appears to be affected by DNA damage. Using a ubiquitin::GFP reporter system, they provide evidence that DNA damage leads to a general increase in protein turnover (Figure 1). The finding that RNAi-mediated depletion of core proteasome components blocks DNA-damage-induced heat shock resistance bolsters this hypothesis. All in all, Ermolaeva et al. [2] provide strong support for the notion that ‘anything that doesn’t kill you makes you stronger’. What makes this study particularly appealing is the fact that phenomena that have been largely studied in tissue culture and in single-celled organisms have now been explored in intact animals. It will be interesting to study more broadly the interaction of the DNA-damage response with general stress responses and aging pathways. It will also be essential to assess the generality of these findings in other organisms. Finally, it will be important to investigate how DNA-damage signalling is emitted from the germ line to somatic tissues. Clearly there are many follow-up studies to come.

References

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Development: Sketch for a Theory of Oct4

How is it that Oct4, a transcription factor that controls pluripotency in stem cells, also controls lineage specification? A recent study investigating common Oct4 targets in vertebrate species indicates an evolutionarily conserved role in mediating cell adhesion. This finding may help decipher Oct4’s versatility in governing stem cell behaviors.

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Stem cells are defined by two different qualities: they can either divide endlessly, maintaining their pluripotent state, or they can differentiate into myriad specific cell types. This dual potential is mirrored in the behavior of the three canonical transcription factors — Oct4, Sox2, and Nanog — that both govern stem cell self-renewal and determine cell fate decisions [1]. For example, Oct4 is induced during TGF-β signaling to promote the specification of cardiac mesoderm through cooperation with canonical Wnt signaling [2,3]. How can one factor promote both the maintenance of stem cell identity and determine specific cell fates? More puzzling still, these transcription factors bind to thousands of targets in the genome, and in fact bind to many of the same targets [4]. Genomic analysis has been instrumental in detailing unique gene regulatory networks and epigenetic states in pluripotent cells [1,5], but the cellular milieu in which these factors are expressed likely impacts context-dependent activity that is less accessible to high-throughput sequencing technology. To provide a complementary perspective to the question of how developmentally relevant transcription factors exert cell fate control, Livigni et al. [5] took an evolutionary approach, as reported in this issue of Current Biology, by studying Oct4 targets conserved across three vertebrate species. Oct4, a homeodomain transcription factor of the Pou family, has been conserved to some degree throughout vertebrate development. PouV factors expressed in Xenopus and Axlotl not
only are sufficient to rescue the self-renewal of mouse embryonic stem (mES) cells upon ablation of Oct4 [7] but can replace Oct4 in the reprogramming of somatic cells as well [8]. Livigni et al. took advantage of this conservation to devise a forward screen for functionally relevant Oct4 targets by determining which genes are similarly regulated in human embryonic stem (hES) cells, mES cells, and Xenopus embryos [6].

Using this approach, the authors were able to narrow the large pool of conserved Oct4 targets identified in mES and hES cells by an order of magnitude.

POUV signaling in the Xenopus embryo involves three Oct4 homologs: Xlpou-25, 60, and 90, whose differential expression is needed to maintain POUV activity during early embryonic development through gastrulation. Microinjection of Xenopus embryos with antisense morpholinos targeting Xlpou-25, 60, and 90 ablates all POUV activity and causes a defect in gastrulation, namely, the convergent extension of neural and mesodermal tissues [6]. Analysis of the transcriptional profile of the xPOUV-depleted (xPVD) embryos at the late blastula and early gastrula stages identified 307 POUV-responsive genes, 201 of which have known or putative mammalian homologs. To identify which targets are conserved across vertebrate species, Livigni et al. cross-referenced their list of 201 targets with previously published chromatin immunoprecipitation (ChIP)-sequencing data, identifying Oct4 targets in mES cells (9,486 genes) and hES cells (4,649 genes), ultimately revealing 57 POUV-responsive genes that are conserved across all three vertebrate species. Strikingly, of the 57 genes that comprise this ‘conserved network’, over half are known regulators of cell adhesion and migration. This constitutes an 11-fold enrichment of this gene class over what would be expected by chance alone.

