

7.340 – Avoiding Genomic Instability: DNA replication, the Cell Cycle and Cancer Mid-term Assignment

Assignment:

You will be assigned two abstracts from articles in the literature about topics we have already discussed. You will write a short (2-3 pages) paper describing one or two experiments with appropriate controls that would allow the researchers to make their conclusions.

Abstract #1

The initiation of eukaryotic DNA replication involves origin recruitment and activation of the MCM2-7 complex, the putative replicative helicase. Mini-chromosome maintenance (MCM)2-7 recruitment to origins in G1 requires origin recognition complex (ORC), Cdt1, and Cdc6, and activation at G1/S requires MCM10 and the protein kinases Cdc7 and S-Cdk, which together recruit Cdc45, a putative MCM2-7 cofactor required for origin unwinding. Here, we show that the *Xenopus* BRCA1 COOH terminus repeat-containing Xmus101 protein is required for loading of Cdc45 onto the origin. Xmus101 chromatin association is dependent on ORC, and independent of S-Cdk and MCM2-7. These results define a new factor that is required for Cdc45 loading. Additionally, these findings indicate that the initiation complex assembly pathway bifurcates early, after ORC association with the origin, and that two parallel pathways, one controlled by MCM2-7, and the other by Xmus101, cooperate to load Cdc45 onto the origin.

Abstract #2

Cdc6p and the origin recognition complex (ORC) are essential for assembly of a pre-replicative complex (preRC) at origins of replication, before the initiation of DNA synthesis. In the absence of Cdc6p, cells fail to initiate DNA replication and undergo a “reductional” mitosis, in which the unreplicated chromosomes are randomly segregated to the spindle poles. We show here that the cells harboring a mutation in the essential Cdc6p Walker A-box arrest in late mitosis, probably at anaphase. This cell cycle block requires either the three Cdc28p phosphorylation sites within the N terminus of Cdc6p or a short region (aa 8–17) that contains a Cy (Cyclin) interaction sequence. These same two Cdc6p mutants that allow a reductional mitosis are defective in binding Cdc28p kinase. In addition to Cdc6p, ORC also binds to cyclin-dependent kinases (CDKs). Interestingly, Sic1p, a CDK inhibitor protein, blocked the S phase-specific Cdc28p-Cln5p kinase from interacting with ORC, but did not prevent the G1-specific Cdc28p-Cln2p kinase-ORC interaction. We suggest that ORC, Cdc6p, and Sic1p bind to different CDKs in a cell cycle-dependent manner to temporally regulate events that (i) allow preRC formation after mitosis, (ii) prevent mitosis before DNA replication can occur, and (iii) promote initiation of DNA replication.

Glossary of Terms:

1. BRCA COOH-terminus repeat (BRCT domain):

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2. S-Cdk:

S-phase Cyclin Dependent Kinase

3. Walker A Box:

A motif (GxxGxGKT/S) found in nucleotide-binding proteins along with the Walker B Motif (DD/E). Important for nucleotide binding.

4. Cy-interaction sequence:

Cyclin-binding motif. Found in substrates of CDK. The sequence is bound by the cyclin, which is complexed with a cyclin-dependent kinase (Cdk). The Cdk then phosphorylates Cy-interaction-sequence-containing protein.