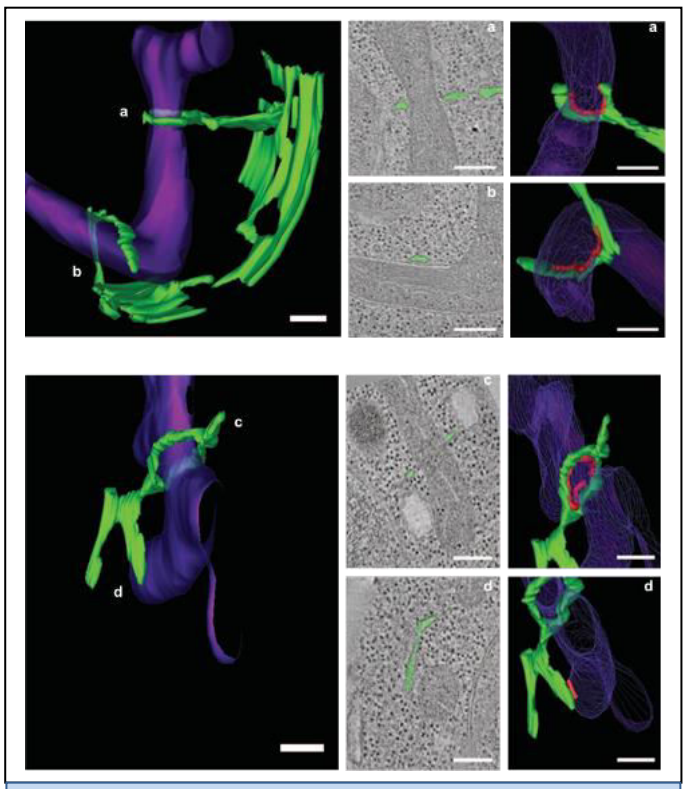

Figure 7: (from [38]) 3D models (left images) of ER (green) and mitochondria (purple) at contact. Middle images: 2D tomographs of contact sites (ER drawn in green). Right images: corresponding 3D models. Contacts in red. Scale bars 200 nm.

In this figure, ER tubules are shown in green and MT in purple. The second column shows 2D images of contact sites (green). The third column has the corresponding 3D models. Contacts (red) are defined as regions where the ER tubule comes within 39 nm of the MT membrane. Scale bars are 200 nm.

The wrapping around of the ER tubules is strongly reminiscent of the engulfment process shown in Figures 3 and 4, suggesting memory retention. What are the features of these tubules? They were investigated in [39], employing diffraction limited and super-resolved fluorescence microscopy. It was determined that the average persistence length of the ER tubules was 3.03 ± 0.24 μm, much larger than the ER diameter, so that one can apply the flexible chain polymer model. The average radius of the tubules was 44.1 ± 3.2 nm, and the bending rigidity of the ER tubule membranes was found to be 10.9 ± 1.2 kT. This value is roughly one order of magnitude below that found for neurons in [40], which is consistent with tubules being pure membrane, with no cortex interaction.

Compliance with Ethical Standards

ACKNOWLEDGMENTS

This work was supported by the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). The author is a member of the Instituto Nacional de Ciência e Tecnologia de Fluidos Complexos.

CONFLICT OF INTEREST

Author declares that he has no conflict of interest

FUNDING

This work was funded by the Brazilian agency Conselho Nacional

de Desenvolvimento Científico e Tecnológico (CNPq) (no grant number)

Ethical approval: This article does not contain any studies with human participants or animals performed by the author

REFERENCES

1. Onfelt B, Nedvetzki S, Benninger RK, Purbhoo MA, Sowinski S, et al. (2006). Structurally distinct membrane nanotubes between human macrophages support long-distance vesicular traffic or surfing of bacteria. J Immunol. 177: 8476-8483.
2. Osswald M, Jung E, Sahm F, Solecki G, Venkataramani V, et al. (2015). Brain tumour cells interconnect to a functional and resistant network. Nature. 528: 93-98.

