Int. J. Dev. Biol. 36: 567-571 (1992)

Short Contribution

Differential expression of the mouse and human *Thy-1* gene in embryonal carcinoma cells

PETR MALÝ*, JARMILA KRÁLOVÁ and PETR DRÁBER

Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

ABSTRACT **Mouse P19 embryonal carcinoma (EC) cells express on their surfaces a Thy-1 glycoprotein. The expression of *Thy-1* at the mRNA and protein levels is down-regulated during differentiation induced by retinoic acid (RA). *Thy-1* is also expressed in human NTERA-2 EC cells, but its expression is not down-regulated during RA-induced differentiation. As a first step towards understanding differential regulation of the mouse and human *Thy-1* gene in EC cells, we have introduced genomic DNA fragments encompassing the mouse or human *Thy-1* gene into NTERA-2 and P19-derived cells and analyzed surface properties of the transfectants. In the transient transfection assay, both mouse and human *Thy-1* genes were expressed on cell surfaces at comparable levels. P19-derived stable transfectants exhibited great clonal variations in the expressions of the transfected *Thy-1* gene products, which in part reflected copy numbers. There was no simple correlation between the expression of the transfected *Thy-1* gene and two stem cell surface markers, TEC-1 and TEC-4. In the course of differentiation induced by RA several clones with a surface phenotype of EC cells exhibited a significant decrease in the expression of the transfected mouse *Thy-1*, whereas expression of the human *Thy-1* was less efficiently down-regulated. The results suggest the presence of multiple *cis*- and *trans*-acting elements controlling expression of the mouse and human *Thy-1* genes in P19 EC cells and their differentiated derivatives.**

KEY WORDS: *Thy-1, retinoic acid, cell differentiation, embryonal carcinoma*

Embryonal carcinoma (EC) cells are multipotent stem cells of teratocarcinomas whose developmental potential closely resembles that of early embryonic cells. EC cells express on their surfaces several antigenic determinants which disappear during differentiation *in vitro* (for review see Muramatsu, 1988). The epitopes, defined by monoclonal antibodies, are carbohydrates and are carried predominantly by a high molecular weight glycoconjugate, embryoglycan (Muramatsu, 1988). Recently we have shown that one of our previously prepared monoclonal antibodies, 1aG4, reacts with P19 EC cells but not with their differentiated derivatives (Dráber *et al.*, 1989a). This antibody recognizes the Thy-1 antigen, which has been extensively characterized, and the corresponding gene has been cloned and sequenced (Giguère *et al.*, 1985; Seki *et al.*, 1985). The *Thy-1* gene is a member of the immunoglobulin superfamily of genes and codes for a 112 amino acid surface glycoprotein that is anchored to the membrane through a phosphatidylinositol-containing membrane domain (Williams and Gagnon, 1982; Low and Kincade, 1985; Tse *et al.*, 1985).

The expression of Thy-1 on teratocarcinoma-derived cells was first reported by Stern *et al.* (1975). These authors found that a fraction of cells of the heterogeneous teratocarcinoma cell line SIKR bound Thy-1-specific antibody, whereas homogeneous SIKR-derived EC cells were Thy-1 negative. Thy-1 antigen was found on differentiated derivatives of the EC cells after their long-term culturing *in vitro*. These results were confirmed by analyzing the mRNA isolated from F9 cells. Undifferentiated F9 cells had no detectable *Thy-1* mRNA, which was switched on after differentiation induced by RA (Giguère *et al.*, 1985). The reverse pattern of Thy-1 antigen expression was observed in P19 EC cells; undifferentiated P19 cells were Thy-1$^+$ whereas their differentiated derivatives were Thy-1$^-$ (Dráber *et al.*, 1989a). In human EC cells, the Thy-1 antigen was found to be expressed in both the undifferentiated and differentiated cells (Andrews *et al.*, 1983). Thus, expression of Thy-1 glycoprotein in EC cells depends on the origin of the corresponding gene (human versus mouse) and on the cell type.

As a first step towards understanding the differential regulation of the mouse and human *Thy-1* genes in EC cells, we have introduced an 8.2 kilobase (kb) fragment encompassing the mouse or human *Thy-1* gene into P19 EC cells. Previous studies from several

Abbreviations used in this paper: DMSO, dimethylsulfoxide; EC, embryonal carcinoma; RA, retinoic acid.

