Chromosomal gene transfer as described in this report may be used for the mobilization of *S. citri* chromosomes and consequently the establishment of a mapping system. Moreover, the development of *Spiroplasma* genetics, studied in vivo or in vitro through recombinant DNA technology, will provide genetic tools to investigate problems specific to *Spiroplasma*, such as helical morphology and pathogenicity, as well as the nature of genes providing the minimal requirements for the life of these simple organisms.

Finally, this report is a contribution to Morowitz's proposal (*20*) for a thorough understanding of all the functions of these simplest of prokaryotic cells.

REFERENCES AND NOTES

1. J. M. Bové, *Annu. Rev. Phytopathol.* **22**, 361 (1984).
2. E. A. Freundt and D. G. Edward, in *The Mycoplasmas*, M. F. Barile and S. Razin, Eds. (Academic Press, New York, 1979), vol. 1, p. 1.
3. H. J. Morowitz, *Prog. Theoret. Biol.* **1**, 35 (1967).
4. S. Razin, *Microbiol. Rev.* **49**, 419 (1985).
5. K. Dybvig and G. H. Cassell, *Science* **235**, 1392 (1987).
6. M. C. Roberts and G. E. Kenny, *J. Bacteriol.* **169**, 3836 (1987).
7. J. Labarère and G. Barroso, *Isr. J. Med. Sci.* **20**, 826 (1984).
8. G. Barroso, thesis, Université de Bordeaux II (1988).
9. J. G. Tully, in *Methods in Mycoplasmology*, J. G. Tully and S. Razin, Eds. (Academic Press, New York, 1983), vol. 1, p. 173.
10. J. M. Bové *et al.*, *C. R. Acad. Sci.* **D286**, 57 (1978).
11. E. L. Wollfman, F. Jacob, W. Hayes, in *Papers on Bacterial Genetics*, E. A. Adelberg, Ed. (Methuen, London, 1960), p. 300.
12. J. Maniloff *et al.*, in *The Mycoplasmas*, M. F. Barile and S. Razin, Eds. (Academic Press, New York, 1979), p. 411.
13. K. Fodor and L. Alföldi, *Proc. Natl. Acad. Sci U.S.A.* **73**, 2147 (1976).
14. P. Schaeffer, B. Cami, R. D. Hotchkiss, *ibid.*, p. 2151.
15. K. N. Kao and M. R. Michayleck, *Planta* **115**, 355 (1974).
16. L. S. Lerman and L. J. Tolmach, in *Papers on Bacterial Genetics*, E. A. Adelberg, Ed. (Methuen, London, 1960), p. 177.
17. M. L. Morse, E. M. Lederberg, J. Lederberg, in *ibid.*, p. 209.
18. M. M. Panicker and E. G. Minkley, Jr., *J. Bacteriol.* **162**, 584 (1985).
19. C. R. Woese, J. Maniloff, L. B. Zablen, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 494 (1980).
20. H. J. Morowitz, *Isr. J. Med. Sci.* **20**, 750 (1984).

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Concerted Nonsyntenic Allelic Loss in Human Colorectal Carcinoma

DAVID J. LAW, SYLVIANE OLSCHWANG, JEAN-PHILIPPE MONPEZAT, DANIELLE LEFRANÇOIS, DAVID JAGELMAN, NICHOLAS J. PETRELLI, GILLES THOMAS, ANDREW P. FEINBERG*

Familial polyposis coli (FPC) is caused by an autosomal dominant gene on chromosome 5, and it has been proposed that colorectal cancer in the general population arises from loss or inactivation of the FPC gene, analogous to recessive tumor genes in retinoblastoma and Wilms' tumor. Since allelic loss can be erroneously scored in nonhomogeneous samples, tumor cell populations were first microdissected from 24 colorectal carcinomas, an additional nine cancers were engrafted in nude mice, and nuclei were flow-sorted from an additional two. Of 31 cancers informative for chromosome 5 markers, only 6 (19%) showed loss of heterozygosity of chromosome 5 alleles, compared to 19 of 34 (56%) on chromosome 17, and 17 of 33 (52%) on chromosome 18. Therefore, it appears that (i) FPC is a true dominant for adenomatosis but not a common recessive gene for colon cancer; and (ii) simple Mendelian models involving loss of alleles at a single locus may be inappropriate for understanding common human solid tumors.

AN IMPORTANT CLUE TO THE MECHanism of human carcinogenesis is provided by inherited disorders that predispose to cancer. The paradigm for these tumors is the category of childhood malignancies that appear epidemiologically to arise from two successive mutations (*1*). The two mutations have been shown to be allelic in the case of retinoblastoma (*2*), and appear to be so in the case of Wilms' tumor (*3*). It has been generally assumed that allelic loss in a cancer, as detected by restriction fragment length polymorphisms, suggests a recessive mechanism of carcinogenesis in that tumor, the presumption being that the remaining allele is mutated or microdeleted. This assumption seems reasonable in the case of retinoblastoma and Wilms' tumor, where allelic loss occurs on the same chromosomal arm as known germline, karyotypic, interstitial deletions in patients predisposed to these cancers (*2, 3*).

