8. R. Levi-Montalcini and P. U. Angeletti, *Physiol. Rev.* **48**, 534 (1968); H. Thoenen and Y. A. Barde, *ibid.* **60**, 1284 (1980); H. Thoenen, C. Bandtlow, R. Heumann, *Rev. Physiol. Biochem. Pharmacol.* **109**, 145 (1987); S. R. Whittemore and Å. Seiger, *Brain Res. Rev.* **12**, 439 (1987).
9. G. P. Harper, R. W. Glanville, H. Thoenen, *J. Biol. Chem.* **257**, 8541 (1982); H. D. Hofmann and K. Unsicker, *Eur. J. Biochem.* **128**, 421 (1982).
10. C. Ayer-LeLievre, L. Olson, T. Ebendal, F. Hallböök, H. Persson, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2628 (1988).
11. L. Olson, C. Ayer-LeLievre, T. Ebendal, Å. Seiger, *Cell Tissue Res.* **248**, 275 (1987).
12. P. Ernfors *et al.*, *Neuron* **1**, 983 (1988).
13. M. J. Radeke, T. P. Misko, C. Hsu, L. A. Herzenberg, E. M. Shooter, *Nature* **325**, 593 (1987).
14. H. Persson and M. J. Villar, unpublished results.
15. A. E. Jackson, P. C. O'Leany, M. Ayers, D. M. de Kretser, *Biol. Reprod.* **35**, 425 (1986).
16. P. Poyet and F. Labrie, *Mol. Cell. Endocrinol.* **42**, 283 (1985).
17. P. Leblond and Y. Clermont, *Ann. N.Y. Acad. Sci.* **55**, 548 (1952).
18. D. B. Lubahn *et al.*, *Science* **240**, 327 (1988); C. Chang, J. Kokontis, S. Liao, *ibid.*, p. 324; *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7211 (1988); D. B. Lubahn *et al.*, *Mol. Endocrinol.* **2**, 1265 (1988).
19. R. M. Evans, *Science* **240**, 889 (1988); F. G. Berger and G. Watson, *Annu. Rev. Physiol.* **51**, 51 (1989).
20. L. Williams, C. McDonald, S. Higgins, *Nucleic Acids Res.* **13**, 659 (1985).
21. A. Sehgal, N. Patil, M. Chao, *Mol. Cell. Biol.* **8**, 3160 (1988).
22. M. Parvinen, *Endocrine Rev.* **3**, 404 (1982); ———, K. K. Vihko, J. Toppari, *Int. Rev. Cytol.* **104**, 115 (1986).
23. H. B. Waynforth, *Experimental and Surgical Techniques in the Rat* (Academic Press, New York, 1980), pp. 147–150.
24. P. Ernfors, A. Henschen, L. Olson, H. Persson, *Neuron* **2**, 1605 (1989).
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Induction of Cellular Senescence in Immortalized Cells by Human Chromosome 1

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The control of cellular senescence by specific human chromosomes was examined in interspecies cell hybrids between diploid human fibroblasts and an immortal, Syrian hamster cell line. Most such hybrids exhibited a limited life span comparable to that of the human fibroblasts, indicating that cellular senescence is dominant in these hybrids. Karyotypic analyses of the hybrid clones that did not senesce revealed that all these clones had lost both copies of human chromosome 1, whereas all other human chromosomes were observed in at least some of the immortal hybrids. The application of selective pressure for retention of human chromosome 1 to the cell hybrids resulted in an increased percentage of hybrids that senesced. Further, the introduction of a single copy of human chromosome 1 to the hamster cells by microcell fusion caused typical signs of cellular senescence. Transfer of chromosome 11 had no effect on the growth of the cells. These findings indicate that human chromosome 1 may participate in the control of cellular senescence and further support a genetic basis for cellular senescence.

Normal human fibroblasts in culture have a limited life span, beyond which the cells cease proliferation, enlarge in size, and undergo a process termed cellular senescence that results in cell death (*1*). The life span of human cells in culture decreases proportionately with the age of the donor (*1, 2*). Many, but not all, tumor cells can be grown indefinitely in culture and therefore have escaped senescence and are termed immortal (*3, 4*). Many carcinogenic agents, including chemical carcinogens, viruses, and oncogenes, can immortalize normal cells (*3–5*). Immortality is not sufficient for neoplastic transformation, but most immortal cells have an increased propensity for spontaneous, carcinogen-induced or oncogene-induced neoplastic progression (*3–5*). Therefore, escape from senescence can be a preneoplastic change that predisposes a cell to neoplastic conversion. Cellular senescence has been proposed as being one of the mechanisms by which tumor suppression occurs (*6*).

