Vol. 202, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1994 Pages 88-93

DIPLOID EXPRESSION AND TRANSLATIONAL REGULATION OF RAT ACROSIN GENE

Karim Nayernia, Kerstin Reim, Heike Oberwinkler and Wolfgang Engel[1]

Institute of Human Genetics, University of Göttingen, Gosslerstrasse 12D, 37073 Göttingen, Germany

Received May 16, 1994

Summary: Acrosin, a sperm acrosomal serine protease has been implicated in the recognition, binding and penetration of the zona pellucida of the ovum. Biosynthesis of acrosin was found to start in early round spermatids which are haploid germ cells. Here, we report that acrosin gene transcription occurs as early as at day 19 of rat spermatogenesis which contains diploid but not haploid spermatogenic cells. Translational control of the acrosin gene may be due to cytoplasmic protein factors which through RNA-bandshift experiments were found to bind to the 5'UTR of the acrosin mRNA. In order to differentiate between diploid and haploid spermatogenic cells at the molecular level, transcription of the protamine 2 gene during rat testicular development was evaluated. Protamine 2 transcripts could be demonstrated for the first time in 25-day-old testes which contain diploid as well as haploid spermatogenic cells.

Spermatogenesis is a complex developmental process which involves amplification of germinal stem cells, their differentiation into spermatocytes, meiotic division and finally transformation into mature spermatozoa. Therefore, spermatogenesis provides an interesting system for examining the regulation of gene expression during development and differentiation. An important gene specifically expressed during mammalian spermatogenesis is that for the serine protease acrosin (EC 3.4.21.10). Acrosin is stored in the sperm acrosome as an enzymatically inactive zymogen, proacrosin and it is released in the vicinity of the oocyte as a consequence of the acrosome reaction. In different mammalian species, biosynthesis of the zymogen proacrosin was found to start in early round spermatids (1) which are haploid spermatogenic cells. While during mouse spermatogenesis proacrosin gene transcription is first observed in pachytene spermatocytes (2,3), which are diploid spermatogenic cells, in rat, bull and boar

[1]To whom correspondence should be addressed. Fax: 49-551-399303.

0006-291X/94 $5.00

proacrosin gene transcription is reported to first occur in haploid round spermatids (4,5). We report here that at least in rat spermatogenesis the proacrosin gene is also diploid expressed. Thus, in mouse and rat the proacrosin gene is under translational control.

We have recently demonstrated a delay between transcription and translation of a proacrosin -CAT transgene containing only the 5'flanking region of rat proacrosin gene (6). During spermatogenesis CAT mRNA first appears in pachytene spermatocytes but with increasing abundance in postmeiotic round spermatids. While transcription of the CAT gene occurs in diploid spermatogenic cells, translation only occurs in haploid spermatids. This suggests translational control of CAT mRNA in transgenic mice, that only contains the 5'untranslated region of proacrosin mRNA. In order to study the mechanism involved in the translational regulation of the proacrosin gene, RNA-protein mobility shift assays were performed with the 5'UTR of the rat proacrosin mRNA and testicular cytoplasmic protein. A RNA-protein complex was found with cytoplasmic proteins from testis, but not with proteins from other organs.

MATERIALS AND METHODS

RNA preparation and RNA-PCR: Testes of 10 to 60 days old rats and kidney, liver, spleen and heart of adult rats were used for RNA isolation by RNaid kit from Dianova (Hamburg, Germany). RNA was treated with DNase I and tested on a 2% agarose gel. The components of PCR were tested for contaminants without added RNA template. RNA-PCR was carried out by the rTth kit from Perkin Elmer Cetus (Vaterstetten, Germany). 50-100 ng of total RNA was reverse transcribed (RT-PCR) into cDNA at 72°C for 10 min. The amplification profile involved 2 min at 95°C for 1 cycle, 1 min at 95°C and 1 min at 60°C each for 35 cycles and 7 min at 60°C for 1 cycle. For all transcripts studied, the PCR products were found to be of the expected size. The identity of the PCR products was confirmed by hybridization to rat proacrosin (7) and protamine 2 (8) cDNA probes.

