the characteristic crystalline shape of glycogen, and lesser amounts of smooth membrane vesicles.

Two to four grams (wet weight) of starting material (10 to 20 litters of neonatal mice) yielded a final pellet of about 5 mg (wet weight)—a quantity sufficient for further biochemical characterization. Since neonatal skin possesses the full complement of organelles found in the adult, this technique should be directly applicable to adult epidermis.

This preparation should make it possible to ascertain the precise functions of the epidermal lamellar body (*5, 6*). Although a comprehensive survey of the organelle's contents is not yet completed, preliminary examination indicates that it has a lipid-to-protein ratio of about 40 to 60, that it has a higher ratio of glycosphingolipids to ceramides (1.29) than crude fractions (0.69), and that it is not richer than other membrane fractions in lysosomal enzymes. Finally, we believe that the method described here will have many applications in cell biology and biochemistry, particularly in situations where a particularly fragile or labile structure must be preserved.

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Evidence for the Recessive Nature of Cellular Immortality

Abstract. *Fusion of immortal cell lines with normal human fibroblasts or certain other immortal cell lines yields hybrids having limited division potential. Cellular immortality was found to be a recessive phenotype in hybrids. It was also found that at least two separate events in the normal cell genome can result in immortality. In fusions involving certain immortal parent cells, these events can be complemented to result in hybrids with finite division capacity.*

The phenotype of limited division of the normal human cell has been reported to be dominant over the immortal phenotype of HeLa (*1*) and SV40-transformed cells (*2, 3*) in hybrids. We now report that hybrids resulting from the fusion of diploid human fibroblasts with several other immortal cell lines also have limited division potential. Our results suggest that cellular immortality is a result of recessive dysfunctions or alterations in the genetic program that limits the division of normal cells. If immortality can occur via more than one set of dysfunctions or alterations in the normal cell program, it should be possible to identify "complementation groups" of immortal cells, which when fused with each other would yield hybrids having finite division potential. We have found that fusion of certain types of immortal cells result in immortal hybrids, whereas fusion of other combinations of immortal cell lines yield hybrids with finite division capacity. These results suggest that there are at least two complementation groups for cellular immortality.

Somatic cell hybridization has been used extensively for analysis of the dominant versus recessive nature of the phenotypes of tumorigenicity and transformation. The results are controversial since the phenotypes have been suppressed in some hybrids (*4*) and expressed in others (*5*). The transformation phenotypes that have been studied include morphology, density-dependent inhibition of growth, the requirement for serum, and anchorage-independent growth in soft agar and methyl cellulose. The phenotype of immortality (that is, the capacity for indefinite division in culture) has rarely been considered or studied. Nevertheless, the notion that cellular immortality is a dominant trait has been accepted. This idea has been based primarily on the observation that in fusions of various species of normal cells with immortal cells, it is possible to obtain hybrids with indefinite proliferative potential (*4*). Since the objectives (for example, chromosomal analysis, tumorigenicity, or anchorage-independent growth) of previous experiments required extensively proliferating hybrids, the methodology used for hybrid isolation restricted analysis to such hybrid clones. Scant attention was paid to the overwhelming majority of hybrid cells that ceased to proliferate. In the few studies (*1–3*) in which careful attention was given to the proliferative behavior of hybrids, the fusion of normal human cells with the immortal cell lines HeLa or SV40-transformed human fibroblasts resulted in hybrids having finite division potential. These results indicated that the phenotype of immortality was recessive in hybrids. Variant immortal cells occurred in some of the nonproliferating hybrid populations at a frequency of about one in 10^5 cells (*1, 3*). These variant cells probably account for the widespread belief that such cell fusions do not yield hybrid cells with finite division capacity.

