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Research Article

Tunneling nanotubes: Emerging view of their molecular components and formation mechanisms

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ABSTRACT

Cell-to-cell communication is essential for the development and maintenance of multicellular organisms. The tunneling nanotube (TNT) is a recently recognized distinct type of intercellular communication device. TNTs are thin protrusions of the plasma membrane and allow direct physical connections of the plasma membranes between remote cells. The proposed functions for TNTs include the cell-to-cell transfer of large cellular structures such as membrane vesicles and organelles, as well as signal transduction molecules in a wide variety of cell types. Moreover TNT and TNT-related structures are thought to facilitate the intercellular spreading of virus and/or pathogenic proteins. Despite their contribution to normal cellular functions and importance in pathological conditions, virtually nothing is known about the molecular basis for their formation. We have recently shown that M-Sec (also called TNFaip2) is a key molecule for TNT formation. In cooperation with the RalA small GTPase and the exocyst complex, M-Sec can induce the formation of functional TNTs, indicating that the remodeling of the actin cytoskeleton and vesicle trafficking are involved in M-Sec-mediated TNT formation. Discovery of the role of M-Sec will accelerate our understanding of TNTs, both at the molecular and physiological levels.

Introduction

To develop and maintain homeostasis, cells of a multicellular organism must communicate with each other in various ways. Communication between remote cells is mediated mainly by secretion of signaling molecules, such as cytokines, from the stimulatory cells and their subsequent binding to specific receptors on the responding cells. Another form of communication with remote cells is mediated by small membrane vesicles, exosomes, secreted from a broad range of cell types as a consequence of fusion of multivesicular late endosomes/lysosomes with the plasma membrane [1].

By contrast, adjacent cells often communicate with each other via junctional complexes, such as gap junctions and synaptic junctions [2]. The gap junctions are narrow channels directly connecting the cytoplasm of neighboring cells, which allow inorganic ions and small water-soluble molecules to pass from the cytoplasm of one cell to another, thereby coupling the cells both electrically and metabolically. Neurological and immunological synapses also transmit

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cell–cell signals through the extracellular space, relying on mechanisms of ligand–receptor signaling across the closely apposed cell–cell junctions [3].

In addition to these well-established examples, tunneling nanotubes (TNTs), sometimes referred to as membrane nanotubes, and related structures are newly-emerged distinctive mechanisms for cell–cell communication in a wide variety of cell types. These structures can directly connect cells even over relatively long distances [4,5]. While accumulating evidence from recent studies indicates their importance for a variety of cellular functions, we still have a limited molecular understanding of TNTs regarding their structures and biogenesis. TNTs have become a very active research area, however, and new evidence on molecular components and formation mechanisms has been forthcoming. Here we summarize current findings on molecular aspects of TNTs and related structures.

What are TNTs?

TNTs can be recognized as thin membranous structures connecting two or more cells and, importantly, these structures are not attached to the substratum (Fig. 1A–F) [6,7]. A different sort of cytoplasmic connection is observed after cell division as a structure containing the midbody; it persists temporarily as a tether between the two daughter cells, contains dense matrix material, and is visible by light microscopy. By contrast, TNTs do not contain the midbody. Gerdes and colleagues first described TNTs as a structure that provides plasma membrane continuity between connected cells and facilitates the selective transfer of membrane vesicles and organelles to neighboring cells [7]. They discovered these structures in rat pheochromocytoma PC12 cells and rat kidney NRK cells, but subsequent studies have identified TNTs or similar structures in various types of cells, including T24 urothelial cells, Jurkat T cells, THP-1 human monocytes and human primary natural killer (NK) cells (Table 1).

