

Gene expression pattern

# Expression of mouse *HES-6*, a new member of the Hairy/Enhancer of split family of bHLH transcription factors

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## Abstract

We studied the expression of mouse *HES-6*, a new member of the Hairy/Enhancer of split family of basic helix-loop-helix transcription factors. *HES-6* is expressed in all neurogenic placodes and their derivatives and in the brain, where it is patterned along both the anteroposterior and dorsoventral axes. *HES-6* is also expressed in the trunk, in the dorsal root ganglia and in the myotomes. In the limb buds *HES-6* is expressed in skeletal muscle and presumptive tendons. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *HES-6*; Hairy/Enhancer of split; bHLH; Sensory placodes; Olfactory placode; Otic placode; Epibranchial placodes; Trigeminal placode; Sensory ganglia; Dorsal root ganglia; Hindbrain; Muscle; Tendons; Limb buds; Branchial arches

## 1. Results

Expression studies of the Hairy/Enhancer of split [H/E(spl)] family of bHLH transcription factors (for review see Fisher and Caudy (1998)) have led to the discovery and better understanding of several remarkable developmental processes, such as the periodic expression of genes in space (Ingham et al., 1985) and time (Palmeirim et al., 1997). It is therefore of considerable interest to identify and characterize new members of this family. We searched the GenBank EST database for sequences similar to chick *hairyl* and identified mouse and human clones encoding two closely related new members of the H/E(spl) family of genes. Since the mouse gene is the sixth murine member of this family, and because of the high sequence identity between the two genes, we named them mouse and human *HES-6*, respectively.

Sequence analysis of the cDNAs predicts proteins of 224 (mouse) and 222 (human) amino acids. Both contain basic helix-loop-helix domains with a proline residue in the 6th position of the basic region. They also contain the orange and carboxy-terminal WRPW domains. These are unique features of the H/E(spl) family of bHLH transcription factors (Atchley and Fitch, 1997). The mouse and human *HES-6* protein sequences are 85% identical and the corresponding bHLH and orange domains are 92 and 98% identical, respectively. All of the differences within the bHLH domain are contained in the loop portion of the domain. Phylogenetic

tree analyses of bHLH (Fig. 1) and orange (data not shown) domains suggest that while *HES-6* genes belong to the H/E(spl) family, they are not particularly closely related to any other member of this family described so far.

We studied the expression of *mHES-6* in 8.5- through 13.5-day-old mouse embryos, using whole-mount in situ hybridization. No expression was detected in E8.5 embryos. In E9.5 and E10.5 embryos, *mHES-6* is most prominently expressed in all neurogenic placodes (Fig. 2A–K,O) – the olfactory, trigeminal, otic (otic vesicle) and the three epibranchial placodes (geniculate, petrosal and nodose). In the otic vesicle expression is restricted to the anterior-ventral region, which contributes neurons to the vestibulo-acoustic ganglion (VIII). Expression was not detected in the lens placode, which does not give rise to neuronal cells.

Expression was also detected in mesenchymal cells adjacent to each placode (Fig. 2A–I), with the exception of the nasal placode. These may represent cells migrating from the placode to the corresponding ganglion (Noden, 1993; Hatini et al., 1999), because the staining often spans the gap between the two. Consistent with this, *mHES-6* expression was also detected in sensory placode-derived ganglia: trigeminal (V), facio-vestibulo-acoustic complex (VII–VIII), petrosal (IX) and nodose (X). By E11.5, expression in the cranial placodes is no longer detectable, while expression appears in the mesenchyme within the branchial arches (Fig. 2D).

