Transcriptional enhancer factor 1 disruption by a retroviral gene trap leads to heart defects and embryonic lethality in mice

Zhi Chen, Glenn A. Friedrich, and Philippe Soriano

Program in Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 USA

We have used a retroviral gene trap in embryonic stem (ES) cells to derive a recessive embryonic lethal mouse strain, ROSAβ-geo5. Mutant embryos display an enlarged pericardial cavity, brachycardia, a dilated fourth ventricle in the brain, and die between embryonic days 11 and 12. Whereas heart development in the mutant embryos is extensive, the ventricular wall is abnormally thin with a reduced number of trabeculae. Cloning of the trapped gene indicates that proviral insertion creates a null mutation in the transcriptional enhancer factor 1 (TEF-1) gene. Although transcription of a number of muscle-specific genes believed to be TEF-1 targets appears normal, the defect in cardiogenesis is likely attributable to diminished transcription of one or several cardiac-specific genes.

[Key Words: Transcription factor; TEF-1; cardiogenesis; gene trap]

Received July 19, 1994; accepted in revised form August 12, 1994.

Targeted gene disruption in embryonic stem (ES) cells has become a prominent approach to study the role of genes critical in mammalian development, but it is limited to analyses of known genes. To identify and mutate new genes involved in vertebrate development, one potential strategy is insertional mutagenesis. To improve on the efficiency of this approach, we and others have devised gene trap approaches that rely on the ability of an endogenous promoter to drive the expression of a promoterless reporter gene, typically β-galactosidase (Gosler 1989; von Melchner and Ruley 1989; Friedrich and Soriano 1991; von Melchner et al. 1992). When the gene trap integrates into a transcriptional unit, the normal expression pattern of the tagged gene can be followed by expression of the reporter, and the insertion creates a mutation as well (for review, see Gridley 1991; Joyner et al. 1992).

Several groups have reported using gene traps in ES cells to screen for genes essential for mouse development (Friedrich and Soriano 1991; Skarnes et al. 1992; von Melchner et al. 1992). In a previous study from this laboratory, we used a retroviral gene trap vector, ROSAβ-geo, in which a bifunctional β-galactosidase/neomycin phosphotransferase gene (β-geo) is used as a reporter (Friedrich and Soriano 1991). Whereas many mutant strains can be derived using gene traps, criteria to select which mutant to study need to be defined. One possibility is to select mutations based on the expression pattern of the trapped gene during development. However, targeted mutagenesis of some genes has shown that expression pattern is often a poor indicator of what phenotype might be expected. As a result, we have taken the approach of breeding gene trap insertions to homozygosity to identify developmental mutants, particularly those resulting in defects in early embryo development or organogenesis. Among 42 strains derived in our laboratory, 18 strains have a recessive embryonic lethal phenotype and 2 strains are recessive male sterile [Friedrich and Soriano 1991; unpubl.]. In this study we report the characterization of the ROSAβ-geo5 mutant in which the transcription enhancer factor 1 (TEF-1) gene has been disrupted.

Transcription factors play a vital role in basal level and tissue-specific regulation of gene expression, as well as in establishing cell differentiation during embryo development. TEF-1 was initially isolated from human HeLa cells and shown to bind specifically to the GT-IIC and Sph motifs in the SV40 enhancer. It is broadly expressed in many cell types but not in some hematopoietic cells (Xiao et al. 1991). TEF-1 belongs to a class of transcription factors that contains a DNA-binding motif, the TEA domain (Burglin 1991), in the amino-terminal end of these proteins. Included in this family are the TEC1 gene from Saccharomyces cerevisiae (Laloux et al. 1990), the ABA regulatory gene from Aspergillus nidulans (Mirabito et al. 1989), and the scalloped (sd) gene of Drosophila melanogaster (Campbell et al. 1992). These TEA genes...
domain-containing proteins regulate transcription of multiple genes. Both TEF-1 and TEC1 were isolated during analysis of enhancer elements [Laloux et al. 1990; Xiao et al. 1991]. The _AbaA_ gene controls development of the asexual apparatus of the filamentous fungus _A. nidulans_. It binds at _cis_-acting regulatory elements of several developmentally regulated genes and acts as a genetic switch to control development of the asexual spore [Andrianopoulos and Timberlake 1994]. Mutation of the _sd_ gene in _Drosophila_ causes early embryonic lethality, implying that the _Drosophila_ TEF-1 homolog plays an essential role in early development [Campbell et al. 1992]. The diversity of functions displayed by these proteins suggests that TEA domain-containing proteins have numerous regulatory roles in development among different species.

