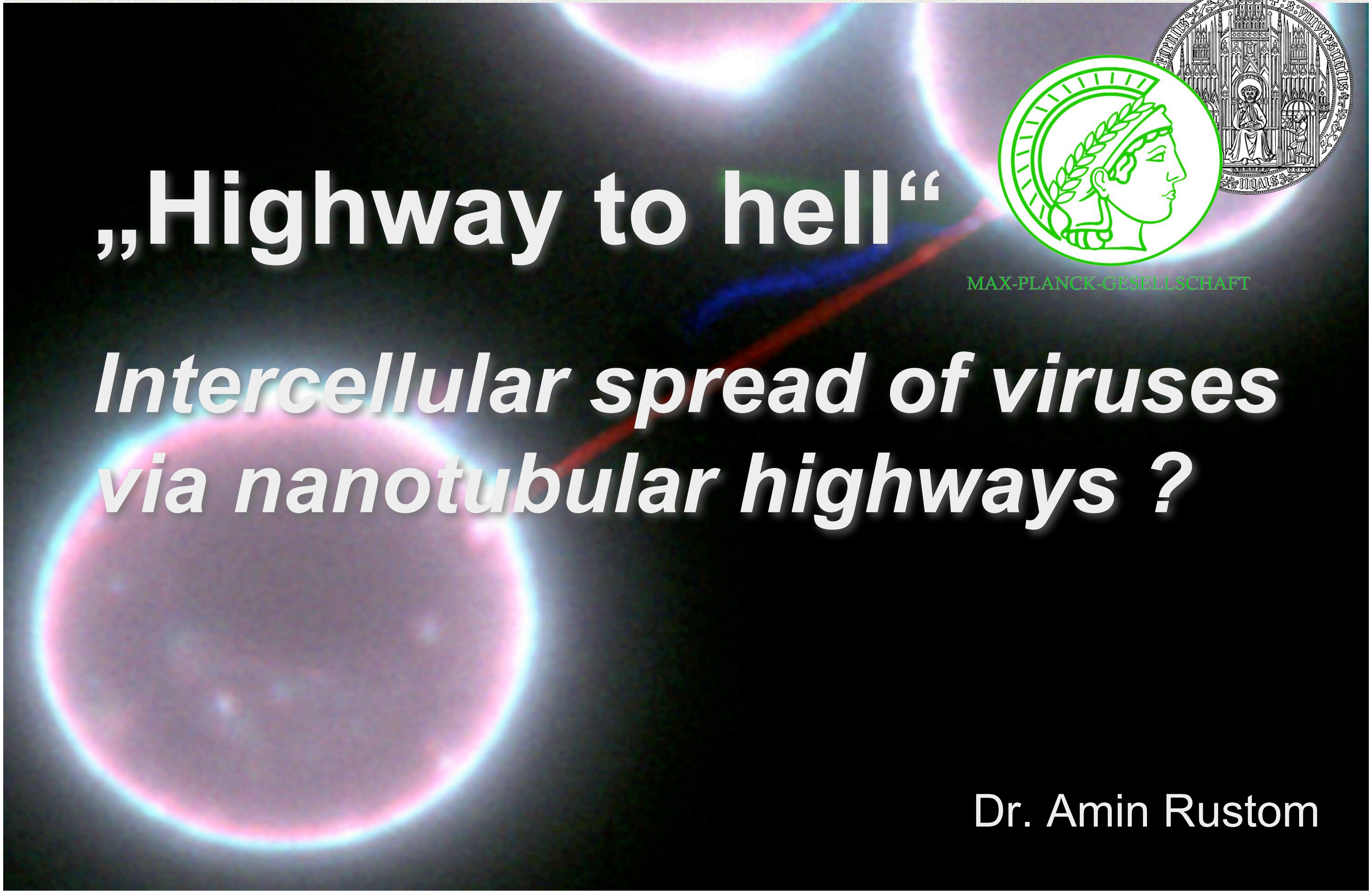
GenConText Research

SYMPOSIUM

Natural Genetic Engineering and Natural Genome Editing
3-6 July 2008. Salzburg-Austria



„Highway to hell“

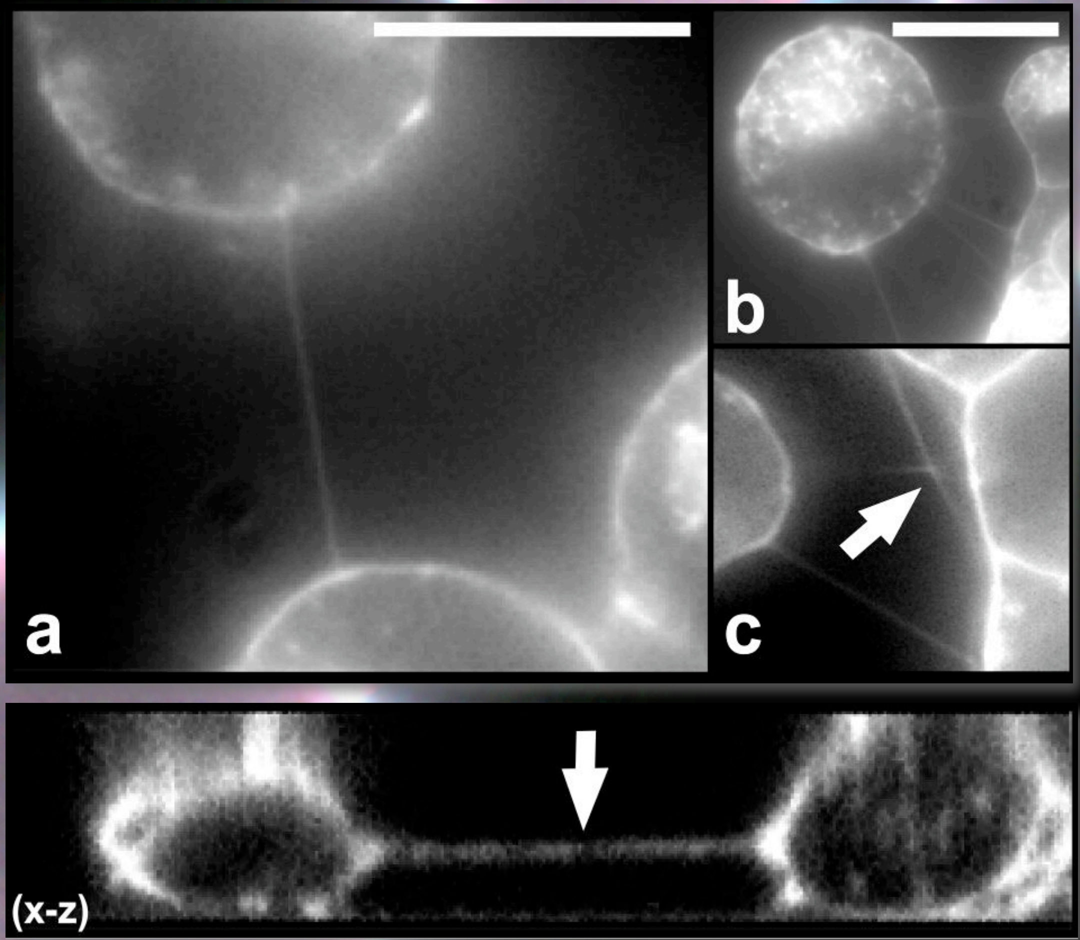
MAX-PLANCK-GESELLSCHAFT

Intercellular spread of viruses via nanotubular highways ?

