METALS

Spying on the CIA

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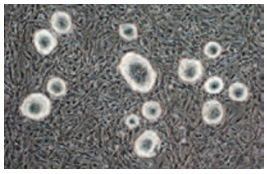
In eukaryotes, iron-sulfur cluster assembly is controlled by machinery specific to either the mitochondria or the cytosol and nucleus. Stehling *et al.* now expand our understanding of the poorly understood cytosolic iron-sulfur protein assembly (CIA) machinery, identifying new protein factors, unexpected substrate specificities and links to other iron regulatory mechanisms. The human homologs of known CIA components, CIA1 and CIA2B, have been shown to form a complex with MMS19, which assists in cluster insertion. The authors also identified CIA2A as related to CIA2B, but the distinction between these proteins was unclear. Immunoblotting confirmed all three of the CIA proteins were present in the cytosol. Knockdowns and pulldowns of each of the three proteins demonstrated that CIA2A and CIA2b both bind CIA1, though not simultaneously, and defined distinct but overlapping pools of substrates for the assembly proteins. For example, CIA2A, but not CIA1 or CIA2B, was required for the maturation of iron regulatory protein 1 (IRP1), involved in the post-transcriptional regulation of proteins involved in iron homeostasis. CIA2B did, however, effect iron regulation via IRP2; interestingly, CIA2A and CIA2B caused the same IRP2-mediated changes, even though the two proteins had opposing effects on IRP2 stability and its ability to bind the iron-responsive element in mRNA. These results define a branched pathway in Fe-S cluster maturation and identify unexpected cross-talk in iron regulation. *CG*

STEM CELLS

A chemical recipe

Science, published online 18 July 2013; doi:10.1126/science.1239278



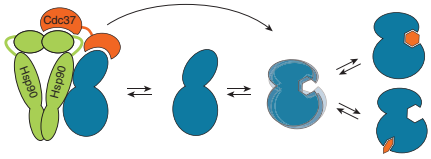
PINGPING HOU

The finding that viral transfection of four transcription factors was sufficient to convert fibroblasts into induced pluripotent stem cells has dramatically altered the field of regenerative medicine. However, a major concern in using this approach is the potential for cancer-generating outcomes and effects. The use of chemical compounds to induce pluripotency has been proposed as a desirable alternative. To search for a chemical cocktail that required no genetic factors, Hou *et al.* performed a chemical screen in fibroblasts and found that the addition of four compounds designated as C6FZ:CHIR99021, 616452, Forskolin and DZNep produced chemically induced pluripotent stem cells (CiPSCs). CiPSCs had similar characteristics to virally induced pluripotent stem cells and embryonic stem cells in their gene expression profiles, ability to differentiate into tissues representing all three germ layers and germline transmission. Mechanistically, the authors observed that treatment with these small molecules induced endogenous components of the pluripotency transcriptional circuitry, even though none of the compounds directly target transcription. In particular, C6F promoted *Sall4* and *Sox2* expression, whereas DZNep altered the epigenetic state of the *Oct4* promoter. Using this approach to generate CiPSCs from human cells could provide new inspiration for clinical applications. *GM*

KINASES

Thermodynamic sensors

Nat. Biotechnol. **31**, 630–637 (2013)



Determining the specificity of small molecules for their targets in cells is challenging. Taipale *et al.* establish that chaperones can serve as thermodynamic sensors to quantify interactions between their clients and small molecules in cells. In cells stably expressing a luciferase-HSP90 chaperone fusion protein, the authors introduced BCR-ABL with or without the ATP-competitive BCR-ABL inhibitor imatinib, immunoprecipitated the complex and quantified the amount of kinase-chaperone interaction on the basis of luciferase activity and an ELISA for the kinase. Imatinib dose-dependently disrupted the chaperone-kinase interaction with a half-maximum effective concentration of 180 nM. In comparable experiments of mutated variants of BCR-ABL and the kinase inhibitors developed to target them, the expected selective activities were observed. The authors also demonstrated that the assay effectively quantified BCR-ABL interaction with allosteric inhibitors. Applying the assay against their full kinase panel, including 565 kinases, they were able to identify the primary in-cell target for 26 of 30 ATP-competitive kinase inhibitors, including new targets for some of the compounds. Similarly, the assay validated the specificity of seven allosteric modulators for their intended kinase target. Finally, the authors applied their assay to predict that crizotinib, a recently approved drug, interacts with tyrosine kinases in addition to the intended targets ALK and MET. These off-target hits were validated in secondary assays. Thus this chaperone-based thermodynamic assay combines the throughput of *in vitro* experiments with the physiological relevance of in-cell approaches. *AD*

RECEPTORS

A painful difference

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Glycine receptors (GlyRs) are pentameric ligand-gated ion channels that bind the neurotransmitter glycine at each of five extracellular subunit interfaces. Phosphorylation of Ser346 of GlyR α3 subunits by protein kinase A leads to chronic inflammatory pain sensitization and represents a focal point for designing drugs with analgesic activity. To identify unique structural features of α3 subunits that might permit selective drug design, Han *et al.* examined how α3 subunits differ from the widely expressed α1 GlyR subunits, which are not involved in pain sensitization. The authors monitored glycine-induced conformational changes in the two subunit types by voltage-clamp fluorometry, where the requisite environmentally sensitive fluorophore was placed at a common extracellular location in each subunit. These experiments revealed that, despite sharing a high amino acid sequence identity, α1 and α3 GlyRs adopt distinct quaternary structures. Using chimeras of the two subunits, the authors found that structural variations in the intracellular domain housing the Ser346 phosphorylation site were responsible for the different structures. Further voltage-clamp fluorometry experiments, including with the competitive antagonist tropisetron, showed that phosphorylation of Ser346 produced conformational changes that propagated all the way to the α3 glycine-binding site. These results suggest that chronic inflammatory pain sensitization produces a unique conformational change in the α3 glycine-binding site that could be exploited in the design of new therapeutics for chronic pain. *MB*