MICROSCOPY UNIT

Head: Jan Malínský, PhD
E-mail: malinsky@biomed.cas.cz
Phone: +420 241 062 597

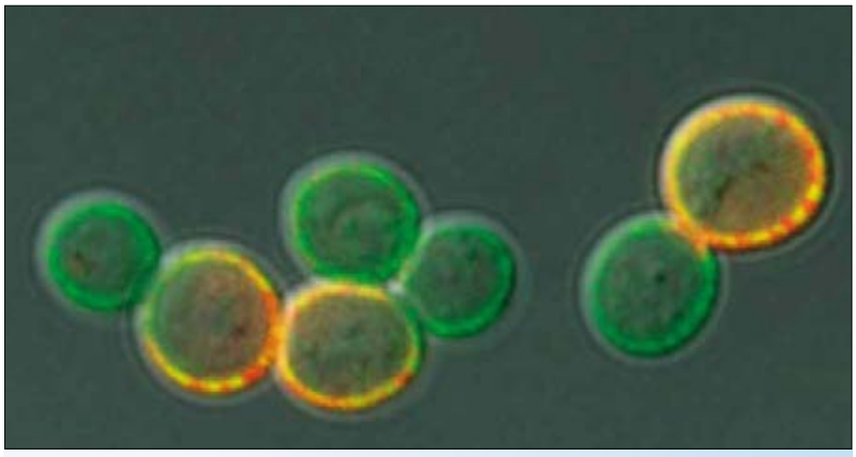
Jan Malínský, PhD | Research Scientist
Michaela Blažíková, MSc | PhD Student
Vendula Strádalová, MSc | PhD Student
Tomáš Červinka, Bc | Technician
Lenka Hlavínová | Technician
Petra Veselá, Bc | Technician
Jitka Eisensteinová | Secretary

RESEARCH TOPICS

We study the assembly and maintenance of cellular structures not bound by a membrane. These highly dynamic, steady state organelles engaged in specific functions communicate with their surroundings directly by diffusion, and the mechanisms controlling their structure and function are still poorly understood. Many of these compartments, such as the nucleolus or Cajal body in the cell nucleus, are easily seen under a microscope and have been known for a long time. Others have been described only recently – for instance lipid raft-based compartments in membranes. Morphological changes or the disintegration of these cellular structures often accompany pathologic phenotypes. Knowledge about the composition, formation and maintenance of these structures is limited. Thus, a great deal is open for studies using contemporary microscopy techniques, capable not only of exactly localizing cellular components, but also of detecting their movement and potential interactions at the molecular level as well.

Zeiss LSM 5 DUO confocal microscope.
We are well equipped for a variety of fluorescence and electron microscopy approaches. Besides our own research, methodological support for other groups is provided by the Unit staff.

