Volume 16 Number 15 1988 Nucleic Acids Research

Proopiomelanocortin cDNA sequences from the bovine ovary indicate alternative non-functional transcriptional initiation and a new polymorphism

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Submitted May 24, 1988

Using Northern hybridization analysis it can be shown that the single copy proopiomelanocortin (POMC) gene is expressed at low levels in rat (1,2) and bovine (Ivell, unpublished) ovarian tissue as a 800-900 bp transcript. This is some 250 bp shorter than in the pituitary, the main site of production of ß-endorphin and ACTH. ß-endorphin has also been located in the ovary and it would be logical to assume local gene expression via an albeit shorter mRNA.

We have isolated and sequenced a total of three independent clones out of a large cDNA library (complexity >$2x10^6$) made in lambda gt11 using mRNA from an early bovine corpus luteum (3). EcoRI inserts were subcloned into Bluescribe (Stratagene, California) plasmids for double-stranded DNA sequencing (4). All clones are 5'truncated just downstream of the gamma-MSH encoding region (Fig.1). In two clones (pBP-4M and pBP-5A) the sequences were similar to that published for the pituitary (5); in clone pBP-7M a small sequence discrepancy is evident with one silent change and a deletion of precisely four codons encoding small neutral amino acids (Fig.1).

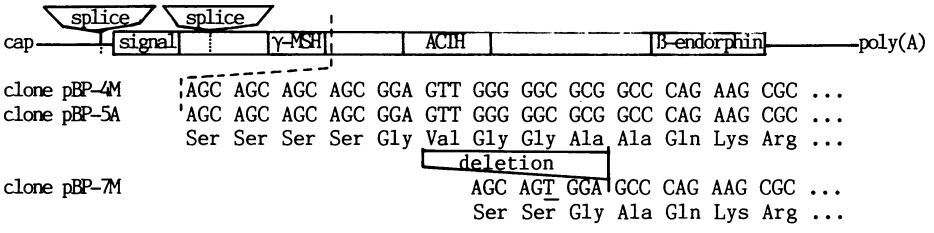

Fig.1 5' Sequences of POMC-encoding cDNA clones from the bovine ovary.

The library had been prepared using size-selected cDNA, and of ca. 4000 oxytocin-encoding clones analysed 96% were full-length (3). Recently, using nuclease mapping, it could be shown for human (6) and rat (7) testicular mRNA that 90% of in vivo transcripts were also 5'truncated to a similar extent as in Fig.1. Together with the Northern hybridization evidence, these data suggest that the cDNA structures reflect neither a cloning nor an mRNA degradation artefact but a gonadal specific alternative transcription initiation close to the gamma-MSH encoding region of exon 3. This initiation cannot lead to physiological peptide production since the signal sequence responsible for channeling the precursor into the endoplasmic reticulum and the secretory pathway is not encoded: these transcripts are therefore functionally defective. A similar conclusion was recently reached also for vasopressin in the bovine ovary (3).

The sequence discrepancy in pBP-7M is consistent with an allelic polymorphic variation in the POMC gene. The silent change and the loss of four small neutral amino acids in an inter-peptide region of the precursor is unlikely to affect the physiology of the POMC polyprotein or its post-translational processing.

Acknowledgements: We should especially like to thank Ms Beate Brackmann for excellent technical assistance and Prof. S. Numa for generously providing the pituitary clone pSNAC-20 for the library screening.

References: 1) Chen, CLC et al (1986) Endocrinology 118:2382-2389. 2) Jin, DF et al (1988) Endocrinology 122:1466-1471. 3) Morley, S. and Ivell, R. (1987) Mol.Cell. Endocrin. 53:255-258. 4) Chen, E.J. and Seeburg, P.H. (1985) DNA 4:165-170. 5) Nakanishi, S. et al (1979) Nature 278:423-427. 6) Lacaze-Masmonteil, T. et al (1987) Proc.Natl.Acad.Sci.USA 84:7261-7265. 7) Jeannotte, L. et al (1987) Mol.Endo.1: 749-757.