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Expression of the two mannose 6-phosphate receptors is spatially and temporally different during mouse embryogenesis

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Summary

Mammalian cells express two mannose 6-phosphate receptors, MPR46 and MPR300, both of which mediate the targeting of lysosomal enzymes to lysosomes. Additionally the receptors mediate the secretion (MPR46) and the endocytosis (MPR300) of lysosomal enzymes and the binding of IGFII (MPR300). We have analyzed the distribution of MPR46 and MPR300 transcripts during mouse embryogenesis by in situ hybridization. Up to day 15.5 of embryonic development we found a non-overlapping distribution of the transcripts for the two receptors. High expression of MPR46 was observed at sites of hemopoiesis and in the thymus while MPR300 was highly expressed in the cardiovascular system. Late in embryogenesis (day 17.5) a wide variety of tissues expressed the receptors, but still the expression pattern was almost non-overlapping. This unexpected complementary expression pattern points to specific functions of the two mannose 6-phosphate receptors during mouse embryogenesis.

Key words: development, MPR46, MPR300, in situ hybridization.

Introduction

Mannose 6-phosphate receptors (MPRs) mediate the targeting of newly synthesized soluble lysosomal enzymes. The receptors recognize mannose 6-phosphate residues which are specifically added to lysosomal enzymes during their passage through the Golgi apparatus. Receptor ligand complexes migrate from the trans Golgi network to prelysosomal compartments, where the complexes dissociate due to acidic pH. The ligands are delivered to lysosomes, while the MPR can recycle to the Golgi apparatus (Kornfeld and Mellman, 1989).

Two MPRs are known, both of which mediate the transport of newly synthesized mannose 6-phosphate-containing ligands from the Golgi apparatus to prelysosomal structures of the endocytic pathway. Furthermore, the larger of the two receptors with an apparent M_r of 300×10^3 (MPR300) mediates the endocytosis of mannose 6-phosphate-containing ligands, while the smaller of the two receptors with an apparent M_r of 46×10^3 (MPR46) mediates the secretion of part of its ligands. The two receptors bind essentially the same mannose 6-phosphate-containing ligands although with different affinities and pH optima (Dahms et al., 1989; Chao et al., 1990). The insulin-like growth factor (IGF) II is another ligand of mammalian MPR300 (Morgan et al., 1987). The physiological function of IGFII binding to MPR300 is unknown. Most of the metabolic effects of IGFII can be explained by its binding to the IGFI receptor (Czech, 1989; Roth, 1988). While IGFII mediated coupling of the MPR300 to a G_i-protein suggests a function in transmembrane signalling (Murayama et al., 1990) other studies indicate a function of MPR300 in controlling the level of IGFII by its uptake and degradation (Czech, 1989; Haig and Graham, 1991).

With the exception of a few tumor-derived cell lines that lack the MPR300 (Gabel et al., 1983; Mainferme et al., 1985) all mammalian cell lines and tissues examined contain both receptors. However, the concentrations and the relative ratios of the two receptors vary among several human cell lines and tissues by about one order of magnitude (Wenk et al., 1991).

Why do cells express two different MPRs? The most likely reason is that the two receptors have different or at least partly different functions, e.g. binding of IGFII. MPR300 has acquired the IGFII binding site fairly recently in evolution, since in non-mammalian species, such as chicken and frog, MPR300 lacks the IGFII binding site (Canfield and Kornfeld, 1989; Clairmont and Czech, 1989). It is not clear whether the acquisition of IGFII binding or the need for other functional differences has provided the evolutionary force for the development and expression of two MPRs.

In an attempt to further compare the properties of the two MPRs we have investigated their expression during mouse embryonic development from d9.5 to d17.5. To our surprise we observed an almost complementary pattern of expression of the two receptors.

Materials and methods

Mouse embryos and cryosectioning

Mice, strain NMRI, were mated overnight. Females showing vaginal plugs on the next morning were kept separately and the beginning of gestation was defined as midnight. The embryos were sectioned, washed in ice cold 1×PBS (0.13 M NaCl, 7 mM Na_2HPO_4, 3 mM $NaH_2PO_4 \cdot 2H_2O$, pH 7.0), and blotted on a paper towel. For preparation of RNA, embryos were subsequently homogenized. For cryosectioning they were immediately frozen at the surface of dry ice, embedded in tissue-tec freeze medium (Reichert-Jung) and stored at −70°C for up to several weeks. Cryosectioning was done with the cryostat Modell 2700 Frigocut (Reichert-Jung).

Isolation of RNA

Total RNA was prepared according to the protocol of Chirgwin et al. (1979). Poly(A)$^+$ RNA was isolated as described by Gonda et al. (1982).

Preparation of probes

RNA probes for Northern blot analysis and in situ hybridization were obtained by in vitro transcription of receptor gene fragments cloned into the pGem1 vector (Promega). The MPR46 mouse cDNA *Eco*RI fragment was 1397 bp in size and contained 99 bp of 5′ untranslated region, the entire coding region and 360 bp of 3′ non-translated sequences (Köster et al., 1991). The MPR300 mouse cDNA *Eco*RI fragment was 1263 bp in size (EMBL accession number X60389) and encoded a part of the luminal domain of the receptor corresponding to nt 5041 to 6303 of the human MPR300 gene (Oshima et al., 1988). Antisense and sense RNA probes were transcribed from a *Bam*HI linearized vector using T7 RNA polymerase. For Northern blot analysis (Maniatis et al., 1982) RNA was labelled with [^{32}P]UTP, for in situ hybridization with [^{35}S]UTP using the in vitro transcription protocol of Holland (1986). The transcripts were precipitated twice with ammonium acetate to remove free nucleotides. Before hybridization of sections, riboprobes were hydrolyzed to an average size of 150 bases (Cox et al., 1984).

