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# The mammalian gene *pecanex 1* is differentially expressed during spermatogenesis

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**Abstract**

Using mRNA differential display and cDNA library screening approaches we have identified differential gene expression of *pecanex 1* – a mammalian homologue of *pecanex* gene from *Drosophila* – in the testes of the rat. Northern blot analyses showed that the transcript is only present in the germ line and not in the somatic cells of the testis, reaching its peak at the pachytene stage of the meiotic prophase. Moreover, nonradioactive in situ hybridization did not detect the expression of the gene in any cell type of the testis other than pachytene spermatocytes. Northern blot assays did not allow the detection of the transcript in nine other tissues. Remarkably, although *pecanex* exerts a neurogenic role in *Drosophila*, the transcript was not detectable by Northern blotting in the nervous tissue of adult rats, nor in the brain of neonate and embryonal stages. The protein product of the *pecanex 1* gene was detected by immunoblotting in pachytene spermatocytes and round spermatids as well, but not in liver nor brain. From genomic analysis we conclude that, although only one *pecanex* gene exists in *Drosophila*, mammalian *pecanex 1* belongs to a gene family with three related genes in different chromosomes. We speculate that *pecanex 1* could play an important role in the testis, related to spermatogenesis.


*Keywords:* Spermatogenesis; Meiosis; Gene expression; Differential display; *Pecanex*

## 1. Introduction

Mammalian spermatogenesis can be divided into three stages: mitotic proliferation of spermatogonia, meiosis and spermiogenesis. During meiosis, a program of gene expression that provides the spermatocyte I with the molecular apparatus for chromosome pairing, recombination and segregation is executed. The genetic program that proceeds along spermiogenesis is responsible for the biochemical and structural changes that haploid spermatids undergo in order to become a mature sperm [1–3]. The whole process of spermatogenesis is under the complex regulation of different molecules, including hormones and growth factors [4].

Some of the genes that encode spermatogenic cell-specific proteins are not homologous to any genes expressed in somatic cells. Besides, a remarkable feature of mammalian spermatogenesis is the high number of genes that encode spermatogenesis cell-specific isoforms [3]. Spermatogenesis-specific isoforms can be generated by the usage of different transcription start sites, differential polyadenylation sites or alternative splicing [5]. Post-transcriptional

*Abbreviations:* DD, differential display; ORF, open reading frame; EST, expressed sequence tag; pcx, *Drosophila's pecanex*; RT, reverse transcriptase; PCR, polymerase chain reaction; 7-TM, seven transmembrane receptors (refers to receptors with seven transmembrane domains); C-terminal, carboxy-terminal; N-terminal, amino-terminal; o.n., overnight; GST, gluthation-*S*-transferase

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regulation has a relevant importance in spermatogenic cells and has been proposed as a mechanism of selection for novel testis-specific functions [3].

The aim of our work was the identification and characterization of differentially expressed genes during mammalian spermatogenesis. For that purpose, mRNA differential display (DD) [6,7] was applied to compare RNA populations obtained from highly purified pachytene spermatocytes (meiotic prophase cells) and round spermatids (post-meiotic cells) of the rat. Using thirteen different combinations of primers [8; Geisinger, A., unpublished data], we have isolated a majority of differential cDNA fragments (74%) that appeared as specific for spermatids; the others (26%) were spermatocyte-specific (i.e. differential for meiotic prophase) [8]. Among the latest, some have been characterized and turned out to represent 3′ ends of genes that code for products that are exclusively expressed during spermatogenesis [9]. Others corresponded to cDNAs that represent testis-specific isoforms of previously known genes (Geisinger, A., unpublished data).

The *pecanex* (*pcx*) locus has been described several years ago in *Drosophila* as containing a maternal-effect neurogenic gene. The product of this gene would be involved in the normal development of the nervous system of the fly, since it has been shown that in the absence of maternal expression the embryo develops severe hyperneuralization similar to that characteristic of *Notch* mutants [10,11]. Expression analyses of the *pcx* gene showed the existence of a main 9 kb transcript and two minor transcripts of 3.7 and 2.3 kb. The three transcripts were detectable in males as well as in females at all developmental stages examined [10]; however, the maximum levels of the 9 kb species accumulated between 5 and 10 h of development, corresponding to the period of neuroblast differentiation and division [12]. More recently, a partial homologue of *pcx* from rat brain has been reported [13]. Besides, sequences of some other cDNAs and ESTs with partial homology to *Drosophila*'s *pcx* isolated from different mammalian tissues as well as developmental stages have been submitted to the GenBank (see below).

In the present study we report that *pecanex 1*, a mammalian gene that shows high similarity to *Drosophila*'s *pcx*, is differentially expressed during spermatogenesis of the rat. We also provide evidence that *pecanex 1* is a member of a family composed of three different genes in mammals. We speculate that the product of *pecanex 1* may play a regulatory role during spermatogenesis.

## 2. Materials and methods

### 2.1. Animals and tissues

Nine tissues (testis, epididymis, brain, heart, lung, liver, intestine, spleen and kidney) were harvested from 40 day old male Wistar rats (*Rattus norvegicus*; Charles River Wiga, Sulzfeld, Germany). Ovaries were collected from females of the same age. Neonates and embryos of the same strain were obtained from the Faculty of Medicine (Montevideo) and used for tissue collection. All tissues were washed in 0.8% NaCl, immediately frozen in liquid nitrogen and kept at −80 °C for further processing, except for testes, which were processed for elutriation immediately after extraction.

### 2.2. Isolation of stage-enriched cell populations of rat testis

Cell suspensions were prepared from the testes of 40 day old rats and submitted to centrifugal elutriation in order to obtain highly enriched populations of spermatogenic cell types, essentially as already described [14]. Fractions with the highest concentration of pachytene spermatocytes and round spermatids were collected separately. The minimum purity percentages obtained were 86% and 81% for the pachytene spermatocytes and round spermatids fractions respectively.

### 2.3. RNA isolation and differential display

RNA was extracted from frozen tissues or elutriated cell populations with the RNA Isolation Kit from Stratagene (Heidelberg, Germany). Differential display was carried out with RNA from elutriated cell populations according to Liang et al. [7], using the RNA Map kit, oligo-dT primers and arbitrary decamers AP1-9 from GenHunter (Nashville, TN, USA). Differentially expressed fragments were eluted from the acrylamide gel and reamplified as already described [7,8].

### 2.4. cDNA library screening

A cDNA fragment derived from a differentially expressed RNA was reamplified and cloned into a pGEM-T vector II (Promega, Madison, WI, USA), labeled by random priming with the DIG High Prime DNA Labeling and Detection Starter Kit II (Boehringer Mannheim, GmbH, Germany), and used as a probe to screen a λ-ZAPII cDNA library made from a highly enriched population of pachytene spermatocytes [15]. Library screening and chemiluminescent detection with CSPD were performed following Boehringer's indications, as previously described [9].

### 2.5. Northern blot analysis

cDNA probes were labeled by random priming (Megaprime DNA Labeling System, Amersham, Buckingamshire, UK) with α [$^{32}$P]dATP (Amersham). Northern blot assays were carried out essentially according to Sambrook et al. [16]. Gels with 40 μg of total RNA per lane were transferred to positively charged nylon membranes, immobilized by baking 15 min at 120 °C, prehybridized for 1 h and