Cloning and genetic characterization of *sep10* és *sep11* genes in *Schizosaccharomyces pombe*

Ph.D Thesis

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Cell division in eukaryotic cells is accomplished and maintained by a series of tightly regulated events, the cell cycle, which is believed to express its activity through several hundreds of genes and subsequent proteins and their interacting regulatory networks. Mutations of genes implicated in cell cycle result in impairment of correct progression of cell division, thus can lead to cancer in humans. Therefore understanding the processes and control of cell cycle has been a major task for biological research to solve.

In the past thirty years, considerable advances have been made to reveal the key processes of cell cycle, leading to the recognition of pivotal molecules that control G1/S and G2/M progression. These achievements were honoured by the Nobel price in 2001. In spite of that discovery, little is known about the myriad of genes required for cell cycle and about their interactions.

The above discovery has pointed out that events and key molecules in cell cycle are conservative, and acting in a similar manner in a broad spectrum of species. Consequently, model systems applied extensively in biology are also important for cell cycle research. Yeasts have been emerged as key model organisms for studying the processes and control of cell cycle, and enormously contributed to the above-mentioned exploration.

One of the two major yeast models is *Schizosaccharomyces pombe*, which is highly suitable to cell cycle research, mainly because of its mode of cell division and amenability to genetics, cell and molecular biology. *S. pombe* has also proved to be a good model for studying cytokinesis, the physical division of one cell into two, which is the last step in the cell division cycle.

Cytokinesis in *S. pombe* is executed analogically to mammalian cytokinesis, but it also has properties similar to those of plant cell division. As mitosis is initiated, a medial ring consisting of actin, myosin and a number of associated proteins is assembled at the midline. After mitosis is completed, the ring constricts and gradually pulls the cytoplasm toward the midpoint, physically separating the daughter cells. As the ring is constricting, a primary division septum containing cell wall material is synthesised to the space, appearing between the cells membrane and the cell wall. Later two lateral layers known as secondary septa are also

added. The actual physical division is accomplished by the degradation of the primary septum. A considerable number of genes were identified to participate in the assembly of the medial ring and in the initiation of septum formation, but little is known about the process and regulation of septum dissolution.

Mutants were isolated at the Department of Genetics and Molecular Biology, University of Debrecen, affecting the process of septum degradation. Since the mutants were cell separation defective, they were named *sep* (separation) mutants. The genetic analysis of the large number of mutants revealed 16 novel genes, implicated in the process of septum dissolution. The *sep* genes can be divided into a few subgroups. Subsequent genetic and cytological analysis of the mutant phenotype of a large subgroup containing eleven mutants revealed that they have impact on sexual differentiation besides cytokinesis and exhibit complex phenotypes. This finding indicated that the involvement of these genes in cytokinesis is indirect, and they might participate in a regulatory network affecting the control and execution of several important cell cycle events. Accordingly, the first gene cloned form the subgroup, *sep15*, turned out to encode a homologue of a subunit of the Mediator complex, which is involved in transcription regulation (see below). To further explore the function of the *sep* genes, this Ph.D research project was aimed to clone two other genes from the eleven, s*ep10* and *sep11*, and characterise their roles and possible interactions.

Cloning and sequencing of *sep10* revealed that it encodes an intronless, conservative protein, which exhibits significant (more than 50% identity) sequence homology to known and unknown proteins from a wide variety of organisms, including yeast (*S. cerevisiae*), mouse and human. The two characterised homologous proteins are involved in transcription. The human homologue (hsoh1) is a subunit of a transcription complex called Mediator and the yeast homologue (SOH1) is also interacts with a transcription complex and exhibits transcription regulator activity. This finding is consistent with the anticipation that this subgroup of *sep* genes is possibly involved in a regulatory network, coupling gene regulation to the correct accomplishment of cytokinesis and other processes. Cloning of *sep11* disclosed that the gene has three introns and its putative product shows no homology to known or yet uncharacterised proteins, thus providing no information on its possible function. Nevertheless, the *sep11-556* mutant's phenotype and other experimental data suggest that it may have similar function to that of *sep10*.

The Mediator complex was first identified in *S. cerevisiae*, and is required for signal transfer from sequence specific transcription regulators to the basic transcription apparatus consisting of RNA polymerase II and general transcription factors. This implies that its major role is to regulate transcription of subsets of genes, whose activation or repression signals depend on specific transcription activators or repressors.

Functional homologues of the *S. cerevisiae* Mediator were recently identified in mouse and human, indicating that this manner of gene regulation is much more conservative among species than previously anticipated. The Mediator complex was very recently identified also in *S. pombe*. Product of *sep15*, cloned earlier, was found as subunit of the complex, and sep11p was also recognised as subunit. This result confirmed the previous conception that the *sep* genes may participate in a regulatory network in the correct regulation of cytokinesis and sexual differentiation. Furthermore, it also favours that sep11p has transcriptional regulatory role possibly similar to that of sep10p.

During the Ph.D project, it was shown that processes, in which the *S. cerevisiae* homologue of *sep10,* SOH1 is involved, are most probably different from mechanisms that require sep10p. Unlike SOH1, mutation of sep10 did not increase the mitotic recombination between direct repeats and did not show sensitivity to mutagenes, thus it is unlikely that *sep10* is involved in transcription-coupled recombination events and DNA repair, as is thought about SOH1, but suggests the involvement of sep10p in transcription regulation of cytokinesis and sexual differentiation.

Disruptions of both genes were also carried out, which resulted in no lethality, indicating that neither *sep10* nor *sep11* is essential for cell viability. Interestingly, the cells require *sep10* and *sep11* at higher temperatures, as the disruptants are unable to grow at higher temperatures, indicating that they may be implicated in regulation of genes that become essential at higher temperatures. Inactivation of both genes in the same cell was also accomplished, and resulted in lethality at all temperatures, suggesting that the simultaneous inadequate transcription of the sets of genes controlled by these genes can not be tolerated at any temperatures.

In studying the role of *sep10* and *sep11* in sexual differentiation, we obtained results suggesting that the major defect of sexual differentiation in *sep10* and *sep11* mutants is the omission of activation of the transcription of *ste11,* a positive regulator of differentiation, whose activation normally occurs after commitment to sexual development. This result suggested

that both sep10p and sep11p participate in the correct activation of *ste11*. We also revealed that lack of *ste11* activation in *sep10* cells can be substituted by overproducing ste11p, indicating that the possible major role of *sep10* in sexual differentiation is the indirect regulation of *ste11* activity. On the contrary, the overproduction of *ste11* has no suppression effect on defects of sexual differentiation in *sep11*$^{-}$, suggesting that sep11p performs roles not only in *ste11* activation, but possibly in other downstream processes in sexual development.

All above-mentioned results seem to corroborate the hypothesis that *sep10* and *sep11* encode transcription regulators. sep11p is part of the Mediator complex. Both proteins perform regulatory roles in cytokinesis and sexual differentiation. One of their target gene is *ste11*, whose transcription is influenced probably indirectly.

Further research is necessary to explore the roles of *sep10* and *sep11* in the regulation of cytokinesis and differentiation by identifying other target genes (utilizing the soon-available DNA microarrays). Cloning and characterisation of the hitherto uncloned *sep* genes and studying the genetic and biochemical interactions of *sep10* and *sep11* with the already cloned *sep15* would also contribute to a better understanding of the regulatory network that seems to control cell separation and sexual differentiation in *S. pombe*.