In situ hybridization

In situ hybridization was performed according to the protocol of Holland (1986) with the following modifications. Embryos were not fixed before sectioning. Sections were mounted on coated slides. For coating, the slides were cleaned in 10% Extran (Merck, Darmstadt, FRG) overnight, rinsed with warm water for 2 hours, washed three times with diethylpyrocarbonate (DEPC)-treated water and dried for 2 hours at 120°C. The slides were coated (2% aminopropyltriethoxysilan in acetone, 2× acetone, 2× DEPC-treated water, 30 seconds each), dried at 45°C, and stored at room temperature for up to several weeks.

The pretreatment with pronase was omitted. The ^{35}S-labelled riboprobes were diluted in hybridization buffer [300 mM NaCl, 10 mM Tris-HCl pH 7.5, 10 mM sodium phosphate buffer pH 6.8, 5 mM EDTA pH 8, 1× Denhardt's solution, 10% dextran sulfate, 100 mM DTT, 50% deionized formamide, 3 μg/μl tRNA (*E. coli*), 10 μM α-thio-UTP, 0.5 μg/μl heparine] to a specific activity of 5×10^4 counts per minute/μl. Hybridization was performed at 45°C.

The sections were washed 4×30 minutes in 50% formamide, 2×SSC, 10 mM β-mercaptoethanol at 45°C, digested with 8 μg pancreatic RNAse A/ml RNAse buffer (0.5 M NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA) for 30 minutes at 37°C and washed again 4×30 minutes in 2×SSC, 10 mM β-mercaptoethanol at 60°C, 2×30 minutes in 0.1×SSC, 10 mM β-mercaptoethanol at 60°C and 2×5 minutes in 0.1×SSC at room temperature.

Following dehydration in graded ethanol containing 0.3 M ammonium acetate, sections were covered with NTB 2 photoemulsion (Kodak) and exposed for 2 to 3 weeks. Slides were developed, stained with cresyl violet and embedded with Eukitt (Kindler, Freiburg, FRG).

Results

Northern blot analysis of MPR46 and MPR300 expression

The probes for the MPR46 and MPR300 hybridize to specific RNA species of mouse Ltk$^-$ cells as shown by the Northern blot analysis (Fig. 1). The MPR46 riboprobe detects a main 2.3 kb transcript and two minor RNA species of 5 kb (not visible in Fig. 1) and 1.3 kb. The MPR300 riboprobe recognizes a unique RNA of about 10 kb. MPR46 and MPR300 transcripts of the same size are detectable in RNA from mouse embryos from d11 to d17 and in adult mouse organs (shown for brain in Fig. 2). While the relative amount of MPR46 RNA is nearly constant from d11 to d17 that of MPR300 RNA increases about 2.5-fold from d11 to d13 and then remains fairly constant up to d17.

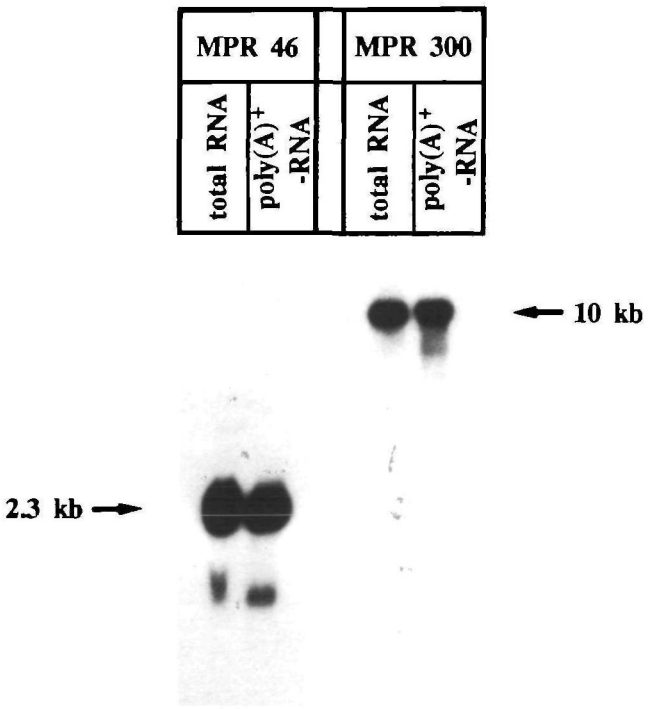


Fig. 1. Northern blot analysis showing specifity of the MPR46 and MPR300 probes. Total and poly(A)$^+$ RNA, 15 and 1.5 μg respectively, isolated from mouse Ltk$^-$ cells, was loaded per lane, electrophoresed, transferred to nylon filters and hybridized with ^{32}P-labelled riboprobes specific for MPR46 and MPR300 (see Maniatis et al., 1982 and methods). Autoradiography was for 24 hours.

