MECHANISMS OF DEVELOPMENT 130 (2013) 381–387

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/modo

Tunneling nanotubes, an emerging intercellular communication route in development

Hans-Hermann Gerdes [a,*], *Amin Rustom* [b], *Xiang Wang* [a]

[a] *University of Bergen, Department of Biomedicine, Jonas Lies vei 91, N-5009 Bergen, Norway*
[b] *Max Planck Institute for Intelligent Systems, Heisenbergstraße 3, 70569 Stuttgart, Germany*

ARTICLE INFO

Article history:
Available online 14 December 2012

Keywords:
Tunneling nanotube
Gap junction
Electrical coupling
Intercellular communication
Neuron
Astrocyte

ABSTRACT

The development of multi-cellular organisms involves a comprehensive and tightly regulated cell-to-cell communication system to coordinate the activity and behavior of individual cells. Diverse signaling pathways ranging from receptor-mediated signal transduction to contact-dependent communication via gap junctions achieve these complex interactions. In this review, we will focus on a new type of intercellular connection, the tunneling nanotube (TNT), which has been observed in many cell types *in vitro* and recently also in developing embryos of different species *in vivo*. We will summarize the latest insights into their functional roles in cell-to-cell signaling with a particular focus on the TNT-dependent electrical coupling between developing embryonic cells. Finally, potential implications of these new findings in the light of developmental processes, particularly in cell migration, will be discussed.

Contents

* Corresponding author. Tel.: +47 55586849; fax: +47 55586360.
E-mail address: hans-hermann.gerdes@biomed.uib.no (H.-H. Gerdes).
0925-4773/$ - see front matter
http://dx.doi.org/10.1016/j.mod.2012.11.006

1. Introduction

Until the end of the last millennium it appeared that the different mechanisms by which cells of multi-cellular organisms communicate with each other have been identified. This includes secretion of molecules followed by receptor-mediated signal transduction in target cells as well as direct communication of closely associated cells via gap junctions. During the last few years, however, new principles of intercellular communication have come into the limelight. One is the secretion of small vesicles, so-called exosomes, which contain instructive molecules and are thought to enter surrounding cells (Fevrier and Raposo, 2004). Another is the formation of thin membranous bridges, termed tunneling nanotubes (TNTs), which connect cells over long distances and transfer various cellular components from cell to cell (Rustom et al., 2004). In this review we will focus on TNTs as the underlying structure of an emerging new mechanism of cell-to-cell communication and will summarize recent data with relevance to developmental processes in neurons and other cell types.

2. Structure and formation of TNTs

TNTs were first described in 2004 as a new type of cell-to-cell connection between rat pheochromocytoma (PC12) cells, a frequently used neuronal cell model (Rustom et al., 2004). These nano-scaled membranous tubes of about 50–200 nm in diameter and up to several cell diameters long contained F-actin but no microtubules. As a distinguishable criterion from other protrusive cell structures TNTs had no contact to the substrate but were hovering freely in the medium. Subsequent studies showed that TNT-like structures were present in cultures of different cell types including fibroblasts, epithelial cells, immune cells, and neurons (Davis and Sowinski, 2008; Gerdes and Carvalho, 2008). The structures varied to some degree in diameter, giving rise to terms like “thin” and “thick” nanotubes (Onfelt et al., 2006), and sometimes contain microtubules in addition to F-actin. Live cell imaging showed that TNTs are transient structures, lasting for minutes to several hours, and form *de novo* by two different mechanisms. According to the first suggestion, cells protrude a filopodium, which makes contact to the neighboring cells, and subsequently converts into a TNT bridge. This mechanism prevails in PC12 cells and primary neurons (Rustom et al., 2004; Wang et al., 2012). A selective block of TNT formation in the presence of the F-actin-depolymerizing drug cytochalasin B further proved the precursor role of filopodia in TNT formation (Bukoreshtliev et al., 2009). A more detailed investigation by Ohno and colleagues in HeLa cells revealed that TNT formation from filopodia is mediated through the Ral-exocyst pathway (Hase et al., 2009). According to the second mechanism, cells form TNTs by retaining a thin thread of membrane upon dislodgement. This mechanism is found in most cell types studied so far such as T cells, normal rat kidney (NRK) and neural crest cells (NCCs) (Sowinski et al., 2008; Wang et al., 2010).

3. Functions of TNTs

3.1. *Intercellular transfer of cellular components*

As mentioned above the first insights into the function of TNTs were obtained using PC12 cells co-cultures of donor and acceptor cell populations. We showed that TNTs accomplish the intercellular transfer of diverse cellular components: cytoplasmic molecules such as EGFP-actin, organelles/vesicles of endocytic origin and plasma membrane molecules such as farnesylated-EGFP (Rustom et al., 2004). Since then a growing number of cells have been demonstrated to utilize TNTs for the exchange of cellular cargos, including significant molecules such as the MHC I in the case of immune cells (Onfelt et al., 2004) and calcium ions in immune and other cell types (Watkins and Salter, 2005; Wittig et al., 2012). Using similar approaches it was shown that mitochondria could be exchanged between cells via TNTs (Abounit and Zurzolo, 2012; Davis and Sowinski, 2008; Gerdes and Carvalho, 2008). Moreover, pathogens such as the human immunodeficiency virus (HIV) and prions were found to exploit TNT-like structures when spreading between cells (Gousset et al., 2009; Sowinski et al., 2008).

Analysis of the translocation of endocytic vesicles through TNTs of PC12 or NRK cells revealed that the vesicles moved in one direction only with a speed in the range of actin-dependent transport (Gurke et al., 2008; Rustom et al., 2004). This was consistent with the finding that TNTs connecting these cell types contained only F-actin and no microtubules, and that myosin Va was detected in these structures (Rustom et al., 2004). In support of an active transport mechanism, we found that depletion of ATP strongly reduced the intercellular transfer of endocytic vesicles (Gurke et al., 2008). Based on these data we proposed an actomyosin-dependent transport mechanism for endocytic vesicles through TNTs.

3.2. *Electrical coupling*

Besides molecular signals exchanged between cells via TNTs, our study on NRK cells revealed that TNTs enable intercellular transfer of depolarization signals for distances of up to at least 70 μm (Wang et al., 2010). The coupling was bidirectional and its strength depended on the length and number of TNT-connections. Importantly, our data suggested that only TNTs with interposed connexin 43 (Cx43) as the main gap junction forming connexin participated in electrical coupling (Wang et al., 2010). Subsequent studies on different cell types demonstrated that TNT-dependent electrical coupling is a general characteristic of animal cells with functional gap junctions (Wang and Gerdes, 2012). In search of physiological changes induced by this long-distance coupling, we found that the TNT-mediated depolarization can elicit transient calcium signals in connected HEK293 cells through activation of low-voltage calcium channels (Wang et al., 2010).

Inspired by the pioneering studies on the importance of electrical signaling in embryogenesis (Levin, 2012), we investigated the possibility of a TNT-dependent electrical coupling