***Address for reprints**: Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Vídenská 1083,142 20 Prague 4,Czechoslovakia. Fax: 422-471 3445.

0214-6282/92/$03.00

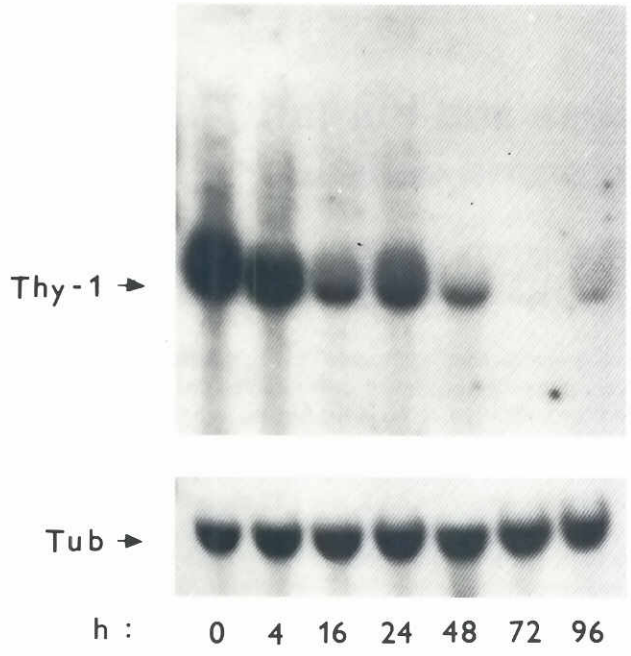

Fig. 1. Northern blot analysis of *Thy-1* mRNA isolated from P19X1 cells cultured for different periods of time with 1 μM RA. *Total RNA (20 μg) was electrophoresed, transferred to nylon filters, and hybridized to ^{32}P-labeled Thy-1. 2 cDNA probe (570 bp Pst I fragment) as described in Experimental Procedures. Blots were autoradiographed at -70° C for three days with an enhancing screen, after which they were stripped and rehybridized to an α-tubulin cDNA probe. Thy-1 and Tub indicate position of the respective mRNAs.*

laboratories have shown that these fragments contain sufficient sequences to direct expression of the genes to the appropriate tissues in transgenic mice with the exception of peripheral T cells (Chen *et al.*, 1987; Gordon *et al.*, 1987; Kollias *et al.*, 1987; Vidal *et al.*, 1990). In this paper, we describe properties of P19-derived cell lines with the transfected mouse or human *Thy-1* gene and changes in *Thy-1* gene expression during RA-induced differentiation.

To determine whether the decrease in the surface expression of Thy-1 in RA-treated P19 cells reflects changes at the mRNA level, we have analyzed *Thy-1* mRNA in cells cultured for different periods of time with RA. Northern blot analysis indicated that *Thy-1* mRNA is a sensitive indicator of RA-induced differentiation (Fig. 1). Rapid down-regulation of *Thy-1* mRNA in RA-treated cells suggests that the complex of RA with its receptor(-s) may be directly involved in the inhibition of *Thy-1* gene transcription. Alternatively, the complex of RA with its receptor(-s) may activate other genes whose products (transcriptional factors) may regulate Thy-1 gene expression in a negative manner. Because changes in the expression of *Thy-1* mRNA, as detected by Northern blot analysis, correlated with changes in the expression of surface Thy-1 antigen, as detected by 1aG4 antibody binding (Dráber *et al.*, 1989a), we employed a simple direct radioantibody binding assay to analyze the expression of both endogenous and exogenous *Thy-1* genes in transfected cells.

To determine whether the 8.2 kb *Eco* RI genomic fragments encompassing the human or mouse *Thy-1* gene can be expressed in mouse and human EC cells, we evaluated the expression of the transfected genes in transient expression assays (Table 1). Transfer of human *Thy-1* or mouse *Thy-1.1* gene into P19S1801A1 and NTERA-2 cells resulted in significant binding of the corresponding antibodies to the transfected cells. Antibodies recognizing the transfected gene products bound to the transfected cells at equal or greater levels than antibodies to the endogenous *Thy-1* gene products. These data indicated that the transfected DNA fragments carried all regulatory sequences necessary for expression of the *Thy-1* genes in EC cells and, therefore, we prepared stable cell lines expressing the transfected gene products.

