The Princess and the P: Pluripotent Stem Cells and P-Bodies

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In this issue of Cell Stem Cell, Di Stefano et al. (2019) identify the RNA helicase DDX6 as a regulator of stem cell pluripotency and differentiation. DDX6 depletion leads to P-body disruption, which, in turn, inhibits or promotes stem and progenitor cell differentiation, depending on their cellular context.

Processing bodies (“P-bodies”) are membraneless cytoplasmic mRNP granules that form via liquid-liquid phase separation. P-bodies were originally reported as cytoplasmic sites of mRNA degradation that contain the key enzymes responsible for decapping and 5’ to 3’ degradation of mRNA molecules (Sheth and Parker, 2003). However, since their original discovery, P-bodies have been found to carry out additional important functions including storage sites for mRNAs (Brenquez et al., 2005), cell signaling, stress regulation, and protein homeostasis (Zhang and Herman, 2019). The observation that P-bodies control both mRNA decay and long-term storage raises interesting questions regarding the regulation of these processes (Wang et al., 2018) and whether these functions might also be temporally separated (e.g., during different stages of development or cell differentiation).

In this issue of Cell Stem Cell, Di Stefano et al. (Di Stefano et al., 2019) report that P-bodies control stem cell exit from pluripotency, as well as adult progenitor cell differentiation, in a context-dependent manner. To search for potential regulators of pluripotency, the authors analyzed previous datasets of RNA binding proteins and identified the RNA helicase DDX6, an essential P-body component, as a key factor in the exit from pluripotency. Among the identified candidates, they found DDX6’s involvement in regulating pluripotency to be the most prominent, as well as conserved in mouse and human pluripotent cells. In human pluripotent cells, DDX6 depletion resulted in the complete dissolution of P-bodies, which caused the cells to be entrapped in a “hyper-pluripotent,” naïve-like state and prevented differentiation (Figure 1A).

Further analyses revealed the identity of several RNA transcripts that are kept translationally repressed inside the P-bodies, many of which encode for chromatin regulators.

Chromatin, composed of DNA, histone proteins, and additional structural molecules, regulates essentially all cellular processes including pluripotency. This occurs by changes in DNA methylation, post-translational modifications of histones, and chromatin compactness, all of which ultimately regulate gene expression. In pluripotent cells specifically, chromatin was shown to assume a unique, more open conformation, which contributes to stem cell function and potency (Schlesinger and Meshorer, 2019). Once liberated upon P-body disruption, mRNAs encoding for chromatin regulators are then free to produce functional proteins.

To test the functional consequences of increased translation of these chromatin regulators, Di Stefano et al. analyzed several chromatin features. Using ATAC-seq, they first probed chromatin accessibility across the genome and found over 7,400 sites that gained accessibility versus −4,000 where accessibility was lost. Not only does this demonstrate a global chromatin reconfiguration, but also a shift into a globally more open chromatin structure, supporting the idea that the cells become hyper-pluripotent. Consistent with the observation that loss of DDX6 and the ensuing P-body disruption lead to a hyper-pluripotent state, sites...
of increased chromatin accessibility were enriched with pluripotency-related binding motifs. Furthermore, mapping a specific histone modification associated with active enhancers (H3K27ac) across the genome, the authors found a significant increase in H3K27ac-marked enhancers including, once again, several pluripotency-related genes. However, a more complex pattern was observed for the heterochromatin-associated histone modification H3K9me3, which was prominently redistributed across the genome, with over a thousand regions gaining methylation and over a thousand regions losing methylation. In contrast, and somewhat unexpectedly, H3K4me3, another histone modification that mostly marks promoter and active regions, remained largely unaltered. To seek potential chromatin-related candidates mediating these effects, the authors searched for DDX6 targets that are both consistent among different cell types and showed increased translational rates upon DDX6 depletion. One of the identified proteins was the Jumonji-domain-containing lysine demethylase KDM4B, which specifically demethylates H3K9, explaining, at least partially, the redistribution of H3K9me3 marks across the genome.

So far, the results suggest that P-bodies are required for stem cell differentiation by arresting mRNA transcripts and either preventing or inhibiting their translation. To test whether this holds true for other, non-pluripotent stem cell types, Di Stefano et al. suppressed DDX6 in different lineage differentiation trajectories of the three germ layers. Neural progenitor cells behaved similarly to pluripotent cells, displaying differentiation resistance when induced to differentiate in the absence of DDX6 (Figure 1B). Intestinal stem cells (ISCs) within intestinal organoids also showed similar behavior: knocking down Ddx6 led to an increased pool of Lgr5-positive ISCs, again suggesting that the stem cells within the organoids are refractory to differentiation (Figure 1B). However, when the authors tested mesodermal progenitors, things became more complicated and interesting. When Ddx6 expression was suppressed, both myoblasts and iPSC-derived mesenchymal stem cells displayed a remarkably increased propensity to differentiate into myotubes and chondrocytes, respectively (Figure 1C). This suggests that P-bodies may have different functions in different cell lineages, and/or that P-bodies are temporally regulated and the different stem cells the authors tested appear at different stages of development.

Intriguingly, a crucial role for DDX6 was recently identified in human development. Rare missense mutations in DDX6 were found in a developmental intellectual disability (Balak et al., 2019). Importantly, all identified mutations were found in DDX6 domains involved in RNA binding and helicase activity, resulting in P-body disruption in vivo. The fact that the human phenotypes associated with DDX6 mutations are mostly brain and face related (Balak et al., 2019) does not rule out potentially important roles for DDX6/P-bodies in other organs and tissues, but rather suggests that our nervous system is more susceptible to DDX6 dysregulation, or that it reflects earlier manifestations. Although it is clear that P-bodies are important for development, their exact roles in the various stages of development and in different tissues remain to be studied.

Several other interesting questions remain. Is DDX6 depletion tantamount to P-body disruption or does DDX6 have other, non-related, functions? The authors began to address this by depleting other DDX6-interacting P-body components. Depletion of LSM14A, which also results in P-body disruption, phenocopied the hyper-pluripotent phenotype of DDX6 depletion, while depletion of DCP1A, a DDX6-interacting protein, which has no effect on P-bodies, also had no effect on the cells. While this simple experiment nicely demonstrates the importance of P-bodies, it does not rule out other possible functions for DDX6 or address this question in other non-pluripotent stem cell systems. Interestingly, a recent study demonstrated that knockout of Ddx6 in mouse embryonic stem cells leads to the translational upregulation of miRNA targets without affecting mRNA stability, effectively mimicking ESCs deficient for miRNA processing (Freimer et al., 2018). The next step would be to analyze miRNAs and their potential targets in a human stem cell system.

Finally, since loss of P-bodies was observed in the maturation process of mouse oocytes (Fliemr et al., 2010), it would be intriguing to test whether the hyper-pluripotent state the authors uncovered following P-body dissolution resembles the previously identified rare and transient totipotent “2-cell-like” state in mouse ESCs (Macfarlan et al., 2012).

Overall, this elegant study by Di Stefano et al. identifies a yet unrecognized role for P-bodies in stem cell pluripotency and differentiation and opens the door for elucidating the relationship between P-bodies and other cytoplasmic granules and chromatin organization and function.
REFERENCES