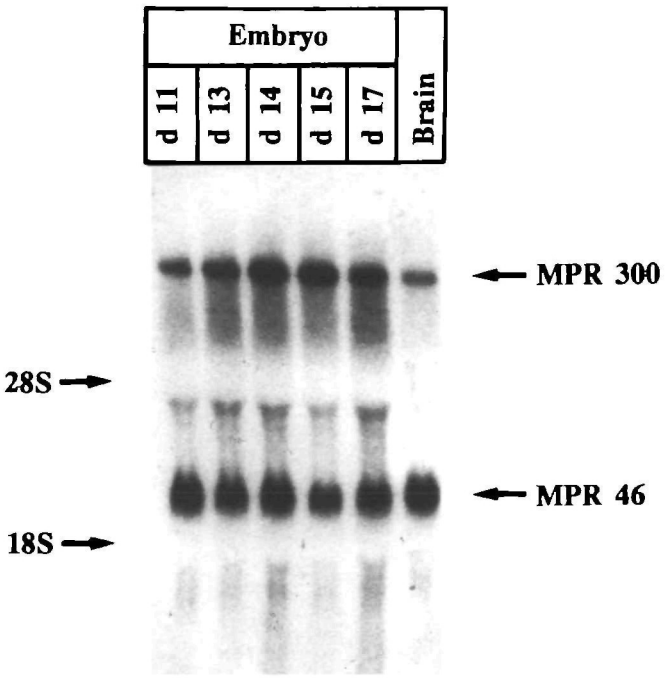


Fig. 2. MPR46 and MPR300 mRNA expression in embryos of different developmental age. Total RNA (15 μg per lane) of embryos from day 11 to 17 (d11-d17) and of brain from 5-weeks-old mice was hybridized to a 1:1 mixture of ^{32}P-labelled antisense riboprobes specific for MPR46 and MPR300. The specific activities of the two probes were identical. Autoradiography was for 24 hours.

MPR46 and MPR300 expression during mouse development from d9.5 to d17.5

Mid- and parasagittal sections of embryos from d9.5 to d17.5 were analyzed. High levels of MPR46 transcription are detectable for the first time at d11.5 in liver, while MPR300 transcripts are detectable already at d9.5 in the heart and in umbilical vessels (Fig. 3). This pattern changes to a more complex distribution towards the later stages of embryogenesis (Fig. 3 and Fig. 4).

At higher magnification than shown in Fig. 3 it becomes apparent that the signal for MPR46 in liver from d13.5 onwards originates mainly from cell aggregates surrounded by erythrocytes which give no signal. These aggregates are assumed to represent blood-forming foci. In addition heavily labelled single cells morphologically identifiable as megakaryocytes are seen in liver from d15.5 onwards. The intervening patches of parenchmyal liver cells give much weaker signals.

Next to liver, MPR46 RNA is most abundant in the thymus (from d13.5 onwards) and in the upper intestinal tract (from d15.5 onwards). In the thymus the silver grains are initially distributed homogenously. At d17.5 they are concentrated in the subcapsular region which is enriched in large lymphoid cells and in the central part of the thymus above round cell patches.

Weaker MPR46 RNA signals are seen in the lung (d13.5), the somites and sklerotomal bodies (d11.5 to d13.5), the pancreas (d13.5), the submaxillary and sublingual glands (d15.5) and the skin (d15.5). The expression in the central nervous system is weak and has a maximum between d11.5 to d13.5.

At d17.5 the MPR46 expression reaches a complex pattern (Fig. 4A). Table 1 summarizes the tissues, in which MPR46 transcripts are detectable. Among the tissues that start to show prominent MPR46 labelling at d17.5 are brown adipose tissue, thyroid, tooth anlage, hypophysis, adrenal gland, urogenital and lower intestinal tract, bone marrow and zones of ossification (see below).

At all stages of embryonic development the most prominent signals for MPR300 were seen in the cardiovascular system. Lower levels of MPR300 tran-

Table 1. *Distribution and relative abundance of MPR46 and MPR300 transcripts in d17.5 old mouse embryo*

Tissue	MPR46	MPR300
Nervous system		
Brain	(+)	(+)
Rathke's pocket	+	−
Choroid plexus	++	++
Leptomeninx	+	++
Ganglion coeliacum	−	++
Skin		
Epidermis, hair follicles	+	−
Dermis	−	+
Branchiogenic tissues		
Thyroid, thymus, mucous glands	++	−
Infundibulum	+	−
Tongue	−	++
Respiratory system		
Nasal cavity, trachea (epithelia), lung	++	−
Bronchi, bronchioli	−	+
Digestive system		
Pharynx, oesophagus, (epithelia)	++	−
Small intestine, rectum (epithelia)	++	++
Liver *parenchymal cells*	(+)	(+)
hemopoietic foci	++	−
Pancreas	++	+
Urogenital system		
Gonade	+	+
Kidney	+	+
Urether, urinary bladder, urethra		
epithelia	++	−
walls	−	+
Circulatory system		
Heart, vessels	−	++
Skeletal system, connective tissue		
Perichondrium/periosteum	+	++
Ossifying zones		
desmal (skull)	++	++
enchondral (vertebrae)	−	++
Tooth anlage	+	(+)
Bone marrow	++	−
Connective tissue	−	+
Other tissues		
Brown adipose tissue	++	−
Adrenal gland	+	−

The relative expression was estimated by visual inspection. −, signal not above background; (+), low; +, moderate; ++, high.

