

The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit

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Tissue homeostasis in metazoans is regulated by transitions of cells between quiescence and proliferation. The hallmark of proliferating populations is progression through the cell cycle, which is driven by Cyclin-dependent kinase (CDK) activity. Here we develop a live-cell sensor for CDK2 activity and unexpectedly found that proliferating cells bifurcate into two populations as they exit mitosis. Many cells immediately commit to the next cell cycle by gradually building up CDK2 activity from an elevated level, while the others lack CDK2 activity and enter a transient state of quiescence. This bifurcation is directly controlled by the CDK inhibitor p21 and is regulated by mitogens during a restriction window at the end of the previous cell cycle. Thus, cells decide at the end of mitosis to either start the next cell cycle by immediately building up CDK2 activity or to enter a “transient G0 state” by suppressing CDK2 activity.

I. PURPOSE

THE number of cells in each tissue must be tightly controlled during development and throughout adult life. Much of this remarkable feat of balanced tissue homeostasis is achieved by switching cells between two different states: proliferative and quiescent. However, the detailed mechanisms that control the transitions between these two states are still poorly understood. In one prominent model, cells are thought to commit to the cell cycle at a "restriction point" in late G1 [1], defined as the time when the presence of mitogens is no longer necessary to complete the cell cycle. Since cycling cell populations are asynchronous, biochemical analysis of this commitment mechanism cannot readily be performed, and synchronization methods may trigger stress responses or alter the natural behavior of cells. We therefore developed a fluorescent sensor to monitor the change in CDK2 activity as single asynchronously cycling cells make a proliferation-quiescence decision. Using time-lapse microscopy and customized cell tracking, we are able to track CDK2 activity in MCF10A, Hs68, and Swiss 3T3 cells over several cell cycles.

II. RESULTS

We discovered that cycling cells cross a bifurcation point at the end of mitosis where they either immediately build up CDK2 activity and start the next cell cycle (CDK2^{inc} cells) or enter a transient G0 state characterized by low CDK2 activity (CDK2^{low} cells). In the continued presence of

mitogens, many cells in the transient G0 state will re-enter the cell cycle by increasing CDK2 activity. However, if mitogens are withdrawn while CDK2 activity is still low, cells assume a prolonged G0 state. CDK2^{inc} cells have a relatively short and invariant G1 phase of approximately 5-7 hr duration. In contrast, CDK2^{low} cells spend variable amounts of time in G0 that lengthens the total cell cycle time and explains most of the observed variability in inter-mitotic time. We further show that the path a cell takes in the bifurcation is directly controlled by the CDK inhibitor, p21. We demonstrate the central role of p21 by showing 1) that the CDK2^{inc} vs CDK2^{low} decision closely correlates with the endogenous level of p21 in single cells, 2) that p21-null MCF10A cells lack the G0 state and 3) that rapid induction of p21 protein immediately prior to mitosis causes cells to enter G0 upon completion of mitosis.

We next tested the timing of cell cycle commitment by removing mitogens and following the cellular response. We demonstrate that CDK2^{inc} cells are insensitive to mitogen withdrawal at all times during an ongoing cell cycle. We also show that the last point in the cell cycle where mitogen withdrawal promotes entry into G0 at the end of mitosis is approximately five hours before the end of the preceding cell cycle. The effect of mitogen withdrawal is not instantaneous but rather displays significant cell-to-cell variability over an eight hour window before mitosis during which time the probability of entering quiescence after mitosis steadily increases. This shows that the integration of mitogenic signals at the end of the preceding cell cycle is critical for the ultimate decision of which path a cell will take.

III. CONCLUSIONS

Together, our results argue that cells integrate mitogenic inputs during a newly identified restriction window at the end of the previous cell cycle (R_1) and then bifurcate into CDK2^{inc} or CDK2^{low} states upon completion of mitosis. Only the cells in the CDK2^{low} state experience a second restriction window (R_2) in which they can decide to re-enter the cell cycle by building up CDK2 activity. This second restriction window maps to the classic restriction point whose existence has been postulated since 1974.

REFERENCES

- [1] Pardee AB (1974) A restriction point for control of normal animal cell proliferation. *PNAS* **71**, 1286-1290.

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