Nonlinear summation of the effects of clustered terminals could also account for the lack of fits observed in other preparations, including when analyzing fluctuating PSP's evoked in motoneurons by Ia synapses [7; see also (1)].

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### References and Notes

1. Reviewed in E. M. Mc Lachlan, *Int. Rev. Physiol.* **17**, 49 (1978).
2. H. Korn, A. Triller, A. Mallet, D. S. Faber, *Science* **213**, 899 (1981).
3. H. Korn, A. Mallet, A. Triller, D. S. Faber, *J. Neurophysiol.* **48**, 679 (1982).
4. A. Triller and H. Korn, *ibid.*, p. 708.
5. C. E. Shannon, *Bell Syst. Techn. J.* **27**, 379 (1948); *ibid.*, p. 623; S. Kullbach, in *Information Theory and Statistics* (Wiley, New York, 1959).
6. The potential values were allocated into $n + 2$ classes (where $n$ is the binomial parameter) with ranges equal to the quantum size $q$. Let $D_i$, $i = 1, \ldots, n + 2$ be the $n + 2$ intervals covering $R$ (the real numbers) defined as:

$$D_1 = (-\infty, q/2]$$
$$D_i = ((2i - 3)\, q/2, (2i - 1)\, q/2],$$
$$i = 2, \ldots, n + 1$$
$$D_{n+2} = ((2n + 1)\, q/2, + \infty)$$

Then the probabilities $p_i$ were computed as follows:
(i) Predicted probabilities, noted as $\hat{p}_i$

$$\hat{p}_i = \int_{D_i} f(v)dv$$

(ii) Experimental probabilities

$$p_i = \frac{1}{S} \sum_{j=1}^{s} 1_{D_i}(v_j)$$

where $v$ is the recorded potential and $s$ is the number of stimulations, the corresponding (PSP's) recordings being $v_j$, for $j = 1, \ldots, s$. Then

$$E_{exp} = -\sum_{i=1}^{n+2} \hat{p}_i \log \hat{p}_i$$

$$E_{th} = -\sum_{i=1}^{n+2} \hat{p}_i \log \hat{p}_i$$

The discrepancies between the distributions were quantified as $100\,(E_{th} - E_{exp})/E_{th}$, to allow comparisons between experiments.
7. T. H. Brown, D. H. Perkel, M. W. Feldman, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2913 (1976); S. B. Barton and I. S. Cohen, *Nature (London)* **268**, 267 (1976); J. J. B. Jack, S. J. Redman, K. Wong, *J. Physiol. (London)* **321**, 65 (1981)
8. S. Roper, J. Diamond, G. M. Yasargil, *Nature (London)* **223**, 1168 (1969); J. Diamond, S. Roper, G. M. Yasargil, *J. Physiol. (London)* **232**, 87 (1973).
9. D. S. Faber, H. Korn, A. Triller, *Soc. Neurosci. Abstr.* **9**, 456 (1983).
10. D. S. Faber and H. Korn, *J. Neurophysiol.* **48**, 654 (1982).
11. For any amount $x$ of transmitter released, the probability that $r(x)$ is less than a given concentration $y$ must equal the probability that the recorded potential $v$ is less than the same concentration $y$, that is,

$$\text{Prob}\,[r(x) < y] = \text{Prob}\,[v < y].$$

As $r$ is a monotonically increasing function

$$\text{Prob}\,[x < r^{-1}(y)] = \text{Prob}\,[v < y],$$

or, with the notation in the text:

$$F\,[r^{-1}(y)] = \Phi\,[y].$$

Finally, $r = \Phi^{-1} \circ F$, where $\circ$ stands for composition of functions and $r^{-1}$ and $\Phi^{-1}$ express the reciprocals of $r$ and $\Phi$.
12. C. Koch, T. Poggio, V. Torre, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2799 (1983).
13. T. Furshpan and T. Furukawa, *J. Neurophysiol.* **25**, 732 (1962); Y. Fukami, T. Furukawa, Y. Asada, *J. Gen. Physiol.* **48**, 581 (1965).
14. Cooperation between adjacent synapses has also been described [B. L. Mc Naughton, R. M. Douglas, G. V. Goddard, *Brain Res.* **157**, 277 (1978); H. Korn and D. S. Faber, *Soc. Neurosci. Abstr.* **9**, 456 (1983)].
15. H. B. Barlow, in *Sensory Communication*, W. A. Rosenblith, Ed. (MIT Press, Cambridge, Mass., 1961), p. 217; S. Laughlin, *Z. Naturforsch.*, **36c**, 910 (1981).
16. We thank D. S. Faber and T. Heidmann for helpful comments in interpretation of the data.

