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Localization of the peptidylglycine α-amidating monooxygenase gene (*Pam*) introduces a region of homology between human Chromosome 5q and mouse Chromosome 1

A.C. Lossie,[1] B.A. Eipper,[2] T.A. Hand,[2] S.A. Camper[1]

[1]Department of Human Genetics, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0618, USA
[2]Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

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The peptidylglycine α-amidating monooxygenase gene (PAM; EC 1.14.17.3) encodes a multifunctional protein that is composed of two enzymatic subunits, peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidyl-α-hydroxyglycine α-amidating lysase (PAL; Eipper et al. 1993; Ouafik et al. 1992). PHM and PAL act sequentially to catalyze the COOH-terminal α-amidation of many neuroendocrine peptides. The first step in generating biologically active peptides is cleavage from a larger inactive precursor protein, often generating a glycine residue at the COOH-terminus. PHM converts the glycine residue into a peptidyl-α-hydroxyglycine intermediate, which is subsequently converted to the α-amidated product at physiological pH by PAL. The PHM and PAL subunits of PAM are required for the activation of all peptides bearing an α-amide modification at their COOH-terminus, including vasopressin, gastrin, and oxytocin. In addition, PAM transcripts have been detected in many tissues, including the heart, anterior pituitary, hypothalamus, and submaxillary gland (Ouafik et al. 1992).

The human PAM gene was assigned to Chr 5 by analysis of somatic cell hybrids and localized on Chr 5q14-21 by in situ analysis (Ouafik et al. 1992, 1993). We mapped *Pam* to mouse Chr 1 in three individual backcrosses using RFLPs: (DF/B-*df/df* × CASA/Rk)F_1 × DF/B-*df/df* (Cross 1; Buckwalter et al. 1991), (C57BL/6J × *M. spretus*)F_1 × C57BL/6J (Cross 2; Rowe et al. 1994), and (C57BL/6J × *M. spretus*)F_1 × *M. spretus* (Cross 3; Rowe et al. 1994). We report the localization of *Pam* on mouse Chr 1 in relation to three previously reported simple sequence repeat markers: *D1Mit8, D1Bir18,* and *D1Mit30* (Dietrich et al. 1992; Rowe et al. 1994).

The *Pam* gene was mapped by RFLPs with standard Southern blot procedures. Genomic DNA from crosses 1, 2, and 3 was digested with *Ssp*I, *Hin*dIII, and *Pst*I respectively. Filters were hybridized at 65°C with a radiolabeled 655-bp fragment of the rat PAM cDNA, rPAM-1. This portion of the cDNA is contained entirely within exon 27 and encompasses nucleotides 3231 through 3886. Blots were washed to a final stringency of 0.1 × SSC; 0.1% SDS at 57°C. In Cross 1, *D1Mit8* and *D1Mit30* were mapped by PCR amplification in a 96-well MJ machine under previously established conditions (Dietrich et al. 1992), except that amplified products were size fractionated on 2% agarose, 1% NuSieve (FMC Bioproducts) gels and visualized by ethidium bromide staining.

By haplotype analysis and minimization of crossover frequency, we determined the unambiguous gene order: centromere–*D1Mit8*–*Pam*–*D1Bir18*–*D1Mit30*–telomere. The distances in cM and estimated standard deviation between loci were: *D1Mit8*–7.3 ± 3.5–*Pam*–9.1 ± 3.9–*D1Mit30* (Cross 1), *D1Mit8*–4.6 ± 2.6–*Pam*–3.2 ± 1.8–*D1Bir18* (Cross 2), and *D1Mit8*–7.8 ± 3.3–*Pam, D1Bir18* (Cross 3).

We created a composite map of mouse Chr 1 based on these data (Fig. 1 C). Genes surrounding *Pam* on mouse Chr 1 have been assigned to human Chrs 6, 18, 2 and 1 (Fig. 1 A,B). The localization of *Pam* to this region of mouse Chr 1 is surprising, since all other genes on human Chr 5q that have previously been mapped in the mouse have been assigned to Chrs 11, 13, and 18, forming several large segments of synteny and linkage conservation. However, in all three crosses the rPAM-1 cDNA probe detected a single band on Southern blots, unambiguously demonstrating the assignment of the *Pam* gene, not a pseudogene, to Chr 1 (Fig. 1 D). Additionally, in crosses 1 and 2, a *Hin*dIII polymorphism detected with an rPAM-1 cDNA probe encompassing exons 2 through 16 cosegregated with the polymorphic fragments detected with the 3′ UTR probe. This further confirms the localization of the *Pam* gene on mouse Chr 1. The unambiguous assignment of *Pam* to this region of Chr 1 displaying disrupted synteny homology suggests the introduction of a novel homology group between human Chr 5q and mouse Chr 1.

Correspondence to: S.A. Camper

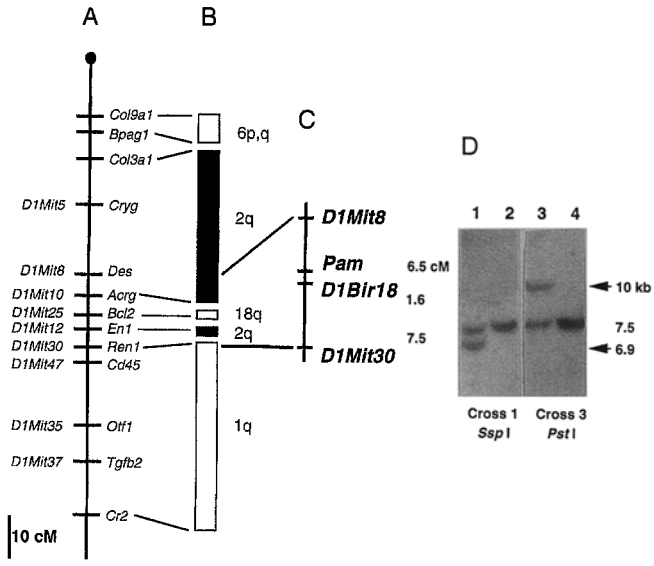

Fig. 1. Localization of *Pam* on mouse Chr 1. The composite map of mouse Chr 1 shows the co-localization of simple sequence repeat markers with molecular markers (panel A; Copeland et al. 1993; Seldin et al. 1993). The human chromosomal assignment of each of the genes depicted in panel A defines the regions of mouse-human synteny homology (panel B; Seldin et al. 1993). Our analysis of three backcrosses places *Pam* relative to *D1Mit8, D1Bir18,* and *D1Mit30* (panel C). The *Ssp*I RFLP revealed a CASA/Rk-specific allele of 6.9 kb and DF/B-*df/df*-specific allele of 7.5 kb in Cross 1 (panel D; lanes 1,2). Digestion with *Pst*I produced a 10-kb C57BL/6 allele and 7.5-kb *M. spretus* allele in Cross 3 (lanes 3, 4). The **arrows** indicate the informative alleles present in heterozygous progeny (lanes 1, 3), but absent in homozygotes (lanes 2, 4).

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Data from Cross 2 and Cross 3 are deposited in MGD accession # E1010 and # E1035.

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