We have analyzed the proliferative potential of hybrids from various fusions involving normal, virally transformed, and tumor-derived human cells. The technique we use for hybrid isolation (*3, 6*) allows us to analyze hybrids having a small proliferative potential (fewer than eight population doublings) and a more extensive proliferative potential (more than eight population doublings). We found that the phenotype of limited division is dominant in hybrids obtained after fusion of normal human cells with other human cells, whether the other cells are normal (*6*) or are derived from immortal tumor or immortal SV40-transformed cells (*3*). In the fusions of normal cells with SV40-transformed cells we had found earlier that the hybrids having limited division capacity expressed T

antigen (*3*). Using ^{32}P-labeled SV40 DNA to probe Southern blots of Eco RI–cleaved DNA isolated from these hybrids, we found that the viral DNA band patterns in the hybrids and the SV40-transformed parent used for fusion are the same. Thus, the phenotype of limited cell division of the normal cell is dominant over immortality even in the presence of a stably integrated viral genome that is being expressed.

Table 1 summarizes the results obtained from fusion of normal human cells with various immortal human cell lines. The normal cells had a proliferative potential of 12 to 17 population doublings remaining at the time of fusion. The most significant result is that all hybrids analyzed ceased dividing, even after proliferating through approximately 80 population doublings. Since we used immortal cell lines that varied greatly in tissue of origin as well as the process of immortalization (tumor-derived versus virally transformed), it appears that the recessive nature of the phenotype of immortality may be a general phenomenon. Rare variant immortal cells did occur during the period of division cessation in some hybrids, at very low frequencies of about one in 10^5 cells (*7*).

To test whether the fusion of various immortal cell lines might result in complementation to yield hybrids with limited division potential, we fused an SV40-transformed human fibroblast line with various other immortal human cell lines (Table 2). When a oubain-resistant, thioguanine-resistant (O^RTG^R) mutant SV40-transformed cell line was fused with the cell line from which it was derived or with other independently derived SV40-transformed lines, there was no complementation to yield hybrids with limited life-span. All hybrids analyzed could divide indefinitely. This indicates that in the three SV40-transformed cell lines we have studied, immortality is the result of either the same or noncomplementary genetic defects. In all the other fusions, most of the hybrid clones were capable of very limited division (fewer than eight population doublings). We feel confident that these small clones are hybrid clones and not contact feeding colonies because the number of cells inoculated is low (ten cells per square centimeter), and no clones were obtained on the control dishes. Two controls were used (*3, 6*): parent cells that were mixed and not treated with polyethylene glycol and parent cells individually treated with polyethylene glycol and mixed before inoculation into selective medium. However, since highly aneuploid parents were used in the fusions, the possibility exists that some events other than true complementation resulted in hybrids with low proliferative potentials. We therefore base our major conclusions on the results obtained with extensively proliferating hybrid clones (that is, those achieving at least 15 population doublings). When HT 1080 or its subclone were fused with SV40-transformed human fibroblasts, the hybrids that achieved more than eight population doublings could divide indefinitely (more than 100 population doublings) and exhibited no complementation for mortality. In all other fusions with SV40-transformed human fibroblasts, there was complementation, and division ceased in all hybrid clones analyzed. In the fusions of HeLa and T98G cells with SV40-transformed cells there was a resumption of division in some instances after foci of dividing cells appeared in the hybrids (*7*).

Fusion with 143B TK$^-$ (thymidine kinase–deficient) cells yielded no clones capable of indefinite proliferation. All clones that had limited division potential showed positive staining for SV40 T antigen by indirect immunofluorescence.

Since the immortal phenotype of 143B TK$^-$ was recessive in fusion with normal cells, and since 143B TK$^-$ yielded hybrids of limited life-span when fused with SV40-transformed cells, we fused 143B TK$^-$ to a thioguanine-resistant mutant of HT 1080, which gave immortal hybrids when fused with SV40-transformed cells. In this fusion experiment (Table 2), three of the five hybrid clones that could be subcultured ceased dividing, and division was not resumed. The other two hybrid clones were able to attain more than 100 population doublings and are therefore considered immortal.