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## Activation of a c-K-*ras* Oncogene by Somatic Mutation in Mouse Lymphomas Induced by Gamma Radiation

Abstract. *Mouse tumors induced by gamma radiation are a useful model system for oncogenesis. DNA from such tumors contains an activated K-*ras *oncogene that can transform NIH 3T3 cells. This report describes the cloning of a fragment of the mouse K-*ras *oncogene containing the first exon from both a transformant in rat-2 cells and the brain of the same mouse that developed the tumor. Hybrid constructs containing one of the two pieces were made and only the plasmid including the first exon from the transformant gave rise to foci in NIH 3T3 cells. There was only a single base difference (G → A) in the exonic sequence, which changed glycine to aspartic acid in the transformant. By use of a synthetic oligonucleotide the presence of the mutation was demonstrated in the original tumor, ruling out modifications during DNA-mediated gene transfer and indicating that the alteration was present in the thymic lymphoma but absent from other nonmalignant tissue. The results are compatible with gamma radiation being a source of point mutations.*

Certain oncogenes can change the normal morphology of cultured rodent fibroblasts toward an oncogenic phenotype, enabling those that carry the genes to grow in soft agar and form tumors when injected into nude mice (*1*). Such oncogenes have been found in human tumors and cell lines derived from them (*1*) and, more recently, in model systems in which tumors were induced by carcinogens or γ-radiation (*2*, *3*). The oncogenes that appear to be most frequently activated in humans and in animal model systems belong to the *ras* family: H-, K-, and N-*ras* (*4*). H- and K-*ras* were first found in rat retroviruses (*5*). N-*ras* was first found in a human tumor cell line (*1*), and in other species we found it associated with carcinogen-induced mouse lymphomas (*2*). All three genes encode closely homologous proteins with a molecular weight of about 21,000 (p21), and each gene, when activated, differs from its normal cellular homolog by a single point mutation that alters the 12th or 61st amino acid of the protein (*6*).

We recently reported the activation of cellular *ras* (c-*ras*) oncogenes in vivo, in mice treated with a carcinogen or γ-radiation (*2*). Both treatments cause a high incidence of thymomas in crosses between AKR and RF/J mice, but the carcinogen activates N-*ras* whereas radiation activates K-*ras*. To further characterize the activated K-*ras*, we have studied one of the γ-radiation–induced tumors (tumor X). DNA from tumor X was used to obtain 3T3 primary transformants (Fig. 1A, lane a). With DNA from this primary transformant, secondary transformants were obtained in rat-2 cells (Fig. 1A, lane c) to facilitate the identification and subsequent cloning of mouse sequences (*2*). The two bands of 17.3 and 14.4 kb on top of lane a represent the endogenous 3T3 band (17.3 kb, compare Fig. 1A, lane e) plus the new information acquired from the tumor (14.4 kb). This band has a different molecular weight because of the rearrangements that frequently occur during gene transfer. If one compares Fig. 1A, lanes a and c, it is clear that only the 14.4-kb band is transferred concordantly with the oncogenic phenotype. Since the point mutations associated with *ras* activation have been found in only two positions, at amino acids 12 and 61 (*6*), predominantly in the former, we examined the fragment containing the first exon and therefore the sequences for the 12th codon. We found the first exon in the 17.3-kb band in normal mouse DNA and in the 14.4-kb band in the rat transformant by using a specific probe obtained from a Kirsten rat sarcoma virus complementary DNA (cDNA) clone (data not shown) (*7*). The 17.3-kb band from the mouse brain that developed tumor X and the 14.4-kb from the secondary rat transformant were extracted from agarose gels and shown to be pure (Fig. 1A, lanes f and d). They were subsequently ligated to phage λ 47.1 Hind III arms and cloned as described (*8*).