We reported specific loss of heterozygosity of chromosome 11 alleles in 40% of human bladder cancers (*4*), and thus suggested that recessive cancer genes are commonly involved in adult malignancies. Similar losses of heterozygosity have subsequently been reported in tumors of the breast (*5, 6*), kidney (*7*), and lung (*8*); as well as in acoustic neuroma (*9*). However, only in the case of retinoblastoma has homozygous deletion been proved by cloning the gene (*2*).

Recently, two laboratories have identified a genetic marker on chromosome 5 that is tightly linked to the gene for familial polyposis coli (FPC), a rare autosomal dominant disorder that predisposes to the development of hundreds of premalignant colonic adenomas (polyps), and eventually to colorectal carcinoma unless the colon is removed (*10*). In addition, Solomon *et al.* reported loss of heterozygosity on the long arm of chromosome 5 in 20 to 40% of sporadically occurring colorectal carcinomas, and they proposed that the FPC gene, like Wilms' tumor and retinoblastoma, is a recessive tumor gene, with inactivation of this gene occurring in a relatively high proportion of sporadically occurring colorectal cancers in the general population (*11*). Okamoto *et al.* also reported loss of heterozygosity of chromosome 5 alleles in three of five colorectal carcinomas (*12*), and Wildrick and Boman reported the same phenomenon in 3 of 11 carcinomas (*13*).

To address the question of a putative role of a recessive tumor gene on chromosome 5, we analyzed 35 colorectal cancers, including two from a patient with FPC and five from patients with Lynch syndrome, a more common cause of hereditary colon cancer than FPC. We also individually examined 42 premalignant adenomas. Chromosomes 5, 17, 18, and several other chromosomes that have been implicated cytogenetically were studied with polymorphic DNA markers.

Typically, loss of heterozygosity is determined by the loss of one of the two germline alleles of a polymorphic locus, as detected by loss of a band on a DNA blot. However, this analysis is complicated by the fact that the

D. J. Law and A. P. Feinberg, Howard Hughes Medical Institute and the Departments of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109.
S. Olschwang, J.-P. Monpezat, G. Thomas, Laboratoire de Génétique Moleculaire des Tumeurs, Institut Curie, 75231 Paris, Cedex 05, France.
D. Lefrançois, Laboratoire de Structure et Mutagenèse Chromosomiques, Institut Curie, Paris, France.
D. Jagelman, Department of Colorectal Surgery, Cleveland Clinic, Fort Lauderdale, FL 33309.
N. J. Petrelli, Surgical Developmental Oncology, Roswell Park Memorial Institute, Buffalo, NY 14263.

*To whom correspondence should be addressed.