In the brain (Fig. 2L–Q), we first detect expression of *mHES-6* at E9.5. At E10.5 and E11.5, the expression domains

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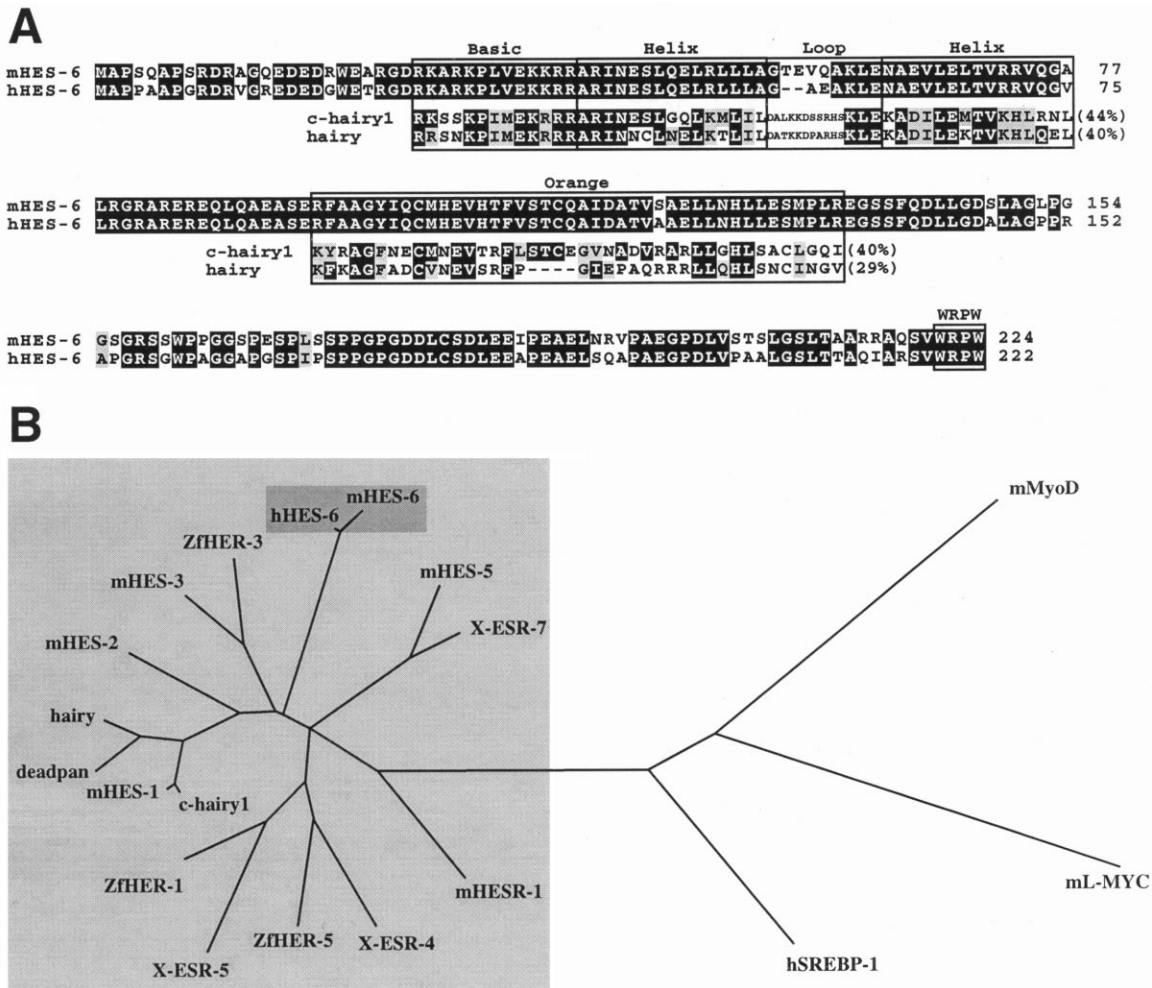


Fig. 1. (A) Alignment of the predicted amino acid sequences of mouse and human HES-6 proteins. (B) A neighbor-joining phylogenetic tree of bHLH domain sequences shows that mouse and human HES-6 proteins (dark box) belong to Hairy/Enhancer of split family (light box) and are less related to the bHLH proteins from other families (outside of the light box). Prefixes before the protein names indicate species: c, chick; h, human, m, mouse; X, frog; Zf, zebrafish; no prefix, fruit fly.