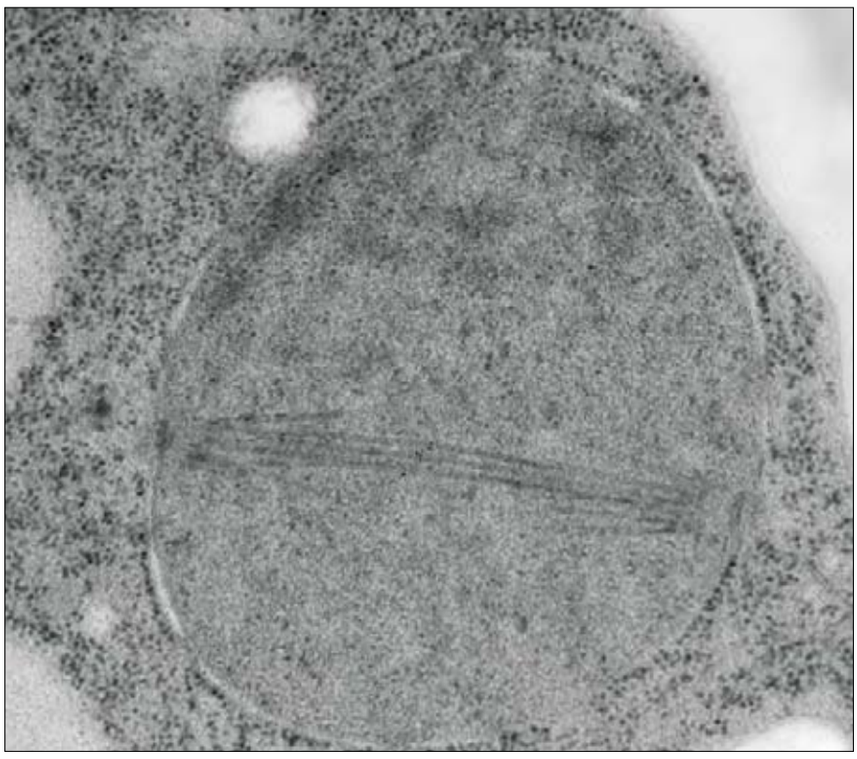
We were able to distinguish distinct domains in the plasma membrane of the yeast S. cerevisiae, the Membrane Compartments MCC (containing can1) and MCP (with Pma1). These domains are large enough to be resolved by fluorescence microscopy (Malinska et al., MBC14: 4427-36, 2003). We showed that MCC consists of stable, uniform, isolated patches within the plasma membrane. To date, twenty integral and soluble proteins have been shown to co-localize with the MCC pattern. The composition of the MCC patches depends on the membrane potential. We have hypothesized a specific role for them in protein turnover. Using various approaches of electron microscopy as well



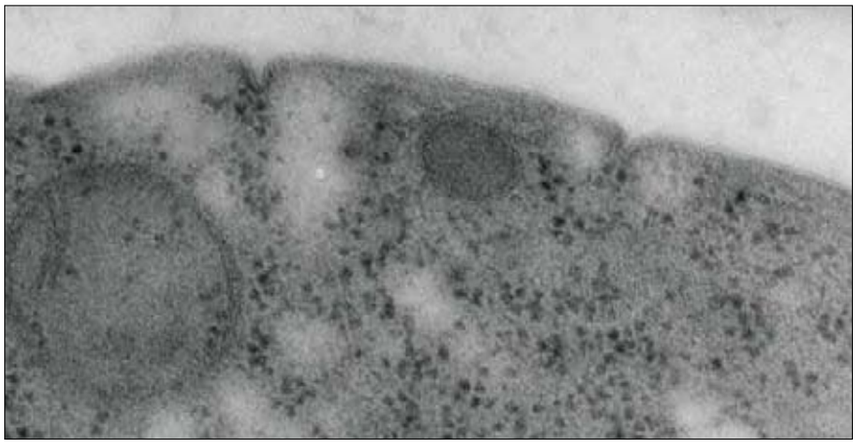
Plasma membrane domains in S. cerevisiae.
Transversal confocal section of living cells co-expressing the plasma membrane proton ATP-ase Pma1p-ds (red) and the proton-arginine symporter Can1-GFP (green) combined with a DIC image (grayscale). Pma1 and Can1 are laterally segregated within the plasma membrane; Can1 is confined to isolated patches, while Pma1 fills the surrounding area. Note the absence of a red signal in the growing buds caused by the slow maturation of the dsRed molecule.

as goal-directed mutant yeast strains, we are now attempting to describe the fine structure of MCC and to localize its protein components with a resolution of 20 nm. The specific lipid composition of MCC reported in fluorescence microscopy studies is indicative of the specific ultrastructure of this organelle.