In the first series of experiments, circular plasmids or isolated linear *Eco* RI fragments containing the mouse or human *Thy-1* gene were introduced into P19S18O1A1 cells by co-transfection with a vector, pSTneoB, transferring resistance to G418 and the transfected cells were selected in culture medium supplemented with G418. Individually growing colonies, visible after approximately two weeks, were expanded and analyzed for the surface expression of the transfected gene products (Fig. 2). The majority of colonies (19 of 22) isolated after transfection with the isolated *Eco* RI fragment of human *Thy-1* gene expressed hThy-1 antigen detectable by radioantibody binding assay. *Thy-1* antigen expression varied greatly. When the h*Thy-1* gene in the form of a circular plasmid was transfected into P19S18O1A1 cells, the corresponding Thy-1 antigen was found on fewer colonies (13 of 48) and the antibodies bound to the positive cells less efficiently. A significant difference between linearized and circular form of *Thy-1* was also observed in cell lines with the transfected mouse *Thy-1.1*. These results were confirmed in a second series of experiments, in which the linearized or circular form of human or mouse *Thy-1* genes were transfected into P19XT.1.1 cells, which are embryoglycan-defective derivatives of P19X1 cells (Dráber and Maly, 1987).

After cloning and selection of the cell lines with stable expression of the transfected *Thy-1* genes, we analyzed copy numbers of the genes transfected. Southern blotting analysis revealed considerable variation in the number of copies of the transfected genes (Table 2). There was no simple relationship between the gene copy number and surface antigen expression.

Stable cell lines expressing the transfected gene products were analyzed in detail for expression of TEC-1 and TEC-4. We have

TABLE 1

TRANSIENT EXPRESSION OF THE TRANSFECTED MOUSE OR HUMAN *Thy-1* GENE IN P19 AND NTERA-2 CELLS

Cells	Gene transfected	^{125}I-Antibody bound (%)[a]		
		αThy-1.2	αThy-1.1	αhThy-1
P19S18O1A1	None	6.4	0.2	0. 2
P19S18O1A1	Thy-1.1	6.4	14.0	0. 2
P19S18O1A1	hThy-1	7.2	0.3	13.5
NTERA-2	None	0.2	0.2	3.7
NTERA-2	Thy-1.1	0.2	12.1	3.4

[a]The cells were analyzed for expression of surface antigens using a direct radioantibody binding assay as described in Experimental Procedures. Numbers represent percentage of radioactivity bound to cells. Mean values of at least two experiments performed in triplicates are shown.