RNA-protein mobility shift assay: S100 cytoplasmic extracts were prepared from testis, liver, kidney and spleen by the procedure of Dignam et al. (9). The ^{32}P-labeled RNA containing 590 nt upstream of AUG of rat proacrosin mRNA (7) were transcribed from pSPT 19 plasmid with SP6 RNA polymerase using the protocol of the supplier (Boehringer, Mannheim, FRG). In vitro transcribed RNA was heated at 95°C for 15 min and cooled on ice. This produced uniform secondary structures in the RNA and yielded one major band in the nondenaturing polyacrylamide gel. Radiolabeled RNA was incubated with 80 μg of S100 cytoplasmic extracts from testis, liver, kidney or spleen, respectively, in 10 mM Hepes/100 mM KCl/10 mM EDTA in a volume of 25 μl for 20 min at 30°C. After addition of heparin (50 mg/ml) the samples were incubated again 10 min at 30°C. RNA-protein complexes were resolved in 6% nondenaturing LMP-polyacrylamide geles run at 50 mA for about 4 hrs at 4°C. After electrophoresis the gels were autoradiographed.

RESULTS AND DISCUSSION

Biosynthesis of proacrosin has been studied in spermatogenesis of various mammals and was found to start in early round spermatids (1) which are haploid germ cells. cDNAs as well as genomic clones have been characterized from mouse, rat, boar, bull and human (3-5,7,10) and were used for in situ hybridization experiments with testes sections and for Northern blot experiments with RNA of isolated germ cell fractions. In all species

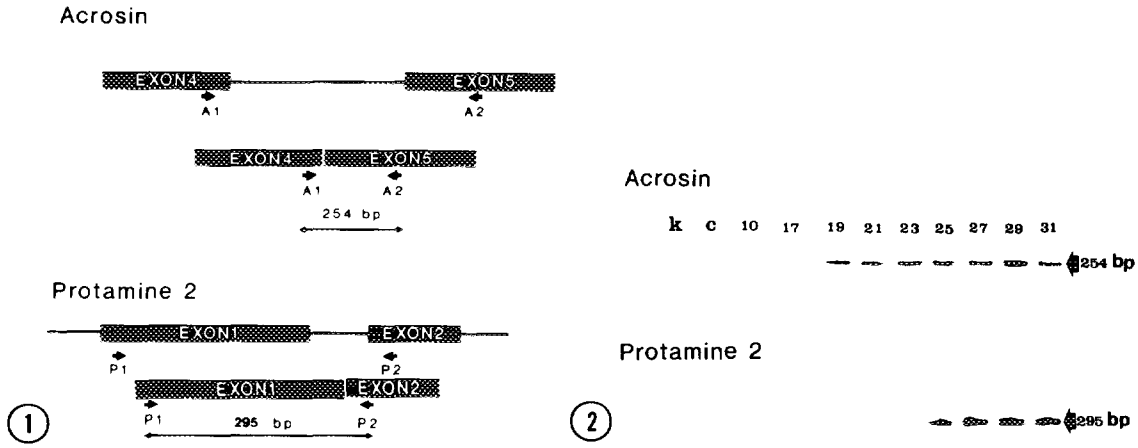

Fig. 1.
Schematic representation of oligonucleotide primers used for reverse transcription and amplification of RNA (RT-PCR) for rat acrosin and protamine 2 genes. Solid bars represent the coding sequences, thin lines represent introns or flanking regions. Numbers indicate the expected size of the PCR-amplified fragments derived from cDNA templates. The acrosin primers were as follows: A1 = 5'-CGCA GGG TAT CCT GAA GGC AAG-3'; A2 = -GTTG CTG GGT TGT AGG TGG GTG-3'. The protamine primers were: P1 = 5'-GGT TCG CTA CCG AAT GAG GAG-3'; P2 = 5'-GCA TTT CCT GCA CCT GCA GCTC-3'.

