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Graham F L & van der Eb A J. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-67, 1973.
[Laboratory for Physiological Chemistry, State University of Leiden, The Netherlands]

This paper describes the development of a technique for introducing DNA into mammalian cells. The method involves formation of DNA-calcium phosphate precipitates that, upon addition to cell cultures, become adsorbed to the cells. DNA uptake occurs during incubation at 37 degrees in the presence of excess calcium ions. [The *SCI*® indicates that this paper has been cited in over 2,350 publications.]

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In 1969 I was nearing the completion of my PhD at the Ontario Cancer Institute (OCI) in Toronto and planning my postdoctoral studies. My wife suggested that we spend this period in Europe, and through contacts between Marv Gold at OCI and Adrian de Waard in The Netherlands, I arranged a postdoctoral position in Leiden with Alex J. van der Eb, who was interested in human adenoviruses. As part of an application for a fellowship from the National Cancer Institute of Canada (NCI), I needed an interesting problem involving adenoviruses and cancer. I thought it would be useful to have an assay for infectious adenovirus DNA so that we could analyse various fractions and fragments of the viral DNA for ability to infect and transform cells, and this was the problem that I proposed in my NCI application.

I arrived in The Netherlands (with an NCI fellowship) in April of 1970. Lex gave me some DEAE-dextran, at that time the reagent most commonly used for getting nucleic acids into mammalian cells,[1] showed me how to grow and plaque adenovirus and how to prepare adenovirus DNA, and I set to work. I was soon able to assay infectivity of SV40 DNA using DEAE-dextran but took nearly a year to detect a very low level of adenovirus DNA infectivity. I spent the second year trying to improve the DEAE-dextran technique, but whatever I tried, the assay remained insensitive and irreproducible for adenovirus DNA. I was nearing the end of my patience, my NCI fellowship, and probably my scientific career when Lex suggested looking at cellular uptake of labelled DNA as a measure of the efficiency of the assay. This had at least the merit of being faster than the two-week plaque assays needed to titrate adenoviruses.

Since it was well known, even to me, that bacterial transformation needed divalent cations, I decided to look at the effect on DNA uptake of $MgCl_2$ and $CaCl_2$ in combination with DEAE-dextran. I added various concentrations of the salts to DNA solutions, added the mixtures to cell cultures, and measured cell-associated, DNase-resistant counts. The first results were encouraging: $CaCl_2$, but not $MgCl_2$, gave a dramatic enhancement in apparent DNA uptake. Enhancement of infectivity was also obtained and found to be independent of DEAE-dextran. Soon after the first couple of successful experiments, I noticed that addition of $CaCl_2$ to DNA solutions resulted in the formation of a fine precipitate. It so happened that the solutions we used for dilution of DNA were Tris buffered tissue culture media, which contain phosphate ions.

It was easy to guess, and then to show, first, that the precipitate was the result of adding high concentrations of $CaCl_2$ to solutions containing phosphate; second, that DNA was coprecipitated with the calcium phosphate that was formed; and, third, that both DNA uptake and infectivity were dependent on precipitation. Free calcium ions also played a role in uptake of DNA into cells since, if the excess calcium ions were removed from the culture media after adsorption of coprecipitates, infectivity was abolished even though the DNA remained cell associated. Once a superficial understanding of the mechanism was achieved, fine-tuning of the assay went very quickly, and within a year we published a detailed description of the method.

In many ways the discovery of the technique was serendipitous. Had the DNA solutions not contained just the right amount of phosphate and been at the right pH, no biologically active coprecipitate would have formed. Had the DNA-$CaPO_4$-$CaCl_2$ cocktail not been left in the culture dishes when medium was added back onto the cells, DNA would not have been taken up because the calcium ion concentration would have been too low. Finally, had I seen a precipitate in the first experiment, I may have chucked the mixture down the drain and tried something else. Who would have believed that precipitating DNA out of solution could enhance its biological activity?

Obviously, the reason this paper is highly cited is not because it describes merely an assay for adenovirus DNA infectivity, useful as that may be for adenovirologists. Of much more general importance was the fact that we were able to transform cells,[2] something we had been unable to do using DEAE-dextran. We showed that DNA fragments could transform and determined the size and location of the adenovirus transforming region,[3] setting the stage for hundreds of subsequent studies involving DNA-mediated gene transfer. During the past decade the technique has become popular as a simple, general method of introducing DNA into a variety of eukaryotic cells for assays of infectivity, transforming activity, or transient expression of pure DNA, and many of the people who use this method are still citing the original paper. Douglas E. Brash recently provided a brief overview of human cells in genetic research.[4]

1. **McCutchan J H & Pagano J S.** Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. *J. Nat. Cancer Inst.* 41:351-7, 1968. (Cited 405 times.)
2. **Graham F L & van der Eb A J.** Transformation of rat cells by DNA of human adenovirus 5. *Virology* 54:536-9, 1973. (Cited 165 times.)
3. **Graham F L, Abrahams P J, Mulder C, Heijneker H L, Warnaar S O, de Vries F A J, Fiers W & van der Eb A J.** Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and simian virus 40. *Cold Spring Harbor Symp.* 39:637-50, 1974. (Cited 350 times.)
4. **Brash D E, Mark G E, Farrell M P & Harris C C.** Overview of human cells in genetic research: altered phenotypes in human cells caused by transferred genes. *Somat. Cell Molec. Genet.* 13:429-40, 1987.

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