

MECHANISMS OF CELL CYCLE PROGRESSION IN NORMAL AND CANCER CELLS

In my group we aim to understand how cell division works in both normal and cancer cells. We use biochemistry, molecular genetics and automated live-cell fluorescence imaging in human cells for this purpose. We are focussing on three larger biological processes in the cell cycle: i) how cells decide to enter mitosis or stay in G₂ phase; ii) how cells use regulated protein destruction to exit mitosis and divide successfully and iii) how cells that exit mitosis re-initiate DNA replication. Increasingly, we are also studying the mechanisms by which cells respond to insults or mistakes in G₂ or mitosis, which could be critical for cancer therapy.

Mitosis: Activation of cyclin B₁-CDK₁ and other mitotic kinases Cells should delay mitotic entry when the conditions are unfavourable for safe division, such as after DNA damage, or when cells are too small. The decision to enter mitosis is controlled by signalling pathways that direct the activation of the principal mitotic kinase, cyclin B₁-CDK₁. Cyclin B₁-CDK₁ activity is intensely regulated by changing protein levels, intracellular localisations and intrinsic activities of the WEE₁/MYT₁ inhibitory kinases and CDC25 activating phosphatases. We aim to gain further insights into the mechanisms that trigger abrupt cyclin B₁-CDK₁ activation in late

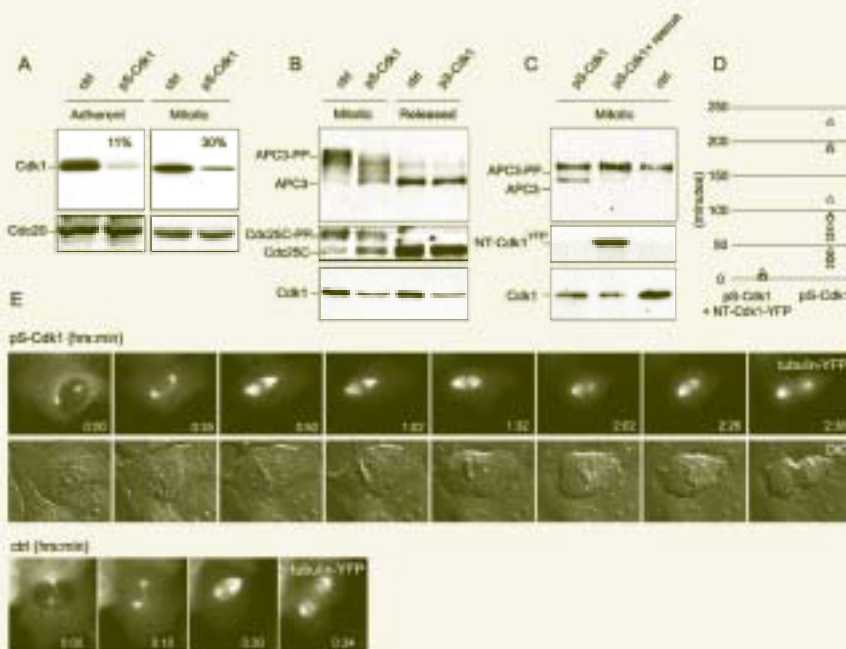


Figure 4: Distinct requirement of Cdk1 for mitotic entry and mitotic progression in human cells (A) Cells selected for Cdk1 shRNA expression were synchronized in G₂/M by thymidine release and mitotic cells were isolated. Separated G₂ and mitotic pools were analyzed for Cdk1 expression by Western blotting. Cdc20 protein levels serve as loading control. The percentage of remaining Cdk1 protein is indicated in the figure. (B) Cells collected by mitotic shake-off were lysed (lanes 1 and 2) or released from nocodazole and incubated in fresh medium for 3 h, recollected, and lysed (lanes 3 and 4). Differences in mitotic phosphorylation shift of APC₃ (human Cdc27 ortholog) and Cdc25C, depending on the Cdk1 levels, are shown (lanes 1 and 2). (C) The impaired phosphorylation of APC₃ in Cdk1-attenuated mitotic cells (lane 1) was rescued by coexpression of a Cdk1-YFP construct containing a silent mutation in the RNAi targeting region (lane 2). Lane 3 are mitotic cells transfected with a control shRNA, revealing normal endogenous Cdk1 levels. (D) Distribution of metaphase duration, measured as time between chromosome alignment at the metaphase plate and onset of sister chromatid separation, in Cdk1 RNAi cells (right) or Cdk1 RNAi cells rescued by coexpression of non-RNAi-sensitive Cdk1-YFP (left). (E) Time-lapse microscopy analysis of mitotic progression after entry with normal or impaired Cdk1 levels. Bottom panels are consecutive images of tubulin-YFP in a pS-control cell in mitosis; top panels show delayed chromosome alignment (frames 2 and 3) and stalled metaphase (frames 4–6) after Cdk1 shRNA. From 'Cyclin B₁-Cdk1 activation continues after centrosome separation to control mitotic progression.' (2007) Lindqvist A, van Zon W, Karlsson Rosenthal C, Wolthuis RMF. *PLoS Biology*:e123.