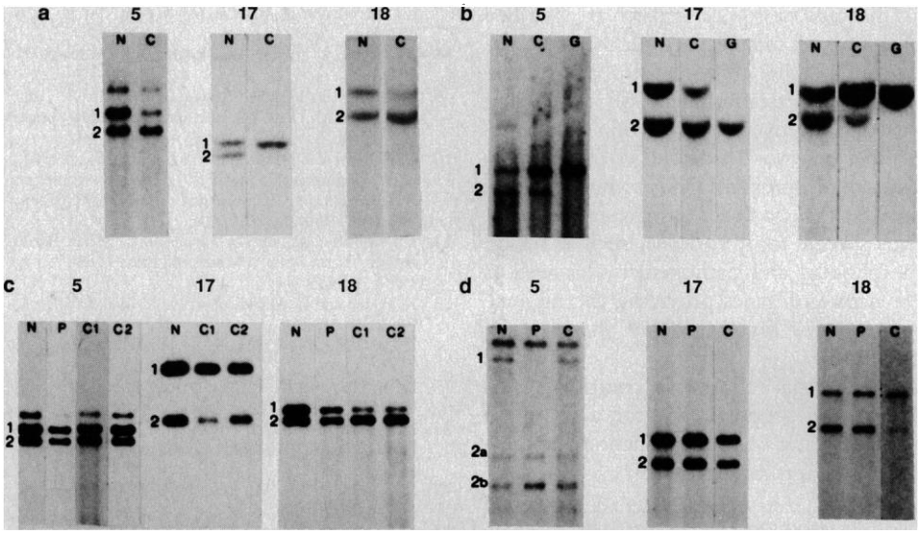

Fig. 1. Allelic losses in human colorectal carcinomas. DNA was prepared (*28*) from normal colonic mucosa (N), from cryostat-sectioned primary colon cancers (C), and from colon tumors after engraftment onto nude mice (G). The sizes of the polymorphic restriction fragments are as described (*29*). Multiallelic systems are numbered arbitrarily with allele 1 representing the larger of the two alleles in a given patient. All unnumbered bands correspond to constant fragments. (a and b) Concerted nonsyntenic allelic losses on chromosomes 5, 17, and 18. (**a**) Patient 25; chromosome 5 probe pC11P11 (D5S71), Taq I polymorphism; chromosome 17 probe pYNZ22.1 (D17S5), Taq I; chromosome 18 probe OLVIIE10 (D18S8), Msp I. (**b**) Patient 34; chromosome 5 probe pC11P11, Taq I; chromosome 17 probe pYNZ22.1, Msp I; chromosome 18 probe OS-4 (D18S5), Taq I. (**c**) Tumors from patient 19, with familial polyposis coli. The polyp (P) is representative of six analyzed. The cancers (C1, C2) arose independently. Chromosome 5 probe pC11P11, Taq I; chromosome 17 probe pYNZ22.1, Bam HI; chromosome 18 probe OLVIIE10, Msp I. (**d**) Tumors from patient 22, with Lynch syndrome. The polyp (P) and cancer (C) arose independently. Chromosome 5 probe CRI-L45, Msp I (allele 2 is comprised of two fragments, marked a and b); chromosome 17 probe pYNZ22.1, Taq I; chromosome 18 probe OLVIIE10, Msp I.

Table 1. Allelic zygosity in human colorectal tumors–cryostat sections. For headings under locus, the top, middle, and bottom lines represent the locus name, its location, and the probe used to identify it, respectively. Filters revealing allelic loss were rehybridized with a nonsyntenic probe, and autoradiographs of both filters were densitometrically scanned (Hoefer GS-300) in the linear range of the film, in order to score changes at each allele independently. Probes have been described (*29*). P, polyp, studied individually; C, cancer (C1/C2 = concurrent, anatomically distinct); —, homozygous, not informative; 1, normal mucosal DNA heterozygous, ≥70% loss of allele 2 in tumor with >70% nuclear homogeneity of tumor sample; (1), normal mucosal DNA heterozygous, 40 to 70% loss of allele 2 in tumor with >70% nuclear homogeneity of tumor sample; 11, normal mucosal DNA heterozygous, loss of allele 2 with duplication of allele 1 in tumor; 12, tumor heterozygous; 2, loss of allele 1; (2), partial loss of allele 1; 22, loss of allele 1 with duplication of allele 2.

Patient	Tumor	Locus											
		D5S71 5q21–22 pC11P11	5qter CRI-L45	5qter CRI-L1265	D17S1 17p13 pHF12-2	D17S5 17p13.3 pYNZ22.1	D18S7 18q11 OLVIIA8	D18S8 18q21.3 OLVIIE10	IGLC 22q11 C_λ	IGLV 22q11–2 V4A	PDGFB 22q12–13 SisR12	D22S10 22q 22c1-18	D22S10 22q 22c1-18
		Taq I	Msp I	Taq I	Msp I	Bam HI	Msp I	Msp I	Eco RI	Taq I	Hind III	Pst I	Taq I
1*	C	12	12	12	—	11	—	12	—		—	12	
2	C	—	—	—	12	12	—	12	—		12	—	
3	C	—	12	12	12	—	—	12	12		—	12	
4	C	—	12	12	12	12	—	—	12		—	12	
5	C	—	12	—	—	12	12	12	—		—	12	
6	C	12	12	—	1§	2§	—	2§	12		—	12	
7	C	12	12	—	12	12	—	—	12		12	—	
8	C	12	—	12	1	—	—	11	—		—	—	—
9	C	—	12	—	2	1	—	1	—		—	—	—
10	C	—	12	12	—	12	—	12	12		—	—	
11	C	—	1	2	2	2	—	(1)	—		—	12	
12	C	—	12	12	—	2	(2)	12	12		—	—	12
13	C	—	12	12	12	12	—	12	—		—	—	12
14	C	—	12	—	—	—	2	—	—		—	—	12
15	C	—	12	—	—	2	—	(2)	—			12	
16*	P‡	—	12	—	—	12	—	12	12		12	12	
	C	—	12	—	—	12	—	12	12		12	12	
17	C	—	12	12	—	2	—	2	12		—	—	—
18†	P1–P15‡	—	12	12	12	—	—	12	—		12	—	
19†	P1–P6‡	12	—	12	—	12	—	12	—		12	—	
	C1	12	—	12	—	(1)	—	2	—		12	—	
	C2	12	—	12	—	12	—	2	—		12	—	
20†	P1–P4‡	12	12	12	—	12	—	12	12		12	—	
21†	P1–P15‡	12	—	12	12	12	12	12	—		12	—	
22*	P‡	—	2			12		12					
	C	—	12			12		1					
23	C	—	—	12	—	2‖	12	12		12			
24	C	—	12		12	12‖	12	12		12			
25	C	2	—		—	(1)‖	—	(2)		12			
26*	C	—	—	—		12‖	12	12		12			