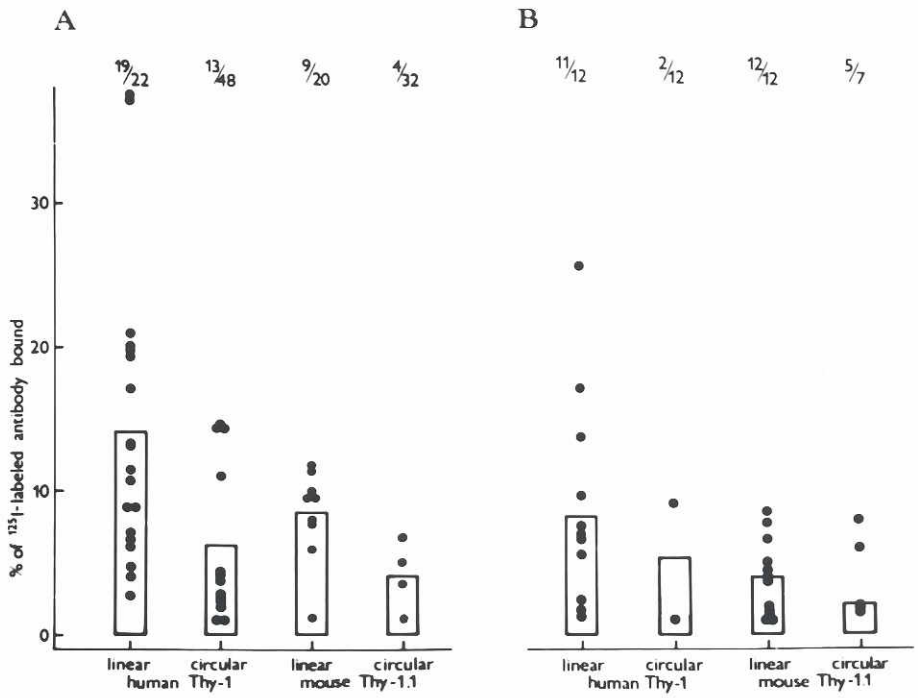

Fig. 2. The expression of the human and mouse *Thy-1* gene in colonies isolated after transfection of P19S18O1A1 (A) or P19XT.1.1 (B) *with circular plasmids or linear Eco RI fragments containing Thy-1 gene of mouse or human origin. The cells were transfected as described in Experimental Procedures and colonies were selected by growth in medium supplemented with G418. Numbers at the top indicate numbers of colonies with detectable surface expression of the transfected gene product/ total number of colonies tested. Percentage of radioactivity bound to individual colonies, as determined in a direct radioantibody binding assay, and means of positive values (hight of rectangles) are shown.*

previously shown that TEC-4 is a sensitive marker of EC cell differentiation and that its decrease precedes the decrease in the expression of TEC-1 (Dráber *et al.*, 1989a,b). According to the expression of these markers, the transfected cell lines presented in Table 2 can be divided into several groups. It should be noted that because P19S18O1A1- and P19XT.1.1-derived cell lines behaved similarly in the tests employed, we did not divide the transfected cell lines into subgroups according to their origin and combined them only according to their differentiation state. Among the P19S18O1A1-derived cell lines with the transfected human *Thy-1* gene, we have found only one, HLP-1, with the surface phenotype of undifferentiated EC cells. Several other cell lines expressing hThy-1 and with the morphology of EC cells exhibited a significant decrease in the expression of TEC-1 epitope (clones 5-9). Surprisingly, these cells showed a relatively high level of TEC-4 epitope, compared to TEC-1 epitope. Only in HLP-10 cells was the TEC-1 more strongly expressed than TEC-4, suggesting an early stage of differentiation. All P19XT.1.1-derived cell lines (clones 2 -4) had the morphology and antigenic makeup of undifferentiated EC cells except for the absence of TEC-1, which was removed genetically (Dráber and Maly, 1987). Three cell lines (11-13) with the morphology of differentiated fibroblast-like cells and with a relatively high level of the exogenous Thy-1 had very weak expression of both TEC-1 and TEC-4 epitopes.

Cell lines with an exogenous Thy-1.1 gene exhibited weaker expression of the Thy-1.1 antigen compared to cells with transfected human Thy-1 and, again, most of them, although having the morphology of EC cells, exhibited relatively low expression of TEC-1 compared to TEC-4 expression. MLP-14 was the only cell line with high expression of both TEC-1 and TEC-4 epitopes. The MLP-20 cells on the other hand had the morphology of differentiated cells and exhibited decreased expression of both stem cell markers.

To determine whether mouse and human *Thy-1* genes will respond to changes induced by RA, the transfected cells were cultured in the presence of 1 μM RA for 4 days and their surface antigenic phenotype was determined. Again, the expression of surface antigens varied considerably among individual clones after differentiation. In EC cells expressing human *Thy-1* gene (clones 1-9), treatment with RA induced a marked decrease in the expression of TEC-4; if expression of TEC-4 in untreated cells is taken as 100%, then the mean decrease was by 94%. In the same cells the mean decrease in hThy-1 antigen expression was only by 14%.

Similar analysis of cell lines with the integrated mouse Thy-1.1 gene (clones 14-19) showed a decrease in TEC-4 antigen expression (mean decrease by 92%); mean decrese in expression of the transfected Thy.1.1 gene product was by 57%.

In several cell lines with low expression of TEC-4, different results were obtained. In the TEC-1$^+$ cell line (HLP-10), there was only a weak down-regulation of both the endogenous and transfected Thy-1 gene products. However, these cells were able to respond to RA as determined by pronounced changes in the expression of TEC-1 and TEC-4 epitopes. RA had no significant effect on the expression of *Thy-1* genes in cells with morphology of differentiated cells and expressing low amounts of the TEC-1/TEC-4 epitopes (clones 11-13 and 20). HLP-11 cells resembled 3T3 fibroblasts in their relatively high levels of the endogenous *Thy-1* which were slightly up modulated after treatment with RA. Other clones of differentiated cells expressed low levels of the endogenous *Thy-1* and relatively high levels of the transfected *Thy-1* gene product and responded to RA treatment weakly or not at all.

