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Expression of the L14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development

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Summary

A cDNA encoding L14, the lactose-binding, soluble lectin of relative molecular mass 14 × 10^3, has been isolated in a differential screen designed to identify genes that are regulated during the differentiation of murine embryonic stem cells in vitro. The expression patterns of the gene and of the encoded protein during mouse embryogenesis are consistent with the lectin playing a role at several stages of development. Firstly, it is initially synthesised in the trophectoderm of expanded blastocysts immediately prior to implantation, suggesting that it may be involved in the attachment of the embryo to the uterine epithelium. Secondly, in the postimplantation embryo, the lectin is abundantly expressed in the myotomes of the somites. This observation, when taken together with data indicating a role for the lectin in myoblast differentiation in culture, suggests that the protein is important in muscle cell differentiation. Finally, within the nervous system expression of this gene is activated early during the differentiation of a particular subset of neurones.

Key words: L14 lectin, mouse development, in situ hybridisation, implantation, muscle differentiation, nervous system, mesenchyme

Introduction

Lectins are generally defined as non-enzymatic, non-immunoglobulin proteins, which bind selectively to specific carbohydrate structures (Goldstein et al., 1980). Many of the lectins isolated from vertebrate tissues fall into one of two distinct structural classes (Drickamer, 1988). The first comprises a large family of integral membrane and secreted proteins that require Ca^{2+} for carbohydrate binding. The second class consists of smaller, Ca^{2+}-independent lectins characterised by two important properties that led to their original isolation: solubility in aqueous solution and preferential binding of lactosamine-based structures. This latter class of molecule has been given several names: soluble lectins (Barondes, 1984), S-type lectins (Drickamer, 1988) and S-Lac lectins (Leffler et al., 1989). The size of this class is not yet established but in the rat it encompasses at least four members with relative molecular masses of 14, 17, 18 and 29 × 10^3 (Leffler and Barondes, 1986; Leffler et al., 1989). The best characterised of these is the smallest, L14, which is neither phosphorylated nor glycosylated and appears to be a dimer in vivo (Sparrow et al., 1987). Like a number of other proteins that are found outside the cell (Muesch et al., 1990), L14 lacks a discernible signal peptide and it has been shown that it is externalized by a novel mechanism (Cooper and Barondes, 1990).

The expression patterns of carbohydrate-binding proteins are of particular interest in the context of development, since potential ligand molecules, i.e. glycoconjugates, are known to be regulated. The rapidity and complexity of the changes in carbohydrate residues suggest that they play important roles during embryogenesis (reviewed by Muramatsu, 1988; Thorpe et al., 1988; Fenderson et al., 1990). During fertilization, the binding of sperm to the egg depends upon oligosaccharides carried on the ZP-3 glycoprotein of the zona pellucida (Wassarman, 1987; Bleil and Wassarman, 1988). Similarly, glycoconjugates are implicated in the process of compaction in which adhesion between individual blastomeres greatly increases at the morula stage (Surani, 1979; Atienza-Samols et al., 1980; Rastan et al., 1985). More specifically, the stage-specific embry-

onic antigen 1 (SSEA1) has been shown to play a role in stabilising compaction (Bird and Kimber, 1984; Fenderson et al., 1984) and it is highly regulated both in space and time throughout embryogenesis, suggesting that it may also be important at several other stages (Richa and Solter, 1986). The carbohydrate moieties of glycoproteins, glycolipids and proteoglycans, which together comprise an immense array of structural conformations, may be directly involved in such cell surface functions as adhesion and migration or they may provide signals necessary for morphogenesis. Such carbohydrate-dependent processes can be mediated either by interactions among glycoconjugates or by recognition by carbohydrate-binding proteins such as lectins. Indeed, several studies on the expression of various soluble lectins in the developing slime mould and in *Xenopus* and chick embryos have suggested that lectins may be important during the development of these species (reviewed by Zalik and Milos, 1986).

One possible function for soluble lectins is suggested by the fact that L14 (Zhou and Cummings, 1990; Cooper et al., 1991), and other proteins of the same family (Mecham et al., 1989; Woo et al., 1990), bind specifically to the oligosaccharides carried on the extracellular matrix protein laminin. Indeed, it has recently been shown that in an in vitro culture system L14 can regulate myoblast detachment from laminin, thereby promoting the formation of myofibres (Cooper et al., 1991). These data raise the possibility that one of the in vivo functions of this lectin is to play a role in cell-matrix interactions.

While it has generally been assumed that the principal roles of carbohydrate-protein recognition events are in cell-cell or cell-substratum interactions, both glycoconjugates (Hart et al., 1988) and lectins (Hubert et al., 1989) are also found in the cytoplasmic and nuclear compartments. For example, L14 is intracellular in myoblasts and extracellular in myotubes (Barondes and Haywood-Reid, 1981; Cooper and Barondes, 1990), while another soluble lectin is found in ribonucleoprotein complexes (Laing and Wang, 1988). Glycoconjugates and carbohydrate-binding proteins may therefore also participate in processes other than those taking place at the cell surface. Furthermore, the carbohydrate recognition and binding properties of lectins may only represent part of their functional capabilities (Barondes, 1988). This has already been shown for two soluble lectins, the slime mould lectin discoidin (Gabius et al., 1985) and the mammalian elastin receptor (Hinek et al., 1988), both of which have protein-binding activity. The work of Wells and Mallucci (1991) suggests that L14 itself may be bifunctional. These authors identified an extracellular factor that exerts a cytostatic effect on cultured mouse fibroblasts as L14 and showed that its effect on the cell cycle is apparently not mediated via carbohydrate binding. Earlier work had suggested that this lectin is mitogenic for certain cell types (Lipsick et al., 1980; Pitts and Yang, 1981), and it has recently been reported that it can act as a transforming growth factor (Yamaoka et al., 1991), raising the possibility that it can have opposite effects on cell proliferation depending on the cell type used and the experimental conditions, as is well established to be the case for TGF-β (see review by Moses et al., 1990).

We have been interested in isolating genes that are regulated during the early stages of mouse development, more specifically at the time of implantation. Our approach exploited the properties of embryonic stem (ES) cells (Evans and Kaufman, 1981; Martin, 1981) which can differentiate in vitro following a programme that mimics events occurring during early mouse embryogenesis. Using differential cDNA cloning techniques, we have isolated a number of genes that are regulated during ES cell differentiation (Poirier et al., 1991) and are therefore likely to be important during embryogenesis. One of these encodes L14. We have performed a systematic in situ hybridisation analysis of the expression of this gene and determined the distribution of the encoded protein in developing mouse embryos. Our results indicate that this lectin may play a role at several stages of embryogenesis, most notably in the process of implantation, during muscle development and in the differentiation of particular classes of neurones.

Materials and methods

Recombinant plasmids

The cDNA clone LC1 was isolated from a λgt10 cDNA library constructed from poly(A)$^+$ cytoplasmic RNA from ES cells and shown to hybridise to a 0.6kb mRNA which is present in ES cells but strongly down-regulated upon differentiation to embryoid bodies (Poirier et al., 1991) LC1 was used to rescreen the ES cell cDNA library and we thus isolated a longer clone, LC1.13.

LC1.13 was used to screen a cosmid library of strain 129/Sv mouse DNA in the vector pcos2EMBL (the kind gift of B.G. Herrmann) and two overlapping cosmid clones, pcos 1.4 and pcos 1.2, were isolated Both contain the entire coding sequence of the gene.

Nucleotide sequence determination

Nucleotide sequences were determined in both orientations using the dideoxynucleotide termination method of Sanger et al (1977) as modified for double-stranded templates. The *Eco*RI insert fragments of LC1.13 were sub-cloned into the plasmid Bluescript KS$^+$ and clones with overlapping deletions were generated using exonuclease III and mung bean nuclease (Henikoff, 1984). As none of the cDNAs extended to the 5′ end of the mRNA, we also sequenced an *Apa*I-*Xba*I fragment derived from pcos 1.2 which contains the first exon of the gene (C.-T. J. C. and P. W. J R., unpublished data).

Mouse strains

C57BL/6Pas, SPE/Pas (an inbred strain of the *Mus spretus* species) and the interspecific backcross progeny (C57BL/6 × SPE) F_1 × C57BL/6 were raised in Institut Pasteur, Paris.

Probes

The probe used for northern blots and for in situ hybridisation was a T7 RNA polymerase transcript of a 247 base pair fragment at the 3′ end of the cDNA.

