

Optical investigations of the mating-type region in fission yeast

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We use biophotonic methods such as fluorescence confocal microscopy and optical tweezers to study cellular processes. More specifically we look at the repression and localization of the mating-type region (MAT-region) in fission yeast (*Schizosaccharomyces Pombe*). *S. Pombe* is a well characterized eukaryote sharing many cellular mechanisms with human cells.

Background

The MAT-region is crucial in the regulation of the cell cycle. As long as there is enough nutrients in the cellular environment the cells undergo mitosis and divide happily. If the growth conditions deteriorate the yeast cells differentiate into either P-type cells or M-type cells, become diploids and mate in order to mix their genetic material. They then form four haploid spores that can stay dormant until better times.[1] Figure 1 shows a sketch of this region on the second of *S. Pombe*'s three chromosomes [2].

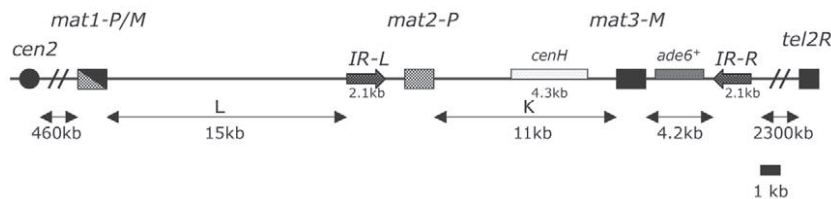


Figure 1: Schematic representation of the mating-type region that is located on the right arm of chromosome II in *S. pombe*. The mating-type region consists of three linked loci; *mat1* (checkered/black box), *mat2-P* (checkered box) and *mat3-M* (black box). *mat2/3* is surrounded by two inverted repeats, *IR-L* and *IR-R*, with perfect sequence identity (block arrows). In the K-region separating *mat2-P* and *mat3-M* there is a 4.3 kb sequence, denoted *cenH*, with 96% homology to the repeats at *cen2* (white box). The inserted *ade6+* reporter gene is shown as a grey box. [2]

All strains we work with have the MAT-region marked with enhanced green fluorescent protein (EGFP). Additionally the strains we look at have either the plasma and nuclear membrane marked with EGFP as well, the nucleolus marked with cyan fluorescent protein (CFP) or the nucleolus and the spindle pole body (SPB) marked with CFP. The group of Geneviève Thon at the Department of Biology (University of Copenhagen) with whom we collaborate, has for each of these strains made four mutants. One was left as such with a wild type MAT-region, one has a ribosomal DNA (rDNA) sequence instead of the right repeat (*IR-R*), a third has a *IR-R* deletion (Δ *IR-R*) and the last is a control with the *ade6+* reporter gene (present in the two other mutants as well) added to the wild type strain (see fig1). The repressing rDNA sequence was found by using random insertion mutagenesis. In all figures the YFP channel of the fluorescence microscope was used to visualize the GFP dot.

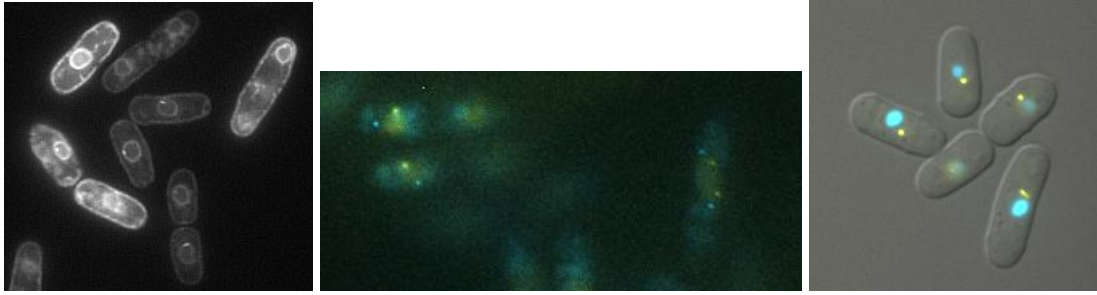


Figure 2: Left: Wild type strain with the MAT-region and its lipid membranes marked with EGFP. Middle: rDNA mutant strain having the MAT-region marked with EGFP (yellow) and the SPB marked with CFP (blue). Right: Wild type strain with MAT-region marked with EGFP (yellow) and the nucleolus marked with CFP (blue). Pictures taken by Marie Domange Jordö (MDJ).

Investigation of the role of localization in the silencing of parts of the MAT-region

In the wild type strains the MAT-region is localized in the proximity of the SPB, near the plasma membrane (see fig2 left) usually opposite to the nucleolus (see fig2 right and fig3 left). We wanted to investigate whether the exchange of the IR-R by a piece of silencing rDNA would lead to a relocalization of the MAT-region within the nucleus. We are still in the investigation phase, but our preliminary data would indicate a relocalization of the MAT-region closer or into the rDNA-rich nucleolus (see fig3).

