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PDIP46 is Involved in Replication Timing Control

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PDIP46 Is Involved in Replication Timing Control

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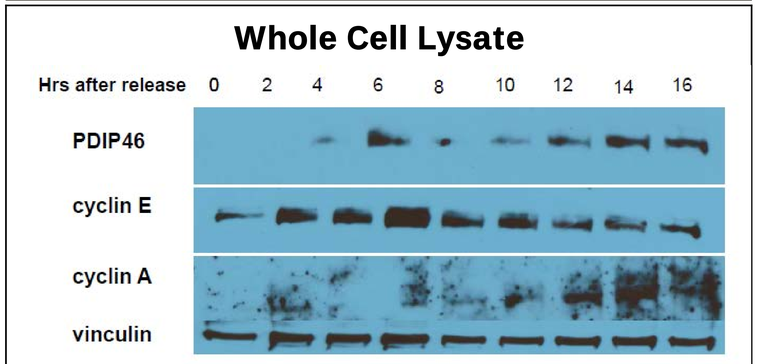
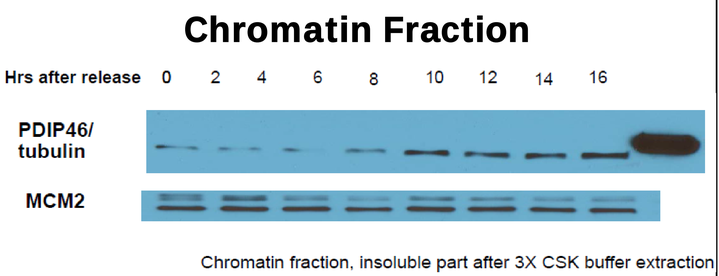
BACKGROUND

1. PDIP46 (SKAR, POLDIP3) was discovered through its interaction with the p50 subunit of human DNA polymerase δ.
2. Singly primed M13 assay revealed that PDIP46 is a potent activator of Pol δ activity. It also stimulates primer extension by Pol δ through template with complex hairpin structure.
4. Last year, we reported that PDIP46 increases the fidelity of Pol δ.
3. PDIP46 also interacts with PCNA via multiple copies of a novel PCNA binding motif, the APIMs.

PDIP46 Osillates with Cyclin E



A549 wild type cells were synchronized at G1/S border with double-thymidineblock. Hrs indicates time after release.