Figure 1B shows that the restriction sites for the brain and the transformant clones on the right-hand side are identical and therefore belong to the same gene, mouse c-K-*ras*. At the left-hand side there is no similarity between the two maps, presumably because the clone isolated from the transformant was modified during the DNA-mediated gene transfer. It is now linked to carrier or rat DNA in the recipient cell and therefore the limit of true mouse c-K-*ras* gene and adjacent sequences is a maximum of 8 kb at the right end of the transformant clone. By using different digests of the clones in Southern blots probed with the sequence specific for the first exon mentioned above, this exon was located in a 0.7-kb Eco RI fragment (Fig. 1B), close to the right end of the insert.

It was then important to show that the K-*ras* recombinants from brain and the transformant contained the area of functional significance. With that aim, we used a plasmid harboring the second, third, and fourth exons from a normal human H-*ras* gene (*9*). In front of these human exons we inserted (Fig. 2) the Bgl II–Hind III fragment from our transformant clone λMKT-142 (Fig. 1B). When this chimeric plasmid (pBKT-142 in Fig. 2) was used in the conventional NIH 3T3 transformation assay (*1*), its efficiency was about five foci per nanogram, which is the expected efficiency for a pure fully active oncogene (*1*). When the Sst II–

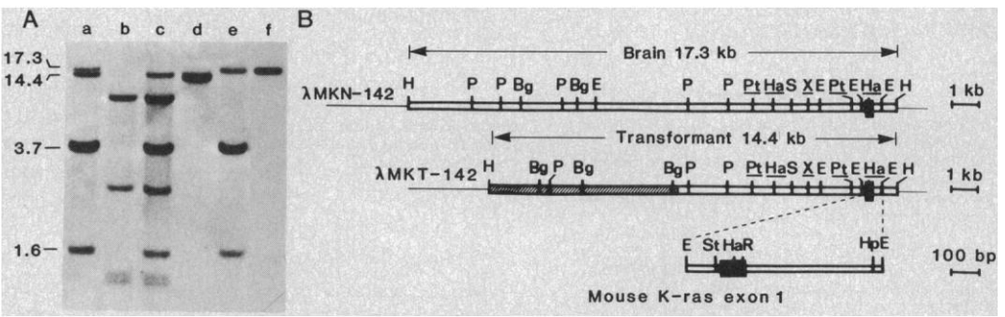


Fig. 1. Cloning and restriction map of a mouse DNA fragment containing the first exon of K-*ras* (A) Southern blot of Hind III–digested DNA's from normal and transformant DNA's together with the purified fragments isolated from them. The Southern conditions were as described (*2*). The hybridization probe was a Sst II–Xba I fragment from the HiHi3 plasmid which contains cDNA sequences from the Kirsten rat sarcoma virus (*7*). The isolated fragments were electroeluted from the agarose gel and purified through an Elutip column (S and S). (Lane a) 3T3 primary transformant generated with DNA from tumor X; (lane b) rat-2; (lane c) rat secondary transformant obtained with DNA from cells shown in lane a; (lane d) 14.4-kb fragment isolated from a preparative gel of DNA from lane c; (lane e) brain DNA from the mouse that developed tumor X; and (lane f) 17.3-kb fragment isolated from a preparative agarose gel of DNA from lane e. (B) Restriction map of the recombinant clones obtained from the transformant and brain DNA's. The restriction maps were obtained by a combination of single and double digests with the enzymes indicated. The box represents the insert in the clones; the adjacent thin lines are λ 47.1 arms. The open box indicates sequences from the mouse K-*ras* locus. The hatched box represents sequences in the transformant clone belonging to carrier or rat DNA. The black box is the first coding exon. The letters are H, Hind III; P, Pvu II; Bg, Bgl II; E, Eco RI; Pt, Pst I; Ha, Hae II; S, Sst II; X, Xba I; St, Stu I; R, Rsa I. The underlined restriction sites are not mapped through all the clone.