Dr. Amin Rustom

The discovery of „*tunneling nanotubes*“

A new type of intercellular communication ...

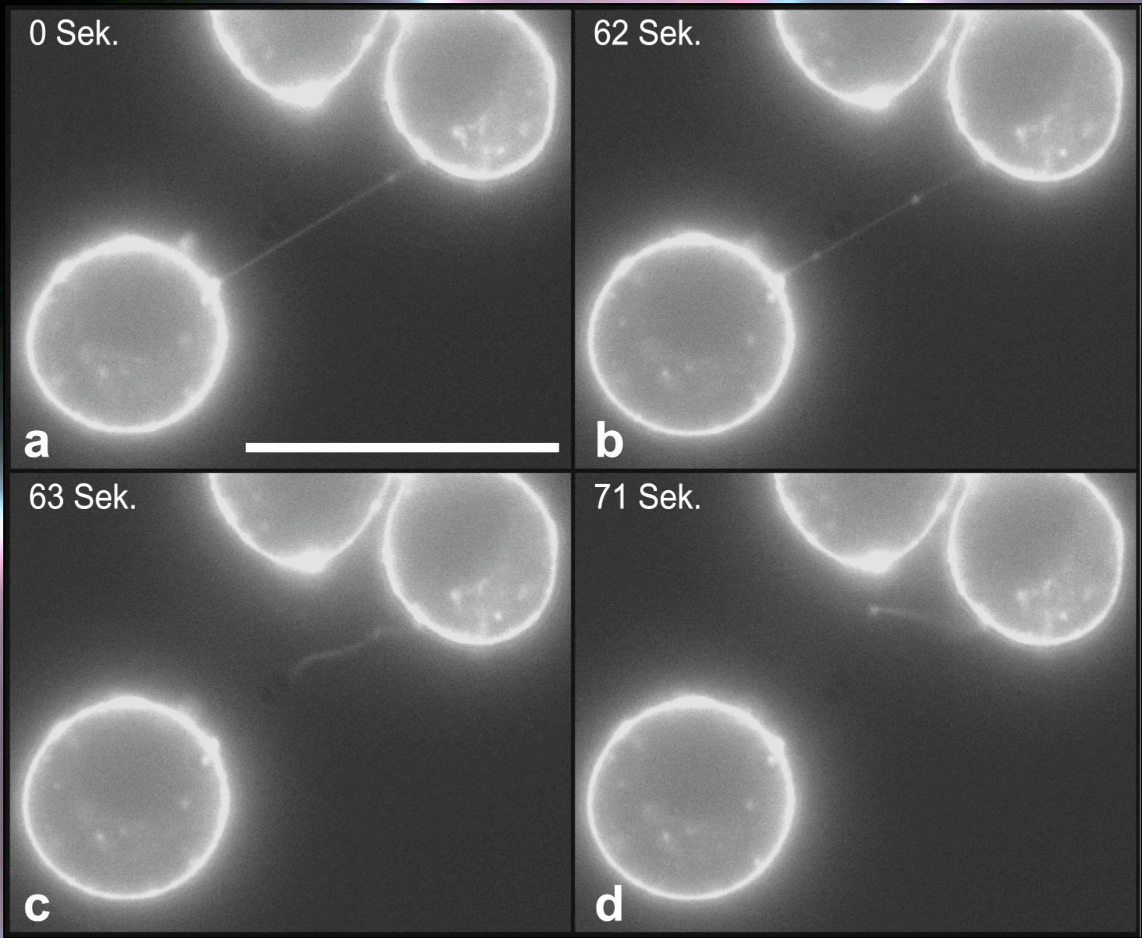


Rustom et al. Science 2004

Cultured rat Pheochromocytoma-Cells (PC12), analysed by
high resolution 3D microscopy - plasmamembrane staining with wheat germ agglutinin

The characterization of TNTs

TNTs represent extremely sensitive structures ...

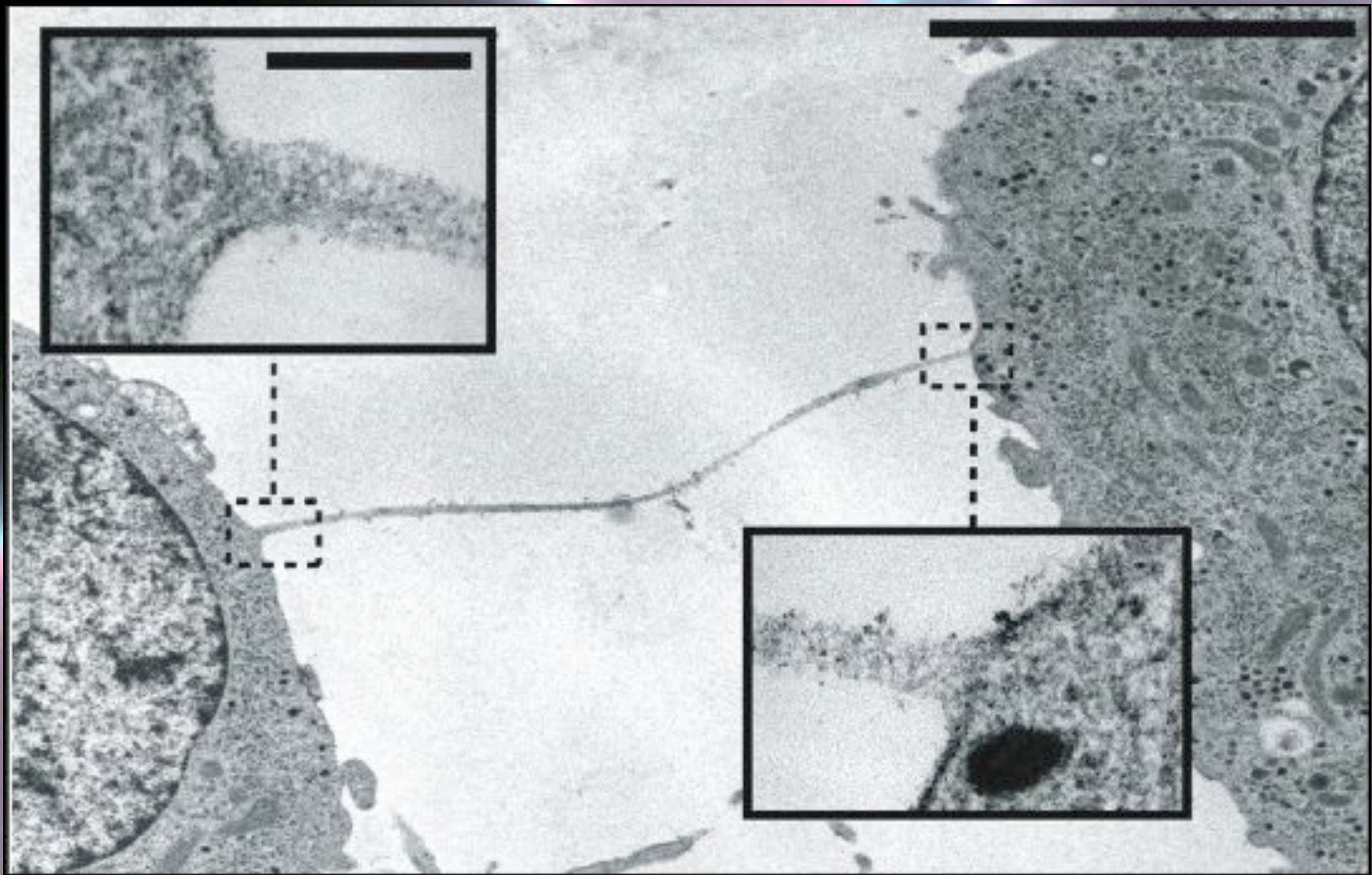


Rustom et al. Science 2004

Cultured rat Pheochromocytoma-Cells, plasmamembrane staining
with wheat germ agglutinin - videomicroscopic analysis with excitation at 565 nm

The characterization of TNTs

TNTs mediate membrane continuity ...

