Genetical and microscopical investigation of dimorphism in *Schizosacharomyces japonicus* var. *japonicus*

ANIKÓ BOZSIK

Schizosaccharomyces japonicus var. *japonicus* is a model organism in yeast genetic researches, which is not yet really prevalent, so first of all it was necessary to elaborate the basic methods for its classical and molecular study. In this aim we could greatly rely on the well-known genetics of *Schizosaccharomyces pombe*, the closest relative of *S. japonicus*: we could adapt the routinly used methods and protocols of *S. pombe* to *S. japonicus* with slight alterations. The close relation between the two *Schizosaccharomyces* species was prooved not only by their similar structural and physiological characteristics, but we also reinforced it with the molecular sequence analysis of their homologue gene products.

The first auxotophic mutants of this species were isolated from the wild type 7-1 strain through induced mutagenesis (UV irradiation). These mutations serve as excellent, stable and easily detectable markers for the classical genetic survays. These auxotrophic mutant strains we distributed into groups, where groups represent genes; so the number of the groups found gives the minimal number of the enzymatic steps of the respective synthetic pathways.

Applying intermediers of the different biosynthetic pathways we determined the approximate place of these auxotrophic groups, or genes in the respective biosynthetic pathways. Because anabolic ways are largely conservative processes throughout the organisms we took that of *S. pombe* as an example. We could also elucidate the exact place of some groups in the biosynthetic pathway relying on their special features, so we found in these cases the functional homologues to *S. pombe*. For example, in uracil biosynthesis we found the exact function of the group 4: this corresponds with the *ura4* gene of *S. pombe*. Similarly, the 3rd uracil group is homologue with *S. pombe ura5*, and 1st arginine group of *S. japonicus* is the functional homologue of *arg1-1* in *S. pombe*. One further homology could be stated with the help of molecular experiments. According to this, the complementation of the mutation in 7-63 *leu3-10* strain of *S. japonicus* was successful with *S. pombe leu1* gene, as well as *Saccharomyces cerevisiae* LEU2 gene, so they must be homologues.

We made comparisons between the two morphological states (unicellular yeast cells and multicellular hyphae) in cytological point of wiew. With respect of the mitotic events of the cells we did not notice any significant difference in the main stages of division between the two shapes, howewer, in the case of hyphae in the last step of division the nuclei seem to elongate opposite direction, which is presumably due to the fact, that they try to keep up quickly with the intense elongation of the cell. The other possible explanation may be that a spindle pole body or some similar cytoplasmic organel can pull the nuclei.

Since *S. japonicus* is a homotallic organism (it can find mating partner in the same colony) self-conjugation may disturb the the figures for recombination. In order to avoid this influence we applied protoplast fusion, which helps to block conjugation between identic cells. This method, which is frequently used in *S. pombe* was successfully adapted to *S. japonicus*. Since we failed to isolate heterotallic mutants in the case of each cross performed we applied protoplast fusion. The spores originated from the sporulation of the diploids we segregated with micromanipulation method and determined the auxotrophy of each colony developing from the spores respectively. The distribution ratio of the auxotrophic alleles turned to be 4:4 ,which correspons wery well with the mendelic rule. So this species is a

suitable model organism for classical genetics. Measuring the recombination frequences from the crosses of the different auxotrophic strains we found linkage groups. This means that in certain pairs the recombination frequency was much less than 0,5 in other word 50%, so the genes are situated close to each other on the chromosome. We could identify 2 linkage groups with 4 members in each. In the first group we could also clarify their exact order on the chromosome.

Applying further mutagenesis we isolated mutant strains, that showed mycelial growth different from the wild type. We selected for mutants, whose hyphae are larger or smaller than the wild type, or do not develop hypha at all. We called these mutants myc, after the word of mycelium. So we isolated myc^{++} (hyperactive hypha developers), myc^{-} (strains, that develop hyphae weaker than the wild type), and myc^{--} (unable to generate hyphae). We studied the genetical background of these mutants and stated, that myc morphology is independent of the auxotrophies as well as other background mutations in each strain and each myc morphology is determined by only one mutation.

We crossed the myc^{-} strains with each other through protoplast fusion, so we performed a fusion matrix. Analysing the myceliar growth activity of the progenies arising from the crosses we devided the myc strains into groups. Each group represent a gene. With this method we yielded, that all the myc^{-} strains examined belong to different group, so each myc^{-} phenotype is determined by different genes. According to these results we suspect, that several independent mutations of the intracellular processes can affect hypha development. In contrast among the four myc^{--} strains that we examined in a similar way we found only two groups. This means that the number of the mutant genes causing entire block in hypha formation are restricted, so we suspect that we must search for the genes responsible directly for dimorphic change among these strains. When we crossed two myc^{--}strains harmed at different loci the resulting diploid was able to develop hyphae smilar to the wild type. These hyphae were also diploids, so this is the phenomenon of real complementation. This can give us several deductions: diploid cells are also able to form hyphae. These hyphae are larger and thicker in diameter but considering other parameters, such as cellular organisation they are just the same as the haploid ones. The other conclusion is, that since real complementation occurs the mutant allels were recessive in both myc^{--}strains. Real complementation also occurs in the case of diploids originating from $myc^{--}$and myc^{++} strains, so myc^{++} mutation is also recessive. During the crosses of $myc^{--}$and myc^{++} strains we frequently obtained double mutants through recombinations: where the two mutation were together in one strain. The phenotype of these strains was myc^{--}, so the myc^{--} locus is dominant epistatic over the myc^{++} locus.

During the cytologycal study of myc mutants we observed, that $myc^{-}$and myc^{--}strains have strange vacuolar pattern, different from the wild type. They possess more smaller, miniature vacuolues while the wild strain have fewer, large, fused vacuoles. The first step of the dimorphic change is the fusion of the vacuoles: this is reduced or missing in the case of myc mutants. Furthermore, myc strains were more rounded in shape.

We tried to transform *S. japonicus* with several methods – auxotrophic-prototrophic transformation, introduction of resistances as dominant marker into the cells – but all the forces to transform this species were futile. We blamed the autonomic replication sequence (ars), in other words replication origo originating from *S. pombe (ars1)* and *S. cerevisiae* for the failure. We suspected that *S. japonicus* is unable to utiltze these foreign ars, that we applied for the transformations. So we searched for proper *ars* from *S. japonicus* in its genom. We generated *S. japonicus* genomic library in an integrative plasmid containing *S. pombe leu1* marker. We transformed the leucine auxotrophs of *S. japonicus* with this library. We selected for leu+ transformants with strong plasmid loss. We regained these plasmid from the

transformed strain. After restriction analyses and subcloning procedures we yielded a 1,5 kb long stretch with slight ars activity in *S. japonicus*. This hypotetic ars 10 fold rises the transformational efficiency in *S. japonicus*, and also slightly effective in *S. pombe*. We sequenced this 1,5 kb long fragment and made computer analysis to find homologues. We found significant homology only on protein level. These are all Cut1-like proteins, which function is the sister chromatide separation in the anaphase of the cell division.

Using computer analysis we performed amino acid sequence comparison in the conservative domains of the homologue proteins. This way we could make deductions considering the evolutionary distance between the species, with whom we found homology. This study show that the closest relative of *S. japonicus* is *S. pombe* (it is quite evident because of the strong morphological and physiological similarity) but suprisingly *Aspergillus nidulans* seems to be closer to *S. japonicus* in the evolutional tree than *Saccharomyces cerevisiae*. So filamentous form seems to be more ancient in fungi and yeast form evolved later.