Human TEF-1 activates transcription through the SV40 and papillomavirus-16 enhancers [Davidson et al. 1988; Ishiji et al. 1992]. Biochemical and immunological experiments indicate that TEF-1 is probably identical to MCAT binding factor [M-CBF; Farrance et al. 1992], which binds MCAT sequence motifs [5'-CATTCCT-3'] present in a number of cardiac and skeletal muscle-specific genes, such as cardiac troponin C, T, and I, as well as myosins [Nikovits et al. 1986; Mar and Ordaith 1988, 1990; Mar et al. 1988; Thompson et al. 1991; Flink et al. 1992; Parmacek et al. 1992; Shimizu et al. 1992; Kariya et al. 1993]. As the MCAT motif has been implicated in regulation of these genes, TEF-1 may play a role in the regulation of MCAT-dependent muscle-specific genes.

The ROSAβ-geo5 strain characterized in this report, in which the TEF-1 gene has been disrupted, exhibits an embryonic lethal phenotype associated with heart dysfunction and homozygous embryos die between embryonic day 11 (E11) and E12 of gestation. Histological examination reveals an abnormally thin ventricle wall in the mutant embryo heart. However, cardiogenesis is initiated and expression of several potential TEF-1 targets appears to be normal. Thus, our analysis of the ROSAβ-geo5 mice indicates that TEF-1 plays an essential role in the maturation stage of cardiogenesis.

**Results**

The trapped gene is broadly expressed

The ROSAβ-geo5 mutant line was derived by infection of ES cells with the ROSAβ-geo retroviral vector, as described previously [Friedrich and Soriano 1991], and will be referred to henceforth as ROSA5. Expression of the trapped gene in the ROSA5 strain was followed by X-gal staining of embryos at different stages of development. Before implantation, lacZ expression was observed in cleavage-stage embryos [Fig. 1A] and blastocysts in both the inner cell mass and the trophectoderm [Fig. 1B]. At the primitive streak stage, X-gal staining was observed both in embryonic and extraembryonic compartments with higher expression in extraembryonic ectoderm [Fig. 1C]. At E12, β-galactosidase staining was observed throughout the embryo [Fig. 1D]. Histological sections showed β-galactosidase expression in all cells of the developing heart [data not shown]. In the adult, staining was observed in kidney, lung, uterus, heart, and skeletal muscles. Other tissues, including brain, liver, thymus, or spleen, displayed lower levels of staining [data not shown]. The connective tissues in certain organs, such as capsule of spleen and interlobular tissue of liver, were stained positive by X-gal, but lymphocytes did not exhibit β-galactosidase activity. Blood from these animals had no detectable β-galactosidase activity [data not shown]. These findings indicate that the trapped gene in ROSA5 is expressed broadly, but not ubiquitously, during embryogenesis and in adult mice.

ROSA5 mutants die at midgestation

A single-copy probe flanking the provirus insertion was isolated from a ROSA5 genomic library and used to distinguish the mutant and wild-type alleles on Southern blots [data not shown]. No homozygous animals were found among 240 offspring from crosses between hetero-
zygous parents, indicating that the proviral integration causes embryonic lethality. To determine the time of lethality, embryos were genotyped using yolk sac DNA between E9.5 and E14.5 (Table 1). At E9.5, mutants were recovered in the expected Mendelian ratio and could not be distinguished from their wild-type or heterozygous littermates by size or gross morphology. Starting at E10.5, the yolk sacs of most of the homozygotes were pale, although the vascular system was clearly visible [Fig. 2A]. The fourth ventricle of the brain was also enlarged [Fig. 2D]. By E11.5, mutant embryos had dilated pericardial cavities [Fig. 2B] and a reduction of the heart rate to about half that of wild-type or heterozygous embryos. In most living mutants at this stage, pulsation of the heart was weak such that red blood cells were retained in the ventricle even after contraction. At E11.5, about half of the mutant embryos were necrotic or in the process of resorption. At E12.5, no viable homozygous mutant embryos were observed. These results indicate that proviral insertion in ROSA5 causes embryonic lethality between E11.5 and E12.5 of gestation.

