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# Contribution of filopodial bridges to Ectromelia virus infection

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A b s t r a c t

Cell-to-cell interaction is the basis of all biology in multicellular organisms. Though biochemical interactions occur over distances, physical continuity remains the most direct means of cellular communication. Cellular bridging through thin cytoplasmic channels—plasmodesmata in plants and tunneling nanotubes in animals—creates direct routes for transfer of signals and components, even pathogens, between cells. The aim of this study was to evaluate if filopodia-like protrusions induced by ectromelia virus (ECTV, mousepox virus) in infected cells *in vitro* could be classified as membrane bridges. In our experiments Vero and BALB/3T3 cells were used and changes in cellular morphology and the presence of filopodial bridges were visualised by fluorescence microscopy. In both cell lines ECTV infection induces production of two kinds of actin-rich protrusions by infected cells: short-actin tails and long-filopodia-like. It appears that both types contribute to the spread of ECTV particles, although their nature is currently unclear.

K e y W o r d s: actin tails, ectromelia virus, filopodia, membrane bridges, tunneling nanotubes

## Introduction

Communication with the environment is essential for any biological system. In multicellular organisms, most cells do not exist as independent units, but are organized in specialized tissues. To coordinate physiological processes, cells communicate with each other in different ways. Much of this interaction occurs at the cell-to-cell contact and is regulated by complex structural interfaces. Tight cell-cell junctions, found in neurological and immunological synapses, transmit signals through extracellular space, relying on ligand-receptor mechanisms. On the other hand, direct exchange of cytoplasmic contents can be found in animal cells (gap junctions) and plants (plasmodesmata), which can efficiently propagate an intracellular signal between cells.

With the advance of fluorescence microscopy and imaging techniques, new types of cell-to-cell communication have been described. These are based on the formation of thin, elongated, intercellular membrane channels, described also as membrane bridges. In a broad sense, two distinct types of thin membrane bridges can be distinguished, based on the ability of cytosolic content to be exchanged between cells. Structures that physically connect two cytoplasms, permitting direct intercellular transfer of cytoplasmic molecules, organelles and membrane components are referred to as tunneling nanotubes. In these structures, exchange of signals or cargo can be made via the central channel or along the surface of continuous outer membrane. By contrast, in non-tubular bridges, two membranes are tightly adjoined at the site of cell-cell contact, but no cytoplasm connection is present and signals are transducted by the transport of ligands across the outer membrane. These bridges are referred to as cytonemes or filopodial bridges. Both kind of connections are characterized as filopodia-like elongated structures, which are stretched between cells at their nearest distance and F-actin being their main cytoskeletal component [4, 6].

There is growing evidence that efficient spread of pathogens, like bacteria and mammalian viruses, is mediated by cell membrane protrusions. By using established or induced filopodial bridges between cells, infectious agents avoid rate-limiting step of

diffusion and efficiently accelerate their spreading. These mechanisms are universally actin-dependent, but vary between particular pathogens. Among the viruses shown to exploit cellular protrusions, to transfer directly from cell-to-cell are the murine leukemia virus, pseudorabies virus, herpes simplex virus, human immunodeficiency virus type 1 and vaccinia virus [4, 6]. The latter, the prototypic poxvirus, is known for its well-described ability to induce the formation of specific kind of cell protrusions known as actin tails, which propel the virus towards the target cell [5]. Among other poxviruses, Myxoma virus was found to induce formation of cytoplasmic corridors, used for efficient virus spread [3]. Both types of these structures (actin tails and cytoplasmic corridors) have been described by us recently [2], as being used by ectromelia virus (mousepox virus, genus: *Orthopoxvirus*) for direct transmission between cells *in vitro*.

Thus, in the light of these findings and new nomenclature being used, we wanted to investigate further if ECTV-induced cytoplasmic corridors, formed in infected cells, have the properties of membrane bridges, and if so, what type of protrusions are they.

## Materials and Methods

Vero (ATCC CCL-81) and BALB/3T3 clone A31 cells (ATCC CCL-163) were infected with highly virulent Moscow strain of ectromelia virus (ECTV-MOS; ATCC 1374). Infections were carried out with MOI = 5 per cell, at 37°C in DMEM with lowered serum content. Cells were fixed at 21 h p.i. with buffered 4% PFA. For immunofluorescence polyclonal FITC-anti-ECTV antibodies were used. F-actin was stained with TRITC-phalloidin (Sigma), and DNA with Hoechst 33342 (Calbiochem). Images were recorded and analysed using Olympus BX60 fluorescence microscope equipped with Color View III cooled CCD camera and cell^F software, with further post-processing with Image-J software [1].

