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GCCAGCTCATTTGGCGAGCGTTGATTGGTGGATCAA
CGAGTAAACCGCTCGCAACTAACCACCTAGTTCGGT
19. The buffer consisted of 4 m*M* tris (*p*H 8), 60 m*M* KCl, 2 m*M* $MgCl_2$, 4% glycerol, 1 m*M* DDT, bovine serum albumin (100 μg/ml), salmon sperm DNA (2.5 μg/ml), and 0.1% Brij 58 at 23°C.
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21. Provided by R. Brent.
22. We thank B. Arcangioli, A. Brunelle, R. Schleif, and K. Pfeifer for advice on the gel-binding assay; E. Furfine, J. Greene, A. Ninfa, and L. Chodosh for advice on protein purification; and R. Brent for a gift of LexA antiserum. This work was supported by a Damon Runyon–Walter Winchell Cancer Fund Postdoctoral Fellowship to S.H. and a grant to L.G. from the National Institutes of Health (5RO1 GM3044-05).

11 December 1987; accepted 26 February 1988

Interleukin-1 Immunoreactive Innervation of the Human Hypothalamus

CHRISTOPHER D. BREDER, CHARLES A. DINARELLO, CLIFFORD B. SAPER*

Interleukin-1 (IL-1) is a cytokine that mediates the acute phase reaction. Many of the actions of IL-1 involve direct effects on the central nervous system. However, IL-1 has not previously been identified as an intrinsic component within the brain, except in glial cells. An antiserum directed against human IL-1β was used to stain the human brain immunohistochemically for IL-1β–like immunoreactive neural elements. IL-1β–immunoreactive fibers were found innervating the key endocrine and autonomic cell groups that control the central components of the acute phase reaction. These results indicate that IL-1 may be an intrinsic neuromodulator in central nervous system pathways that mediate various metabolic functions of the acute phase reaction, including the body temperature changes that produce the febrile response.

INTERLEUKIN-1 (IL-1) IS A CYTOKINE that is elaborated by cells involved in host defense, such as macrophages (*1*). It mediates several components of the acute phase reaction, including the febrile response, secretion of adrenocorticotropic hormone (ACTH), and the synthesis of acute phase proteins. Plasma IL-1 is thought to enter the hypothalamus at the organum vasculosum of the lamina terminalis (OVLT), a circumventricular organ with no blood-brain barrier, located at the anteroventral tip of the third ventricle (*2*). IL-1 injection into the brain causes fever, secretion of adrenal corticosteroids, and synthesis of acute phase proteins by the liver (*3*). However, the site of action of IL-1 within the brain has not yet been determined.

A factor with IL-1–like activity has been isolated from the brain (*4*), but IL-1 has been demonstrated so far only in glial cell lines (*5*). We used immunohistochemistry to examine the cellular localization of IL-1 in the human brain. Our results indicate that IL-1 is contained in neural elements within the hypothalamus, where it may serve as a neuromodulator in the central component of the acute phase reaction.

Five normal human brains were collected at autopsy (*6*) and the basal forebrains were cut at 50 μm on a freezing microtome. Sections were stained immunohistochemically with an antiserum against human IL-1β (*7*). Control sections were prepared by omitting the primary antiserum or by using antiserum that had been adsorbed with recombinant human IL-1β (*8*). There was no fiber staining in this control material (Fig. 1, D and E). In addition, the IL-1β antiserum was adsorbed against a number of neuropeptides that have been identified in immunohistochemical studies of the hypothalamus (*8*), none of which reduced the intensity of staining. Sections through the hypothalamus were also stained with an antiserum to human IL-1α, which has approximately 30% amino acid identity with IL-1β (*8*). Only a few scattered fibers were stained by this antiserum, an indication that the staining with the IL-1β antiserum was specific.

IL-1β–like immunoreactive (ir) varicose fibers were seen in a characteristic distribution within the hypothalamus in each of the brains (Fig. 2). The densest accumulations of fibers were found in the periventricular regions that participate in anterior pituitary control. Immunoreactive fibers were found throughout the periventricular and arcuate nuclei of the hypothalamus, as well as in the parvocellular part of the paraventricular nucleus (Figs. 1C and 2). IL-1βir fibers could be traced into the infundibulum, including the region of the median eminence containing the hypophyseal portal vessels (Fig. 1B). In addition, IL-1βir innervation was seen in the magnocellular part of the paraventricular nucleus, and to a lesser extent in the supraoptic nucleus, among the cell bodies of neurons that secrete oxytocin and arginine vasopressin (AVP) from the posterior pituitary gland (Fig. 1A).

