

Differential expression of the HMG box factors *TCF-1* and *LEF-1* during murine embryogenesis

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SUMMARY

The recent identification of a number of T lymphocyte-specific enhancers has allowed the cloning of several novel transcription factors. Two of these, *TCF-1* and *LEF-1*, contain a virtually identical DNA-binding domain of the High Mobility Group (HMG-1) box type. *TCF-1* and *LEF-1* originate from a recent gene duplication event as evidenced by comparison with the chicken homologue, *chTCF*. We have now analyzed the differential expression of these two transcription factors. In a panel of lymphoid cell lines, *TCF-1* was exclusively expressed in the T cell lineage. In contrast, *LEF-1* mRNA was detected at equivalent levels in pro- and pre-B cells and in all T lineage cells.

In situ hybridization on murine embryos revealed that *TCF-1* and *LEF-1* were widely expressed at day 7.5 of gestation. At later stages, the expression patterns were complex and only partially overlapping. The expression

of *TCF-1* and *LEF-1* coincided until day 10.5, when mRNAs were detected in limb buds, neural crest, pharyngeal arches and nasal process. At later time points (day 13.5 to 14.5), sites of overlapping expression included lung, the urogenital system, tooth buds, thymus and choroid plexus. Unique expression sites for *TCF-1* included Reichert's membrane and trophoblast-derived cells, the ribs and thoracic prevertebrae, craniofacial structures, the adrenal gland and meninges. Unique *LEF-1* expression was observed in the tail prevertebrae, brain and inner ear. Postnatally, expression of both genes could only be detected in lymphoid tissues. These observations suggest that *TCF-1* and *LEF-1* exert differential functions during murine embryogenesis.

Key words: *TCF-1*, *LEF-1*, transcription factors, HMG box, T cell differentiation, mouse

INTRODUCTION

In recent years, a number of T lymphocyte-specific enhancers have been identified that control transcription of structural T cell genes (reviewed in Clevers and Owen, 1991). This has allowed the cloning of several transcription factors, that are candidate-regulatory genes of lymphoid differentiation. Two closely related factors, *TCF-1* (van de Wetering et al., 1991) and *LEF-1* (Travis et al., 1991), are the subject of the present study.

TCF-1 was originally identified as a T cell-specific DNA-binding protein in gel retardation assays using the enhancer of the *CD3 γ* gene. Subsequently, human *TCF-1* was cloned from a Lambda-GT11 expression library (van de Wetering et al., 1991). *TCF-1* contains a DNA-binding HMG box, an 80 amino acid region homologous to High Mobility Group (HMG-1) proteins (Jantzen et al., 1990), which specifically binds in the minor groove of the A/T A/T C A A A G motif (van de Wetering and Clevers, 1992). *TCF-1* binding sites were also identified in the *TCR α* , *TCR β* and *TCR δ* enhancers (Oosterwegel et al., 1991a).

LEF-1 was originally cloned from pre-B cells by a subtraction strategy, and was subsequently found to be expressed also in mature T cells. No expression was observed in the B lineage beyond the pre-B cell stage (Travis et al., 1991). The HMG boxes of *TCF-1* and *LEF-1* share 75 out of 78 identical amino acid residues and apparently bind the same consensus motif. *LEF-1* has been shown to bend DNA and is proposed to act as an architectural element in the control of transcription (Giese et al., 1992). The human homologue of *LEF-1*, termed *TCF-1*, was independently characterized and cloned based on its affinity for the TTCAAAG motif in the *TCR δ* enhancer (Waterman et al., 1991).

We arbitrarily distinguish three different phenotypic stages in early T cell development with regard to rearrangement and expression of the genes encoding the constituent chains of the antigen receptor, the T cell receptor (TCR)/CD3 complex (Clevers et al., 1988). Prothymocytes express the early T cell markers Thy-1 and CD2. They do not express *TCR* or *CD3* genes other than *CD3 γ* and have undergone no *TCR* gene rearrangements. Prethymocytes

express all *CD3*-chains as well as partially rearranged *TCR*-chains. Thymocytes/T cells have fully rearranged *TCR* genes, and carry TCR/CD3 complexes on the cell surface. Corresponding stages are defined in the B cell lineage (Alt et al., 1981).

The limited number of studies reported to date precludes a direct comparison of the expression profiles of *TCF-1* and *LEF-1* at various stages of lymphoid differentiation (Leiden, 1992). Such information is clearly important for the understanding of the individual roles of these factors as well as their hierarchical relationship in this differentiation process. We have, therefore, performed northern blot analyses on a panel of precursor and mature lymphoid cell lines.

From studies on other tissue-specific transcription factors, it has emerged that such factors usually display a wider tissue-distribution than the structural genes originally used to identify the factor. For example, the POU-domain genes *Oct-2* and *Pit-1*, which are believed to control specific expression of genes in the B cell lineage and pituitary cells respectively, both are expressed in the developing nervous system (Xi et al., 1989). In addition, *LF-B1* is thought to be involved in liver-specific gene expression, but is expressed in a variety of other tissues (Baumhueter et al., 1990). We have addressed this issue for *TCF-1* and *LEF-1* by analyzing mRNA expression of these two genes by in situ hybridization of murine embryos from day 7.5 to day 14.5.

MATERIALS AND METHODS

Cell lines

Cells were grown in RPMI-1640 supplemented with 5% fetal calf serum and antibiotics. All cell lines are of murine origin. Pro- and prethymocyte cell lines were derived from day 14 fetal thymus organ culture, and transformed with a retroviral myc-raf oncogene construct (J2) (A. K., unpublished data). These prothymocyte lines, 34.1.E and 35.1.E, express CD2, Thy-1 and the *CD3*- gene. They are CD4/CD8 negative and their T cell receptor genes are in germline configuration. No *CD3*- and *CD3*- mRNA is detectable. This phenotype is consistent with that described by Palacios and Pelkonen (1988). Prethymocytes, 34.1.L and 35.1.L, express *CD3*-, *CD3*- and *CD3*- mRNA as well as partially rearranged *TCR*-mRNA and *TCR*- mRNA; they are surface TCR/CD3 and CD4/CD8-negative. T cell lines derived by transformation of day 17 fetal thymocytes, A1 and B2, display a mature T cell phenotype, including the surface expression of the TCR/CD3 complex and CD4 and CD8. 1881 is a μ -negative mutant pre-B cell line; 38B9 represents a pro-B cell line; 40E1 pre-B cells express μ and δ . 38B9 and 40E1 are derived from fetal liver, while 1881 is derived from adult bone-marrow by transformation with Abelson virus (Alt et al., 1981). All other cells were obtained from the American Type Culture Collection. Briefly, EL-4 is a mature TCR- / surface-positive Thymoma line. The phenotype of BW5147 is similar to that of EL-4, but as a result of the absence of *CD3*- mRNA, no surface expression of the TCR/CD3 complex occurs. Ag8 and Ns1 are B lineage cell lines (non-Ig-secreting myeloma cells). NIH-3T3, 3T6 and L-cells are fibroblast cell lines.

