Localization of the Genes Encoding the Melanocortin-2 (Adrenocorticotropic Hormone) and Melanocortin-3 Receptors to Chromosomes 18p11.2 and 20q13.2–q13.3 by Fluorescence *in Situ* Hybridization

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Adrenocorticotropic hormone (ACTH) and α-, β-, and γ-melanocyte-stimulating hormone (MSH) are products of pro-opiomelanocortin post-translational processing (1). These compounds are collectively labeled as melanocortins (MC). Aside from their established effects on the regulation of the adrenal cortex (ACTH) and melanocytes (α-MSH), the melanocortins have been implicated in a broad array of physiological events. In the central nervous system, melanocortins have been shown to affect behavior, learning and memory, control of the cardiovascular system, thermoregulation, the release of other neurohumoral agents, and analgesia (2, 3). Peripherally, melanocortins have been identified to have immunomodulatory and neurotrophic properties and to be involved in the events surrounding parturition (4–6). Melanocortins mediate their effects through cell membrane receptors belonging to the superfamily of seven transmembrane G-protein-linked receptors. Using the technique of polymerase chain reaction with primers based on conserved areas of the seven transmembrane G-protein-linked receptor family, we recently isolated an "orphan" subfamily of this receptor group. Within the past year, two of these receptors were identified as specific for α-MSH (MC1) (7, 8) and ACTH (MC2) (7). We have recently described a third melanocortin receptor (MC3) that appears to recognize the core heptapeptide sequence of melanocortins with equal potency and efficacy and identified its presence in the brain, placenta, and gut (9). We have also characterized a fourth melanocortin (MC4) receptor primarily present in the brain (10). Unlike the α-MSH (MC1) and ACTH (MC2) receptors, the MC3 and MC4 receptors do not appear to be expressed in either the adrenal gland or melanocytes. In this paper we report the chromosomal localization of the ACTH (MC2) and the melanocortin-3 (MC3) receptors.

The technique of fluorescence labeled *in situ* hybridization (FISH) according to a protocol modified from Pinkel *et al.* (11) Lichter *et al.* (12), and Lemieux *et al.* (13) was used for the chromosomal localization of the MC2 and MC3 receptors. DNA of EMBL3 phage clones containing the genomic sequences of the MC2 and MC3 receptors was isolated and biotinylated using a Bionick kit (BRL, Gaithersburg, MD). Metaphase chromosomes from a normal female were prepared from peripheral blood lymphocytes following overnight synchronization with 5-bromodeoxyuridine and thymidine release. Cells were harvested and slides were prepared using standard cytogenetic techniques. Probe labeling, preannealing, hybridization, and washing were performed as described using 330 ng biotinylated DNA per slide (11, 12). Signal detection was achieved by incubations of 30 min at 37°C with fluorescein goat anti-biotin and fluorescein-labeled anti-goat IgG (Vector, Burlingame, CA) in 4× SSC/0.1% Tween/1% BSA. Each incubation was followed by washes in 4× SSC/0.1% Tween at 37°C. Slides were counterstained with propidium iodide (PI), rinsed in phosphate-buffered saline, and coverslipped with PPD11 anti-fade solution (13). Photographs were taken on Kodak ASA 400 Gold film using a Zeiss Axioskop epifluorescence microscope equipped with a Zeiss filter set allowing simultaneous visualization of FITC and PI. The results of hybridization are shown in Fig. 1.

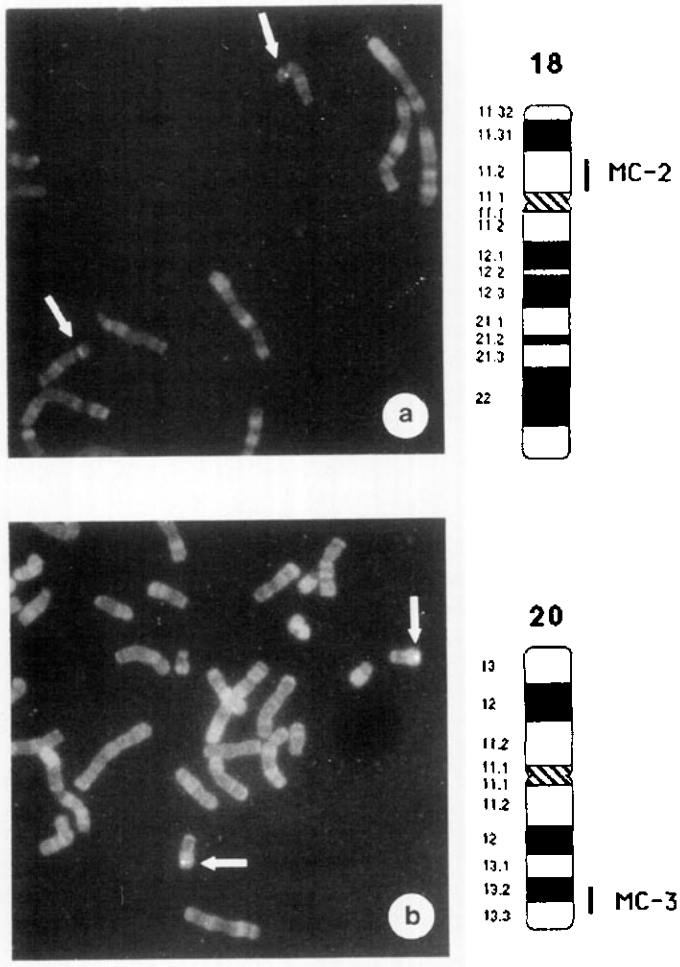

FIG. 1. Regional mapping of (**a**) the melanocortin-2 (ACTH) receptor gene to 18p11.2 and (**b**) the melanocortin-3 receptor gene to 20q13.2–q13.3 using the FISH technique. Hybridization signals were detected with FITC on propidium iodide-stained R-banded chromosomes.

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Using the FISH technique, we localized the ACTH and the melanocortin-3 receptors to chromosome loci 18p11.2 and 20q12.3–q13.2, respectively. We have reported previously the localization of the melanocortin-4 receptor to chromosome 18q21.3 (10). The scattered chromosomal distribution of the melanocortin receptors MC2, MC3, and MC4 appears to conform to a similar pattern of chromosomal localization seen in other human multisubtype seven transmembrane receptors (α_2, β_1, β_2-adrenergic receptors, m1-5 muscarinic cholinergic receptors, D1, D2, and D4 dopamine receptors) (14). Only the human α-1A, α-1B adrenergic receptors share a common arm on the same chromosome. Apparent exceptions are the chemotactic receptors (complement 5A receptor, *N*-formyl peptide receptor, and two "orphan" receptors that are structural homologues of the *N*-formyl peptide receptor), which have all been mapped to chromosome 19 (15), and the receptors for the glycoproteins luteinizing hormone and follicle-stimulating hormone, which have both been localized to 2p21 (16). According to the Genome Database, the only gene previously localized at 18p11.2, the site of the melanocortin-2 receptor, encodes a putative receptor-like protein tyrosine phosphatase (17). No genes have been previously localized to 20q13.2–q13.3, the site of the melanocortin-3 receptor. The gene encoding the melanocortin-4 receptor shares 18q21 with the genes for *bcl*-2 gene products (18) and plasminogen activator inhibitor, type II (19).

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