Fig. 2.
RT-PCR analysis of acrosin and protamine 2 mRNAs in testes of rats at various ages. PCR products were electrophoresed and transfered onto nitrocellulose filters. The blots were probed by a rat acrosin cDNA and a human protamine 2 cDNA-fragment, respectively. k = RT-PCR with RNA from kidney; c = RNA without reverse transcription as control.

except in the mouse, hybridization results supported a transcription of the proacrosin gene in haploid male germ cells (4,5). In mouse testis and germ cell fractions the first expression of the proacrosin gene was found in pachytene spermatocytes (2,3) which are diploid germ cells. Using the reverse trancribed PCR-method (RT-PCR) with testicular RNA from rats 10 to 60 days old, it is shown here that during rat spermatogenesis the proacrosin gene is also diploid expressed. As a control RT-PCR was also performed for the rat protamin 2 gene which is known to be transcribed only in haploid germ cells (11) (Fig. 1).

Figure 2 shows an autoradiogram of the resulting PCR products. The proacrosin mRNA was first detected at postnatal day 19, the protamine 2 mRNA at postnatal day 25. At these developmental stages late pachytene spermatocytes and early round spermatids, respectively, first appear in the rat testis (12). These results clearly demonstrate that the rat proacrosin gene is diploid expressed while the rat protamine 2 gene is haploid expressed. Proacrosin and protamine 2 transcripts are both translated several days later after gene expression. The proacrosin mRNA is stored for 5 days and translated in early round spermatids while the protamine mRNA is translated in elongating spermatids one week later (11). These results indicate that both, the proacrosin and the protamine genes are under translational control.

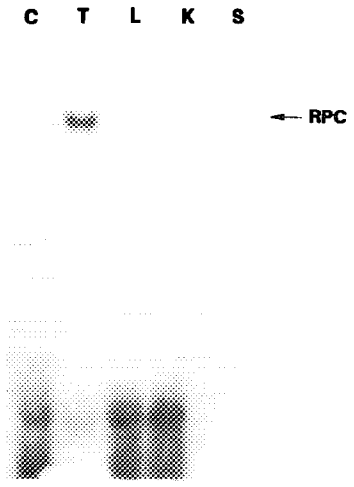

Fig. 3.
Formation of a specific RNA-protein complex between the 5'UTR of rat proacrosin mRNA and mouse testicular cytoplasmic extract. S100 cytoplasmic extracts from testis (T), liver (L), kidney (K) and spleen (S) were incubated with the ^{32}P-labeled in vitro transcribed RNA containing 5'UTR (590 nt upstream of AUG) of the rat proacrosin mRNA. A specific RNA-protein complex (RPC) is detected only with the testicular extract. C = In vitro transcribed RNA without tissue extract as control.

Many eukaryotic mRNAs contain regulatory elements that control their posttranscriptional utilization. These regulatory elements often reside within the 5' and 3' untranslated regions (UTRs) of mRNAs and interact with specific cytoplasmic proteins that modulate stability or translational competence of mRNAs. It has been suggested that 60-75 percent of testis mRNA may be subjected to translational control (13,14). Such control has been clearly demonstrated for the mRNAs of protamines (15), phosphoglycerate kinase 2 (14), mitochondrial capsule selenoprotein (16) and transition proteins (16,17).

In the case of protamines Kwon and Hecht (15, 18) reported several binding motifs for cytoplasmic proteins in the 3'UTR of the mRNA which regulate translation. Three of these proteins, with molecular weights of 18, 48 and 52 kDa have been further characterized. Three highly conserved cis-acting elements, Y,H and Z have been analyzed in the 3'UTR of protamine 2. In round spermatids the phosphorylated 18 kDa protein binds to a putative stem-loop structure formed by the Y and H elements and represses the translation in round spermatids. At a later stage of spermatid differentiation, as a result of dephosphorylation, the 18 kDa protein no longer binds to the mRNA and thus enables translation. In the proacrosin mRNA the respective binding motifs and the proteins have not yet identified. However our results in mice which are transgenic for a 2.3 kb 5'flanking region of rat proacrosin gene, the CAT reporter gene and the SV40 polyadenylation signal demonstrated that proacrosin gene translational control is maintained by sequences residing in the 5'UTR (6). Although the proacrosin-CAT fusion gene lacks the 3'UTR of the endogenous proacrosin gene, this transgene is

