caused by a defect in the HPRT gene (*6*). One of the goals of our research is to test the utility of retroviral vectors as potential agents for correction of this defect in somatic cells. We have shown that lymphoblasts from Lesch-Nyhan patients can be infected with the HPRT-virus in vitro (*7*). Results presented here show that the HPRT-virus can be transferred into intact animals because (i) bone marrow and spleen cells of mice receiving infected bone marrow became long-term producers of HPRT-virus, and the released virus appeared to be identical to the virus used for infection of the engrafted bone marrow and (ii) human HRPT was detected in the spleen of a recipient mouse.

From these results we cannot estimate the number of hematopoietic cells infected by HPRT-virus. Because we could detect HPRT-protein clearly in only one animal, transfer may have been relatively inefficient. However, several lines of evidence suggest that $HPRT^+$ hematopoietic cells have a selective advantage over $HPRT^-$ hematopoietic cells. The tissue distribution of $HPRT^-$ cells in partially $HPRT^-$ mosaic mice suggests that selection against $HPRT^-$ cells is particularly strong in blood (*8*). A similar conclusion was made in studies of human heterozygotes carrying the defective HPRT gene (*9, 10*). In addition, myeloid progenitor cells from patients with HPRT deficiency grow poorly in culture compared with those from normal patients (*11*). These results suggest that, even though the HPRT-virus may not be expressed in every cell in virus-treated marrow, retroviral transfer of the HPRT gene into somatic cells from Lesch-Nyhan patients may be effective because of the presumed selective advantage conferred by the synthesis of HPRT in some infected cells.

Note added in proof: Expression of human HPRT protein activity has also been detected in the spleen of another mouse transplanted with amphotropic HPRT-virus obtained from the cell clone c7cl.

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Characterization and Localization of Proopiomelanocortin Messenger RNA in the Adult Rat Testis

Abstract. *Northern blot analysis of total RNA and polyadenylated RNA isolated from adult rat testes showed that a proopiomelanocortin (POMC)-like messenger RNA molecule is present in these extracts. The testicular POMC messenger RNA is comparable in length to amygdala and midbrain POMC messenger RNA and appears to be at least 200 nucleotides shorter than POMC messenger RNA found in the hypothalamus and anterior and intermediate lobes of the pituitary gland. Hybridization in situ showed that POMC messenger RNA is located in Leydig cells, which are the only testicular cells that contain immunostainable POMC-derived peptides. These results suggest that local synthesis of POMC occurs in the testis.*

The potential physiological role for peptide hormones originally isolated from the pituitary gland has increased recently with numerous demonstrations that these hormones are present in a number of mammalian nonpituitary tissues (*1*). For example, peptides related to proopiomelanocortin (POMC) have been identified immunocytochemically at various sites in the reproductive tract of the adult male rat, including the Leydig cells of the testis (*2*). Electrophoretic and chromatographic techniques, combined with radioimmunoassay, have been used to determine the molecular species and concentrations of POMC-related peptides in the testis (*3*). Fragments of sizes corresponding to those of endorphin and α-melanocyte–stimulating hormone (α-MSH) are the major POMC-derived peptides present in adult testis extracts; however, in contrast to those found in the adult rat intermediate pituitary lobe (*4*), these testicular POMC-derived peptides are present in their nonacetylated forms (*3*). These results show that the end points of POMC processing in the reproductive tract are similar to those in the hypothalamus, where nonacetylated forms of these peptides are also present (*5*). The concentrations of POMC-related peptides present in the adult rat testis are not altered by hypophysectomy (*3*), suggesting that testicular POMC peptides are not derived from pituitary secretion and subsequent uptake by testicular cells. This observation, however, does not eliminate the possibility that these peptides may be synthesized elsewhere.

Complementary DNA (cDNA) probes can be used to determine whether messenger RNA (mRNA) that can direct local synthesis of specific peptides or proteins is present in specific tissues. Thus far, POMC cDNA probes have been used to demonstrate POMC-like mRNA in specific brain regions (*6*) and to locate cells containing POMC-like mRNA in the periarcuate region of the hypothalamus (*7*). We have investigated whether a cDNA probe for POMC can detect POMC-like mRNA in testis extracts and whether this probe can locate specific cells containing POMC mRNA after hybridization in situ.