Two main theories of cellular senescence have been proposed. One is the error catastrophe model, which proposes that accumulation of random damage or mutations in protein and RNA synthesis results in the loss of proliferative capacity (*7*). A second hypothesis is that senescence is a genetically programmed process (*8*). The experimental evidence supporting the error accumulation hypothesis has been criticized (*1–9*), whereas recent support for a genetic basis of senescence has emerged (*10*). The majority of hybrids between human cells with a finite life span and immortal cells with an indefinite life span were found to senesce, indicating that senescence is dominant over immortality (*10, 11*). Certain hybrids between different immortal human cell lines senesce, indicating that different complementation groups exist for the senescence function lost in these cells (*10*). Four complementation groups were established, suggesting that multiple genes might be lost or inactivated during escape from senescence. With the use of hamster × human cell hybrids we have now mapped a putative senescence gene (or genes) to human chromosome 1.

For these studies, an immortal Syrian hamster cell line (10W-2) was fused with MRC-5 cells, which are normal, human, fetal lung fibroblasts previously used for studies of cellular senescence (*12*). The 10W-2 line was chosen because it has a near-diploid and a stable karyotype, and it is nontumorigenic (*13*). The MRC-5 cells were used at passage 25 and had undergone an estimated 40 population doublings in culture. When these cells were subcultured as controls, they reproducibly senesced after 21 to 24 additional population doublings in four independent experiments. The MRC-5 cells were fused with the 10W-2 cells as described in Table 1. From two independent experiments, 27 hybrid clones were isolated after fusion in medium containing HAT and ouabain. After 2 to 3 weeks in selective media, healthy colonies consisting of >1000 cells were isolated from cloning cylinders and passaged until the clones either ceased proliferation (that is, they senesced) or achieved >100 cell doublings as calculated from measurements of cell number at each passage. Fifteen of the 27 hybrid clones exhibited limited life spans. Each of these clones grew rapidly for the first three passages, underwent 15 to 20 total population doublings, and then displayed signs of cellular senescence characteristic of the parental MRC-5 cells at the end of their life span. Criteria for senescence included cellular enlargement and flattening, cessation of proliferation as measured by the failure to increase cell number during a 2-week period, failure to subculture, failure to form colonies at clonal density, and lack of significant incorporation of [^{3}H]thymidine [as measured by the presence of labeled nuclei (<2%) after autoradiography]. The limited life span of the majority of the hybrids indicates that cellular senescence is dominant in these hamster × human hybrids. A similar con-

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clusion was drawn from studying human (*10, 11*) and hamster (*13, 14*) intraspecies hybrids.

Twelve of the 27 hybrid clones ultimately escaped senescence. These clones showed signs of senescence after two to three passages, but some cells in the culture continued to proliferate and achieved >100 population doublings. These hybrid cells at later passages showed other features of immortal cells. After the cultures had achieved approximately 40 population doublings, the cells had a high labeling index with [^{3}H]thymidine, similar to the parental 10W-2 cells (>80% after a 24-hour labeling period) and grew with colony-forming efficiencies of >10%. As human chromosomes are usually lost in interspecies hybrids (*15*), we examined the possibility that escape from senescence was due to loss of an essential chromosome (or chromosomes) by karyotypic examination of the hybrids after escape from senescence (approximately 40 population doublings).

A summary of the human chromosomes present in the hybrids that escaped senescence is given in Table 1. The modal number of hamster and human chromosomes in each hybrid varied between 90 and 95. The diploid MRC-5 cells have 46 chromosomes, and the parental 10W-2 cells had a modal chromosome number of 44 to 46. The number of human chromosomes per hybrid ranged from 6 to 20; thus, the hybrid clones that escaped senescence contained a near-tetraploid number (70 to 89) of hamster chromosomes. The increased number of hamster chromosomes could have arisen by fusion of two hamster cells with one human cell or by fusion of a human cell with a 10W-2 cell that had become near-tetraploid. We previously found that immortal hybrids between hamster tumor cells and normal Syrian hamster embryo cells predominately formed from fusion of two tumor cells with one normal cell (*13, 14*), which suggests that two genome equivalents of the immortal parent cell increases the probability that a hybrid will escape senescence. This could be due to an increased dosage of a critical gene in the immortal parent cell or, alternatively, the increased chromosome number may result in karyotypic instability in the hybrid cells leading to loss of genes from the normal cells. These mechanisms are not mutually exclusive and both may play a role in the escape from senescence.