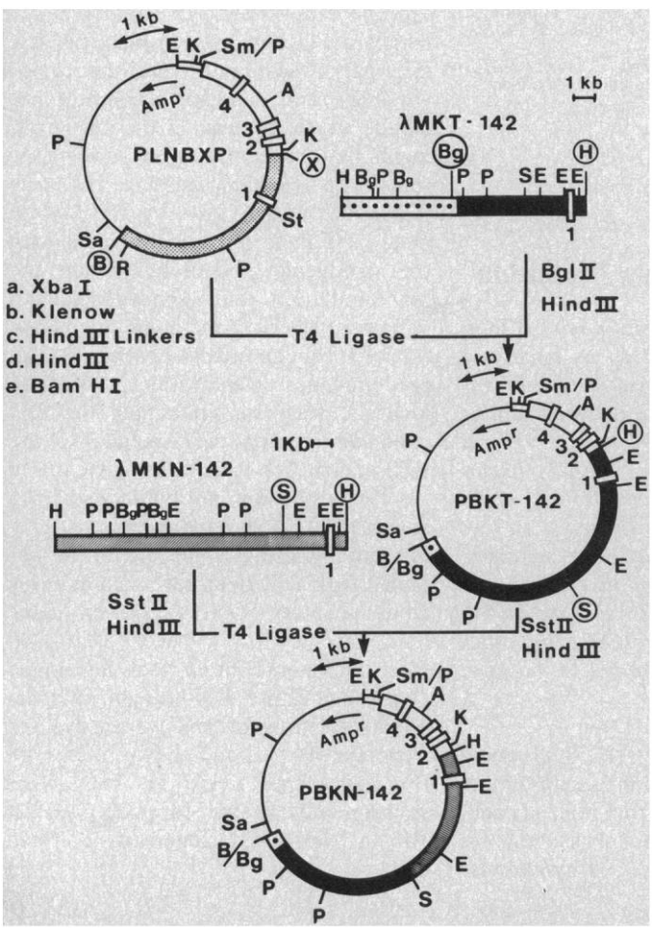


Fig. 2. Hybrid *ras* constructs with brain or transformant first exon. Plasmid PLNBXP (*9*) contains the second, third, and fourth coding exons from a normal human H-*ras* gene. The exons are represented by small rectangular open boxes numbered 1 to 4. The thin line is for plasmid sequences. The boxes are as follows: open, human H-*ras* sequences; stippled, human K-*ras* sequences from the original PLNBXP; single dots, sequences from carrier or rat DNA present in λMKT-142; striped, sequences from the mouse brain K-*ras* locus; solid, sequences of the mouse K-*ras* locus isolated from the transformant. The operations conducted for the construction are indicated in the figure and were performed under standard conditions (*20*). The symbols are for restriction endonucleases: E, Eco RI; K, Kpn I; Sm, Sma I; P, Pvu II; A, Apa I; X, Xba I; St, Stu I; R, Rsa I; B, Bam HI; Sa, Sal I; H, Hind III; Bg, Bgl II; S, Sst II. The circled endonuclease symbols represent the restriction sites involved in each particular construction step. λMKT-142 and λMKN-142 are described in Fig. 1B.

