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A Pluripotency Platform for Prdm14

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The tripartite network of *Prdm14, Blimp1*, and *AP2γ* is essential for the important process of germ cell specification, but their precise molecular mechanisms of action remain lacking. Tu and colleagues (2016) report in *Nature* that the transcriptional co-repressor CBFA2T2 is an essential interactor protein regulating PRDM14 function, shedding light into the mechanisms directing germline formation and pluripotency.

The question of how a few cells are set aside in the developing embryo in order to generate the germline is of central importance in biology. The founder cells that give rise to the germ cells are called primordial germ cells (PGCs). In mice, PGCs first appear between embryonic day (E) 6.25 and E7.25 as a small subpopulation of around 30–40 cells in the posterior epiblast located proximally to the extraembryonic ectoderm. These cells are unique in their developmental properties. They are set apart from the embryonic program to transmit the genetic and potentially epigenetic information to the new generation. They are highly specialized and unipotent and resist the differentiation program that occurs in the rest of the embryo. They also re-induce the pluripotency gene network and ultimately have the potential to generate a totipotent zygote by undergoing extensive epigenetic changes, including global loss of the repressive DNA methylation and acquisition of active histone marks (reviewed in Surani et al., 2007). Three transcriptional regulators form a tripartite network that is critical for establishment of the PGCs: *Blimp1*, *Ap2γ*/*Tfap2c*, and *Prdm14* (Magnúsdóttir et al., 2013; Ohinata et al., 2005; Yamaji et al., 2008). In a recent issue of *Nature*, Tu and colleagues (2016) sought to investigate further the fundamental question of PGC specification, focusing on PRMD14, which has the earliest specific expression in the germ cell lineage (Yamaji et al., 2008).

Prdm14 is exclusively expressed during three crucial developmental windows characterized by reprogramming events: the 2- to 4-cell stage mouse preimplantation embryo, the inner cell mass of the early blastocyst, and during PGC development (Figure 1A) (Burton et al., 2013; Yamaji et al., 2008). In order to learn more about the function of PRDM14 in PGC development, Tu and colleagues (2016) identified interacting partners of PRDM14 using a proteomics screen in the human germ cell tumor line NCCIT. The top interactor was CBFA2T2, a co-repressor that they subsequently found to be highly correlated with PRDM14 in terms of chromatin binding, gene regulation, and function. They show that the two proteins co-localize broadly across the genome in both NCCIT and mouse embryonic stem cells (mESCs) and, as is the case for PRDM14, many targets of CBFA2T2 are genes involved in pluripotency (*Pou5f1*, *Klf4*, *Dax1*), lineage allocation (*Elf3*, *Cdx1*, *and Pitz2*) and chromatin modification (*Ehmt1*, *Dnmt3a*, *Dnmt3b*, *Dnmt3l*, *Tet2*, *Jarid 2*). However, very limited correlation with the Polycomb repressive complex was identified in contrast to previous reports in mESCs (for an extensive review on Prdm14, see Nakaki and Saitou, 2014). These results suggest that CBFA2T2 might act as a co-factor to PRDM14 in the regulation of PGC development.

In support of this, the authors determine that the ability of PRDM14 and CBFA2T2 to bind to chromatin each depends on the presence of the other factor and that the two factors exist in a large 600 kDa complex, suggesting the possibility for further interactors, which are likely to be functionally important for germ cell biology. Seven conserved amino acids predicted, based on studies of the CBFA2T2 homolog RUNX1T1 (Liu et al., 2006), to be required for