All of the MRC-5 × 10W-2 hybrid clones that escaped senescence lost both copies of human chromosome 1. A total of 120 metaphases were examined and none contained a human chromosome 1. All other human chromosomes were present in one or two copies in at least one of the immortal hybrids (Table 1).

To determine whether the loss of chromosome 1 in the hybrids that escaped senescence was the fortuitous consequence of human chromosome loss or an indication that a gene or genes on this chromosome influenced the senescence process, we undertook two additional approaches. The 10W-2 cells used in the experiment lack hypoxanthine phosphoribosyl transferase (HPRT) gene activity. Hamster-human hybrid clones were selected in HAT-containing medium, which means that surviving cells must retain the HPRT gene located on the long arm of human chromosome X; karyotypic analysis confirmed that all immortal hybrids retained a human chromosome X (Table 1). We used normal, human fibroblasts with a translocation between chromosome X and either chromosome 1 or chromosome 11 (obtained from the National Institute of General Medical Sciences human genetic mutant cell repository, Cam-

Table 1. Correlation of loss of human chromosomes with escape from senescence in hybrids between normal human (MRC-5) and immortal hamster (10W-2) cells. The MRC-5 cells (passage 25) were obtained from the American Type Culture Collection and 10W-2 cells were derived in our laboratory as described (*13*). The 10W-2 cells were selected for loss of hypoxanthine phosphoribosyl transferase (HPRT) gene activity by selection of cells resistant to 6-thioguanine (5 μg/ml); these cells failed to grow in HAT-containing medium (complete medium containing 0.1 m*M* hypoxanthine, 0.01*M* aminopterin, and 16 μ*M* thymidine) (*13*). The cells were grown in IBR medium with 10% fetal bovine serum (FBS) plus penicillin (100 IU/ml) and streptomycin (0.1 mg/liter) at 37°C in humidified air containing 10% CO_2. For cell fusions, normal MRC-5 human cells (2×10^6) and immortal 10W-2 cells (1×10^6) were plated together in 75-cm^2 flasks and allowed to grow overnight. These cells were then treated with polyethylene glycol (PEG) as described (*13*). After 24 hours, cells were plated on plastic at a density of 10^4 cells per 100-mm dish. Hybrid cells were selected in HAT-containing medium (20% FBS) containing $10^{-5}M$ ouabain, which is toxic to human but not hamster cells, with a change of medium every 4 days. Hybrid clones were isolated with glass cloning cylinders after 2 to 3 weeks and were expanded to a sufficient cell number for further analysis. Hybrid clones were passaged weekly by determining the cell number with a Coulter cell counter and replated at a density of 10^5 cells per 100-mm dish. Cells were prepared for karyotypic analysis by treatment with Colcemid (0.1 μg/ml) for 2 hours, followed by a hypotonic solution (0.075*M* KCl) for 20 min, and fixation in methanol:glacial acetic acid (3:1). Air-dried chromosome preparations were stained with quinacrine mustard plus Hoechst 33258 (*26*) and examined microscopically under fluorescence, which allowed distinction of human and hamster chromosomes in the hybrids. Ten well-banded metaphases were analyzed per hybrid clone. The colony-forming efficiencies of the clones on plastic were determined by plating 10^2 hybrid cells per 100-mm dish (five dishes); colonies containing more than 50 cells were scored 1 to 2 weeks later. To determine the percentage of cells synthesizing DNA (labeling index), we plated cells at a density of 10^4 cells per dish, 2 days later incubated them with [^{3}H]thymidine (1 μCi/ml in the nonselective medium and 5 μCi/ml in the HAT-selective medium) for 24 hours, and then fixed them with methanol and processed them for autoradiography. Over 1000 cells were counted at random for the presence of grains over the nucleus.

