This Week's Citation Classic®

Szybalska E H & Szybalski W. Genetics of human cell lines. IV. DNA-mediated heritable transformation of a biochemical trait. *Proc. Nat. Acad. Sci. USA* 48:2026-34, 1962.
[McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI]

This paper describes the selective HAT system (hypoxanthine, aminopterin, thymidine) designed for quantitative scoring and isolation of hypoxanthine-guanine-phosphoribotransferase-positive (HPRT+ or IMPPase+) cells, and its application for the first demonstration of DNA-mediated genetic transformation of human cells, at the *HPRT* locus. No HPRT+ revertants were ever observed with the D98/AH-2 HPRT- recipient cells, but with increasing HPRT+ DNA concentrations the transformation frequency increased linearly, up to 1,000-fold over the detection limits. [The *SCI*® indicates that this paper has been cited in more than 370 publications.]

A Forerunner of Monoclonal Antibodies and Human Gene Therapy

Waclaw Szybalski
McArdle Laboratory for Cancer Research
University of Wisconsin
Madison, WI 53706

In 1957-1958, I extended our work in microbial genetics to analogous studies with human cell lines by adapting the Detroit 98 human bone marrow cell line and isolating various mutants, including azahypoxanthine-resistant (D98/AH;*HPRT*-) strains, which we showed to be missing the HPRT enzyme (designated IMPPase in the early 1960s; *Proc. Amer. Assoc. Cancer Res.* 3:272, 1961). When attending a lecture of Van Potter, my colleague at McArdle Laboratory, on the purine pathways, it occurred to me that one could also select for gain of the HPRT function using a medium containing aminopterin (A; to block de novo purine synthesis), thymidine (T; to compensate for thymidilate synthesis inhibition by aminopterin), and hypoxanthine (H; as a source of purine nucleotides providing that HPRT is regained). More significantly, we also developed a system to select for HPRT gain, using a medium which my wife, Elizabeth, named HAT. Using this powerful selection and a mutant, D98/AH-2, which has never produced any revertants on HAT medium, we began to look for DNA-mediated transformation of *HPRT*- cells to the HPRT+ phenotype. We exposed D98/AH-2 (*HPRT*-) cells suspended in high-phosphate buffer (to retain favorable pH) to increasing concentrations of *HPRT*+ DNA purified from D98S cells, and plated the treated cells on HAT medium. Early experiments were not successful, but when spermine was added and enough time for phenotype expression provided, we obtained nearly a 1,000-fold increase in transformants over the detection limit. We included all the appropriate controls and even fractionated and assayed the transforming DNA in a shallow CaCl gradient.

Although first puzzled by the formation of a precipitate when spermine was used with high-phosphate buffer, we soon found that this was Ca•phosphate, since our spermine preparation contained more than 10 percent $CaCl_2$ (as detected by spectroscopic analysis). Thus, Ca•phosphate was vital for transformation, although spermine still improved its efficiency by two- to threefold.[1]

In 1962-1963, I constructed a hybridoma line by fusing my own skin cells with D98/AH-2 and using HAT selection. Similarly, using our HAT selection system, J.W. Littlefield[2,3] selected cell hybrids in 1964, and, 14 years after we designed HAT selection, G. Kohler and C. Milstein[4] applied it to making monoclonal antibodies, for which they were awarded a Nobel Prize.

When presenting our data at seminars and symposia in 1962-1964, we coined the terms "gene surgery" and "gene therapy" to stress the clinical potential of our work, but there was little interest in our results (except among poultry breeders),[1] probably because at that time prokaryotes, DNA synthesis, and the genetic code were at the center of attention. Thus in 1964, I decided to limit our studies to prokaryotic systems, especially phage λ, while depositing all of our cell lines, including D98/AH-2, with ATCC in Washington.

Transformation of mammalian cells is now routine, but as often happens with standard procedures, secondary publications rather than our nearly 30-year-old paper are usually cited. To our surprise, a patent was issued in 1983 for transformation of mammalian cells[5] using our HAT selection procedure. Of several reviews and books referring to our 1962 *PNAS* paper, space permits citing only two,[6,7] published at the beginning and the end of the past decade.

1. **Szybalski W.** DNA-mediated genetic transformation of human cell lines. *Proceedings of the 12th Annual Session National Poultry Breeders Roundtable*, 1963, Kansas City, MO. p. 90-109.
2. **Littlefield J W.** Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. *Science* 145:709-10, 1964. (Cited 1,990 times.)
3. ----------. Medium for hybrid selection. *Science* 180:256, 1973.
4. **Kohler G & Milstein C.** Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 356:495-7, 1975. (Cited 6,900 times.) [See also: **Garfield E.** The 1984 Nobel prize in medicine is awarded to Niels K. Jerne, César Milstein, and George J.F. Kohler for their contributions to immunology. *Essays of an information scientist*. Philadelphia: ISI Press, 1986. Vol. 8. p. 416-31.]
5. **Axel R, Wigler M H & Silverstein S J.** *Processes for inserting DNA into eukaryotic cells and for producing proteinaceus materials*. US patent 4,399,216. 16 August 1983.
6. **Scangos G & Ruddle F H.** Mechanisms and applications of DNA-mediated gene transfer in mammalian cells—a review. *Gene* 14:10, 1981. (Cited 140 times.)
7. **Old R W & Primrose S B.** *Principles of gene manipulation. An introduction to genetic engineering*. Oxford, England: Blackwell, 1989. p. 255-6; 418.

Received October 17, 1990