Reprogramming

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Modulating RNA condensates to control cell fate

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RNA sequestration in P-bodies stabilizes cell-fate transitions by repressing past identities — a process that can be harnessed to control cell fate.

The ability to control cellular identity is a central goal in biotechnology. In a paper in *Nature Biotechnology*, Pessina et al.[1] investigate how developmental cell-fate transitions are influenced by RNA sequestration in cytoplasmic processing bodies (P-bodies). Comparing RNA profiles in the cytoplasm and in P-bodies for six cell types, including embryonic and differentiated states, the authors found that P-bodies are enriched for transcripts of the previous cell state across vertebrates. At a mechanistic level, cell-type-specific localization of RNA can be regulated by microRNA (miRNA). By manipulating RNA sequestration in P-bodies, the authors converted human embryonic stem cells into totipotent cells or germ cells. Future work could apply this localization strategy to enhance conversion to other cell states of interest or to improve temporal control of synthetic circuits.

During development, waves of progenitor cells transition to new cell states[2–4]. Activation of lineage-specific genes allows cells to initiate different gene regulatory networks, supporting the cells' adoption of new identities. Simultaneously, regulatory networks from the preceding cell fate are repressed through transcriptional and post-transcriptional mechanisms. Models of cell-fate transitions often approximate cell states and fates using transcriptional profiles, integrating vast amounts of data from single-cell RNA sequencing atlases[5,6]. Potentially, such models may generate a comprehensive map of how cells transition identities, offering insight into fragile points exploited by disease and suggesting interventions to improve therapies. One challenge to this vision is an increasing awareness of the role of RNA processing and translational regulation in controlling cellular processes and cell fate[7,8].

Although profiles of RNA offer snapshots of cell state, regulation of RNA properties beyond abundance can control cell identity, confounding models based on RNA profiles alone. For example, processing of RNA during and after transcription alters the levels of proteins produced from transcripts. Subcellular localization of RNA transcripts into condensates can be a timely, effective mechanism for modulating the translation of mRNA into protein and thus cell state. Ribonucleoprotein complexes, composed of RNAs and associated regulatory proteins, can assemble into higher-order granules such as P-bodies in the cytosol. Initially thought to be hubs of RNA decay, P-bodies may primarily act to translationally repress localized transcripts. Recent work reveals that P-bodies contribute to stem cell potency, and dissolution of P-bodies releases repression of cell-fate regulators, leading to changes in identity[9]. However, the broader role of P-bodies during development and the mechanisms governing cell-type-specific localization of biomolecules to P-bodies were poorly understood.

To investigate the role of RNA sequestration in cell-fate transitions, Pessina et al. in their new study[1] examined the RNA profiles of P-bodies in several vertebrate embryonic stem (ES) and derived cell types. The authors applied fluorescence-activated particle sorting to isolate P-bodies and perform downstream sequencing. Compared to cytoplasmic RNA profiles, P-body-enriched RNAs were translationally repressed but did not show differences in half-life, truncation or poly(A) sequence length. This result agrees with those of prior studies highlighting a sequestration function for P-bodies. Strikingly, comparing the cytosolic and P-body-enriched profiles across six human cell types spanning developmental stages, Pessina et al.[1] found that P-body contents reflected the cytoplasmic transcripts of the prior cell state. This pattern also appeared in mouse and chicken ES cell states, suggesting a regulatory mechanism conserved across vertebrates. Dissolution of P-bodies in ES cells via knockdown or knockout of the RNA helicase DDX6 — essential for P-body assembly[10] and previously shown to affect cell identity[9] — induced the expression of markers associated with the two-cell embryo, an earlier developmental state. These findings suggest that P-bodies translationally repress transcripts instructive for the prior cell state to facilitate developmental transitions (Fig. 1a).

This model requires a mechanism for cell-type-specific targeting of transcripts to P-bodies. Exploring the P-body RNA profiles, Pessina et al. observed enrichment of targets of the Argonaute (AGO) protein family, of specific miRNAs and of targets of those miRNAs. Thus, they hypothesized that regulation by miRNAs contributes to cell-type-specific sequestration in P-bodies. To confirm P-body sequestration via miRNA sequence, the authors demonstrated altered P-body RNA profiles upon AGO2 knockout, treatment with a miRNA inhibitor or suppression of distal polyadenylation, which shortens the 3′ untranslated region containing miRNA target sites. To directly manipulate miRNA targeting, they constructed a synthetic *Nanog* transcript containing wild-type or mutant target sites for the miRNA let-7, a regulator of stem cell differentiation. In *Nanog*-knockout mouse ES cells, the wild-type let-7 construct accumulated in P-bodies and decreased self-renewal capacity compared to the mutant let-7 transcript, matching predictions. To expand the engineering of cell identity via P-bodies, the authors knocked down *DDX6* in primed human pluripotent cells during induction of the primordial germ cell fate, finding that this enhanced conversion rates threefold (Fig. 1b). Thus, insights into the mechanism of endogenous P-body sequestration can be leveraged to modulate cell fate.

