Acta Biochimica Polonica

Vol. 41 No. 2/1994

QUARTERLY

Functional analysis of spermatocyte-specific hst70 gene promoter in transgenic mice

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Key words: heat shock, transgenic mice, testis, hsp70

During differentiation of spermatogenic cells a number of genes show a unique expression pattern [1]. One of the genes which are specifically expressed during spermatogenesis is a novel gene (hst70) which belongs to the heat shock (hsp70) multigene family [2, 3]. In contrast to other members of the family which are ubiquitously expressed, transcription of the hst70 gene has been found so far only in pachytene spermatocytes [3, 4].

The basic problem in characterization of the tissue- and cell-specific promoters is to determine the localization of *"cis"* regulatory elements which confer the specificity of the transcription initiation. In the case of genes which are specifically activated in male germinal cells the functional analysis of promoters is hampered by the lack of permanent spermatogenic cell lines and primary cells in culture. In such a situation the transgenic mice technology seems to be a method of choice.

Recently, transgenic mice in which the 5′ end flanking sequences of rat hst70 gene were ligated to bacterial β-galactosidase reporter gene were constructed. The analysis of the transgene expression suggested that DNA regulatory sequences which are responsible for its testis-specific expression are localized in the 780 bp fragment of the hst70 gene upstream sequences [5].

However, the use of hybrid gene described above was found to have important drawbacks limiting the utility of the tgHST/β-gal transgenic mice for future investigations. First, in many tissues the endogenous activity of β-galactosidase is rather high giving rise to background problem. Second, high expression of β-galactosidase in spermatogenic cells may give rise to degeneration of seminiferous epithelium. Third, the DNA fragment ligated to the β-galactosidase gene contained not only 5′ end flanking sequences but also the entire leader region and a part of 3′ end nontranslated sequences of the hst70 gene. Thus, participation of extra-promoter sequences in the regulation of testis specific expression of the hst70 gene cannot be excluded.

To avoid disadvantages mentioned above we constructed new lines of transgenic mice named tg-hst(780)/CAT in which the rat hst70 gene upstream sequences were ligated to bacterial CAT (chloramphenicol acetyltransferase) reporter gene. This new hst70-CAT hybrid gene contains no other hst70 gene sequences except the 5′ end flanking region. The structure of the construct used to obtain transgenic mice is shown in Fig. 1. Glass-purified *XbaI-KpnI* restriction fragment (Fig. 1) was microinjected into pronuclei of the one-cell-stage embryos obtained from C57BL/6 × DBA/2J F1 mouse females mated with the same F1 males. Then, embryos were transferred into the oviducts of pseudopregnant F1 females. Transgenic founders were selected by PCR-based assay.

Seven lines of transgenic mice were obtained. The copy number of the transgene were from approx. 20 to 1 copies per diploid genome in different lines. For further analysis, heterozygous offspring of transgenic founders (lines tg-hst(780)/CAT 7-1, tg-hst(780)/CAT 9-4 and

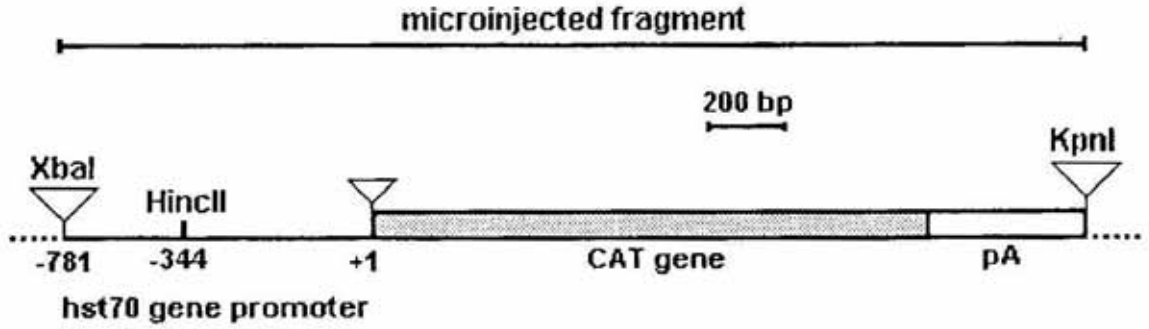

Fig. 1. Structure of the hst70-CAT hybrid gene.
The plasmid pHST(780)-CAT was constructed by ligation of the 780 bp fragment of the rat hst70 gene 5' end flanking sequences in front of the CAT gene cloned in pBL-CAT6 vector (details of the construction described in [6]). Broken lines are vector sequences, solid line is the hst70 gene promoter, filled box is a CAT reporter gene, open box represents SV40 sequences in which the polyadenylation signals are localized. Triangles mark positions of pBL-CAT6 polylinker fragments. *Xba*I and *Kpn*I restriction sited within the polylinker were used to obtain the DNA fragment for microinjection. The position of the transcription start site of the hst70 gene *in vivo* has been re-mapped by us using the primer extension method (not shown) and been shifted by 4 nucleotides upstream the site reported earlier [2]. Numbers below the map of the construct are new, corrected coordinates of the nucleotide sequence of the hst70 gene. Nucleotide +1 refers to the transcription initiation site.