Northern blot analysis

All RNA samples were prepared by precipitation with LiCl (Auffray and Rougeon, 1979) RNA was electrophoresed in 1.5% (w/v) agarose gels containing 20 mM MOPS, pH 7.0, 5 mM sodium acetate, 1 mM EDTA and 0.7% (w/v) formaldehyde (Maniatis et al., 1982) and transferred to Amersham Hybond-N nylon paper

For antisense RNA probes, hybridisation was carried out in 60% (v/v) formamide, 1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10× Denhardt's solution, 1% (w/v) SDS, 0.1% (w/v) sodium pyrophosphate, 100 μg/ml yeast RNA, 10% (w/v) dextran sulphate at 65°C. Filters were washed in 0.2× SSC, 0.2% (w/v) SDS at 70°C for 1 hour

For oligolabelled cDNA probes (Feinberg and Vogelstein, 1983), hybridisation was performed in 50% (v/v) formamide, 50 mM sodium phosphate, pH6.8, 2× Denhardt's solution, 0.5× SSC and 0.05% (w/v) SDS at 42°C Filters were washed in 0.2× SSC, 0.2% (w/v) SDS at 65°C for 1 hour

In situ hybridisation

The procedure of Wilkinson and Green (1990) was used throughout. Sections of morula-stage embryos were prepared by processing whole oviducts at 2 5 days post coitum (dpc). Blastocysts at 3 5 dpc and expanded blastocysts at 4.5 dpc were flushed from the uterus, fixed for 30 minutes in 4% (w/v) paraformaldehyde/phosphate-buffered saline (PBS), and introduced into the swollen oviductal ampulla of a host female on the day after mating (procedure of Copp, 1978). The host oviduct, containing embryos at 0 5 dpc plus reintroduced embryos at 3.5 or 4.5 dpc, was then processed and sectioned as a whole. Spinal cords were dissected from newborn and adult animals after perfusion with 4% paraformaldehyde/PBS. 6 μm paraffin wax sections were hybridised with ^{35}S-labelled antisense RNA probe. Autoradiographic exposure times were either 4 or 7 days, except as detailed in the legend to Fig 3.

Immunohistochemistry

Embryos were fixed in 4% (w/v) paraformaldehyde for either 30 minutes (blastocysts) or 3 hours (10.5 dpc embryos) Blastocysts were introduced in the swollen ampulla (see above) and fixed again for a further 30 minutes. Samples were transferred to a solution of 30% (w/v) sucrose in 0.1 M sodium phosphate buffer, pH 7 5, and incubated overnight prior to embedding in Tissue Tek 13 μm frozen sections were prepared

The anti-L14 antibody used in these experiments was an affinity purified rabbit serum raised against the rat protein (Cooper and Barondes, 1990) and was the kind gift of D.N.W Cooper (University of California, San Francisco).

Detection of the lectin in frozen sections was performed using a 1:250 dilution of the affinity purified serum and fluorescein-conjugated, goat anti-rabbit antiserum according to the procedure of Dodd et al. (1988).

Results

Isolation and sequence of LC1 cDNA

We have used differential screening of a cDNA library constructed from poly(A)$^+$ cytoplasmic RNA from ES cells to isolate clones corresponding to mRNAs which are expressed in ES cells and down-regulated during in vitro differentiation. One of these clones, named LC1, hybridises to a 0.6kb transcript which is abundant in ES cells but barely detectable in embryoid bodies (Poirier et al., 1991). The complete nucleotide sequence of this mRNA, derived from both cDNA and genomic clones, is shown in Fig. 1. Computer-assisted searching of the databases showed that this sequence corresponds to L14, a soluble lectin that has been previously characterised in a number of species: mouse (Wilson et al., 1989; Cooper and Barondes, 1990; Wells and Mallucci, 1991), rat (Clerch et al., 1988; Hynes et al., 1990), human (Hirabayashi et al., 1988; Couraud et al., 1989), cow (Abbott et al., 1989) and chick (Ohyama et al., 1986; Hirabayashi et al., 1987). The sequence contains a single open reading frame of 405 nucleotides, which can encode a protein of relative molecular mass 14.9 × 10^3. This protein sequence does not contain a signal peptide. The gene encoding this protein has been assigned the symbol *Lect14*.

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1   CGTCTCTCGGGTGGAGTCTT CTGACTGCTGGTGGAGCAGG TCTCAGGAATCTCTTCGCTT   60

61  CAGCTTCAATCATGGCCTGT GGTCTGGTCGCCAGCAACCT GAATCTCAAACCTGGGGAAT  120
                M  A  C   G  L  V  A  S  N  L   N  L  K  P  G  E

121 GTCTCAAAGTTCGGGGAGAG GTGGCCTCGGACGCCAAGAG CTTTGTGCTGAACCTGGGAA  180
    C  L  K  V  R  G  E   V  A  S  D  A  K  S   F  V  L  N  L  G  K

181 AAGACAGCAACAACCTGTGC CTACACTTCAATCCTCGCTT CAATGCCCATGGAGACGCCA  240
      D  S  N  N  L  C   L  H  F  N  P  R  F   N  A  H  G  D  A  N

241 ACACCATTGTGTGTAACACC AAGGAAGATGGGACCTGGGG AACCGAACACCGGGAACCTG  300
      T  I  V  C  N  T   K  E  D  G  T  W  G   T  E  H  R  E  P  A

301 CCTTCCCCTTCCAGCCCGGG AGCATCACAGAGGTGTGCAT CACCTTTGACCAGGCTGACC  360
      F  P  F  Q  P  G   S  I  T  E  V  C  I   T  F  D  Q  A  D  L

361 TGACCATCAAGCTGCCAGAC GGACATGAATTCAAGTTCCC CAACCGCCTCAACATGGAGG  420
      T  I  K  L  P  D   G  H  E  F  K  F  P   N  R  L  N  M  E  A

421 CCATCAACTACATGGCGGCG GATGGAGACTTCAAGATTAA GTGCGTGGCCTTTGAGTGAA  480
      I  N  Y  M  A  A   D  G  D  F  K  I  K   C  V  A  F  E

481 GCCAGCCAGCCTGTAGCCCT CAATAAAGGCAGCTGCCTCT GCTCCCCATATAAAAAAAAA  540

541 AAAAA  545
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Fig. 1. Nucleotide 1 corresponds to the cap site. The 545 nucleotides include an open reading frame of 384 nucleotides with an ATG codon at position 72 and a stop codon at position 477. The sequence of this cDNA is identical to the partial murine sequence of Wilson et al. (1989) except at position 499 (−C) in the non-coding region. However, it differs from the sequence of Wells and Mallucci (1991) as follows: T not C at 304, C not T at 308, 317, C not A at 401, +A at 507 and T not A at 531 The difference at 304 leads to a change of a phenylalanine codon to serine but the changes at 308, 316 and 401 only represent third position conservative changes of the proline codon while those at 507 and 531 are in the non-coding region

Mapping of Lect14 *to chromosome 15*

High molecular weight DNA samples were prepared from the spleens of 65 (*Mus spretus* × C57BL/6)F$_1$ × C57BL/6 backcross animals. DNA was digested with *Sau*3A, an enzyme that allowed the detection of a restriction fragment length polymorphism, using the cDNA as a probe, between the *Mus spretus* DNA (a 5

Table 1. *Linkage data for* Lect14

	Distribution of haplotypes in the (C57BL/6×SPE) F_1×C57BL/6 progeny															
Pvt-1	B	S	B	S	B	S	B	S	B	S	B	S	S	B	B	S
Pva	B	S	B	S	B	S	S	B	B	S	S	B	B	S	S	B
Lect14	**B**	**S**	**B**	**S**	**S**	**B**	**S**	**B**	**S**	**B**	**B**	**S**	**B**	**S**	**B**	**S**
D15Pas3	B	S	S	B	S	B	S	B	B	S	B	S	S	B	S	B
Numbers	24	33	0	1	0	1	3	2	0	0	1	0	0	0	0	0

A panel of 65 DNA samples prepared from the offspring of a (C57BL/6×*Mus spretus*) F_1×C57BL/6 backcross have been checked for their phenotypes with the four DNA probes *Pvt-1* (Banerjee et al , 1985), *Pva* (Zulke et al., 1989), *Lect14* and *D15Pas3* (anonymous DNA segment). When considered altogether these data give a linear arrangement of the genes as follows:
– *Pvt-1* – 9.2±3 5 cM – *Pva* – 3.0±2.1 cM – *Lect14* – 1 5±1.5 cM – *D15Pas3* –

kb fragment) and the C57BL/6 DNA (a 4.5 kb fragment). The 5 kb fragment was thus considered diagnostic for the *Mus spretus* contribution and the DNA from each individual backcross mouse was then typed as heterozygous when this fragment was detected or homozygous when it was not. The pattern of the segregation of the 5 kb fragment among the 65 animals was then matched to previously collected data to find evidence for linkage (Guénet et al., 1988). We found that the *Lect14* gene maps to chromosome 15, to a locus 3.0±2.1 cM distal of *Pva* (Zuhlke et al., 1989) (Table 1). So far this site is not associated with any known mutation.

Northern blot analysis of Lect14 *expression*

We initially studied the expression pattern of the *Lect14* gene in both embryos and adult tissues by northern blotting. Fig. 2 shows that the *Lect14* transcript, of about 0.6 kb, was barely detectable in RNA from embryos at 9.5 days post coitum (dpc) but subsequently increased in abundance until 12.5 dpc. The high level of transcript detected at this stage was maintained throughout the rest of embryonic development. The gene was expressed in adult tissues including thymus, kidney, heart, muscle, lung and testis, and at lower levels in intestine, spleen and stomach. Very low amounts of mRNA were detected in adult brain, salivary gland and liver.

We performed a detailed in situ hybridisation analysis in order to study expression during earlier stages of embryogenesis and to establish the cell-type specificity of the *Lect14* transcripts detected in whole embryo and organ RNA. We have chosen to present a detailed account of three aspects of *Lect14* expression during embryogenesis, namely its regulation prior to gastrulation, during the second half of gestation and in the developing nervous system.