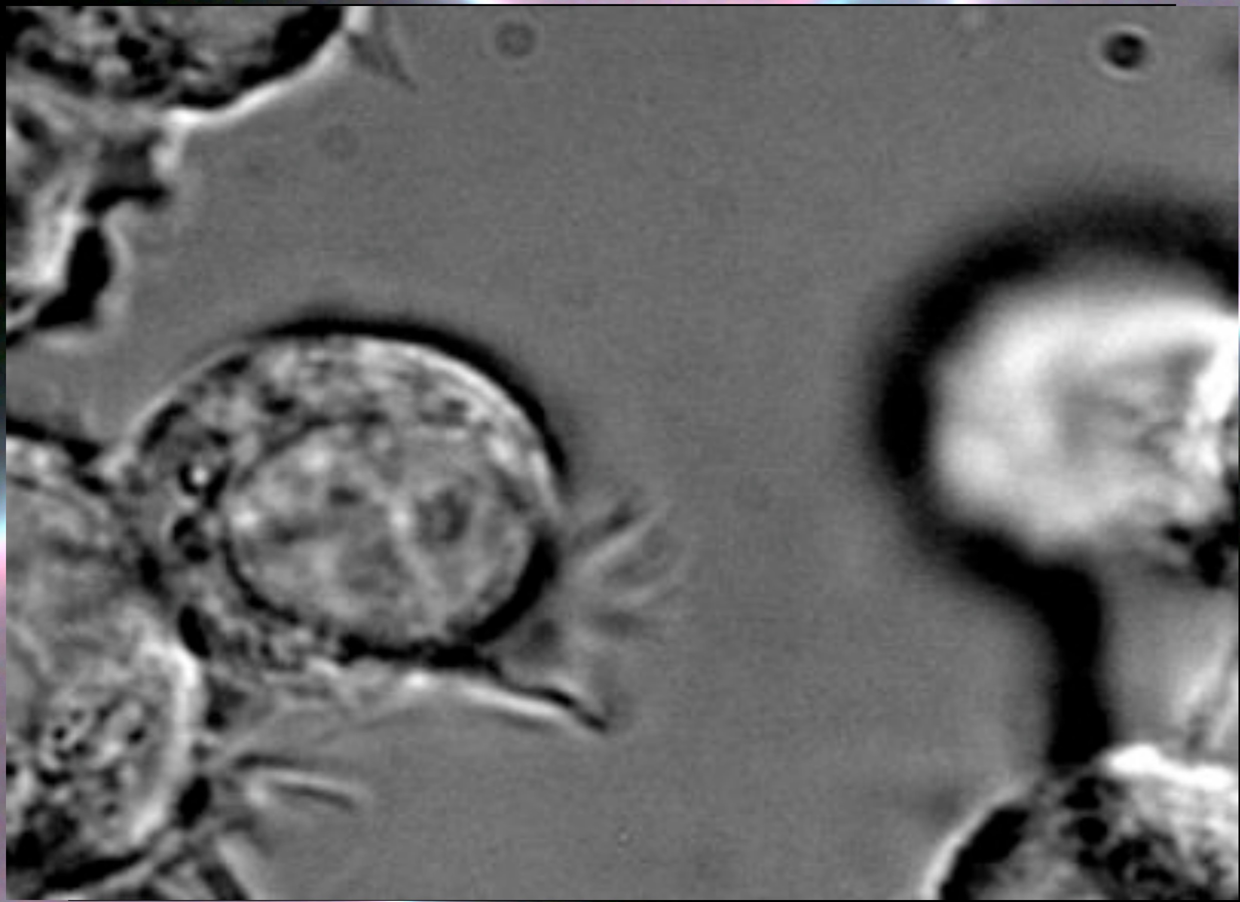


Rustom et al. Science 2004

Cultured rat Pheochromocytoma-Cells (PC12), analysed by transmission electron microscopy

The characterization of TNTs

TNTs can be formed *de novo* ...



Rustom et al. Science 2004

Cultured rat Pheochromocytoma-cells - time laps videomicroscopic analysis

The characterization of TNTs

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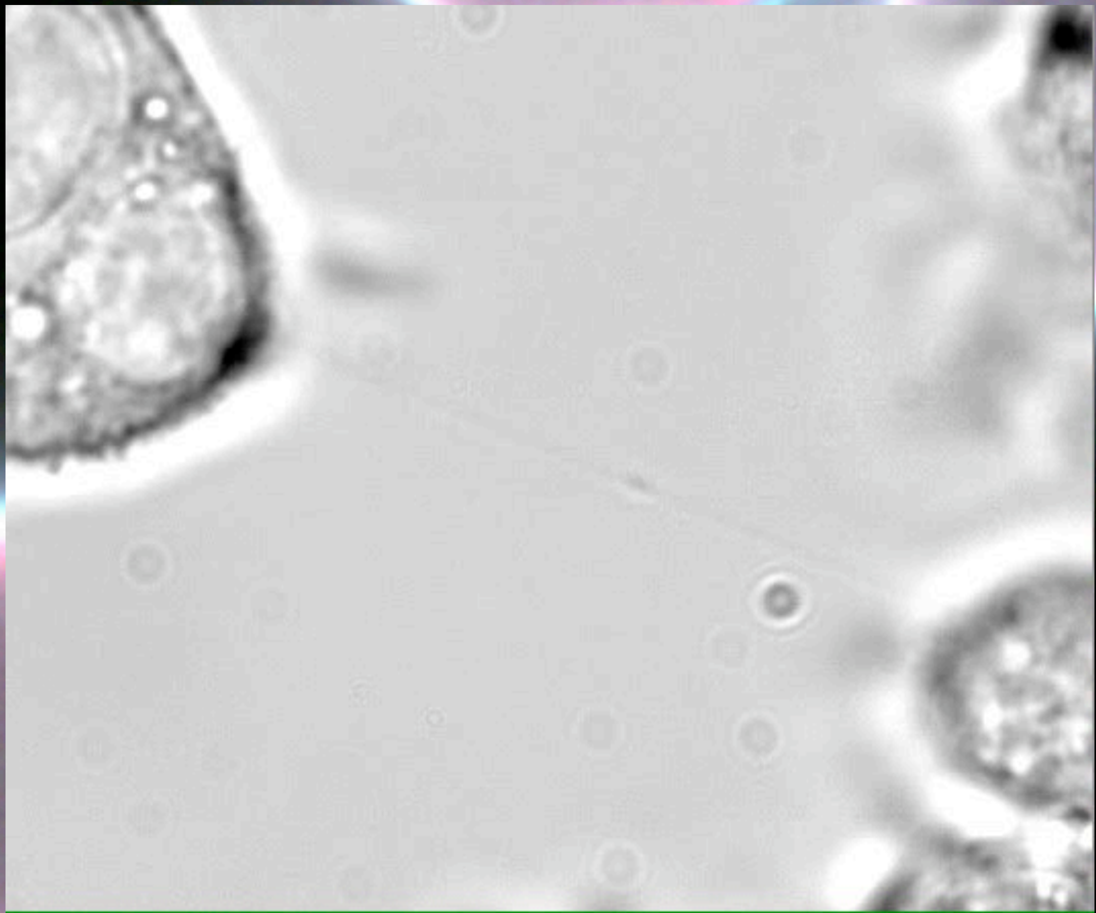