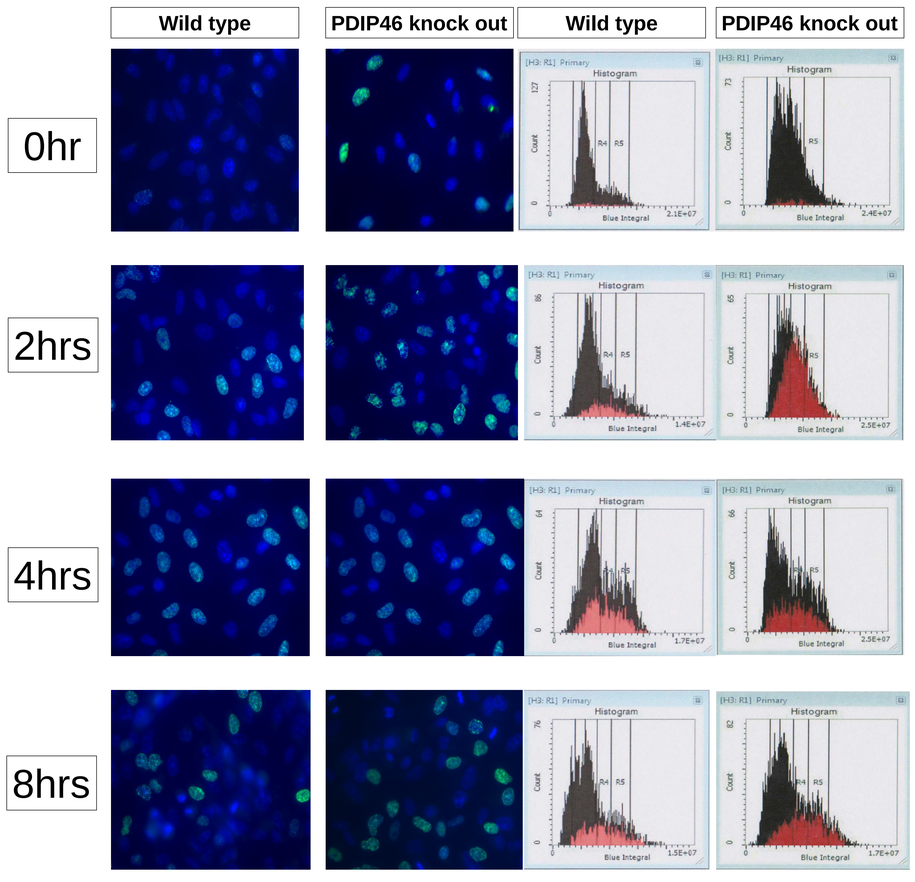


Chromatin fraction, insoluble part after 3X CSK buffer extraction

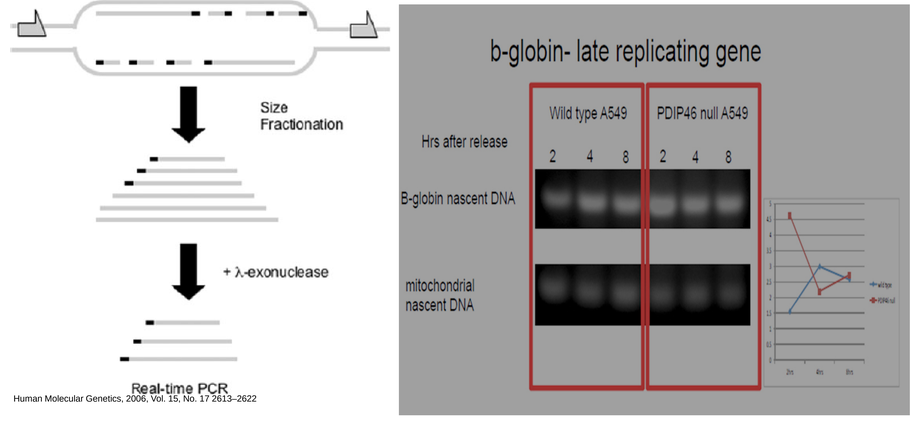
Colocalization of PDIP46 with BrdU Labeled Nascent DNA

Replication Timing Is Deregulated in PDIP46 Knock Out A549 Cells

A549 wild type and PDIP46 knock out cells were synchronized at G1/S border with double-thymidine block. After release, cells were pulse-labeled with 50uM of BrdU and subsequently stained with anti-BrdU antibody. Nuclei were counter-stained with DAPI. Spatial-temporal patterns and BrdU incorporation/cell cycle are analyzed with fluorescent microscopy and laser scanning cytometry respectively.

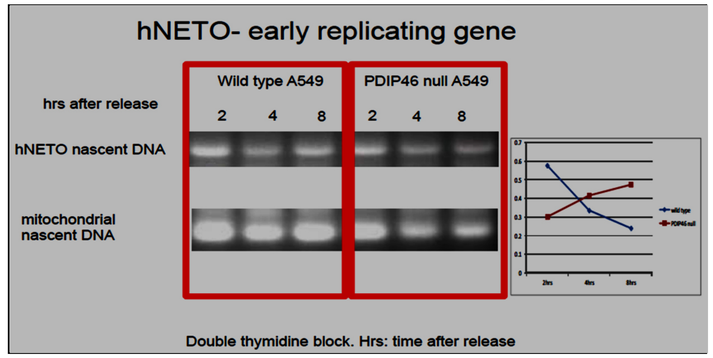


Nascent DNA Abundance Assay – beta-globin (late replicating gene)



Human Molecular Genetics, 2006, Vol. 15, No. 17 2613–2622

Nascent DNA Abundance Assay – hNETO (early replicating gene)



Mechanisms