## Results and Discussion

In our previous studies [2], we observed that ECTV, similarly to VACV [5], induced alterations in the microfilament organization of the studied Vero and BALB/3T3 cells. These changes manifested in the abnormal cell morphology and production of protrusions.

In present experiments, we have found that at 21 h.p.i. both Vero and BALB/3T3 ECTV-infected cells produced filopodia-like protrusions - either numerous and small, or individual, long projections. Both types of these filopodia were apparently used by virus for spread directly from cell to cell. Small, microvilli-like protrusions, were recognized as actin tails with their characteristic feature of propelling single viral particle from the infected cell. These structures were observed mainly in BALB/3T3 cells (Fig.1). Long filopodia projections attributed mainly to Vero cells (Fig. 2), were found to connect cells on longer distances, enabling spread of numerous ECTV particles directly from cell to cell. These projections were rich in F-actin, and both cells seemed to be connected, with virus entering uninfected cell directly, without exposure to external environment. Nevertheless, we were unable to determine if the membrane bridges were open for cytoplasmic material.

Apparently, both types of protrusions fall into the category of filopodial bridges. However, without clear criteria of distinguishing filopodia from tunneling nanotubes and definition of their molecular determinants [6], it is impossible to conclude what type of projections is produced by ECTV-infected Vero cells. Further investigation is needed, as these structures are obviously contributing to efficient ECTV spread.

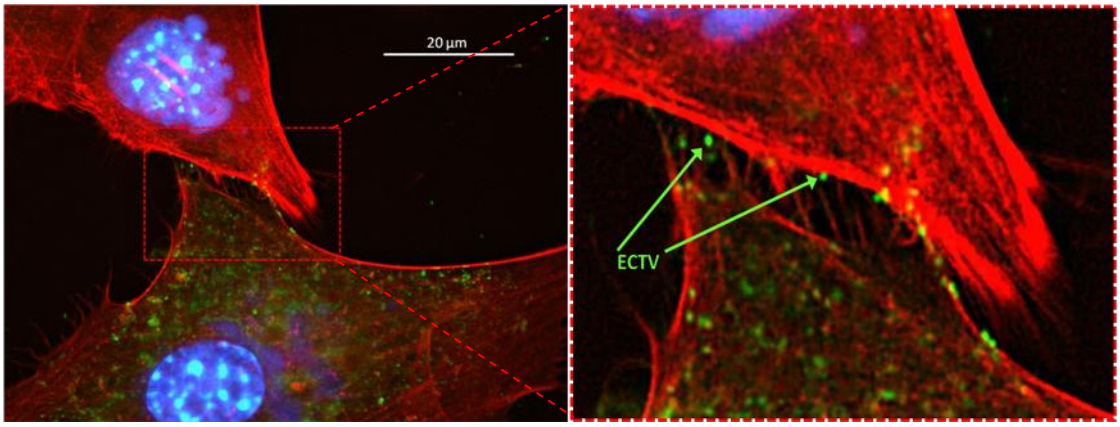


Fig.1 BALB 3T3 cells, 21 h p.i. Fluorescence microscopy. Staining for: actin (red), ECTV-MOS (green), DNA (blue). Actin tails formed by infected cell transfer single ECTV-MOS particles on their tips (indicated by arrows) to uninfected cell. Red dashed box is expanded on the right.

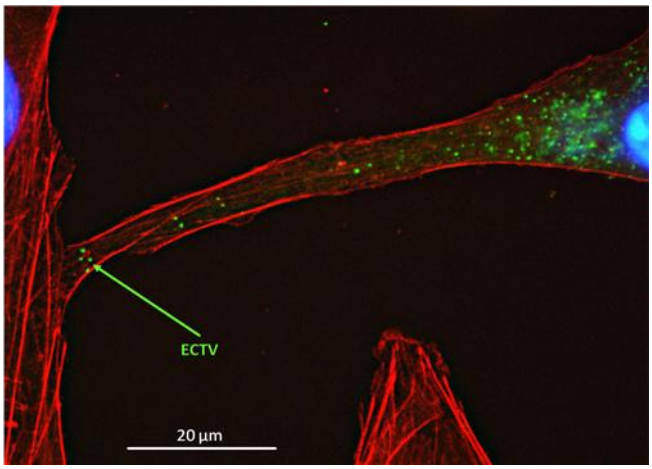


Fig.2. Vero cells, 21 h.p.i. Fluorescence microscopy. Staining for: actin (red), ECTV-MOS (green), DNA (blue). Filopodium produced by infected cell serves as a "corridor" for ECTV-MOS virions (indicated by arrows) to infect neighbouring cell.

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