Rustom et al. Science 2004

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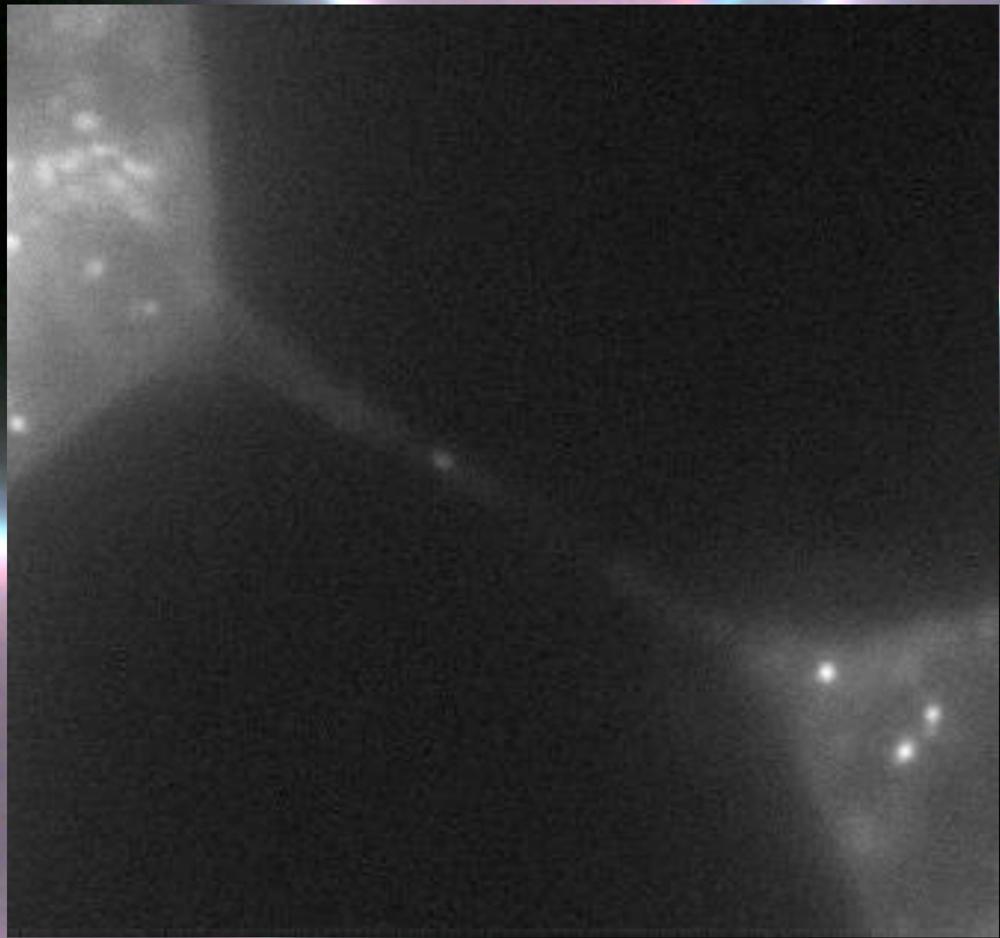
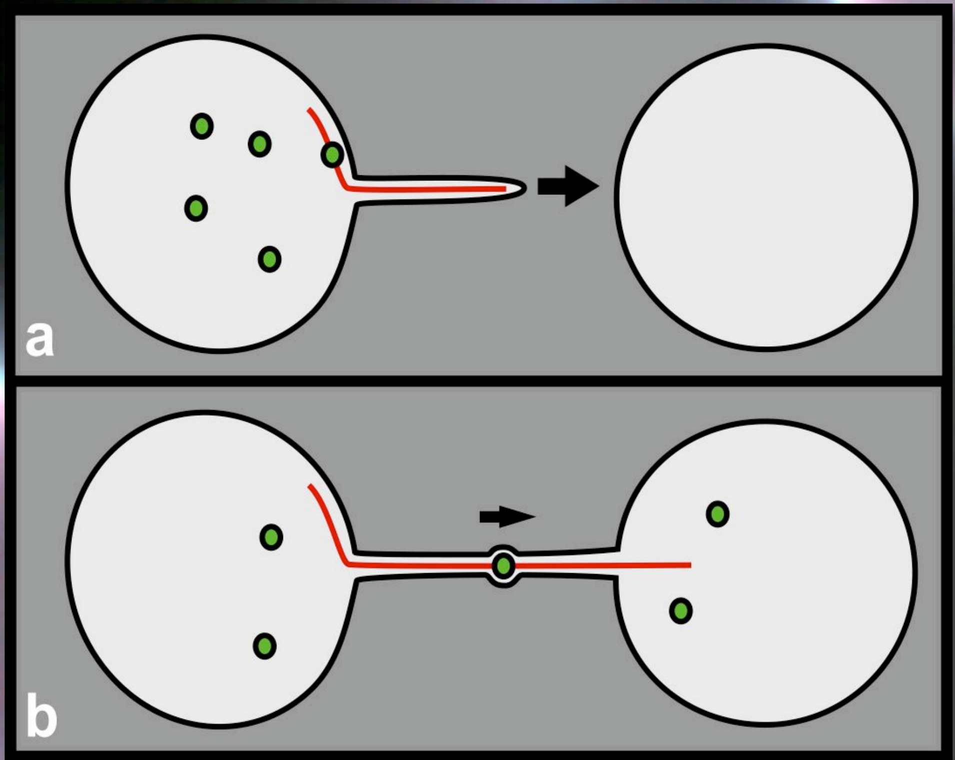


Rustom et al. Science 2004

Cultured rat Pheochromocytoma-cells - time laps videomicroscopic analysis

The TNT model

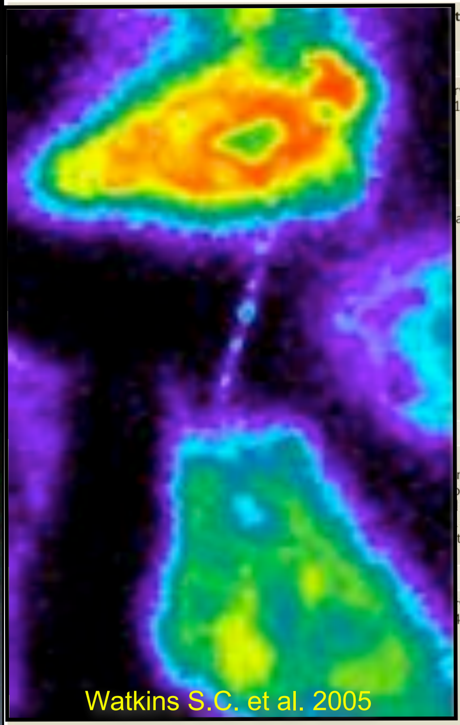
A new principle of intercellular communication ...



Rustom et al. Science 2004

The physiological relevance of nanotubes...

Examples from around 130 citations ...