RNA was isolated from adult male pituitary, liver, testis, amygdala, and midbrain by the guanidinium isothiocyanate procedure (*8*); polyadenylated

[poly(A)] RNA from testis, midbrain, and amygdala was subsequently isolated by use of an oligo-dT cellulose column (*9*). Total and poly(A) RNA samples were separated on denaturing formaldehyde gels (*10*), transferred to Gene Screen (New England Nuclear), and hybridized (*11*) with a 550-base-pair ^{32}P-labeled POMC cDNA insert, I-13 (*12*).

Radiolabeled bands were present in filter lanes containing RNA isolated from testis, pituitary, and brain, but not in the lane containing liver RNA (Fig. 1). The hybridizing bands in testicular poly(A) RNA (Fig. 1) and in total testicular RNA (not shown) were estimated to be between 850 and 900 nucleotides long—at least 200 nucleotides shorter than pituitary and hypothalamic POMC mRNA and similar in length to POMC-like mRNA in amygdala, midbrain, and cerebral cortex (Fig. 1) (*6*). The abundance of POMC mRNA in testis was compared with that in other nonpituitary sites of POMC mRNA synthesis by estimating the intensities of the radiolabeled POMC bands from RNA samples hybridized on the same blot. The concentration of testicular POMC mRNA was estimated to be at least 200 times that of amygdala and midbrain POMC mRNA (Fig. 1) and similar to that of hypothalamic POMC mRNA (*6*).

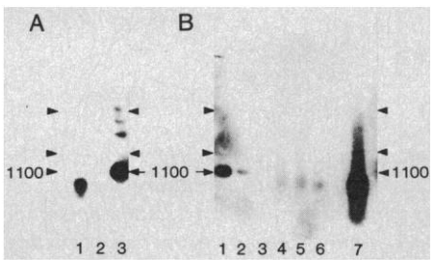

Fig. 1. Northern blot analysis of testis, pituitary, brain, and liver RNA. Total and poly(A) samples were prepared (*8, 9*), separated on 1.5 percent agarose gels (*10*), and transferred to Gene Screen. Since RNA electrophoretic mobility is affected by the total amount of nucleic acid in the sample, liver RNA was added to some samples (see below) to ensure that all lanes contained similar amounts of RNA. The blots were baked for 3 hours at 80°C and prehybridized overnight. The blots were then hybridized (*11*) overnight at 42°C with ^{32}P-labeled I-13 insert (*12*). The specific activity of nick-translated (*17*) I-13 used for blot hybridization was 5×10^8 cpm/μg; 3×10^7 cpm were used per hybridization. Blots were then washed in double-strength standard saline citrate (SSC) (SSC is 0.15*M* sodium chloride and 0.015*M* sodium citrate) containing 0.1 percent sodium dodecyl sulfate (SDS) and 0.05 percent sodium pyrophosphate for 30 minutes at 22°C and 0.2-fold strength SSC containing 0.1 percent SDS and 0.05 percent sodium pyrophosphate for 30 minutes at 55°C. The positions of the 28*S* and 18*S* ribosomal subunits and the known length of pituitary POMC mRNA (1100 nucleotides) are indicated. (A) Overnight exposure of a region of a blot containing testis, liver, and pituitary samples: (lane 1) 10 μg of testis poly(A) RNA; (lane 2) 10 μg of total liver RNA; (lane 3) 5 μg of total pituitary RNA plus 5 μg of total liver RNA. The radiolabeled band in the testis sample is at least 200 nucleotides shorter than pituitary POMC mRNA and is approximately half as abundant as the pituitary sample. (B) Five-day exposure of a partially overlapping region of the same blot: (lane 1) 100 ng of total pituitary RNA plus 10 μg of total liver RNA; (lane 2) 20 ng of total pituitary RNA plus 10 μg of total liver RNA; (lane 3) blank; (lane 4) 12 μg of amygdala poly(A) RNA from pooled male and female adult rats; (lane 5) 12 μg of female midbrain central gray poly(A) RNA; (lane 6) 12 μg of male midbrain central gray poly(A) RNA; (lane 7) 10 μg of testis poly(A) RNA (same as lane 3 in Fig. 1A). The intensity of hybridization is similar in brain samples and the pituitary sample containing 20 ng of total pituitary RNA.