Expression of Lect14 *before gastrulation*

Fig. 3 shows the expression of this gene during the first seven days of development. No hybridisation signal was observed over fertilized eggs (0.5 dpc, panels 1,2), morulae (2.5 dpc, panels 3,4) or early blastocysts (3.5 dpc, panels 5,6). The first detectable *Lect14* transcripts appeared in the trophectoderm cells of hatched blastocysts at 4.5 dpc; the inner cell mass (ICM) cells of these

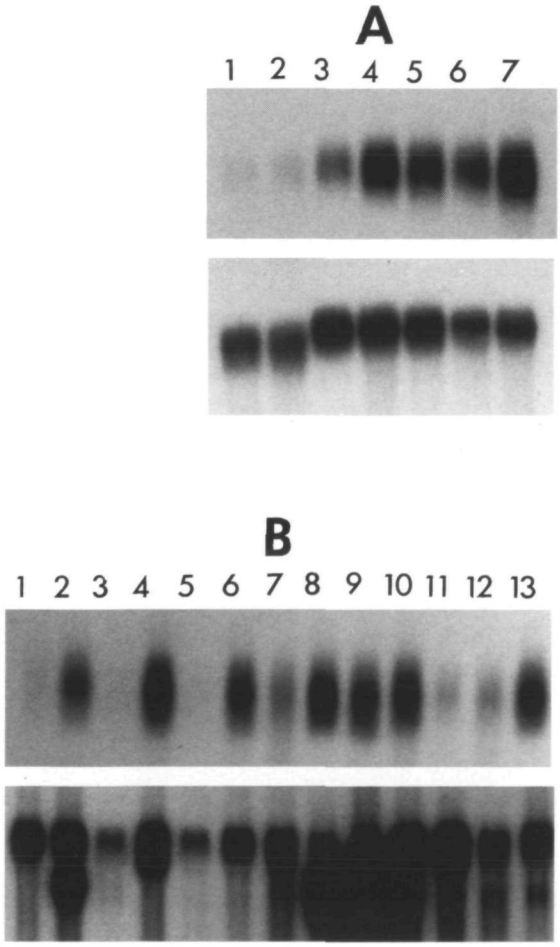


Fig. 2. RNA blot analysis of *Lect14* expression during embryogenesis and in adult tissues. (A) Expression during embryogenesis. 10 μg of total RNA were loaded in each lane. The filter was hybridized with an α-tubulin probe as a control (lower panel). (1) 9.5 dpc, (2) 10 5 dpc, (3) 11 5 dpc, (4) 12.5 dpc, (5) 13.5 dpc, (6) 14.5 dpc, (7) 17.5 dpc. (B) Expression in adult tissues 10 μg of total RNA were loaded in each lane.(1) brain, (2) eye, (3) salivary glands, (4) thymus, (5) liver, (6) kidney, (7) spleen, (8) heart, (9) skeletal muscle, (10) lung, (11) intestine, (12) stomach, (13) testis.

expanded blastocysts remained negative (panels 7,8). Moreover, immunofluorescence studies show that L14, the lectin encoded by the *Lectl4* gene, accumulates in the trophectoderm cells of the 4.5 dpc blastocyst whereas it is not present in the cells of the ICM, nor in any cells of earlier embryos (Fig. 4, panels 1 to 4). Hatching of the blastocyst from its zona pellucida immediately precedes attachment of the trophectoderm cells to the uterine epithelium at the site of implantation. The cells of this epithelium do not contain detectable *Lectl4* mRNA (data not shown).

At 5.5 dpc, when implantation is complete, *Lectl4* transcripts were found in the ectoplacental cone, the trophoblastic giant cells and, to a lesser extent in the extraembryonic ectoderm of the conceptus (Fig. 3, panels 9,10). The pattern of expression in these trophectoderm-derived cell types is similar at 6.5 dpc (Fig. 3, panels 11,12). In contrast, there was no detectable expression at these stages in any of the ICM-derived cells which give rise to the embryo proper. During subsequent development, expression of *Lectl4* persisted in certain extraembryonic tissues of the conceptus. In particular, we observed expression in the placenta, the amnion and the mesoderm of the visceral yolk sac (VYS) at 12.5 dpc, while the VYS endoderm and the parietal endoderm were both devoid of *Lectl4* transcripts at this time (data not shown).

Expression of Lect14 *during the second half of gestation*

Fig. 5 shows that in a 10.5 dpc embryo high levels of *Lectl4* RNA were specifically found in the myotome of each somite and in the wall of the dorsal aorta. These sites were also positive in adjacent sections which were hybridised with a probe for cardiac α-actin (Sassoon et al., 1988) in order to identify the myotome cells (not shown). Low levels of *Lectl4* expression were detectable in most of the other cells of the embryo at this stage, except for the brain and neural tube which were negative. We again observed that the distribution of the protein parallels that of the mRNA. In 10.5 dpc embryos only the myotome region of each somite contained detectable lectin immunoreactivity (Fig. 4, panels 5 to 7).

The expression pattern of *Lectl4* at later stages of development is illustrated at 14.5 dpc in Fig. 6. Panels 1 and 2 show that most organs contain *Lectl4* transcripts and that these are differentially expressed by the constituent tissues. In the kidney, gut and lung (also see panels 3,4), it is clear that expression is high in mesodermal cell types, but undetectable in endodermal (epithelial) cells. A more homogeneous signal distribution was observed over the liver and muscle blocks.

The only mesodermal tissue where we did not detect *Lectl4* expression at this stage is cartilage. The specific down-regulation that occurs when this tissue differentiates from mesenchyme is exemplified in the development of the vertebral column. In the 10.5 dpc embryo the cells of the sclerotomes contain low levels of *Lectl4* transcripts (Fig. 6). When these cells differentiate to form the cartilaginous vertebrae and the intervertebral discs, only the latter continue to express this gene (Fig. 6, panels 3,4). In addition, we did not detect *Lectl4* RNA in the chondrifying vertebrae, ribs and appendicular skeleton (data not shown).

Lectl4 transcripts do not accumulate in the epidermis or in the majority of the cells of the brain and spinal cord (see below). Thus most cells of ectodermal origin are devoid of *Lectl4* transcripts.

Expression of Lect14 *in the developing nervous system*

Strong hybridisation signal was detected in the ventral horns of embryonic and adult mouse spinal cord, over regions that are occupied by motor neurones (Fig. 7). This restricted pattern of expression is already established at 12.5 dpc, when neuronal differentiation has just begun (panels 3,4). It is maintained throughout embryogenesis (panels 5,6), in the newborn (panels 7,8) and in the adult animal (panels 9,10). A section through the brain stem of a 12.5 dpc embryo reveals that the signal is also restricted to motor neurones in this area of the central nervous system (panels 1,2).

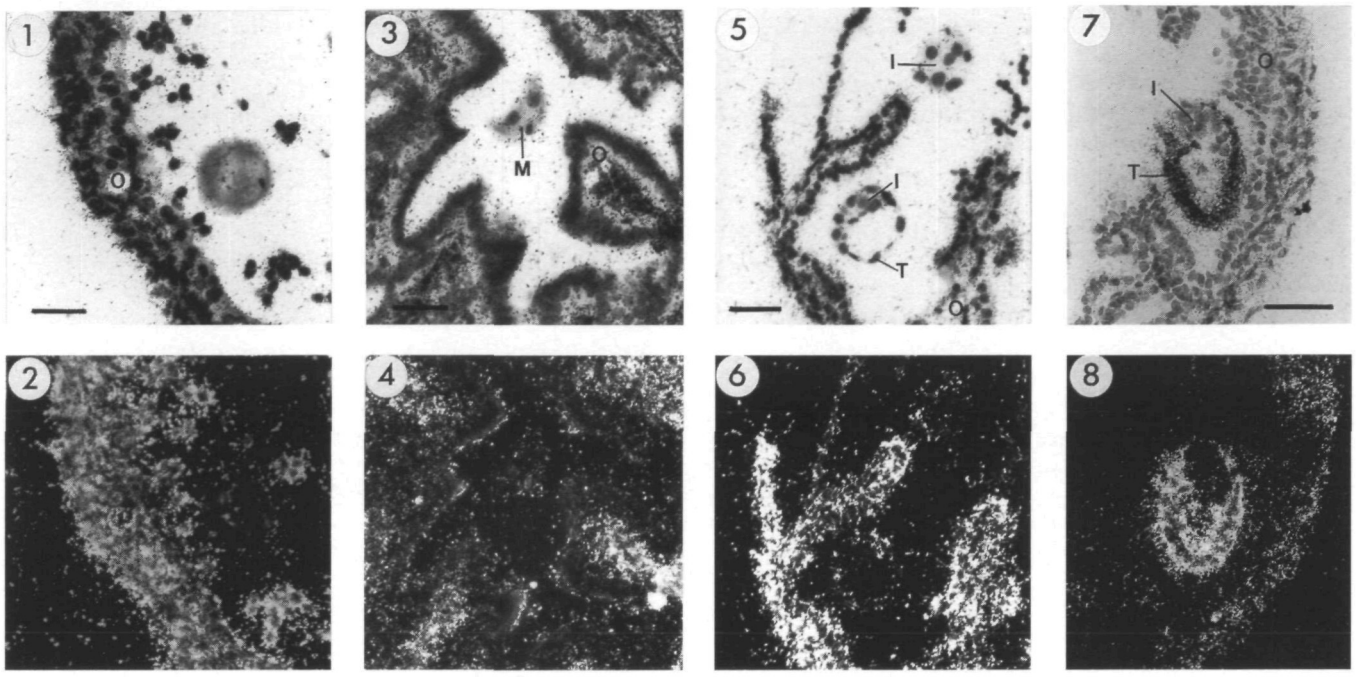
Lectl4 transcripts were also detected in the sensory neurones of the dorsal root ganglia (DRG) of embryos and newborn animals (panels 3 to 8). The density of grains was very high but not uniform, the strongest signal being associated with neurones of small and intermediate diameter.