There was also IL-1βir innervation of hypothalamic structures that participate in autonomic control. In addition to the autonomic portions of the paraventricular nucleus, IL-1βir fibers were found in several other cell groups whose neurons directly innervate autonomic preganglionic nuclei (*9*), including the dorsomedial nucleus of the hypothalamus and the lateral hypothalamic area. IL-1βir fibers were also seen within the subfornical organ (Fig. 2E), the bed nucleus of the stria terminalis (Fig. 1G), the substantia innominata, the ventromedial nucleus of the hypothalamus (Fig. 1H), the posterior hypothalamic area (Fig. 1D), and the paraventricular nucleus of the thalamus, all structures thought to have a role in central autonomic control (*10*).

Our results indicate that the human hypo-

C. D. Breder, Committee on Neurobiology, University of Chicago, Chicago, IL 60637.
C. A. Dinarello, Departments of Medicine and Pediatrics, Tufts University School of Medicine, New England Medical Center Hospitals, Boston, MA 02111.
C. B. Saper, Departments of Pharmacological and Physiological Sciences and Neurology, Committee on Neurobiology, and the Brain Research Institute, University of Chicago, Chicago, IL 60637.

*To whom correspondence should be addressed.

thalamus is innervated by a network of IL-1βir fibers. We did not observe immunoreactive cell bodies in the hypothalamus or elsewhere in the brain. This is not surprising, as the visualization of peptide-immunoreactive cell bodies in the brains of experimental animals often requires the administration of an inhibitor of axonal transport such as colchicine. Furthermore, the quality of the staining in human autopsy brains is limited by the relatively long but unavoidable delay between death and fixation of the brain (4 to 20 hours in our series). Nevertheless, the distribution of IL-1βir axons is quite similar to the pattern of axonal projections that we and others have traced from the periventricular preoptic region (*11*). These data suggest that the IL-1 cell bodies are located near the anteroventral tip of the third ventricle.

IL-1βir fibers in the human hypothalamus innervate structures that are thought to mediate the central components of the acute phase reaction. Several groups of investigators have shown that IL-1 injected either systemically (*12*) or intracerebroventricularly (*13*) causes increased secretion of ACTH. This response is primarily mediated by the secretion of corticotropin-releasing factor (CRF) from terminals of neurons in the hypothalamus into the hypophyseal portal circulation (*13*). Most of the CRF cell bodies are located in the paraventricular nucleus of the hypothalamus (*7, 14*) (Fig. 2). Our results demonstrate that, in the human brain, the areas containing the CRF cell bodies are among the most heavily innervated by IL-1βir fibers.

In addition, IL-1 has been reported to act directly on anterior pituitary cells that secrete ACTH, as well as luteinizing hormone and prolactin (*15*). We found dense IL-1βir innervation of the median eminence, suggesting that IL-1 may be secreted directly into the hypophyseal portal vessels, where it may affect anterior pituitary secretion.