Northern blot analysis

Northern blot analysis was performed according to standard procedures. RNA was isolated by lithium chloride/urea precipitation. For each sample, 10 μ g of RNA was run on a 1.2% agarose gel containing 2% formaldehyde. RNA was blotted onto reinforced nitrocellulose filters (BAS 85, 0.45 μ m, Schleicher and Schuell).

Hybridization mix contained 5 \times SSPE, 5 \times Denhardt, 0.1% SDS, 50% formamide, 10% dextran sulfate and 100 μ g/ml salmon sperm DNA. Hybridization was performed overnight at 42°C. High stringency washing was performed using 0.2 \times SSC/0.1% SDS at 65°C. Kodak X-AR5 films were exposed up to seven days with amplifying screens at -80°C.

cDNA probes

Human *TCF-1* was cloned from an HPB-ALL T cell cDNA library by oligoprobeing based on published sequences (Waterman et al., 1991). Subsequently, murine cDNA clones were obtained from an EL-4 phage cDNA library under conditions of low stringency using the human cDNA clone as a probe. The identity of all cDNAs was confirmed by sequencing. For northern blotting, DNA

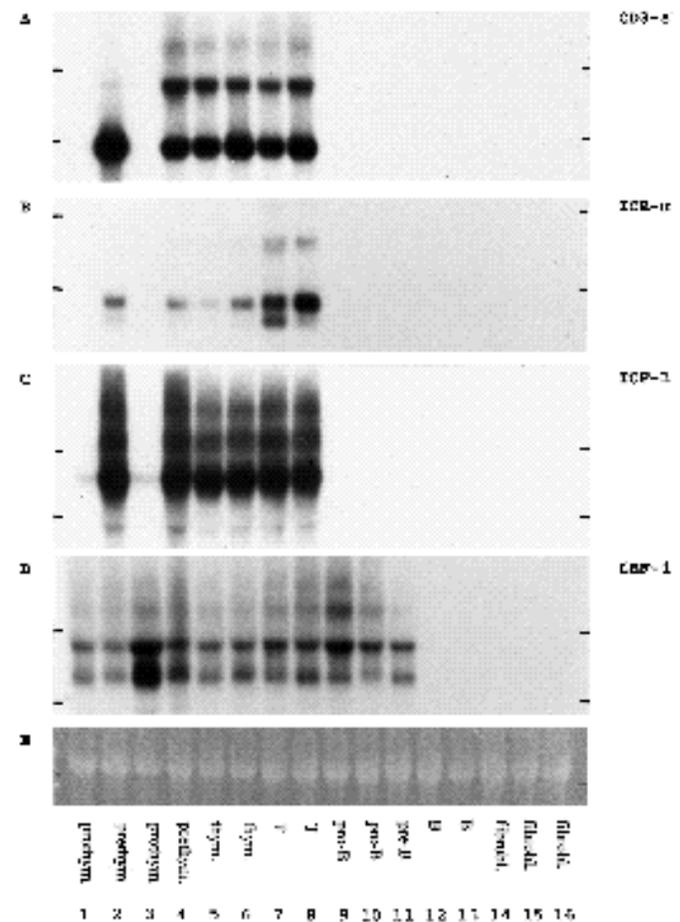


Fig. 1. Northern blot analysis of *TCF-1* and *LEF-1* in a panel of lymphoid (progenitor) cells. Northern blots were probed for *CD3*- (A), *TCR*- (B), *TCF-1* (C), *LEF-1* (D). Hyphens mark the ribosomal bands. Equal amounts of RNA were analyzed as is evident from the ethidium bromide-stained nitrocellulose blot (E). (1) 34.1.E and (3) 35.1.E are day 14 fetal thymus-derived prothymocyte cell lines; (2) 34.1.L and (4) 35.1.L are day 14 fetal thymus-derived prethymocyte cell lines; (5) A1 and (6) B2 are day 17 fetal thymus-derived T cell lines; (7) EL-4 and (8) BW5147 are mature TCR- T cell lines; (9) 38B9 is a pro-B cell line; (10) 40E1 and (11) 1881 are pre-B cell lines; (12) Ag8 and (13) Ns1 are myeloma-derived B cell lines; (14) NIH-3T3, (15) L-cells and (16) 3T6 are fibroblast cell lines. Dominant transcript sizes were 2.9 kb for *TCF-1*; 2.7 and 4.2 for *LEF-1*; 1.3 for *CD3*-; 1.5 and 1.8 for *TCR*-.