TNTs and the immune system

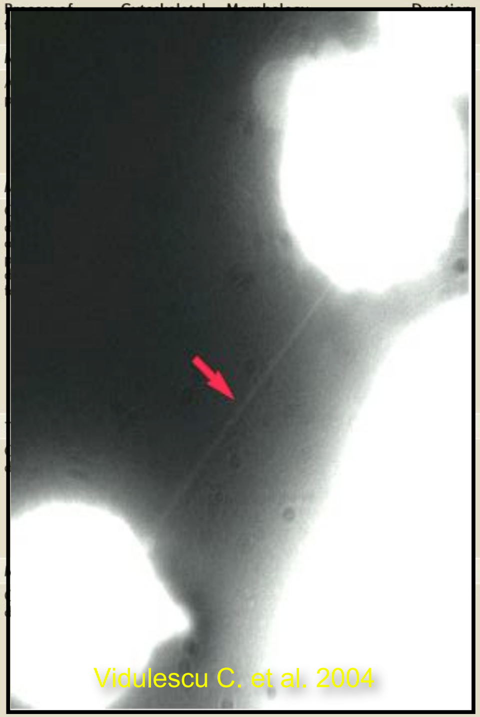
They fulfill important functions at a.o. the Immunological Synapse

e.g. intercellular transfer of MHC molecules or Ca^{2+} signals

				…tion	Membrane continuity	Cargo transfer	Mechanism of cargo transfer	Functional consequences
				…rved …1 h	Yes	Endosome-derived vesicles; membrane-anchored proteins	(~26 ± 8 nm s⁻¹)	ND
				…al	Yes[4]	Bacteria[5]; vesicles[5]; calcium flux[4]; cytoplasmic molecules[4]	ATP-dependent movement along surface (~ 0.1 µm s⁻¹)[5]; ATP- and microtubule-dependent vesicle transfer (~ 1 µm s⁻¹)[5]; calcium flux (~ 35 µm s⁻¹, slowed to 10–15 µm s⁻¹ after 3 s[4])	Can communicate activation … bacteria for phagocytosis[5]
				…ty)	No	Viral proteins	HIV-1 Gag–GFP …	Potential route … HIV-1
				…rved …0	ND	Activating NK-cell receptor 2B4 clusters at nanotube connections and can transfer to target cells[10,65]	ND	Transfer of activating ligands can lead to NK cells killing each other[32] and reduced NK cell cytotoxicity[65]
Cytotoxic T cells and target cells[8]								
Cell-contact dependent	ND	Ø ND (~ 10 µm)	Straight, above the substratum	ND	ND	Possible route for transfer of MHC proteins to T cells	ND	Acquisition of antigen can lead to T cells killing each other
Virally infected fibroblast or epithelial cells (viral cytonemes)[46,66]								
Actin-driven protrusion stabilized by retroviral Env	F-actin[66]	Ø 75–200 nm (~ 6 µm)	Curved, adherent to substratum, makes many connections	> 4 h	No	Viral particles	Viral particles transfer ~ 0.01 µm s⁻¹	Intercellular transmission of retroviruses
Synthetic vesicles and cells[67,68]								
A wide range of micro-manipulation techniques have been used to artificially create membrane tethers from vesicles and cells	No cytoskeleton	Ø Generally 100–300 nm (several hundred µm)	Straight, complex networks easily made, branched, above the substratum	Several hours	Yes	Membrane proteins[68], vesicles or beads	Membrane flow, diffusion, electrophoretic transport[69]	Used to study membrane physics and other applications such as reaction parameters in restricted geometries

The physiological relevance of nanotubes...

Examples from around 130 citations ...



TNTs and cancer biology

They are involved in intercellular communication of different tumor cells

e.g. transfer of tumor-resistence proteins or vesicles

Process of [illegible]	Cytoskeletal [illegible]	Morphology [illegible]	[illegible]	Duration	Membrane continuity	Cargo transfer	Mechanism of cargo transfer	Functional consequences
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Yes	Endosome-derived vesicles; membrane-anchored proteins	Actin-based unidirection[illegible] vesicle trans[illegible] (~26 ± 8 nm s^{-1})	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Yes[4]	Bacteria[5]; vesicles[5]; calcium flux[4]; cytoplasmic molecules[4]	ATP-dependent movement along surface (~ 0.1 µm s^{-1})[5]; ATP- and microtubule-dependent vesicle transfer (~ 1 µm s[illegible]) calcium flux (~ 35 µm s^{-1}, slowed to 10–15 µm s^{-1} after 3 s[4])	Can communicate activation si[illegible] calcium [illegible] spread[4]; captur[illegible] bacteria for phagocytosis[5]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	No	Viral proteins	HIV-1 Gag–GFP [illegible] 0.0[illegible] ± 0.03 µm s[illegible]	Potential rout[illegible] [illegible] transmission of HIV-1
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ND	Activating NK-cell receptor 2B4 clusters at nanotube connections and can transfer to target cells[10,65]	ND	Transfer of activating ligands can lead to NK cells killing each other[32] and reduced NK cell cytotoxicity[65]
Cytotoxic T cells and target cells[8]								
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The physiological relevance of nanotubes...

Examples from around 130 citations ...

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				d	Yes	Endosome-derived vesicles; membrane-anchored proteins	[illegible] (~26 ± 8 nm s^{-1})	ND
					Yes[4]	Bacteria[5]; vesicles[5]; calcium flux[4]; cytoplasmic molecules[4]	ATP-dependent movement along surface (~0.1 µm s^{-1})[5]; ATP- and microtubule-dependent [illegible] calcium flux (~35 µm s^{-1}, slowed to 10–15 µm s^{-1} after 3 s[4])	Can communicate activation signals by calcium flux spread[4]; capture bacteria for [illegible]
					No	Viral proteins	HIV-1 Gag–GFP (~0.08 ± 0.03 µm s^{-1})	Potential route for intercellular transmission of HIV-1
				d	ND	Activating NK-cell receptor 2B4 clusters at nanotube connections and can transfer to target cells[10,65]	ND	Transfer of activating ligands can lead to NK cells killing each other[32] and reduced NK cell cytotoxicity[65]
Cytotoxic T cells and target cells[8]								
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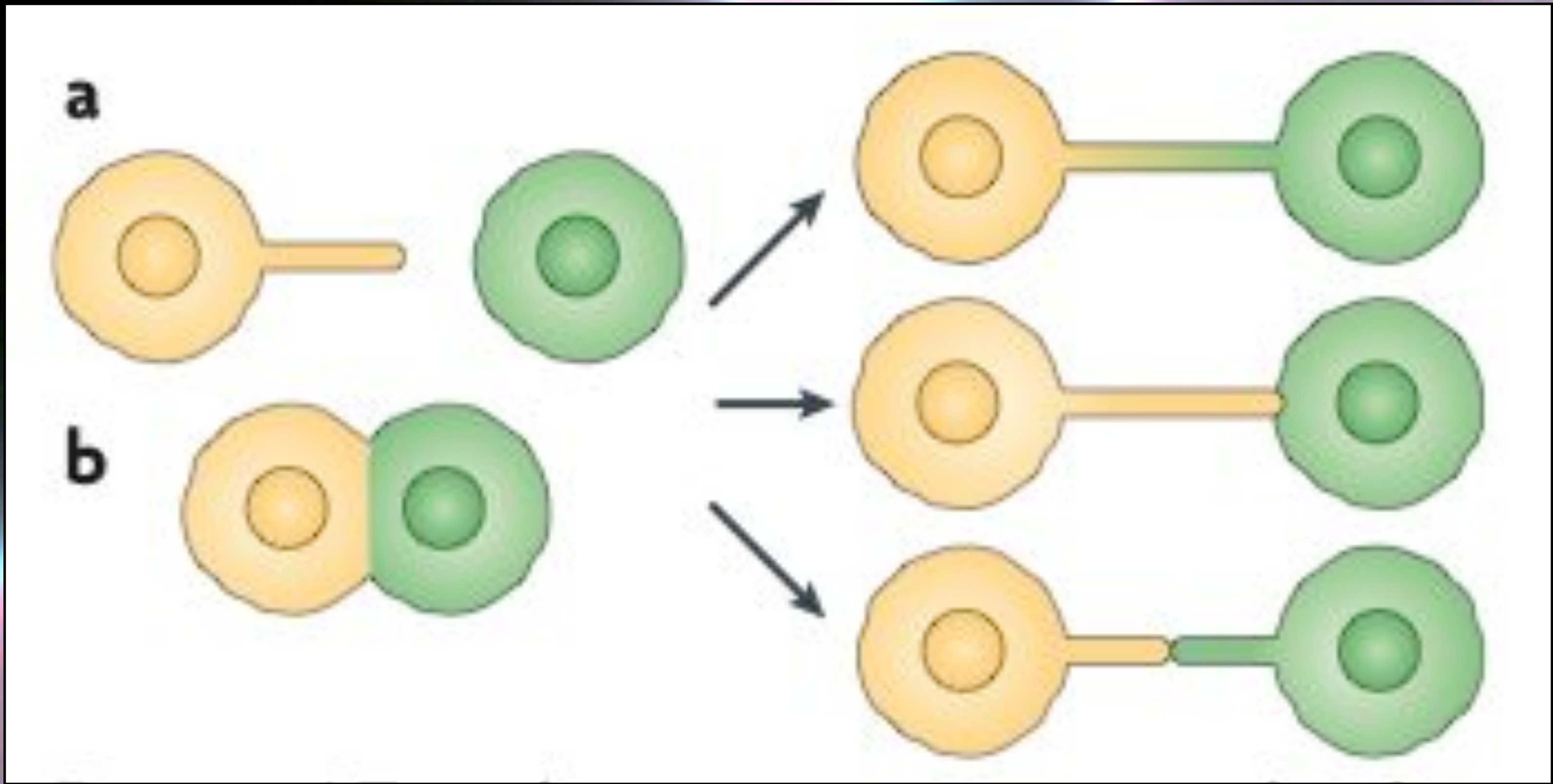
TNTs in developmental biology and stem cell differentiation