The results reported here indicate that down-regulation of the Thy-1 antigen expression on the surface of RA-treated P19 EC cells reflects changes at the *Thy-1* mRNA level. This implies that this

TABLE 2

THE EXPRESSION OF THE TRANSFECTED AND ENDOGENOUS *Thy-1* GENE PRODUCTS AND TEC-1 AND/OR TEC-4 ANTIGENS IN THE CELL LINES ISOLATED AFTER TRANSFECTION WITH A HUMAN OR MOUSE *Thy-1* GENE

Clone[a]	Copy number[b]	^{125}I-Antibody bound (%)[c]				
		αhThy-1 -/+	αThy-1.1 -/+	αThy-1.2 -/+	αTEC-1 a -/+	αTEC-4 -/+
HLP- 1	(20)	27.2/17.4	NT	5.8/2.3	28.6/1.8	17.7/0.8
HLX- 2	(NT)	29.3/25.5	NT	5.5/1.0	NT	12.7/0.6
HLX- 3	(NT)	15.7/15.3	NT	1.2/0.5	NT	14.2/0.5
HCX- 4	(NT)	6.8/2.4	NT	10.3/0.6	NT	16.7/0.3
HLP-5	(13)	9.4/16.6	NT	3.6/1.1	9. 0/1. 5	13. 9/0. 8
HCP-6	(3)	14.0/10.0	NT	3.6/0.2	5. 3/0. 3	13. 0/0. 8
HCP-7	(1)	4.8/5.1	NT	1.3/0.5	2. 9/0. 5	13. 8/0. 8
HCP-8	(8)	12.7/12.0	NT	0.8/1.0	1. 4/1. 6	22. 1/1. 2
HCP-9	(2)	5.6/3.6	NT	3.6/0.6	3. 8/4. 1	26. 8/3. 0
HLP-10	(22)	22.5/21.2	NT	4.1/3.8	20.2/2.2	3.2/0.7
HLP-11	(25)	41.1/27.0	NT	14.4/17.4	1.5/4.7	0.7/1.2
HCP-12	(3)	21.6/21.3	NT	0.7/1.1	0.3/1.3	1.1/3.6
HCP-13	(3)	20.7/18.0	NT	1.8/1.3	0.3/1.1	1.0/5.5
MLP-14	(12)	NT	12.6/3.9	9.1/1.3	20.6/0.8	17.6/1.4
MLP-15	(19)	NT	10.5/3.7*	3.3/2.8	7.5/2.5	22.1/2.3
MCX-16	(NT)	NT	9.4/1.6*	1.9/0.1	NT	10.2/0.2
MCX-17	(NT)	NT	5.1/1.9*	10.2/1.4	NT	10.2/1.3
MLP-18	(10)	NT	6.2/3.5*	1.4/0.3	8.9/5.2	14.9/0.5
MLP-19	(23)	NT	11.9/9.4*	5.4/3.3	10.1/2.8	16.9/1.1
MLP-20	(22)	NT	7.6/10.5*	1.5/3.5	3.3/0.3	1.4/0.9
Controls:						
P19S18O1A1		NT	NT	6.4/0.1	33.7/0.2	17.0/0.2
P19X1		NT	NT	7.8/0.4	26.4/2.6	16.6/0.6
P19XT.1.1		NT	NT	5.2/0.2	0.2/0.3	14.7/0.4
3T3		NT	NT	14.6/19.7	0.2/0.3	0.5/1.4
NTERA-2		4.7/4.2	NT	NT	NT	NT

[a]Name of a clone involves an origin of the transfected *Thy-1* gene [human (H) or mouse (M)], its form [linear (L) or circular (C)], origin of the parental cells [P19S18OIAI (P) or P19XT. 1. 1 (X)], and a clone number (1-20).
[b]Numbers in parenthesis indicate copy numbers of the transfected gene determined by a densitometric measuring of Southern blot radiograms.
[c]The cells were analyzed for expression of surface antigens using a direct radioantibody binding assay as described in Experimental Procedures. Numbers represent percentage of radioactivity bound to cells cultured in the absence (-) or presence (+) of retinoic acid (10^{-6} M) for 4 days. Mean values of at least two experiments performed in triplicate are shown.
NT not tested.

