CC/NUMBER 8
FEBRUARY 24, 1992

This Week's Citation Classic®

Graham F L, Smiley J, Russell W C & Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36:59-72, 1977.
[Depts. Biology and Pathology, McMaster Univ., Hamilton, Ontario, Canada; and, Div. Virology and Biochemistry, Nat. Inst. Med. Res., Mill Hill, London, England]

This paper describes the establishment and characterization of a human cell line transformed by human adenovirus type 5 DNA: the 293 cell line. [The *SCI*® indicates that this paper has been cited in more than 735 publications.]

Cell Line Transformation

Frank L. Graham
McMaster University
Department of Biology
1280 Main Street West
Hamilton, Ontario L8S 4K1
Canada

From 1970 to 1972, I worked in the laboratory of Alex van der Eb in Leiden on the development of a technique for assaying the infectivity and transforming activity of Ad5 DNA.[1] By the end of 1972, we had shown that we could transform primary rat embryo and rat kidney cells, that fragmented Ad5 DNA was active, and that by shearing the viral DNA to eliminate its infectivity we could transform permissive hamster cells that were otherwise refractory to transformation by nondefective virus or intact DNA.[2] We decided to attempt to transform primary human embryonic kidney cells. Thanks to the aid and coaching of Marie Alice Salgado, a technician in van der Eb's lab, I had gained the skill and patience at cell culture which would prove essential for success. The first experiment was encouraging in that a single transformed focus was seen in one of a total of 40 transfected dishes, but we failed to establish a permanent line from this transformant. The next experiment, initiated at the beginning of 1973, was more successful. A couple of colonies appeared and one of these was eventually, with some difficulty, induced to grow. Not until the end of June, 1973, were there enough cells to freeze away a few ampoules, and I felt sufficiently secure to entrust Salgado and van der Eb with the care of the cells while my wife and I went on holiday in Italy. Fortunately, they kept the cells happy and uncontaminated in my absence and on my return I could freeze away a few more ampoules and continue passaging the cells.

For the first 300 days, the cell population grew at a frustratingly slow pace, doubling every week-to-10 days. Then things got worse as the cells entered a classical crisis and refused to grow at all for another two-to-three months. A few cells survived the crisis, and the growth rate increased dramatically to about one population doubling every two-to-three days, and we could start using the cells for experiments. Because the original transformant was derived from dish number 3.1 in experiment 293, I named it cell line 293-31, now shortened to just 293.

Why such a monumental effort to establish an Ad5 transformed human cell line? In 1970, T.L. Benjamin had shown that transformation defective mutants of polyoma virus could be isolated by selecting for host range mutants able to replicate in polyoma virus transformed mouse cells but restricted for growth in normal mouse cells.[3] I wanted to be able to do the same with adenovirus and, using 293 cells, Tim Harrison, Jim Williams, and I were able to isolate Ad5 host range mutants with the desired phenotype[4] as have many other adenovirologists since.[5]

In the spring of 1973, I received an EMBO travel grant that allowed me to carry a culture of 293 cells to Willy C. Russell at Mill Hill, England, where we did a few studies on proteins in 293 cells. On my return to Canada at the beginning of 1975, I brought the line with me for my own studies. At McMaster, Jim Smiley, who was then a graduate student in Stan Mak's lab, used Southern analysis to show that the cells produced mRNA specific for the left end, transforming region of the viral genome—an important result since it provided a basis for assuming that host range mutants would be defective in genes encoded by this region. By this time, the cells had been distributed to a number of labs throughout Europe and North America, and we thought we had better publish something by combining our results with those of Russell and Rod Nairn.

Most of the citations of this paper come from adenovirologists, since almost everywhere adenoviruses are studied, 293 cells are used.[5] Not only are they useful for isolation and propagation of transformation defective adenovirus mutants, but they are excellent recipients of transfected DNA and support the growth of all kinds of human adenovirus, including so-called fastidious serotypes which replicate poorly on most other human cell lines.[6]

1. **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. (Cited 3,730 times.) [See also: **Graham F L.** Citation Classic. *Current Contents/Clinical Medicine* 16(46):16, 14 November 1988, and *Current Contents/Life Sciences* 31(46):16, 14 November 1988.]
2. **Graham F L, van der Eb A J & Heijneker J L.** Size and location of the transforming region in human adenovirus type 5 DNA. *Nature* 251:687-91, 1974. (Cited 170 times.)
3. **Benjamin T L.** Host range mutants of polyoma virus. *Proc. Nat. Acad. Sci. USA* 67:394-9, 1970. (Cited 200 times.)
4. **Harrison T, Graham F L & Williams J.** Host range mutants of adenovirus type 5 defective for growth in HeLa cells. *Virology* 77:319-29, 1977. (Cited 215 times.)
5. **Ginsberg H & Young C H S,** eds. *The adenoviruses. Comprehensive virology.* New York: Plenum, 1984.
6. **Wadell G, Allard A, Johansson M, Svensson L & Uhnoo I.** Enteric adenoviruses. *SO: Ciba Found. Symp.* 128:63-91, 1987.

Received January 23, 1991