They can fulfill important functions during a.o. differentiation of stem cells

e.g. speculations about RNA transfer

The current „nanotube“ model

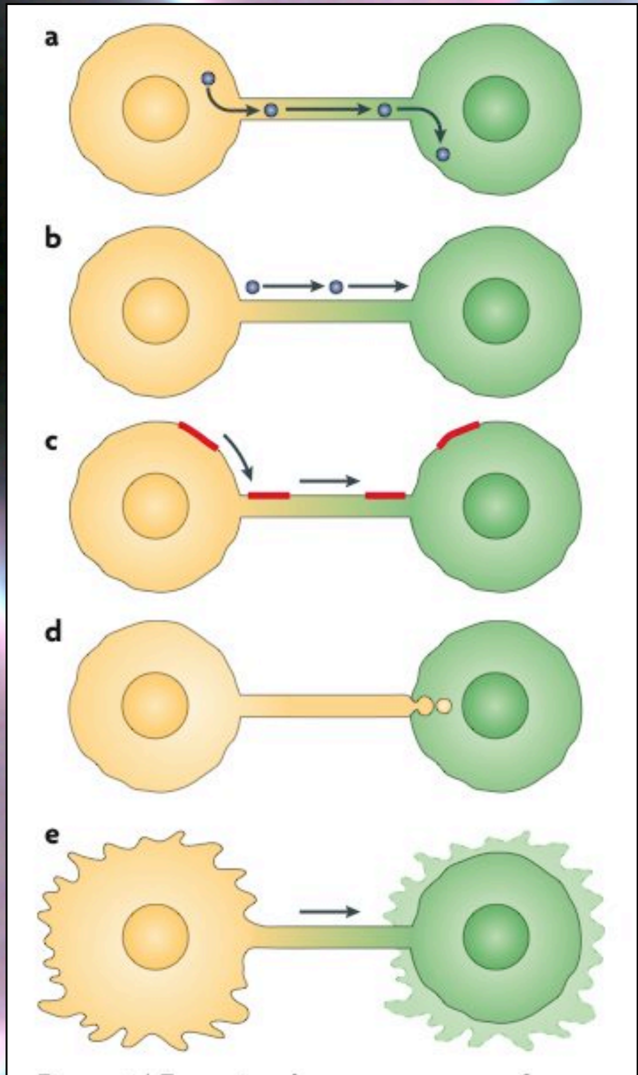
Formation ...



Daniel M. Davis et al. Nature Reviews 2008

The current nanotube model

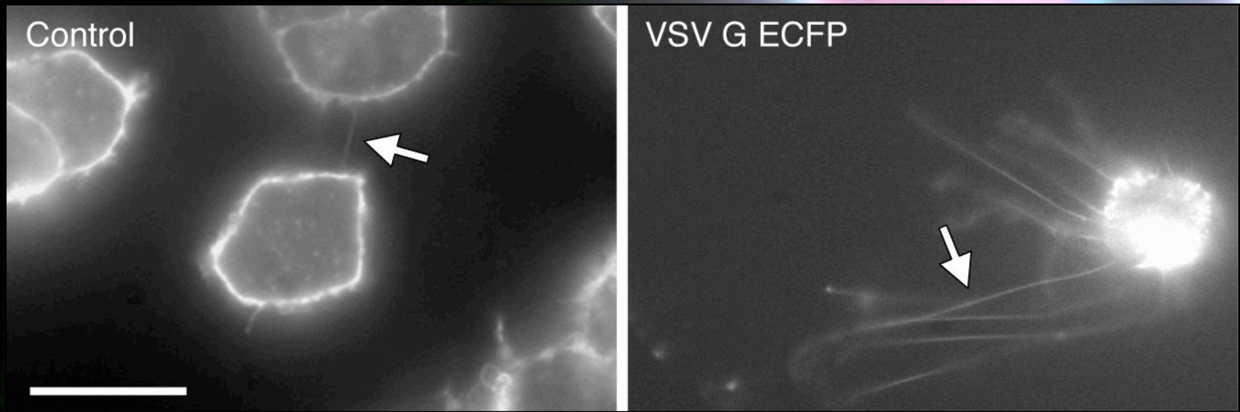
Function ...