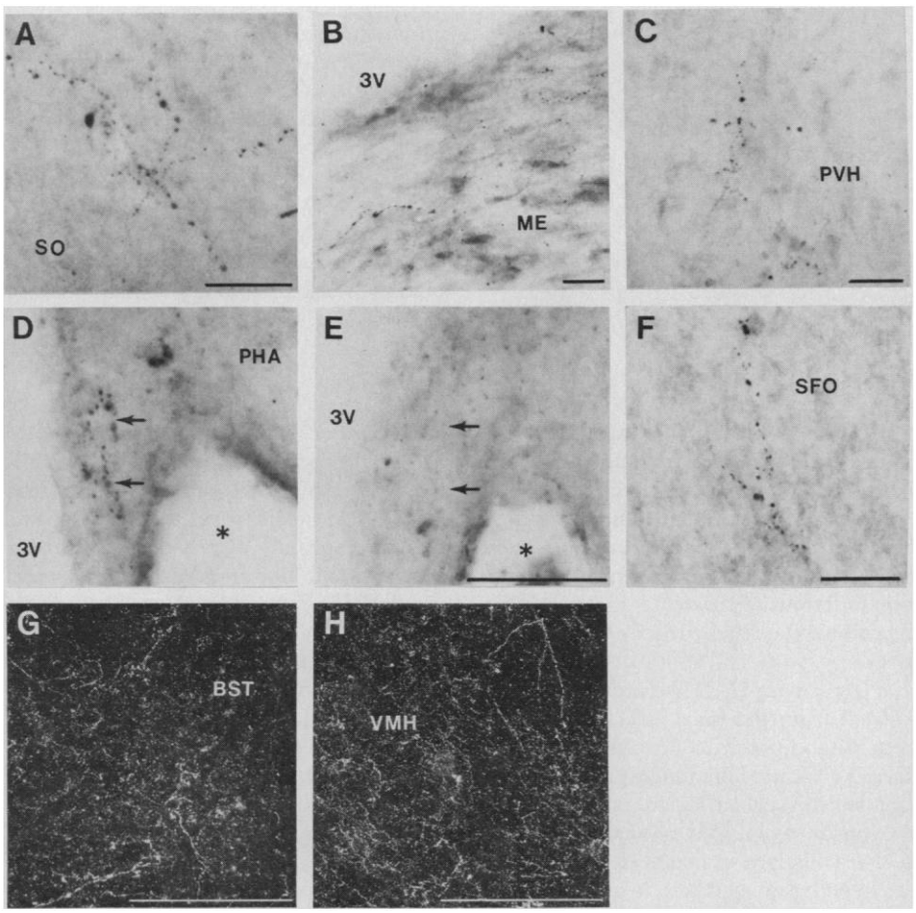

Fig. 1. Photomicrographs to illustrate the appearance of IL-1βir fibers in the human basal forebrain. Individual IL-1βir fibers are shown at high magnification, ramifying among the cells of the (**A**) supraoptic nucleus (SO), (**C**) paraventricular nucleus (PVH), and (**F**) subfornical organ (SFO), and (**B**) coursing into the median eminence (ME). (**D**) A fiber ramifying in the area adjacent to a blood vessel (asterisk) in the wall of the third ventricle, at the level of the posterior hypothalamus (PHA). (**E**) An adjacent section through the same blood vessel incubated with primary antiserum that had been preadsorbed with recombinant human IL-1; no fiber staining was seen in this material. (**G** and **H**) Darkfield, low magnification photomicrographs illustrating the density of IL-1βir fiber labeling in the bed nucleus of the stria terminalis (BST) (compare with Fig. 2A) and along the ventrolateral edge of the ventromedial nucleus (VMH) (compare with Fig. 2B). Scales for (A) to (F), 50 μm; for (G) and (H), 500 μm.

The febrile response depends on the activation of various endocrine and autonomic thermogenic mechanisms. In addition to the increase in adrenal corticosteroids, there is alteration of the secretion of thyroxine and vasopressin and adjustment of blood flow from cutaneous to deep vascular beds (*16*). The periventricular preoptic nucleus, which contains most of the thyrotropin-releasing hormone neurons (*17*), is densely innervated by IL-1βir fibers. The portions of the paraventricular and supraoptic nuclei that contain the vasopressin neurons are also innervated (*7*) (Fig. 2). In addition, there is substantial IL-1βir innervation of the hypothalamic cell groups involved in central cardiovascular regulation (*10, 11*).

The acute phase reaction is marked by the increased secretion of several proteins by the liver, including C-reactive protein, serum amyloid A, and ceruloplasmin (*1, 18*). The secretion of these acute phase reactants is in part under neural control (*3*). In particular, the ventromedial nucleus and lateral hypothalamus are believed to have a role in neural regulation of hepatic metabolism (*19*). Both of these areas receive IL-1βir innervation.

Another effect of IL-1 is to induce drowsiness accompanied by synchronization of the electroencephalogram (*20*). The lateral hypothalamus innervates the entire cerebral cortex, and lesions in this area also produce somnolence and electroencephalographic synchronization (*21*). The lateral hypothalamic area, too, is innervated by IL-1βir fibers.