**Homozygous ROSA5 embryos display abnormalities in the heart**

Homozygous embryos were dissected and prepared for histological sections. The defects found at E10.5 were tissue edema and an abnormality in the ventricle wall of the heart. In wild-type embryos, the heart had developed both atrial and ventricle chambers separated by the endocardial cushion tissue [Fig. 2E]. Each chamber was lined with endocardium, consisting of myocardium and an endothelial layer, and the heart was surrounded by the pericardium. The trabeculation process, in which myoblast cells derived from pericardial mesoderm differentiate into myocytes, was initiated and extensive by E10.5. The interventricular foramen and septum were being formed. In contrast, in the mutant embryos, the ventricle wall was thinner and there were only one to two layers of trabecular lining the wall, as compared with three to four layers in wild-type or heterozygous littermates [Fig. 2E,F]. In addition, periodic-acid-Schiff's [PAS] positive staining "ghost" particles were observed underlying endocardial cells in the mutant, possibly representing degenerating myocytes [Fig. 2G,H]. No other defects were detected in the mutant embryos by histological examination of other tissues, including the liver, somites, and nervous system. No histological abnormality was observed in brain except for the dilation of the fourth ventricle [data not shown].

To examine the subcellular structures of differentiating myoblasts, transverse sections of heart ventricles from E11.5 homozygous mutant embryos and control embryos were examined by transmission electron microscopy. Myofibers appeared normal with alternate A and I banding patterns, and Z lines were also observed [Fig. 3A-D]. In the longitudinal axis, orderly arrangements of actin filaments around the large myosin filaments were observed in cross section through the A bands [Fig. 3E,F]. Mitochondria appeared normal and desmosomes and fascia adherens were also present [data not shown]. Thus, the mutation in ROSA5 does not lead to inherent defects in the formation of myofibrils in embryonic myocardial cells.

**The trapped gene in the ROSA5 mutant encodes TEF-1**

To identify the disrupted gene in the ROSA5 mutant, a cDNA library was constructed from liver RNA of ROSA5 using a lacZ reverse primer. The library was screened with another lacZ primer adjacent to the splice acceptor site. Among five clones characterized, the longest one contained 410 bp of cDNA sequence 5' of the splice acceptor site. Comparison of the cDNA sequence with the GenBank data base indicated that it shared 70% nucleotide identity with the 5'-untranslated region of the human TEF-1. A 260-bp DNA fragment from this cDNA clone was used to screen a randomly primed ES cell cDNA library, and two cDNA fragments with 2.4- and 1.4-kb inserts were sequenced. The cDNA sequence has an open reading frame encoding a protein of 426 amino acids [Fig. 4] that shares 421 identical amino acids with the human TEF-1 protein [Xiao et al. 1991]. The AUU codon at nucleotide 463 rather than an AUG codon with the human TEF-1 protein [Xiao et al. 1991]. The AUU codon at nucleotide 463 rather than an AUG codon to position 517 is presumed to be the site of translation initiation of mouse TEF-1 as the human TEF-1 gene uses an AUU codon exclusively as an initiator. The TEA domain, which has DNA-binding activity, is identical between mouse and human TEF-1. About 1.4-kb of mouse TEF-1 cDNA has been cloned independently [Blatt and DePamphilis 1993] and encodes a protein almost identical to that shown in Figure 4 except for two amino acid differences at the 5' end.