*Patients with Lynch syndrome. †Patients with FPC. ‡Cryostat sectioning was not performed on polyps. §This tumor was only 50% homogeneous and showed 50% allelic loss. ‖Taq I digest, which detects the same polymorphism as Bam HI.

tumors themselves are heterogeneous and usually contain a substantial number of normal cells. Therefore, studies of loss of heterozygosity comparing tumor and normal samples assign a threshold of allelic intensity, below which the tumor is scored as having lost an allele. It is usually assumed that incomplete loss of an allele is due to infiltration of normal cellular elements (*6, 8, 9, 11–13*). Rather than make this assumption, we histologically microdissected relatively homogeneous islands of malignancy from 24 of the colorectal cancers. In most cryostat-sectioned preparations, we could find tumor islands that were >90% homogeneous, while in some, 70 to 90% was the maximum obtainable purity. As an additional approach to purifying tumor cell populations, we explanted nine tumors into nude mice, and flow-sorted hyperdiploid nuclei from an additional two.

With this strategy, of the 35 colorectal adenocarcinomas studied, 31 were informative for at least one of three chromosome 5q probes. Only six of these tumors (19%) showed loss of heterozygosity on 5q (Fig. 1 and Tables 1 and 2). We also examined several other chromosomes implicated by cytogenetic studies of colorectal carcinomas, including chromosomes 1 (*14, 15*), 12 (*16*), 13 (*15*), 17 (*17*), and 18 (*17*). In addition, we examined chromosome 22 because of reported allelic losses (*12*) and chromosome 11. A total of 19 of 34 (56%) informative colorectal cancers showed loss of chromosome 17 alleles as determined with polymorphic probes, and 17 of 33 (52%) cancers showed loss of chromosome 18 alleles (Fig. 1 and Tables 1 and 2). The other chromosomal markers showed alterations infrequently (Tables 1 and 2) (*18*).

As a whole, these data are in marked contrast with those of Solomon *et al.* (*11*) and Okamoto *et al.* (*12*), who observed loss of heterozygosity of chromosome 5 alleles in 20 to 40% and 60% of colorectal cancers, respectively, and loss of chromosome 17 (*11, 12*) and 18 (*12*) alleles in none. Okamoto *et al.* also reported frequent allelic loss on chromosome 22 (*12*), but we saw such alterations in none of 33 informative cancers. The precise chromosomal localization of the probes does not account for these differences, since both D17S1 and D18S1 were used in those studies, and comparable markers were used on 5q and 22q. However, our data for chromosomes 17 and 18 are consistent with those of Monpezat *et al.* (*19*) and Fearon *et al.* (*20*). The latter two studies, like ours, involved physical or biological purification of tumor samples, which can prevent both underscoring and overscoring of apparent allelic losses; however, those studies did not examine chromosome 5.

Not only were chromosome 5 allelic losses less frequent than those on chromosomes 17 and 18 in our samples, but in the six cases of chromosome 5 allelic loss, chromosomes 17 and 18 were also involved (Tables 1 and 2). Coexisting allelic losses on chromosomes 5, 17, and 18 did not appear to represent generalized losses, however, since only one of the six tumors (patient 33) showed loss of an allele on another chromosome (Table 2) (*18*). Thus, a simple recessive model of chromosome 5 allelic loss does not appear to apply to colon carcinogenesis. The region of overlap of allelic losses on chromosome 17 was the distal short arm. Chromosome 18 allelic losses appeared to involve an entire chromosome.

We could infer the timing of allelic loss in three cases. In patient 11, there was complete loss of chromosome 5 and 17 alleles,

Table 2. Allelic zygosity in human colorectal tumors–xenografted and flow-sorted carcinomas. Locus and tumor designations are as in Table 1.

Patient	Locus										
	D5S71 5q21–22 pC11P11	5qter CRI-L45	5qter CRI-L1265	D17S5 17p13.3 pYNZ22.1	D17S28 17p pYNH37-3		D17S1 17p13 pHF12-2	MYH2 17p13.1 p10-5	TK1 17q21 pHTK9	GH2 17q22-24 pGH800	D18S1 18p13.2 pHF12-62
	Taq I	Msp I	Taq I	Msp I	Taq I	Msp I	Msp I	Msp I	Taq I	Msp I	Taq I
27	—	12	12	2	—		—	—	12		—
28	12	12		2	—		—	12	—		12
29	12	12		2	—	—	—	12	—		—
30	12	12		—	—		12	12	—		12
31	—	—	12	12	—	12	—	12	12	12	12
32	—	—	—	12	12	12	—	—	—	—	—
33	11	—		—	—	—	—	2	—	—	1
34	1	—		2		—	—		—	12	—
35*	—	2		2	—	—	—	1	12	12	11
36†	—	—	—				2	—	—	—	—
37†	1	—					(1)	—	—	—	2