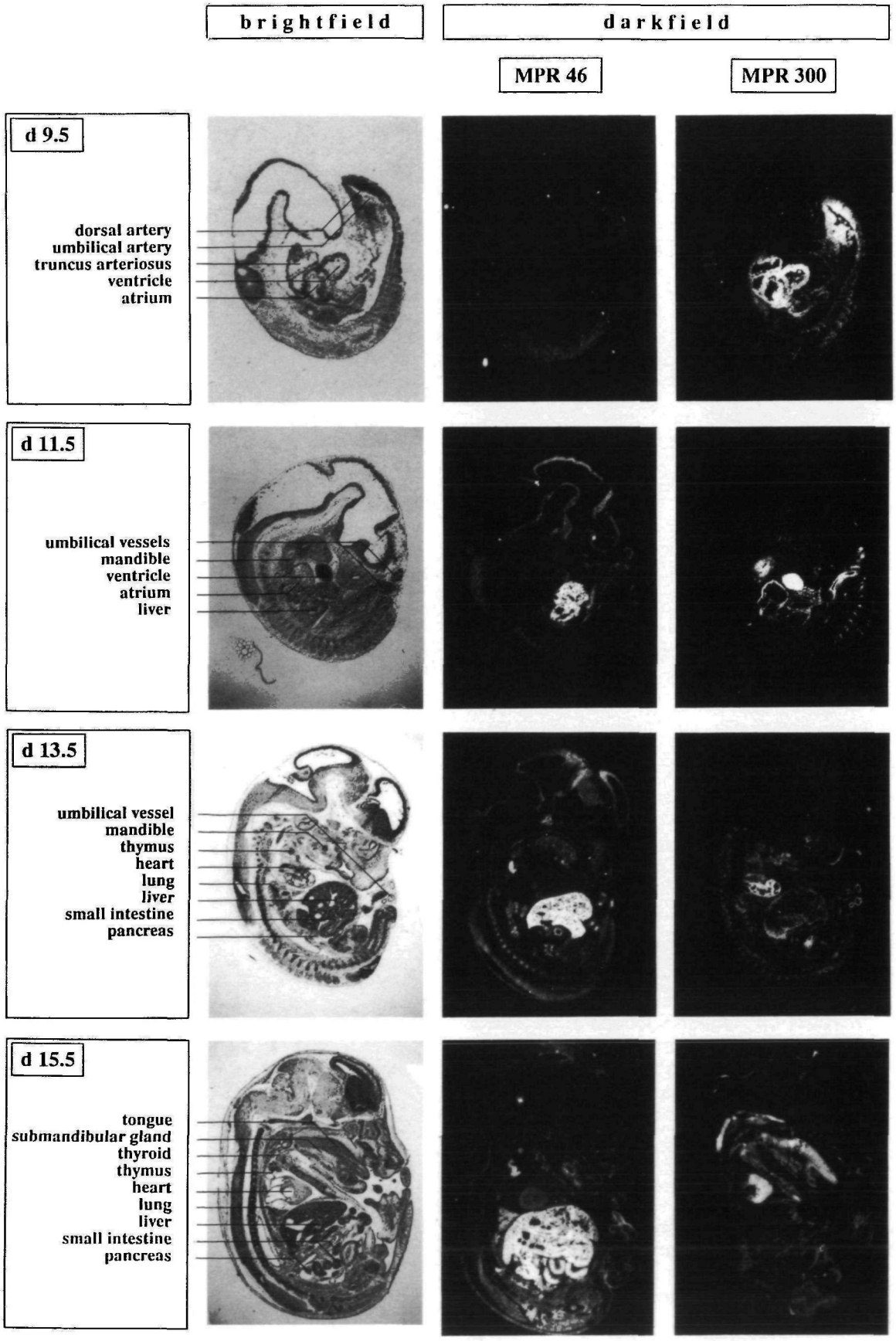

brightfield
darkfield
MPR 46
MPR 300
d 9.5
dorsal artery
umbilical artery
truncus arteriosus
ventricle
atrium
d 11.5
umbilical vessels
mandible
ventricle
atrium
liver
d 13.5
umbilical vessel
mandible
thymus
heart
lung
liver
small intestine
pancreas
d 15.5
tongue
submandibular gland
thyroid
thymus
heart
lung
liver
small intestine
pancreas

Fig. 3. In situ hybridization of MPR46 and MPR300 in mouse embryos of d9.5 to d15.5. Sagittal cryosections were hybridized with antisense and sense ^{35}S-labelled riboprobes for both receptors. The sense probes, which served as a control, yielded no signal under the conditions used for visualizing the sections hybridized to the antisense probes (not shown). The sections are shown under brightfield (left) and darkfield (right) illumination. Tissues with high levels of MPR transcripts appear white under darkfield illumination. The main tissues with MPR expression are indicated on the left.

scripts are detectable in the somites (d9.5), intersomitic clefts (d11.5) and in the liver close to the capsular surface and around blood vessels (d11.5). A strong labelling of the mandible becomes apparent at d11.5. At later stages Meckel's cartilage (d13.5) and tongue (d15.5) show prominent signals for MPR300. The tissues that express MPR300 transcripts at d17.5 (Fig. 4B) are listed in Table 1. Strong labelling is now apparent in the intestinal tract, in smooth and striated muscles, the perichondrium/periosteum and zones of ossification in the vertebrae and the skull.

Complementary pattern of MPR46 and MPR300 expression

During organogenesis an almost mutually exclusive pattern of MPR46 and MPR300 expression was observed in liver, thymus, lung, brown adipose tissue, thyroid, submaxillary and sublingual glands, pituitary gland (all positive for MPR46), tongue and cardiovascular system (positive for MPR300). Even in tissues that express both receptors the expression of MPR46 and MPR300 can often be ascribed to different cell types. For example in the urinary bladder (Fig. 4) expression of MPR46 is restricted to the epithelial layer, while MPR300 transcripts are most abundant in the muscular wall. Further examples are shown in Fig. 5A-D. In the skin (Fig. 5A) the expression of MPR46 is concentrated in the ectodermally derived stratum germinativum and hair follicles, while MPR300 expression is most prominent in mesodermally derived