The detailed morphological and structural characteristics of TNTs are substantially different among cell types [4]. TNTs of PC12 cells are 50–200 nm in diameter, and they can be up to several cell diameters long [7]. In the case of immune cells such as macrophages, Epstein Barr Virus-transformed B cells and human peripheral blood NK cells, the average length of TNTs reaches 30 μm with some measuring over 140 μm [8]. TNTs contain an F-actin backbone and lack microtubules in most cell types [4,7]; however, some exceptions do exist [4]. For example, NK cells have TNTs containing microtubules [9]. Macrophages have two types of TNTs, and the thicker ones (>0.7 μm in diameter) contains both F-actin and microtubule backbones [10]. This heterogeneity probably represents cell-specialized functions and features of TNTs as described below. More recent studies have also shown that the termini of some TNTs and associated structures are not continuous with the plasma membrane of connected cells but instead have junctions [9,11,12–14], as we will also describe later.

Proposed functions of TNTs

Transmission of intercellular signaling via TNTs

Calcium flux is the best-characterized signal transmitted between remote cells via TNTs. Upon mechanical or chemical stimulation, myeloid-linage dendritic cells (DCs) and monocytes propagate their calcium signals within seconds to other cells connected by TNTs (Fig. 1G) [12]. TNT-medicated intercellular transmission of calcium signals induced morphological changes such as lamelipodia extension in recipient DCs, one of the earliest responses seen in phagocytes following stimulation [12]. This observation demonstrates that the cell–cell interaction via TNTs facilitates transduction of signals between remote cells (Table 1).

Watkins and Salter [12] performed a rigorous examination to exclude the possibility that the propagation of calcium flux between DCs is dependent on ATP released from these cells following mechanical stimulation as described previously [15]. The Ca^{2+} flux between DCs was not induced by addition of BzATP, a stabilized form of ATP, and not blocked by suramin, an antagonist of P2 purinergic receptors responsible for ATP-mediated calcium responses in mast cells. In addition, physical disruption of the TNT-network by scraping with a micropipette tip resulted in abrogation of the intercellular propagation of the calcium flux (Fig. 2B). These investigators further demonstrated that the small cytoplasmic dye Lucifer yellow could readily transfer via TNTs between THP-1 myeloid cells, whereas the larger cytoplasmic molecule dextran could not [12]. Thus, there must be some junctions and/or gating mechanisms for TNT-based transport of intercellular material, which may imply the association of a gap-junction that can pass low molecular weight molecules but not macromolecules. Intriguingly, however, calcium transmission via TNTs in THP-1 cells was insensitive to conventional gap-junction inhibitors, suggesting the presence of previously unknown gating mechanisms. On the other hand, Gerdes and colleagues recently showed that in various cell types, including HEK293, NRK, human umbilical vein endothelial cells (HUVEC) and neural crest cells, TNTs have gap junctions at their distal ends which mediate electrical coupling between distant cells through the interposed gap junction channels [11]. Furthermore, they also showed that the electrical signals transferred from one cell to another via TNTs are sufficient to induce a transient calcium elevation in the recipient cell by activating low voltage-gated calcium channels [11]. Taken together, these studies suggest that different mechanisms of intercellular calcium signaling likely exist, and may reflect the diversity of TNT structures and functions in different cell types.

Another type of TNT-mediated signal transduction was reported in NK cells. NK TNTs contain a submicron scale junction similar to the intercellular lytic synapse between NK cells and target cells, where a proximal signaling protein of NK-cell activating receptor NKG2D and its ligand MHC Class I chain-related protein A (MICA) accumulates [9]. One function of NK cells is recognition and elimination of cells undergoing different forms of stress, such as microbial infection and malignant transformation [16]. This study raises the intriguing possibility that NK cells might eliminate target cells via an immunological synapse located at the terminus of their TNTs; thus, TNTs could aid the lysis of distant cells by NK cells. NK-cell cytotoxicity involves the secretion of cytolytic effector molecules from specialized organelles known as lytic granules. Intracellular trafficking of the lytic granule is dependent on microtubules [16], and the NK-cell TNTs contain a microtubule backbone. Thus, the microtubule within NK-cell TNTs may act as a railway for delivering lytic granules to the distal end of the TNTs.