Hybrid clone	Presence (+) or absence (−) of indicated human chromosome																							Number of chromosomes
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
1	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+	−	+	−	+	−	+	+	+	−	88–98
6	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	−	+	+	+	+	+	78–91
7	−	−	+	−	−	+	+	+	−	+	+	+	+	+	+	−	+	−	+	−	+	+	+	+	87–102
8	−	−	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	80–102
12	−	−	+	+	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	101–109
A-1	−	+	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	85–99
B-2	−	−	−	+	−	+	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	−	+	+	83–90
B-3	−	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	79–92
B-4	−	−	+	+	−	+	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	91–98
D-1	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	82–98
F-2	−	−	+	−	+	+	+	−	−	−	−	+	−	+	+	+	+	−	+	+	+	+	+	+	77–92
J-2	−	−	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	−	+	−	+	+	+	−	68–90
	Percentage of metaphases having indicated human chromosome																							
	0	3	73	46	20	53	54	27	9	19	56	63	43	49	84	34	47	24	39	41	87	39	90	39	
	Number of discordant clones per 12 examined																							
	0	1	10	8	5	11	10	6	2	4	9	11	8	10	12	9	11	6	9	9	12	10	12	7	

den, New Jersey) to make cell hybrids with the 10W-2 cells. GM3552 cells have a 46,X,t(X;11) (Xpter>Xq26::11q23>11qter; 11pter>11q23::Xq26>Xqter) karyotype and GM4618 cells have a 46,X,t(X;1) (Xqter> Xp22.1::1q23>1qter;1pter>1q23::Xp22.1> Xpter) karyotype. The translocated portion of the X chromosome in each case contained the HPRT gene located on Xq. Both cell strains had a finite life span, and hybrids between these cells and 10W-2 cells senesced. The percentage of senescent hybrids was 40% (10 out of 25) in the case of fusions between 10W-2 and GM3552 cells [with the t(X;11) translocation], similar to the value obtained for MRC-5 × 10W-2 cell (56% or 15 out of 27). In contrast, nearly 90% (14 out of 16) of the hybrids senesced in fusions of GM4618 and 10W-2 cells [with the t(X;1) translocation]. This increased frequency of senescent hybrids is consistent with the hypothesis that human chromosome 1 contains a gene or genes that regulate the senescence process. Further, this gene or genes must be on the long arm of chromosome 1. The two GM4618 × 10W-2 hybrids that escaped senescence were examined karyotypically, and no intact t(X;1) chromosome was observed. We interpret these results to indicate that a deletion of the critical portion of chromosome 1 occurred, which allowed these hybrids to escape senescence. As these cells grew in HAT-containing media, the HPRT gene on chromosome X must have been retained in these cells.

To further confirm the role of human chromosome 1 in the senescence of 10W-2 cells, we transferred a single copy of chromosome 1 into 10W-2 cells by the microcell transfer technique (*16, 17*). Mouse A9 cells containing a single human chromosome 1 or chromosome 11 tagged with a dominant selectable marker (*neo*) were isolated (*17*), and the single human chromosomes were transferred by microcell fusions to 10W-2 cells, two other immortal Syrian hamster cell lines, and mouse A9 cells. The frequency of colonies resistant to G418 antibiotic was 2.3×10^{-6} to 5.9×10^{-6} after transfer of chromosome 11 into the various cell lines, and no colonies senesced (Table 2). The frequency of G418-resistant colonies was 2.6×10^{-6} to 7.4×10^{-6} after transfer of chromosome 1 into the mouse A9 cells and two hamster cell lines, but only one colony with >100 cells was observed in ten experiments with 10W-2 cells (frequency, 3×10^{-8}). This clone, however, senesced after 4 weeks and failed to grow to >1000 cells. Several small, senescent colonies (8 to 20 cells) were observed after transfer to chromosome 1 into 10W-2 cells, but these colonies ceased proliferating and sometimes eventually detached from the dish. No small colonies were seen in control cultures after selection with G418.