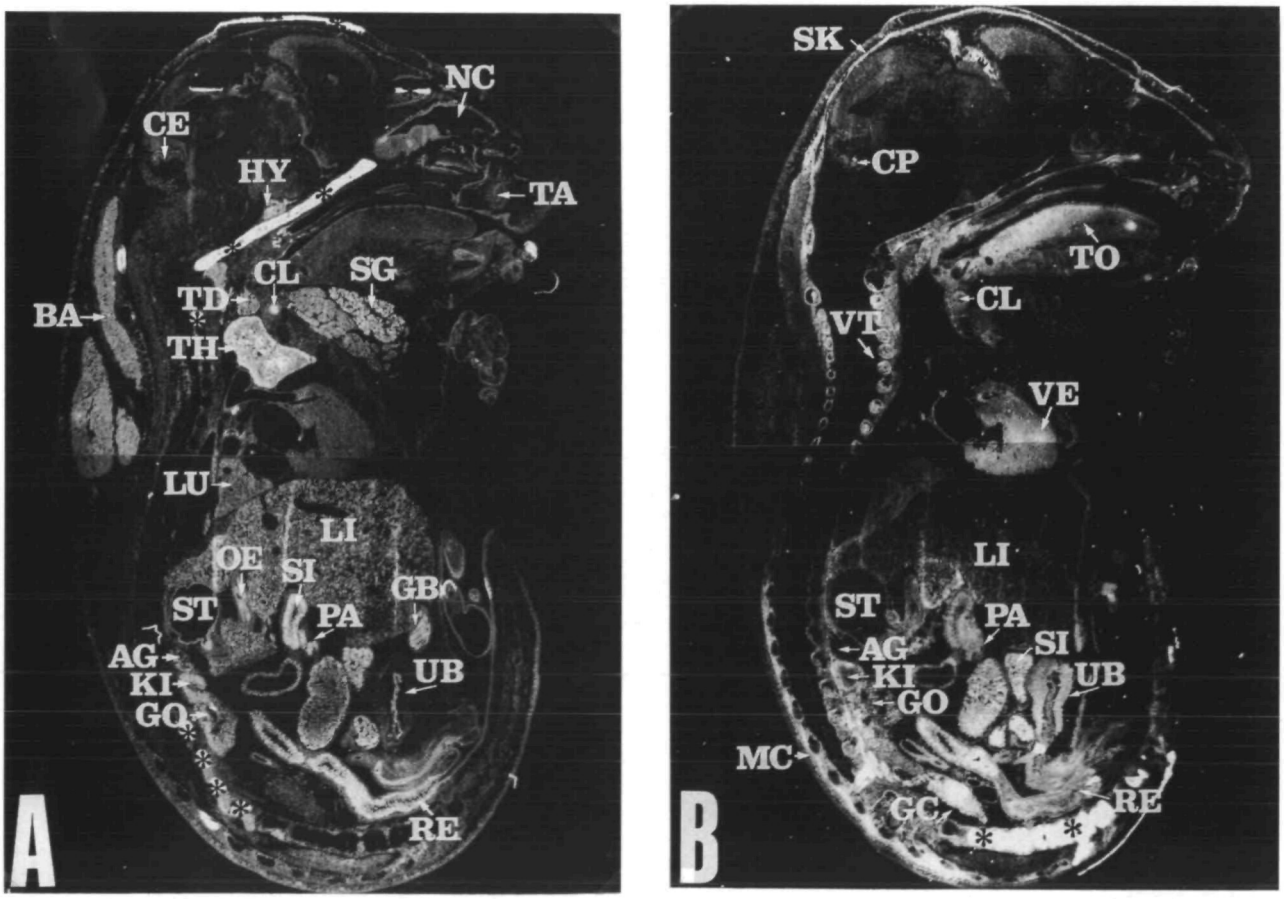


Fig. 4. In situ hybridzation of MPR46 (A) and MPR300 (B) in mouse embryos of d17.5. The sites of major MPR expression are indicated: BA, brown adipose tissue; CL, clavicle; CP, choroid plexus; GB, gall bladder; GC, ganglion coeliacum; GO, gonade; HY, hypophysis; KI, kidney; LI, liver; LU, lung; MC, condensed mesenchymal connective tissue; NC, nasal chamber; OE, oesophagus; PA, pancreas; RE, rectum; SG, submandibulary and sublingual gland; SI, small intestine; SK, skull; AG, adrenal gland; ST, stomach; TD, thyroid; TH, thymus; TO, tongue; UB, urinary bladder; VE, ventricle; VT, vertebra. Artefacts that appear white under darkfield illumination and which are caused by local ablation of the sections from the slide are marked by black asterisks.

layers of condensed connective tissue below the epidermis.

In the intestinal tract (shown for the small intestine in Fig. 5B) the expression of MPR46 and MPR300 is highest in the mucosal layer. MPR46 is most frequent in the epithelia of the forming crypts with significantly lower labelling of the epithelia covering the villi. MPR300 expression is scarce in the crypts, uniformly high in the epithelial layer of the villi and intermediate in the smooth muscle layer.

At zones of enchondral ossification MPR300 transcripts are abundant in the ossifying tissue, while MPR46 transcripts are absent or - if present - restricted to single cells. In the surrounding perichondrium/periosteum a strong ubiquitous expression of MPR300 is found, while the expression of MPR46 is restricted to single cells of unknown nature, which show a massive labelling (see Fig. 5C). In the clavicle (Fig. 4), which is one of the sites of early bone marrow formation, the bone marrow is heavily labelled for MPR46 while MPR300 transcripts are not seen. In the cerebellum at d17.5 (Fig. 5D) the external and internal granular layer are positive for MPR46 while strong MPR300 RNA signals appear above the pia mater and the Purkinje cells.

