ABSTRACT

Plasma membrane (PM) of living cells hosts variety of important cellular functions that must be precisely coordinated in space and time. Recent research shows that the plasma membrane is organized into specific domains to accomplish all these tasks. Our laboratory is focused on the organization of the plasma membrane in *Saccharomyces cerevisiae* where several distinct lateral compartments were identified at the fluorescence microscopy level. One of them is the Membrane Compartment occupied by arginine transporter Can1 (MCC) which consists of isolated, highly stable, ergosterol enriched, 300nm patches containing specific proton symporters and proteins of unknown function (Sur7- and Nce102-like). These membrane domains are organized by cytosolic protein complexes called eisosomes, composed mainly of proteins Pil1 and Lsp1.

This work is a continuation of studies that tried to elucidate the composition, structure and function of MCC. In the first section of this work we concentrated on ultrastructural characterization of MCC domains. Foremost, we developed a protocol preserving the plasma membrane ultrastructure. The comparison of cryofixed and chemically crosslinked cells clearly showed that cryofixation by high pressure freezing together with freeze substitution and low temperature resin embedding leads to superior sample structure preservation and significantly increased labeling density as compared to conventional aldehyde fixation. We took advantage of these findings in the main part of the thesis that proved that MCC domains correspond to furrow-like plasma membrane invaginations, reported by freeze fracture studies in early sixties. We showed that the plasma membranes of *nce102Δ* and *pil1Δ* strains, defective in segregation of MCC-specific proteins, lacked the characteristic furrow-like invaginations. Conversely, mutants exhibiting elongated MCC patches in confocal microscope possessed accordingly elongated invaginations under electron microscope. And last but not least, the immunolocalization of Sur7-GFP and Pil1-GFP proteins on ultrathin resin sections confirmed the localization of both markers to furrow-like invaginations.

We showed that Nce102p is an important MCC constituent. Therefore, we next focused on the elucidation of its role in MCC formation. Determination of the Nce102p membrane topology suggested that this protein does not span the membrane four times as predicted but that it rather adopts a hairpin conformation which could contribute to furrow formation. We also demonstrated that close and distant Nce102 homologs can substitute this protein in tethering Can1p to MCC and concluded that the function of Nce102-like proteins was conserved in *Ascomycota*. Our data suggested that the C-terminus is necessary for the Nce102p function.

The last part of this work was stimulated by our observations that MCC does not colocalize with the cortical ER network occupying a substantial part of the plasma membrane in yeast. We showed that the deletion of the main MCC organizer, Pil1p, leads to an aberrant redistribution of cortical ER network. As we also found that cortical ER redirects the vesicular transport into distinct, ER-free, plasma membrane areas it is obvious that: 1) cortical ER contributes to a functional compartmentalization of the yeast plasma membrane and 2) MCC is also involved in this functional partitioning of the plasma membrane.

In summary, this work has brought new crucial details on the organization of the plasma membrane in budding yeast, with apparent general consequences for other organisms. We ascribed a specific structure to MCC domain which helped to clarify its unusual stability and led to a discovery of eisosome structure. The analysis of Nce102p then suggested how this protein directly affects MCC formation and shaping. And the investigation of cortical ER network showed that its distribution beneath PM is regulated by MCC presence and that this organelle is involved in positioning of various dynamic processes in PM.