Although Pessina et al.[1] effectively perturbed P-bodies to engineer cell state in ES lineages, other cell-fate conversions may not be manipulatable via P-bodies. P-body RNA profiles reflect cytoplasmic profiles of the immediately preceding identity, and this overlap diminishes over prolonged culture. Therefore, P-body dissolution is unlikely to enhance conversion between distant lineages, such as terminally differentiated cells and ES cells. Nevertheless, increasing populations of totipotent cells through the method described in this study may be

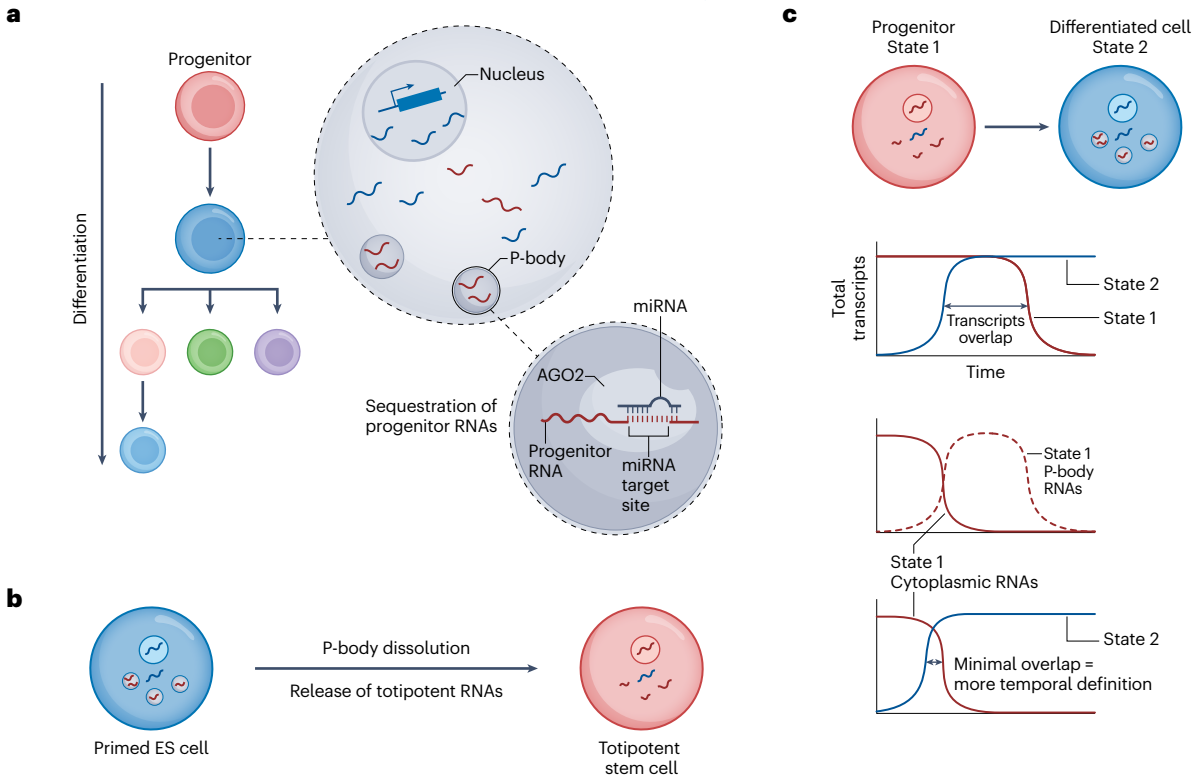

Fig. 1 | RNA sequestration stabilizes cell-fate transitions by repressing past identities. a, During development, cells differentiate from pluripotent cells to downstream progenitors and somatic cell types. As they differentiate, RNAs from the preceding cell fate localize to P-bodies in a process mediated by miRNA sites, miRNAs and AGO2. **b**, Dissolution of P-bodies allows primed ES cells to transition toward a totipotent stem cell state. Storage of transcripts in P-bodies may facilitate plasticity during cell-fate transitions, allowing cells to revert to previous states. **c**, Transitions in cell state may become more temporally defined by sequestration of mRNA transcripts into P-bodies. Although the overall amounts of transcripts from state 1 and state 2 overlap (top), sequestration of mRNAs in P-bodies may render a fraction of mRNAs unavailable to translational machinery in the cytoplasm during transition (middle). The resulting profile of cytoplasmic mRNAs – and their protein products – more quickly matches that of state 2, as there is less time in which the transcripts of both states overlap in the cytoplasm (bottom). Thus, sequestration may speed the transition of cell fates.

useful for both studying development and generating cells for potential therapeutic applications.

Additionally, the role of RNA sequestration in the context of native development remains unclear. By acting as a storage depot, P-bodies may allow cells to rapidly alter their state. Localization to P-bodies may facilitate a faster change in cytoplasmic RNA profiles from the initial to subsequent state (Fig. 1c). As the authors suggest, storage of RNAs from the previous state may confer plasticity, helping to balance pluripotency and differentiation in stem cells. Alternatively, the transit of endogenous transcripts through P-bodies may be unidirectional, with negligible reversible transcript release. In this model, P-bodies could act as a cellular 'dust bin' for collection and eventual degradation. Further investigation of the role of P-bodies in native developmental programs, as well as comparison to other state changes such as those during stress response or disease, may reveal how P-bodies are used across diverse cell types and states.

Overall, this study reveals one avenue through which cells modulate RNA localization to control cell state. The function of P-bodies illustrates the fine spatiotemporal complexity of gene regulation, highlighting cellular processes beyond control of steady-state levels of biomolecules. As our understanding of endogenous regulatory mechanisms increases, our ability to coopt them improves, as Pessina et al. demonstrate[1]. Future work could engineer synthetic gene circuits with miRNA target sites for localization to P-bodies. Such a strategy would leverage a native mechanism for enhanced temporal resolution to improve the performance of circuits controlling differentiation, multicellular patterning and expression dynamics. More broadly, engineering localization to condensates beyond P-bodies could expand the methods for and precision of engineered cell fates.

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Competing interests

The authors declare no competing interests.