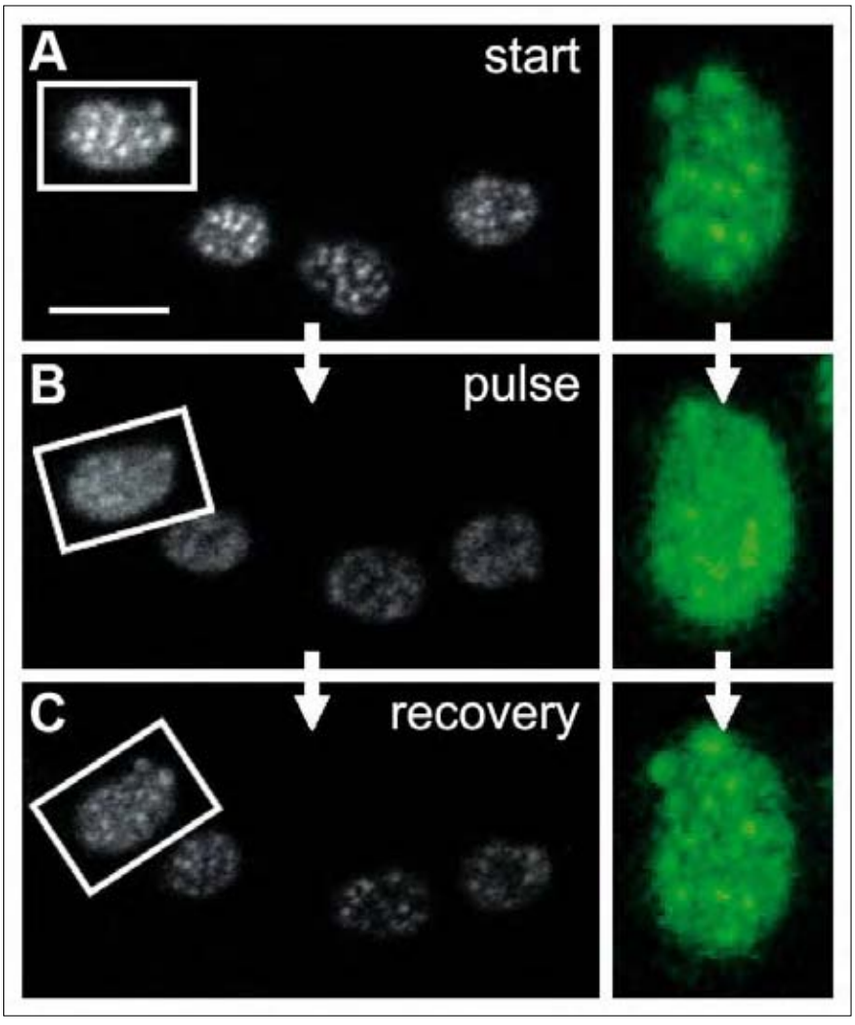
We also focus on the formation, distribution and dynamics of structures coupled to chromatin function – DNA replication sites, nucleoli etc. Using fluorescently-labeled components of the replication machinery, we visualize the dynamics of DNA replication. We also focus on the formation, distribution and dynamics of structures coupled to chromatin function – DNA replication sites, nucleoli etc. Using fluorescently-labeled components of the replication machinery, we visualize the dynamics of DNA replication. By kinetic analysis of the continuous exchange of small nuclear ribonucleoprotein particles between the nucleoplasm and Cajal bodies, we contribute to the understanding of mRNA splicing. Our aim is to formulate mathematical models describing the behavior of various nuclear compart-ments in general.



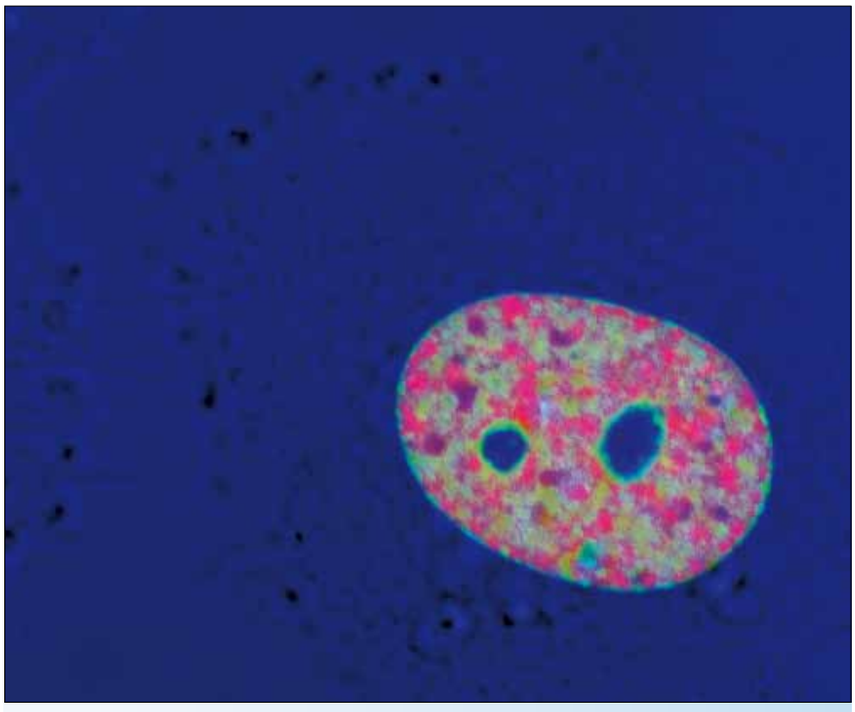
Yeast cell nucleus during mitosis.
During endomitosis, the nuclear envelope remains intact. Within the nucleus, the mitotic spindle with spindle pole bodies immersed in the nuclear envelope can be clearly recognized. A living yeast cell was frozen under high pressure, embedded into a synthetic resin and sectioned. High contrast of protein-rich structures (ribosomes, microtubuli) was achieved by the uranylacetate stain.