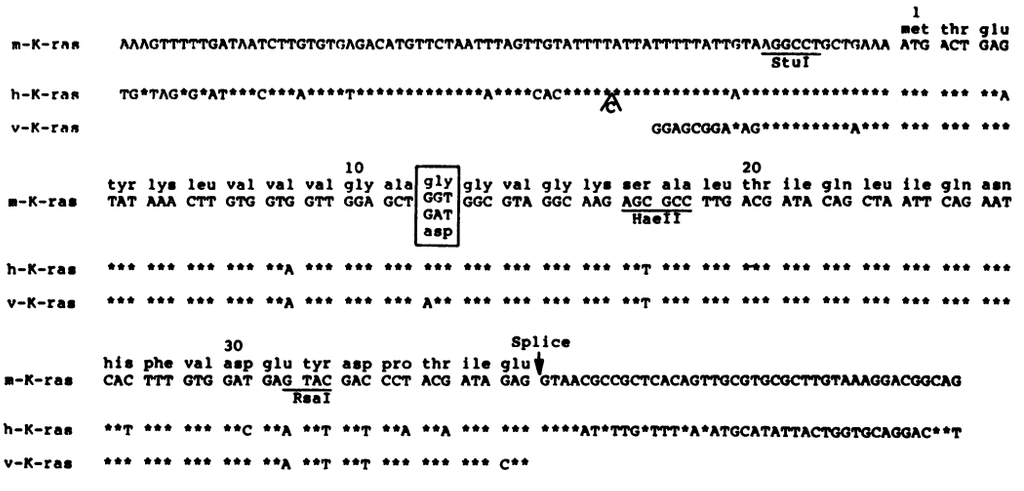


Fig. 3. Sequence comparison of the K-*ras* first coding exon from mouse brain, mouse transformant, human and viral origin. The 0.7-kb Eco RI fragment from both brain and transformant clones containing the exon (Fig. 1B) was subcloned into the plasmid pUC8 (*12*) and sequenced by the method of Maxam and Gilbert (*13*). A, adenine; G, guanine; T, thymine; C, cytidine. Stars indicate identity with the mouse sequence (m). The amino acids are represented on top of the coding region. Some restriction sites are given. The location of the 3′ splice site and the human (h) sequence is from McGrath *et al.* (*10*). The viral sequence (v) is from Tsuchida *et al.* (*11*). The G → A mutation in the transformant clone and change of glycine to aspartic acid in the 12th coding position are shown. The two sequences were otherwise identical over the entire region shown. For the best match between human and mouse sequences, a one-base gap has been inserted in the mouse sequence 30 bases upstream from the first methionine.

Hind III fragment containing the first exon was replaced with its counterpart from the normal clone (Fig. 2), the transformation efficiency dropped to undetectable values, indicating that the tumor-derived first exon carries the ability to transform.

These constructs raise the possibility of exon exchange among the *ras* genes not only between K-*ras* and H-*ras* but also between mouse and human. Moreover, with the hybrid construct pBKT-142 that transforms 3T3 cells very efficiently, we were able to locate the approximate 5′ end of the gene. Pvu II–digested plasmid retains its transformation ability, while Sst II–digested plasmid loses it, indicating that the 5′ end must be located between these two sites (see Fig. 1B).

Our next step was to determine the primary structure of the first exon. We therefore compared the sequences of the first exons of the human gene (*10*), the transformant mouse clone, the normal gene from brain DNA of the same animal, and the Kirsten sarcoma virus gene (*11*) (Fig. 3). We found only a single base difference between the normal and transformant clones derived from the same mouse. This difference was in the second base of the triplet coding for amino acid 12, changing glycine to aspartic acid. The brain contained the normal sequence coding for glycine, showing that the mutant tumor gene arose somatically and was not a heritable germ-line variant. Compared with the mouse gene, the human gene had ten base differences, none of which affected the amino acid sequence of the p21 protein. Seven of these ten differences were in the last third of the exon. The disparity in the conservation of the sequences upstream and downstream from the coding region was striking. While downstream only four bases were identical between the human and mouse genes, 59 of the first 67 bases upstream were identical [the known 5′ splice point is located in humans 11 bases upstream from the methionine (*10*)]. This degree of conservation, which is unusual for intronic sequences, suggests that the sequences have a regulatory role and have therefore been fixed in evolution. The remarkable A-T richness of this region might be considered in this context. We might otherwise speculate that the first exon has an alternative splicing expanding 5′ up to 65 bases beyond the methionine codon.

Only one protein-coding exon in the fragment confers on the hybrid construct the ability to transform 3T3 cells, and we found a single base difference between active and inactive forms of the exon. This finding together with evidence in other systems that mutations in the codon for the 12th amino acid are causally related to the ability of *ras* genes to become activated (*6*), supports the claim that the substitution of glycine for aspartic acid is responsible for activating this mouse K-*ras* oncogene derived from a γ-radiation–induced lymphoma.

Since we had cloned the activated K-*ras* gene exon from a transformant and not directly from tumor X, we wanted to demonstrate the presence of the mutation in the original tumor DNA used to produce the transformed foci. Figure 3 shows that no useful restriction enzyme recognition sites were affected by the point substitution in the activated K-*ras* gene that we had cloned. We therefore used the technique of differential hybridization with a single mismatch (*14*).

We prepared a "nineteenmer" complementary to the transformant sequence. The oligonucleotide was centered in the second base of the 12th codon. Figure 4A shows a reconstruction experiment in which we used increasing amounts of the cloned DNA's