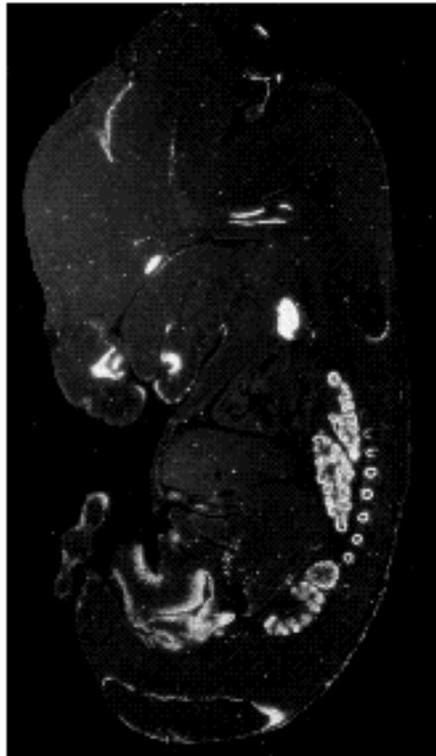
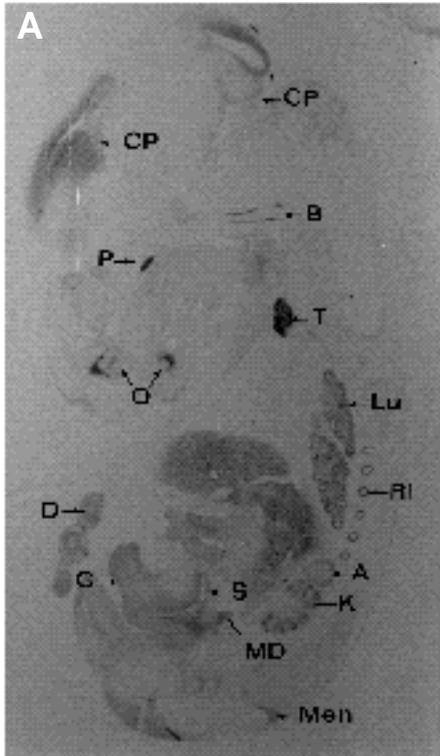
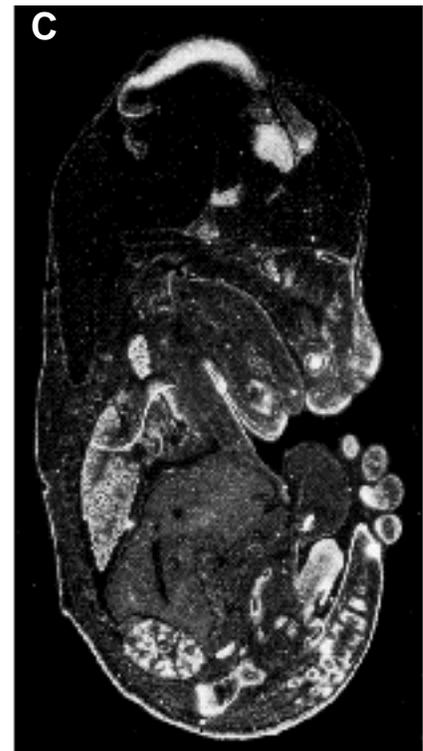
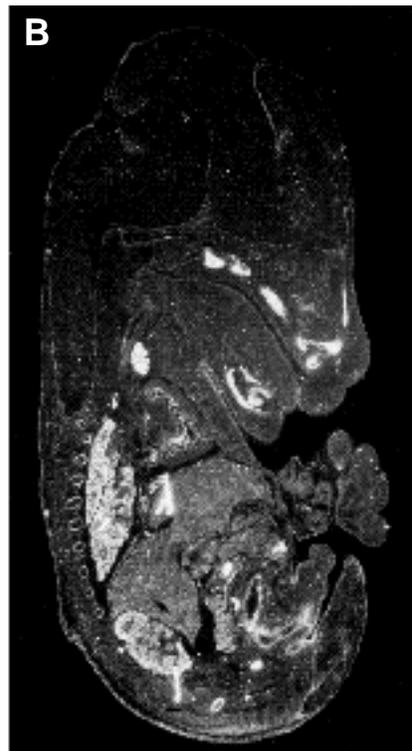
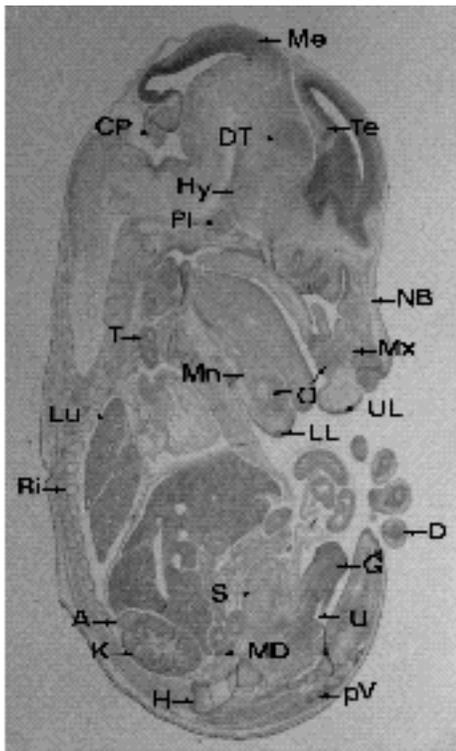


Fig. 2. In situ hybridization analysis of *TCF-1* and *LEF-1* expression on parasagittal murine embryo sections. All figures represent typical examples of multiple in situ experiments on serial sections of several different embryos. Bright-field (left) and the accompanying dark-field (right) pictures are shown. (A) Day 13.5; *TCF-1*. Expression of *TCF-1* was observed in thymus (T); odontoblasts of the tooth bud (O); cartilage primordium of the basioccipital bone (B) and the palate (P); meninges surrounding the neural canal (Men); choroid plexus (CP); mesenchymal cells of the lung (Lu), kidney (K) and urogenital sinus (S); (para)mesonephric duct (MD); cortex of the adrenal gland (A); anterior and posterior sites of the genital eminence (G); digits of the hindlimb (D) and around the thoracic ribs (Ri).



(B) Day 14.5, *TCF-1*: expression pattern was similar to that in Fig. 2A. In addition, *TCF-1* expression was observed in the cartilage primordium of the nasal bone (NB), maxilla (Mx) and mandible (Mn). (C) Day 14.5, *LEF-1*: Expression was observed in thymus (T), odontoblasts of the tooth bud (O), choroid plexus (CP), lung (Lu), kidney (K), urogenital sinus (S), (para)mesonephric duct (MD) and in the digits of the hindlimb (D). Diffuse expression was observed throughout the genital eminence (G) and around the urethra (U). *LEF-1* was exclusively present in tail prevertebrae (pV), osteogenic cells of the hipbone (H), lower (LL) and upper lips (UL) and in distinct parts of the brain: the pituitary gland (Pi), the roof of the mesencephalon (Me), dorsal thalamus (DT), hypothalamus (Hy) and part of the telencephalon (Te).

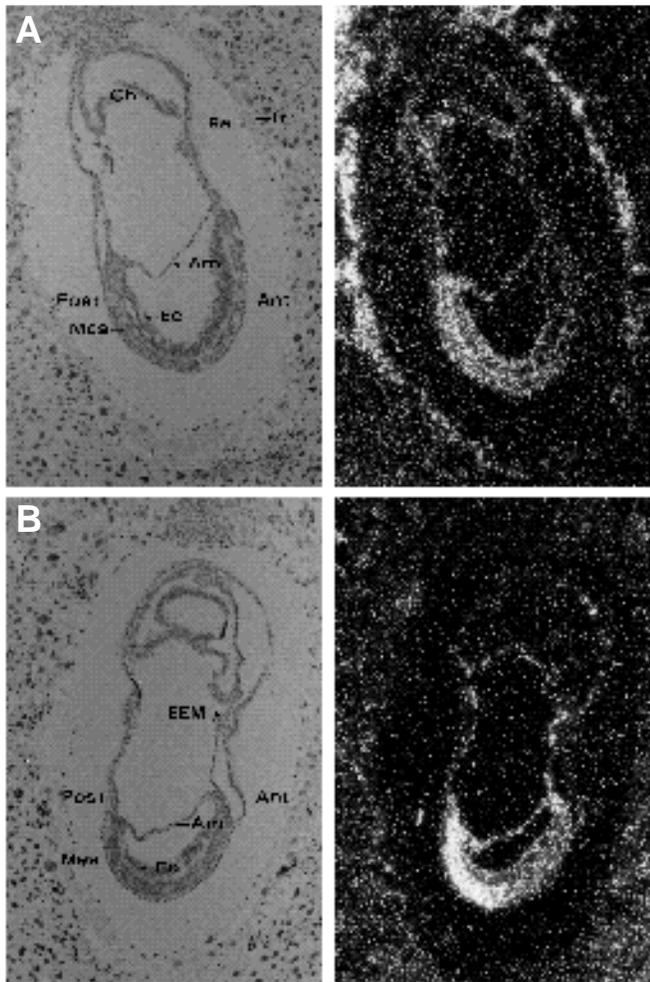


Fig. 3. Expression of *TCF-1* and *LEF-1* in day 7.5 embryo sections. At day 7.5, expression of *TCF-1* and *LEF-1* was observed in the embryonic mesoderm (Mes) and ectoderm (Ec) layers and extra-embryonic cells of the amnion (Am), chorion (Ch) and allantois (not shown). The orientation of the embryo is based on analysis of serial sections. The expression displayed a posterior-anterior gradient. No mRNA was detected in the endodermal layer. *TCF-1* was also present in the Reichert's membrane (Re) and in trophoblast-derived cells (Tr). (A) day 7.5, *TCF-1*; (B) day 7.5, *LEF-1*. EEM, extraembryonic mesoderm.

probes were labeled by random oligonucleotide priming (Pharmacia). For *TCF-1*, *CD3 ϵ* and *TCR α* , full-length cDNA inserts were used. The *LEF-1* probe was a 422 base pair *NdeI-Sall* fragment (1716-2138 bp; Travis et al., 1991).

