Talk

Optimization of super-resolution microscopy to the visualization of biotechnological samples

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ABSTRACT

Motivation: Super-resolution microscopy (SRM) allows the resolution of cellular structures in the range of tens of nanometers being able to useful for biotechnological researchs, like the study of the chromosomal structure of the fission yeast Schizosaccharomyces pombe. Nevertheless these techniques need an optimization and the preservation of the structures of study.

In this investigation we want optimize the SRM protocol to be useful to the visualization of structures in the mencionated scale. Specifically, we want define more precisely the position of telomeres and centromeres that make the Rabl configuration of chromosomes. Commonly is used the fluorescence microscopy (FM) for the cell research, but it shows less foci than the real number of this structures. However, using cells with the inactivated Linker of nucleoskeleton and cytoskeleton complex (LINC) the Rabl configuration can change, realeasing the centromere-LINC contacts. This separation could allow discern more centromeres with FM than in wild type cells, but could affect the telomeres.

In this study we will compare the chromosomal distribution between wild type cells and inactivated LINC cells using FM and SRM.

Methods: Different strains are generated with centromeres, telomeres and nuclear membrane tagged with fluorescence proteins to study through FM the possible alterations in the Rabl configuration in inactivated LINC cells.

This strains together with inmunofluorescence techniques allow an approach to the optimization of a SRM protocol. Then to study the structures of interest with SRM, strains with HA-tag are used in combination with the inmunofluorescence protocol. In addition, protein extraction and western blots was performed to test the union of the antibody to the target protein and check the efficient of the union of primary and secondary antibody.

Results: Optimization of the immunofluorescence protocol has been achieved with the tag GFP, necessary for the refinement of the SRM. The best results in FM have been reached with cells fixed with 4% paraformadehyde and tagged with nanobodies, being unnecessary the cellular wall digestion to permeabilize it.

Using MF to analyze the Rabl configuration, wild type and inactivated LINC cells shown a similar distribution of telomeres, being the most common the cells with 2 telomeres, followed by 1 and 3 telomeres. Moreover the 27% of LINC mutants show 2 centromeres versus the wild type that only show 1 centromere.

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