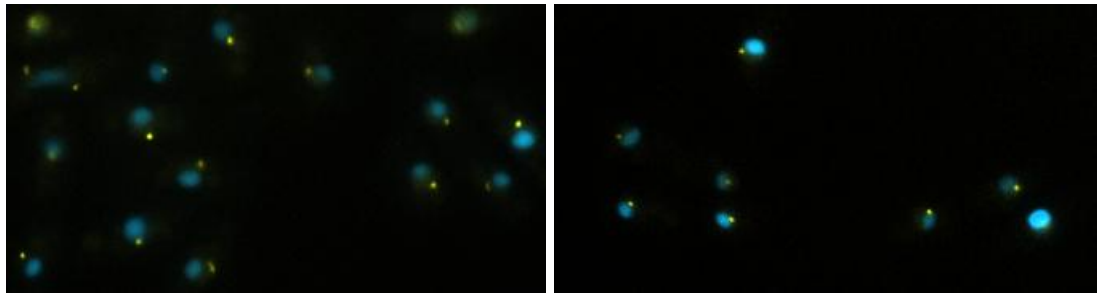


Figure 3: Left: The wild type strain with the MAT-region marked with EGFP (yellow) and the nucleolus marked with CFP (blue). Right: The rDNA mutant strain with the MAT-region marked with EGFP (yellow) and the the nucleolus marked with CFP (blue). The MAT-region seems located closer to the nucleolus in the rDNA mutant than in the wild type strain. Pictures taken by MDJ.

This is in agreement with the relocalization of the MAT-region with regard to the SPB shown in the middle image of figure 2. The question is now whether it is the heterochromatic structure of the gene sequence or its relocalization within the cell nucleus that silences the P and M loci of the MAT-region. More generally, the position of a particular gene sequence within the nucleus could maybe both influence and be influenced by e.g. its degree of compaction.[3] It would be nice to determine how big a part of the MAT-region of the different mutants is composed of heterochromatin. Maybe this could be possible using electron microscopy.

Diffusion of MAT-region

We would also like to monitor the diffusion of the MAT-region inside the nucleus, see how strongly confined it is and compare it for the different mutants. This could be used to investigate a possible tethering of the MAT-region to the nuclear envelope and maybe also bring some light on its possible affiliation with the spindle pole body [2]. This part of the project is in the very beginning.

Inserting gold nanoparticles into living yeast cells

Measuring forces inside living cells is a challenge from many aspects. To use optical tweezers could be one way of approaching this. It is then necessary to find means to calibrate the trap in a complex visco-elastic media [4]. For any good calibration one needs a well defined handle. Quantum dots as well as gold or silver nanoparticles would be good candidates [5][6].

One of our ongoing projects is to insert 80nm gold particles into living yeast cells. The idea would be to incorporate the gold into lipid vesicles and then, using an electrofusion method [7], fuse them together with yeast protoplasts. The figure 4 shows a giant unilamellar vesicle (GUV) grown by electroformation in the presence of 80nm gold particles. In the 3D-projection of the z-stack obtained by confocal microscopy one can clearly see a confinement of the particles inside the vesicle.

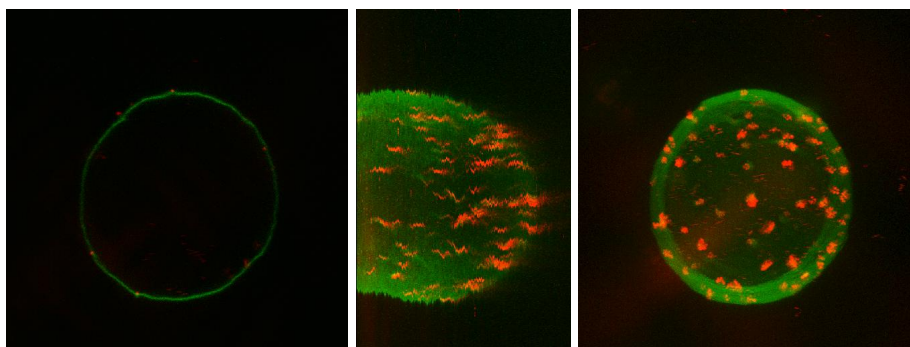


Figure 4: EggPC GUV (lipid membrane in green) grown by electroformation in the presence of 80nm gold particles (red). Left: Cross-section. Middle: Side view of the 3D-projection of the vesicle. Right: Top view of the 3D-projection. Pictures taken by MDJ.

Some issues still have to be addressed like the influence of a bio-coating of the handles on their incorporation into lipid vesicles. Also the size of the vesicles is crucial; fusing a too large vesicle to a cell would probably severely disturb it. Nevertheless, the big advantages of mass insertion of nanoparticles into living cells makes this project worth pursuing. What we aim at is to develop tools that would allow us to study processes that are difficult or hard to mimic *in vitro* combining both observation and actual force measurements.

References

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