RESEARCH HIGHLIGHTS

Inactive X in human iPS cells

Maternal and paternal X chromosomes are active in mouse embryonic stem cells isolated from blastocysts, although human embryonic stem cells have greater variability in X activation. In mouse and human development, and embryonic stem cell differentiation, the non-coding RNA, XIST, coats one randomly selected X chromosome resulting in its inactivation. In mice, X chromosome reactivation accompanies induced reprogramming to the pluripotent stage by expressing the four factors, oct4, nanog, sox2 and c-myc.

Plath and colleagues now show that hiPSCs (human induced pluripotent stem cells) carry an inactive X, still coated by XIST (*Cell Stem Cell*, doi:10.1016/j.stem.2010.06.024). Recruitment of the polycomb complex subunit, PRC2, which promotes the addition of repressive heterochromatin marks, is an early step in X inactivation; however, in the maintenance phase, PRC2 leaves inactive X (Xi). The authors found that PRC2 still coats Xi in hiPSCs, suggesting that reprogramming induces an early X inactivation-like stage. Prolonged passaging of hiPSCs nevertheless leads to loss of XIST coating without any effect on X silencing. The authors also demonstrate that owing to clonal selection, the same X is inactivated in a given hiPSC line. Using fibroblasts from a female carrier of the X-linked mutant dystrophin gene, which is associated with Duchenne muscular dystrophy, the authors isolated one hiPSC line expressing only the wild-type allele and another expressing the mutant allele. Thus, the non-random X inactivation seen in hiPSCs has potential implications for using hiPSC technologies to study X-linked human diseases. NLB

Protein sequences reveal membrane structure

Eukaryotic cell membranes differ in lipid and protein composition, but it is unclear if these differences alter their physical properties and in turn determine the selection of resident transmembrane proteins. To gain insights into plasma, Golgi and endoplasmic reticulum membranes, Sharpe *et al.* (*Cell* **142,** 158–169; 2010) identified a set of single transmembrane domain (TMD) proteins that have experimentally determined topology and location, and then used these to find orthologues in fungal and vertebrate genomes. Sequence comparison of the aligned TMDs showed that they share common characteristics based simply on which membrane they reside in, irrespective of sequence or function of the protein. For example, plasma membrane TMDs are significantly longer than those found in the Golgi or endoplasmic reticulum and the distribution of specific residues differs between TMDs localized in the plasma and Golgi membranes. Additionally, an artificial neural network was able to accurately predict the location of yeast membrane proteins based on TMD sequences alone.

Thus, the composition of eukaryotic TMDs does not depend solely on protein function, but also on the physical properties of the membrane in which they reside, which differs between organelles and the plasma membrane. GD

Chemotaxis: TORCing to Ras

In *Dictyostelium discoideum*, chemotaxis towards cAMP is governed by GPCR-mediated signalling at the leading edge. The most well-studied mechanism involves phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P3). Devreotes and colleagues now identify a parallel pathway, where RasC triggers TORC2 (target of rapamycin complex 2)-mediated activation of the pleckstrin homology (PH) domain-containing protein, PKBR1. (*J. Cell Biol.* **190,** 233–245).

The authors found that phosphorylation of the hydrophobic motif of PKBR1 (necessary for its activation) is severely reduced in cells lacking RasC, but not in cells lacking RasG. Inducible expression of RasC restores PKBR1 phosphorylation. Expression of an activated RasC mutant prolongs the phosphorylation of RKBR1 and of PKB substrates, and induces prolonged activation of adenylyl cyclase and increased actin polymerization. Chemotaxis is defective in cells expressing activated RasC, suggesting that RasC also needs to be inactivated at the leading edge. Importantly, all the effects of activated RasC are abolished if the TORC2 component PiaA is simultaneously deleted, suggesting that TORC2 is an essential RasC effector. Finally, the authors demonstrate that RasC is required for PKB activation *in vitro* and that activated RasC interacts, either directly or indirectly, with the Rip3 component of the TORC2 complex. Thus, RasC works upstream of TORC2 in *D. discoideum* migration. It will be interesting to see if TORC2 is also under Ras control in other systems. CKR

Stress response: AMPKing up transcription

Under conditions of hypoxia or low-nutrient availability, the AMP-activated protein kinase (AMPK) modulates the transcriptional programme to promote energy conservation and cell survival. However, the AMPK substrates that are important for such transcriptional regulation have remained unclear. Berger and colleagues now report that AMPK directly phosphorylates histone H2B to promote transcription in response to metabolic or genotoxic stress (*Science* doi:10.1126/science.1191241).

AMPK mediates the stress response in part through phosphorylation of p53 and activation of p53-dependent transcription. Berger and colleagues sought to clarify whether AMPK also has a direct role in transcriptional regulation. Chromatin immunoprecipitation assays revealed that AMPK and its upstream activator LKB1 associated with the *p21* promoter following ultraviolet irradiation or glucose withdrawal. Histone phosphorylation is known to regulate gene transcription, and AMPK-dependent histone H2B phosphorylation was detected in response to cell stress *in vivo*.

H2B was phosphorylated at the p53 binding sites in the promoters of the p53-target genes *p21* and *cpt1c*, as well as along their transcribed regions. Incorporation of a phosphorylation-deficient H2B mutant into these regions abrogated stress-induced transcription of *p21* and *cpt1c* and decreased cell viability, supporting the importance of AMPK-mediated H2B phosphorylation in the cellular response to metabolic and genotoxic stress. These data reveal a central and multifaceted role for AMPK in transcriptional regulation following cell stress. EJC

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