The cellular location of POMC mRNA in testicular tissue sections was determined by hybridization in situ (*13*). Tissue sections were prepared for hybridization in situ, hybridized overnight with ^{3}H-labeled I-13 DNA, and washed (Fig. 2). Slides were then processed for autoradiography and exposed for 6 weeks at 4°C. The slides showed silver grains localized in the cytoplasm of most Leydig cells, which were identified by their location outside the seminiferous tubules. Relatively few background grains were seen over neighboring cell types (Fig. 2). Similar results were obtained after in situ hybridization of POMC cDNA probes for mouse testicular sections (*14*). Leydig cells were never labeled when sections were exposed to hybridization solution containing radiolabeled pBR322 DNA.

Taken together, these observations indicate that the POMC-related peptides immunocytochemically demonstrated in the adult rat Leydig cells (*2*) are synthesized within these cells. Thus, Leydig cells, like hypothalamic neurons (*7*), are specific extrapituitary cell types showing POMC gene expression.

These observations raise interesting points concerning transcriptional and translational control of POMC synthesis. First, although testis POMC mRNA is at least as abundant as hypothalamic POMC mRNA, the steady-state content of POMC-related peptides in the testis is about 0.1 percent of that in the hypothalamus (*3*). The reason for this difference is not yet known but may represent either a relatively inefficient translation of POMC mRNA or rapid degradation or secretion (or both) of POMC-related peptides.

Second, the concentration of the shortened POMC-like mRNA is much higher in testis than in brain regions such as amygdala, midbrain, cerebral cortex, and cerebellum (*6, 11*); thus testis should be an appropriate tissue in which to investigate transcriptional or posttranscriptional events leading to production of the shortened POMC mRNA. Since there appears to be just one POMC gene in the rat (*15*), the shortened transcript in nonpituitary tissues does not result from expression of a slightly different POMC gene from the one expressed in the pituitary. Thus, the testicular POMC mRNA may be shortened because of an alternative starting point for transcription, an alternative splicing pattern during processing of the initial transcript, possibly leading to a deletion of a specific region in the mature mRNA, or a significant

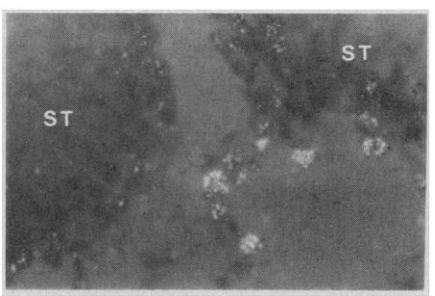

Fig. 2. Hybridization in situ of a testicular tissue section. An adult male rat testis was immersion-fixed for 2 hours at room temperature in freshly prepared 4 percent paraformaldehyde-phosphate buffered saline (*p*H 7.0), equilibrated overnight in 20 percent sucrose, and embedded in OCT. Cryostat sections (8 μm) were hydrated and treated with 0.2*N* HCl for 10 minutes. Sections were then prehybridized at 24°C for 1 hour with prehybridization buffer containing 50 percent formamide, quadruple-strength SSC, 20 m*M* tris (*p*H 7.5), yeast total RNA (0.1 mg/ml), yeast transfer RNA (0.05 mg/ml), herring sperm DNA (0.1 mg/ml), 1 m*M* EDTA, 0.02 percent Ficoll, 0.02 percent polyvinylpyrrolidone-20, and bovine serum albumin (0.2 mg/ml). Prehybridization buffer was removed and replaced with hybridization buffer containing the above components plus 10 percent dextran sulfate and ^{3}H-labeled I-13 DNA (specific activity, 10^7 cpm/μg; 2×10^4 cpm per section). The hybridization mix was heat-denatured at 100°C for 10 minutes before it was applied to the tissue section. Incubation of tissue section with hybridization mix continued overnight at 24°C. Sections were washed for 1 hour each time in two changes of double-strength SSC containing 0.1 percent SDS at room temperature and for 5 hours in 0.2-fold strength SSC containing 0.1 percent SDS at 37°C. Sections were air-dried and coated with emulsion (Ilford L-4) that had been diluted 1:1 with 0.6*M* ammonium acetate. Sections were then exposed at 4°C for 6 weeks, developed with D-19, and counterstained with hematoxylin and eosin. Silver grains were observed in the cytoplasm of most Leydig cells in the interstitial space of the testis; cells within the seminiferous tubules (*ST*) are unlabeled.

