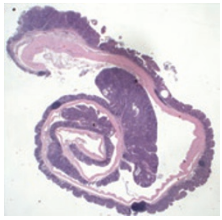
Switching c-Jun



Jun N-terminal kinases (JNKs) regulate the transactivation activity of c-Jun, and therefore the activity of the AP-1 transcription factor formed by c-Fos and c-Jun family members, but the mechanism for this regulation has remained unclear. Previous research suggested that phosphorylation of c-Jun by JNKs leads to the dissociation of a repressor from AP-1 target genes, in a process involving modulation of histone acetylation. Now, Behrens and colleagues have identified Mbd3, a component of the NuRD repressor complex, as a protein that interacts with the c-Jun transactivation domain. JNK-mediated phosphorylation of c-Jun decreased this interaction, and Mbd3 was necessary for repression of c-Jun target gene transcription when JNK signaling was inhibited. Moreover, Mbd3 plays an essential part in the epigenetic regulation of c-Jun transcription by recruiting histone deacetylase HDAC1. Conditional deletion of *mbd3* in the mouse gut resulted in increased proliferation of intestinal progenitor cells and led to the identification of the stem cell marker *lgr5* as a c-Jun target gene regulated by Mbd3–NuRD. Additionally, these animals developed more frequent and larger tumors in response to an experimental tumorigenesis assay. Deletion of one allele of *c-jun* rescued this phenotype, indicating that the effects of *mbd3* deletion are due to the loss of its interaction with c-Jun. These results provide a mechanistic explanation for the ability of JNKs to activate c-Jun and suggest an interesting model where unphosphorylated c-Jun actively represses AP-1 target genes by promoting histone deacetylation via HDAC1 recruitment by Mbd3. (*Nature* doi:10.1038/nature09607, published online 2 January 2011) *SM*

Letting transcription factors work

Although transcription factors are key to deploying programs that underlie particular cell fates, the ability of such factors to trigger a cell type–specific phenotype can be restricted to specific contexts. Tursun, Hobert and colleagues have now tested what might inhibit reprogramming by examining the *Caenorhabditis elegans* transcription factor CHE-1, which is involved in the development of a specific glutaminergic neuron fate called ASE. They found that CHE-1 expression in all cells can induce expression of a reporter driven by CHE-1 binding sites. However, CHE-1 expression did not lead to expression of ASE-specific terminal differentiation factors (for example, gcy-5 and ceh-36) beyond head neuronal cells. An RNAi screen targeting chromatin regulators was then used to identify factors that could induce expression, and knockdown of lin-53 was found to lead to gcy-5 and ceh-36 expression in mitotic germ cells. Not only did these cells express ASE-specific, pan-neuronal and glutaminergic markers, but they also adopted aspects of neuronal nuclear morphology, sent out axon-like projections and contained transport-like particles. Moreover, these cells did not express markers of other neuronal types, suggesting that these outcomes are CHE-1 specific. Expression of other neuronal-specific transcription factors during lin-53(RNAi) led to GABAnergic (caused by unc-30 expression) and A- and B-type cholinergic (upon unc-3 expression) differentiation, but germ cells could not be driven to a muscle cell fate by MyoD expression under these conditions. Because lin-53 orthologs are involved in chromatin regulation, the authors also tested the effect of global HDAC inhibition, observing an outcome similar to that for lin-53(RNAi). These results suggest that chromatin regulators may be an obstacle to reprogramming in particular cells. Exactly which complexes are involved and how they mediate these effects are inviting avenues for further investigation. (*Science* doi:10.1126/science.1199082, published online 9 December 2010) *SL*

Written by Inês Chen, Maria Hodges, Sabbi Lall & Steve Mason

Improving single molecules

Single-molecule methods are revealing the dynamics of molecules without the need to interpret large ensembles. The usefulness of this approach is restricted by the time resolution of single-molecule fluorescence studies, currently at the millisecond timescale. The theoretical limit for fluorescence single-molecule studies with the instrumentation available is near to 10 microseconds, but it is the fluorescent dyes themselves that slow down the timescale. FRET dyes saturate at 100 kHz because of transient blinking and bleaching when they are highly irradiated. Many researchers have attempted to protect these dyes, and the main approach has been to eliminate oxygen in solution using antioxidants together with organic triplet quenchers. However, these molecules are much less efficient triplet quenchers than dissolved oxygen, leading to triplet buildup and no decrease in timescale. A new method from Munõz and colleagues substitutes the antioxidants by the oxygen free radical scavenger 2-mercaptoethylamine (cysteamine). This way they keep the highly efficient triplet quenching of dissolved oxygen (reinforced by Trolox) while they manage to reduce dye bleaching caused by oxygen free radicals. The authors tested their setup by using it to monitor the microsecond changes in the folding of an SH3 domain of α-spectrin, and the folding and unfolding of the α-helical protein BBL, which occurs on a microsecond timescale. This method is likely to be widely adopted for studying processes occurring at the microsecond scale. (*Nature Methods* doi:10.1038/nmeth.1553, published online 9 January 2011) *MH*

Grounds for toxicity

Protein misfolding and aggregation can harm cells and play a role in neurodegenerative conditions such as Huntington's, Parkinson's and Alzheimer's diseases. Familial forms of these diseases are caused by mutations in specific proteins that render them more prone to aggregate and form amyloid deposits. The identities of the pathologic species are still unclear, though there is evidence suggesting that soluble oligomeric aggregates cause cytotoxicity. The other big question is how these aggregates cause toxicity, and this has now been tackled by Hayer-Hartl, Hartl, Vabulas and colleagues. To avoid the issue of loss-of-function or other effects that would be protein specific, the authors used artificially designed β-sheet polypeptides that can form amyloids *in vitro*. After showing that these so-called 'β-proteins' behaved as amyloidogenic proteins in human cells in culture, with similar aggregation and toxicity phenotypes, the authors used quantitative proteomics to identify the cellular proteins associated with the aggregates. The β-protein interactome was enriched in proteins with intrinsically disordered regions. Pulse-labeling experiments also revealed a bias toward newly synthesized proteins, particularly large, multidomain ones, which would fold more slowly and hence present partially folded regions that would be preferentially targeted by the β-proteins. The identified proteins included many with central roles in essential cellular functions such as transcription, RNA processing and ribosome biogenesis, translation, chromatin organization, cytoskeleton and vesicle transport. Amyloid-like aggregation thus causes cellular toxicity by sequestering proteins with disordered regions and those not yet fully folded—which the authors call 'metastable subproteome'—and by interfering with a multitude of cellular processes. (*Cell* **144**, 67–78, 2011) *IC*