Curved membranes in yeast.
Various biological membranes, including the invaginated plasma membrane and the outer and inner membranes of mitochondria, were visualized on an ultrathin section of a yeast cell. The cytoplasm is filled with dark ribosomes and lucent round-shaped aggregates of glycogen.



Transient re-distribution of plasma membrane components upon membrane depolarization.
Some proteins are able to leave the membrane compartment of Can1 in a reversible manner after membrane depolarization. If heterologously expressed in S. cerevisiae, the glucose transporter HUP1 of C. kessleri is one of these proteins. A tangential section of living cells in three consecutive snapshots is presented. A pulse of an external electric field was applied prior to (B), while a 20 minute recovery preceded (C). Note that the patches in (C) are re-forming in their original positions (if compared to A).



Localization of replicating chromatin.
The DNA replication marker PCNA-mRFP (red) was co-localized with the histone H3. A confocal section of a mid-to-late S-phase cell, combined with a DIC image (blue), is shown. Note the spatial separation of the condensed chromatin and the active process of DNA replication.

CURRENT GRANT SUPPORT

GA CR, 204/07/0133, Self organization principles of nonmembrane-bound organels in eukaryotic cells.

GA CR, 204/08 J024), Lateral membrane compartments: Formation, functional relevance and genomics.

GA AS CR, KAN200520801, Targeted expression and transport of bioactive molecules.

SELECTED RECENT PUBLICATIONS

1. Mašata M, Malínský J, Fidlerová H, Smirnov E, Raška I. (2005) Dynamics of replication foci in early S phase as visualized by cross-correlation function. J Struct Biol 151(1): 61–68.

2. Smirnov E, Kalmarova M, Koberna K, Zemanová Z, Malínský J, Mašata M, Cvačková Z, Michalová K, Raška I. (2006) NORs and Their Transcription Competence during the Cell Cycle. Folia Biol. -Prague 52: 59–70.

3. Grossmann G, Opekarová M, Malínský J, Weig-Meckl I, Tanner W. (2007) Membrane potential governs lateral segregation of plasma membrane proteins and lipids in yeast. EMBO J 26(1): 1–8.

4. Staněk D, Přidalová J, Novotný I, Huranová M, Blažíková M, Wen X, Sapra AK, Neugebauer KM. (2008) Spliceosomal snRNPs repeatedly cycle through Cajal bodies. Mol Biol Cell 19(6): 2534–2543.

5. Střádalová V, Gaplovská-Kyselá K, Hozák P. (2008) Ultrastructural and nuclear antigen preservation after high-pressure freezing/freeze-substitution and low-temperature LR White embedding of HeLa cells. Histochem Cell Biol 130(5): 1047–1052.

6. Grossmann G, Malínský J, Loibl M, Stahlschmidt W, Weig-Meckl I, Frommer WB, Opekarová M, Tanner W. (2008) Plasma Membrane Microdomains Regulate Turnover of Transport Proteins in Yeast. J Cell Biol 183(6): 1075–1088.