Discussion

The present study provides the first evidence that soluble lectins may play a role in mammalian development at stages as early as implantation. The possible importance of soluble lectins during early embryogenesis has already been shown in two other species. In *Xenopus* embryos a soluble lectin of relative molecular mass 43×10^3 is first detected in fertilised eggs and later secreted into the extracellular matrix during gastrulation (Roberson and Barondes, 1983; Outenreath et al., 1988), while in chick embryos two similarly sized lactose-binding proteins (one of them being L14) are detected in some migrating cells during gastrulation (Zalik et al., 1987, 1990; Levi and Teichberg, 1989). These authors have suggested that interactions between lectins and associated glycoconjugates may play a role in the dynamic changes of the extracellular environment during embryogenesis in these species. Our work indicates that this may also be true during mouse development.

Isolation of the Lect14 *gene*

We isolated the *Lectl4* cDNA by virtue of the fact that the corresponding mRNA is expressed at high levels in ES cells and is strongly down-regulated during in vitro differentiation to embryoid bodies (Poirier et al., 1991). Our in situ hybridisation analyses show that the gene is first activated in the trophectoderm of late blastocysts and that it is not detectably expressed in the ICM cells, or in any of their immediate derivatives. Although ES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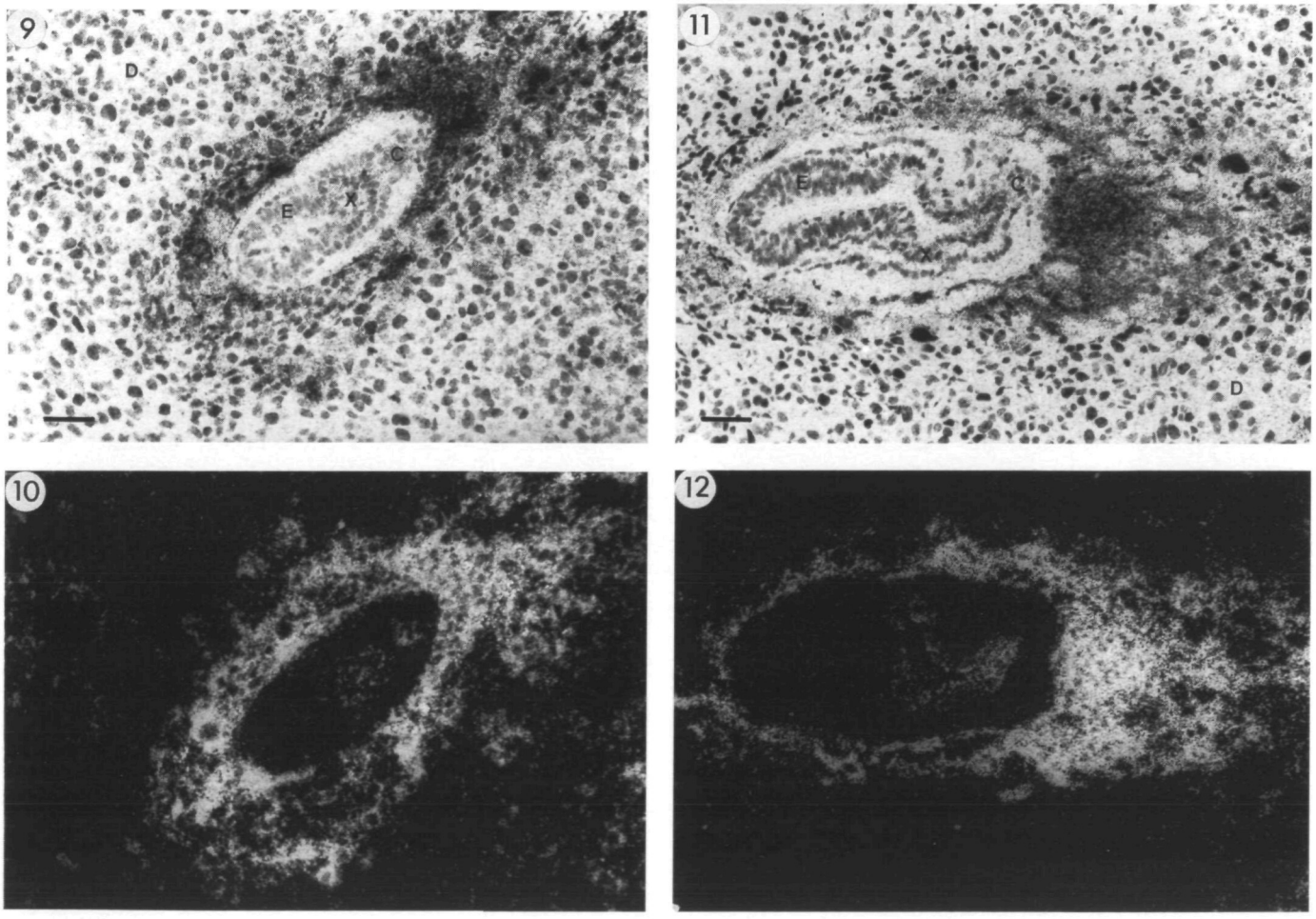
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Fig. 3. In situ hybridisation of ^{35}S-labelled antisense *Lect14* RNA probe to sections of mouse embryos from fertilisation to gastrulation. Even-numbered panels are dark-field views of the sections shown in the corresponding odd-numbered bright-field panels. Panels 1-8 show embryos at various preimplantation stages. The surrounding tissue in these panels is oviduct (see Materials and Methods). Panels 9-12 show implanted embryos within their decidua. (1,2) 0.5 dpc; (3,4) 2.5 dpc; (5,6) 3.5 dpc (early blastocyst); (7,8) 4.5 dpc (late, expanded blastocyst); (9,10) 5.5 dpc; (11,12) 6.5 dpc. C, ectoplacental cone; D, deciduum; E, embryonic ectoderm; I, inner cell mass; M, morula stage embryo; O, oviduct; T, trophectoderm; X, extraembryonic ectoderm. Scale bars=50 μm. Autoradiographic exposure times were 4 days for panels 7-12 and 2 weeks for panels 1-6.

cells have the same developmental potential as the cells of the ICM once introduced into an embryo (Bradley et al., 1984), these results, and others that we have obtained (Poirier et al., 1991), indicate that the maintenance of ES cells in culture perturbs gene expression. It is possible that expression of the *Lect14* gene in ES cells is functionally significant in that L14 may play a role in attachment to the substratum in vitro.

Receptor-carbohydrate interactions in implantation

Lect14 transcripts are not detectable during the first four days of gestation but the gene is activated by 4.5 dpc in the trophectoderm cells of the hatched blastocyst, the ICM cells remaining negative. We have used antibodies raised against the rat L14 to show that the