Reverse transcription–polymerase chain reaction (RT–PCR) was used to determine whether the disruption of TEF-1 in ROSA5 creates a null mutation. E9.5 embryos derived from heterozygous crosses were used to isolate RNA, and DNA fragments representing the wild-type [570 bp] or mutant [258 bp] alleles were amplified by RT–PCR using two different pairs of primers. As shown in Figure 5A, only the 570-bp DNA fragments were amplified in wild-type embryos [lanes 5,6,9,10], both the 570- and the 258-bp products were produced in heterozygous embryos [lanes 1,4,7,8,13,14], and only the 258-bp fragment was amplified in homozygous mutant embryos [lanes 11,12]. The absence of the wild-type frag-

---

**Table 1. Genotype of ROSA5 embryos**

| Age (days) | Wild type | Heterozygote | Homozygote |
|-----------|-----------|--------------|------------|
| 9.5       | 9         | 29           | 12         |
| 10.5      | 15        | 35           | 14         |
| 11.5      | 15        | 24           | 12         |
| 12.5      | 13        | 27           | 7          |
| 14.5      | 2         | 5            | 1          |

Embryos were isolated at different stages of gestation and genotyped by Southern blots of yolk sac DNA using a genomic flanking probe.
Figure 2. Phenotype of the ROSA5 mutant embryos. (A) E11.5 mutant and wild-type embryos were dissected from the uterus, and the decidua was removed to expose embryos with the yolk sac intact. The arrow indicates a homozygous mutant embryo. Note the paleness of the yolk sac. (B) The yolk sac was removed to display the enlarged pericardial cavity and the dilated hindbrain of the mutant embryo. Another pair of wild-type E11.5 embryos (C) and ROSA5 mutant E11.5 embryos (D) are shown to display dilation of the fourth ventricle in the brain prior to the enlargement of pericardial cavity. Histological analysis of heart tissue from heterozygous and homozygous ROSA5 embryos (E–H). Transverse sections of heart from heterozygous (E,G) and mutant (F,H) embryos are shown. Similar planes of sections are shown in E and F and in G and H. Note the thin ventricle wall [F] and the PAS-positive particles underlying endocardial cells [H] indicated by arrows. (EC) Endocardial cushion; (IVS) interventricular septum; (LV) left ventricle; (RV) right ventricle. Magnification, (E,F) 59× (G,H) 236×.

ment in the mutant embryos indicates that the gene trap insertion prevents the production of 5' exons of TEF-1.

To address the possibility that splicing bypasses the reporter gene to create aberrant TEF-1 messages, another set of primers was used to determine whether TEF-1 exons 3' to the reporter gene are still produced. A pair of TEF-1 primers were designed to amplify a 366-bp fragment at the 3' end of TEF-1 cDNA, spanning the translation termination codon and the 3'-untranslated region. RT–PCR reactions with this set of primers failed to detect TEF-1 message from homozygous mutant ROSA5 embryos [Fig. 5B, lanes 7–10]. Thus, TEF-1 messages 3' to the insertion site of the gene trap vector are not produced. Taken together, these RT–PCR analyses indicate that the gene trap insertion in ROSA5 creates a null allele of the TEF-1 gene.

Potential targets of TEF-1 are not down-regulated in ROSA5 mutants

TEF-1 is a transcription factor that has been proposed to be involved in the regulation of several cardiac muscle-specific genes [see Discussion]. Genes such as cardiac troponin T (cTnT; Mar and Ordahl 1988), cardiac troponin C (cTnC; Parmacek et al. 1992), cardiac troponin I (cTnl; Nikovits et al. 1986), and myosin heavy chains (Thompson et al. 1991; Flink et al. 1992; Shimizu et al. 1992; Kariya et al. 1993) contain TEF-1 binding sites within their regulatory regions. Thus, it is conceivable that the phenotype observed in the ROSA5 mutants might be attributable to abnormal regulation of one or more of these potential TEF-1-regulated genes.

To test this hypothesis, Western blot and RT–PCR
analyses were performed to analyze the expression of these genes in E10 mutant embryos. Proteins isolated from mutant embryos were separated on SDS–polyacrylamide gels, transferred and bound to membrane, and reacted with antibodies against cTnT, cTnI, and myosins. Proteins in the size range of 200 kD (myosin), 41 kD (cTnT), and 31 kD (cTnI) are all detected in the homozygous embryos and in the blood. Characterization of the ROSA5 mutant demonstrates that TEF-1 plays an essential role in cardiac muscle development in mammals. The homoygous mutants die between E11 and E12 of gestation, a critical period for cardiogenesis. The heart is the first organ to appear during organogenesis, and rapid growth of embryos requires efficient removal of metabolites and growth of embryos requires efficient removal of metabolites in the brain. Histological analysis of these embryos revealed defects in the heart, including an abnormally thin ventricular wall and a reduced number of trabeculae.