Daniel M. Davis et al. Nature Reviews 2008

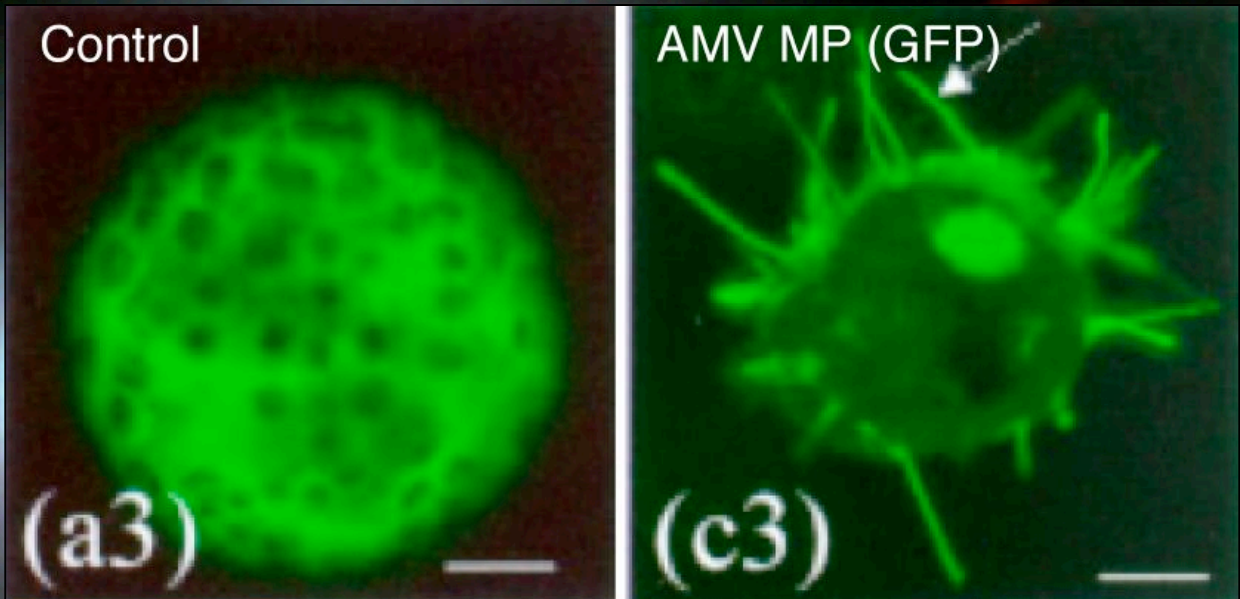
Reactions of different cell-systems

Surprising similarities after the expression of viral proteins ...



„animal“

Expression
of
Viral protein



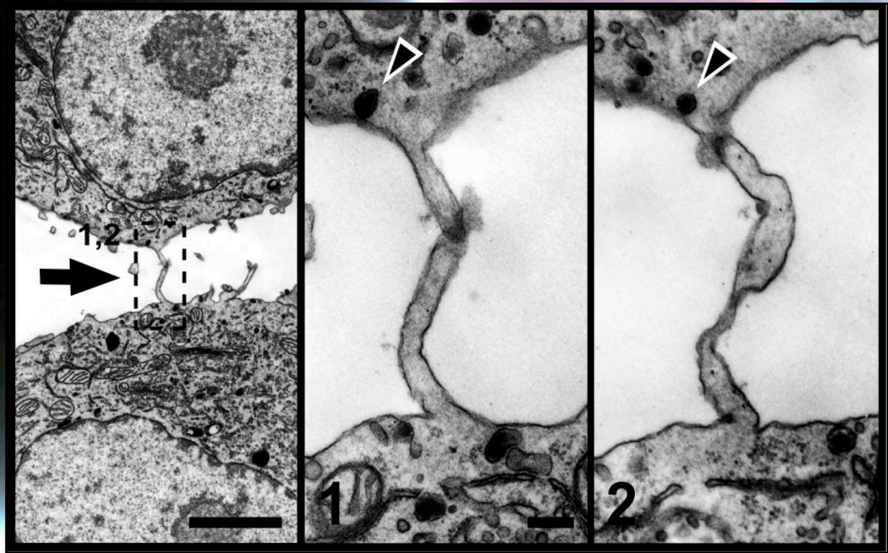
„plant“

Expression
of Alfalfa Mosaik
Virus Movement
Protein (AMV MP)

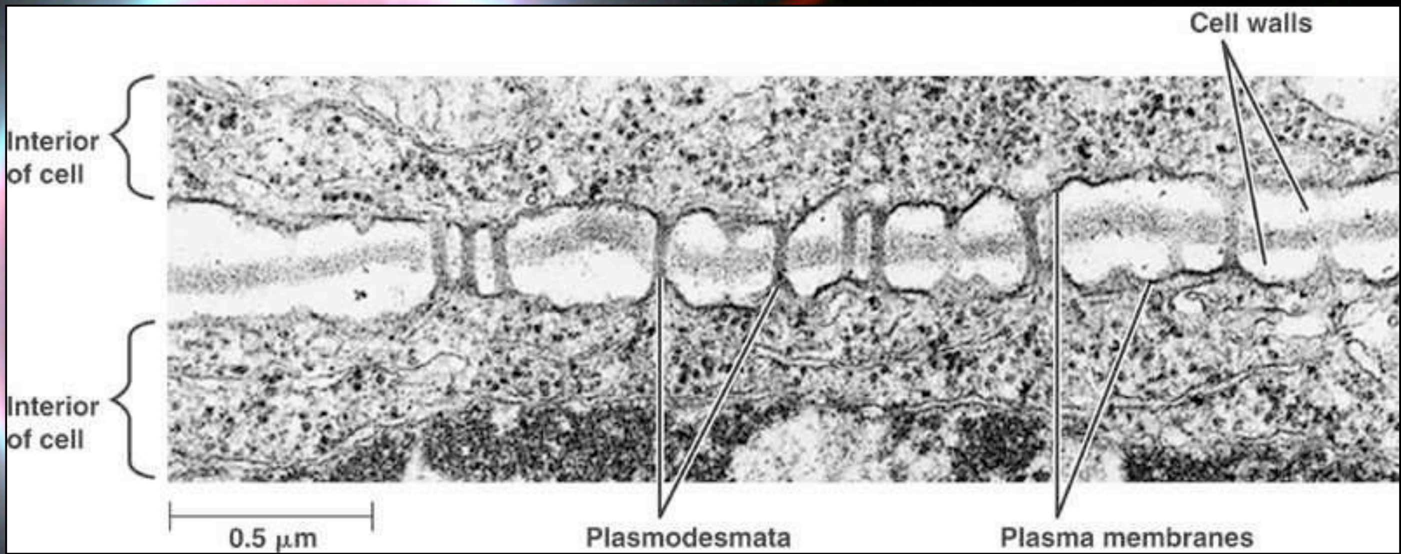
Jesús A. Sánchez-Navarro et al. Molecular Plant-Microbe Interactions; 14(9):1051-1062) 2001

Comparison between TNTs and Plasmodesmata

Striking similarities ...

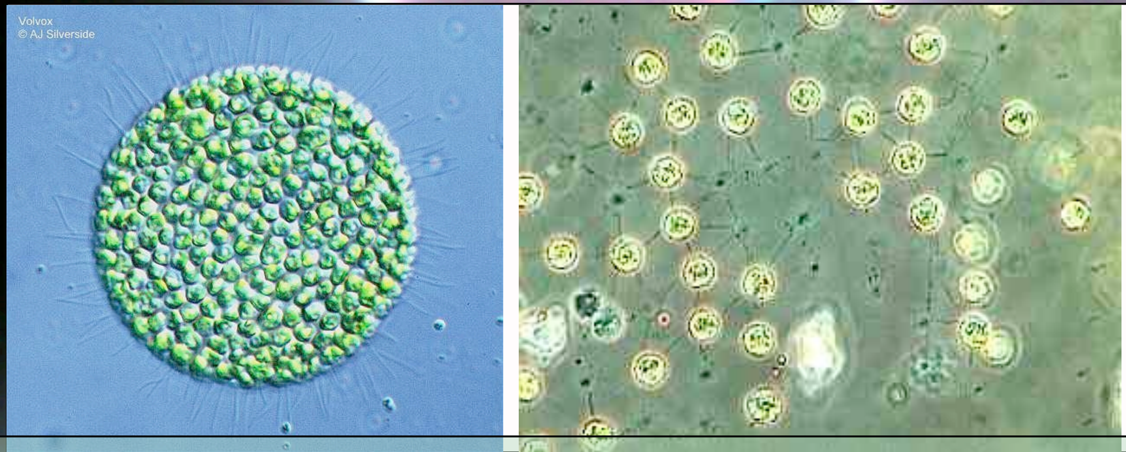


„animal"

