STEM CELLS

Conquering the unknown

A group of scientists has fully defined all the reagents needed for the derivation and maintenance of hES cells.

Black boxes can be dangerous when it comes to human embryonic stem (hES) cell research. Therefore James Thomson and his colleagues at the University of Madison have devoted considerable time and effort to defining all the components needed for culturing hES cells.

hES cells have the potential to differentiate into any cell type, but the task of propagating them in culture is fraught with challenges. Researchers used to grow hES cells on a layer of feeder cells in the presence of a commercially available serum substitute, which made culturing the cells less reproducible and introduced potentially harmful molecules. This was highlighted recently by the identification of contamination in all the hES cell lines currently admissible for federally funded research with a mouse sialic acid which they acquired from mouse feeder cells or serum components.

Previous research in the Thomson and other laboratories showed that they could get rid of the feeder cells by supplementing the medium with high concentrations of basal fibroblast growth factors. Now Thomson and colleagues took on the challenge of finding an alternative to the commercial serum replacement and the matrix on which the cells grow.

They developed a medium with clearly defined ingredients and showed that this medium supported growth of the federally approved hES cell lines. Notably, they found that these cell lines lost the mouse sialic acid after some time in culture. When asked whether this gave these cell lines a new chance in therapeutic application Thomson was hesitant: "You have to jump through a lot of hoops to show safety, and if you had new cell lines derived in clearly defined conditions, it would simply be safer."

His lab successfully derived two new hES cell lines in the defined medium, but both new lines unexpectedly showed karyotypical abnormalities after prolonged periods in culture. Thomson's plan is to repeat the process to see whether the abnormal karyotypes are related to the new medium itself, or to how the cells were handled.

The other undefined component in the currently used culture system is the matrix, often derived from mouse tumors. Thomson's lab showed an alternative with exclusively human products. The downside of this fully defined matrix is its prohibitive cost. Thomson views this issue very practically: "We got defined; now we need cheap." Coming up with a cost-effective way of producing this matrix will be the next challenge.

Despite these hurdles, Thomson is confident that the question of hES cell derivation and culture will soon be largely answered, leaving the much greater challenge of working out differentiation conditions. "Good developmental biologists are needed", concludes Thomson, but he also points to another significant roadblock to progress: "I think currently the bottleneck is predominantly political not scientific."

Nicole Rusk

RESEARCH PAPER

Ludwig, T.E. *et al.* Derivation of human embryonic stem cells in defined conditions. *Nat. Biotechnol.*; **24**, 185–187 (2006).

PROTEIN BIOCHEMISTRY

MICHAELIS-MENTEN FOR SINGLE ENZYME MOLECULES

The foundation of enzyme kinetics has been shown to hold true even at the single-molecule level.

Anyone who has ever taken an introductory biochemistry course probably learned the Michaelis-Menten equation in class and then tested it in the laboratory with a classic enzymatic assay. First discovered in 1913, this equation has been rigorously applied and has proven to accurately describe the kinetic behavior of ensembles of thousands of different enzymes.

With recent advances in protein dynamics technologies, researchers have discovered that proteins are not static entities but are actually in constant motion. For enzymes, these fluctuations can result in active site conformational changes, potentially hindering or abetting catalysis. Armed with this understanding, Sunney Xie and lead authors Brian English and Wei Min of Harvard University set out to answer the question, 'Does the Michaelis-Menten equation hold true for single enzyme molecules?'

Xie and coworkers first had to modify the Michaelis-Menten equation to describe stochastic single-molecule behavior, and then carefully construct an experiment to measure the catalytic turnovers of their test enzyme, β-galactosidase. Though many groups including Xie's had attempted to use fluorescence to study single enzyme molecules, this new experiment took the technique to the next level, as Xie explains, "The key was to use a fluorescent product instead of a fluorescent substrate. The fluorescent product allows continuous replenishing; it is generated and detected in the probe volume and then it quickly diffuses away." They monitored the turnover of a single immobilized β-galactosidase molecule by measuring the photon 'burst' of each product molecule generated from the fluorogenic substrate, resorufin–β-D-galactopyranoside. By constructing and comparing Lineweaver-Burke linear plots of the traditional ensemble data and the single-molecule data, they found excellent agreement, demonstrating that the Michaelis-Menten equation holds true for single enzyme molecules.

Yet Xie and coworkers discovered that the enzymatic rate constant is not actually constant, but fluctuates broadly on a single molecule basis. This finding is masked in ensemble-averaged experiments because slower individual enzyme molecules yield a long tail generally ignored in kinetics analyses. "If you only care about 90% of the population, it's not even important," explains Xie. "But inside a live cell you might only have one or a few copies of a particular enzyme, so then these fluctuations become very important." Moreover, Xie believes that this work really explains why the classic Michaelis-Menten is so accurate, as he says, "It works so well because it works even in the presence of these fluctuations."

Allison Doerr

RESEARCH PAPERS

English, B.P. *et al.* Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. *Nat. Chem. Biol.* **2**, 87–94 (2006).