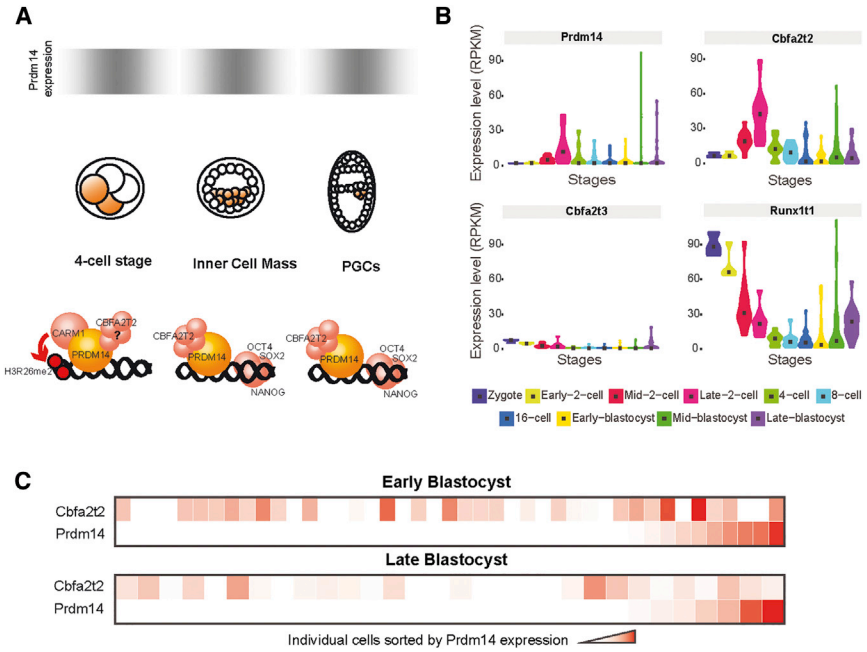

Figure 1. Proposed Mode of Action and Expression of PRDM14 and Its Functional Interactor CBFA2T2

(A) Prdm14 has a highly restricted expression pattern during development, as shown in the simplified schematic. It is expressed in waves that correlate with stages and cell types in which epigenetic and transcriptional reprogramming events occur: the 2- to 4-cell stage, the inner cell mass, and PGCs. A model of predicted PRDM14 molecular mechanism of action in these cell types is presented at the bottom.

(B) Violin plots showing expression of *Prdm14*, *Cbfa2t2*, and family members *Cbfa2t3* and *Runx1t1* during mouse preimplantation development. The single-cell RNA sequencing data used for this analysis is from Deng et al. (2014). *Cbfa2t2*, but not *Cbafa2t3* or *Runz1t1*, shows very similar expression dynamics to *Prdm14*.

(C) Co-expression of *Cbfa2t2* and *Prdm14* in single blastocyst cells, aligned according to level of *Prdm14* expression in early and late blastocysts (Deng et al., 2014). *Prdm14* is only enriched in a subpopulation of Inner Cell Mass (ICM) cells during the blastocyst stage, and *Cbfa2t2* expression is heterogeneous. Note that only some ICM cells express both *Prdm14* and *Cbfa2t2*, suggesting that in some ICM cells PRDM14 may be functioning together with CBFA2T2, whereas in others, alternative partners may exist. We thank Diego Rodriguez-Terrones for the analysis of expression data shown in Figures 1B and 1C.

oligomerization of CBFA2T2 were also found to be required for PRDM14 localization on chromatin, but not for interaction between CBFA2T2 and PRDM14. As PRDM14 is able to bind to DNA in a sequence-specific manner (Ma et al., 2011), the requirement of CBFA2T2 and specifically the requirement for CBFA2T2 oligomerization for PRDM14 chromatin binding is surprising and provides an example of a more active role for a co-repressor protein in transcription factor recruitment. One possibility is that the large CBFA2T2 complex is required for chromatin modification, which subsequently stabilizes PRDM14 and OCT4 on chromatin. This could be addressed through detailed biochemical analysis of the large CBFA2T2-containing complex.

Cbfa2t2 had previously been shown to be upregulated during PGC specification and iPS cell reprogramming. To verify the in vivo significance of CBFA2T2 activity in PGC specification, the authors show that *Cbfa2t2* knockout results in a strikingly similar phenotype to *Prmd14* knockout. *Cbfa2t2*−/− mice are infertile, and PGC specification and development is severely affected with a greater than 2-fold decrease in the number of PGCs observed as early as E8.75, very similar to the effects observed in *Prdm14* knockout mice (Yamaji et al., 2008). *Cbfa2t2*−/− mESCs displayed a failure to maintain pluripotency and proliferate in the absence of 2i conditions, again similarly to *Prdm14*−/− cells (Ma et al., 2011).

