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JOURNAL CLUB FOR CHAPTER 4: CHROMOSOME STRUCTURE AND FUNCTION

EXPERIMENTAL APPROACH 4.3

Clarke, L. and Carbon, J. Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature* 1980; 287: **504-509**

1. What was the goal of the experiments described in this paper?
2. How did the authors know where in the chromosomes to begin to look for centromeric DNA?
3. The authors knew that yeast plasmids are frequently lost in cell division. How did they use this fact to identify a centromere?
4. Describe how the authors identified which plasmids were mitotically stable.
5. What is meiotic stability? How did the authors screen for this property?
6. Once the DNA fragments containing the centromere were identified, how did the authors isolate the actual centromere within that DNA fragment?
7. Why did the authors use Southern blot analysis to identify which cells the centromere DNA was in?
8. Clarke and Carbon do not actually identify the exact DNA sequence that constitutes a centromere. What experiments could you conduct to delineate the minimal sequence that constitutes the centromere?

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Clarke, L. and Carbon, J. Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature* 1980; 287: **504-509**

1. What was the goal of the experiments described in this paper?

   The authors sought to identify the DNA region that defined a yeast centromere.

2. How did the authors know where in the chromosomes to begin to look for centromeric DNA?

   The region in which the centromere was located had previously been genetically mapped.

3. The authors knew that yeast plasmids are frequently lost in cell division. How did they use this fact to identify a centromere?

   The authors knew that typical yeast plasmids are lost at a high rate. They reasoned that, if a plasmid had a centromere, it would be lost at a lower rate than typical plasmids. Clark and Carbon therefore inserted different pieces of DNA into yeast plasmids and compared the rate at which the resulting plasmids were lost after multiple rounds of mitosis. The authors tested a variety of DNA fragments derived from the region of chromosome III to which the centromere had been genetically mapped, as shown in Figure 1 in the paper.

4. Describe how the authors identified which plasmids were mitotically stable.

   The different DNA fragments were cloned into yeast plasmids containing the TRP1 gene and an origin of replication. Yeast cells with a mutation in the TRP1 gene were then transformed with the plasmids. Individual colonies, each representing a single transformant, were grown for 20-30 generations in rich medium and then plated on non-selective media or on selective medium. The number of colonies on the nonselective plate gave a measure of the total number of cells present. Yeast that had lost the plasmid were not able to grow on the trp- medium, so the number of colonies on the selective plate gave a measure of how many cells had retained the plasmid after successive cell divisions. The proportion of cells that retained a given plasmid was determined simply by comparing the number of colonies on the selective and non-selective plates. The more colonies present on the selective plate, the more mitotically stable the plasmid.

5. What is meiotic stability? How did the authors screen for this property?

   Prior to meiosis, a transformed diploid yeast cell contains a homologous pair of each chromosome plus one plasmid. The first round of DNA replication yields two replicated copies of each chromosome (each a set of sister chromatids) and two copies of the plasmid. After the first meiotic division, the pair of sister plasmids containing centromeres should NOT separate and thus both should segregate to only one of the daughter cells. After the next round of meiotic division, the two sister chromatids marked with his+ or his- segregate

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to daughter cells and the sister plasmids segregate, as well. Of the four haploid spores that result from meiosis, two will contain the centromeric plasmid.

The authors tested whether the plasmid segregated at the first division or at the second division by scoring the HIS marker on the chromosomes. If correct meiotic segregation had occurred, the plasmids would either both be in the his+ spores or both be in the his- spores. If one plasmid were in the his- spore and one in the his+ spore, it would indicate that the segregation had occurred inappropriately at the first meiotic division.

6. Once the DNA fragments containing the centromere were identified, how did the authors isolate the actual centromere within that DNA fragment?

   The authors began with the DNA fragment containing the centromere. They then further cleaved this DNA fragment with restriction enzymes, and plasmids containing these smaller fragments were each tested again for both mitotic and meiotic stability.

7. Why did the authors use Southern blot analysis to identify which cells the centromere DNA was in?

   Southern blot analysis was performed to verify by a second, physical, method that the DNA containing the centromere was segregating as expected based on the genetic experiments described above.

8. Clarke and Carbon do not actually identify the exact DNA sequence that constitutes a centromere. What experiments could you conduct to delineate the minimal sequence that constitutes the centromere?

   First, the location of the centromere within the sequence Clarke and Carbon identified can be further defined by testing ever-smaller regions for centromere function. Next, random mutagenesis could be used to introduce mutations at various locations in the sequence that confers centromere activity. Mutations in the essential sequence required for centromere function should give rise to a defect in mitotic stability, while those that lie outside the centromere should not affect mitotic stability.