In situ hybridization

In situ hybridization was performed essentially as described by Wilkinson et al. (1987) with modifications introduced by Kress et al. (1990). Briefly, dissected embryos were fixed 4 to 18 hours in 4% paraformaldehyde, dehydrated in ethanol and butanol and embedded in paraffin. Embryos were sectioned at 6 μ m thickness and mounted on 3-aminopropyl-triethoxy-silane-coated slides. Slides were deparaffined, digested with proteinase K, acetylated with acetic anhydride dissolved in triethanolamine, dehydrated and hybridized overnight at 55°C. Subsequently, high stringency washing was performed in 50% formamide/2 \times SSC/10 mM DTT at 65°C. Slides were treated with ribonuclease A, rinsed in 2 \times SSC,

0.1 \times SDS, dehydrated and dipped in Ilford emulsion G.5. After drying, the slides were exposed for 1 to 10 days at 4°C, developed in Kodak D-19 and fixed with Kodak LX-24.

RNA probes

Antisense radiolabeled RNA probes were generated from *TCF-1* and *LEF-1* cDNA subcloned in pBluescript SK, using T7 RNA polymerase and [³⁵S]UTP (Stratagene). Constructs were linearized (*NcoI* for *TCF-1*, and *PvuII* for *LEF-1*) to yield 449 bp and 494 bp labeled probes respectively, both covering 3 untranslated sequence. Probes were used for hybridization at 1 \times 10⁵ cts/minute/ μ l.

RESULTS

Northern blot analyses of lymphoid cell lines

At the initiation of this study, all available evidence indicated that *TCF-1* and *LEF-1* are uniquely expressed by lymphoid cells (Waterman et al., 1991; Travis et al., 1991; van de Wetering et al., 1991; Oosterwegel et al., 1991b). To document the expression profiles of *TCF-1* and *LEF-1* at various stages of lymphoid differentiation, we performed northern blot analysis on a panel of well-defined precursor and mature lymphoid cell lines. As illustrated in Fig. 1, high level expression of *TCF-1* mRNA was restricted to the T cell lineage and correlated with the expression of the T cell-specific genes *CD3 ϵ* and *TCR α* (Fig. 1A,B and C). The prothymocyte lines expressed approximately 50-fold less *TCF-1* than their more mature counterparts, as determined by densitometrical analysis. No *TCF-1* mRNA was detectable in the pro/pre-B cell, B cell and fibroblast cell lines. *LEF-1* was expressed at roughly equivalent levels in all T cells including the *TCR α* ⁻/*CD3 ϵ* ⁻ negative prothymocyte lines and in the pro- and pre-B cell lines (Fig. 1D). The two myeloma-derived cell lines did not contain *LEF-1* mRNA. The expression in early B cell-stages was in line with the reported expression patterns for *LEF-1* (Travis et al., 1991). Expression of *LEF-1* mRNA in prothymocytes had not previously been reported. Thus, the two highly related genes were differentially expressed in the lymphoid lineage. Notably, the appearance of *LEF-1* mRNA preceded the onset of expression of *TCF-1* and of the putative target genes *CD3 ϵ* and *TCR α* in the T cell lineage. The banding pattern obtained with the two probes was in good agreement with northern blot data in the original reports (Oosterwegel et al., 1991b; Travis et al., 1991).

In situ hybridization during murine embryogenesis

In order to address the issue whether *TCF-1* and *LEF-1* are expressed outside the lymphoid system during embryogenesis, in situ hybridization was performed on mouse embryos from day 7.5 to day 14.5 of gestation. Embryo sections were hybridized with 3'-untranslated antisense RNA probes under conditions of high stringency. The specificity of the selected probes was confirmed by Southern blot analysis (not shown).

Both *TCF-1* and *LEF-1* were found to be expressed at several sites outside the immune system. At the early stages, day 7.5 to day 10.5 of gestation, the overall expression patterns observed for *TCF-1* and *LEF-1* were