Most of the sites mediating the neural response to plasma IL-1 are located at some distance from the OVLT, which is thought to be the site at which IL-1 enters the hypothalamus to induce the febrile response (*2*). It is therefore unlikely that these hypothalamic neurons are affected directly by circulating IL-1. Current theory holds that IL-1 injected either into the circulation or into the cerebral ventricles acts on IL-1–receptive neurons near the OVLT. These neurons would then activate hypothalamic mechanisms that produce the cerebral component of the acute phase reaction, presumably by using other neurotransmitters as intermediate messengers.

Our results suggest an alternative hypothesis: IL-1 may serve, at least in part, as its own intermediate messenger. IL-1 neurons in the preoptic area may respond to circulating IL-1 that crosses the OVLT. These neurons may then use IL-1 as a neuromodulator to cause the autonomic and endocrine adjustments that result in the cerebral com-

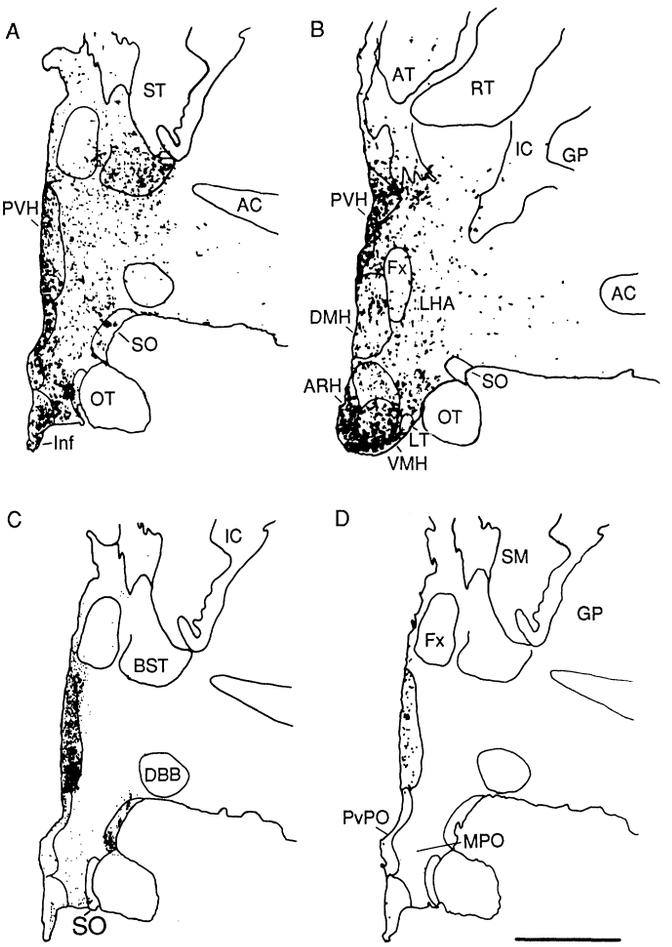

Fig. 2. Line drawings of sections through the human hypothalamus. (**A** and **B**) Pattern of IL-1βir fiber staining at the preoptic and tuberal levels of the hypothalamus, respectively. (**C** and **D**) Cell bodies at the preoptic level that were AVPir and CRFir, respectively. The IL-1βir fibers innervate the key endocrine and autonomic areas of the hypothalamus, critical in elaborating the acute phase reaction, including the CRF and AVP cell groups. Scale bar, 5 mm. Abbreviations: AC, anterior commissure; ARH, arcuate nucleus; AT, anterior thalamic nuclei; BST, bed nucleus of the stria terminalis; DBB, nucleus of the diagonal band of Broca; DMH, dorsomedial nucleus; Fx, fornix; GP, globus pallidus; IC, internal capsule; Inf, infundibulum; LHA, lateral hypothalamic area; LT, lateral tuberal nucleus; MPO, medial preoptic area; OT, optic tract; PVH, paraventricular nucleus; PvPO, periventricular preoptic nucleus; SM, stria medullaris; SO, supraoptic nucleus; ST, stria terminalis; VMH, ventromedial nucleus.

ponent of the acute phase reaction.

