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Yeast Meiotic Chromosome Structure Revealed By Deconvolution Microscopy

Z. Zhang,* M. N. Conrad,** and M. E. Dresser**

* Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071
** Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

In the yeast *Saccharomyces cerevisiae*, as well as in many other organisms, dramatic chromosome structure changes have been observed during the first meiotic prophase. These structure changes play a crucial role for homolog pairing, alignment and recombination, which in turn, are important for the faithful transmission of genome to the next generation. Deconvolution microscopy was used in this study to examine the chromosome behavior and dynamics during the first meiotic prophase.

Yeast cells were induced into meiosis described elsewhere [1]. Cells entering the meiotic prophase were fixed with 4% paraformaldehyde, briefly rinsed with 30% ethanol, stained with DAPI and mounted on a coverslip. Cells were then examined using a Zeiss fluorescent microscope equipped with a 12-bit CCD camera. A piezoelectric nanopositioner was used for optical sections, driven along with image acquisition, by MetaMorph software. The spacing sizes in X-, Y- and Z-axis are 0.08, 0.08 and 0.16 μm, respectively. This is achieved by using a 100 X, 1.4NA objective and a Zeiss variable zoom. An X-Cite illuminator (EXFO Photonic Solutions) is used to eliminate lamp flicker. Deconvolution of the 3D datasets was achieved using Auto-Deblur software.

DAPI stained nuclei demonstrated that in meiotic prophase, telomeres migrate from inside the nucleus to the nuclear periphery, as shown by GFP tagged Ndc1p, a nuclear pore complex protein. Chromosomes are often seen forming loops, with telomeres attached to the nuclear membrane, as indicated by CFP tagged Rap1p, which accumulates mainly at telomeres (Fig. 1). As telomeres migrate from inside the nucleus to nuclear periphery, they appear to form two or three relative large clusters; presumably, these two clusters then migrate towards SPB, forming the so-called bouquet structure [2]. An interesting observation is that during the bouquet stage, not only telomeres migrate and attach to the nuclear envelope, but also most of the chromosome mass accumulates close to the nuclear envelope, forming a barrel-like structure (Fig. 2). The function of this barrel orientation, however, is unknown.

Deconvolution microscopy has proven a powerful imaging tool for cellular and molecular biology research. It has several advantages over confocal microscopy, such as, cost efficient, easy to be modified to meet one's specific needs, and most importantly, it can be accomplished at very low light level, therefore more suitable for light-sensitive living specimens over long time periods[3]. One critical issue we found that affects the quality of deconvolution is the fluorescent light source. Figure 3 shows a X-Z section of a fluorescence dye imaged using either Zenon lamp or X-Cite illuminator. Lamp flicker was found even with a relatively new Zenon lamp but not with X-Cite illuminator. The severe lamp flicker caused some of the images unusable for deconvolution.

References:

[1] M. E. Dresser *et al.* Genetics 138 (1994) 633
[2] E Trelles-Sticken *et al.* J. Cell Sci. 122 (1999) 651
[3] J.G. McNally *et al.* Methods 19 (1999) 373