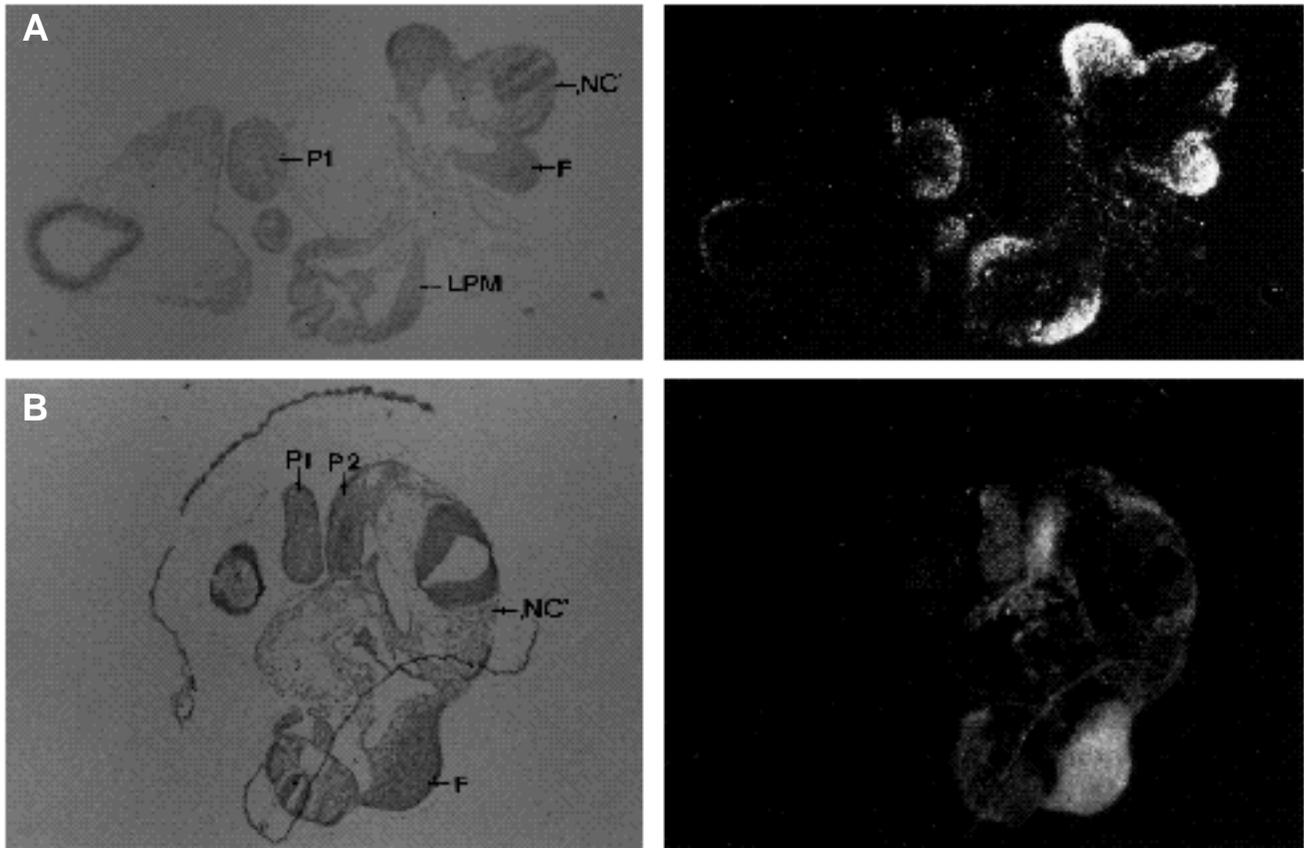


Fig. 4. *TCF-1* and *LEF-1* expression at days 9.5 of gestation. At day 9.5 *TCF-1* and *LEF-1* were observed in the forelimb bud (F) and in part of the lateral plate mesoderm (LPM). Both transcripts were present in the first (P1) and the second pharyngeal arch (P2), and mesenchymal cells that most likely derive from neural crest ('NC') adjacent to the neural tube. The second pharyngeal arch is not shown for *TCF-1* in this sections. (A) day 9.5, *TCF-1*; (B) day 9.5, *LEF-1*.

very similar. At later time points, the expression patterns partially diverged. Fig. 2A depicts a typical experiment for *TCF-1*, hybridized to a near mid-sagittal section of a day 13.5 embryo. Most sites of *TCF-1* expression are visible on this whole-embryo section. Fig. 2B and C depict two consecutive sections of day 14.5 embryos probed for *TCF-1* and *LEF-1* respectively, allowing a direct comparison of the expression sites of the two genes. Below, we discuss *TCF-1* and *LEF-1* expression in individual organ systems, illustrated by higher magnification pictures when appropriate.

Expression at day 7.5

At the earliest time point examined, day 7.5 (Fig. 3), inspection of serial sections revealed that highest expression of both genes was observed in mesoderm. A lower expression level was seen in ectoderm, whereas no expression was apparent in endoderm. The expression of both genes occurred along an anteroposterior gradient, the highest levels being expressed posteriorly. Furthermore, the genes were expressed in extraembryonic layers, i.e. the allantois, amnion and chorion. In addition, *TCF-1* (but not *LEF-1*) was expressed in Reichert's membrane and in trophoblast-derived cells. No expression of the two genes could be detected in embryonic stem cells, which derive from preimplantation inner cell mass (not shown).

Limbs

At day 9.5, widespread expression of both genes was observed in the forelimb and in part of the lateral plate mesoderm (Fig. 4). At day 10.5, expression of both *TCF-1* and *LEF-1* was particularly high in the mesenchymal cells at the rim of the limb buds between the apical ectodermal ridge and the marginal vein (Fig. 5A). This area may correspond to the progress zone (Tabin, 1990). Expression of both genes proceeded in essentially the same fashion in the hindlimb (not shown). Fig. 2A,C illustrate expression at the apex of the digits of the hindlimb at day 14.5. No later time points were examined.

Neural crest

At day 10.5, both genes were expressed in patches of cells in and beside the neural tube. These cells are clustered at both sides of the neural tube, suggesting a neural crest origin (Fig. 5B). Derivatives of neural crest cells, like mesenchymal cells of the pharyngeal arches, odontoblasts and meninges expressed high levels of both mRNAs at later stages.

Pharyngeal arches

Both *TCF-1* and *LEF-1* were expressed in the first and second pharyngeal arches at day 9.5 (Fig. 4) and 10.5 (Fig.

5C,D). As neural crest contributes significantly to the mesenchyme of the arches, it is likely that the observed expression occurs in neural crest-derived cells. At day 10.5, the epithelial lining between the maxillary and the mandibular component of the first pharyngeal arch and the epithelial lining of the third pharyngeal arch were also clearly positive for both genes (Fig. 5C,D). As discussed below, *TCF-1* and *LEF-1* were differentially expressed in several osteogenic cells at day 14.5, which derive from the first pharyngeal arch.

Facial structures

From day 10.5 onwards, both genes were expressed in the mesenchymal cells of the nasal process. A representative example of expression is shown for *LEF-1* in Fig. 7B. Both the medial and the lateral nasal process expressed *TCF-1* and *LEF-1* at this stage, while differential expression was observed at later time points: At day 13.5, weak expression of *TCF-1* was observed in the lower and upper lips, which became undetectable at day 14.5 (Fig. 2A,B). In contrast, *LEF-1* was highly expressed in both lips at day 14.5 (Fig. 2C).

Fig. 2 displays unique expression of *TCF-1* in several other craniofacial structures. *TCF-1* mRNA was observed in the primordium of the basioccipital bone and the palatal process at day 13.5 (Fig. 2A) and in the cartilage primordium of the nasal bone, the palate, the maxilla and the mandible at day 14.5 (Fig. 2B).