Patient	D18S3 18p11.3 B74		D18S5 18q21.3 OS-4		D18S6 18pter–p11 L2.7		D18S7 18q11 OLVIIA8	D18S8 18q21.3 OLVIIE10	D18S10 18 Fr8-12	MBP 18q22 pHBP-2	D22S9 22q11 p2234	IGLV 22q11 V4A
	Taq I	Msp I	Taq I	Pst I	Taq I	Pst I	Msp I	Msp I	Pst I	Pst I	Taq I	Taq I
27	—	—	—	—	—	—	—	12		—		12
28	—	12	—	12	—	12	12	12	12	—	12	12
29	—	—	—	12	—	—	—	—	12	—	—	12
30	—	—	—	—	12	—	12	12	12	—	12	12
31	—	12	12	—	—	—	—	—	12	—	12	12
32	—	—	12	12	—	—	—	12	12	—	—	12
33	—	—	—	2	—	—	—	2	2	—	—	12
34	2	'2	1	—	—	—		2	1	2		12
35*	—	11	11	—	—	11		22	—	—	12	—
36†	—	—					—	2				12
37†	—	—					—	1				12

*Patients with Lynch syndrome. †Flow-sorted nuclei.

but only partial loss of chromosome 18 alleles (Table 1), suggesting that the chromosome 18 loss occurred relatively late in this tumor (and was thus present in only some of the cells). Similarly, in patient 37, there was complete loss of chromosome 5 and 18 alleles, but only partial loss of chromosome 17 alleles (Table 2); and in patient 25, there was complete loss of chromosome 5 alleles, but only partial loss on chromosomes 17 and 18. That the tumors were not substantially contaminated by normal cells was confirmed molecularly by demonstrating complete allelic loss at other loci. Thus, both chromosomes 17 and 18 appear to be able to play a relatively late role in tumor progression, and may augment events at other loci.

Even in hereditary colon cancer, chromosome 5 did not appear to play an independent role in progression to malignancy. Separate sigmoid and transverse colon carcinomas from a patient with FPC (patient 19, Table 1) lost chromosome 18 alleles, and one of the carcinomas also lost chromosome 17 alleles, but neither cancer lost chromosome 5 alleles (Fig. 1c). This includes D5S71, which is linked to the FPC gene (*10*). Five patients developed cancer in the setting of Lynch syndrome, an autosomal dominant that is much more frequent than FPC, and that accounts for up to 10% of colon cancer in the general population (*21*). The only allelic loss on chromosome 5 among the 42 polyps we examined occurred in a Lynch syndrome patient (patient 22, Table 1, and Fig. 1d). The separately arising cancer from this patient showed allelic loss on chromosome 18 and not on chromosome 5 (Table 1 and Fig. 1d).

We therefore propose the following model of allelic loss in colon carcinogenesis. Mutation of the FPC gene causes benign adenomatosis, but not carcinoma per se. While alteration of a gene on chromosome 5 is not sufficient to cause cancer, it may cooperate with genetic alterations at other loci in tumor progression. The gene on chromosome 5 need not necessarily be at the FPC locus. The karyotypic finding of 5q− is common to many leukemias and myelodysplastic syndromes, particularly in the advanced stages (*22*). A family of growth factors is present on 5q, distal to the FPC gene, and structural alterations in the vicinity of these genes is one postulated mechanism by which 5q− is involved in tumor progression (*23*).

Allelic losses on chromosomes 17 and 18 occur much more frequently in colorectal cancer than losses on chromosome 5, suggesting that recessive colon cancer genes reside on these chromosomes. Thus, colon cancer appears to arise from multiple alterations at nonsyntenic loci. This model does not preclude involvement of additional loci on other chromosomes, and the loci on chromosomes 17 and 18 might play an additive role with other events that have been described in colon cancer, such as oncogene mutations (*24*) or alterations in DNA methylation (*25*).

A classic Mendelian model of dominant or recessive genes may be an oversimplification in the case of multistep carcinogenesis in a clonal somatic cell lineage. It is important to note that the most frequent chromosomal alteration in solid tumors is a change in chromosome number (*26*). Colon cancers, like most solid tumors, are frequently hyperdiploid (*27*) and the cumulative effect of altered gene dosage at multiple loci may be an important determinant of the transformed phenotype.

