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Corticotropin-releasing hormone (*Crh*) maps to mouse Chromosome 3

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The mammalian stress response is mediated in large part via the hypothalamus–anterior pituitary–adrenal (HPA) axis. The key hypothalamic releasing factor in this axis is corticotropin-releasing hormone (CRH) (Vale et al. 1983). This 41-amino-acid peptide is released from the median eminence and transported via the hypophyseal portal blood system to the anterior pituitary, where it increases synthesis of pro-opiomelanocortin mRNA and secretion of adrenocorticotropin (ACTH; Vale et al. 1981). ACTH stimulates the production of glucocorticoids in the adrenal cortex. In addition to the role of CRH within the HPA stress axis, CRH is expressed in many other regions within the central nervous system where it is thought to influence behavioral, autonomic, and immunological responses to stress (Brown and Fisher 1985; Irwin et al. 1992; Koob and Bloom 1985).

CRH has been assigned to human Chromosome (Chr) 8 by Southern blot analysis of somatic cell hybrids and localized to band 8q13 by in situ hybridization of metaphase chromosomes (Arbiser et al. 1988). Synteny homology between mice and humans suggested that *Crh* would map to mouse Chr 3, 4, 8, or 15 (Nadeau et al. 1992).

We mapped *Crh* to mouse Chr 3 using a *M. spretus* interspecific backcross (C57BL/6J-tg9257 × SPRET/Ei) × C57BL/6J (Bain et al. 1993; Barrow et al. 1993). This cross was previously typed at four loci on distal Chr 3, *Gba, Hsd3b, Tshb,* and *Amy-1* (Bain et al. 1993). We extended the characterization of this cross by typing two markers on proximal Chr 3, carbonic anhydrase 2 (*Car-2*) and interleukin 2 (*Il-2*). Southern blot hybridization was used to map *Crh* and *Car-2,* an enzyme that catalyzes the reversible hydration reaction of carbon dioxide to bicarbonate, (Fig. 1; Venta et al. 1985). *Il-2,* a lymphokine that induces activated T-cells to complete the cell cycle, was mapped by PCR (Fig. 1; Dietrich et al. 1992; Fiorentino et al. 1989). Haplotype analysis of 53 animals and minimization of crossover frequency was used to deduce the gene order and genetic distance (cM): *Crh, Car-2*–11.3 ± 4.4–*Il-2*–22.6 ± 5.7–*Gba*. The map constructed from these data (Fig. 2) is consistent with the location presented for *Car-2, Il-2,* and *Gba* on the consensus map (Meisler and Seldin 1991), although these genes have not been previously localized relative to one another. Three carbonic anhydrase genes have been localized in close proximity on human Chr 8 and mouse Chr 3 (Beechey et al. 1990; Eicher et al. 1976; Nakai et al. 1987). Our observation that *Car-2* and *Crh* cosegregate extends the synteny conservation between mouse Chr 3 and human Chr 8.

It is difficult to predict the phenotypes that might result from *Crh* mutations because of the multiple roles of CRH within the HPA axis and central nervous system. Human clinical studies suggest that hypothalamic CRH deficiency can result in adrenocortical insufficiency and hypopituitarism (Fehm et al. 1976). Animal studies suggest that other consequences of CRH deficiency are possible. For example, impaired synthesis and secretion of CRH in the paraventricular nucleus of the hypothalamus of the Lewis (LEW/N) rat leads to an increased susceptibility to streptococcal cell wall-induced arthritis (Sternberg et al. 1989). The expression of CRH in multiple regions of the mouse brain and in some peripheral tissues beginning at embryonic day 13.5 suggests that CRH expression may also play an important role in development (C. Keegan, unpublished data). Therefore, a deficiency of CRH could result in a variety of phenotypes, and a total loss of CRH function could potentially be lethal. Increased CRH activity often results in hypercortisolism. In humans, hypercortisolism is associated with Cushing's syndrome and is characterized by truncal obesity, muscle wasting, and decreased fertility (Nelson 1989). A similar phenotype was observed in transgenic mice that overexpress CRH (Stenzel-Poore et al.

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1992). High levels of ectopic CRH expression in transkaryotic rats caused marked adrenal cortical hyperplasia and anterior pituitary corticotrope hyperplasia and hypertrophy (Asa et al. 1992; Hammer et al. 1992). Finally, hypersecretion of CRH is thought to play a role in clinical depression and anorexia nervosa, suggesting that animals with increased CRH levels would exhibit altered motor activity, feeding, and sexual behavior (Gold and Chrousos 1985; Krahn et al. 1986, 1990; Levine et al. 1983; Nakahara 1983; Nemeroff et al. 1984; Sirinathsinghji et al. 1983; Sutton et al. 1982).

Two mutations are located on proximal mouse Chr 3, cocoa (*coa*) and subtle gray (*sut*) (Meisler and Seldin 1991). The phenotypes of these two mutants do not correlate with those suggested for CRH deficiency from human and animals studies.