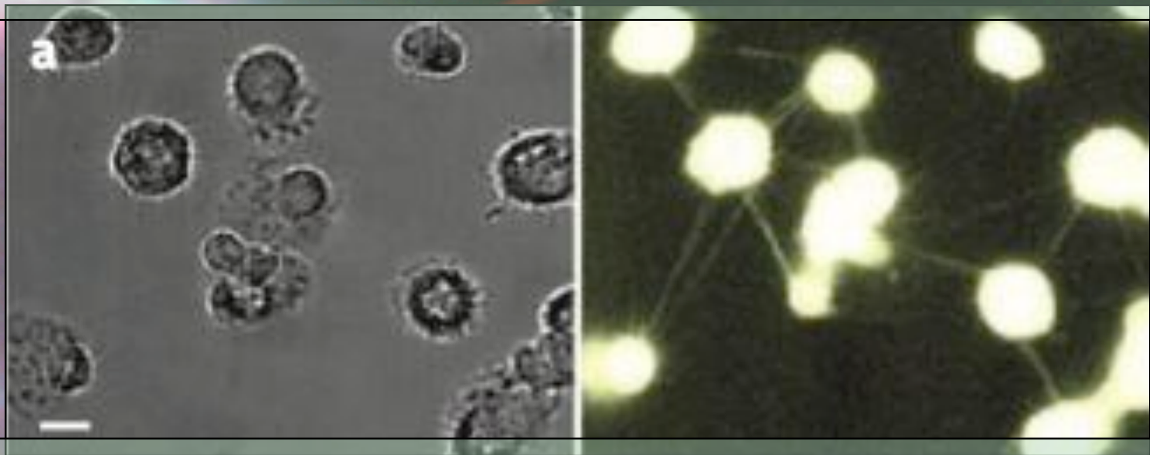


„plant"

New aspects for the evolution of multicellular organisms ?

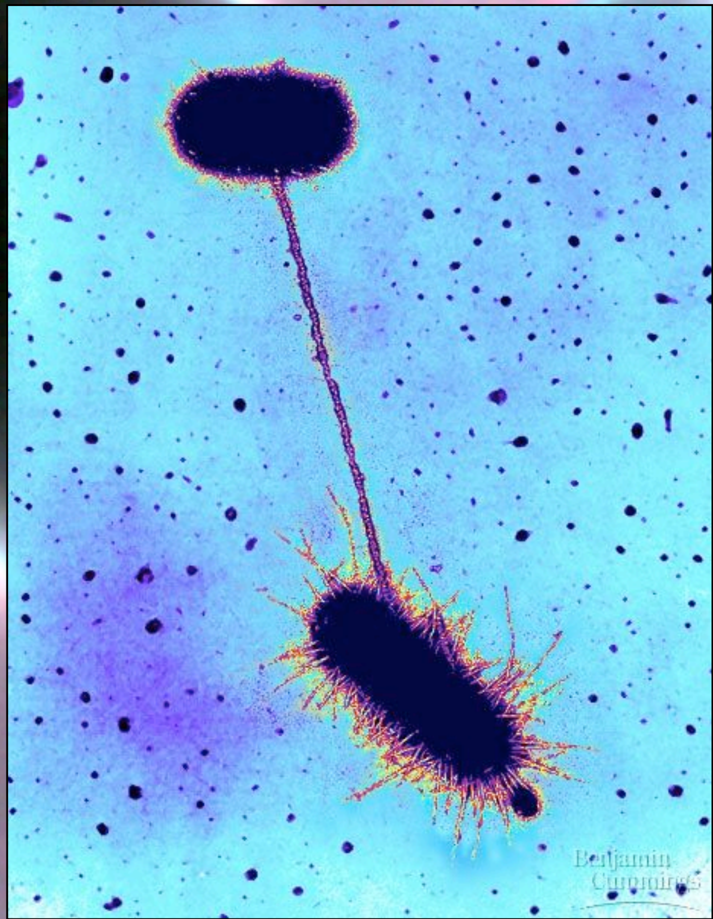


Volvox
A colony of green algae and model system for the development of multicellular organisms ...



Human monocyte-derived macrophages
Önfeld et al. J. Immunologie 2006

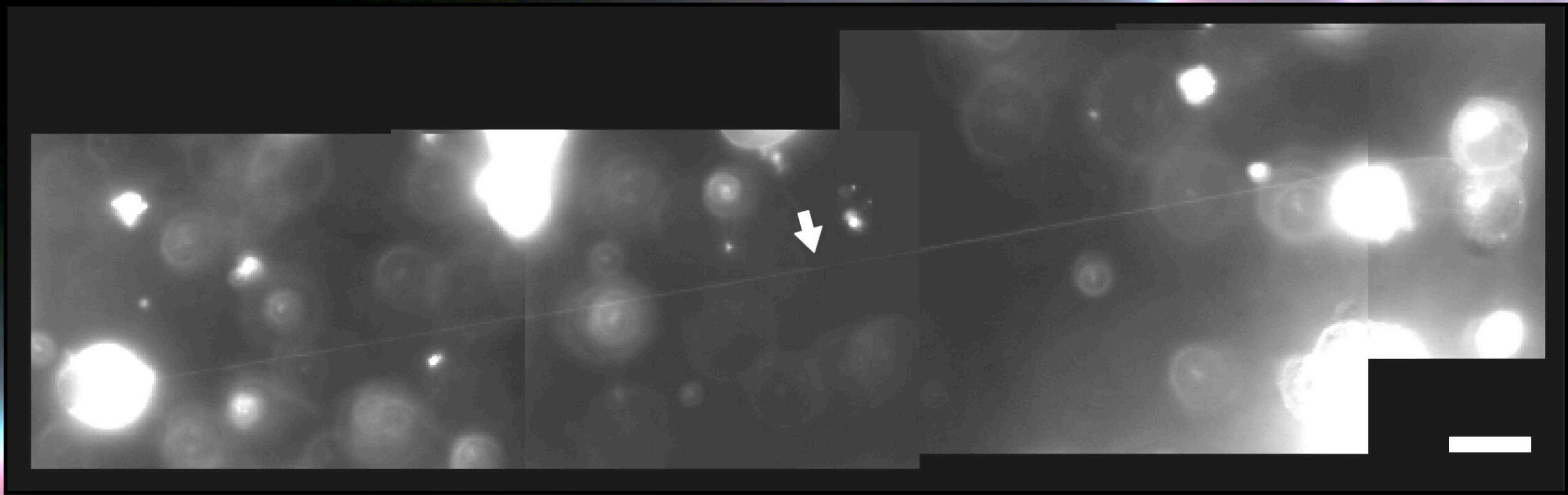
A conserved mechanism throughout evolution ?



Bacterial conjugation

Transfer of genetic information along cytoplasmic bridges ...

Nanotubes: Far from beeing understood...



Cultured rat Pheochromocytoma-Cells
(„any disturbance avoided“)

Acknowledgements

Collaborations and Interactions

- Professor Dr. Hans Hermann Gerdes (Universität Bergen, Norwegen)
- Professor Dr. Hilmar Bading (Institut für Neurobiologie, Heidelberg)
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- Professor Volker Storch (Zoologie, Universität Heidelberg)
- Dr. Momburg (DKFZ, Heidelberg)

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- Marcus Abel

Thanks for your attention!