become stronger and more distinct, and patterned along the dorsoventral and anteroposterior axes of the brain. For example, in the hindbrain, adjacent rhombomeres express *mHES-6* in stripes at different dorsoventral levels (Fig. 2L,M). The strongest expression is detected in the ventral hindbrain with a sharp anterior boundary at the isthmus. The isthmus region shows a gap in expression that gradually becomes smaller between E9.5 and E11.5 (Fig. 2N,P,Q). Anterior to the isthmus, expression continues to the thalamic region, with decreased intensity (Fig. 2N,P,Q). Since the expression of *mHES-6* appears to be punctate in most regions, the differences in level of expression could be due either to different densities of expressing cells or to different levels of expression in individual cells. *mHES-6* is also expressed in the dorsal telencephalon and in the optic stalk at E10.5 (Fig. 2J,K,P,Q) and in the diencephalon at E11.5 (Fig. 2Q).

*mHES-6* is strongly expressed along most of the dorsal neural tube. During E10 and E11 this expression extends caudally throughout the spinal cord (Fig. 3), while in the

head there are regions of lower expression such as in rhombomeres 2–4 (Fig. 2L,M). While we detect no expression in migrating neural crest or in the autonomic nervous system, *mHES-6* is expressed in the dorsal root ganglia (Fig. 3A,B,D–F). In the spinal cord, ventral and dorsal domains of expression are observed, as well as several dorsoventrally-restricted bands at different levels of the cord (e.g. Fig. 3D).

Outside the nervous system, *mHES-6* is expressed in the myotome (Fig. 3). In older embryos (E12.5–E13.5) *mHES-6* is expressed in at least some of the body wall muscles including the intercostals and the diaphragm (data not shown). In the limb buds, *mHES-6* expression also appears to mark muscles and tendons and their precursors (Fig. 4). In the forelimbs expression is first detected at E10.5 in two domains, dorsal and ventral, in the central area of the limb buds in the mesenchyme underlying the surface ectoderm (Fig. 4A,H). As the limb buds grow this expression domain elongates (E11.5, Fig. 4B,C,I) and starts to separate into distinct subdomains (E12.5, Fig. 4D,E) such that by E13.5

