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M-Sec

Emerging secrets of tunneling nanotube formation

Hiroshi Ohno,* Koji Hase and Shunsuke Kimura

Laboratory for Epithelial Immunobiology; Research Center for Allergy and Immunology; RIKEN; Tsurumi; Yokohama, Kanagawa Japan

Tunneling nanotubes (TNT) are the latest addition to the array of strategies used for intercellular signaling. TNTs are continuous conduits of the plasma membrane that allow direct physical connection of plasma membranes and cytosol among remote cells. They are important for intercellular communication by mediating exchange of cellular components as well as signal transduction molecules. Despite ample evidence suggesting the pathophysiological importance of TNTs, virtually nothing is known about the molecular basis for their formation. With the lack of specific TNT markers, their study has relied solely on morphological analyses, and the precise identity of TNT and TNT-like structures have been difficult to define. We have now shown that M-Sec is a TNT marker and a central factor 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 is 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.

Key words: tunneling nanotube, M-Sec, RalA, exocyst complex, F-actin, virus infection

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*Correspondence to: Hiroshi Ohno; Email: ohno@rcai.riken.jp

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Immune cells employ various ways to communicate with other cells. Direct cell-cell contact, for example, takes place during antigen presentation by dendritic cells for T-cell activation. The interface between the two cells is referred to as the immunological synapse.[1] Gap junctions are another class of direct intercellular contacts that can mediate transfer of small molecular weight solutes between the cytoplasm of two connected cells.[2] In contrast, interactions between remote cells are often mediated by humoral factors such as cytokines where the signal donor cell secretes the factor and the acceptor cell expresses the cognate receptor on its cell surface to receive signals. Yet another form of intercellular communication is mediated by membrane vesicles, in particular exosomes.[3] Exosomes are small vesicular structures contained in late endosomes or multivesicular bodies. These multifunctional vesicles are produced by invagination of the limiting membrane of these organelles and are released upon fusion of the organelles with the plasma membrane. Tunneling nanotubes (TNTs) and related structures are the latest addition to the lineup of cell-cell interaction platforms.[4-6]

TNTs were first described in rat pheochromocytoma PC12 cells,[7] but subsequent studies have revealed that many cell types, including those in the immune system such as dendritic cells and macrophages, also form TNTs.[4-6] TNTs are a continuous conduit of the plasma membrane that allows direct physical connections of plasma membranes and the cytosol of remote cells. Hence, TNTs are thought to be important for intercellular communication by mediating exchange of materials, ranging in size from as small as Ca^{2+} ions to as large as cytoplasmic vesicles and/or small organelles of endosomal/lysosomal origin. Moreover, TNTs are occasionally 'hijacked' by viruses for intercellular spreading, a strategy that protects them from exposure to the extracellular space and enables them to evade host humoral immunity.

Despite the ample evidence suggesting their pathophysiological importance, virtually nothing has been known about the molecular basis for TNT formation. Moreover, owing to the lack of specific TNT markers, the identification of these structures has been mainly based on morphological criteria and the presence of cytoskeletal elements. This type of analysis has revealed some heterogeneity in TNT and TNT-like structures, such as the existence of microtubule-containing TNTs and close-ended filopodial bridges. We have now shown that M-Sec is a TNT marker and central factor for TNT formation.[8] M-Sec was first described as tumor necrosis factor alpha-induced protein 2 (also called B94),[9,10] but its function has long been unknown. We found that M-Sec can induce functional TNTs in cooperation with the RalA small GTPase and the exocyst complex, indicating that remodeling of the actin cytoskeleton is involved in M-Sec-mediated TNT formation. Despite its sequence homology with Sec6, a component of the exocyst complex, M-Sec itself does not seem to be included in the complex. M-Sec likely coordinates with RalA and the exocyst complex to initiate TNT formation whereas cdc42 seems to be required for TNT elongation, since short protrusions of membrane tubules accumulate without further elongation in the presence of dominant negative cdc42.[8]

Involvement of actin remodeling in M-Sec-mediated TNT formation is consistent with a previous observation that TNTs are associated with F-actin. In fact, M-Sec-induced TNTs are associated with F-actin but not with microtubules.[8] It has been reported that there are two types of TNTs in macrophages, thin TNTs only associated with F-actin and thicker ones with both F-action and microtubules.[11] The formation of the thick microtubule-containing TNTs may be totally independent of M-Sec, or it may require M-Sec in combination with other, as-yet-unidentified, factor(s).

Although TNT-like structures were observed upon transient expression of M-Sec in HeLa cells, functional TNTs mediating Ca^{2+} flux only formed when M-Sec was stably expressed (our unpublished observation). These results suggest that the TNT-like structures induced by transient M-Sec expression are close-ended membrane protrusion, the so-called filopodial bridges.[5,6,12] This observation could be explained if functional TNT formation requires the expression of M-Sec in both of the cells connected by a TNT, as would be the case for stable M-Sec transfectants. After transient transfection, by contrast, a substantial portion of the cells would not express M-Sec; accordingly, TNT-like protrusions formed by a cell expressing M-Sec would be abortive, resulting in formation of filopodial bridges instead of a complete TNT upon reaching a nearby cell without M-Sec expression. Another possibility is that sustained expression of M-Sec is required for membrane fusion at the tips of the nanotubes to form TNTs. Recently, human immunodeficiency virus type 1 (HIV-1), the causative agent of aquired immune deficiency syndrome (AIDS), was reported to utilize TNT or filopodial bridges for intercellular transmission, not only in macrophages[13] but also in T lymphocytes.[14] Steady-state expression of M-Sec is essentially restricted to myelomonocytic lineage cells in normal adult tissues, thus normal T cells are largely devoid of M-Sec (ref. 10; and RefDIC,[15] http://refdic.rcai.riken.jp/welcome.cgi). However, human T-cell leukemia virus type 1 is known to induce M-Sec expression in T cells.[16] Therefore, it is possible that HIV-1 may also induce M-Sec expression to promote TNT-like structures in T cells, resulting in the enhancement of intercellular HIV transmission. Intriguingly, Xu et al.[17] have reported recently that HIV-1-infected macrophages transfer viruses and/or the viral protein Nef to surrouding B cells via TNT. This process ultimately suppresses the production of virus-specific IgG2 and IgA and results in the evasion of humoral immune responses by HIV-1. Taken together, these observations suggest that TNTs play a critical role in viral pathology including HIV-1.

The discovery of M-Sec as a marker for TNTs and a promoter of TNT formation will help clarify the mechanisms of formation of these structures as well as their structural and functional properties. We have recently established M-Sec knockout mice, which should be a useful model to uncover the physiological role of TNTs. In addition, the identification of M-Sec-targeted drugs may provide a new strategy for containment of viral infections such as HIV.

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