Fig. 5. Cell-specific expression of MPR46 (left) and MPR300 (right) at d17.5 in skin (A), small intestine (B), ossifying vertebrae (C) and cerebellum (D). (A) Sections of the skin of the back region (brightfield). High levels of MPR46 transcripts are seen in the stratum germinativum (SG) of the epidermis (EP) and in the hair follicles (HF). MPR300 transcripts are abundant in condensed layers of connective tissue (CT). (B) Section of the small intestine (brightfield). MPR46 transcripts are abundant in epithelia of differentiating crypts (CR), while MPR300 transcripts are frequent in epithelia of villi (VI) and in smooth muscle cells (SM) of the intestinal wall. LU, lumen of the gut. (C) Section of a vertebra with beginning enchondral ossification (brightfield). MPR46 transcripts are abundant in single cells located in the perichondrium (PC), while MPR300 transcripts are more frequent in the perichondrium and in the ossifying cartilage (OC). (D) Section of the cerebellum (darkfield). MPR46 transcripts are concentrated in the external (EG) and internal (IG) granular layer, while MPR300 transcripts are seen in the pia mater (PM) and Purkinje cells (PC).

Discussion

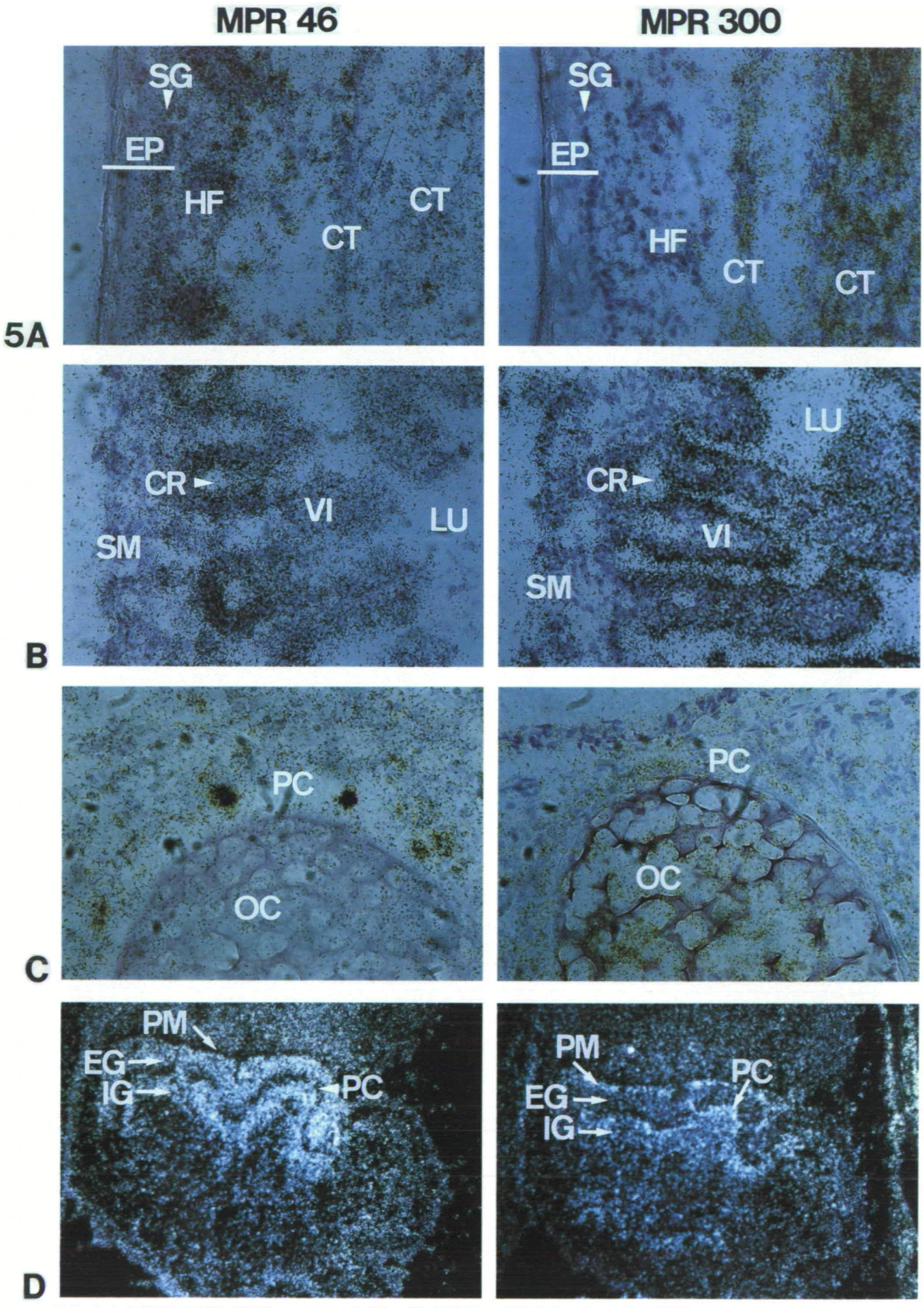
The unexpected finding of this analysis of MPR46 and MPR300 expression during mouse development by in situ hybridization is the spatially and temporally differential expression of the two receptors. Even at late stages of embryogenesis, where MPR transcripts are detectable in most tissues, the expression pattern of the two MPRs is almost complementary. In several tissues that express both MPRs higher resolution showed that expression occurred either in different cell types or at different developmental stages of a cell type (e.g. intestinal mucosa, Fig. 5B). This differential expression pattern strongly argues for specific, yet unidentified functions of the two MPRs during organogenesis in mouse.

