VRG14-001 - Chromosome Biology

Zusammenfassung

Cells use multiple mechanisms to ensure that chromosomes are segregated with high fidelity by the microtubule-based mitotic spindle. The vast majority of cancer cells are aneuploid (contain the wrong number of chromosomes), indicating that one or more of these segregation fidelity mechanisms has failed. This leads to a massive increase in the rate at which cells missegregate their chromosomes, a state termed chromosomal instability. Despite major recent advances in the genomic characterization of cancer cells, very little is known about how and why cancer cells missegregate their chromosomes. In addition, a basic understanding of the consequences of chromosomal instability and how cells adapt to a genome in flux is lacking. As an independent investigator, I will fill these gaps in our understanding of chromosomal instability using a combination of cell biology, genetics, biochemistry and the budding yeast S. cerevisiae as a model organism.

Aim I. Regulation of Chromosome Segregation by the Chromosomal Passenger Complex. Aneuploid cancer cell lines have defects in the strength of attachments between microtubules and the kinetochore (the microtubule-attachment site on chromosomes). The strength of these attachments is regulated by the kinase Aurora B, which provides the catalytic activity of the chromosomal passenger complex (CPC). The CPC is a four-subunit complex that detects improper microtubule-kinetochore connections and weakens them via phosphorylation of various targets on the kinetochore. One fundamental question that I wish to address is: how does the CPC preferentially destabilize aberrant kinetochore-microtubule connections? My post-doctoral research has shown that error correction by the CPC occurs independently of the complex's association with centromeric chromatin (Campbell and Desai, Nature 2013). This result challenges fundamental assumptions for how the CPC identifies incorrect attachments, and opens up new lines of investigation. Additionally, these results provide evidence for a new, kinetochore-centric model for how the CPC functions in chromosome segregation. I have now begun testing various aspects of this new model through a combination of genetic and cell biological approaches. To determine how misoriented chromosomes are detected, I will attack the problem by determining the contributions of both the CPC and the kinetochore. My research has revealed that the CPC can function efficiently with only two of the four members of the complex: Aurora B and INCENP. I will next test which domains and activities associated with these two proteins are required for the CPC's essential functions. In a parallel line of experimentation, I will determine which kinetochore proteins contribute to the identification of misoriented chromosomes. My research also provides evidence for a link between the CPC and maintaining cohesion between sister chromatids. I will determine how the CPC contributes to sister-chromatid cohesion via a combination of genetic profiling and localization studies.

Aim II. Cellular Adaptation to Chromosomal Instability. Some cancers have chromosome segregation errors in every cell division, which would be detrimental to the growth of normal cells. Little is known about how cancers are able to thrive with high levels of chromosomal instability (CIN). In order to determine how cells cope with CIN, I have been evolving yeast strains that missegregate chromosomes at an extremely high rate due to the removal of the CPC component Bir1 (Survivin in humans). Although BIR1 deletion strains initially grow very slowly, I have discovered that these strains quickly evolve to grow at much faster rates despite maintaining high levels of CIN. I will sequence these strains to determine how they evolved to cope with CIN. Mutations identified in this assay will provide valuable information about how cells adapt to CIN and create starting-points for future projects. In addition to identifying specific mutations that lead to CIN tolerance, this line of investigation will provide insight into the complex relationship between chromosomal

instability, aneuploidy, and cell viability. By measuring chromosome loss rates, chromosome number, and cellular fitness of evolved CIN cell lines, I will correlate these phenomena over a large collection of evolved yeast strains. These experiments will provide insight into the potential repercussions of both CIN and aneuploidy on the viability and evolvability of cells.

Wissenschaftliche Disziplinen:
Cell biology (50%) | Genetics (30%) | Biochemistry (20%)

Keywords:
Chromosomes, Kinetochore, Genomic Instability

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Status: Laufend (01.05.2015 - 30.04.2023)

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