Table 2. Microcell transfer of human chromsome 1 or 11 into different immortal cell lines. Microcell-mediated chromosome transfers were performed as described (*17*). Briefly, mouse A9 cells containing a single human chromosome (1 or 11) with an integrated pSV2*neo* plasmid DNA were plated at a density of 1.5×10^6 to 1.8×10^6 cells per 25-cm^2 flask and treated for 48 hours with Colcemid (0.02 μg/ml) for micronucleus formation. Micronuclei were harvested by filling the flasks with serum-free medium containing cytochalasin B (10 μg/ml), and the flasks were centrifuged at 23,000*g* for 60 min. Microcell pellets were resuspended into serum-free medium, and filtered through 8-μm, 5-μm, and 3-μm polycarbonate filters in series to remove whole cells and large microcells. The filtered microcells were centrifuged at 1500 rpm for 5 min, resuspended in serum-free medium containing phytohemagglutinin-p (50 μg/ml), and incubated with donor cells for 15 min. After aspiration of medium from the flasks, the recipient cells were fused with microcells by treatment with PEG. After 24 hours, cells were plated at a density of 10^5 or 10^6 cells per 100-mm dish with selective medium containing G418 antibiotic (800 μg/ml) (Gibco). Cells were cultured for 2 to 4 weeks, and colonies of >100 cells were scored. A9, mouse cell line; BP6T, immortal, tumorigenic hamster cells transformed by benzo[*a*]pyrene (*12*); BHK-A, baby hamster kidney cells that became tumorigenic spontaneously; *n*, number of experiments.

Recipient cells	Chromosome 1			Chromosome 11		
	n	Number of colonies (>50 cells)	Frequency	*n*	Number of colonies (>50 cells)	Frequency
10W-2	10	1	3.2×10^{-8}	12	70	2.3×10^{-6}
A9	2	23	7.4×10^{-6}	2	17	4.6×10^{-6}
BP6T	7	34	3.1×10^{-6}	5	101	3.1×10^{-6}
BHK-A	6	51	2.6×10^{-6}	4	65	5.9×10^{-6}

The presence of human chromosome 1 in the senescent microcell hybrids was confirmed by in situ karyotypic analyses of colonies (*18*) and by in situ DNA hybridization with a human chromosome 1–specific α satellite DNA probe (*19*). Furthermore, similar microcell transfer experiments with tumorigenic derivatives of 10W-2 cells occasionally yielded surviving colonies after transfer of human chromosome 1. The cells grew very slowly at first, but after a few passages showed an increased growth rate. Karyotypic analyses clearly revealed human chromosome 1 at early passages and a progressive loss of chromosome 1 associated with increased growth of the clones (*20*). These observations are consistent with this chromosome containing a growth arrest or senescence gene or genes.

The mapping of a normal cellular gene involved in cellular senescence to a specific chromosome provides support for the hypothesis that senescence is a genetically programmed event. This does not imply, however, that a single gene controls senescence in normal cells. Cellular senescence is undoubtedly a multigenic process, and escape from cellular senescence (immortality) appears to require defects in one or more of a relatively small number of genes. These defects can be corrected by fusion of immortal cell lines with normal diploid cells (*10*), by fusion with certain other immortal cell lines (*10*), and by introduction of a single human chromosome (for example, chromosome 1 in the case of 10W-2 cells). The demonstration of at least four complementation groups by fusion of immortal × immortal cell lines (*10*) supports the notion that defects in different genes can lead to escape from senescence. Thus, introduction of human chromosome 1 would not be expected to result in senescence in all recipient cell lines, and some cells can be grown indefinitely following transfer of a normal human chromosome 1 (Table 2). However, microcell transfer of human chromosome 1 into a human endometrial cancer cell line also resulted in senescence of these cells (*21*).

The gene or genes involved in the senescence of 10W-2 cells are located on the long arm of human chromosome 1. Alterations of chromosome 1q occur in a variety of human tumors including intestinal, breast, ovarian, uterine, and colon and myeloproliferative disorders (*22*). Alterations in chromosome 1q are also associated with the acquisition of immortality in vitro of colorectal adenomas (*23*). Suppression of tumorigenicity or transformed properties has also been associated with the presence of human chromosome 1 in cell hybrids (*24*).

The findings that cellular senescence is dominant in cell hybrids and that inactivation of specific genes is possibly important in escape from senescence are not necessarily incompatible with the notion that activation of specific oncogenes results in escape from senescence. Simian virus 40 (SV40)–induced immortal human cell lines (*25*) as well as chemically induced, immortal rodent cell lines (*3*) often appear to arise by a multistep process. Thus, both activation of oncogenes and inactivation of possible senescence genes may be necessary for escape from senescence (*3*).