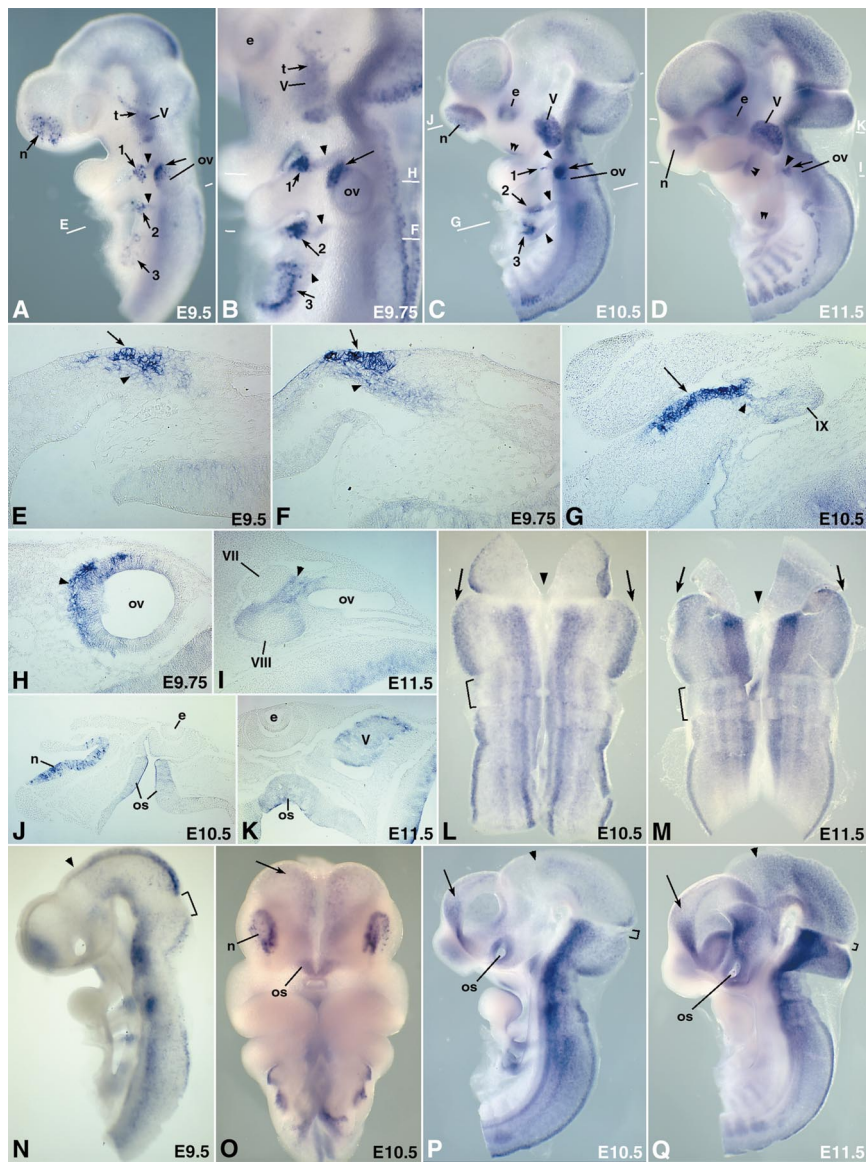


Fig. 2. Expression of *mHES-6* in the head. (A–D) Whole-mount staining of the heads of E9.5–11.5 embryos. *mHES-6* is detected in each of the neurogenic placodes (ectodermal expression indicated by arrows) and the associated subectodermal domains (arrowheads). Within the otic vesicle *mHES-6* is expressed in the anterior-ventral domain. During E10.5 (C) and E11.5 (D) *HES-6* is up-regulated in the mesenchyme within the branchial arches (double arrowheads). The staining in the eye region in (C,D) is within the optic stalk (J,K,P,Q). White lines on each side of the embryo represent the planes of sections in (E–K). (E–G) Transverse sections through the 2nd branchial placode of embryos in (A–C). Ventral is to the left and lateral is to the top. *mHES-6* is expressed in the ectodermal placode (arrows) and the cells within the underlying mesenchyme (arrowheads). At E9.75 (F) this expression in mesenchyme extends dorsally. By E10.5 (G) *mHES-6* expression is also detected in the glossopharyngeal (IX) ganglion. (H,I) Transverse sections through the otic vesicle region in E9.75 (H) and E11.5 (I) embryos. Orientation as in (E–G). *mHES-6* is expressed in the anteroventral aspect of the vesicle and in the adjacent mesenchymal cells (arrowheads). In (I) expression is also seen in the acoustic (VIII), but not the facial (VII) component of the facio-acoustic ganglion complex (VII–VIII). (J,K) Transverse sections through the eye region of E10.5 (J) and E11.5 (K) embryos. Orientation as in (E–G). *mHES-6* is expressed in the nasal placode, optic stalk, and trigeminal (V) ganglion. No expression is seen in the lens or the retina at either stage. (L,M) Ventricular views of the flat mounted hindbrains of E10.5 (L) and E11.5 (M) embryos. Arrowheads and arrows point to ventral and dorsal midlines, respectively. Anterior is to the top. *mHES-6* is expressed in anteroposteriorly extending stripes of varying intensities. Expression in the dorsal stripe is downregulated within rhombomers 2–4 (bracketed). (O) Ventral view of the head of E10.5 embryo showing *mHES-6* expression in the rostral telencephalon (arrow) as well as in the olfactory placode and the optic stalk. (N,P,Q) Ventricular view of the neural tubes of embryos in (A,C,D). *mHES-6* is expressed in the hindbrain with a sharp anterior boundary at the isthmus region. The non-expressing isthmus region (bracketed) becomes narrower as the embryo develops from E9.5 to E11.5. Further anteriorly *mHES-6* is expressed in the midbrain with an anterior boundary in the diencephalon (arrowheads) which is followed by another non-expressing domain. *mHES-6* is also expressed in the dorsal telencephalon (arrows) and in the optic stalk. e, eye; n, olfactory placode; os, optic stalk; ov, otic vesicle; t, trigeminal placode; 1,2,3, 1st (geniculate), 2nd (petrosal) and 3rd (nodose) epibranchial placodes; V, trigeminal ganglion, VII, facial and VIII, acoustic components of the facio-acoustic ganglion complex; IX, glossopharyngeal ganglion.

