Research Roundup

Cloning confusion

Every cloning pronouncement has one guaranteed ingredient: controversy. This time, Atsuo Ogura (National Institute of Infectious Diseases, Tokyo, Japan) and Rudolf Jaenisch (Massachusetts Institute of Technology, Cambridge, MA) are duking it out. Ogura claims that most of the defects that Jaenisch has seen in his cloned mice were caused by the genetic instability of the embryonic stem (ES) cells that Jaenisch used as a cloning source. In turn, Jaenisch calls Ogura’s claims of faithful gene expression “ludicrous” and “unbelievable.” Let the cloning wars continue.

At stake is an explanation of the various defects seen in some cloned offspring. Jaenisch believes that a number of things may be going wrong, but initially he has focused his attention on a subset of developmental genes called imprinted genes. Jaenisch found that a number of these genes were expressed at wildly varying levels in both his ES cells and the cloned progeny derived from these cells.

An imprinted gene is defined as one whose expression level varies depending on whether the gene was inherited from the father or mother. Jaenisch did not test parent-specific effects. But Ogura found that his clones expressed the correct allele of a number of imprinted genes: some were produced only from the paternal allele and others only from the maternal. Furthermore, a number of genes showed correct expression levels in cloned fetuses.

All is not sunny, however, even in Ogura’s world. The placentas of his cloned mice have variable and generally low expression of a wide range of genes, both imprinted and nonimprinted. Ogura believes that this is a general failure in reprogramming of gene expression, which might make it easier to tackle than the allele-specific effects of imprinting. He is now using gene chip analysis to see if there is any pattern in the misexpression data, and to extend the current analysis. “We must know what is a cloning effect and what is a culture effect and what is a donor cell effect,” he says.

If cloning protocols are improved, deciding whether to allow human cloning will become that much more difficult. But for now, on this point, Ogura and Jaenisch agree. “If the scientific evidence changes we can change our views,” says Jaenisch. “Right now it is totally out of the question.”

Reference: Inoue, K., et al. 2002. Science. 295:297.

What makes Ran run?

Changes in the nucleotide state of Ran, a small GTPase, provide the energy that drives transport into the nucleus. But so many proteins affect Ran’s status or localization that it has been difficult to work out which, if any, are the key control points. Now, Alicia Smith and Ian Macara (University of Virginia, Charlottesville, VA) and colleagues have coupled real-time measurements of Ran transport with a computational model to come up with an answer.

“There’s been a lot of hype about computational biology, but it’s actually only useful to address certain types of questions,” says Macara. “This was a compartmental problem, so it was relatively easy to set up the model.”

Macara did so with the help of Virtual Cell, a program developed by Leslie Loew and colleagues at the University of Connecticut Health Center, Farmington, CT. Macara plugged in a lot of rate constants, binding constants, and protein concentrations, many of which had been determined in earlier biochemical experiments. The resulting model matched the response of live cells when injected with labeled Ran, even when the levels of certain binding proteins and exchange factors were altered before injection.

There was little effect on the steady-state transport kinetics after changing the levels or behaviors of a number of import factors. And yet the transport rate in vivo falls far short of the maximal rate seen in vitro, suggesting a control point. That control point may be Rcc1. This guanine nucleotide exchange factor converts recently imported RanGDP into RanGTP, thus triggering the discharge of Ran from its import carrier. The model showed that altering the levels of Rcc1 had the most profound effect on the rate of Ran transport. According to Macara, this kind of result “is something that’s very hard to determine in a system where everything is coupled to everything else.” Now that he has the working model, however, Macara can determine how accessory factors might alter import, and ask why the cell uses adapters as well as carriers during import.

Reference: Smith, A.E., et al. 2002. Science. 295:488–491.