REFERENCES AND NOTES

1. L. Hayflick and P. S. Moorhead, *Exp. Cell Res.* **25**, 585 (1961); L. Hayflick, *ibid.* **37**, 614 (1965); *Mech. Ageing Dev.* **28**, 177 (1984); A. Macieira-Coelho, *Interdiscip. Top. Gerontol.* **23**, 1 (1988); B. M. Stanulis-Praeger, *Mech. Ageing Dev.* **38**, 1 (1987).
2. G. M. Martin, C. A. Sprague, C. J. Epstein, *Lab. Invest.* **23**, 86 (1970); E. L. Schneider and Y. Mutsui, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3584 (1976).
3. J. C. Barrett and W. F. Fletcher, in *Mechanisms of Environmental Carcinogenesis: Multistep Models of Carcinogenesis*, J. C. Barrett, Ed. (CRC Press, Boca Raton, FL, 1987), vol. 2, pp. 73–116.
4. H. S. Smith *et al.*, *J. Natl. Cancer Inst.* **78**, 611 (1987); J. C. Barrett, *Cancer Res.* **40**, 91 (1980).
5. R. A. Weinberg, *Science* **230**, 770 (1985); R. F. Newbold, R. W. Overell, J. R. Connell, *Nature* **299**, 633 (1982); R. F. Newbold and R. W. Overell, *ibid.* **304**, 648 (1983).
6. W. O'Brien, G. Stenman, R. Sager, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8659 (1986).
7. L. E. Orgel, *ibid.* **49**, 517 (1963); R. Holliday and G. M. Tarrant, *Nature* **238**, 26 (1972); S. J. Fulder and R. Holliday, *Cell* 6, 67 (1975).
8. J. R. Smith and C. K. Lumpkin, *Mech. Ageing Dev.* **13**, 387 (1980).
9. J. H. Buchanan, C. L. Bunn, R. I. Lappin, A. Stevens, *ibid.*, p. 339; C. B. Harley, J. W. Pollard, J. W. Chamberlin, C. P. Stanners, S. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* 77, 1885 (1980); T. E. Johnson and G. McCaffrey, *Mech. Ageing Dev.* **30**, 285 (1985).
10. O. M. Pereira-Smith and J. R. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6042 (1988); *Science* **221**, 964 (1983).
11. R. H. Norwood, W. R. Pendergrass, C. A. Sprague, G. M. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2231 (1974).
12. J. P. Jacobs, C. M. Jones, J. P. Baille, *Nature* **170**, 168 (1970).
13. M. Oshimura, T. W. Hesterberg, J. C. Barrett, *Cancer Genet. Cytogenet.* **22**, 225 (1986); M. Koi, C. Jones, L. A. Annab, J. C. Barrett, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8773 (1989); M. Koi and J. C. Barrett, *ibid.* **83**, 5992 (1986).
14. M. Oshimura *et al.*, *Cancer Res.* **48**, 1623 (1988).
15. M. C. Weiss and H. Green, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1104 (1967).
16. R. E. K. Fournier and F. H. Ruddle, *ibid.* **74**, 919 (1977).
17. M. Koi *et al.*, *Mol. Carcinogen.* **2**, 12 (1989); M. Koi, *Jpn. J. Cancer Res.* **80**, 122 (1989).
18. M. Oshimura *et al.*, *Cancer Res.* **48**, 702 (1988).
19. J. S. Waye, *Genomics* **1**, 43 (1987).
20. O. Sugawara, M. Oshimura, M. Koi, L. A. Annab, J. C. Barrett, unpublished data.
21. H. Yamada *et al.*, in preparation.
22. G. Kovacs, *Int. J. Cancer* **21**, 688 (1978); *Cancer Genet. Cytogenet.* **3**, 125 (1981); L.-C. Chen, C. Dollbaumand, H. S. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7204 (1989); N. B. Atkin and V. J. Pickthall, *Hum. Genet.* **38**, 25 (1977); N. B. Atkin, M. C. Baker, A. Ferti-Passantonopoulou, *Acta Cytol.* **27**, 450 (1983); J. D. Rowley, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5729 (1977); J. T. Mascarello, C. Osborn, R. P. Kadota, *Cancer Genet. Cytogenet.* **38**, 9 (1989); A. Reichman, P. Martin, B. Levin, *ibid.* 7, 51 (1982); J. K. V. Willson, G. N. Bitner, T. D. Oberley, L. F. Meisner, J. L. Weese, *Cancer Res.* **47**, 2704 (1987); C. Paraskeva, B. G. Buckle, D. Sheer, C. B. Wigley, *Int. J. Cancer* **34**, 49 (1984).
23. C. Paraskeva, S. Finerty, S. Powell, *Int. J. Cancer* **41**, 908 (1988); C. Paraskeva, A. Harvey, S. Finerty, S. Powell, *ibid.* **43**, 743 (1989).
24. W. F. Benedict, B. E. Weissman, C. Mark, E. J. Stanbridge, *Cancer Res.* **44**, 3471 (1984); A. Stoler and N. Bouck, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 570 (1985).
25. A. J. Girardi, F. C. Jensen, H. Kiprowski, *J. Cell Comp. Physiol.* **65**, 69 (1965).
26. M. C. Yoshida, T. Ikeuchi, M. Sasaki, *Proc. Jpn. Acad.* **51**, 181 (1975).