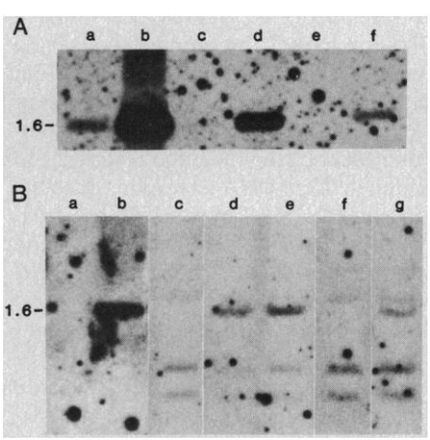


Fig. 4. Detection of the point mutation with a synthetic oligonucleotide. DNA's were digested with Hind III and Pst I, run in a 1 percent agarose gel, hybridized, and washed according to Kidd *et al.* (*14*) with the following modifications: the filter was washed for 1 hour at room temperature, 5 minutes at 60°C, and 1 minute at 63°C. Autoradiograms were exposed for 3 days. Molecular weights are in kilobases. The sequence of the oligonucleotide (Chemgenes) used in these experiments is 5′-TTGGAGCTGATGGCGTAGG-3′. (A) Reconstruction experiment: lanes a, c, and e contain DNA from the phage λMKN-142 and lanes b, d, and f DNA from λMKT-142. Lanes a and b, 100 ng of DNA; lanes c and d, 10 ng; lanes e and f, 1 ng. (B) Lanes a and b contain 5 ng of λMKN-142 (normal) and λMKT-142 (transformant) respectively. The other lanes contain 20 μg of the following genomic DNA's: lane c, NIH 3T3; lanes d and e, two independently derived 3T3 primary transformants from tumor X; lane f, brain from the mouse that developed tumor X; lane g, tumor X.

from the brain and the transformant. Even at the equivalent of 100 gene copies, there was no detectable signal in the normal DNA, but the transformant band hybridized strongly with the probe (Fig. 4A, lane d). Using these conditions, we compared DNA's from primary transformants and the parent 3T3 cells, and tumor X and the brain from the same animal. As we reported earlier (*2*), in the transformants the activated K-*ras* oncogene was amplified three to five times. It showed a strong band of 1.6 kb (Fig. 4B, lanes d and e). The appearance of the band could not be due to the increased number of normal gene copies, because even 100 copies of the normal gene gave no signal under these conditions. More important, we obtained evidence that the mutation in the transformant (Fig. 3) was also present in the original tumor from which it was obtained (Fig. 4B, lane g). As expected from the sequence in Fig. 3, DNA from the brain of the same animal did not hybridize under these conditions. The lower bands appearing in all lanes with genomic DNA's (Fig. 4B, lanes c to g) showed that approximately equal amounts of DNA were loaded in each track.

The presence of the mutation in DNA obtained directly from tumor X rules out the possibility of a mutation during DNA-mediated gene transfer (*15*). Its absence from brain DNA again confirms that the mutation was not genetically inherited but arose de novo in the tumor. Two out of the other three remaining positive tumors had the same mutation G → A in the second base of the 12th codon (data not shown). We have not yet determined the alteration in the fourth positive tumor.

Genes of the *ras* family activated by somatic point mutations have been reported in a variety of human tumors (*6, 9, 10*), and it has been possible to link a genetic alteration in human tumors with a functional assay that transforms cells to the oncogenic phenotype. Ionizing radiation causes the formation of strand breaks in DNA, the release of bases from the DNA backbone, and modification of the bases themselves (*16*). The carcinogenic effect of the radiation is thought to be due to these DNA alterations or to the subsequent DNA repair mechanisms. Radiation has long been implicated in the generation of tumors in humans and experimental animals (*17*). We have now shown that at least three radiation-induced lymphomas have in their DNA's a point mutation that is able to activate normal *ras* genes so that they can transform rodent fibroblasts into tumorigenic cells. This does not demonstrate that *ras* activation is the primary event in radiation-induced lymphomas, but it raises that possibility. That viruses are not the driving force in our system was shown by Mayer and Dorsch-Hasler (*18*).

Similar point mutations are associated with many human tumors and carcinogen-induced malignancies in experimental animals (*2, 3, 19*), and our results suggest a means by which it might be possible to study the association between point mutations and K-*ras* activation in kinetic experiments involving analysis of bone marrow and thymic cells at different times after tumor induction.