Tu and colleagues (2016) identify a strikingly similar correlation in terms of genomic binding, gene regulation, and function between PRDM14 and CBFA2T2 in both NCCIT and mESCs. These findings provide significant insight into the molecular mechanism of PRDM14 action and the regulation of ESC pluripotency and of germ cell fate specification. However, *Prdm14* is also expressed transiently at the 2–4 cell stage of mouse preimplantation development, a second period when epigenetic reprogramming occurs and totipotency is acquired and subsequently lost. PRDM14 is involved in lineage allocation in association with CARM1 at this stage (Burton et al., 2013). *Cbfa2t2* also shows a very similar expression pattern to *Prdm14* (Figure 1B) (Deng et al., 2014), with transient expression starting at the late 2-cell stage through to the 8-cell stage. Subsequently, like *Prdm14*, it reappears in a subpopulation of cells in the early blastocyst. However, single-cell mRNA sequencing data in the blastocyst revealed only a partially overlapping expression pattern of *Cbfa2t2* and *Prdm14* throughout the blastocyst stage, such that the subpopulation of cells expressing *Prdm14* do not always co-express *Cbfa2t2* (Figure 1C) (Deng et al., 2014). Thus, these results, taken together with the current study by Tu et al. (2016), which clearly demonstrates a dependence of PRDM14 on CBFA2T2, suggest a different functionality for PRDM14 in the two distinct populations of cells in the blastocyst, depending on whether or not they co-express CBFA2T2. In all, the work published by Tu and colleagues (2016) reveals a deeper understanding of the molecular mechanisms governing germ cell formation and cell fate of pluripotent cells.

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Right Place Right Time: Heterogeneity-Driven Organ Geometry

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How do plants make species-specific leaves and flowers with stereotypical size and shape? A new study in *Developmental Cell* finds that local spatiotemporal variability in cell growth rate is essential for robustness in organ geometry control, and reactive oxygen species act to suppress this local heterogeneity during organ maturation.

From tulip petals to human arms, size and shape are tightly regulated during organ development. This regulation is so consistent, species-specific organ geometry can be used to define taxonomic relationships. Even if organ growth is perturbed, inherent cellular responses can buffer the effects and stably maintain the robustness of organ geometry (Day and Lawrence, 2000). The mechanisms behind the robustness have long been elusive but are now starting to unfold.

Both in animals and plants, the final configuration of organs results from differential cellular growth rates that generate mechanical signaling between neighboring cells to laterally limit or promote growth (Uyttewaal et al., 2012; Heisenberg and Bellaïche, 2013). Particularly in plants, mechanical signals influence the directionality of cell and tissue growth. The mechanical force field guides the alignment of the cortical microtubules (CMTs). CMTs in turn direct the deposition of cellulose fibrils, which are the load-bearing constituent of the cell wall that determines the deformability of the cell (Landrein and Hamant, 2013).

The *Arabidopsis* sepal is a powerful model to study plant organogenesis, because it is a relatively simple representative of all lateral organs (i.e., leaf-like organs) that is naturally accessible throughout flower development. By correlating cell growth and CMT orientation, combined with computational simulation of mechanical feedback, Hervieux et al. (2016) proposed that the arrow-like sepal shape is determined via reorientation of CMTs at the tip. This CMT reorientation is caused by a growth-driven shift in mechanical force fields, such that cell growth is restricted at the tip in maturing sepals. In this issue of *Developmental Cell*, Hong et al. (2016) further disentangle the puzzles behind sepal geometry control, highlighting the importance of spatiotemporal variations of growth at both the cellular and organ levels.

The authors first found that individual cells in expanding sepals vary their growth rates in a spatially and temporally dynamic manner. A mechanical model of sepal growth was created to test the roles of the heterogeneity in growth rate. The growth rate was modeled as being determined by cell wall stiffness, with cells that have softer cell walls growing more than cells with stiff cell walls. Locally variable growth rates enhanced growth at the tissue level, as previously shown at the *Arabidopsis* shoot apex (Uyttewaal et al., 2012). Interestingly, spatial variability alone was not enough to confer consistent organ geometry; rather, this was achieved with temporal variability in addition to spatial variability, with each cell changing its stiffness over time. Stiffening of the cells was counterbalanced by subsequent softening, with stiffer cells being compensated for by softer neighboring cells. Such "spatiotemporal averaging" of local growth suggests both autonomous and non-autonomous negative feedback loops acting to regulate cell wall stiffness and cell growth.

In order to understand the molecular mechanisms underlying the variability-driven organ geometry control, Hong and colleagues (2016) isolated mutants with irregular sepal geometry. Six of them were allelic and were further characterized to have reduced cell division rate and relatively homogenous cellular growth rate and cell wall stiffness. The irregularity in shape was independent of size and was apparent in later stages of sepal development, when the organs expand rapidly. Other lateral organs were affected similarly.