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A Potent GAL4 Derivative Activates Transcription at a Distance in Vitro

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Transcription of a typical eukaryotic gene by RNA polymerase II is activated by proteins bound to sites found near the beginning of the gene as well as to sites, called enhancers, located a great distance from the gene. According to one view, the primary difference between an activator that can work at a large distance and one that cannot is that the former bears a particularly strong activating region; the stronger the activating region, the more readily the activator interacts with its target bound near the transcriptional start site, with the intervening DNA looping out to accommodate the reaction. One alternative view is that the effect of proteins bound to enhancers might require some special aspect of cellular or chromosome structure. Consistent with the first view, an activator bearing an unusually potent activating region can stimulate transcription of a mammalian gene in a HeLa nuclear extract when bound as far as 1.3 kilobase pairs upstream or 320 base pairs downstream of the transcriptional start site.

GAL4 IS A TRANSCRIPTIONAL ACTIVATOR found in the yeast *Saccharomyces cerevisiae* (*1*). The 881–amino acid protein binds as dimers to 17-bp dyad sites to activate transcription of a nearby gene (*2*). GAL4 will also activate transcription of a gene in many other eukaryotic cells, including mammalian tissue culture cells (*3*), when its binding sites are placed in the vicinity of the target gene. A fragment comprising the first 147 amino acids of GAL4 [GAL4(1–147)] binds DNA but fails to activate transcription in vivo because, according to our current picture, it lacks an activating region (*4*). We used two fusion

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Table 1. Summary of activation on the different templates. Different exposures of the autoradiographs in Figs. 1 and 2 were scanned with a densitometer and quantified. AH refers to GAL4(1–147)+AH and VP16 indicates GAL4(1–147)+VP16. The stimulations listed are all relative to the level of transcription seen with the TATA box alone in the absence of activator: this amount was assigned a value of 1. Addition of a single TATA-proximal ATF site generally gives a twofold stimulation under our reaction conditions. The experiments were repeated on average three times per template with similar results. The ratio of the transcription signal in the presence of GAL4(1–147)+VP16 to that in the presence of GAL4(1–147)+AH is indicated. The size of the insertions between the GAL4 binding sites and the TATA box are indicated as are the actual distances from the edge of the GAL4 sites to the first T of the *E4* TATA; the sites in pG_5E4T already begin 23 bp from the first T of the *E4* TATA box and the ATF site contributes an additional 25 bp.

Template	Insert (bp)	Distance of GAL4 sites from TATA (bp)	ATF site	Activator	Fold stimulation	Fold stimulation VP16/AH
				–	1	
pG_5E4T	0	23	–	AH	50	4.4
				VP16	216	
				–	1	
$pG_5I54E4T$	54	77	–	AH	15	14.7
				VP16	212	
				–	1	
$pG_5I201E4T$	201	224	–	AH	2	10
				VP16	20	
				–	2	
$pG_5I54AE4T$	54	102	+	AH	50	6.6
				VP16	328	
				–	2	
$pG_5I201AE4T$	201	249	+	AH	7	13.1
				VP16	92	
				–	2	
$pG_5I1300AE4T$	1270	1318	+	AH	2	>100*
				VP16	12	
				–	2	
$pAE4TI320G_5$	320	320	+	AH	4	12.5
				VP16	50	

*We are unable to measure activation by GAL4(1–147)+AH on this template and therefore the ratio cannot be determined accurately, but it is probably very high.