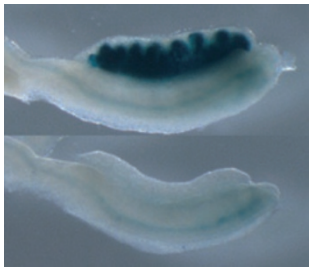
## SRY XY is male



Sex determination in mammals is known to depend on the Y chromosome, and a single factor in particular, *Sry*, promotes male development. The mechanism by which SRY acts has been unclear, though chromatin modulation, splicing regulation and both activation and repression have been proposed. Sekido and Lovell-Badge now tie SRY activity during the period of male gonadal development commitment in XY individuals to the *Sox9* enhancer. In a heavy-duty analysis of elements driving expression, the authors started with a BAC-based reporter carrying sequences upstream of *Sox9* to recapitulate its expression in transgenic mice: a 120 kb region drove expression in 10.5-day embryonic gonads, and by day 12.5 this expression became upregulated in developing testes and downregulated in developing ovaries. They then further narrowed this region to a 3.2-kb testis-specific enhancer. Further analyses in mice indicated that both SRY and SF1 (steroidogenic factor 1, an orphan nuclear receptor) are required for *Sox9* expression, and binding sites for both factors were defined by ChIP and mutation of candidate binding elements. However, maintenance of *Sox9* expression after SRY disappears is dependent on SF1 and autoregulation by SOX9 itself. Thus, this profound switch in development has been narrowed down to a 3.2-kb enhancer that is sufficient to drive relevant gene expression, opening up this process to further definition of the regulation mediated by this region, analysis of the molecular mechanisms underlying pathways downstream of SOX9 in males and examination of how *Sox9* expression is downregulated in XX individuals. (*Nature,* advance online publication 4 May 2008, doi:10.1038/nature06944) *SL*

## HDX, improved

Hydrogen/deuterium exchange (HDX) has been used to probe protein structure, as it provides information about the solvent accessibility of amide bonds. HDX can be monitored by NMR analysis because the deuterium nucleus is invisible in the proton NMR spectrum. HDX can also be used to determine the contact sites between a protein and a binding partner, as these sites will be protected from solvent and thus show a slower rate of HDX compared to the free protein. However, if the ligand is large, it will impair the detection of the amide signals in the complex. Conversely, if the complex is dissociated in an aqueous solvent before NMR measurement, there will be isotope exchange and the information will be lost. Such limitations have now been overcome by Dyson and colleagues by using a solvent that does not exchange protons, such as DMSO, in which the complex is dissociated and the proteins fully unfolded but the pattern of HDX retained. The authors applied this method to probe the contacts of co-chaperone protein Aha1 with Hsp90 and found that the results were in excellent agreement with the crystal structure of the complex. In particular, they identified a secondary binding site, and it corresponded to a crystal contact of Aha1 with an Hsp90 molecule from an adjacent asymmetric unit, indicating that this interaction occurs in solution. It will be exciting to see this technique applied to study the contacts between chaperones and their client proteins. (*FEBS Lett.* **582**, 1495–1500, 2008) *IC*

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## A gran-d ol' time

Granzymes are serine proteases that are secreted by cytotoxic T lymphocytes and natural killer cells to destroy mammalian cells that have been 'targeted' for elimination. There are five isoforms of these proteins in humans, and it has been known that granzyme A (GzmA) induces cell death in a caspase-independent manner that involves reactive oxygen species (ROS). In a recent paper from Martinvalet *et al.*, two-dimensional gel electrophoresis was used to identify potential mitochondrial targets of this protease. These experiments suggested that NDUFS3—a subunit of the NADH:ubiquinone oxidoreductase ETC complex I—is a direct target of GzmA in human cells. Further experiments showed that this protease, but not granzyme B, cleaves NDUFS3 after Lys56; mutation of this key lysine residue to alanine rendered the protein resistant to GzmA cleavage. ROS inhibitors were unable to prevent the GzmA-induced degradation of NDUFS3, suggesting that ROS production occurs after GzmA cleaves the protein. Because GzmA does not have a mitochondrial import signal peptide and does not disrupt the mitochondrial outer membrane, it is not clear how it reaches NDUFS3 (which lies in the mitochondrial matrix). One potential mechanism would involve Hsp70 or Hsp90, as one of these chaperone proteins could bind the protease and deliver it to the outer mitochondrial membrane translocase. The authors determined that both chaperones coimmunoprecipitate with GzmA, and preliminary experiments suggest that the Tom/Tim system may be involved, but more work is needed to definitively determine exactly how GzmA enters the mitochondrial matrix. As the proteomics approach used by the authors required an analysis of only a small subset of the cellular proteome, it may be possible to use a similar approach to identify the mitochondrial substrates of other Gzms. (*Cell* **133**, 681–692, 2008) *JMF*

## Synergy signature

Malignant cell transformation typically depends on the cooperation of a few oncogenic mutations that lead to complex changes in gene expression patterns. This has made the identification of critical downstream target genes a difficult and serendipitous process. Thus, despite considerable effort, only a few such targets have been identified, and so intervention strategies have been exclusively focused on mutant gene products. Focusing instead on synergies downstream of cancer mutations, McMurray *et al.* identified genes regulated in a cooperative manner on a genomic scale by comparing messenger RNA expression profiles of colon cells expressing mutant p53, activated Ras or both mutant proteins together using Affymetrix microarrays. They termed those genes that responded synergistically 'cooperation response genes', or CRGs. The CRGs identified encode proteins that are involved in the regulation of cell signaling, transcription, apoptosis, metabolism, transport or adhesion, and a large proportion are misexpressed in human cancer. Normalizing the expression of 14 of the 24 CRGs, through either cDNA re-expression or short hairpin RNA–mediated knockdown, reduced tumor formation. In contrast, only 1 out of 14 non-CRG perturbations showed a significant reduction in tumor formation. Thus, it seems that such synergistic behavior in gene expression data can be exploited as an important new strategy for identifying cancer genes, as well as targets for intervention in cancer cells harboring oncogenic gain- and loss-of-function mutations. (*Nature*, advance online publication 25 May 2008, doi:10.1038/nature06973) *BK*