

Human-Mouse Hybrid Cell Lines and Susceptibility to Species-specific Viruses¹

R. POLLACK, J. SALAS,² R. WANG,³ T. KUSANO⁴ AND H. GREEN
*Departments of Pathology and Cell Biology, School of Medicine,
 New York University, New York, New York*

Human-mouse somatic cell hybrids contain apparently complete chromosome complements of the mouse, but the human chromosome complements are only partial. It is therefore possible to study how species-specific viruses which depend on human or on mouse genes behave in hybrids which contain partial or complete complements derived from the permissive species, and in which genes of the non-permissive species are also present.

A number of viruses showing a high degree of species specificity were examined — polio, SV40 and adeno, for which human cells are permissive and mouse cells are not; and polyoma virus, for which mouse cells are permissive and human are not. The mouse parental cell type in all cases was 3T3-4E (Matsuya and Green, '69). The human parental cell was the established line D98/AH₂ (Szybalski et al., '62) which gave rise to the HLE series of hybrids reported previously (Matsuya and Green, '69), and two strains of human diploid fibroblasts, WI38 (Hayflick and Moorhead, '61) and KL, whose hybrids with 3T3-4E have not previously been reported. Since 3T3-4E, like diploid mouse cells, contains only telocentric and acrocentric chromosomes, all biamed chromosomes found in the hybrid cells were of human origin. Generation of biamed chromosomes by centric fusion of mouse chromosomes is well known in established mouse lines but the rate of this process would be negligible during the short period involved in these experiments. No attempt was made to distinguish human acrocentrics from mouse chromosomes, so the number of biamed chromosomes gives a minimal value for the human contribution. The mouse contribution will be slightly overestimated by inclusion of the human acrocentrics.

Five hybrid clones obtained from three different hybridizations were infected with polio virus type I (Sabin). The parental 3T3-4E was not infectable by this virus. The hybrids supported polio infection and generally gave viral yields comparable to those of the respective human parental lines (table 1). Not all human-mouse hybrids are able to support polio virus multiplication (Weiss and Green, '67). Some, usually containing lower numbers of human chromosomes, were found to be resistant to infection. However, it is clear from table 1 that a hybrid clone, if it possesses a suitable human complement may also possess up to seven times the haploid number of mouse chromosomes and still be fully permissive for polio multiplication.

SV40 at high multiplicity is able to initiate an infection in a considerable fraction of the cells of D98/AH₂, an established human line. In one experiment 24% of the cells synthesized T-antigen and in another, 40% synthesized capsid antigen, both being determined by immunofluorescence. The mouse parental line synthesized T-antigen with lower frequency than the human line, and did not support late functions at all, as no cells showed the presence of capsid antigen. The hybrid line HLE-C, when infected under comparable conditions, consistently made T-antigen with a frequency higher than that of the mouse parental line, suggesting that the human genes contributed to some extent to the support of this viral function. As no cells of the same line were found to make viral capsid antigen, the human

Received July 14, '70. Accepted Sept. 11, '70.

¹ Aided by grants from the National Cancer Institute.

² Fellow of the International Agency for Research on Cancer.

³ Fellow of the National Cancer Institute.

⁴ Present address: Research Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University, Sendai, Japan.

