Embryonal totipotent cells can produce both embryonic and extraembryonic tissues and can generate whole organisms. In mice this level of genome plasticity is preserved in the 2-cell embryos, but is absent in embryonic cells from later stages of development. Recently it has been demonstrated that totipotent-like cells spontaneously appear in embryonic stem cell cultures and that the depletion of the histone chaperone Chromatin Assembly Factor I (CAF-I) increases the abundance of 2-cell-like cells. On the other hand, earlier studies have demonstrated that CAF-I is necessary for epigenetic conversions at the telomeres of S. cerevisiae. This commentary proposes that the absence of CAF-I confers totipotency of embryonic cells and that its activation triggers chromatin changes that reset the epigenome toward cell differentiation.

Totipotent cells (TCs), unlike pluripotent embryonic stem cells (ESC), can generate whole organisms and give rise to both embryonic and extraembryonic tissues. During murine development this level of unhindered genome plasticity is lost at the 2-cell (2 C) stage. It is known that the transitions from 2 C to blastocyst stages is accompanied by a massive rearrangement of heterochromatin domains. These rearrangements are correlated with the more restricted pluripotent state and arguably lead to a lineage commitment and differentiation.

It has been previously shown that 2C-like cells spontaneously emerge in ESC cultures. These cells resemble true 2 C cells in several aspects, including the lack of chromo-centers (these are the first highly condensed heterochromatin structures seen in embryonal cells) and the transcription of a subset of endogenous retrovirus-like sequences (MERVL).

In a recent paper Ishiuchi et al. reported that the depletion of 2 different subunits of a histone chaperone called Chromatin Assembly Factor I (CAF-I) dramatically increases the proportion of 2C-like cells in the ESC cultures. The authors demonstrated that the 2C-like promoting activity of CAF-I requires its nucleosome assembly function and a passage through early S-phase. Furthermore, domain analysis of the CAF-1 p150 subunit clearly showed that the regions required for histone deposition were also required to maintain low levels of 2C-like cells. Hence, the data strongly suggests that it is the histone deposition by CAF-1 that restrains totipotency in mouse ESC cultures. Importantly, the authors also demonstrate that the p60 subunit of CAF-I is absent from 2C-like cells and that its absence correlates with the timing of expression of MERVL. To the knowledge of the author of this commentary the 2 C totipotent cells are the only proliferating metazoan cells that normally lack CAF-I.

The paper by Ishiuchi et al. is following the findings of an earlier study, which has shown that the depletion of CAF-I in mouse embryos causes a developmental arrest at the 16-cell stage. The same study has reported that the depletion of CAF-I in cultured ESC cells leads to de-condensation and loss of heterochromatin clustering, but such modifications are not seen in lineage committed embryonic fibroblasts. Altogether, these finding suggest that in the absence of CAF-I the embryonal 2 C cells do not proceed to ESC and to lineage committed cells and that chromatin
assembly and passage through S-phase are required for these effects. However, the precise role of CAF-I in these processes remains unclear.

CAF-I is a well-known factor that participates in the first step of re-assembly of chromatin in the wake of the replication fork. Its key role is to deposit Histones H3/4 into the newly built nucleosomes. In S.cerevisiae, the destruction of the Cac1p subunit of CAF-I (the yeast homolog of the mammalian p150CAF-I subunit) leads to massive de-repression of subtelomeric genes. These genes normally alternate between a fully silenced or fully active state of expression. This interesting epigenetic phenomenon is referred to as Telomere Position Effect. However, a recent study has pointed out that the loss of telomeric gene silencing in Δcac1 cells should be attributed to low frequency of conversions between the active and silenced states rather than to overall reduced silencing. Hence, this paper suggested that in S.cerevisiae, CAF-I is involved in epigenetic conversions.

Ishiuchi et al. have summarized their exciting findings in a model (Fig. 7 in their paper and Fig. 1A in this manuscript), which emphasizes the causative effect of the depletion of CAF-I in the generation of 2C-like cells. However, this model does not thoroughly consider the possible mechanisms by which 2C-like cells appear and disappear in the ECS cultures. We can safely assume that the low abundance of 2C-like cells (0.5%) is maintained by an equilibrium of transitions between ECS and 2C-like cells. If this was not the case, a gradual increase in the proportion of 2C-like cells in the ECS culture would be expected. This conjecture suggests that the higher proportion of 2C-like cells could be due to low frequency of conversions between the active and silenced states rather than to overall reduced silencing.

In the alternative model (Fig. 1), the loss of CAF-I does not promote the conversions to 2C-like state. Instead, it reduces the rate of reversion of 2C-like cells back to the ECS state. In this situation, the normal role of CAF-I is to trigger chromatin rearrangements and reset the epigenetic landscape of the 2C-like cell toward ECS. In the absence of CAF-I, these chromatin conversions are impeded and the cells remain in 2C-like state. Such an interpretation is in agreement with the proposed role of CAF-I as a regulator of epigenetic conversions and can be supported by the findings in. A very important implication of this interpretation is that CAF-I is generally involved in epigenetic conversions in eukaryotic cells. Another very important implication is that the lack of CAF-I (as is the case in 2C cells) contributes to an uncommitted epigenetic landscape and ultimately to a totipotence.

