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The *tabula rasa* of cells

It isn't often that a scientific publication get its author on the cover of *Time*, but that's what happened to Jamie Thomson when his laboratory at the University of Wisconsin derived the first human embryonic stem (hES) cell lines[1]. Two years later, *Nature Biotechnology* published the second paper describing the production of hES cell lines by Benjamin Reubinoff, Martin Pera and their colleagues at Monash University in Melbourne[2]. This paper confirmed Thomson's results, and went further, showing that hES cells could be differentiated *in vitro*.

The first cells were derived with a medium containing mouse fibroblasts, "which is kind of a standard tissue culture that you use when you don't know what you're doing," explains Thomson, "because they secrete a lot of stuff. Then over the years, we've found that the things that do support mouse ES cells don't support hES cells." Pera says hES cell culture has progressed on many fronts, particularly "refining the culture system to make it easier to expand the cells and defining the medium to eliminate animal products." The remaining challenges, he says, are scaling up the culture and creating reliable techniques to grow up an entire culture from a single ES cell. Already, Pera says, many promising culture methodologies have been described; now they need to be assessed by multiple research groups. In January of this year, Thomson's group described the first fully defined xeno-free medium for culturing human stem cells and isolated two cell lines derived in fully defined medium, though both lines had chromosomal abnormalities[3].

But Thomson thinks that the major barriers for culturing hES cells have already been overcome. "There will be improvements," he says, "but it will be kind of diminishing returns from now on." In particular, the twin specters of genetic instability and tumorigenicity will be mastered, though not exorcised, through better technique, says Thomson, who was part of the team that first described the emergence of chromosoma abnormalities in cultured hES cells[4]. "If you're really careful with the culture conditions, the cells are quite stable. It's a concern that has to be managed," he adds, "but it is ultimately manageable."

"The odd abnormal cell may not be that much of a problem if it doesn't have an advantage" that lets it outcompete healthy cells, agrees Pera. Nonetheless, abnormal cells will arise even in ideal culture conditions; the key will be identifying and removing potentially dangerous cells before they are used in patients. In this issue, Pera and colleagues[5] show that five hES cell sublines with chromosomal abnormalities all express the CD30 receptor and that the protein's expression in normal hES cells prevents apoptosis, but Pera says additional biomarkers will be essential to weed out cells likely to run amok. Understanding, and preventing, the process that sets them down that path is more important.

Of course, deriving and culturing hES cells is really a means to an end. The ultimate goal is understanding and controlling differentiation well enough that the cells can be used to generate tissue for human therapies. "We just basically need more people beavering away at it," says Thomson, who believes that developmental biologists will be able to make most clinically relevant cell types within a decade. "Ultimately, we're going to arrive at a molecular blueprint of the pluripotent stage and then we'll know what switches we need to tweak," agrees Pera, though he is quick to point out that the ability to create differentiated cells is a very far cry from the ability to use the cells for therapy. But he warns that there will be many bumps in the road towards creating differentiated tissues, stemming largely from our ignorance of basic biology. "Stem cell culture isn't an absolute reflection of embryonic development: it's sort of a caricature."

Martin Pera: "Stem cell culture isn't an absolute reflection of embryonic development; it's sort of a caricature."

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Beacons of light

Ten years ago, a pair of scientists described a new kind of probe for quantifying nucleic acids that could do it in real-time and in solution[1]. No purification, no gels, no radioactivity (and no background smudges to contend with). They did it by combining the specificity of nucleic acid hybridization with the ability of nucleic acids to take on different conformations. This was the birth of molecular beacons. In the intervening years, beacons (which are fluorogenic hairpin oligonucleotide probes) have been applied in various ways—for quantitative PCR, for pathogen detection, for assaying single nucleotide polymorphism and mutations, for incorporation into self-reporting arrays, to name a few. The simple beacon itself has evolved by adding gold particles to broaden its specificity and sensitivity, primers so that it can both prime and detect nucleic acid amplification in a unimolecular and hence faster reaction, and even enzymes so that it can potentially prime, amplify and detect a sequence all in one tidy little package.

Sangi Tyagi and Fred Kramer of the Public Health Research Institute (Newark, NJ, USA) were working at the time on Qβ replicase amplification of RNA, which, like the polymerase chain reaction that later supplanted it, had unacceptably high backgrounds when working with low-abundance RNAs. They had the idea, based on Paul Lizardi's (now at Yale University) work on conformational changes of nucleic acids, to create probes that would allow amplification only when they changed their shape.

Thus, Tyagi, Kramer and Lizardi designed a probe with a stem-loop structure that unfolds when it reacts with its target sequence, the complement for which is encoded in the loop. In the first paper, Tyagi, Kramer and Lizardi showed how their fluorogenic probe, which they dubbed molecular beacon, could assay the accumulation of a target molecule in solution (a PCR fragment of the HIV invertase gene), one of the first

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