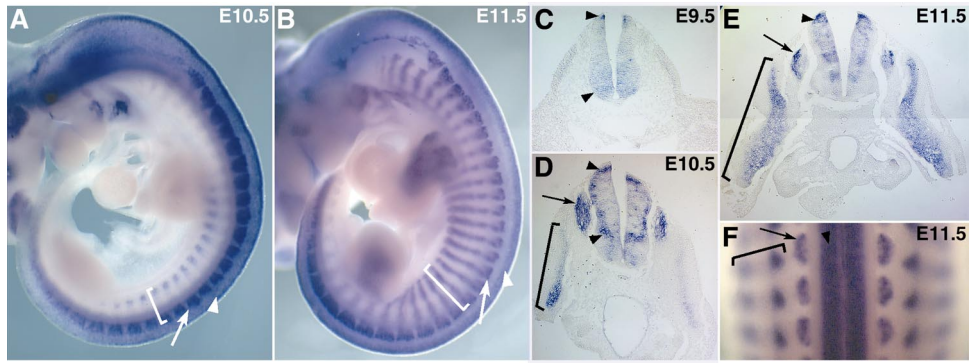


Fig. 3. Expression of *mHES-6* in the trunk. *mHES-6* is expressed in the myotomes (bracketed), dorsal root ganglia (arrows) and spinal cord (arrowheads). (A,B) Lateral view of E10.5 (A) and E11.5 (B) embryos. (C–D) Transverse sections of the trunk at the forelimb level (C) and inter-limb level (D,E). (C) At E9.5 *HES-6* is expressed in the dorsal-most region and in the ventral half of the spinal cord. By E10.5 (D) *mHES-6* is expressed throughout the spinal cord with higher levels of expression in the dorsal-most region and in a band within the ventral half of the neural tube. At this time *mHES-6* is also expressed in the dorsal root ganglia and myotomes. Similarly, at E11.5 (E) *mHES-6* is expressed in the neural tube, dorsal root ganglia and myotome. (F) Close-up of the inter-limb region of E11.5 embryo (dorsal view, anterior is at the top).

distinct muscle masses are stained (Fig. 4F,J). At E12.5 *mHES-6* also starts to be expressed in the handplate in what appear to be the forming tendons (Fig. 4 E,G,K). In the hindlimbs *mHES-6* is expressed similarly, but expression is delayed with respect to the forelimbs (Fig. 3A,B).

Thus during the 9th–13th days of mouse embryonic development, *mHES-6* is predominantly expressed in the nervous system and in muscle precursors and muscle cells. This pattern resembles the expression of the neural-

and muscle-specific bHLH proteins (Lee, 1997; Fode et al., 1998; Ma et al., 1998; Tajbakhsh and Buckingham, 2000, and references therein).

## 2. Methods

Mouse and human *HES-6* genes were identified by searching the GenBank EST database for sequences similar