Similar proposals have been put forward for the coordination of the central and systemic actions of several other peptide hormones. For example, it has been suggested that cholecystokinin acts both as a systemic hormone and a central neurotransmitter to control feeding (*22*), and that angiotensin (*23*) and atrial natriuretic peptide (*24*) function in both capacities in the regulation of blood volume and pressure. In each case, cell bodies with peptide immunoreactivity are found close to a circumventricular organ, where the circulating peptide may enter the brain to produce central effects.

Our hypothesis is also consistent with the recent demonstration that IL-1 can act on monocytes to induce the further secretion of IL-1 (*7*). In the brain, IL-1 may act in an analogous manner to cause the secretion of IL-1 from neurons that set into action the various autonomic and endocrine responses that constitute the central components of the acute phase reaction.

Note added in proof: Since submitting this report we have obtained another specimen with improved immunohistochemical staining, in which the IL-1βir cell bodies can be demonstrated in the preoptic and hypothalamic periventricular nuclei, the arcuate nucleus, and the retinochiasmatic area.

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7. The antisera used for immunohistochemistry of IL-1β and IL-1α were rabbit polyclonal sera against recombinant human protein; their preparation and characterization have been reported [C. A. Dinarello *et al.*, *J. Immunol.* **139**, 1902 (1987)]. Antisera to CRF and arginine vasopressin (AVP) were obtained from Immunonuclear Corporation. Immunoadsorption controls for these sera are described elsewhere [D. F. Cechetto and C. B. Saper, *J. Comp. Neurol.*, in press]. For immunohistochemistry, sections were incubated for 30 minutes at room temperature in 0.1*M* phosphate-buffered 0.9% saline, *p*H 7.4 (PBS) containing 3% H_2O_2 and 0.25% Triton X-100; they were washed in PBS and incubated for 1 hour in PBS containing 5% nonfat dried milk (PBS-M), and then overnight at 4°C with primary antiserum diluted 1:500 in PBS-M. The next day the sections were incubated for 1 hour at room temperature in goat antiserum to rabbit immunoglobulin G conjugated to horseradish peroxidase (Tago) diluted 1:50 in PBS-M. Sections were then washed in PBS and stained with 0.05% diaminobenzidine and 0.01% H_2O_2 in PBS for 10 minutes.
8. Recombinant IL-1β, donated by Immunex, has been characterized [S. R. Kronheim *et al.*, *Biotechnology* **4**, 1078 (1986)]. For adsorption controls, diluted IL-1β antiserum was incubated overnight with 50 μg/ml of IL-1β, atriopeptin III, CRF, luteinizing hormone–releasing hormone, α-melanocyte–stimulating hormone, neuropeptide Y, somatostatin, thyrotropin-releasing hormone, β-endorphin, ACTH, or AVP before it was used in the immunohistochemical protocol.
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30 November 1987; accepted 16 February 1988

Molecular Cloning of Human and Rat Complementary DNA Encoding Androgen Receptors

CHAWNSHANG CHANG, JOHN KOKONTIS, SHUTSUNG LIAO

Complementary DNAs (cDNAs) encoding androgen receptors were obtained from human testis and rat ventral prostate cDNA libraries. The amino acid sequence deduced from the nucleotide sequences of the cDNAs indicated the presence of a cysteine-rich DNA-binding domain that is highly conserved in all steroid receptors. The human cDNA was transcribed and the RNA product was translated in cell-free systems to yield a 76-kilodalton protein. The protein was immunoprecipitable by human autoimmune antibodies to the androgen receptor. The protein bound androgens specifically and with high affinity.

IN TARGET CELLS, STEROID HORMONES can form specific complexes with intracellular receptors (*1*). The hormonal regulation of gene expression appears to involve interaction of steroid receptor complexes with certain segments of genomes and modulation of specific gene transcription (*2*). The structural and functional analyses of different steroid receptor domains that participate in steroid and DNA binding are now possible largely as the result of the successful cloning of complementary DNAs (cDNAs) that code for various steroid receptors (*3–8*). Isolation of a cDNA that encodes the androgen receptor (AR), however, has not been clearly demonstrated. Cloning of cDNA for the AR has been difficult because monospecific antibodies against the AR have not been available for screening cDNA libraries.