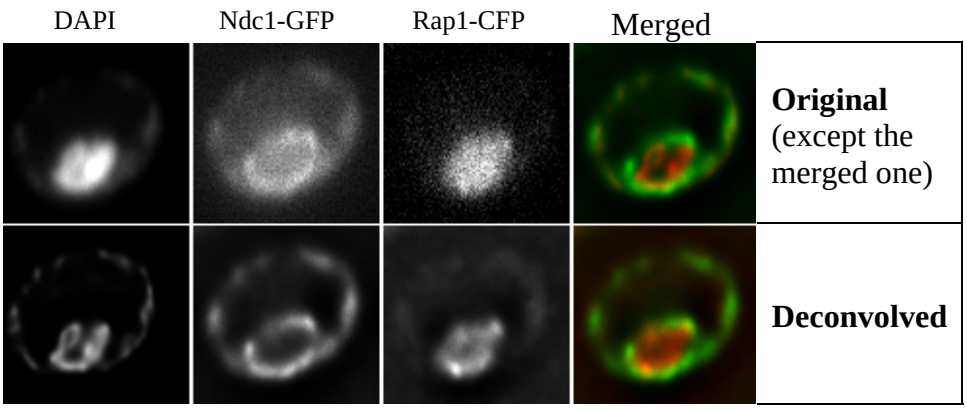

Fig. 1 Meiotic prophase nucleus, showing the middle slice of a Z series section. Nucleus DNA is stained with DAPI, Rap1-CFP and Ndc1-GFP. Original slices were shown on the top panel and deconvolved slices were shown on the lower panel.

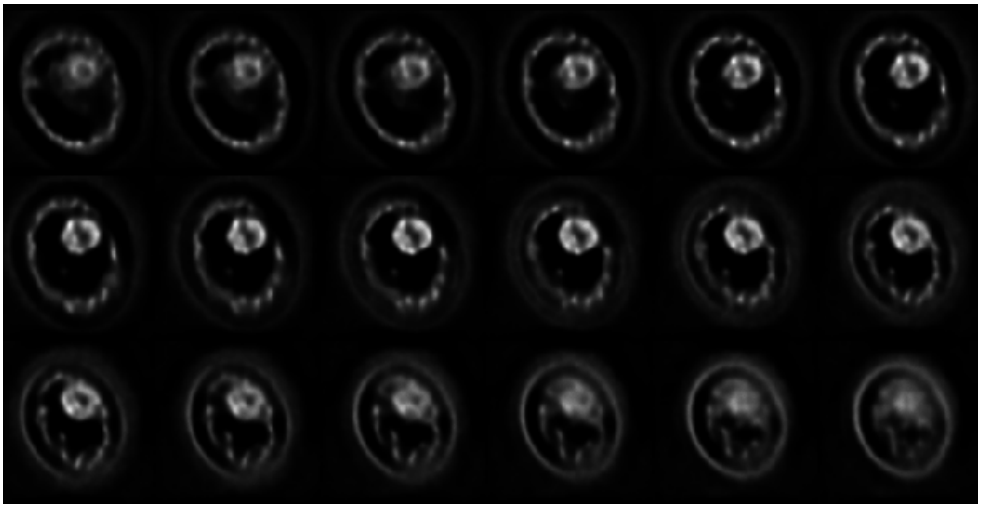

Fig. 2. A z-series section of DAPI stained meiotic prophase nucleus, showing the barrel orientation

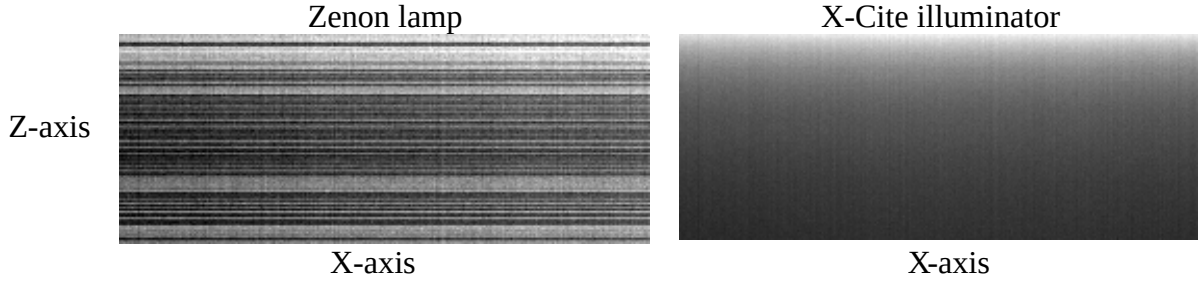

Fig. 3. Comparison of different illumination system for deconvolution microscopy. A drop of fluorescence dye was put onto a slide covered with a coverslip. 60 frames (100 ms/frame) of a single focus plane were taken continuously using either Zenon lamp or X-Cite illuminator. X-Z series of the time-lapse was shown.

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