Tissues that are active in hemopoiesis and T-cell differentiation were the first that expressed high levels of MPR46 RNA. The MPR46 transcripts became detectable in liver (d11.5), thymus (d13.5) and bone marrow (d17.5) at the developmental stage when these tissues begin their function in hemopoiesis and blood cell differentiation. Hemopoiesis begins in liver at d11 and in bone marrow at d16, while the thymus is formed at d12 and involved in T-cell maturation and selection from d15 onwards (Rugh, 1991; Cristanti et al., 1986). Clearly it will be of interest to analyse the expression, distribution and function of MPR46 in hemopoietic and accessory cells of the bone marrow and thymus to obtain insight in the specific functions of MPR46 in these cells.

At later stages of embryonic development (d15.5-d17.5) MPR46 transcripts are abundant in tissues of endodermal origin (e.g. epithelia of the respiratory, digestive and urogenital tracts, salivary glands, thyroidea, pancreas), but are also found in a few derivatives of the mesoderm (e.g. kidney) and ectoderm (e.g. epidermis). No information is available about the protein level of MPR46 in any of the embryonic tissues.

The expression of the MPR300 during the pre- and the postimplantation period of mouse (Harvey and Kaye, 1991) and rat (Senior et al., 1990; Tollefsen et al., 1989) has been the subject of several studies. Transcripts of the MPR300 are produced only from the maternal chromosome, but not from the paternal (Barlow et al., 1991) and transcription is observed as early as the two cell stage of mouse development (Harvey and Kaye, 1991). Using in situ hybridization of developing rat, MPR300 transcripts were first detected in the extraembryonic membranes. In the embryo proper high levels of expression were observed during organogenesis and histodifferentation in mesoderm-derived tissues such as the cardiovascular system, developing muscle, perichondrium and periosteum and a few endoderm-derived tissues, such as the respiratory endoderm (Senior et al., 1990). This pattern agrees with that in mouse observed in this study. While in rat none of the ectoderm-derived tissues showed MPR300 expression, in mouse MPR300 transcripts were clearly detectable in the cerebellum, plexus choroideus and ganglion coeliacum. Hence MPR300 is expressed in derivatives of all three germ layers as is MPR46.

For a number of tissues high expression of MPR300 transcripts correlates with a high expression of IGFII transcripts (Senior et al., 1990; Tollefsen et al., 1989; Ohlsson et al., 1989). The coordinately regulated expression has suggested a role of the receptor in a paracrine/autocrine function of IGFII. There are however exceptions, and the most notable is liver. The liver parenchyma is one of the major sites of IGFII expression during embryonic development (Beck et al., 1987; Stylianopoulou et al., 1988), but expresses only low levels of MPR300 as shown in this and other studies (Senior et al., 1990).


MPR 46
MPR 300
SG
EP
HF
CT
CT
SG
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5A
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For the cardiovascular system it is known that the high levels of MPR300 transcripts are paralleled by high levels of immunoreactive receptor (Senior et al., 1990; Tollefsen et al., 1989; Sklar et al., 1989). The functional importance of MPR300 for the cardiovascular system is suggested by the following observation. Mice that maternally inherit a deletion of the Tme locus and hence lack MPR300 expression become highly oedematous and die at d15 of embryogenesis (Barlow et al., 1991). The development of severe oedems points to a malfunction of the cardiovascular system, which from d11 onwards maintains a circulation of blood and hence is important for the provision of embryonic tissues with nutrients and the disposal of metabolic waste.

The most pertinent question raised by the present study certainly concerns the role of MPR46 and MPR300 during organogenesis and histodifferentiation. The few data available for the frequency of the two receptors in adult tissues (Wenk et al., 1991) suggest that differential expression patterns are also maintained in the postnatal period and this assumption is supported by preliminary data of in situ hybridization of adult mouse tissues (U. Matzner, unpublished). The additional function of the MPR300 as a receptor for IGFII provides a plausible explanation for the existence of two MPRs only if the evolution of MPR46 is correlated with the acquisition of the IGFII binding site by MPR300. Otherwise yet unknown receptor properties or other known functions that are specific for either type of MPR such as endocytosis and secretion of mannose 6-phosphate-containing ligands, which include growth factors (Kovacina et al., 1989; Dennis et al., 1991; Lee and Nathans 1988), may have necessitated the evolution of two types of MPRs and their differential expression during development.

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References

Barlow, D. P., Stöger, R., Hermann, B. G., Saito, K. and Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature* **349**, 84-87.

Beck, F., Samani, N. J., Penschow, J. D., Thorley, B., Tregear, G. W. and Coghlan, J. P. (1987). Histochemical localization of IGF-I and -II mRNA in the developing rat embryo. *Development* **101**, 175-184.

Canfield, W. M. and Kornfeld, S. (1989). The chicken liver cation-independent mannose 6-phosphate receptor lacks the high affinity binding site for insulin-like growth factor II. *J. Biol. Chem.* **264**, 7100-7103.

Chao, H. H.-J., Waheed, A., Pohlmann, R., Hille, A. and von Figura, K. (1990). Mannose 6-phosphate receptor dependent secretion of lysosomal enzymes. *EMBO J.* **9**, 3507-3513.

Chirgwin, J. J., Przbyla, A. E. and MacDonald, R. J. (1979). Isolation of biological active ribonucleic acid from sources enriched in ribonuclease. *Biochem.* **18**, 5294.

Clairmont, K. B. and Czech, M. P. (1989). Chicken and Xenopus mannose 6-phosphate receptors fail to bind insulin-like growth factor II. *J. Biol. Chem.* **264**, 16390-16392.

