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研究报告

绵羊钙调蛋白调节基因Calponin h1 的分子克隆

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摘要 在一项研究中我们发现雌激素体在胚胎发育后期对绵羊子宫平滑肌Calponin (CaP) 基因的活动有明显上调作用，而CaP一直被作为观察其他基因表达水平变化的基准参照基因（Reference Gene）。迄今为止，绵羊CaP尚未完整克隆，为进一步了解其结构和功能，根据人、小鼠和家猪的同源保守区序列设计锚定寡核苷酸引物，通过5′-RACE及3′-RACE方法克隆了绵羊子宫平滑肌组织全长CaP h1 cDNA (GenBank登录号： AY327118)， 在cDNA序列的基础上， 又通过PCR-SSP方法获得了CaP h1基因除内含子1、2之外的其余4个内含子全部序列 （GenBank登录号分别为：AY771807，AY771808， AY771809， AY771810） 。DNA序列测定和分析表明，绵羊子宫平滑肌CaP h1 cDNA全长1499bp，编码297个氨基酸，5′-UTR及3′-UTR分别为79bp和529bp。CaP h1基因组DNA的克隆和序列分析表明，绵羊CaP全长约8kb，由 7个外显子和6个内含子组成。 同源序列比较发现，该基因外显子在不同物种间相对保守；与人类、野猪、小鼠、大鼠和鸡Calponin mRNA同源性分别为88%、92%、81%、79%和81%，但不同物种间内含子存在较大差异(>50%)。本研究填补了绵羊CaP基因分子克隆的空白，为进一步研究该基因的功能及子宫平滑肌收缩的调节机理奠定了基础。

关键词 绵羊 Calponin 分子克隆

分类号 Q958

Molecular cloning of smooth muscle Calponin h1 in sheep

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Abstract

It was found that the level of Calponin h1 (CaP h1) mRNA was significantly up-regulated by Estrogen in the myometrium of sheep towards the end pregnancy. Although the CaP h1 has been widely used as a reference gene to observe the changes of expression level of other genes, the full-length gene in sheep has not been obtained. With the oligo nucleotide primers according to human, mouse and pig CaP h1 mRNA, the full-length cDNA of CaP h1 was cloned by 5′- and 3′-RACE (Genbank accession number = AY327118). The cDNA was 1499bp in length and contained a complete open reading frame of 891 bp, encoding a protein of 297 amino acid residues. 5′-and 3′-UTR was 79 bp and 529bp, respectively. With PCR-SSP approaches,the genomic DNA of sheep CaP h1 was obtained .It showed that the gene has 7 exons and 6 introns, spanning over 8kb（Genbank accession number of introns：AY771807，AY771808， AY771809， AY771810.） Homologous comparison indicated that the cDNA sequences are highly conserved across the species. The highest homology was found in wild pig (92%), followed by human (88%), rat (81%), mouse (81%) and chicken (79%). The intron sequence and length showed a large variation among species (>50%).

Key words sheep Calponin molecular cloning

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