regulation occurs at the level of *Thy-1* gene expression or *Thy-1* mRNA processing. Comparison of the expression of the Thy-1 gene products in undifferentiated EC cells and their RA-induced differentiated derivatives showed that both exogenous Thy-1 gene products of mouse Thy-1.1 and human Thy-1 are less efficiently down-regulated compared to endogenous Thy-1.2 and that mouse Thy-1 is more efficiently down regulated than human Thy-1. The combined data indicate different regulation of the mouse and human *Thy-1* genes in differentiating mouse EC cells. Recently Vidal *et al.* (1990) have described that tissue-specific enhancer elements in the Thy-1 genes are located downstream from the site of transcriptional initiation and that these elements are different in human and mouse *Thy-1*. At present we do not know which of these elements are important for the expression of *Thy-1* in EC cells. However, significant heterogeneity in *Thy-1* gene expression in EC cells and their differentiated derivatives suggests that other more distant elements are involved and that the site of integration is important for the expression of the transfected genes.

Experimental Procedures

Cell lines

The origin and properties of the mouse EC cell lines (P19X1, P19S18O1A1 and P19X1-derived embryoglycan defective P19XT.1.1), human EC cell line (NTERA-2), mouse fibroblast cell line (3T3) and culture conditions have been described (Dråber, 1987; Dråber *et al.*, 1989b). To induce differentiation, the cells were harvested, washed and plated into tissue culture dishes in culture medium supplemented with all-trans retinoic acid (RA; Sigma Chemical Co., St. Louis, MO).

Monoclonal antibodies

Antibodies specific for mouse Thy-1.1 (clone MRCOX7), mouse Thy-1.2 (clone 1aG4) and human Thy-1 (clone F15-42-1-5) have been described (Dalchau and Fabre, 1979; Dråber *et al.*, 1980; Mason and Williams, 1980). TEC-01 antibody (Dråber and Pokornâ 1984) recognizes the same carbohydrate epitope, Gal(ß1->4)-[Fuc(α1->3)]GlcNAc, as does SSEA-1-specific antibody (Gooi *et al.*, 1981), whereas TEC-04 antibody recognizes an EC cell surface protein epitope (Dråber *et al.*, 1989b). The antibodies were isolated from ascites fluid by precipitation at 50% ammonium sulfate saturation and purified by ion-exchange chromatography on DEAE-Sephadex A-50 (Pharmacia Fine Biochemicals, Uppsala, Sweden).

Plasmids

The 8.2 kb *Eco* RI genomic fragments encompassing the mouse *Thy-1.1* or human *Thy-1* gene were obtained as pBSV-derived plasmids $pBST_1$ (Giguère *et al.*, 1985) and $pBSHT_1$ (van Rijs *et al.*, 1985), respectively, from Dr. F. Grosveld. A plasmid containing a *Pst* I fragment of mouse *Thy-1.2* cDNA, clone TM8 (Hedrick *et al.*, 1984), was obtained from Dr. S. Hedrick. The pCD-29-5A clone (Hiraki *et al.*, 1986), in which *Thy-1.2* cDNA is under the control of SV40 promoter, was a gift from R.L. Coffman. For selection of transfected cells we used the pSTneoB vector, in which the bacterial aminoglycosyl 3' phosphotransferase type II (AGPT) gene is under the control of tandem promoters of SV40 and HSVtk (Katoh *et al.*, 1987). This vector, which confers resistance to the neomycin analog G418 on mammalian cells, was provided by K. Katoh.

Transfection and selection of stable clones

DNA transfection was performed by a modification of the method of Chen and Okayama (1987). EC cells (5×10^5) were seeded into a 60-mm culture dish, incubated in culture medium for one day, washed and then 500 µl of DNA mixture was added to 5 ml of incubation media on the plate. DNA mixture contained 9 µg of DNA of mouse Thy-1.1 or human Thy-1 *Eco* RI genomic fragments encompassing the respective genes (in the plasmid vectors or excised from the plasmid by Eco RI and electrophoretically purified) and 1 µg of pSTneoB plasmid DNA in 0.5 ml transfection buffer containing 125 mM $CaCl_2$, 25 mM N,N-bis(hydroxyethyl)-2-aminoethane-sulfonic acid (pH 7.0), 140 mM NaCl, and 0.75 mM Na_2HPO_4. After 4 h of incubation at 37°C and 3% CO_2 in air, the medium was removed and 2 ml of 20% dimethylsulfoxide (DMSO) in transfection buffer was added. After 4 min of incubation, DMSO was replaced with culture medium. Next day, the cells were harvested and transferred into a 90-mm tissue culture dish in selective medium containing 500 µg/ml of G418 antibiotic (Geneticin, Sigma). Surviving clones were picked out after 14 days and expanded for further analysis. Transient expression assay was performed in the same way except that pSTneoB DNA was omitted from the DNA mix. Two days after transfection, the cells were harvested and analyzed by a direct radioantibody binding assay.