TABLE 1

Virus	Human parental cell ¹	Hybrid chromosome content: Bi-Acro + armed telo	Viral susceptibility						PFU/ml	Ratio ³	HA (dilution titer)	Ratio ³	Ratio ³		
			T-antigen % of cells positive by immunofluorescence		Capsid antigen % of cells positive by immunofluorescence		Hybrid								
			Human	Mouse	Human	Mouse									
Polio I	D98/AH ₂	23	125										1.8 × 10 ⁷	1.2	
	WI38	4	132										2.8 × 10 ⁷	2.3	
	KL	9	130										1.0 × 10 ⁷	5.0	
		KLE-H	12	158										3.6 × 10 ⁶	0.1
		KLE-J	16	77										2.4 × 10 ⁶	0.1
SV40	D98/AH ₂	23	125	24	5.5	12	40	< 0.01	< 0.01	< 0.01					
Adeno: type 2	D98/AH ₂	23	125				40	< 0.01	< 0.01	< 0.01					
		HLE-B	31	125			40	< 0.01	< 0.01	< 0.01					
		HLE-B	31	125	20	< 0.1	< 0.01	25	< 0.01	< 0.01					
		HLE-I	30	108	10	< 0.1	< 0.1	10	< 0.01	< 0.01					
Polyoma	D98/AH ₂	23	125										1000	1.0	
	KL	9	130										1000	1.0	
		KLE-I	12	158									200	0.2	
		KLE-J	16	77									2.0 × 10 ⁷	0.50	
													2.3 × 10 ⁶	0.04	

¹ Mouse parental cell in all cases was 3T3-4E, which contains 69 acrocentric and telocentric chromosomes.
² Mean numbers. Metacentric and submetacentric chromosomes are all of human origin and their number is therefore a minimal number of human chromosomes in the hybrid.
³ Compared to permissive parental cell.

genes appeared to make no contribution adequate to support this function (see also (Swetly et al., '69)).

It is interesting to compare the behavior of SV40 with that of polyoma virus in the same and similar hybrids. Polyoma virus can grow well in 3T3-4E (Basilico et al., '70) and produces yields of 2×10^8 p.f.u./ml or higher, which corresponds to hemagglutination dilution titers of 1:1000 or higher. Human cells support no polyoma multiplication. The hybrids HLE-C, KLE-H and KLE-J produced viral yields approaching those of the parental mouse line and are therefore permissive. Not only are all the mouse genes essential for polyoma multiplication present in these hybrids but the human genes do not interfere appreciably.

The adeno virus types 2 and 12 grow in human cells, and at a multiplicity of 200 PFU per cell were able to induce adeno T-antigen and adeno capsid antigen in 10-40% of the cells of the human parental line D98/AH₂. The mouse parental cells did not support either of these functions. Three clones of HLE hybrids, possessing at least 23 human chromosomes derived from D98/AH₂, behaved like the mouse parental line, as no fluorescence-positive cells were seen for either antigen.

It is clear from this survey that the same clone of hybrid cells (HLE-C) may be fully permissive for viral multiplication (polio and polyoma), may support early

functions but not late ones (SV40), or may be totally non-permissive (adeno). The permissive state of the interspecies hybrids towards both polio and polyoma viruses indicates that the cells can accommodate the necessary viral receptors and other cellular gene products deriving from the two species. Whether a hybrid clone is permissive for any viral function probably depends on (a) whether the chromosomes bearing the necessary cellular genes are included in the hybrid and (b) whether the genes from the non-permissive species exert any suppressive effect. These factors will be described in more detail elsewhere for polio virus and polyoma virus.

ACKNOWLEDGMENTS

We are indebted to Dr. W. Strohl for stocks of the adeno viruses and to Dr. Raymond Gilden for the fluorescent anti-adeno sera.

LITERATURE CITED

- Basilico, C., Y. Matsuya and H. Green 1970 *Virology*, 41: 295-305.
Hayflick, L., and P. Moorhead 1961 *Exptl. Cell Research*, 25: 585-621.
Matsuya, H., and H. Green 1969 *Science*, 163: 697-698.
Swetly, P., G. Brodano, B. Knowles and H. Koprowski 1969 *J. of Virol.*, 4: 348-355.
Szybalski, W., E. H. Szybalska and G. Ragni 1962 In: *Analytical cell culture*. National Cancer Inst. Monograph 7, 1962, pp. 75-87.
Weiss, M. C., and H. Green 1967 *Proc. Natl. Acad. Sci.*, 58: 1104-1111.