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Publications

Van Zon W, Ogink J, ter Riet B, Medema RH, te Riele H, Wolthuis RMF. *The APC/C recruits cyclin B₁-Cdk1-Cks in prometaphase before D box recognition to control mitotic exit.* *J. Cell Biol.* 2010;190:587-602

Voets E, Wolthuis RMF. *MASTL is the human orthologue of Greatwall kinase that facilitates mitotic entry, anaphase and cytokinesis.* *Cell Cycle* 2010;9:3591-3601

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G₂ and the parameters of an 'activation threshold' for mitosis (figure 4). Various other kinases and phosphatases participate in controlling the positive feedback loop that leads to cyclin B₁-CDK₁ activation and the G₂-M transition. A recent project led to the identification a novel human orthologue of the Greatwall kinases, MASTL. MASTL may reduce the mitotic entry threshold and drive mitotic entry of G₂ cells by inhibiting PP₂A activity. By determining the mechanisms that direct mitotic entry, we aim to understand how normal and cancer cells may differ in their decisions to enter mitosis or stay in G₂ phase.

Mitotic Exit: coordination of events by protein destruction Once cells are in mitosis, they need to ensure that all the duplicated chromosomes are attached to the mitotic spindle. This is safe-guarded by the mitotic *Spindle Checkpoint*, which inhibits another key enzyme of our interest, a large ubiquitin ligase called the Anaphase-Promoting Complex (*APC/C*) and its activator CDC₂₀. Mitotic entry first requires accumulation of regulatory proteins, such as cyclin B₁, but these proteins must be destroyed again at distinct, consecutive time points for correct cell division (figure 5). We found that degradation of cyclin A and Nek₂A, followed by loss of cyclin B₁, GEMININ and SECURIN, coordinates mitosis and prepares cells for the next S phase. As such, precise regulation of protein destruction by the APC/C is essential to guarantee cell viability and genomic integrity.

An important question that remains is how the APC/C acts with CDC₂₀ to recognize a critical substrate at the right time in mitosis. Our recent work revealed new roles for APC/C-cooperating E₂ ubiquitin conjugating enzymes, mechanisms by which APC/C substrates are targeted for destruction, and roles of the spindle checkpoint in connecting mitosis to the following S phase. An important future research goal will be to understand how genetic modifications in cancer cells may impinge on critical steps in mitotic progression and mitotic exit. Furthermore, we are trying to resolve the factors that determine the fate of cells that are arrested in mitosis as a result of prolonged spindle checkpoint activity. It is anticipated that answers to these questions could create exciting opportunities to improve anti-cancer therapies.

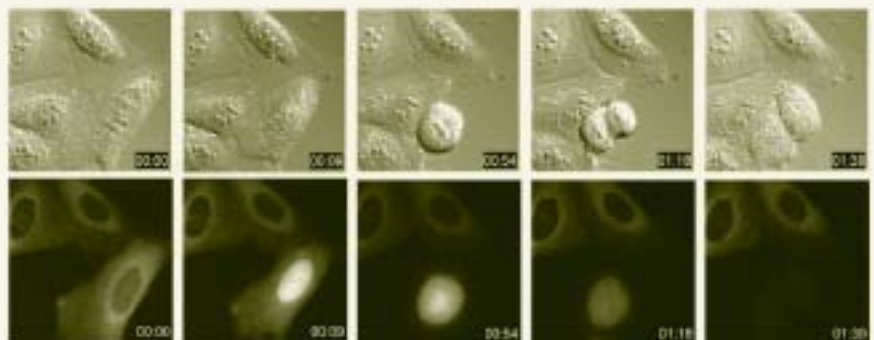


Figure 5: Measuring APC/C activity by time-lapse fluorescence microscopy of Cyclin B₁ destruction A plasmid encoding for fluorescent cyclin B₁ was injected into G₂-phase cells, a few hours before they entered mitosis. The top panel shows a cell undergoing mitotic division, followed over time by differential interference contrast (DIC). The bottom panel shows the localisation and quantitative levels of cyclin B₁ in the fluorescence channel of the same cells. The cell undergoing division rapidly degrades cyclin B₁, as measured by a decrease in fluorescence signal. This assay allows the quantitation of ubiquitin-dependent protein destruction in live cells undergoing division and was first established in the laboratory of Jon Pines, Gurdon Institute, Cambridge, UK.