Table 1. Fusion of human diploid fibroblasts with various immortal cell lines. The immortal cell lines used were SV40-transformed fibroblasts GM 639, VA 13, and GM 847 O^R; cervical carcinoma HeLa; fibrosarcomas HT 1080 and 1080 21A (a clone of HT 1080); glioblastoma T98G; and the Kirsten mouse sarcoma virus–transformed osteosarcoma 143B TK$^-$. The percentage of the immortal cell lines able to produce clones with more than eight population doublings (PD) during a 2-week incubation is shown in parentheses. Extensively proliferating hybrids (more than 15 PD) were examined for immortal foci.

Immortal cell line	Human diploid fibroblast	Number of experiments	Hybrids with PD ≥ 8 (%)	Extensively proliferating hybrids	
				PD range before division ceased	Ratio of clones with immortal foci to total clones
GM 639 (90)	GM 1662 O^R	6	30	16 to 64	9/18
VA 13 (90)	GM 1662 O^R	4	30	16 to 69	5/10
GM 847 O^R (60)	CSC 303 G	2	40	16 to 78	10/20
HeLa (75)	GM 1662 O^R	2	20	16 to 21*	18/89*
HT 1080 (70)	GM 1662 O^R	4	7	16, 22	1/2
1080 21A (60)	GM 1662 O^R	2	10	21 to 32	3/4
T98G (85)	GM 1662 O^R	3	4	17 to 64	2/6
143B TK$^-$ (95)	GM 1662 O^R	2	7	16 to 26	0/6

*Data from Bunn and Tarrant (*1*).

Table 2. Fusions of various immortal cell lines. GM 639 O^RTG^R and GM 847 O^R are SV40-transformed human skin fibroblasts. The percentage of cells in immortal cell lines that are able to produce clones with more than eight population doublings (PD) during a 2-week incubation is shown in parentheses.

Immortal cell line 1	Immortal cell line 2	Hybrids with PD ≥ 8 (%)	Extensively proliferating hybrids				
			Number of clones achieving given PD range before division ceased				Ratio of clones with immortal foci to total clones
			15–19	20–29	30–39	40–70	
GM 639 (90)	GM 639 O^RTG^R (80)	100					12/12
VA 13 (90)	GM 639 O^RTG^R	100					12/12
VA 13 (90)	GM 847 O^R (50)	100					12/12
GM 639 (90)	GM 847 O^R	100					12/12
HeLa (75)	GM 639 O^RTG^R	18	5	3	2	1	2/11
HT 1080 (70)	GM 639 O^RTG^R	10					4/4
1080 21A (60)	GM 639 O^RTG^R	20					8/8
T98G (85)	GM 639 O^RTG^R	20	1	8	3		6/12
143B TK$^-$ (95)	GM 639 O^RTG^R	6	4	5		2	0/11
HT 1080 TG^R (60)	143B TK$^-$ (95)	6	2			1	2/5

Since the fusions are intraspecies, and in many cases involve highly aneuploid lines, it is not possible to perform detailed cytogenetic analyses. However, comparisons of total chromosome counts of hybrid clones with those of the parental cell lines indicate varying degrees of chromosome loss in the hybrids. The median chromosome number in the hybrids from the GM 639 × T98G and GM 639 × GM 639 fusions is very nearly the same as the sum of the median number for each of the parental cell lines. In contrast, the median chromosome number of hybrids from fusions of GM 639 with HeLa, HT 1080 21A, and HT 1080 is 15 to 30 less than the sum of the median chromosome numbers of the parents, but in all cases larger than the number for either parent. Thus, it appears that there is no correlation between the extent of chromosome loss and the proliferative potential of the hybrids. In addition, the hybrids are positive for T antigen and therefore retain chromosome 5 of the SV40-transformed parent to which the viral genome has been mapped (*8*).

