CHARACTERIZATION OF A NOVEL EVOLUTIONARY CONSERVED AND DEVELOPMENTALLY REGULATED GENE DURING MOUSE GAMETOGENESIS.

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To isolate clones corresponding to genes involved in the development of gametogenesis, including those controlling meiosis, a 16 day post coitum (dpc) fetal ovary cDNA library was constructed (López-Alañón and del Mazo, 1995). The clone designed as *Geg-154* was isolated after a double differential screening using single stranded cDNAs from adult testis versus single stranded cDNAs from somatic tissues.

Northern blot analysis on testicular RNA reveals two transcripts. One of 4.2 Kb, constitutively expressed. This transcript is also detected on somatic tissues and fetal and adult ovary RNA but at very low level. A second one of 3.0 Kb, transcribed in testis from day 18 of postnatal life, is detected as testis specific.
Coincident with the appearance of the 3.0 Kb transcript, the level of accumulation of the 4.2 Kb transcript is also significatively enhanced, suggesting a developmental regulation of both transcripts (Fig. 1).

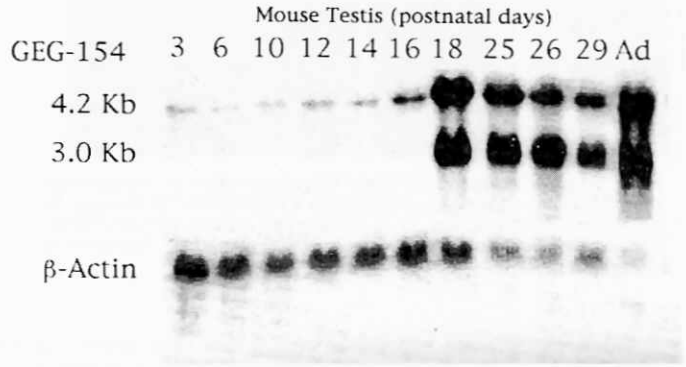

Fig1: Northern blot analysis of Geg-154 on mouse testis RNAs

Complete sequencing of 2821 bp corresponding to *Geg-154* cDNA clone was performed with a transposon based strategy (Strathmann et al., 1991). A 3' untranslated region (UTR) of 1500 bp with a polyadenylation signal and a polyA tail was observed. The most probable open reading frame (ORF) of 1300 bp codifies for a 45 KDa polypeptide (Fig. 2).

Geg-154 sequence was compared to databanks and no homology with any known sequence at the nucleotide or aminoacidic level was found. Recently, many EST sequences from different species such as human, budding yeast, bovine, and mouse have appeared in the databases presenting a high homology to *Geg-154.*

MIIGIPVYVGRKIHNRYEGKDVSKHKRNLAIAGGVTLSVIVSPVVAAVTVGI
GVPIMLAYVYGVVPISLCRSGGCGVSAGNGKGVRIEFDDENDINVGGTNA
AIDTTSVAEARHNPSIGEGSVGGLTGSLSASGSHMDRIGTIRDNLSETASTM
ALAGASITGSLSGSAMVNCFNRLEVQADVQKERCSLSGESGTVSLGTVSDN
ASTKAMAGSILNSYIPLDREGNSMEVQVDIESKPFKFRHNSGSSSVDDSGA
TRGHTGGASSGLPEGKSSATKWSKEATGGKKSKSGKLRKKGNMKINETRE
DMDAQLLEQQSTNSSEFEAPSLSDSMPSVADSHSSHFSEFSCSDLESMRTSC
SHGSSDCHARFTAVNTLPEVENDRLENSPHQCSSSAFQSCFLFRCPTAQPC
CRRAWHQQKWGEAYGGFVFW