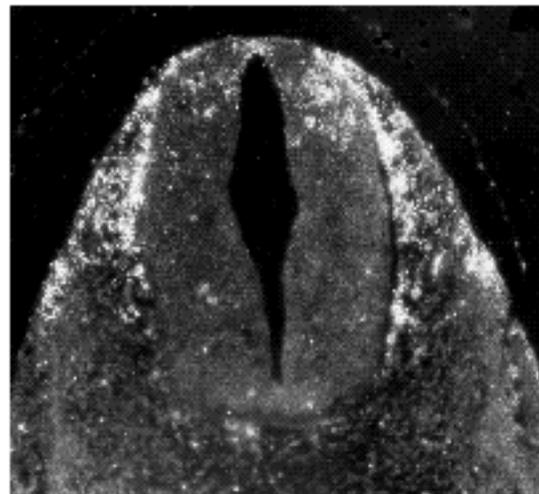
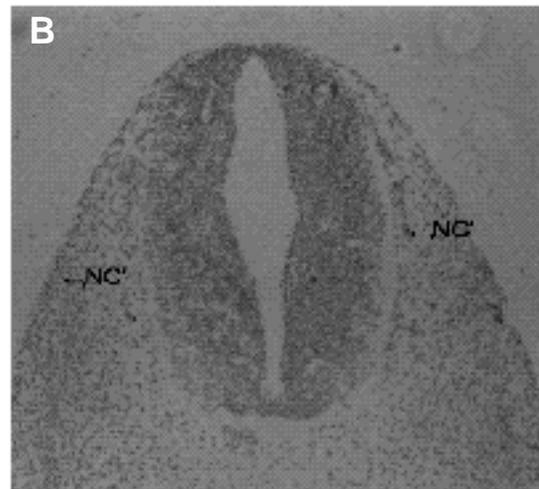
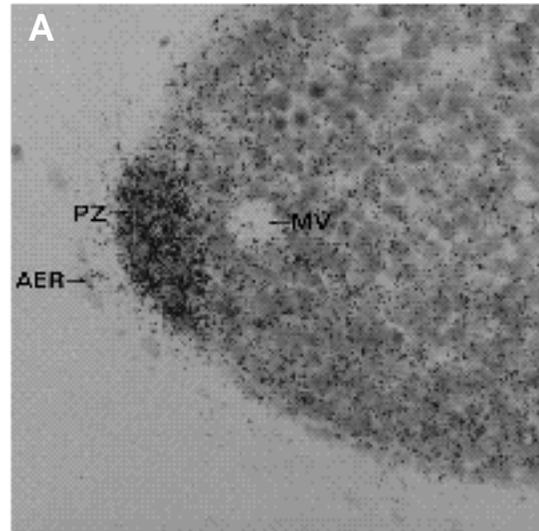
Bone-forming tissues

Both *TCF-1* and *LEF-1* mRNAs were detected in the neural crest-derived mesenchymal cells of the developing jaw, which differentiate into (pre)odontoblasts at day 14.5 (Fig. 2). As discussed above, *TCF-1* was expressed in pre-cartilagenous cells forming the primary and secondary palate, the basioccipital and nasal bones, the maxilla and the mandible (Fig. 2A,B). Expression of *LEF-1* was observed in mesenchymal cells around the cochlea at day 14.5 (Fig. 8).

TCF-1 and *LEF-1* expression was also observed in the vertebral column. Interestingly, vertebral expression occurred in a mutually exclusive fashion. At two subsequent days, day 13.5 and 14.5 of gestation, *TCF-1* was expressed in the thoracic prevertebrae and ribs (Figs 2A,B, 6B), whereas *LEF-1* displayed a complementary expression in tail prevertebrae and, additionally, in the mesenchymal cells forming the hip-bone (Fig. 2C). No rostral-caudal shift in expression was observed in time.

Lung

Expression of *TCF-1* and *LEF-1* in lateral mesenchymal cells of the lung bud was first observed at day 10.5 (Fig. 6A, for *TCF-1*). This mesenchymal pattern of expression was retained throughout the development of the lung. For example, Fig. 6B,C illustrates high level expression of both genes in the mesenchymal cells directly adjacent to the epithelial layer of the bronchioles at day 14.5. The bronchiolar epithelial cells themselves, however, were always negative. One day postnatally, no mRNA for *TCF-1* and *LEF-1* could be detected in lung tissue by either in situ hybridization or northern blot analysis (not shown).



Urogenital system

Analogous to the limb buds and the lung, *TCF-1* and *LEF-1* were expressed in mesenchymal cells directly adjacent to epithelial cells in the urogenital system. In kidney, mesenchymal cells surrounding the mesonephric tubules were

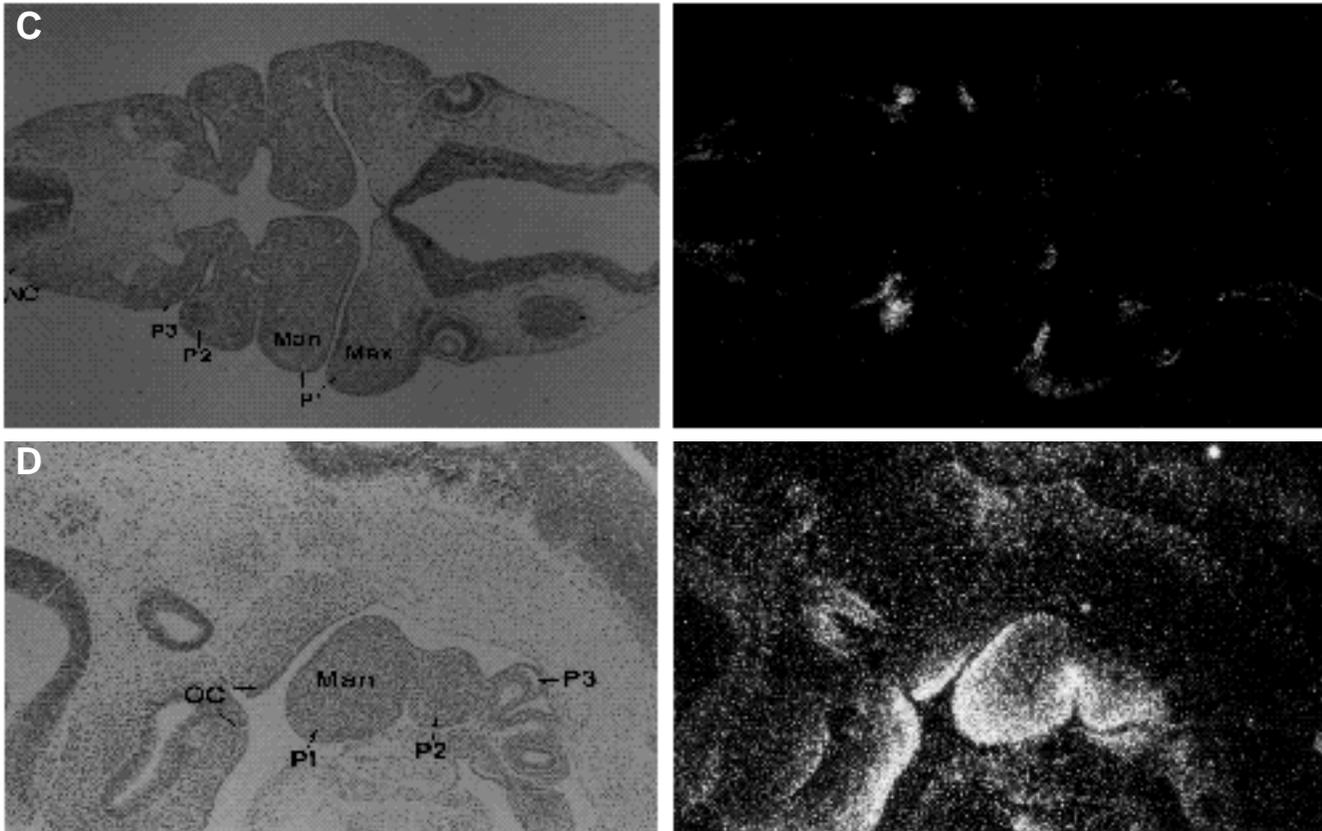


Fig. 5. Expression of *TCF-1* and *LEF-1* at day 10.5. At day 10.5, most prominent sites of expression were limb buds and neural crest cells. The first (P1), second (P2) and third pharyngeal arch (P3) were positive. The epithelial lining between the mandibular (Man) and the (Max) component of the first pharyngeal arch and the epithelium of the third arch also expressed both genes. The roof of the oral cavity (OC) expressed *LEF-1*. (A) day 10.5, *TCF-1*: detail of progress zone (PZ) of the limb bud, between the apical ectodermal ridge (AER) and the marginal vein (MV); (B) day 10.5, *TCF-1*: detail of posterior neural tube with putative migrating neural crest cells ('NC'); (C) day 10.5, transverse section probed for *TCF-1*; (D) day 10.5, *LEF-1*: detail of pharyngeal arches in para-sagittal section.

