Spatial positive feedback at the onset of mitosis

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***Short Abstract* — The Cdk1:Cyclin-B1 complex, at the heart of mitosis, is embedded within two interlinked positive feedback loops that generate a bistable switch. The spatial distribution of the Cdk1:Cyclin-B1 is dynamically regulated: at the onset of mitosis Cdk1:Cyclin-B1 translocates to the nucleus in a switch-like, abrupt manner. We hypothesized that spatial regulation of the Cdk1:Cyclin-B1 complex may be coupled to the control of its activation. By combining mathematical modeling with quantitative single cell imaging we discovered that a spatial-positive feedback triggers the nuclear translocation of active Cdk1:Cyclin-B1. When feedback regulation is compromised, Cdk1:Cyclin-B1 import is delayed and the normal timing of mitotic events is disrupted resulting in a sluggish and variable completion of mitosis. Together, these studies show that coupling spatial feedbacks to the activation feedback loops in the cascades that govern mitosis promotes robust unidirectionality, insulation and synchronicity of cell division.**

***Keywords* —** Spatial feedback, positive feedback, bistability, spatial-temporal regulation, mitosis

EXTENDED ABSTRACT

A. Introduction

Mitosis is unquestionably one of the most spectacular events in cell biology, as it brings unparalleled morphological changes in the cell. The Cyclin dependent kinase 1 (Cdk1) and its regulatory sub-unit (Cyclin-B1) are at the heart of most of these mitotic changes. The activation of the Cdk1:Cyclin-B1 complex results from two interlinked positive feedback loops: Cdk1:Cyclin-B1 activates its own activator, the phosphatase Cdc25, and is in turn inhibited by its own inhibitor, the Wee1 kinase [1]. In addition, a negative feedback loop involving the APC complex, results in Cyclin-B1 degradation. This topology results in a bistable activation of the Cdk1:Cyclin-B1 complex, which oscillates between low and high activity during interphase and mitosis, respectively [1]. The spatial distribution of the regulatory network that embeds Cdk1-Cyclin-B1 is also tightly controlled throughout each division cycle [2]. Wee1 is mostly a nuclear protein while Cdc25 shuttle between cytoplasm and nucleus during interphase and translocates to the nucleus in mitosis. The Cdk1-Cyclin-B1 complex localization is also dynamically regulated. During interphase shuttles between cytoplasm and nucleus with a very rapid nuclear export rate, whereas at the onset of mitosis, the active complex translocates to the nucleus in a very rapid, abrupt manner. The switch like nature of the Cdk1:Cyclin-B1 nuclear import made us hypothesized that its spatial regulation might be coupled to the control of its activation. In other words, is the switch-like nature of the Cdk1:Cyclin-B1 nuclear import an in-build spatial component that confers robustness to Cdk1:Cyclin-B1 bistable switch?

B. Results

We approached this question by combining mathematical modeling with quantitative single cell imaging studies in HeLa cells and found that a spatial-positive feedback triggers the translocation of active Cdk1:Cyclin-B1 from the cytoplasm to the nucleus, where a supra-threshold increment of localized nuclear Cdk1:Cyclin-B1 results in more Cdk1:Cyclin-B1 import. This newly discovered spatial component of the Cdk1:Cyclin-B1 regulatory network confers robustness to the bistable switch architecture. When feedback regulation is compromised by pharmacological or genetic means, that either modulate Cdk1:Cyclin-B1 network components activation or their spatial distribution, Cdk1:Cyclin-B1 nuclear import is delayed and becomes very graded. Importantly, the fidelity of the timing of mitotic events is disrupted rendering completion of mitosis a more variable, sluggish process. The timing of nuclear envelop break down (NEB) was intrinsically linked to the absolute concentration of Cdk1:Cyclin-B1 in the nucleus, and was notably delayed when the switch like nature of Ckd1:Cyclin-B1 import was perturbed, showing that spatial-temporal regulation of the complex is invaluable for timing of mitotic events. Importantly, positive feedback regulation at this level can explain our observations of insulation in the timing between different mitotic events in normal proliferating cells.

C. Conclusions

Together, our studies strongly suggest that coupling of spatial positive feedback regulation to activation control in the cascades that govern mitosis promotes robust unidirectionality, insulation and synchronicity of cell division.

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