Histone Variants Link Chromatin Structure to Chromosome Segregation

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During mitosis, cells must segregate their chromosomes equally to ensure that both cells receive a complete genome. Errors in chromosome segregation lead to aneuploidy (an abnormal number of chromosomes within a cell), which is a hallmark of cancer and causes other conditions such as Down syndrome. Given that aneuploidy is commonly found in cancer and other diseases, it is of great interest to fully understand the mechanisms by which cells segregate their chromosomes.

Chromosome segregation relies on a protein complex called the kinetochore, which provides the point of attachment for the microtubule filaments that separate copies of each chromosome. The kinetochore is assembled on particular DNA sequences called centromeres. Like the rest of the genome in all eukaryotes, centromeric DNA is wrapped around a complex of histone proteins to form the nucleosome, the basic unit of chromatin. As centromeric chromatin is essential for chromosome segregation, graduate student Tessie Ng and colleagues in the laboratory of Dr. Sue Biggins (Basic Sciences Division) undertook a study to identify residues in histones H3 and H4 that are important for kinetochore function and chromosome segregation in budding yeast. This work, published in Genetics, "lays the foundation for us to try to reconstitute centromeric chromatin with different types of histone mutants to gain a mechanistic understanding of how they alter the biochemical and biophysical properties of kinetochore behavior," says Dr. Biggins.

To determine residues of histones H3 and H4 important for kinetochore function, the authors used a library of yeast mutants where every residue in these proteins was systematically changed to alanine. Because it is not bulky and is chemically inert, substitution of alanine for a biologically important amino acid residue in a protein is expected to cause defects in that protein's function. This screen identified 26 mutations in histone H3 and 15 mutations in histone H4 that increased the frequency of losing of a non-essential chromosome. Because histone mutations can affect many cellular processes, the authors also used a modified genetic screen to identify histone mutations important in another chromosome segregation process called spindle biorientation. Mutants identified in both assays were carried forward for further analysis.

To ascertain whether the chromosome loss phenotypes observed might be due to defects in DNA replication, the authors analyzed DNA content in each mutant strain by fluorescence-activated cell

sorting (FACS). None of the mutants exhibited defects in DNA replication, suggesting that the identified histone mutations affect chromosome segregation rather than replication. Indeed, by analyzing the segregation of a fluorescently-labeled chromosome, the authors found that most of the mutants missegregated this chromosome a substantial fraction of the time.

The authors next investigated the basis of chromosome missegregation. Gene expression analysis revealed that the expression of genes encoding segregation-related proteins was unaffected, and that the mutations did not alter binding of the centromeric histone variant Cse4 to centromeres. Several of the mutations did alter the stability of the kinetochore complex, however, as evidenced by reduced interactions among kinetochore protein components in these strains.

The identification of amino acid residues in histones H3 and H4 that are important for proper chromosome segregation may have important implications for understanding certain diseases. "Given that aneuploidy is a major hallmark of cancer cells, this also provides us with new information about how chromatin changes can lead to chromosome missegregation events that might contribute to aneuploidy," says Dr. Biggins. Indeed, mutations in histones are increasingly recognized in cancer (Wu et al., 2012; Behjati et al., 2013), and this work suggests a mechanism by which such mutations might drive carcinogenesis.

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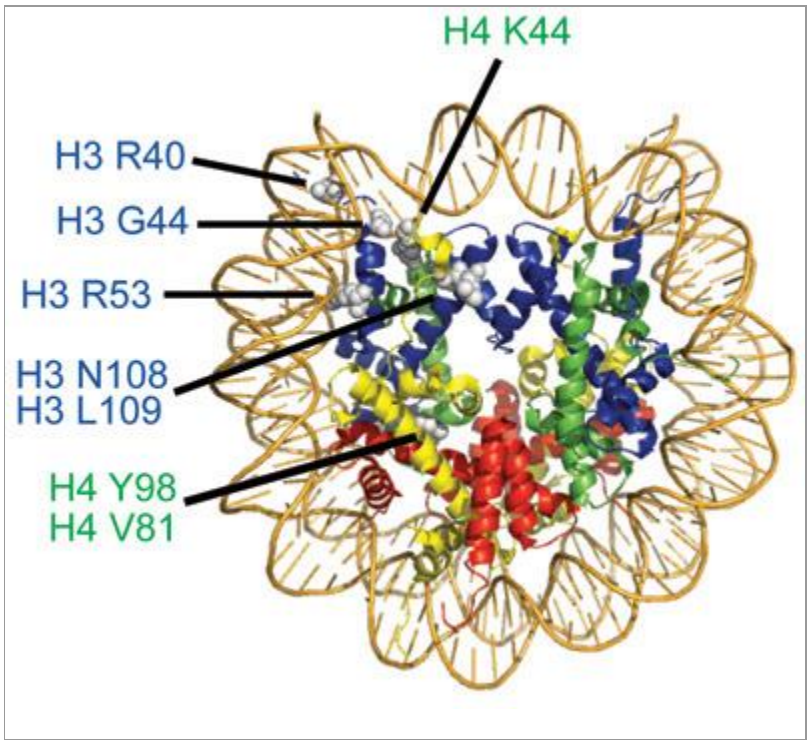

Image provided by Dr. Sue Biggins

Structural model of the nucleosome with residues affecting chromosome segregation and kinetochore function indicated.