weakly positive for both genes on day 10.5 (not shown). Expression levels increased over the next few days. On day 14.5, high levels of expression were observed in the mesenchymal cells surrounding the metanephros (Fig. 2). One day post-partum, minimal amounts of *TCF-1* and *LEF-1* were occasionally detected by in situ hybridization in medullary mesenchymal cells of some of the kidneys examined (data not shown). We interpreted these observations as evidence for the perinatal down-regulation of gene expression. Both genes were expressed in mesenchymal cells of the (para)mesonephric duct and of the urogenital sinus at day 14.5 (Fig. 2). Fig. 2 also depicts the expression of *TCF-1* and *LEF-1* on day 14.5 in the genital eminence: *LEF-1* was expressed in a diffuse pattern throughout the genital eminence and around the epithelial cells of the developing urethra (Fig. 2C), whereas *TCF-1* was mainly observed at the anterior and posterior sides of the genital eminence (Fig. 2A).

Thymus

High expression of *TCF-1* in the fetal thymus was first observed around day 13.5 of gestation (Fig. 2), coinciding with the arrival of prothymocytes from the fetal liver and with the rearrangement and expression of T cell receptor

genes in these cells (Pardoll et al., 1987). Similar observations were made for *LEF-1*, although the latter gene appeared to be expressed at more moderate levels. The two genes are most likely not expressed by thymic stroma, as several murine thymic stromal cell lines were negative in northern blotting (not shown). Furthermore, immunohistochemical studies on human thymus have demonstrated that *TCF-1* expression is restricted to lymphoid cells (J. Castrop and H. C., unpublished).

Central nervous system

TCF-1 and *LEF-1* were both weakly expressed in the choroid plexus (Fig. 2). Fig. 7A depicts a detail of the developing choroid plexus between the telencephalic vesicles and the diencephalon at day 10.5 for *TCF-1*.

Unique *TCF-1* expression occurred in the meninges surrounding the spinal cord (Fig. 2A). Exclusive expression of *LEF-1* occurred in the brain. At day 11.5 of gestation, part of the telencephalon, the diencephalon and mesencephalon displayed high levels of expression, whereas the rhombencephalon was negative (Fig. 7B). At day 14.5, *LEF-1* mRNA was still expressed in part of the telencephalon, in diencephalon-derived structures like the dorsal thalamus and hypothalamus, in the roof of the mesencephalon and in the

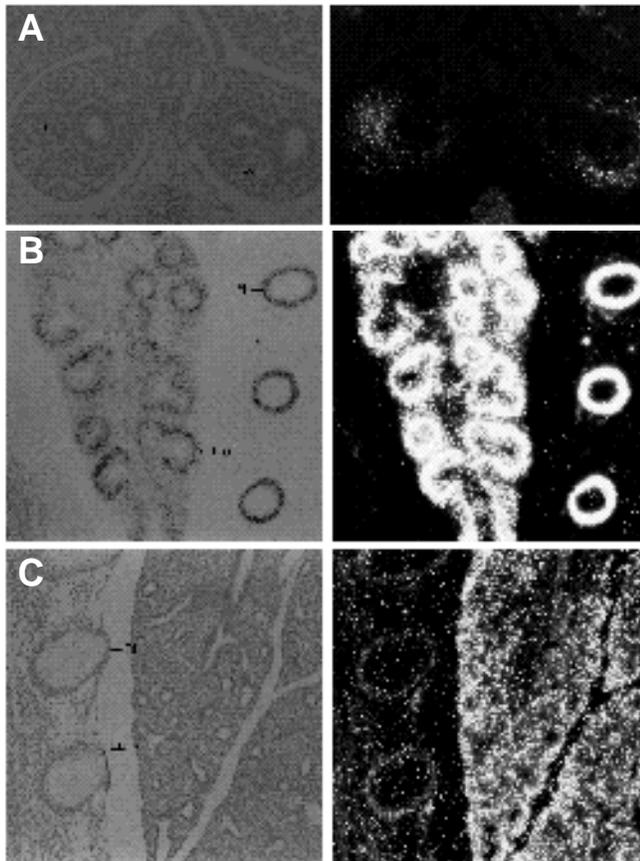


Fig. 6. Expression of *TCF-1* and *LEF-1* in lung. Expression of *TCF-1* and *LEF-1* was observed in the mesenchymal cells around the bronchioles from day 10.5 onwards. Ri, ribs. (A) day 10.5, *TCF-1* expression in the lung bud (Lu); (B) day 13.5, *TCF-1*; (C) day 14.5, *LEF-1*.

pituitary gland (Fig. 2C). One day postnatally, brain had ceased to express *LEF-1* as analyzed by in situ hybridization and northern blotting (not shown).

Adrenal gland

Exclusive expression of *TCF-1* was detected at day 13.5 and 14.5 in the cortex of the adrenal gland, which is of mesodermal origin (Fig. 2). The adrenal medulla, which is derived from neural crest, was negative.

DISCUSSION

The closely related cDNAs for *TCF-1* and *LEF-1* were originally cloned from lymphoid cells. Both genes have been implicated in transcriptional control of several T cell-specific structural genes and thus in the regulation of lymphoid differentiation (Oosterwegel et al., 1991a; Travis et al., 1991). However, there are no experimental data available yet on the in vivo function of these two genes. The HMG box genes most closely related to *TCF-1* and *LEF-1* are mammalian *SRY* and the *Schizosaccharomyces pombe* gene *mat Mc* (Gubbay et al., 1990; Sinclair et al., 1990; Kelly et al., 1988). *SRY* and *mat Mc* both have affinity for