Northern blot analysis

Total RNA was isolated by a modified method of Auffray and Rougeon (1980) using 8 M urea-3 M LiCl. Twenty µg of total RNA was electrophoretically separated on a 1% formaldehyde agarose gel and blotted onto nylon membranes (Hybond N, Amersham, England). A 570 basepair (bp) *Pst* I

fragment of mouse *Thy-1.2* cDNA or a 1617 bp fragment of rat α-Tubulin cDNA (Lemischka *et al.*, 1981) labeled by [α-^{32}P]-deoxyadenosine 5'-triphosphate (3000 Ci/mmol, Du Pont de Nemours, Bad Homburg, Germany) was used for hybridization. Hybridization was performed at 42°C in 50% formamide using Denhardt's solution with 10% dextran sulfate. The filters were washed four times for 10 min at room temperature in 100 ml of 2x standard sodium citrate (SSC), 0.1% sodium dodecylsulfate (SDS), followed by two washes for 30 min at room temperature and then at 60°C in 0.1x SSC, 0.1% SDS. The filters were dried and exposed to X-ray films for 1-5 days.

Radioantibody binding assay and indirect immunofluorescence

These have been decribed (Dráber *et al.*, 1989a). The specific activity of the ^{125}I-labeled antibodies was approximately $2x10^7$ cpm/μg. The antibodies bound specifically to the target cells as has been previously described (Dráber and Maly, 1987).

Southern blot analysis

Southern blot analysis was performed according to Maniatis *et al.*, 1982.

Acknowledgments

We wish to thank Drs. F. Grosveld, R.L. Coffman, S. Hedrick, and K. Katoh for providing us with the cloned genes, Drs. J. Fabre and D.W. Mason for sending us monoclonal antibodies, M. Aulická, and H. Klepalová for technical assistance, and Dr. Marta Rauscherová for help in preparing the manuscript. This work was supported by grant No. 55272 of the Czechoslovak Academy of Sciences.

References

ANDREWS, P.W., GOODFELLOW, P.N. and BRONSON, D.L. (1983). Cell-surface characteristics and other markers of differentiation of human teratocarcinoma cells in culture. In *Teratocarcinoma Stem Cells* (Eds. L.M. Silver, G.R. Martin and S. Strickland). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 579-590.

AUFFRAY, C. and ROUGEON, F. (1980). Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem. 107:* 303-314.

CHEN, C. and OKAYAMA, H. (1987). High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol. 7*: 2745-2752.

CHEN, S., BOTTERI, F., van der PUTTEN, H., LANDEL, C.P. and EVANS, G.A. (1987). A lymphoproliferative abnormality associated with inappropriate expression of the Thy-1 antigen in transgenic mice. *Cell 51:* 7-19.

DALCHAU, R. and FABRE, J.W. (1979). Identification and unusual tissue distribution of the canine and human homologues of Thy-1 (theta). *J. Exp. Med. 149:* 576-591.

DRÁBER, P. (1987). The epitope of mouse embryonic antigen(s) recognized by monoclonal antibody TEC-02 is a carbohydrate carried by high-molecular-weight glycoconjugates. *Cell Differ. 21:* 119-130.

DRÁBER, P. and MALY, P. (1987). Mutants of embryonal carcinoma cells defective in the expression of embryoglycan. *Proc. Natl. Acad. Sci. USA 84:* 5798-5802.

DRÁBER, P. and POKORNÁ, Z. (1984). Differentiation antigens of mouse teratocarcinoma stem cells defined by monoclonal antibodies. *Cell Differ. 15*: 109-113.

DRÁBER, P., MALY, P., HAUSNER, P. and POKORNÁ, Z. (1989a). The Thy-1 glycoprotein is expressed in mouse embryonal carcinoma cells P19. *Int. J. Dev. Biol. 33*: 369-378.

DRÁBER, P., NOSEK, J., and POKORNÁ, Z. (1989b). Unusual stage- specific embryonic antigen (TEC-4) defined by a monoclonal antibody to embryonal carcinoma cells defective in the expression of embryoglycan. *Proc. Natl. Acad. Sci. USA 86:* 9337-9341.