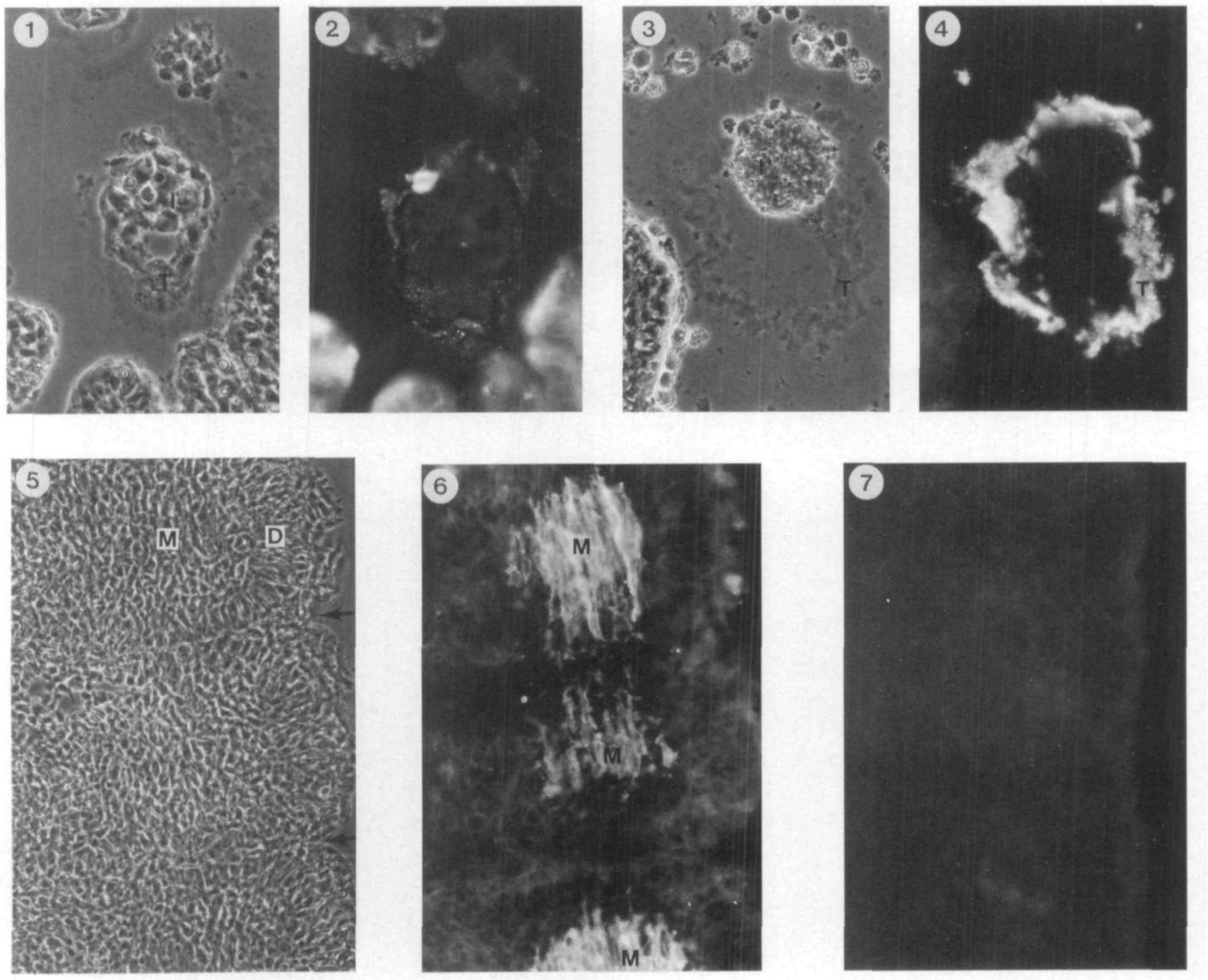

Fig. 4. Immunolocalisation of L14 in mouse embryos. (1,2) 3.5 dpc embryo; (3,4) 4.5 dpc embryo; (5,6,7) 10.5 dpc embryo. Panels 1,3 and 5 show phase-contrast images, panels 2,4,6 and 7 show immunofluorescence images. Panel 7 is a section similar to those in panels 5 and 6: the primary antibody was omitted during processing. The surrounding tissue in panels 1-4 is oviduct (see Materials and Methods). The bright spot in panel 2 is a speck of dirt. D, dermatome, I, inner cell mass; M, myotome; T, trophoblast. Arrows indicate the limits of adjacent somites.

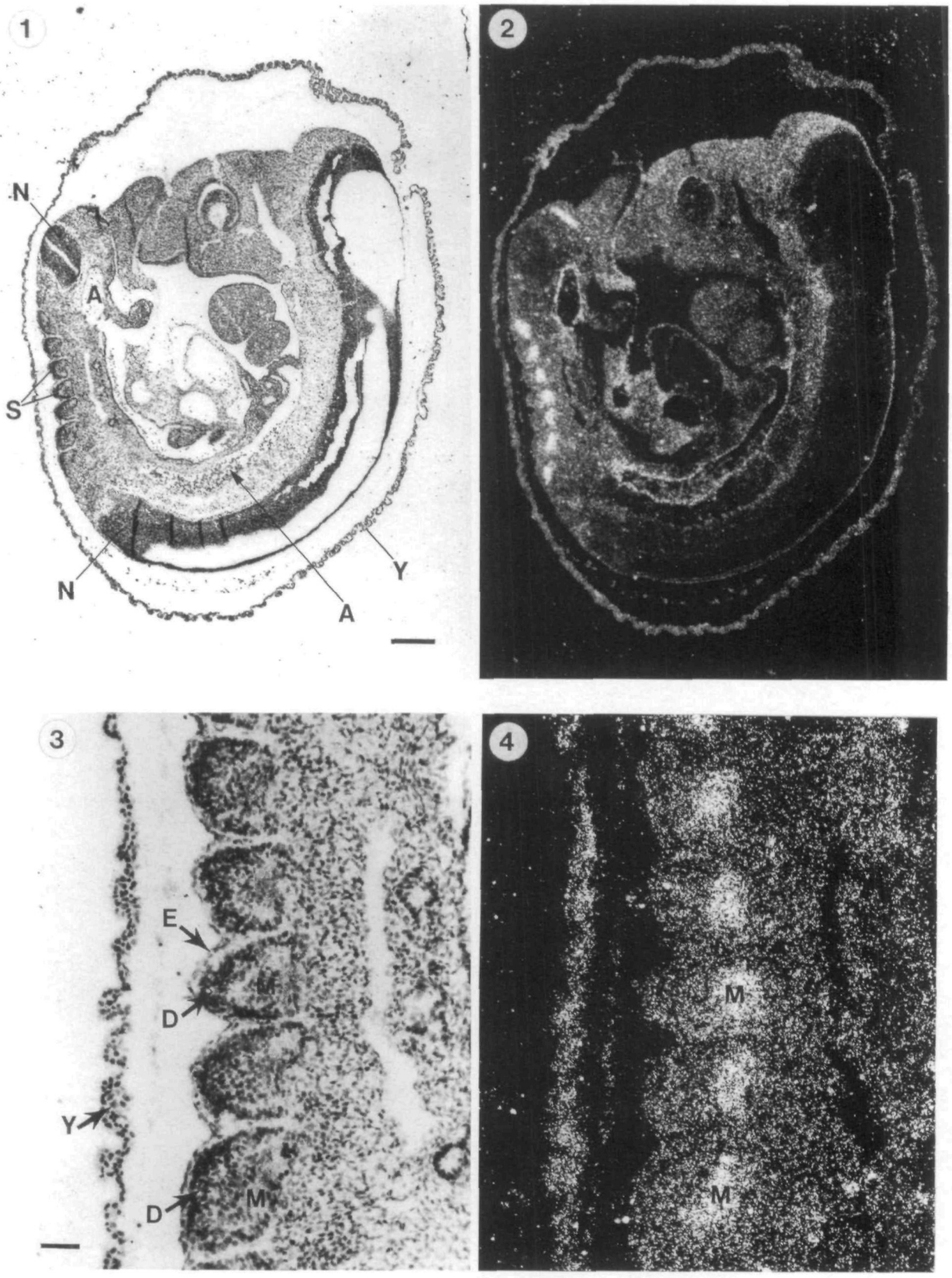

Fig. 5. In situ hybridisation of ^{35}S-labelled antisense *Lect14* probe to a longitudinal section of a 10.5 dpc embryo under bright-field (1,3) and dark-field (2,4) illumination. Panels 3 and 4 show detail of the *Lect14* expression in the myotomes. A, dorsal aorta; D, dermatome; E, epidermis; M, myotome; N, neural tube; S, somites; Y, yolk sac. Scale bar panel 1=0.25 mm; panel 3=50 μm.

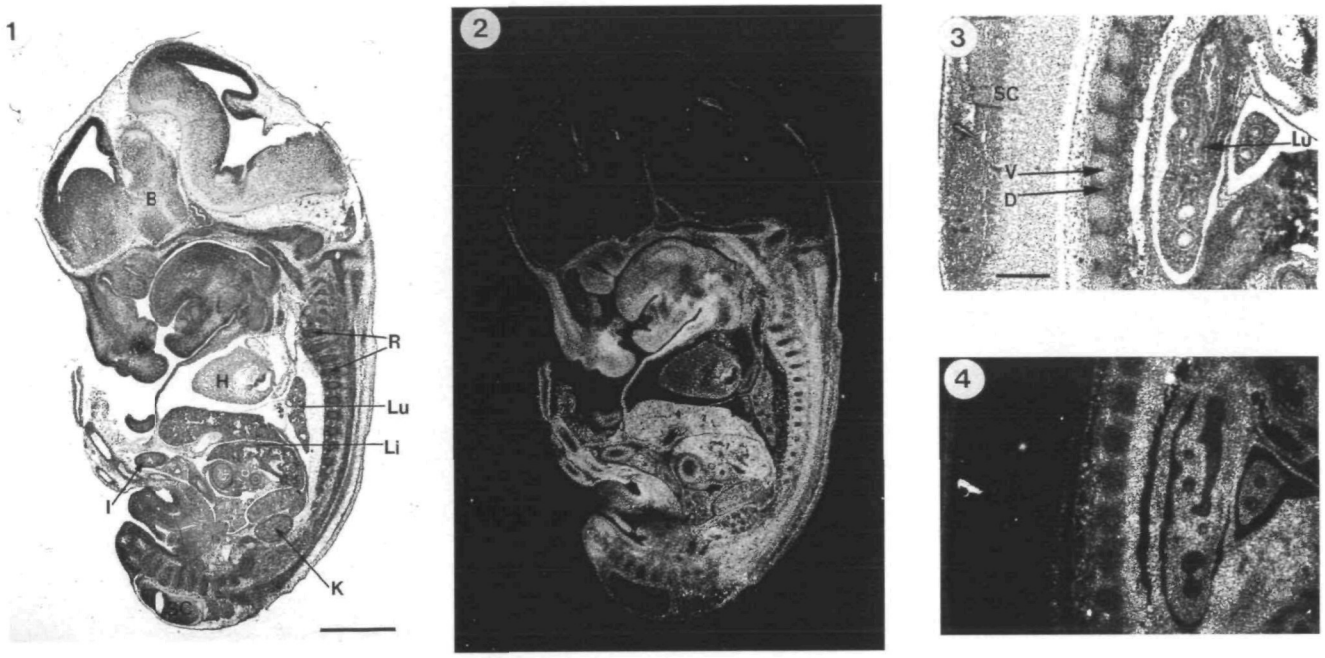

Fig. 6. In situ hybridisation of ^{35}S-labelled antisense *Lect14* probe to longitudinal sections of a 14.5 dpc embryo under bright-field (1,3) and dark-field (2,4) illumination. Panels 3 and 4 show detail of the *Lect14* expression pattern in the developing lung and vertebral column. B, brain, D, intervertebral disc; H, heart; I, intestine; K, kidney; Li, liver; Lu, lung; R, ribs; SC, spinal cord; V, vertebra. Scale bars. panel 1=1 mm; panel 2=0.25 mm

