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Review

Mechanism of membrane nanotube formation by molecular motors

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ABSTRACT

Membrane nanotubes are ubiquitous in eukaryotic cells due to their involvement in the communication between many different membrane compartments. They are very dynamical structures, which are generally extended along the microtubule network. One possible mechanism of tube formation involves the action of molecular motors, which can generate the necessary force to pull the tubes along the cytoskeleton tracks. However, it has not been possible so far to image in living organisms simultaneously both tube formation and the molecular motors involved in the process. The reasons for this are mainly technological. To overcome these limitations and to elucidate in detail the mechanism of tube formation, many experiments have been developed over the last years in cell-free environments. In the present review, we present the results, which have been obtained *in vitro* either in cell extracts or with purified and artificial components. In particular, we will focus on a biomimetic system, which involves Giant Unilamellar Vesicles, kinesin-1 motors and microtubules in the presence of ATP. We present both theoretical and experimental results based on fluorescence microscopy that elucidate the dynamics of membrane tube formation, growth and stalling.

Contents

1. Introduction

Internal membranes in eukaryotic cells are organized in many distinct membrane compartments involved in protein and lipid synthesis, sorting, recycling, etc. Communication between these compartments involves highly dynamical transport intermediates with variable shapes, spherical or tubular in general. They are membrane containers that carry selected proteins and lipids and which can form and detach from one cellular compartment and then, after transport in the cell, fuse with another precisely targeted compartment [1]. The impressive development of imaging of living cells in the past 15 years due to, in particular, the capability to use molecular biology to label-specific proteins with Green Fluorescent Proteins (GFP) and to follow their cellular localization over time has completely transformed our view of the cell [2,3]. Although electron microscopy has long been the best tool for imaging cell sub-compartments and localization of proteins with a very high resolution, very little information can be obtained with this technique on cell dynamics, in particular cellular transport. Mainly based on electron microscopy images, it was accepted that transport intermediates have more or less a spherical shape. With video-microscopy on cell expressing fluorescently labeled proteins, it became clear that dynamic tubular structures also participate to cellular traffic [4–10]. They can be either long and thin membrane tubes still connected to the original membranes or disconnected and moving in the cell or moderately

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extended structures called “tubulo-vesicules”. The very thin tubes have a diameter below optical resolution but much longer extension and can then be detected optically. Conversely, their very polarized shape and very small lateral cross-section explain why they could not be previously detected with electron microscopy techniques, since only very thin 2D sections were used for these studies. These tubular carriers have been observed on different routes of membrane transport, not only in the secretion pathway from endoplasmic reticulum (ER) to the Golgi apparatus [4] and from the Golgi to the plasma membrane [5,6,11,12] but also along the retrograde transport from Golgi to ER [7,8]. Their number increases in the presence of some drugs (such as Brefeldin A [13]) or due to over-expression of some proteins [14], but their existence is now well established.

The cytoskeleton is directly involved in the formation of membrane tubes, as evidenced by dual color visualization of transport intermediates and microtubules (MTs). These dynamical biological polymers MTs are essential for the traffic of vesicular transport intermediates in the cell [15]. In particular, they are involved in the formation of membrane tubes, as tubes have been usually seen to be extended along MT tracks [6]. Besides transport between different parts of the cell, dynamical membrane tube networks are also intrinsically part of the shape of organelles [16,17] such as the ER [18,19], Golgi [20] or endosomes [21,22]. Particularly, the ER consists of a highly dynamic network of membrane tubules and lamellae contiguous to the outer nuclear envelope. Following simultaneously the MTs and ER tubule dynamics, C. Waterman-Storer and E.D. Salmon showed that ER membrane tubes grow in the plus-end direction of MTs either attached to their tip in synchrony with MT polymerization dynamics, or along MTs regardless of their dynamics (Fig. 1) [23]. In the later case, it was proposed that the force required to generate membrane tubes was produced by molecular motors associated to MTs. These motors can either belong to the kinesin family and move towards the plus-end of the MTs or to the dyneins' and move in the opposite direction [24,25]. The dynamic nature of the membrane tubes and their colocalization with the MTs suggested that motor proteins in concert with the cytoskeleton and MT-associated proteins or MT tip tracking proteins [26] are essential in the formation of these tubes. This idea was further supported by *in vivo* experiments in which the expression of kinesin heavy chains was suppressed [27] or MTs were depolymerized [28]. In the absence of active kinesins or MTs, the tubular structures were no longer present in cells. Other mechanisms have been proposed leading to membrane deformation, which usually involve either proteins binding to a membrane and inducing spontaneous curvature, or protein assemblies forming scaffolds on the membrane such as for coat formation (clathrin, COPI or COPII), or a biochemical transformation of the lipids of the membrane (for reviews, see for instance [29], or [30]). Tubule formation has been observed independently of molecular motors and MTs, when Shiga or cholera toxins bind to their lipid receptors [31] and produce a spontaneous negative curvature of the plasma membrane. In the case of ER, additional membrane proteins named “reticulons” have been identified, which probably also contribute to the tubulation of the ER [32]. The role of actin filaments in the traffic involving the Golgi apparatus has been investigated with a growing interest in the past year, as actin cytoskeleton and probably myosin motors have been shown to contribute to the formation of the membrane carriers derived from the Golgi and to the Golgi shape [33]. Eventually, bacteria, such as Salmonella for instance, can hijack MT-related motors and recruit them at the surface of vacuoles in infected cells to induce membrane tube formation in order to control the dynamics of membrane exchanges with their replication compartment [34,35].

