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Klebe R J, Chen T R & Ruddle F H. Controlled production of proliferating somatic cell hybrids. *J. Cell Biol.* 45:74-82, 1970. [Department of Biology, Yale University, New Haven, CT]

A method was described for producing large numbers of somatic cell hybrids,β-propiolactone inactivated Sendai virus was used to fuse cells, and we selected for somatic cell hybrids with HAT medium. To control contacts between cells during the fusion event, we introduced a technique that involved fusing substrate adherent cells. [The *SCI*® indicates that this paper has been cited in more than 225 publications.]

Production of Somatic Cell Hybrids

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This study made possible the production of large numbers of somatic cell hybrids. Prior to this report, the isolation of somatic cell hybrids was more of an art than a science. In earlier studies, hybrid cell lines were isolated by rather unpredictable means. For example, the earliest somatic cell hybrid overgrew both of its parental cell lines in a fortuitous display of hybrid vigor. While viral cell fusion was well described by Y. Okada,[1] in Japan, and J.F Enders,[2] in the US, viral cell fusion resulted in either rapid cell death or permanently infected cultures. Early methods for the production of short-term heterokaryons employed UV-inactivated Sendai virus. Unfortunately, the massive dose of UV required to completely kill a virus preparation would also destroy the cell fusion activity of the virus.

As in the case of many other advances, the present study came about by the combination and modification of methods developed by other groups. First, we used Sendai virus to fuse cells following the very careful studies of Okada and his colleagues. To eliminate the infectivity of Sendai virus, we used the β-propiolactone inactivation method, described by J.M. Neff and Enders, which completely destroys the infectivity of Sendai virus while preserving cell fusion activity. Prior studies of viral cell fusion involved treating dense cultures of ceils growing in suspension. To better control cell contacts during the fusion event and prevent more than two cells fusing at a time, we chose to fuse cells at semiconfluence in monolayer culture. We used the HAT biochemical selection system described by J.W. Littlefield[3] and E.H. Szybalska and W. Szybalski[4] to kill both parental cells. Lastly, according to me approach of F.H. Ruddle,[5] we used isozyme markers as genetic markers to characterize the hybrid nature of the presumptive hybrid cell lines isolated. This paper also introduced the RAG cell line, which became a favorite of somatic cell geneticists, due to its nonreverting HPRT marker and its differentiated traits.

The method we described took the magic out of making somatic cell hybrids. This study demonstrated that the yield of hybrids increased with increasing virus concentration and increasing cell density. As expected, the number of hybrids obtained was greatest when an equal number of each parental cell was employed. Hundreds of hybrid clones could be prepared using this simple, straightforward approach. Cell fusion is now carried out in a fashion similar to that described here, except that poly(ethylene giycol) is used instead of inactivated virus, following the work of E.C. Cocking[6] (plant ceils) and G. Pontecorvo[7] (mammalian cells). And, a recent paper by V.A. McKusick[8] provides a look at current trends in mapping human genes.

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2. **Neff J M & Enders J F.** Poliovirus replication and cytopathogenicity in monolayer hamster cell cultures fused with beta propiolactone-inactivated Sendai virus. *Proc. Soc. Exp. Biol. Med* 127:260, 1968. (Cited 145 times.)
3. **Littlefield J W.** Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. *Science* 145:709-10, 1964 (Cited 2,000 times.)
4. **Szybalska E H & Szybalski W.** Genetics of human cell lines. IV. DNA-mediated heritable transformation of a biochemical trait. *Proc. Nat. Acad Sci. USA* 48:2026-3, 1962. (Cited 370 times.) [See also: **Szybalski W**. A forerunner of monoclonal antibodies and human gene therapy. Citation Classic. *Current Contents/Life Sciences* 34(46): 11. 18 November 1991.]
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6. **Cocking E C**. Plant-cell protoplasts—isolation and development. *Annu. Rev. Plant Physiol.* 23:29, 1972. (Cited 210 times.)
7. **Pontecorvo G**. Production of mammalian somatic cell hybrids by means of polyethylene glycol treatment. *Somat. Cell Genet.* l:397-400, 1975. (Cited 420 times.) [See also: **Pontecorvo G.** Fusing cultured mammalian cells with polyethylene glycol. **Citation Classic.** ***Current Contents/Agriculture. Biology & Environmental Sciences*** **20(10): 16, 5 March 1990. and** ***Current Contents/Life Sciences*** **33:(10): 16, 5 March 1990.]**
8. **McKusick V A.** Current trends in mapping human genes. *FASEB J.* 5:12-20, 1991.

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