protein is also present in the trophectoderm cells. This result is of considerable interest with regard to the new cell-cell interactions that are established at this stage.

Changes in cell surface and, in particular, in glycoconjugate composition are known to be associated with the process of implantation (Kimber, 1990). It has been proposed that components of the extracellular matrix participate in this process (Farach et al., 1987; Carson et al., 1988). However, it is more likely that interactions of this sort are involved in secondary steps, i.e. the invasion of the uterine lining by the trophoblast cells, while the primary step, i.e. attachment of the embryo, probably involves more specific ligand-receptor interactions. One possible ligand is lacto-N-fucopentaose 1 (LNF-1) which Lindenberg et al. (1988) have shown to be able to inhibit the attachment of blastocysts to cultured uterine endometrial cells when added exogenously. LNF-1 is found in vivo on the surface of the uterine epithelium where it becomes more abundant and restricted to specific areas immediately prior to implantation (Kimber et al., 1988). The LNF-1 epitope itself is not present on early embryos but LNF-1 receptors appear on the surface of the blastocyst during the fourth day of gestation immediately before the hatching stage (4.5 dpc) (Lindenberg et al., 1990). Therefore, one of the key events controlling implantation may be an interaction between LNF-1 carrying glycoconjugates on the uterine epithelium and a specific receptor(s) on the surface of the expanded blastocyst. We note that the appearance of L14, which is known to bind LNF-1 (Leffler and Barondes, 1986; Sparrow et al., 1987), in the trophectoderm cells exactly parallels that of the putative LNF-1 receptor, raising the possibility that this lectin is the LNF-1 receptor and that it mediates the attachment of the blastocyst to the uterine wall. It is, however, the case that the rat and human L14s also bind the related carbohydrate LNT (Leffler and Barondes, 1986; Sparrow et al., 1987) and, on the basis of sequence homology, the mouse protein would be expected to also bind this molecule. In a limited set of experiments, Lindenberg et al. (1988) did not observe any inhibition by LNT of the attachment of blastocysts to endometrial cells, an apparent discrepancy which requires further investigation.

Nervous system

In the nervous system, *Lect14* transcripts accumulate in most or all of the motor neurones of the brain stem and spinal cord and in the sensory neurones of the dorsal root ganglia. Our results confirm and extend those previously reported in the developing rat nervous system (Regan et al., 1986; Hynes et al., 1990), which show that L14 and lactoseries glycoconjugates are co-expressed by the same functional set of dorsal root ganglia sensory neurones, suggesting that this molecule may be mediating carbohydrate recognition events that are known to be critical during the development of the nervous system (Hynes et al., 1989). The L14 could be involved in specifying pathways of axon migration by mediating interactions between neurones and carbohydrate structures present either on other cells or in the extracellular matrix or it could be exerting a direct signalling action through binding to carbohydrates on growth cones. It is noteworthy that expression of *Lect14* is detectable in motor neurones at a very early stage of

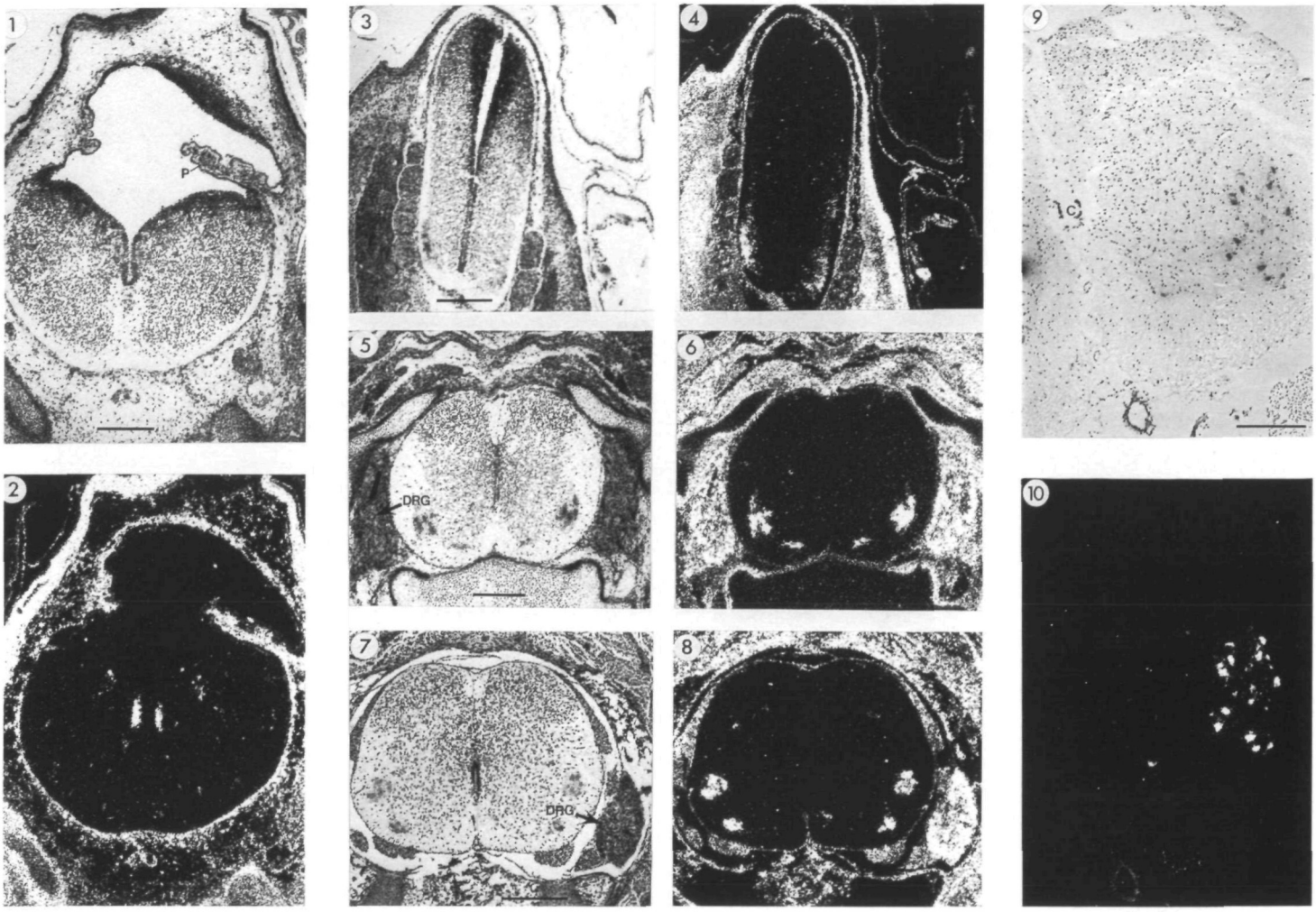

Fig. 7. In situ hybridisation of ^{35}S-labelled antisense *Lect14* probe to sections of embryonic and postnatal mouse central nervous system. Even-numbered panels are dark-field views of the sections shown in the corresponding odd-numbered bright-field panels Dorsal is towards the top of each panel (1,2) Coronal section of the fourth ventricle of the brain at 12.5 dpc. (3,4) Section through spinal cord of 12 5 dpc embryo. (5-10) Transverse sections through the spinal cord: (5,6) 14.5 dpc embryo, (7,8) lumbar region of newborn, and (9,10) lumbar region of adult mouse. C, central canal, DRG, dorsal root ganglion; P, lateral choroid plexus. Scale bars=0.25 mm.

differentiation when neural fibres are growing out of the neural tube (12.5 dpc).

Muscle differentiation

A role for L14 in myogenesis is suggested by the fact that the onset of *Lect14* transcription and translation in the postimplantation embryo occurs in the myotomes that give rise to muscle cells. Myotome cells become visibly distinct within the somites during the tenth day of development by which time they are already expressing some muscle-specific genes (Sassoon et al., 1988) and contain much higher levels of *Lect14* mRNA and L14 protein than any other cells in the embryo. The *Lect14* gene appears to be expressed prior to *myoD* (Sassoon et al., 1989), since we detect expression as early as 9.5 dpc.

