A look back: fast, cheap and under control

Scientists have known since the early 1960s that it was possible to 'persuade' mammalian cells to take up foreign DNA—the difficult part has been getting this to happen with any sort of efficiency or reliability.

A major break came in the late 1960s, when several papers from the lab of Joseph Pagano demonstrated that the positively charged polymer DEAE-dextran, when combined and complexed with viral RNA or DNA, profoundly improved the uptake of these nucleic acids by cultured monkey kidney cells^{1,2}. This technique considerably enhanced the quality of Pagano's viral preparations and represented a major leap forward—this system is still used today, to some extent—but it had several liabilities. Among other shortcomings, DEAE-dextran can be highly toxic to cells, the foreign DNA is vulnerable to environmental nucleases, and the method is limited exclusively to use in transient transfections, in which the cells being manipulated will express the gene of interest only temporarily.

Frank Graham and Alex van der Eb recognized the limitations of this system; armed with data indicating that the presence of divalent cations greatly improves the efficiency of uptake, they tested a variety of compounds to help get adenoviral DNA into human KB cells. They found that their DNA, when combined with CaCl_2 in a phosphate-containing buffer, formed a precipitate, and that the extent of precipitation directly correlated with the extent to which the DNA adsorbed to the surface of cultured cells³. This strategy dramatically improved the uptake of viral DNA by the cells relative to the DEAE-dextran method, and unlike with the older technique, the use of high concentrations of DNA was an apparent boon rather than a liability. Calcium phosphate-mediated transfection also proved particularly well-suited to the generation of stable transfectants, a feature that enhanced the appeal of this approach to other researchers in the community, making it an extremely popular and widely used technique.

This was, of course, a long time ago, and since then a variety of more technically advanced alternative techniques has burst onto the scene, including lipofection, electroporation and biolistics. For today's researchers looking to get their DNA of choice into mammalian cells, the marketplace offers an embarrassment of riches, with virtually every supplier and manufacturer imaginable offering a broad array of solutions to any investigator's transfection woes. So why then would anybody fall back on a simplistic, 30-year-old technique?

According to Florian Wurm, of the Swiss Federal Institute of Technology, that's an easy question

to answer. "We still use calcium phosphate in the lab... it costs us nothing, and we just figured out how to do it so that every cell gets DNA and expresses it. It's a humongously successful DNA delivery vehicle." In 1996, Wurm and his colleagues published an article that—quite surprisingly, for more or less the first time—rigorously tested a broad array of transfection conditions and essentially codified a series of guidelines to which one could adhere to maximize the efficiency of DNA transfer⁴. Traditionally, one of the most routine complaints about calcium phosphate had been that although DNA complexes will adsorb to many cells, the extent to which the DNA actually makes it into the nucleus was highly unpredictable. By carefully identifying the optimal conditions, Wurm found that his transfection efficiency skyrocketed: "Even today, when I go around and mention that calcium phosphate is as good as any of the highly advertised, commercialized vector systems, people say, 'What? That can't be true.' And I say, 'Yes, [and] we're at 100% transfection efficiency—just look at our cells!'"

Another important enhancement was the development of protocols that enabled the calcium phosphate-mediated transfection of cells grown in suspension⁵, which not only increased the range of cell lines for which this method was suitable but also enabled its ultimate incorporation into optimized and considerably scaled-up transient transfection protocols⁶, such as the ones that might be applied in a pharmaceutical or other industrial setting for the bulk production of recombinant protein.

To be sure, there are certainly applications for which more advanced or specialized transfection strategies will be needed. But in many cases, simpler can still be better, and with the level of transfection efficiency that can be attained now, often the only limiting factor will be access to enough of the right DNA. "I'm interested in protein production," explains Wurm, "so for me, the excitement in using calcium phosphate... was that here I have a vehicle that doesn't cost me anything. It's essentially cost-free, and I can go now to any scale of preparation if I just make enough DNA."

Michael Eisenstein

1. Vaheri, A. & Pagano, J.S. *Virology* **27**, 434–436 (1965).
2. McCutchan, J.H. & Pagano, J.S. *J. Natl. Cancer Inst.* **41**, 351–357 (1968).
3. Graham, F.L. & van der Eb, A.J. *Virology* **52**, 456–467 (1973).
4. Jordan, M. *et al. Nucleic Acids Res.* **24**, 596–601 (1996).
5. Chu, G. & Sharp, P.A. *Gene* **13**, 197–202 (1981).
6. Girard, P. *et al. Cytotechnology* **38**, 15–21 (2002).