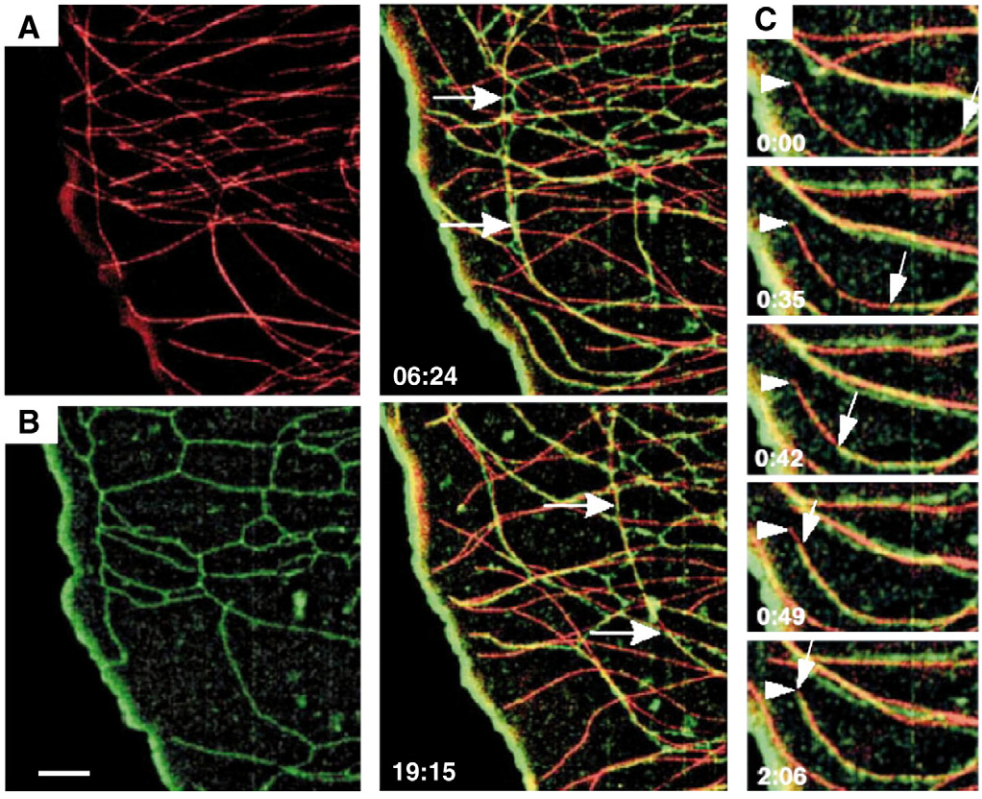

Fig. 1. Structure and dynamics of Endoplasmic Reticulum (ER) in the lamelipodium of a Newt lung cell. (A–B) Fluorescent images showing the colocalization of ER (green, membrane labelled with Dioc6) tubules and microtubules (red, microinjection of X-rhodamin-labelled tubulin). (A) Microtubule network (right) and overlay with ER network (left). The green and red images were taken with A 3-s difference. (B) ER network after image processing (right) and overlay with the microtubule network (right). Bar, 5 μm. (C) Growth of an ER tubule (arrow) along a preexisting microtubule (microtubule tip indicated by an arrowhead). Time lapse serie of images (elapsed time in minute:second). Adapted with permission from [23].