We do not know the subcellular localisation of L14 in the myotome. However, our results are consistent with a role for it in the new cell-cell and cell-substratum interactions which are known to occur in the somites at this time. For example, expression of the cadherins, which mediate cell adhesion, is regulated at this stage of embryogenesis (Duband et al., 1987). It is also of interest that a related soluble lectin is abundant in chick myotomes (Levi and Teichberg, 1989). Myoblasts migrate out of the myotome, invading the peripheral mesenchyme where they terminally differentiate and fuse to form the axial skeletal muscles. During myoblast differentiation in vitro, the lectin is initially localised in the cytoplasm but is then released into the extracellular space (Cooper and Barondes, 1990) where it can bind to laminin thus detaching cells from their substratum and allowing fusion to myofibers (Cooper et al., 1991). These observations strongly suggest a role for the L14 in the later stages of myogenesis.

Expression in other mesodermal cell types

As organogenesis proceeds *Lect14* transcripts become widely but not uniformly distributed. Ectodermal derivatives, namely the epidermis and the nervous system (with the exception discussed above), do not express detectable levels of *Lect14* mRNA. Similarly, the endodermal components (epithelium) of the visceral organs, for example lung, gut, kidney and oviduct, are also negative. In contrast, most of the remaining tissues, which are composed of cells derived from mesoderm, express *Lect14* at significant levels. The obvious exception to this is the developing cartilage, in which the mRNA is undetectable.

These results suggest that L14 is important not only during certain developmental processes, such as implantation and muscle cell differentiation, but also in the maintenance of mature tissue phenotype. Previous studies have shown that the lectin is localized outside the cells in the connective tissues of muscle, intestine and lung (Barondes and Haywood-Reid, 1981; Beyer and Barondes, 1982; Cerra et al., 1984). Such observations have led to the hypothesis that this molecule might play a role in the organization of the extracellular matrix that regulates the differentiation of some tissues of mesodermal origin (Catt and Harrison, 1985). It is notable that, in the chick embryo, another soluble lectin appears to have the opposite pattern of expression in visceral organs, i.e. it is specific for epithelia (Levi and Teichberg, 1989), suggesting that different members of the same family could be involved in the elaboration of different types of extracellular matrix.

Conclusions

We have found that the expression of L14 is tightly regulated during a number of stages of embryogenesis. We believe that the striking regulation of its synthesis at the time of implantation strongly suggests that this carbohydrate-binding protein may play a key role in the attachment of the hatched blastocyst to the uterine epithelium. It is also likely that this lectin is important in the differentiation of both muscle cells and motor neurones. In these latter situations, it may be either the ability of this protein to regulate proliferation or its carbohydrate-binding activity that is significant. To address these questions, it will be necessary to generate highly specific immunological reagents in order to define the exact subcellular localisation of the L14 at each stage of development by immuno-electron microscopy. Antibodies that specifically block either carbohydrate-binding or proliferation regulation will enable the relative importance of these two activities during development to be assessed. Moreover, the role of this gene in the process of implantation can be directly addressed by experiments currently in progress, which seek to inactivate the gene via homologous recombination in ES cells.

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References

Abbott, W. M., Mellor, A., Edwards, Y. and Feizi, T. (1989). Soluble bovine galactoside-binding lectin cDNA cloning reveals the complete amino acid sequence and an antigenic relationship with the major encephalitogenic domain of myelin basic protein *Biochem J* **259**, 283-290

Atienza-Samols, S. B., Razon Pine, P. and Sherman, M. I. (1980) Effects of tunicamycin upon glycoprotein synthesis and development of early mouse embryos *Dev Biol* **79**, 19-32

Auffray, C. and Rougeon, F. (1979). Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA *Eur J Biochem* **107**, 303-314.

Banerjee, M., Wiener, F., Spira, J., Barbonits, M., Nilsson, M. G., Sumegi, J. and Klein, G. (1985) Mapping of the c-myc, pvt-1 and immunoglobulin kappa genes in relation to the mouse plasmacytoma-associated variant (6,15) translocation breakpoint. *EMBO J* **4**, 3183-3188

Barondes, S. H. (1984) Soluble lectins a new class of extracellular proteins *Science* **323**, 1259-1264.

Barondes, S. H. (1988) Bifunctional properties of lectins lectins redefined *Trends Biochem* **13**, 480-482

Barondes, S. H. and Haywood-Reid, P. L. (1981) Externalization of an endogenous chicken muscle lectin with in vivo development *J Cell Biol* **91**, 568-572.

Beyer, E. C. and Barondes, S. H. (1982) Secretion of endogenous lectin by chicken intestinal goblet cells *J Cell Biol* **92**, 28-33

Bird, J. M. and Kimber, S. J. (1984) Oligosaccharides containing fucose linked α(1-3) and α(1-4) to N-acetyl-glucosamine cause decompaction of mouse morulae *Dev Biol.* **104**, 449-460

Bleil, J. D. and Wassarman, P. M. (1988) Galactose at the non-reducing terminus of O-linked oligosaccharides of mouse egg ZP3 is essential for the glycoprotein's sperm receptor activity *Proc natn Acad Sci U S A* **85**, 6778-6782.

Bradley, A., Evans, M. J., Kaufman, M. H. and Robertson, E. J. (1984) Formation of germ-line chimaeras from embryo derived teratocarcinoma cell lines *Nature* **309**, 255-256

Carson, D. D., Tang, J-P. and Gay, S. (1988) Collagens support embryo attachment and outgrowth *in vitro* effects of the Arg-Gly-Asp sequence *Dev Biol* **127**, 368-375.

Catt, J. W. and Harrison, F. L. (1985) Selective association of an endogenous lectin with connective tissues. *J Cell Sci* **73**, 347-359.

Cerra, R. F., Haywood-Reid, P. L. and Barondes, S. H. (1984) Endogenous mammalian lectin localized extracellularly in lung elastic fibres *J Cell Biol.* **98**, 1580-1589

Clerch, L. B., Whitney, P., Haas, M., Brew, K., Miller, T., Werner, R. and Massaro, D. (1988) Sequence of a full-length cDNA for rat lung -galactoside-binding protein primary and secondary structure of the lectin *Biochemistry* **27**, 692-699.

Cooper, D. N. W. and Barondes, S. H. (1990). Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism *J Cell Biol.* **110**, 1681-1691

Cooper, D. N. W., Massa, S. M. and Barondes, S. H. (1991) Endogenous muscle lectin inhibits myoblast adhesion to laminin *J Cell Biol* **115**, 1437-1448.

Copp, A. J. (1978) Interaction between inner cell mass and trophectoderm of the mouse blastocyst *J Embryol exp Morph* **48**, 109-125.

Couraud, P. O., Casentini-Borocz, D., Bringman, T. S., Griffith, J., McGrogan, M. and Nedwin, G. E. (1989) Molecular cloning, characterization and expression of a human 14-kd lectin *J biol Chem* **264**, 1310-1316

Dodd, J., Morton, S. B., Karagogeos, D., Yamamoto, M. and Jessell, T. M. (1988). Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons *Neuron* **1**, 105-116

Drickamer, K. (1988) Two distinct classes of carbohydrate recognition domains in animal lectins *J biol Chem* **263**, 9557-9560

Duband, J. L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G. M. and Thiery, J.-P. (1987). Adhesion molecules during somitogenesis in the avian embryo. *J Cell Biol* **104**, 1361-1374

Evans, M. J. and Kaufman, M. H. (1981) Establishment in culture of pluripotential cells from mouse embryos *Nature* **292**, 154-156

Farach, M. C., Tang, J. P., Decker, G. L. and Carson, D. D. (1987). Heparin/Heparan sulfate is involved in attachment and spreading of mouse embryos *in vitro Dev Biol.* **123**, 401-410

Feinberg, A. P. and Vogelstein, B. (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analyt Biochem* **132**, 6-13.

Fenderson, B. A., Eddy, E. M. and Hakomori, S. I. (1990) Glycoconjugate expression during embryogenesis and its biological significance *BioEssays* **12**, 173-179

Fenderson, B. A., Zehavi, U. and Hakomori, S.-I. (1984) A multivalent lacto-N-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective *J exp Med* **160**, 1591-1596

Gabius, H. J., Springer, W. R. and Barondes, S. H. (1985). Receptor for the cell binding site of discoidin 1. *Cell* **42**, 449-456

Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T. and Sharon, N. (1980) What should be called a lectin? *Nature* **285**, 66

Guénet, J. L., Simon-Chazottes, D. and Avner, P. R. (1988). Genetics of immunological diseases, (ed B Mock and M Potter) In *Current Topics in Microbiology and Immunology*, **137**, p13-17. Springer Verlag Publishers

Hart, G. W., Holt, G. D. and Haltiwanger, R. S. (1988) Nuclear and cytoplasmic glycosylation. novel saccharide linkages in unexpected places. *Trends Biochem* **13**, 380-384

Henikoff, S. (1984) Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing *Gene* **28**, 351-359.