tg-hst(780)/CAT 11-6) in which the number of the transgene copies per diploid genom did not exceed 2 were selected.

Tissue-specific expression of the reporter gene was analyzed by measurement of CAT activity in cell extracts prepared from testis, brain, liver, heart, and lung of adult, transgenic males (Table 1). In all three lines the highest CAT activity was found in testis, albeit a significant CAT activity was detected also in brain (approx. 1%–3% of that in testis). In other tissues analyzed the expression of transgene was insignificant.

In developing mouse testis the expression of endogenous spermatocyte-specific hsp70 gene is activated between the 14th and 16th day postnatally [3] when germinal cells in meiotic prophase appear. To determine the developmental pattern of transgene activation CAT activity was measured in cell extracts prepared from testis of the 14 day, 17/18 day and 21 day old transgenic males. Figure 2 shows that CAT activity was absent from testes of 2 week-old-mice while it was high in testes of 17–21 day-old-animals.

To sum up, the data presented show that the DNA regulatory sequences which confer tissue- and developmentally-specific pattern of the hst70 gene expression are localized within the 780 bp fragment of 5' end flanking region of

Table 1
CAT activity in tissues of hst(780)/CAT transgenic mice
Tissues were homogenized in 0.25 M Tris/Cl buffer, pH 7.8, containing 1 mM EDTA, 0.2% saponine and 1 mM dithiothreitol and centrifuged in a minifuge at 13000 r.p.m. for 10 min.The supernatant was heated for 10 min at 60°C and centrifuged again. CAT activity was measured in supernatants as described before [6, 7]. Protein concentration was determined according to [8]. The values shown in Table 1 are CAT activities normalized against the amount of protein used for the assay and expressed as % conversion/mg of protein $\times h^{-1}$ according to the procedure described in [9]. NA — not analyzed. Mice were 7–12 week old.

Tissue	Relative CAT activity/mg protein $\times h^{-1}$			
	Non transgenic	hst(780)/CAT, 7-1	hst(780)/CAT, 9-4	hst(780)/CAT, 11-6
Brain	NA	16	20	50
Heart	NA	0.7	1.0	1.55
Lung	NA	1.25	1.1	3.44
Liver	NA	0.03	0.075	0.1
Testis	0.17	1600	1700	1800

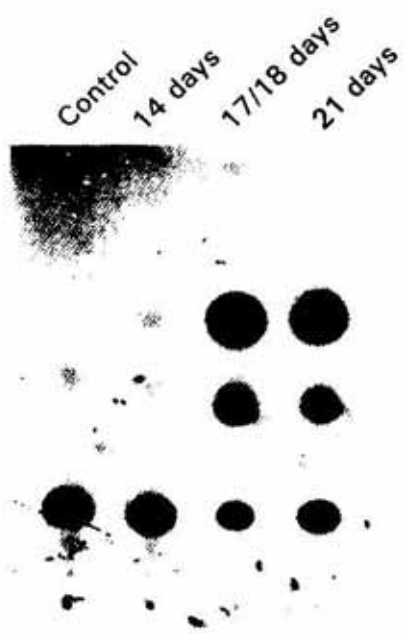

Fig. 2. Developmental activation of the hybrid gene, pHST(780)-CAT, in transgenic mice.
Cell extracts were prepared from the testis of transgenic mice bearing the hst(780)-CAT hybrid gene. Mice were killed at age indicated above the autoradiogram. Control extract was prepared from the testis of nontransgenic, 3-week-old mice. CAT activity was measured as described in the legend to Table 1. A typical autoradiogram of a thin-layer chromatogram of CAT reaction products is shown.

the gene. Preliminary data (not shown, in preparation) indicate that this specific pattern of expression is preserved in transgenic mice bearing the 350 bp fragment of the hst70 gene promoter (5′ end of the fragment is at *Hin*cII site, see Fig. 1) ligated to CAT reporter gene. Our data indicate also that neither leader nor 3′ end nontranslated sequences of the hst70 gene are needed to its tissue-specific expression.

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