Abstract

The SUN family of proteins (Uth1p, Sun4p, Sim1p, Nca3p) is a group of fungal proteins similar to cell wall glucanases and highly homologous in their 256 long C-terminal amino acid domain. Our previous studies on yeast colony development revealed that members of the SUN family of proteins may be involved in the aging process and may play important role for survival during the development and grow of multicellular yeast populations. Our lab implemented a microarray analysis of expression changes in Saccharomyces cerevisiae colonies, which showed significant changes in the expression level of the SUN family member - UTH1. In addition, a strain with a disrupted UTH1 gene displayed a poorer grow and rate of survival in yeast colonies in comparison to the wild type. However, in this work, we focused on identifying and better understanding the functions of particular SUN proteins and determitation of their exact localization. Interestingly, some SUN family proteins have dual localization (Uth1p, Sun4p) to the mitochondria and cell wall and may thus be involved in mitochondrial and cell wall function. In this thesis, the “Results and discussion” section is divided into two parts as follows: the first part addresses questions concerning localization, oxygen-depended regulation and the possible involvement of SUN proteins in cell wall remodeling. The second part was preceded by a novel method of visualization of birth scars and of cell wall localized proteins and concerned participation of Sun4p in birth scar composition. In our study we showed that three members of the SUN family of proteins (Uth1p, Sun4p, Sim1p) are released from cells. In additional, we found that expression of UTH1, SUN4 and SIM1 genes regulated differently under different oxygen levels and during particular phases of yeast culture. We suppose that SUN proteins can be involved in remodeling of the cell wall and changes in its resistance to extracellular toxic compounds. We indicate that Uth1p may be an interesting target for study of the mode of boric acid’s action. We implemented a novel method of visualization of cell wall proteins using antibodies conjugated with fluorescent dyes. This method allowed us to acquire new knowledge about the birth scar: a relatively little-studied yeast cell wall structure of unknown composition. We determined the localization of Sun4p, Dse2p and Dse4p to the birth scar. We determined the precise localization and co-localization of Dse2p, Dse4p and Sun4p within the yeast cell wall. We showed that the specific localization of Sun4p

depends on the presence of Dse2p and that the localization of both Sun4p and Dse2p to birth scars depends on GPI-anchored Egt2p. Deletion or combined double deletion of any of these predicted glucanases leads to cell separation defects. We hypothesize that these proteins are parts of the septum destruction complex which localizes to the daughter side of the bud neck and the birth scar and are required for mother–daughter cell separation at late mitosis. In addition, we showed that the presence of Sun4p within birth scars and the extracellular matrix depends indirectly on the Ace2p transcription factor

Using a novel immunofluorescence approach to yeast birth scar visualization we found that Aim44p is necessary for correct new bud selection. A strain with a disrupted AIM44 gene showed a so called “budding-within-birth scar” phenotype where new buds always appear within birth scars, the zone restricted for budding. A similar phenotype is seen in a strain deleted for gene SWI5, encoding a transcriptional regulator of AIM44. In summary, our results generate new knowledge about the localization of SUN family proteins and their roles in cell wall biogenesis, cell separation and birth scar composition.