Hinek, A., Wrenn, D. S., Mecham, R. P. and Barondes, S. H. (1988) The elastin receptor a galactoside binding protein *Science* **239**, 1539-1541

Hirabayashi, J. and Kasai, K.-I. (1988). Complete amino acid sequence of a β-galactoside binding lectin from human placenta *J Biochem (Tokyo)* **104**, 1-4

Hirabayashi, J., Kawasaki, H., Suzuki, K. and Kasai, K. (1987) Complete amino acid sequence of 14kd-β-galactoside-binding lectin of chick embryo *J. Biochem (Tokyo)* **101**, 775-787

Hubert, J., Seve, A. P., Facy, P. and Monsigny, M. (1989) Are nuclear lectins and nuclear glycoproteins involved in the modulation of nuclear functions? *Cell Diff Dev* **27**, 69-81

Hynes, M. A., Dodd, J. and Jessell, T. M. (1989) Carbohydrate recognition, cell interactions, and vertebrate neural development. In *Neurobiology of Glycoconjugates,* (ed R U. Margolis and R K Margolis), p337-365 New York Plenum Publ Corp

Hynes, M. A., Gitt, M., Barondes, S. H., Jessell, T. M. and Buck, L. B. (1990) Selective expression of an endogenous lactose-binding lectin gene in subsets of central and peripheral neurons *J Neurosci* **10**, 1004-1013.

Kimber, S. J. (1990). Glycoconjugates and cell surface interactions in pre- and peri-implantation mammalian embryonic development *Int Rev Cytol* **120**, 53-167.

Kimber, S. J., Lindenberg, S. and Lundblad, A. (1988) Distribution of some Gal β1-3(4) GlcNAc related carbohydrate antigens on the mouse uterine epithelium in relation to the peri-implantational period *J Reprod Immunol.* **12**, 297-313.

Laing, J. G. and Wang, J. L. (1988) Identification of carbohydrate binding protein 35 in heterogeneous nuclear ribonucleoprotein complex *Biochemistry* **27**, 5329-5334

Leffler, H. and Barondes, S. H. (1986) Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian β-galactosides *J biol Chem.* **261**, 10119-10126

Leffler, H., Masiarz, F. R. and Barondes, S. H. (1989) Soluble lactose-binding vertebrate lectins a growing family. *Biochemistry* **28**, 9222-9229.

Levi, G. and Teichberg, V. I. (1989) Patterns of expression of a 15K β-D-galactoside-specific lectin during early development of the avian embryo. *Development* **107**, 909-921

Lindenberg, S., Kimber, S. J. and Kallin, E. (1990) Carbohydrate binding properties of mouse embryos. *J Reprod. Fert* **89**, 431-439.

Lindenberg, S., Sundberg, K., Kimber, S. J. and Lundblad, A. (1988). The milk oligosaccharide, lacto-N-fucopentose 1, inhibits attachment of mouse blastocysts on endometrial monolayers *J Reprod Fert.* **83**, 149-158

Lipsick, J. S., Beyer, E. C., Barondes, S. H. and Kaplan, N. O. (1980) Lectins from chicken tissues are mitogenic for Thy-1 negative murine spleen cells *Biochim Biophys Res Comm* **97**, 56-61.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning a Laboratory Manual* Cold Spring Harbor, NY Cold Spring Harbor Laboratory Press

Martin, G. R. (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells *Proc. natn Acad Sci USA* **78**, 7634-7638

Mecham, R. P., Hinek, A., Griffin, G., Senior, R. M. and Liotta, L. A. (1989) The elastin receptor shows structural and functional similarities to the 67-KDa tumor cell laminin receptor. *J biol Chem* **264**, 16652-16657

Moses, H. L., Yang, E. Y. and Pietenpol, J. A. (1990). TGF-β stimulation and inhibition of cell proliferation new mechanistic insights. *Cell* **63**, 245-247

Muesch, A., Hartmann, E., Rohde, K., Rubartelli, A., Sitia, R. and Rapoport, T. A. (1990) A novel pathway for secretory proteins? *Trends Biochem* **15**, 86-88.

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

Ohyama, Y., Hirabayashi, J., Oda, Y., Ohno, S., Kawasaki, H., Suzuki, K. and Kasai, K. (1986) Nucleotide sequence of chick 14Kd-galactoside-binding lectin mRNA *Bioch Biophys. Res Comm* **134**, 51-56.

Outenreath, R. L., Roberson, M. M. and Barondes, S. H. (1988). Endogenous lectin secretion into the extracellular matrix of early embryos of Xenopus laevis *Dev Biol.* **125**, 187-194

Pitts, M. J. and Yang, D. C. H. (1981). Mitogenicity and binding properties of beta-galactoside-binding lectin from chick-embryo kidney *Biochem J* **195**, 435-439

Poirier, F., Chan, C.-T. J., Timmons, P. M., Robertson, E. J., Evans, M. J. and Rigby, P. W. J. (1991). The murine *H19* gene is activated during embryonic stem cell differentiation *in vitro* and at the time of implantation in the developing embryo *Development* **113**, 1105-1114

Rastan, S., Thorpe, S. J., Scudder, D. P., Brown, S., Gooi, H. C. and Feizi, T. (1985) Cell interactions in the preimplantation embryo. evidence for involvement of saccharides of the poly-N-acetyllactosamine series *J. Embryol exp Morph* **87**, 115-128.

Regan, L. J., Dodd, J., Barondes, S. H. and Jessell, T. M. (1986) Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. *Proc. natn Acad Sci U S A* **83**, 2248-2252

Richa, J. and Solter, D. (1986) Role of cell surface molecules in early mammalian development In *Experimental Approaches to Mammalian Embryonic Development* (ed J Rossant and R Pedersen) pp293-320 Cambridge Cambridge University Press.

Roberson, M. M. and Barondes, S. H. (1983) Xenopus lectin is localized at several sites in Xenopus oocytes, eggs and embryos *J Cell Biol* **97**, 1875-1881.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors *Proc natn Acad. Sci USA* **74**, 5463-5467.

Sassoon, D. A., Gardner, I. and Buckingham, M. (1988) Transcripts of α-cardiac and α-skeletal actins are early markers of myogenesis in the mouse embryo *Development* **104**, 155-164

Sassoon, D. A., Lyons, G., Wright, W. E., Lin, V., Lassar, A., Weintraub, H. and Buckingham, M. (1989) Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis *Nature* **341**, 303-307.

Sparrow, C. P., Leffler, H. and Barondes, S. H. (1987) Multiple soluble β-galactoside-binding lectins from human lung *J biol Chem* **262**, 7383-7390

Surani, M. A. H. (1979) Glycoprotein synthesis and inhibition of

glycosylation by tunicamycin in preimplantation mouse embryos. compaction and trophoblast adhesion *Cell* **18**, 217-227

Thorpe, S. J., Bellairs, R. and Feizi, T. (1988) Developmental patterning of carbohydrate antigens during early embryogenesis of the chick. expression of antigens of the poly-N-acetyllactosamine series *Development* **102**, 193-210

Wassarman, P. M. (1987) Early events in mammalian fertilization *Ann Rev Cell Biol* **3**, 109-142

Wells, V. and Mallucci, L. (1991) Identification and characterization of an autocrine negative growth factor mouse β-galactoside binding protein is a cytostatic factor and a regulator of cell proliferation *Cell* **64**, 911-97

Wilkinson, D. G. and Green, J. (1990) *In situ* hybridisation and the three-dimensional reconstruction of serial sections In. *Postimplantation Mammalian Embryos A Practical Approach* (ed A L. Copp and D L Cockroft) Oxford. IRL Press

Wilson, T. J. G., Firth, M. N., Powell, J. T. and Harrison, F. L. (1989) The sequence of the mouse 14 kDa β-galactoside binding lectin and evidence for its synthesis on free cytoplasmic ribosomes *Biochem J* **261**, 847-852

Woo, H. J., Shaw, L. M., Messier, J. M. and Mercurio, A. M. (1990) The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2) *J biol Chem* **265**, 7097-7099

Yamaoka, K., Ohno, S., Kawasaki, H. and Suzuki, K. (1991). Overexpression of a β-galactoside binding protein causes transformation of Balb3T3 fibroblast cells *Biochem Biophys Res Comm.* **179**, 272-279

Zalik, S. E. and Milos, N. (1986) Endogenous lectins and cell adhesion in embryonic cells In *Developmental Biology, A Comprehensive Synthesis* **Vol II.** (ed L W Browder). pp145-195. New York Plenum Press

Zalik, S. E., Scheider, W. J. and Ledsham, I. M. (1990) The gastrulating chick blastoderm contains 16KDa and 14KDa galactose-binding lectins possibly associated with an apolipoprotein *Cell Diff Dev* **29**, 217-231

Zalik, S. E., Thomson, L. W. and Ledsham, I. M. (1987) Expression of an endogenous galactose-binding lectin in the early chick embryo *J Cell Sci* **88**, 483-493

Zhou, Q. and Cummings, R. D. (1990) The S-type lectin from calf heart tissue binds selectively to the carbohydrate chains of laminin. *Arch Biophys Biochem* **281**, 27-35

Zuhlke, C., Schoffl, F., Jockusch, H., Simon, D. and Guénet, J.-L. (1989) cDNA sequence and chromosomal localization of the mouse parvalbumin gene, *Pva Genet Res Camb* **54**, 37-43

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