REFERENCES AND NOTES

1. A. G. Knudson, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 820 (1971); A. G. Knudson, H. W. Hethcote, B. W. Brown, *ibid.* **72**, 5116 (1975).
2. S. H. Friend *et al.*, *Nature* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987).
3. A. E. Reeve *et al.*, *Nature* **309**, 174 (1984); E. R. Fearon, B. Vogelstein, A. P. Feinberg, *ibid.*, p. 176.
4. E. R. Fearon, A. P. Feinberg, S. H. Hamilton, B. Vogelstein, *ibid.* **318**, 377 (1985).
5. C. Lundberg, L. Skoog, W. K. Cavenee, M. Nordenskjold, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2372 (1987).
6. I. U. Ali, R. Lidereau, C. Theillet, R. Callahan, *Science* **238**, 185 (1987).
7. B. Zbar, H. Brauch, C. Talmadge, M. Linehan, *Nature* **327**, 721 (1987).
8. J. Yokota, M. Wada, Y. Shimosato, M. Terada, T. Sujimura, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 9252 (1987).
9. B. R. Seizinger, R. L. Martuza, J. F. Gusella, *Nature* **322**, 644 (1986).
10. W. F. Bodmer *et al.*, *ibid.* **328**, 614 (1987); M. Leppert *et al.*, *Science* **238**, 1411 (1987).
11. E. Solomon *et al.*, *Nature* **328**, 616 (1987).
12. M. Okamoto *et al.*, *ibid.* **331**, 273 (1988).
13. D. M. Wildrick and B. M. Boman, *Biochim. Biophys. Res. Commun.* **150**, 591 (1988).
14. F. Shabtai *et al.*, *Cancer Genet. Cytogenet.* **14**, 235 (1985); A. Reichman, P. Martin, B. Levin, *ibid.* **12**, 295 (1984).
15. A. Reichmann, P. Martin, B. Levin, *ibid.* **16**, 229 (1985); B. Levin and A. Reichman, *ibid.* **19**, 159 (1986).
16. R. Becher, Z. Gibas, A. A. Sandberg, *ibid.* **9**, 329 (1983); H. Ochi *et al.*, *ibid.* **10**, 121 (1983); S. Pathak and A. Goodacre, *ibid.* **19**, 29 (1986).
17. M. Muleris, R. J. Salmon, B. Zafrani, J. Girodet, B. Dutrillaux, *Ann. Genet.* **28**, 206 (1985); M. Muleris, R. J. Salmon, B. Dutrillaux, *Int. J. Cancer* **38**, 167 (1986); M. Muleris, A.-M. Dutrillaux, M. Lombard, B. Dutrillaux, *Cancer Genet. Cytogenet.* **25**, 7 (1987).
18. Allelic losses were seen in none of 21 cancers informative for chromosome 1 alleles [probe pYNZ2.1, Msp I digest, D. Barker, M. Schafer, R. White, *Cell* **36**, 131 (1984)]; 3 of 27 informative for chromosome 11 alleles [probe pHINS310 (INS), Pvu II digest, G. I. Bell, M. J. Selby, W. J. Rutter, *Nature* **295**, 31 (1982); and probe pEJ6.6 (HRAS1), Msp I digest, C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982)]; none of 11 informative for chromosome 12 alleles [probes p11-1-7 and p11-2-2 (D12S6), Msp I digest, N. E. Buroker, R. E. Magenis, K. Weliky, G. Bruns, M. Litt, *Hum. Genet.* **72**, 86 (1986); probe pDL32B (D12S7), Taq I digest, L.-C. Tsui *et al.*, *Cytogenet. Cell Genet.* **40**, 763 (1985); and probe pVWE6 (F8VWF), Rsa I and Sac I digests, B. A. Konkle *et al.*, *Nucleic Acids Res.* **15**, 6766 (1987)]; and none of 15 cancers informative for chromosome 13 alleles [probe pTHI62 (D13S37), Msp I digest, M. Leppert *et al.*, *Ninth International Workshop on Human Gene Mapping, Paris* (1987)]. The three tumors with allelic loss on chromosome 11 were those of patient 33, which also lost alleles on chromosomes 5, 17, and 18; patient 15, which lost alleles on chromosomes 17 and 18, but not on chromosome 5; and patient 28, which lost an allele on chromosome 17 but not on chromosomes 5 or 18. Other loci were not affected in these three cases.
19. J.-P. Monpezat *et al.*, *Int. J. Cancer* **41**, 404 (1988).
20. E. R. Fearon, S. R. Hamilton, B. Vogelstein, *Science* **238**, 193 (1987).
