Development

Changes in cell-cycle rate drive diverging cell fates

Changes in the concentrations of transcription factors drive cells to commit to different fates. These concentration changes can be achieved through mechanisms such as transcriptional activation or protein degradation. However, the role of the cell cycle in regulating transcription factor levels and cell fate remains underappreciated. During differentiation, cells often divide before committing to divergent fates, enabling the generation of new cells and maintenance of a self-renewing progenitor population. Differences in the proliferation rate of these populations emerge as their fates diverge. Thus, it remains challenging to separate the role of the cell cycle in establishing cell identity from cell identity itself.

In 2013, to address how cell cycle influences the divergence of myeloid and lymphoid cells from a progenitor population, Kueh et al. examined how expression of the pioneer transcription factor PU.1 generated distinct cell fates in mice. At high levels, PU.1 positively regulates its own transcription to maintain myeloid identity in macrophages. Simultaneously, PU.1 reduces the cycling rate of myeloid cells. Increased transcription of *Spi1*, the gene encoding PU.1, might drive accumulation of PU.1 in progenitors, reinforcing expression and commitment to the myeloid fate. Alternatively, lengthening of the cell cycle may enable PU.1 to accumulate and drive commitment. To examine these competing models of cell-fate regulation, Kueh et al. established a transgenic mouse model with a bicistronic cassette including a fluorescent marker, PU.1–GFP. As PU.1–GFP levels mirror expression of endogenous PU.1, the rate of PU.1 synthesis can be monitored as cells transition from progenitors into B cells and macrophages.

Through time-lapse microscopy, the team observed differences in synthesis rates between emerging populations of B cells and macrophages, as expected. However, synthesis rates of PU.1 were similar in emerging and established macrophages, indicating that the increase in synthesis does not distinguish cells transiting to a macrophage identity. Instead, levels of PU.1 increased in emerging macrophages as they lengthened their cell cycle, enabling the accumulation of PU.1. Similarly, synthetically lengthening the cell cycle increased PU.1 accumulation and differentiation to the macrophage identity. Addition of a competitive inhibitor enabled the accumulation of native PU.1 and PU.1–GFP while inhibiting the activity of PU.1. Loss of PU.1 activity reduced macrophage differentiation, demonstrating that both cell-cycle lengthening and PU.1 activity are required for cell-fate commitment.

Combined, these data demonstrate that positive feedback converging on PU.1 drives commitment towards the macrophage identity via cell-cycle lengthening, not simply through transcriptional activation. In alignment with this model, overexpression of PU.1 increased commitment but did not increase synthesis of endogenous PU.1. Instead, endogenous PU.1 accumulated as the cell cycle lengthened. In modelling this cell-fate transition, Kueh et al. showed that feedback from cell-cycle lengthening stabilizes cell identity and reduces fate switching. Together, their work highlights how a native differentiation system harnesses the cell cycle to implement feedback, driving cells towards a stable cell fate via changes in protein levels.

The effect of the cell cycle on protein levels varies by protein half-life. The long half-life of PU.1 (approximately 70 h) compared to the average cell cycle length (approximately 24 h) makes it particularly sensitive to changes in proliferation rate, whereas less stable proteins will be minimally affected by changes in the rate of division. As the half-lives of many matrix proteins, such as collagens, exceed the average time between cell divisions, the levels of matrix proteins will be substantially affected by changes in proliferation.

Over the past decade, cell-fate transitions have been extensively studied by transcriptional profiling in bulk and in single cells using single-cell RNA-sequencing. Under the hypothesis that activation or loss of specific gene regulatory networks directs cell fate, many studies have sought to identify transitions by transcriptional signatures. Although such work has captured mRNA profiles in exquisite depth, these studies remain blind to changes in protein concentration that can drive changes in cell identity.

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As highly proliferative cells reprogramme at higher rates, it is interesting to speculate whether proliferation can facilitate transitions by expediting the dilution of stable proteins associated with the donor cell identity. Potentially, increased cellular division can speed up the dilution of stable proteins, facilitate cellular remodelling and broadly accelerate the rate and frequency of cell-fate transitions. Although transcriptional activation and direct regulation of proteins remain important, Kueh et al. highlight how the cell cycle indirectly influences protein concentrations to stabilize cell-fate trajectories, suggesting a mechanism to support other native and pathological transitions.

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Competing interests
The author declares no competing interests.

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Related article: Babos, K. N. et al. Mitigating antagonism between transcription and proliferation allows near-deterministic cellular reprogramming. *Cell Stem Cell* **25**, 486–500 (2019)