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### References and Notes

1. C. Shih, C. Padhy, M. Murray, R. A. Weinberg, *Nature (London)* **290**, 261 (1981); M. Perucho *et al.*, *Cell* **27**, 467 (1981); S. Pulciani *et al.*, *Nature (London)* **300**, 539 (1982); C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982); M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **269**, 404 (1982); S. Pulciani *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2845 (1982); K. Shimizu, M. Goldfarb, M. Perucho, M. Wigler, *ibid.* **80**, 383 (1983).
2. I. Guerrero, P. Calzada, A. Mayer, A. Pellicer, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 202 (1984).
3. A. Balmain and I. B. Pragnell, *Nature (London)* **303**, 72 (1983); S. Sukumar *et al.*, *ibid.* **306**, 658 (1983).
4. K. Shimizu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2112 (1983).
5. D. DeFeo *et al.*, *ibid.* **78**, 3328 (1981); H. Langbeheim, T. Y. Shih, E. M. Scolnick, *Virology* **106**, 292 (1980).
6. C. J. Tabin *et al.*, *Nature (London)* **300**, 143 (1982); E. P. Reddy, R. K. Reynolds, E. Santos, M. Barbacid, *ibid.*, p. 149; E. Taparowsky *et al.*, *ibid.*, p. 262; Y. Yuasa *et al.*, *ibid.* **303**, 775 (1983).
7. R. W. Ellis *et al.*, *ibid.* **292**, 506 (1981).
8. I. Guerrero, A. Villasante, P. D'Eustachio, A. Pellicer, *Science*, in press.
9. H. Nakano *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 71 (1984).
10. J. P. McGrath *et al.*, *Nature (London)* **309**, 501 (1983).
11. N. Tsuchida, T. Ryder, E. Ohtsubo, *Science* **217**, 937 (1982).
12. S. Vieira and J. Messing, *Gene* **19**, 259 (1982).
13. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
14. V. J. Kidd, R. B. Wallace, K. Itakura S. L. C. Woo, *Nature (London)* **309**, 230 (1983).
15. M. P. Calos, J. S. Lebrowski, M. Botchan, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3015 (1983).
16. P. A. Cerutti, in *Photochemistry and Photobiology of Nucleic Acids*, S. Y. Wang, Ed. (Academic Press, New York, 1976), vol. 2, pp. 275–401; J. F. Ward and I. Kuo, *Radiat. Res.* **66**, 485 (1976); B. Dunlap and P. Cerutti, *FEBS Lett.* **51**, 188 (1975); G. Scholes, J. F. Ward, J. Weiss, *J. Mol. Biol.* **2**, 379 (1961); H.-J. Rhaese and E. Freese, *Biochim. Biophys. Acta* **155**, 476 (1968).
17. A. S. Kaplan, *Cancer Res.* **27**, 1325 (1967).
18. A. Mayer and K. Dorsch-Hasler, *Nature (London)* **295**, 253 (1982).
19. I. Guerrero *et al.*, in preparation.
20. T. Maniatis, E. F. Fritsh, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
21. We thank M. Perucho for plasmid pLNBXP and discussions, E. Scolnick for the K-*ras* probe, A. Mayer for help in setting up the system, and P. D'Eustachio and R. Dalla-Favera for critical reading of the manuscript. We also thank R. Altman and L. Lloyd for technical assistance. Supported by NIH grants CA-36327 and GM-32036. I.G. is a fellow of the Spanish High Research Council, A.V. is a fellow at the Fogarty International Program, and A.P. is an Irma Hirschl-Monique Weill/Caulier Award recipient.

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## A Novel Nuclear Form of Estradiol Receptor in MCF-7 Human Breast Cancer Cells

Abstract. *Nuclear estrogen receptor from MCF-7 cells undergoes a time-dependent, hormone-inducible transformation to a form that is less extractable from nuclei and less exchangeable with ligand. This receptor-modifying, intranuclear event is independent of receptor loss (processing) and appears associated with hormone responsiveness (progesterone-receptor induction) in these cells. The magnitude of receptor loss, however, is variable and apparently not a prerequisite for hormone action to induce progesterone receptor.*

After treatment of MCF-7 human breast cancer cells with estradiol ($E_2$), translocation of estradiol-receptor complexes (ER) is followed by processing, a time-dependent irreversible decline in the amount of nuclear ER (*1*). Within 3 to 5 hours, the number of ER's has fallen by about 70 percent, and a new steady-state level is reached. Receptor processing in MCF-7 cells correlates with estrogen induction of progesterone receptor (PR) (*2*). A similar depletion in receptor sites is seen in rat uterine cells (*3*). Processing appears to be a prerequisite for estrogen stimulation since it is either impaired or blocked entirely in the presence of antiestrogens. $R_3$, an estrogen-independent variant subline of MCF-7, shows no receptor processing, does not respond to estrogens, and has no detectable PR inducible by $E_2$ (*1, 4*). The molecular mechanism and the precise correlation of this event with hormone-regulated effect in MCF-7 cells remains