21. H. T. Lynch *et al.*, *Cancer* **56**, 934 (1985).
22. H. VandenBerghe *et al.*, *Cancer Genet. Cytogenet.* **17**, 189 (1985); M. Gyger *et al.*, *Leukemia Res.* **10**, 9 (1986); J. Hozier *et al.*, *Int. J. Cancer* **26**, 281 (1980).
23. M. J. Pettenati *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2970 (1987); M. M. LeBeau *et al.*, *ibid.*, p. 5913.
24. K. Forrester, C. Almoguera, K. Han, W. E. Grizzle, M. Perucho, *Nature* **327**, 298 (1987); J. L. Bos *et al.*, *ibid.*, p. 293.
25. S. E. Goelz, B. Vogelstein, S. R. Hamilton, A. P. Feinberg, *Science* **228**, 187 (1985).
26. A. A. Sandberg, *The Chromosomes in Human Cancer and Leukemia* (Elsevier, New York, 1980); L. C. Strong, *Cancer Bull.* **35**, 115 (1983); S. Pathak and A. Goodacre, *Cancer Genet. Cytogenet.* **19**, 29 (1986).
27. A. Reichman, P. Martin, B. Levin, *Int. J. Cancer* **28**, 431 (1981).
28. Tumors were either frozen, engrafted into nude mice, or subjected to flow sorting as described (*19*). Frozen specimens without fixative were warmed to −20°C, mounted in O.C.T. (Miles Scientific) on a cryostat, and serially sectioned at 8-μm intervals. Tumor islands (approximately 3 to 5 mm in diameter) were microdissected and pulverized in liquid nitrogen, and DNA was purified as described (*25*). DNA was digested with restriction enzyme (5 U/μg) overnight, followed by a second digestion (5 U/μg) for an additional 2 hours. After electrophoresis in a 0.6 to 1.0% agarose gel, DNA blot hybridization was performed on GeneScreen (New England Nuclear) by a modification of the technique of G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984). Probes were oligolabeled as described by A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983); *ibid.* **137**, 266 (1984).
29. References describing the probes used are as follows: probe pC11p11 (*10*); probes CRI-L45 and CRI-L1265, J. W. Schumm *et al.*, *Am. J. Hum. Genet.* **42**, 143 (1988), and H. Donis-Keller *et al.*, *Cell* **51**, 319 (1987); probes pHF12-2 and pHF12-62, D. Barker, M. Schafer, R. White, *Cell* **36**, 131 (1984); probe pYNZ22.1, Y. Nakamura *et al.*, *Science* **235**, 1616 (1987), and D. Barker *et al.*, *ibid.* **236**, 1100 (1987); probe OLVIIA8, O. Delattre *et al.*, *Nucleic Acids Res.* **15**, 1343 (1987); probe OLVIIE10, F. Marlhens *et al.*, *ibid.*, p. 1348 (1987); probe C_α, R. A Taub *et al.*, *Nature* **304**, 172 (1983); probe V4A, C. Julier *et al.*, *Cytogenet. Cell Genet.* **40**, 665 (1985); probe SisR12, K. C. Robbins, S. G. Devare, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2918 (1981), and C. Julier *et al.*, *Cytogenet. Cell Genet.* **40**, 665 (1985); probe 22cl-18, M. H. Hofker *et al.*, *Nucleic Acids Res.* **13**, 7167 (1985); probe pYNH37-3, Y. Nakamura *et al.*, *Science* **235**, 1616 (1987); probe p10-5, C. E. Schwartz, E. McNally, L. Leinwald, M. H. Skolnick, *Cytogenet. Cell Genet.* **40**, 740 (1985); probe pHTK9, D. M. Durnam, D. Myerson, J. K. McDougall, R. E. Gelinas, *ibid.* **37**, 458 (1984); probe pGH800, A. Chakravarti *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6085 (1984); probe B74, M. G. Mattei *et al.*, *Hum. Genet.* **69**, 268 (1985), and F. Morle *et al.*, *Cytogenet. Cell Genet.* **37**, 544 (1984); probe OS-4, H. Tateishi, I. Nishisho, T. Miki, S. Takai, T. Honjo, *Nucleic Acids Res.* **14**, 1926 (1986); probe L2.7, M. H. Skolnick, H. F. Willard, L. A. Menlove, *Cytogenet. Cell Genet.* **37**, 210 (1984), and G. A. Geitvik *et al.*, *Int. Cong. Hum. Genet.* **7**, 687