3. Nussenzveig HM. (2018). Cell membrane biophysics with optical tweezers. Eur Biophys J. 47: 499-514.
4. Nussenzveig HM. (2019). Are cell membrane nanotubes the ancestors of the nervous system? Eur Biophys J. 48: 593-598.
5. Moroz LL, Kohn AB. (2016). Independent origins of neurons and synapses: insights from ctenophores. Philos Trans R Soc B Biol Sci. 371: 20150041-20150014.
6. Northcut RG. (2013). Evolution of centralized nervous systems: two schools of evolutionary thought. In: In the Light of Evolution: National Academies Press,Washington, DC.
7. Huang H, Liu S, Kornberg TB. (2019). Glutamate signaling at cytoneme synapses. Science 363: 948-955.
8. Ramirez-Weber FA, Kornberg TB. (1999). Cytonemes: cellular processes that project to the principal signaling center in Drosophila imaginal discs. 97: 599-560.
9. Rustom A, Saffrich R, Markovic I, Walther P and Gerdes, HH. (2004). Nanotubular highways for intercellular organelle transport. Science. 303: 1007-1010.
10. Watkins SC, Salter RD. (2005). Functional connectivity between immune cells mediated by tunneling nanotubules. Immunity. 23: 309-318.
11. Pontes B, Viana NB, Campanati L, Farina M, Moura Neto V, et al. (2008). Structure and elastic properties of tunneling nanotubes. Eur Bioph J. 37: 121-129.
12. Arkwright et al. (2010). Fas stimulation of T lymphocytes promotes rapid intercellular exchange of death signals via membrane nanotubes. Cell Research. 20: 72-78.
13. Da Cunha V, Gaia M, Gadelle D, Nasir A, Forterre P. (2017). Lokiarchaea are close relatives of Euryarchaeota, not bridging the gap between prokaryotes and eukaryotes. PLOS Genetics. 13: e1006810.
14. Sowinski S, Jolly C, Berninghausen O, Purbhoo MA, Chauveau A, et al. (2008). Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. Nat Cell Biol. 10: 211-219.
15. Sherer NM, Lehmann MJ, Jimenez-Soto LF, Horensavitz C, Pypaert M, et al. (2007). Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. Nat Cell Biol. 9: 310-315.
16. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, et al. (2009). Prions hijack tunnelling nanotubes for intercellular spread. Nat Cell Biol. 11: 328-336.
17. Abounit S, Wu JW, Duff K, Victoria GS and Zurzolo C. (2016). Tunneling nanotubes: A possible highway in the spreading of tau and other prion-like proteins in neurodegenerative diseases. Prion. 10: 344-351.
18. Matejka C, Reindl J. (2019). Perspectives of cellular communication through tunneling nanotubes in cancer cells and the connection to radiation effects. Radiation Oncology. 14: 218-228.
19. Chinnery HR, Pearlman E, McMenamin PG. (2008). Cutting edge: membrane nanotubes in vivo: a feature of MHC class II+ cells in the mouse cornea. J Immunol. 180: 5779-5783.
20. Wang X, Veruki ML, Bukoreshtliev NV, Hartveit E, Gerdes HH. (2010). Animal cells connected by nanotubes can be electrically coupled through interposed gap-junction channels. PNAS. 107: 17194-17199.
21. Meldrum BS. (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130: 1007S-1015S.
22. Feinberg EH, Vanhoven MK, Bendesky A, Wang G, Fetter RD, et al. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. Neuron. 57: 353-363.
23. Wuddick MM, Portes MT, Michard E, Rosas-Santiago P, Lizzio MA, et al. (2018). CORNICHON sorting and regulation of GLR channels underlie pollen tube Ca2+ homeostasis. Science. 360: 533-536.
24. Chiu J, DeSalle R, Lam HM, Meisel L, Coruzzi G. (1999). Molecular Evolution of Glutamate Receptors: A primitive signaling mechanism that existed before plants and animals diverged. Mol Biol Evol. 16: 826-838.
25. Whicher AI. (2016). Simulated alkaline hydrothermal vent environments to investigate prebiotic metabolism at the origin of life. Ph D thesis, University College London.
26. Martin W, Russell MJ. (2003). On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Philos Trans R Soc Lond B Biol Sci. 358: 59-83.

27. Kelley DS, Karson JA, Blackman DK, Früh-Green GL, Butterfield DA, et al. (2001). An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. Nature 412: 145-149.
28. Cech TR. (2012). The RNA worlds in context. Cold Spring Harbor perspectives in biology. 4: a006742.
29. Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, et al. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature. 14: 173-179.
30. Khavaja N, Frank Postberg, Jon K Hillier, Fabian Klenner, Sascha Kempf, et al. (2019). Low-mass nitrogen-, oxygen-bearing, and aromatic compounds in Enceladean ice grains. MNRAS. 489: 5231-52316.
31. Taubner RS, Patricia Pappenreiter, Jennifer Zwicker, Daniel Smrzka, Christian Pruckner, et al. (2018). Biological methane production under putative Enceladus-like conditions. Nature Communications. 9: 748-759.
32. Martin W, Müller M. (1998). The hydrogen hypothesis for the first eukaryote. Nature. 392: 37-41.
33. Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, et al. (2019). Isolation of an archaeon at the prokaryote–eukaryote interface. Nature: published online: 15 January 2020. 577: 519-525.
34. Yu L, McPhee CK, Zheng L, Mardones GA, RongY, et al. (2010). Termination of autophagy and reformation of lysosomes regulated by mTOR. Nature. 465: 942-946.
35. Youle RJ, Blick AM. (2012). Mitochondrial Fission, Fusion, and Stress. Science. 337: 1062-1965.
36. Yu S, Melia TJ. (2017). The coordination of membrane fission and fusion at the end of autophagosome maturation. Curr Opin Cell Biol. 47: 92-98.
37. Lee H, Yoon Y. (2016). Mitochondrial fission and fusion. Biochemical Society Transactions. 44: 1725-1735.
38. Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, et al. (2011). ER Tubules Mark Sites of Mitochondrial Division. Science. 334: 358-362.
39. Pantelis G, Allan VJ, Wright GD, Woodman PG, Udommai P, et al. (2018). The flexibility and dynamics of the tubules in the endoplasmic reticulum. Sci Rep. 7: 16474.
40. Pontes B, Ayala Y, Fonseca ACC, Romão LF, Amaral RF, et al. (2013). Membrane Elastic Properties and Cell Function. PLoS ONE. 8: e67708.