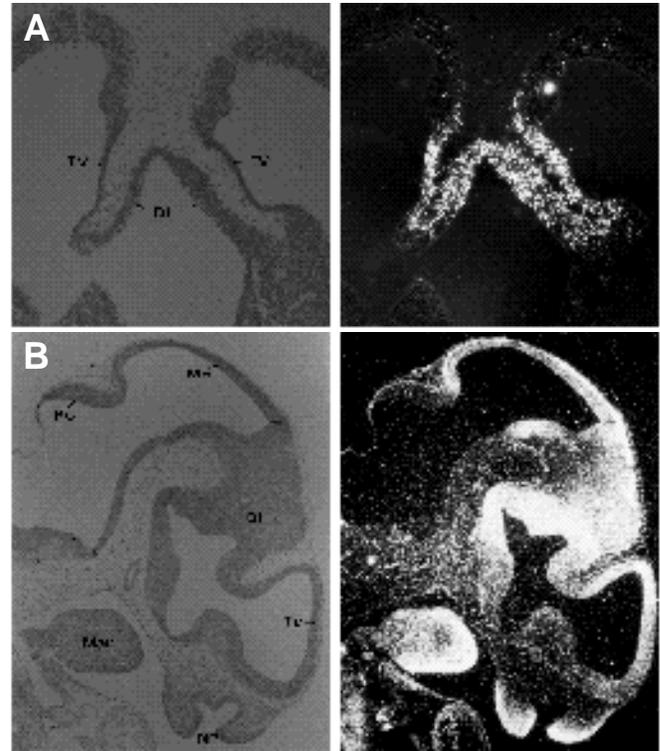


Fig. 7. Differential expression of *TCF-1* and *LEF-1* in the central nervous system. (A) At day 10.5, the developing choroid plexus expressed *TCF-1*. TV, medial wall of the telecephalic ventricle wall; Di, wall of diencephalon. (B) At day 11.5, bright expression of *LEF-1* was observed in telencephalon (Te), diencephalon (Di) and mesencephalon (Me) region of the brain. No *LEF-1* was detected in the rhombencephalon (Ro). Additionally, a representative example of the expression of *TCF-1* and *LEF-1* in the nasal process (NP) and the mandibular process (Man), developing from the first pharyngeal arch, is shown here.

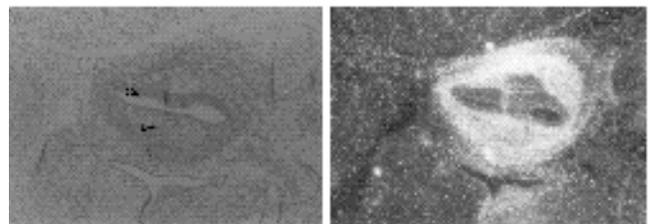


Fig. 8. Exclusive expression of *LEF-1* in inner ear. At day 13.5 and 14.5, mesenchymal cells of the inner ear (IE) around the cochlea (Co) expressed *LEF-1*.

the *TCF-1* motif AACAAAG (Harley et al., 1992; Dooijes and Clevers, in preparation). *SRY* has been demonstrated to control the male phenotype (Koopman et al., 1991) and *Mat Mc* is an *S. pombe* mating type gene (Kelly et al., 1988). By analogy, it is tempting to speculate that *TCF-1* and *LEF-1* exert similarly dramatic effects on the differentiation status of the cell that expresses these genes. For an evaluation of the putative differentional control properties of these two HMG box genes, it is essential to document the timing and the sites of their expression. Our present study provides such

an analysis and directly compares *TCF-1* and *LEF-1* expression.

As studied in a panel of cell lines, the two genes are differentially expressed within the lymphoid lineage. *TCF-1* mRNA is not detectable in any B lineage cell type. In contrast, *LEF-1* mRNA is readily observed in pro- and pre-B cells, as reported previously (Travis et al., 1991). *LEF-1* is also expressed at all stages of T lymphocyte differentiation, whereas high level expression of *TCF-1* only occurs from the prethymocyte stage onwards; prothymocytes express very low levels of *TCF-1*. These observations support the notion that the two genes play differential roles during lymphoid differentiation.

In newborn and adult animals, *TCF-1* and *LEF-1* expression is restricted to lymphoid tissues (Oosterwegel et al., 1991b; Travis et al., 1991; this study). By contrast, our present data reveal that the two genes are expressed at a number of sites outside the immune system during embryogenesis. At day 7.5 of gestation, expression of *TCF-1* and *LEF-1* is detectable throughout the embryo. The expression patterns become focussed from day 8.5 onwards, and are initially very similar for *TCF-1* and *LEF-1*. The most prominent sites for both *TCF-1* and *LEF-1* expression at day 9.5 and 10.5 are the limb bud, putative neural crest, and pharyngeal arches. At later stages, the expression of the two HMG box genes only partially overlap. No common denominator is apparent for the ontogenic origin of these various cell types transcribing the HMG box genes. Thus, expression occurs in cell types of neuroectodermal (e.g. brain, neural crest) as well as in differentiating mesenchymal cells (e.g. in limb bud, pharyngeal arches, lung bud and urogenital system).

The differential expression of the two genes is particularly intriguing considering that *TCF-1* and *LEF-1* derive from a common ancestor gene. We recently reported the cloning of a single chicken homologue of mammalian *TCF-1* and *LEF-1*, termed *chTCF* (Castrop et al., 1992). Based on sequence comparison, *chTCF* represents the evolutionary ancestor which duplicated in mammals to yield *TCF-1* and *LEF-1*. *ChTCF* mRNA was not detectable by northern analysis in the Bursa of Fabricius or a number of non-lymphoid tissues, but very high levels of *chTCF* were expressed in chicken thymus. In situ hybridization experiments are currently in progress in order to compare the expression pattern of *chTCF* with its two mammalian homologues. It is not known at present whether a divergence of function has accompanied the divergence of expression sites of the two mammalian genes. The virtually complete conservation of protein sequence between human and mouse *TCF-1* and between human and mouse *LEF-1* strongly suggest that functional divergence has indeed occurred before the separation of primates and rodents.

The expression of the two genes is shut off around birth in the non-lymphoid tissues, at the moment when the final architecture of most organ systems has been established. As *TCF-1* and *LEF-1* are expressed during (but not after) organogenesis, it appears attractive to postulate that the two genes play a role in the establishment rather than in the maintenance of cellular phenotype. The in vivo function of the two genes remains to be determined. Although the encoded proteins bind in a sequence-specific fashion to a

number of cellular enhancers, they do not act as classical transactivators of transcription when bound to concatamerized versions of their minimal cognate motif (Travis et al., 1991; van de Wetering et al., unpublished). The HMG box of *LEF-1* has recently been shown to induce a dramatic bend in the DNA helix (Giese et al., 1992). Based on this observation, it has been hypothesized that *LEF-1* serves as an architectural element that organizes three-dimensional structure of an enhancer and its associated transcription factors. The HMG box of *TCF-1* similarly bends DNA (van de Wetering and Clevers, unpublished). The highly conserved protein sequences outside the HMG boxes do not appear to contribute to DNA binding or bending, but might provide opportunities for protein/protein interactions with as yet undefined partner molecules.

Single or combined gene knock-out experiments in the mouse germ-line, currently ongoing in several laboratories, will shed light on the differentiation control properties of *TCF-1* and *LEF-1*. The expression data reported here will serve as a starting point for the evaluation of such experiments.

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