Fig 2: Geg-154 cDNA structure and deduced aminoacid sequence

With the aim to study its role during gametogenesis development, *Geg-154* gene product was expressed as a fusion protein with maltose binding protein (MBP) of *E. coli* (di Guan et al., 1988; Maina et al., 1988). After purification, the protein was used to raise antibodies in rabbits.
Western blot analysis on mouse testis protein at different stages of development reveals two bands of 75 KDa and 55 KDa present in all stages. An additional band of 50 KDa. is detected in adult testis where the 3.0 Kb. transcript was detected.
By immunocytochemistry, the protein was located to the centrosome of all cells analyzed including cultured cells, mouse embryo sections, mouse adult ovary and *D. melanogaster* ovary cells (Fig. 3). In seminiferous tubule cells the pattern of immunofluorescence was characterized by a prominent signal on the sex vesicle mainly displayed on pachytene spermatocytes (Fig. 4). Also centrosomes of somatic cells were recognized by the antibody in addition to its presence in residual bodies. The sex vesicle or XY body corresponds to the XY bivalent which presents differential morphology and function (gene inactivation) (Solari, 1974) with respect to the autosome bivalents. In postmeiotic cells, such as round spermatids, a nuclear signal which corresponds to the centromeric heterochromatin was also detected.

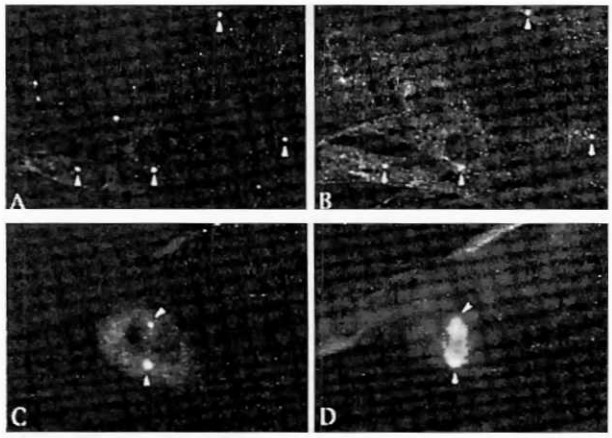

Fig. 3: Immunolocalization of Geg-154 gene product on centrosomes of 15P1 (a Sertoli-like cell culture line). A: anti-GEG-154 staining. B: anti-beta tubulin staining. C: A dividing cell stained with anti-GEG-154. D: anti-beta tubulin staining of the dividing cell. Arrowheads show centrosomes.

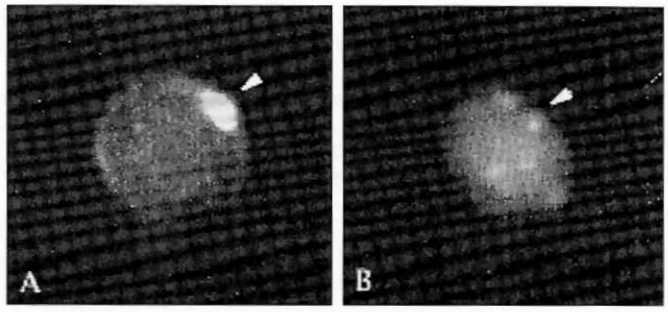

Fig. 4: Immunolocalization of Geg-154 gene product on pachytene spermatocytes.A: anti-GEG-154 staining. B: Nuclear Hoechst staining. Arrowheads show the sex vesicle.

Recently different expressed sequences tags (ESTs) have been reported with high homology with Geg-154. These short cDNA sequences corresponding to genes of a heretofore unknown function have been isolated from various species and tissues (Adams et al., 1995). On the other hand, the presence of a centrosomal signal in all cells of different species analysed suggested an evolutionary conservation of *Geg-154*. This fact was confirmed by southern blots of genomic DNA from different species. Genomic DNA from mouse, rabbit, human, rat, the fission yeast and fly were digested with different enzymes, electrophoresed and transferred to nitrocellulose membranes. Hybridization with a *Geg-154* specific probe was positive with all the DNAs tested.

The isolation of complete 4.2 Kb transcript and the genomic sequence are in progress. Other experimental approaches including generation of transgenic animals with antisense constructions are also being prepared to study the role of this novel gene during cell cycle and spermatogenesis differentiation.

References

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