We report here the preparation and partial structural analysis of cDNA for human (h) and rat (r) AR. For this purpose, we used λGT11 cDNA libraries (*9*) constructed with polyadenylated RNA (*10*) from human testis and the ventral prostate of rats that had been castrated 3 days earlier. The cDNA libraries were initially screened with a 41-bp oligonucleotide probe (*11*) that was highly homologous to nucleotide sequences in the DNA-binding domain of glucocorticoid receptors (GR) (*3*), estrogen receptors (ER) (*4*), progesterone receptors (PR) (*5*), mineralocorticoid receptors (MR) (*6*), and the v-*erb*A oncogene product of avian erythroblastosis virus (*7*).

We obtained 302 and 21 positive clones, respectively, from approximately 3×10^6 human testis recombinants and 6×10^5 rat ventral prostate recombinants. If AR also has a cysteine-rich DNA-binding domain that is highly homologous with the DNA-binding regions of other steroid receptors, some of these positive clones should contain cDNA for AR. To eliminate GR-cDNA clones, we screened these positive clones with two GR-specific 24-bp probes (*11*) that had nucleotide sequences identical to nucleotide segments immediately next to the 5′ end or the 3′ end of the DNA-binding region of hGR-cDNA (*3*). By this method we were able to eliminate 244 and 14 clones, respectively, as hGR- and rGR-cDNA clones. With the same procedure, we did not detect any ER- or PR-cDNA clone in the human testis library. No ER-cDNA clone was detected with the rat prostate library, but one positive clone was obtained with hPR-specific 24-bp probes (*11*). By sequence analysis, we were also able to identify four other clones as hMR-cDNA clones (*6*). All of the remaining clones could be separated into two groups: 30 human testis clones had sequences overlapping to form a 2.1-kb cDNA, whereas 24 human testis and 6 rat prostate clones had sequences overlapping to form a 2.7-kb cDNA. The 2.1-kb and 2.7-kb cDNAs were designated as TR2-type (*12*) and AR-type cDNA, respectively.

Because the distance between the putative polyadenylation signal (AATAAA) and the 5′ end in the TR2-type cDNA was only 2.0

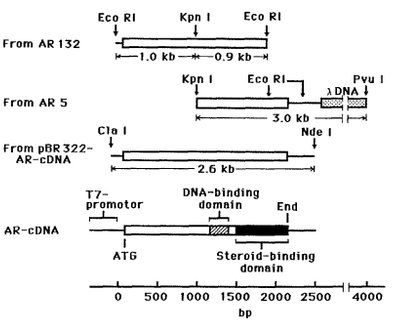

Fig. 1. Strategy used in the construction of cDNA for human androgen receptor. A human testis λGT11 cDNA library that was constructed with *Escherichia coli* Y1090 was screened with a 5′-end ^{32}P-labeled 41-bp oligonucleotide probe (*11*). Positive clones were probed with 5′-end and 3′-end ^{32}P-labeled 24-bp oligonucleotides that were specific for various steroid receptors (*11*). A less stringent hybridization condition was used first to eliminate most of the non-AR clones. The remaining clones were then probed again at the more stringent conditions. After clones for other steroid receptors were eliminated, the DNA inserts in the remaining clones were analyzed by restriction mapping and subcloned into M13 vectors for dideoxy sequence analysis (*10*). Two clones containing DNA inserts that overlapped to form a 2.7-kb cDNA were named AR 132 and AR 5. The cDNA of clone AR 132 was digested with Eco RI to obtain a 1.9-kb fragment that was then digested with Kpn I to obtain a 1-kb Eco RI–Kpn I fragment. This 1-kb fragment was ligated to a 3-kb fragment that was obtained by digestion of another cDNA clone (AR 5) with Kpn I and Pvu I. The ligated 4-kb fragment was inserted into Eco RI– and Pvu I–digested pBR322 vector and used to infect *E. coli* (DH5α). The transformed clones were selected by tetracycline resistance. The plasmid with DNA insert was digested with Cla I and Nde I to obtain a 2.6-kb fragment. The fragment was blunt-ended with the Klenow fragment of *E. coli* DNA polymerase I and ligated to the cloning vector pGEM-3Z plasmid DNA, which had also been blunt-ended by digestion with Sma I. The *E. coli* (DH5α) cells were transformed with the plasmid, and colonies containing the plasmid were selected and amplified. The restriction enzyme sites are shown with arrows.

The Ben May Institute and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637.