difference in the length or position of the poly(A) tail. Immunodeterminants of ACTH, endorphin, and γ-MSH have been detected in Leydig cells (*2, 16*). These determinants are derived from the midportion, carboxyl-terminal, and amino-terminal POMC domains, respectively. Hence the most likely potential splice sites within the coding region would be large nucleotide regions on either side of the γ-MSH determinant in the amino-terminal region. A deletion in the 5′ noncoding region seems unlikely since this region is small and is required for ribosomal binding. Experiments on the shortened POMC-like mRNA in testis might suggest the nature of the shortened POMC-like mRNA observed in specific regions of the brain. Finally, the relatively high concentration of POMC mRNA in testis should allow the role of potential physiological regulators of testis POMC mRNA to be assessed.

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Cellular Transformation by Human Papillomavirus DNA in Vitro

Abstract. *Molecularly cloned DNA's of human papillomaviruses HPV-5 and HPV-1 induced morphological transformation of mouse C127 cells in culture. Single-cell clones of cells transformed by papillomavirus contained multiple persistent episomal copies of the transfected DNA species and were analyzed for growth characteristics indicating malignant potential.*

Papillomaviruses have been identified as the etiological agents of many types of benign warts in both humans and numerous animal species (*1, 2*). Furthermore, some of these viruses have been associated with malignant lesions in their natural host, particularly bovine papillomavirus (BPV) (*2, 3*), cottontail rabbit papillomavirus (CRPV) (*4*), *Mastomys natalensis* papillomavirus (*5*), and human papillomaviruses (HPV). Recent reports have shown HPV-5 DNA in primary and metastatic squamous cell carcinomas of patients with epidermodysplasia verruciformis (*6*) and HPV DNA of several types in genital neoplasias (*7*). Papillomaviruses have not been successfully propagated in culture; however, recent results have shown that papillomavirus genomes can persist in and transform cultured cells (*8–12*). The genome of BPV has been shown to induce morphological transformation of mouse C127 and NIH 3T3 cells (*11*), to replicate

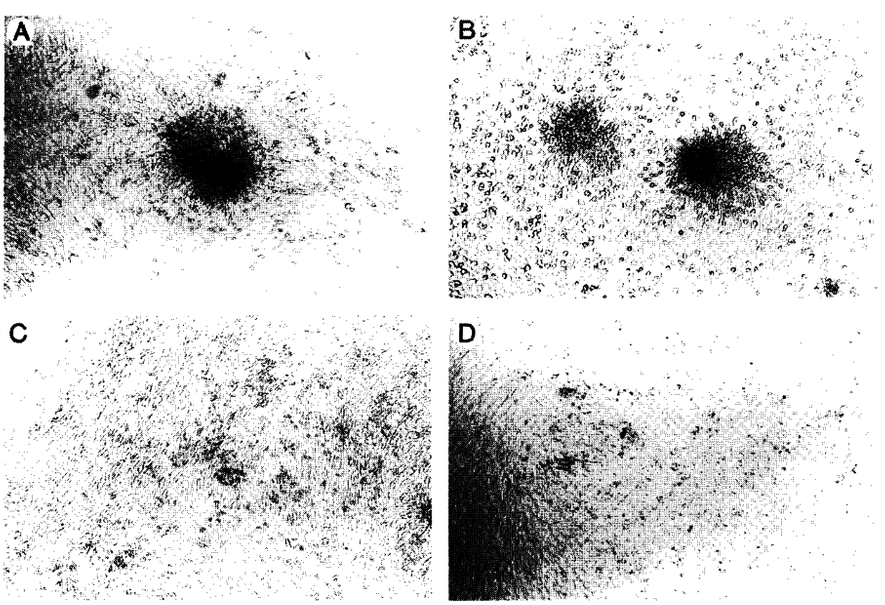

Fig. 1. Transformation of mouse C127 cells with HPV DNA. (A) Focus formation after transfection with pBR322HPV-5 DNA. (B) Focus formation after transfection with pBR322HPV-1 DNA. (C) Transformed clone C112-3A-10-2 isolated from a focus induced by transfection with pBR322HPV-5 DNA. (D) Transformed clone C112-4A-1-3 isolated from a focus induced by transfection with pBR322HPV-1 DNA.