**TEF-1 and heart development**

TEF-1 was initially isolated from HeLa cells based on its ability to bind specifically and cooperatively to the GT-IIC and SphI motifs in the SV40 enhancer [Xiao et al. 1991]. It is broadly expressed in many cell types, but not in some hematopoietic cells, consistent with the lack of β-galactosidase expression that we have observed in lymphocytes and in the blood. Characterization of the ROSA5 mutant demonstrates that TEF-1 plays an essential role in cardiac muscle development in mammals. The homoygous mutants die between E11 and E12 of gestation, a critical period for cardiogenesis. The heart is the first organ to appear during organogenesis, and rapid growth of embryos requires efficient removal of metabolic.

Figure 3. Electron micrographs of myocytes from ROSA5 embryos. Thin sections through trabecular cells in ventricles of developing heart from E11.5 wild-type (A,C,E) and mutant (B,D,F) embryos are shown. (A–D) Transverse sections. Boxed areas in A and B are shown in higher magnifications in C and D. E and F are cross sections through the A band of myofibrils (arrows). (N) Nucleus, (Mt) mitochondrion, (Z) Z lines, (A) A band, and [I] I band are labeled. Magnification, 3060× (A,B); 4290× (C–F).

Discussion

In this report we demonstrate that a mutation of the TEF-1 gene by a retroviral gene trap results in a recessive lethal phenotype. ROSA5 mutant embryos die between E11 and E12 of gestation and show an enlarged pericardial cavity, brachycardia, and a dilated fourth ventricle.
Figure 5. Proviral insertion leads to a null mutation in the TEF-1 gene. RT-PCR analysis was performed to check for the presence of 5' (A) or 3' (B) exons of TEF-1 cDNA in homozygous ROSA5 embryos. (A) In odd-numbered lanes, two TEF-1 5'-end primers, flanking the ROSA β-geo insertion, are used to amplify a 570-bp DNA fragment from wild-type TEF-1 transcripts. In even-numbered lanes, a 5' TEF-1 forward primer and a lacZ reverse oligonucleotide are used to generate a 258-bp DNA fragment from TEF-1-β-geo fusion transcripts. (B) A pair of TEF-1 3'-end oligonucleotide is used in even-numbered lanes to amplify a 366-bp fragment from wild-type TEF-1 transcripts. In odd-numbered lanes, the same TEF-1 5'-forward primer and lacZ reverse primer as used in A were used to detect the 258-bp DNA fragment from TEF-1/β-geo fusion transcripts.

olites and supply of nutrients to all tissues. The earliest phenotype observed in homozygous embryos at late E10 was a pale yolk sac and a dilated fourth ventricle in the brain. This phenotype is consistent with the notion that poor blood circulation may result in increased pressure in the fourth ventricle of the brain attributable to inefficient drainage of the primary head vein. Later, mutant embryos exhibit dilated pericardial cavities as well as slow heartbeats. Histological examination of mutants reveals tissue edema and a thin ventricle wall of the heart, accompanied by a reduced degree of trabeculation and the presence of PAS staining ghost particles underlying endocardial cells. The above findings are consistent with the notion that TEF-1 may be involved in cardiac muscle development. Although a mutation in the N-myc gene (Charron et al. 1992) leads to impaired heart development, it is difficult to conclude whether this nuclear factor operates in a common pathway as TEF-1, as the defects occur at an earlier stage and are associated with a general delay in development. However, some of the defects found in RXRα mutant embryos (Sucov et al. 1994) appear to be similar to those found in ROSA5 mutants.

Because cardiogenesis is initiated at E8, and no obvious phenotype is detected in ROSA5 mutants until late E10, the role of TEF-1 in cardiogenesis is not in initiation of heart development but in maturation of the embryonic heart. The four chambers of the heart in mutant embryos are well developed, trabeculation is initiated, and striated muscle fibers are being formed in myocardial cells. Directing TEF-1 expression to the heart of ROSA5 embryos might rescue the mutant embryos and confirm the critical role of TEF-1 in the maturation stage of cardiogenesis.