translationally regulated in an identical manner as the endogenous mouse proacrosin gene. Thus, the 5'UTR of proacrosin mRNA should contain a sequence for binding a germ cell specific cytoplasmic protein which hinders translation in spermatocytes or activates translation in spermatids. This assumption is supported by the results of our experiments that in vitro transcribed RNA of the 590 nt 5'UTR is able to bind cytoplasmic proteins of the testis (Fig. 3). The proteins are restricted to germ cells. To identify a consensus element in the 5'UTR of acrosin mRNA of different mammals, we compared these regions and have found the motif CAGAGC to be highly conserved. A similar motif (CAGUGC) was found in the 5'UTRs of ferritin and transferrin receptor mRNA (19). It is suggested that this motif plays a role in the post-transcriptional regulation of these genes. Computer modeling of the 5'UTR of acrosin mRNA has indicated that this element may be involved in determination of RNA secondary structure and recognition by the cytoplasmic proteins. Further detailed experiments are in examination to clarify the function of this motif in the post-transcriptional regulation of proacrosin mRNA.

ACKNOWLEDGMENTS

We thank Derek Murphy for reading the manuscript and Mrs. Angelika Winkler for secretarial help. This work was supported by the Deutsche Forschungsgemeinschaft (En84/20-1).

REFERENCES

1. Phi-van, L., Müller-Esterl, W., Flörke-Gerloff, S., Schmid, M. and Engel, W. (1983) Biol. Reprod. 29: 479-486.

2. Kashiwabara, S., Arai, Y., Kodaira, K. and Baba, T. (1990) Biochem. Biophys. Res. Commun. 173: 240-245.

3. Kremling, H., Keime, S., Wilhelm, K., Adham, I.M., Hameister, H. and Engel, W. (1991) Genomics 11: 828-834.

4. Adham, I.M., Klemm, U., Maier, W.M., Hoyer-Fender, S., Tsaousidou, S. and Engel, W. (1989) Eur. J. Biochem. 182: 563-568.

5. Klemm, U., Flake, A. and Engel, W. (1991) Biochim. Biophys. Acta 1090: 270-272.

6. Nayernia, K., Burkhardt, E., Beimesche, S., Keime, S. and Engel, W. (1992) Mol. Reprod. Dev. 31: 241-248.

7. Kremling, H., Flake, A., Adham, I.M., Radtke, J. and Engel, W. (1991) DNA Sequence 2: 57-60.

8.Tanhauser, S.M. and Hecht,N.B. (1989) Nucleic Acids Res. 17: 4395.

9. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11: 1475-1489.

10. Keime, S., Adham, I.M. and Engel, W. (1990) Eur. J. Biochem. 190: 195-200.

11. Hecht , N.B. (1989) Molecular biology of structural chromosomal proteins of the mammalian testis. In "Molecular Biology of Chromosome Function" (K.W. Adolph, Ed.), pp. 396-420, Springer-Verlag, New York/Berlin/Heidelberg.

12.Clermont, Y. (1962) Am. J. Anat. 111: 111-120.

13. Gold, B. and Hecht, N.B. (1983) Biochemistry 20: 4871-4877.

14. Gold, B., Fujimoto, H., Kramer, J.M., Erickson, R.P. and Hecht, N.B. (1983) Dev. Biol. 98: 392-399.

15. Kwon, Y.K. and Hecht, N.B. (1991) Proc. Natl. Acad. Sci. USA 88: 3584-3588.

16. Kleene, C.K. (1993) Dev. Biol. 159: 720-731.

17. Schlüter, G., Schlicker, M. and Engel, W. (1993) Biochem. Biophys. Res. Commun. 197: 110-115.

18. Kwon, Y.K. and Hecht, N.B. (1993) Mol. Cell. Biol. 13: 6547-6557.

19. Klausner, R.D., Ronault, T.A. and Harford, J.B. (1993) Cell 72: 19-28.