# Versatility of tunneling nanotubes and cytoskeletal filaments they have

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Communication between cells is crucial for proper functioning of multi-cellular organisms. The recently discovered membranous tubes, named tunnelling nanotubes (TNTs), that directly bridge neighbouring cells, may offer a very specific and effective way of intercellular transport and communication. Our experiments on RT4 and T24 urothelial cell lines show that TNTs can be divided into two types with respect to their biochemical characteristics and the nature of the process of their formation.

The nanotubes of type I are shorter, usually not longer than 30 μm, more dynamic and contain actin filaments (Figure 1A, 1B). They are formed when cells explore their surroundings in order to make contact with another cell. 1). This type of actin-containing nanotubes can also bridge cells at distances of less than 30 μm and are most likely derived from the adherence cell-cell contacts of cells that move apart as they appear higher of the cell body (Figure 1C).

The nanotubes of type II are longer, more stable and have cytokeratin filaments (Figure 2). They are formed when two already connected cells start to move apart.

On the nanotubes of both types small vesicles were found as an integral part of the nanotubes (i.e. dilatations of the nanotubes). The dilatations of type II nanotubes do not move along the nanotubes, while the nanotubes of type I have frequently dilatations (gondolas) that move along the nanotubes in both directions and are formed in different ways. In some cases the formation of gondolas may be induced by a sudden tension (caused for example by diverging cells) in the membrane nanotubes at specific sites where the local membrane constituents of the nanotubes enable and favour the formation of such dilatations. The tension induced dilatation of the nanotubes may appear anywhere along the nanotube and then travels as a wave along the bridging nanotube in the direction that is energetically favourable. The distension of the nanotubes may be formed also because of a small organelle inside the nanotubes, if the diameter of the organelle is larger than the inner diameter of the nanotube. The organelles inside the nanotubes may be actively transported by different acto-myosindependent mechanisms.

We suggested theoretical models that may explain how these nanotubes are created and stabilized.

1. P. Veranic et al., Biophy. J., **95** (2008) p4416.

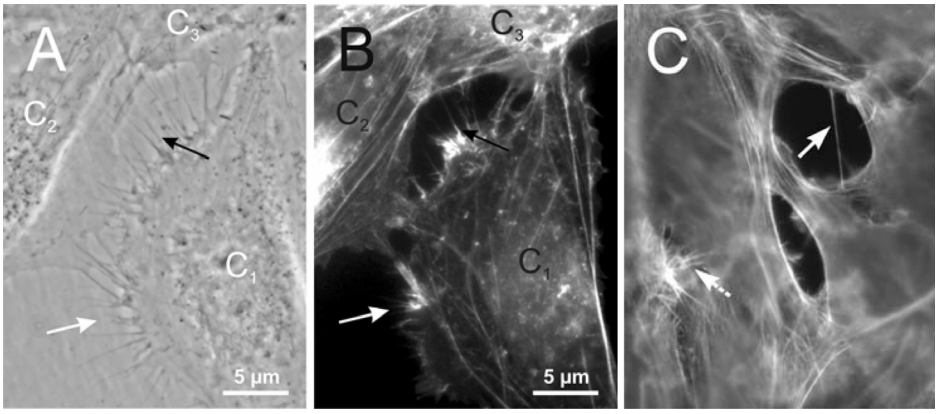


**Figure 1.** Type I nanotubes. Panel A is a phase contrast image of live T24 cells while panel B is a fluorescence micrograph showing actin labelling of the same cells as in *A*, after 15 minutes of paraformaldehyde fixation. Cell C1 is approaching the cells C2 and C3. The white arrows in *A* and *B* indicate short and dynamic membrane protrusion with which the approaching cell explores its surroundings. Black arrow in *A* points at protrusions that have already connected to the target cell. In C arrow is pointing at nanotube that connencts two cells. In all these multiple tubular connections actin filaments are present (↑ in *B, C*).

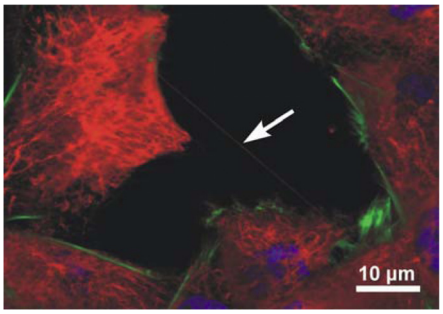


**Figure 2.** Type II nanotubes. A long tubular structure is connecting two cells. It contains thin cytokeratin filaments (arrow). Cytokeratin 7 is labelled in red, actin in green and the nucleus with DAPI in blue.

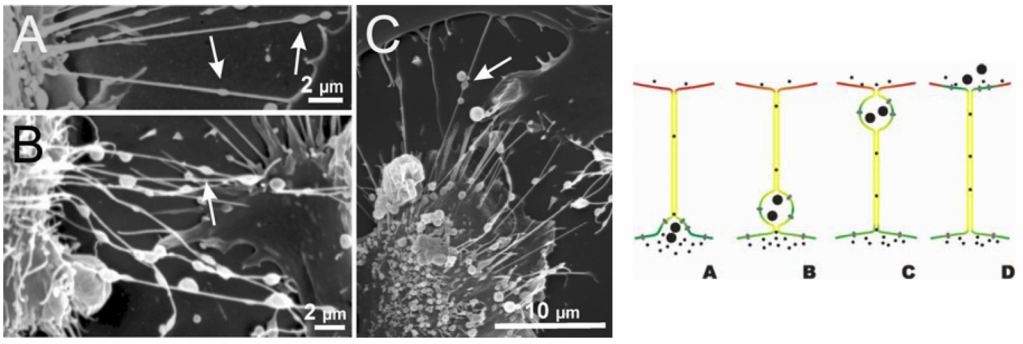


**Figure 3.** Membrane nanotubes with gondolas (white arrows) observed between cells in the human urothelial cell line RT4 (A) and T24 (B and C) by scanning electron microscopy under physiological conditions. Note that the gondolas are an integral part of the tubes. Right: Schematic illustration of nanotubule-directed transport of small carrier vesicles (gondolas) transporting granular content and membrane particles.

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