Cox, K. H., DeLeon, D. V., Angerer, L. M. and Angerer, R. C. (1984). Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. *Dev. Biol.* **101**, 485-502.

Cristanti, A., Colantoni, A., Snodgrass, R. and von Boehmer, H. (1986). Expression of T cell receptors by thymocytes: in situ staining and biochemical analysis. *EMBO J.* **5**, 2837-2843.

Czech, M. P. (1989). Signal transmission by the insulin-like growth factors. *Cell* **59**, 235-238.

Dahms, N. M., Lobel, P. and Kornfeld, S. (1989). Mannose 6-phosphate receptors and lysosomal enzyme targeting. *J. Biol. Chem.* **264**, 12115-12118.

Dennis, P. A. and Rifkin, D. B. (1991). Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 580-584.

Gabel, C. A., Goldberg, D. E. and Kornfeld, S. (1983). Identification and characterization of cells deficient in the mannose 6-phosphate receptor: evidence for an alternate pathway for lysosomal enzyme targeting. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 775-779.

Gonda, T. J., Sheiness, D. K. and Bishop, J. M. (1982). Transcripts from the cellular homologs of retroviral oncogenes: distribution among chicken tissues. *Molec. Cell. Biol.* **2**, 617-624.

Haig, D. and Graham, C. (1991). Genomic imprinting in the strange case of the insulin-like growth factor II receptor. *Cell* **64**, 1045-1046.

Harvey, M. B. and Kaye, P. L. (1991). IGF-II receptors are first expressed at the 2-cell stage of mouse development. *Development* **111**, 1057-1060.

Holland, P. (1986). Localization of gene transcripts in embryo sections: in situ hybridization with RNA probes. In *Manipulating the Mouse Embryo* (ed. B. von Hogan, F. Constantini, E. Lacy). pp. 228-242. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Köster, A., Nagel, G., von Figura, K. and Pohlmann, R. (1991). Molecular cloning of the mouse 46-kDa mannose 6-phosphate receptor (MPR46). *Biol. Chem. Hoppe-Seyler* **372**, 297-300.

Kornfeld, S. and Mellmann, I. (1989). The biogenesis of lysosomes. *A. Rev. Cell Biol.* **5**, 483-525.

Kovacina, K. S., Steele-Perkins, Purduo, A. F., Lioubin, M., Miyazono, K., Heldin, C. H. and Roth, R. A. (1989). Interactions of recombinant ant platelet transforming growth factor-beta 1 precursor with the insulin-like growth factor II/mannose 6-phosphate receptor. *Biochem. Biophys. Res. Commun.* **160**, 393-403.

Lee, S. J. and Nathans, D. (1988). Proliferin secreted by cultured cells binds to mannose 6-phosphate receptors. *J. Biol. Chem.* **263**, 3521-3527.

Mainferme, F., Wattiaux, R. and von Figura, K. (1985). Synthesis, transport and processing of cathepsin C in Morris hepatoma 7777 cells and rat hepatocytes. *Eur. J. Biochem.* **153**, 211-216.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Morgan, D. O., Edman, J. C., Standring, D. N., Fried, V. A., Smith, M. C., Roth, R. A. and Rutter, W. J. (1987). Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature* **329**, 301-307.

Murayama, Y., Okamoto, T., Ogata, E., Asano, T., Jiri, T., Katada, T., Ui, M., Grubb, J. H., Sly, W. S. and Nishimoto, J. (1990). Distinctive regulation of the functional linkage between the human cation-independent mannose 6-phosphate receptor and GTP-binding proteins by insulin-like growth factor II and mannose 6-phosphate. *J. Biol. Chem.* **265**, 17456-17462.

Ohlsson, R., Holmgren, L., Glaser, A., Szpecht, A. and Pfeifer-Ohlsson, S. (1989). Insulin-like growth factor II and short-range stimulatory loops in control of human placental growth. *EMBO J.* **8**, 1993-1999.

Oshima, A., Nolan, C. M., Kyle, J. W., Grubb, J. H. and Sly, W. S. (1988). The human cation-independent mannose 6-phosphate receptor. Cloning and sequence of the full-length cDNA and expression of functional receptor in COS cells. *J. Biol. Chem.* **263**, 2553-2562.

Roth, R. A. (1988). Structure of the receptor for insulin-like growth factor II: the puzzle amplified. *Science* **239**, 1269-1271.

Rugh, R. (1991). *The Mouse: Its Reproduction and Development*, Oxford University Press, Oxford.

Senior, P. V., Byrne, S., Brammar and Beck, F. (1990). Expression of

the IGF-II/mannose-6-phosphate receptor mRNA and protein in the developing rat. *Development* **109**, 67-73.

Sklar, M. M., Kiess, W., Thomas, C. L. and Nissley, S. P. (1989). Developmental expression of the tissue insulin-like growth factor II/mannose 6-phosphate receptor in the rat. Measurement by quantitative immunoblotting. *J. Biol. Chem.* **264**, 16733-16738.

Stylianopoulou, F., Efstradiadis, A., Herbert, J. and Pintar, J. (1988). Pattern of the insulin-like growth factor II gene expression during rat embryogenesis. *Development* **103**, 497-506.

Tollefsen, S. E., Sadow, J. L. and Rotwein, P. (1989). Coordinate expression of insulin-like growth factor II and its receptor during muscle differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1543-1547.

Wenk, J., Hille, A. and von Figura, K. (1991). Quantitation of M_r 46000 and M_r 300000 mannose 6-phosphate receptors in human cells and tissues. *Biochemistry International* **23**, 723-732.

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