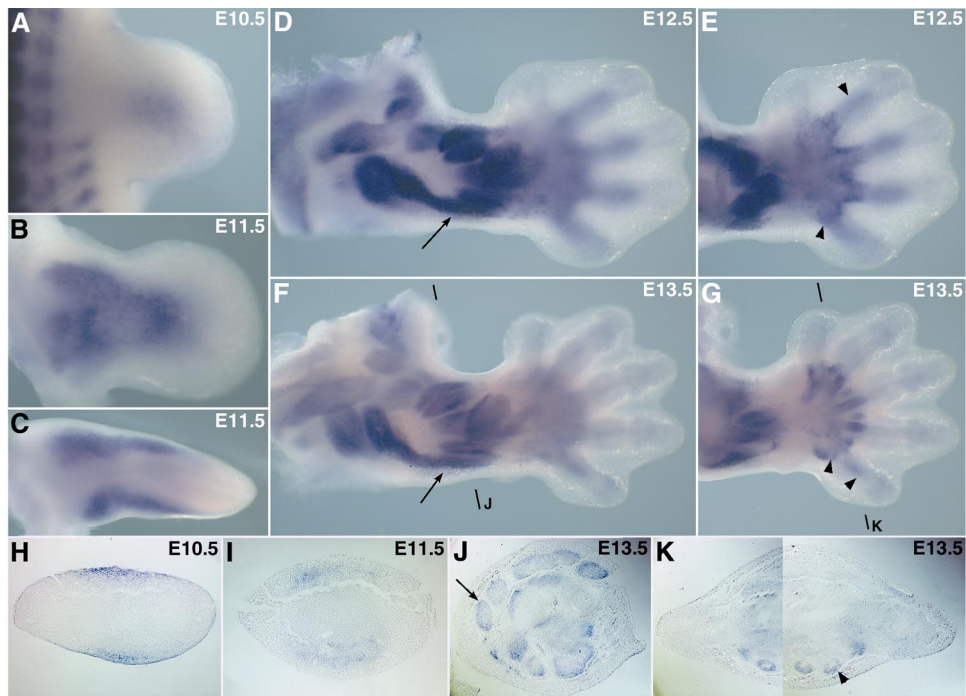


Fig. 4. Expression of *mHES-6* in the limb buds. (A,B,D,F) Dorsal, (C) anterior and (E,G) ventral views of forelimbs of E9.5–E13.5 embryos. (H–K) Transverse sections of E9.5–E13.5 forelimbs (dorsal to the top, anterior to the left). *mHES-6* is first expressed in the limb buds at E10.5 in the central domain (A) in the mesenchyme immediately underneath dorsal and ventral ectoderm (H). At E11.5 this expression extends with the elongating limb (B). There are still two, dorsal and ventral, expression domains (C) but they are in a deeper layer of limb mesenchyme (I). In E12.5 (D,E) and E13.5 (F,G,J) limbs *mHES-6* expression has evolved to mark distinct muscle masses (arrows). At this time *mHES-6* is also expressed in what appear to be the forming tendons (arrowheads in E,G,K) in the future digits and the palm. Lines on each side of the limb buds in (F,G) indicate the planes of section in (J,K).

to chick *hairy1* using BLAST. The EST clones were obtained from I.M.A.G.E. Consortium (mouse – I.M.A.G.E. Consortium CloneID: 385069, GenBank Accession: W62881; human – I.M.A.G.E. Consortium CloneID: 2169827, GenBank Accession: AI564818) (Lennon et al., 1996). The clones were fully sequenced using standard methods and their sequences submitted to GenBank under accession numbers AF260236 for mouse *HES-6* and AF260237 for human *HES-6*.

Sequence alignments were produced using ClustalW (Thompson et al., 1994) and BOXSHADE programs. The phylogenetic tree was generated by the Neighbour Joining method of Saitou and Nei (1987) using ClustalW WWW Service at the European Bioinformatics Institute (<http://www2.ebi.ac.uk/clustalw>). BLOSUM matrix was used when generating the multiple sequence alignment and Kimura correction of distances was applied when calculating the tree branch lengths. The tree was drawn using TREEVIEW (Page, 1996).

Mouse E8.5-E13.5 embryos were collected and whole-mount in situ hybridisation performed as described in Stern (1998). A range of concentrations of proteinase K (10–30 µg/ml) was used for optimal results. The probe was produced by transcription with T3 RNA polymerase using IMAGE clone 385069 (1.3 kb) in pT7T3D-Pac vector (see <http://image.llnl.gov/>). Paraffin sections were prepared from whole-mount stained embryos as described in Stern (1998).

### 3. Note added in proof

Since completion of this manuscript, two other papers have appeared reporting the expression of mouse *HES-6*: Bae, S., Bessho, Y., Hojo, M., Kageyama, R., 2000. The bHLH gene *Hes6*, an inhibitor of *Hes1*, promotes neuronal differentiation. *Development* 127, 2933–2943. Pissarra, L., Henrique, D., Duarte, 2000. Expression of *Hes6*, a new member of the Hairy/Enhancer-of-split family, in mouse development. *Mech. Dev.* 95, 275–278.

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