DRÁBER, P., ZIKÁN, J., VOJTÍSKOVÁ, M. (1980). Establishment and characterization of permanent murine hybridomas secreting monoclonal anti-Thy-1 antibodies. *J. Immunogenet. 7:* 455- 474.

GIGUERE, V., ISOBE, K.-I. and GROSVELD, F. (1985). Structure of the murine Thy-1 gene. *EMBO J. 4:* 2017-2024.

GOOI, H.C., FEIZI, T., KAPADIA, A., KNOWLES, B.B., SOLTER, D. and EVANS, M.J. (1981). Stage-specific embryonic antigen involves α1->3 fucosylated type 2 blood group chains. *Nature 292*: 156-158.

GORDON, J.W., CHESA, P.G., NISHIMURA, H., RETTIG, W.J., MACCARI, J.E., ENDO, T., SERAVALLI, E., SEKI, T. and SILVER, J. (1987). Regulation of Thy-1 gene expression in transgenic mice. *Cell 50*: 445-452.

HEDRICK, S.M., COHEN, D.I., NIELSEN, E.A. and DAVIS., M.M. (1984). Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. *Nature 308:* 149-153.

HIRAKI, D.D., NOMURA, D., YOKOTA, T., ARAI, K.-I. and COFFMAN, R.L. (1986). Isolation and characterization of expressible cDNA clones for mouse Thy-1: a model system for cDNA expression of cell surface proteins. *J. Immunol. 136:* 4291- 4296.

KATOH, K., TAKAHASHI, Y., HAYASHI, S., KONDOH, H. (1987). Improved mammalian vectors for high expression of G418 resistance. *Cell. Struct. Funct. 12:* 575-580.

KOLLIAS, G., SPANOPOULOU, E., GROSVELD, F., RITTER, M., BEECH, J. and MORRIS, R. (1987). Differential regulation of a Thy-1 gene in transgenic mice. *Proc. Natl. Acad. Sci. USA 84:* 1492-1496.

LEMISCHKA, I.R., FARMER, S., RACANIELLO, V.R. and SHARP, P.A. (1981). Nucleotide sequence and evolution of a mammalian α-tubulin messenger RNA. *J. Mol. Biol. 151:* 101-120.

LOW, M.G. and KINCADE, P. (1985). Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein. *Nature 318:* 62-64.

MANIATIS, T., FRITSCH, E.F. and SAMBROOK, J. (1982). *Molecular Cloning, A Laboratory Manual*, 1st Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MASON, D.W. and WILLIAMS, A.F. (1980). The kinetics of antibody binding to membrane antigens in solution and at the cell surface. *Biochem. J. 187:* 1-20.

MURAMATSU, T. (1988). Developmentally regulated expression of cell surface carbohydrates during mouse embryogenesis. *J. Cell. Biochem. 36:* 1-14.

SEKI, T., SPURR, N., OBATA, F., GOYERT, S., GOODFELLOW, P. and SILVER, J. (1985). The human Thy-1 gene: structure and chromosomal location. *Proc. Natl. Acad. Sci. USA 82:* 6657-6661.

STERN, P.L., MARTIN, G.R. and EVANS, M.J. (1975). Cell surface antigens of clonal teratocarcinoma cells at various stages of differentiation. *Cell 6:* 455-465.

TSE, A.G.D., BARCLAY, A.N., WATTS, A. and WILLIAMS, A.F. (1985). A glycophospholipid tail at the carboxyl-terminus of the Thy-1 glycoprotein of neurons and thymocytes. *Science 230:* 1003-1008.

VAN RIJS, J., GIGUERE, V., HURST, J., VAN AGTHOVEN, T., VAN KESSEL, A.G., GOYERT, S. and GROSVELD, F. (1985). Chromosomal localization of the human Thy-1 gene. *Proc. Natl. Acad. Sci. USA 82:* 5832-5835.

VIDAL, M., MORRIS, R., GROSVELD, F. and SPANOPOULOU, E. (1990). Tissue-specific control elements of the Thy-1 gene. *EMBO J. 9:* 833-840.

WILLIAMS, A.F. and GAGNON, J. (1982). Neuronal cell Thy-1 glycoprotein: homology with immunoglobulin. *Science 216:* 696-703.

Accepted for publication: September 1992