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Abstract

During meiosis, homologous chromosomes, one set maternal and the other set paternal, pair with one another. Pairing is a prerequisite for crossing over, where allelic regions on homologs break and recombine with the corresponding homolog. This crossing over results in recombinant chromosomes that in turn increase genetic diversity. What causes the homologs to pair at specific sites is unknown. We are investigating whether specific DNA sequences are involved in pairing. Our first step to identify pairing sites involves mitotic cells of budding yeast in order to isolate pairing regions in sister chromatids. Mitotic cells are used instead of meiotic cells since in haploid vegetative cells pairing can only occur between sister chromatids whereas pairing may occur between sister chromatids and/or homologs in meiotic cells. To isolate pairing regions, it is essential to capture the cells at a stage of the cell cycle when sister chromatids are present in the nucleus. The G2 phase of the cell cycle represents such conditions. Cell cultures were synchronized through initial starvation and then placement in rich growth media. In order to determine whether a sizable portion of the cells were in G2, the culture was analyzed at each time point using microscopy with a fluorescent dye that specifically interacts with DNA (DAPI). A systematic categorization based on cell morphology was used to determine the number of cells in G1, G2, S-phase and mitosis. We found that the optimal time for starving the cells in YPA medium was 17 hours. Following to rich YPD medium, growth for 2.5 hours gave the highest percent of G2 cells. In two vegetative cultures, 28% of cells were at G2 at this point. Given these results, we will be able to enrich for paired homologous sequences utilizing an appropriate molecular assay. Such isolation of paired DNA sequences will assist in decoding the foundations of genetic diversity.