(1986); probe Fr8-12, G.-H. Xiao, K.-H. Grzeschik, G. Scherer, *Ninth International Workshop on Human Gene Mapping, Paris* (1987); probe pHBP-2, J. Kamholz *et al.*, *Am. J. Hum. Genet.* **40**, 365 (1987); and probe p2234, H. McDermid *et al.*, *Cytogenet. Cell Genet.* **40**, 695 (1985).

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Mammalian Glucocorticoid Receptor Derivatives Enhance Transcription in Yeast

MARK SCHENA AND KEITH R. YAMAMOTO

In mammalian cells, the glucocorticoid receptor binds specifically to glucocorticoid response element (GRE) DNA sequences and enhances transcription from linked promoters. It is shown here that derivatives of the glucocorticoid receptor also enhance transcription when expressed in yeast. Receptor-mediated enhancement in yeast was observed in fusions of GRE sequences to the yeast cytochrome c_1 (*CYC1*) promoter; the *CYC1* upstream activator sequences were not essential, since enhancement was observed in fusions of GREs to mutant *CYC1* promoters retaining only the TATA region and transcription startpoints. It is concluded that the receptor operates by a common, highly conserved mechanism in yeast and mammalian cells.

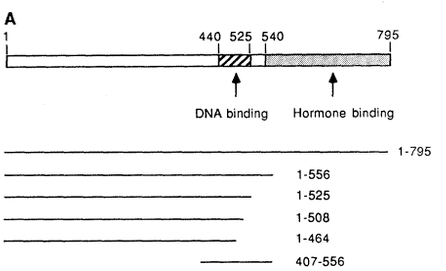
THE GLUCOCORTICOID RECEPTOR selectively regulates gene transcription in animal cells by binding in a steroid-dependent manner to specific DNA sequences termed GREs (*1, 2*). Those GREs associated with the mouse mammary tumor virus (MTV) promoter and with other genes that are regulated positively by glucocorticoids are transcriptional enhancer elements that function only in the presence of bound receptor (*2–4*). Discrete segments of the 795–amino acid rat glucocorticoid receptor have been defined that mediate nuclear translocation, hormone binding, GRE recognition, and transcriptional regulation (*5–9*). These studies also revealed that receptor derivatives lacking the hormone binding domain confer constitutive GRE-dependent enhancement (7); moreover, the DNA binding domain is sufficient for enhancer activation, although its apparent specific activity is low relative to the intact receptor (*8, 9*).

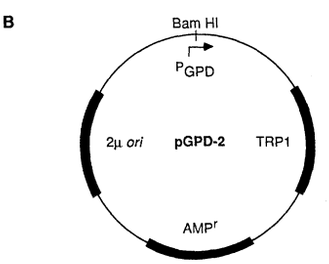
The phenomenon of transcriptional enhancement has now been documented in organisms ranging from bacteria to mammals (*10*). This widespread distribution indicates that enhancement may operate by a common mechanism and that its molecular determinants may have been conserved during evolution. Given the relatively simple physiology and genetic manipulability of *Saccharomyces cerevisiae*, we tested whether the rat glucocorticoid receptor expressed in yeast could enhance transcription from yeast promoters linked to GREs.

We first expressed in yeast a series of receptor derivatives bearing a deletion of the hormone binding domain, amino acids 557 to 795. Studies in tissue culture cells showed that receptor derivatives lacking this region confer high-level constitutive GRE-mediated enhancement. The receptor derivatives were expressed from the yeast glycerol-3-phosphate dehydrogenase promoter in parent plasmid pGPD-2 (*11*) (Fig. 1). All species except X556b, which contains receptor amino acids 407 to 556, are translated from the normal receptor initiation codon, and each terminates translation in downstream linker or vector sequences, resulting in addition of 4 to 13 nonreceptor amino acids. Comparison of receptor derivatives differing only in these COOH-terminal amino acids (for example, see N556a and N556b in Fig. 1) revealed no systematic effects of the short nonreceptor "tails."

Expression and integrity of the various receptor derivatives were assessed by immunoblotting of extracts from strains transformed stably with the receptor expression plasmids. For example, Fig. 2A (lanes 1 and 2) shows accumulation of the predicted 18- and 65-kD proteins, X556b and N556a, respectively, to steady-state levels of about 2500 molecules per cell; these intracellular concentrations are comparable to those in mammalian cells. Similar results were obtained with the other receptor constructs shown in Fig. 1.

As an initial test of DNA binding by receptor derivatives in vivo in yeast, we inserted GRE sequences between functional elements of the yeast cytochrome c_1 (*CYC1*) promoter. As shown in Fig. 3A, pLGΔ312S is a plasmid containing the intact *CYC1* promoter fused to the *Escherichia coli* β-galactosidase (*lacZ*) coding sequences (*12*); two different GRE-containing fragments were inserted at position −178 between the UAS and TATA elements of the promoter (see legend to Fig. 3A). Others have shown that insertions of nonspecific DNA fragments as large as 350 bp at this site have





Plasmid	Receptor	Receptor amino acids	Nonreceptor COOH-terminal amino acids
pGPD-2	None	None	None
pGPD-795	N795	1-795	None
pGPD-556a	N556a	1-556	GELEFPGLEDPST
pGPD-556b	N556b	1-556	GANSRV
pGPD-525	N525	1-525	AGRL
pGPD-508	N508	1-508	AGRL
pGPD-464	N464	1-464	GSRGSVDLDK
pGPD-X556b	X556b	407-556	GANSRV

Fig. 1. Yeast plasmids containing rat glucocorticoid receptor sequences. (**A**) The 795–amino acid rat glucocorticoid receptor, denoting the DNA binding domain (amino acids 440 to 525) and the hormone binding domain (amino acids 540 to 795) (*5, 8*). Receptor segments cloned into yeast plasmids are indicated below the diagram. (**B**) Receptor sequences were inserted as Bam HI fragments (*5, 7*) into the unique Bam HI site 15 bp downstream of the glycerol-3-phosphate dehydrogenase transcription start site in plasmid pGPD-2 (*11*). The resultant expression plasmid and receptor derivatives are indicated, together with the precise receptor amino acids contained in each derivative and the COOH-terminal amino acids contributed by the polylinker (see text). In each case, translation initiates at the normal receptor NH_2 terminus, except for X556b, which uses a seven–amino acid leader sequence (Met-Ala-Ser-Trp-Gly-Ser-Pro) from herpes simplex virus thymidine kinase (*23*). The pGPD-2 vector contains the replication origin and ampicillin resistance gene of pBR322, and the TRP1 selectable marker and 2μ replication origin from yeast.

Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143.