This evolutionary approach is provocative, but we should be cautious in interpreting the results. First, mES cells and hES cells exhibit very different cell biological and developmental properties and therefore may not simply be equivalents obtained from different species [9–12]. It would be interesting to see if additional gene classes would be identified in a more uniform set of samples. Second, at least one study has raised questions regarding the degree to which Oct4 is functionally conserved in vertebrates [13], and other studies have shown that the Oct4 homolog in zebrafish diverged to take on a role in promoting endoderm specification [14]. It would be very interesting to see if a hierarchy of Oct4 functions could be determined by inclusion of additional species.

In translating their findings to mammalian cells, Livigni et al. observed that conditional ablation of Oct4 in mES cells as well as in mEpi-stem cells, which are pluripotent cells derived at the epiblast stage of embryonic development and thus reflect a later cell fate than ES cells, recapitulated the cell adhesion defect observed in xPVD embryos [6]. They identified the E-cadherin gene as a critical Oct4 target in maintaining cell adhesion and stem cell identity in these models. Furthermore, forced expression of E-cadherin could transiently rescue the gastrulation defects observed in xPVD embryos and suppress differentiation upon depletion of Oct4 in mES cells.

Given these tantalizing associations, how might E-cadherin serve as a pivotal molecule in Oct4’s diverse activities? E-cadherin is an epithelial representative of a larger family of cadherin proteins that mediate cell–cell adhesion between cells with a similar extracellular landscape through homotypic interactions at the cell membrane. E-cadherin interactions are also imperative for maintaining epithelial identity, cell compartmentalization, and for mediating cell–cell signaling. A loss of E-cadherin expression correlates with the epithelial–mesenchymal transition, a migratory process that occurs during gastrulation and is recapitulated during tumor metastasis [15]. Induction of E-cadherin expression with LIF/BMP4 permanently reverts stem cells to a fully pluripotent state and restores lineage contribution in teratoma assays [9].

More recently, E-cadherin was reported to substitute for Oct4 in the reprogramming of mouse embryonic fibroblasts (MEFs) [16], thus supporting the findings of Livigni et al. It is unknown how Oct4 regulation of cell adhesion confers stem cell identity, or why disruption of this pathway manifests primarily in defects in differentiation. One possibility is that it promotes compartmentalization of stem cell progenitors, i.e., that Oct4 regulation of E-cadherin expression might spatially restrict a pool of homogenous progenitor cells through multiple stages of cell specification to ensure that there are sufficient numbers to populate each cell lineage. The fact that embryonic development arrests at the initial lineage specification event in Oct4-null and E-cadherin-null mice [17,18], as well as xPVD embryos [6], would seem to support this notion (Xenopus embryos do not form trophectoderm, so the initial specification event may occur during gastrulation). A related possibility is that Oct4 regulation of E-cadherin is important for maintaining the epithelial identity of pluripotent cells, perhaps to make them more receptive to differentiation cues. This hypothesis could be tested by replacing Oct4 with other factors known to maintain epithelial identity in cancer cells, for example, and asking if they can substitute for Oct4 during self-renewal or reprogramming. Lastly, Oct4 regulation of cell adhesion could serve to establish a cell–cell signaling network in the developing embryo. In addition to mediating cell–cell contact, the E-cadherin intracellular domain serves to integrate intracellular Wnt signaling, among other signaling cascades [15]. Such a paracrine signaling network might be important for maintaining homogeneity among pluripotent progenitors as well as enabling the embryo to respond to environmental fluctuations or differentiation cues.

Perhaps the most important conclusion to be drawn from the study of Livigni et al. is that biological functions are not as strictly compartmentalized as we sometimes wish them to be, and that even transcription factors that appear to be highly specialized can fill multiple roles. Even so, it will be necessary to use comprehensive systems biology approaches and modeling to truly understand the functional versatility of Oct4 and other developmentally vital transcription factors.

References


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