We conclude that the phenotype of immortality is recessive in hybrids with normal human cells and that complementation between immortal parent cells can result in hybrids with limited division potential. The data indicate that changes in two or more different events (or sets of events) occurring in the genetic program that limits the division of normal cells can result in immortality. The changes in the normal cell genome that lead to SV40 virus–mediated immortality are different from those occurring in the tumor-derived cell lines studied, with the possible exception of HT 1080 and its subclone; changes leading to immortality in HT 1080 may be the same as those mediated by SV40 virus. The fact that hybrids formed from fusion of 143B TK^- cells with SV40-transformed or HT 1080 cells showed limited proliferation is compatible with this possibility.

Our results support the hypothesis that limited proliferation is a result of a rigorously programmed series of events and that immortality is caused by certain changes—recessive in hybrids—in these events. The results argue strongly against the hypotheses that errors in the protein-synthesizing machinery of cells or recessive mutations are responsible for limited division in normal cells (*9*). The hypotheses suggest that the probability of these events occurring is decreased or eliminated in immortal cells. If this were true, fusion of immortal cell lines should yield only immortal hybrids, which we have experimentally found not to be the case. Smith and Lumpkin (*10*) proposed a hypothesis and model involving changes in gene expression to explain the mechanisms that limit the division potential of normal human cells in vitro. On the basis of this model, one would expect that cells could become immortal by at least two different mechanisms. The results we have described are compatible with this model.

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Aged Rats: Recovery of Motor Impairments by Intrastriatal Nigral Grafts

Abstract. *Dissociated cell suspensions, prepared from the substantia nigra and septal regions of rat embryos, can be grafted to the depths of the caudate-putamen and hippocampus of aged rats. The grafts were rich in dopamine-containing and acetylcholinesterase-positive neurons and had produced extensive new dopaminergic and cholinergic terminal networks in the host neostriatum and hippocampus, respectively. The intrastriatal dopaminergic grafts were associated with a significant improvement in motor coordination in the aged rats. This result suggests that the intracerebral grafting technique may provide a new tool for exploring the role of dopaminergic and cholinergic deficits in the neurological and behavioral impairments associated with aging.*

Aged rats, like aged humans, show a decline in brain functions, such as sensorimotor coordination (*1–3*) and learning and memory (*4, 5*). Recent experiments have attempted to correlate these deficits with age-dependent impairments in synaptic transmission of specific neurotransmitter systems. Thus, for example, deficits of sensorimotor coordination (*2*) and swimming abilities (*3*) in aging rats are similar to those seen after dopamine-depleting brain lesions (*6*), and they may be reduced by the administration of dopamine receptor agonists (*3*). Similarly, the age-related decline in memory and learning (*4, 5*) are reminiscent of the deficits seen after lesions to the septo-hippocampal system (*7*) or after administration of anticholinergic drugs (*8*); some positive results have been obtained in potentiating memory performance in aged rats and humans after administering cholinergic precursors and cholinesterase inhibitors (*4, 9*).

These recent data suggest that impairments in dopaminergic and cholinergic neurotransmission may contribute importantly to the decline in sensorimotor and cognitive function, respectively, associated with the aging process. We have previously shown that lesion-induced impairments of dopaminergic and cholinergic transmission in young rats can be partially reversed both biochemically (*10, 11*) and behaviorally (*12, 13*) by grafts of embryonic dopaminergic or cholinergic neurons; recent reports suggest that neuronal transplants can survive also in the aged rat brain (*14*). We now report that mesencephalic dopaminergic neurons and septal cholinergic neurons, taken from rat embryos, can be grafted with excellent survival rates to the neostriatum and hippocampus, respectively, in the brains of aged rats, and that the intrastriatal dopamine-rich transplants can ameliorate the impairments in motor coordination seen in these animals.

We studied female Sprague-Dawley rats (Anticimex, Stockholm) housed in group cages of three to eight animals