A sensitive measure of repression

Researchers have proposed that complex embryonic patterns are generated by multiple enhancers that respond differentially to gradients of repressor proteins. In a recent report, Dorothy Clyde, Dmitri Papatsenko, Stephen Small, and colleagues (New York University, New York, NY) combine genetics and bioinformatics to demonstrate that this model may be essentially correct.

The pattern of the fly embryo is influenced by combinations of enhancers and repressors that control expression of the pair rule gene eve in seven stripes along the anterior–posterior axis, which foreshadow the segmented body plan of the adult fly. Two enhancers, eve 3+7 and eve 4+6, control two stripes each. Ectopic expression of one of two repressor proteins that normally occur in opposing gradients, hunchback (Hb) or knirps (Kni), uniquely alters eve expression in different stripes. Thus, the enhancers respond differently to the same concentration of the repressors.

Using computational approaches to analyze the Kni and Hb binding sites in the two enhancers, Clyde et al. found that both the number of sites and the predicted binding strength varied between the two regulatory regions in a manner that correlated with the genetic results. Using a position weight matrix that evaluates the relative value of having a particular nucleotide in a specific site within a Kni or Hb binding site, the team was able to make detailed predictions of how an alteration would modify the binding affinity. To test whether the predicted binding strengths reflect biology, they mutated six out of 12 Kni binding sites in the eve 3+7 region, lowering their predicted affinity such that the mutant enhancer should have an intermediary affinity for Kni, relative to wild-type eve 3+7 and eve 4+6. As expected, the mutant enhancer had an intermediate response to a given concentration of Kni.

“We changed a few of these binding sites and we changed what we think is the binding strength of the enhancer,” says Small. “That has really never been shown before. It opens up the real possibility that we can design more intelligent experiments to test how these enhancers work—and that we can predict the activities of DNA sequences just by using the computer.” It also demonstrates the possibility of using such cluster analysis to locate previously unidentified regulatory regions in the genome, which Small calls a Holy Grail of genomics.

Reference: Clyde, D.E., et al. 2003. Nature. 426:849–853.

Repopulating glioma cell lines

Cancer therapies target rapidly dividing cells, which is a problem if tumors contain slow-growing undifferentiated cells that act as stem cell for the malignancy, as accumulating evidence suggests. Now, Toru Kondo, Takao Setoguchi, and Tetsuya Taga (Kumamoto University, Japan, and University of Cambridge, Cambridge, UK) show that this problem may be widespread. They find that several cancer cell lines maintain a small proportion of self-renewing cells.

To search for these potentially dangerous cells, Kondo’s team tested several cancer cell lines for the presence of side populations (SP) of cells containing the breast cancer–resistant protein 1 (BCRP1), an ATP-dependent transporter found in many stem cells. Of six lines tested, four had BCRP1-containing SP cells that accounted for between 0.4% and 2.1% of the total population.

The growth of SP cells requires basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF). “bFGF seems to be essential for the self-renewal of C6 stem cell but is not enough to induce stem cell proliferation” says Kondo. In the presence of both growth factors, the SP cells proliferate but maintain their stem cell–like qualities.

When the authors isolated SP cells and allowed them to repopulate a culture, they found that the cells produce both SP and non-SP cells. By contrast, non-SP cells cannot be induced to take on SP traits, suggesting that the SP cells are the population’s stem cells.

It is these SP cells that seem to be responsible for malignancy. SP cells injected into immunologically compromised mice generate aggressive, invasive cancers, whereas non-SP cells generally give rise only to local tumors. Since both cell types contain the same oncogenic mutations, the difference is probably due to epigenetic differences such as silencing, says Kondo. He and others are already looking for possible stem cell behavior switches, such as Bmi-1, an epigenetic factor recently shown to be concentrated in stem cells.

Reference: Kondo, T., et al. 2004. Proc. Natl. Acad. Sci. USA. 10.1073/pnas.0307618100.