These two models are not mutually exclusive. Indeed, the absence of CAF-I in ESCs can lead to chromatin decondensation and eventually to higher reprogrammability (Fig. 1A) while at the earlier 2C stage of development CAF-I is truly required for the transition from totipotent to pluripotent chromatin state (Fig. 1B). Both possibilities raise exciting and significant questions. For example, it will be very interesting to determine precisely how CAF-I restricts the reversions from ECS to totipotent cells or how it promotes the progression from 2C to ECS and then to lineage committed cells.

Figure 1. (A) The absence of CAF-I promotes chromatin decondensation and reversion of ESCs to totipotent state. This figure depicts Figure 7 in Ishiuchi et al. (B) Alternative model: CAF-I acts in 2C/ECS conversions. In ECS cultures the pluripotent cells spontaneously convert to totipotent 2C-like cells. Under normal conditions the 2C-like cells quickly revert to ECS. The depletion of CAF-I reduces the rate of 2C/ECS conversions and increases the proportion of 2C-like cells.
CAF-I has been postulated to play a critical role in the replication-coupled preservation of chromatin state. However, being a key histone chaperone at the replication fork, it could easily fulfill both preservation and alteration functions. This scenario puts CAF-I in the center of epigenetic reprogramming and in the maintenance of totipotency. These important issues need to be considered and addressed in future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Ishiuchi T, Torres-Padilla ME. Towards an understanding of the regulatory mechanisms of totipotency. Curr Opin Genet Dev 2013; 23:512-8; PMID:23942314; http://dx.doi.org/10.1016/j.gde.2013.06.006
2. Gurard-Levin ZA, Quivy JP, Almouzni G. Histone chaperones: assisting histone traffic and nucleosome dynamics. Annu Rev Biochem 2014; 83:487-517; PMID:24905786; http://dx.doi.org/10.1146/annurev-biochem-060713-035536
3. Macfarlan TS, Gifford WD, Driscoll S, Lettieri K, Rowe HM, Bonanomi D, Firth A, Singer O, Trono D, Pfaff SL. Embryonic stem cell potency fluctuates with endogenous retrovirus activity. Nature 2012; 487:5763; PMID:22722858
4. Ishiuchi T, Enríquez-Gasca R, Mizutani E, Boskovic A, Ziegler-Birling C, Rodriguez-Terrones D, Wakayama T, Vaquerizas JM, Torres-Padilla ME. Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly. Nat Struct Mol Biol 2015; 22:662-71; PMID:26237512; http://dx.doi.org/10.1038/nsmb.3066
5. Houard M, Berliver S, Prohet AV, Quivy JP, Hery P, Almouzni G, Gerard M. CAF-I is essential for heterochromatin organization in pluripotent embryonic cells. PLoS Genet 2006; 2:e181; PMID:17083276; http://dx.doi.org/10.1371/journal.pgen.0020181
6. Kaufman PD. Want reprogramming? Cut back on the chromatin assembly! Nat Struct Mol Biol 2015; 22:648-50; PMID:26333710; http://dx.doi.org/10.1038/nsmb.3081
7. Alabert C, Groth A. Chromatin replication and epigenome maintenance. Nat Rev Mol Cell Biol 2012; 13:153-67; PMID:22358331; http://dx.doi.org/10.1038/nrm3288
8. Jeffrey DC, Wyse BA, Rehman MA, Brown GW, You Z, Oshidari R, Masai H, Yankulov KY. Analysis of epigenetic stability and conversions in Saccharomyces cerevisiae reveals a novel role of CAF-I in position-effect variegation. Nucleic Acids Res 2013; 41:8475-88; PMID:23863839; http://dx.doi.org/10.109/aj/kr623
9. Sharp JA, Franco AA, Osley MA, Kaufman PD. Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in S. cerevisiae. Genes Dev 2002; 16:85-100; PMID:11782447; http://dx.doi.org/10.110/ad.925302
10. Rusche LN, Kirchmaier AL, Rine J. The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem 2003; 72:481-516; PMID:12676793; http://dx.doi.org/10.1146/annurev.biochem.72.121801.161547
11. Yankulov K. Dynamics and stability: epigenetic conversions in position effect variegation. Biochem Cell Biol 2013; 91:6-13; PMID:23442136; http://dx.doi.org/10.113/cb-2012-0048
12. Kaufman PD, Kobayashi R, Kesler N, Stillman B. The p150 and p60 subunits of chromatin assembly factor I: a molecular link between newly synthesized histones and DNA replication. Cell 1995; 81:1105-14; PMID:7600578; http://dx.doi.org/10.101/0092-8674(95)80015-7