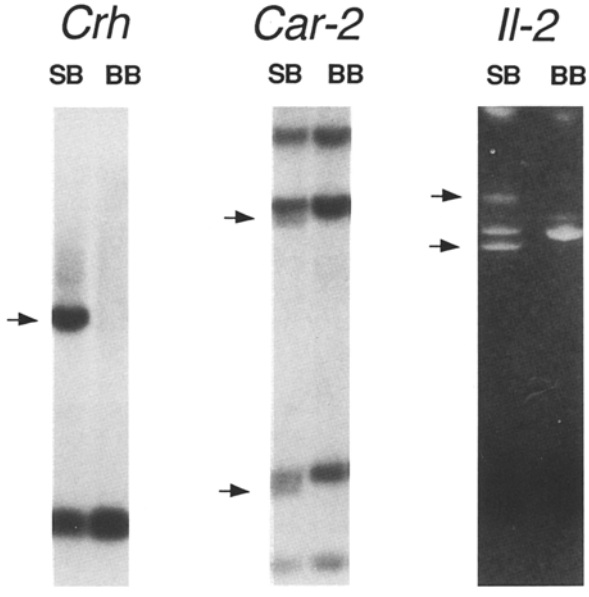

Fig. 1. Polymorphisms used to type backcross progeny on Chr 3. *Car-2* and *Crh* were typed with RFLPs detected by Southern blot analysis, and *Il-2* was typed with a simple sequence repeat polymorphism detected by PCR. *Gba* was previously localized by PCR (Bain et al. 1993). Southern blots were performed as previously described (Sambrook et al. 1989). All gels were transferred to Zeta-Probe nylon filters (Bio-Rad). Probes were labeled by the random hexanucleotide method (Feinberg and Vogelstein 1982). Hybridization was carried out at 65°C. The mouse *Crh* cDNA probe, pGem4ZPst578 (Seasholtz et al. 1991), revealed a polymorphism in genomic DNA digested with *Pst*I. The SPRET/Ei and C57BL/6J alleles produced hybridizing fragments of 2.6 and 0.5 kb, respectively. A mouse cDNA clone for *Car-2*, pBSMACII-Sph, provided by P. Venta, (Venta et al. 1985), detected an *Ssp*I polymorphism. The SPRET/Ei specific alleles revealed fragments of 4.2, 3.1, and 0.9 kb, and the C57BL/6J specific alleles revealed 4.2, 3.2, 1.0, and 0.7 kb fragments. *Il-2* was mapped by PCR using 500 ng of mouse genomic DNA as a template in a 25-μl reaction volume with 10 μmol of each primer and 2 μmol each of dATP, dCTP, dGTP, and dTTP. Primers for *Il-2* (*D3Mit21*) were extended with 1–2 U of Taq polymerase (Dietrich et al. 1992). The amplification products were fractionated through 8% polyacrylamide and visualized by ethidium bromide staining. The amplification products were 217 and 265 bp for SPRET/Ei DNA and 237 bp for C57BL/6J DNA. A subset of animals were typed at *Il-2* by PCR with the same primers radiolabeled with γ^{32} P-ATP and the amplification products visualized by autoradiography as previously described (Buckwalter et al. 1992). **Arrowheads** designate the *M. spretus*-specific alleles present in genomic DNA from (C57BL/6J-tg9257-SPRET/Ei × C57BL/6J) F_1 mice (SB) but absent in C57BL/6J (BB) mice.

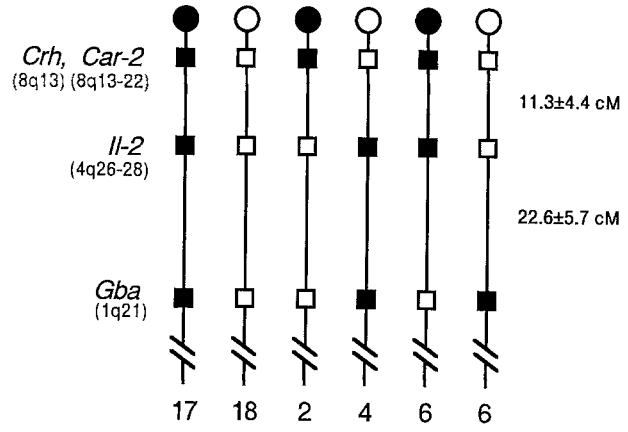

Fig. 2. The haplotype distribution of 53 animals was used to determine gene order. The number of animals observed with each haplotype is listed below the schematic of the chromosome. The C57BL/6J and SPRET/Ei alleles are depicted at each locus tested by **closed** or **open boxes,** respectively. All other gene orders resulted in multiple double-crossover events. The genetic distance (cM) and standard error were calculated as previously described (Buckwalter et al. 1991). The locations of the human genes are given in parentheses (Arbiser et al. 1988; Ginns et al. 1985; Nakai et al. 1987; Seigel et al. 1984).

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