Downstream targets of TEF-1

Although studies on human TEF-1 indicated its role as a transcriptional activator of viral genes (Davidson et al. 1988; Ishiji et al. 1992), the cellular targets were not known until it was shown, based on biochemical analysis and immune cross-reactivity, that TEF-1 is identical to MCBF (Farrance et al. 1992). MCBF binds the M-CAT sequence motifs, 5'-CATTCCT-3' (Mar and Ordahl 1988, 1990). This motif is present in the regulatory re-
functions in vivo. Another interesting question to be addressed is whether other functions of TEF-1 at late stages of development are masked by embryo lethality at E12. Rescuing the heart phenotype may unveil additional functions of TEF-1 during late stages of embryogenesis.

Materials and methods

Derivation of mutant mice

The ROSA5 mutant was derived by infection of ES cells with the retroviral gene trap vector ROSAβ-geo as described previously (Friedrich and Soriano 1991). According to the rules of the Mouse Nomenclature Committee, this strain will be designated TgR(ROSA5)SSor.

Histology

Mouse embryos were dissected in PBS and fixed in Bouin's solution for 6 hr at room temperature. They were then gradually dehydrated in ethanol, cleared with HistoClear, and embedded in paraplast. Sections were cut at 6–8 μm using a Reichert 2030 microtome. Sections of embryos were stained either with hematoxylin and eosin, or PAS stain.

Electron microscopy

E10 embryos were fixed in half-strength Karnovsky's fixative, rinsed in buffer, and postfixed in 2% colloidion-buffered osmium tetroxide. They were dehydrated in graded ethanol and propyleneoxide and embedded in Polybed 812. Sections of 80–90 nm were cut and stained with saturated aqueous uranyl acetate and Millonig's lead stain. They were examined and photographed with a JEOL 100-SX electron microscope operating at 80 kV.

Construction and screening of cDNA library

The TEF-1/β-geo fusion mRNA sequences were isolated from a β-geo primed cDNA library. The cDNA library was constructed with poly[A] + RNA isolated from liver of ROSA5 heterozygotes, using a primer specific for the lacZ sequences [5'-ATCCGCTTACGTCAAATTCAGACGG-3'] and standard procedures (Pharmacia U-prime cDNA synthesis kit). The resulting double-stranded cDNA was ligated into the EcoRI site of λZapII (Stratagene). Plaques (150,000) were screened with a labeled oligonucleotide [5'-GAAAGACCGCGAAGAGTTTGTCCTCAAATTCAGACGG-3'] complementary to sequences immediately 3' to the splice acceptor of ROSAβ-geo. Four positive plaques were purified, and the one with longest insert (cRS-1) was used to recover the cloned insert by phage reshift.

The full-length TEF-1 cDNA was identified by screening a random-primed ES cell cDNA library, constructed as follows. Total cellular RNA was isolated from AB1 ES cells (McMahon and Bradley 1990) grown in the absence of feeder cells in leukemia inhibitory factor (LIF)-supplemented ES medium. Poly[A] + RNA was isolated by purification using the PolyA-Track mRNA isolation system (Promega). Five micrograms of poly[A] + RNA was mixed with random hexamers (Pharmacia) at a final concentration of 6 μg/ml, and cDNA was synthesized according to standard procedures. After ligation to two different adapters (Elldehyde et al. 1991), cDNA was run through a 1% low-melt agarose gel to select cDNA in the size range of 0.6–4 kb. After purification by phenol/chloroform extraction and ethanol precipitation, 0.1 μg of cDNA was ligated to 2 μg of T-filled λYES-R vector (Ellledge et al. 1991) and packaged using a Giga-
Chen et al.

pack Gold packaging extract (Stratagene). The original cDNA library represents 12 × 10^6 individual plaques.

A 260-bp EcoRI-Clal DNA fragment from CR5-1 was used to screen the E5 cDNA library. Among 0.5 × 10^6 colonies screened, 13 positive clones were identified. The inserts from these positive clones were ligated into pKS Bluescript II (Stratagene). Two clones with 2.4- and 1.4-kb inserts were sequenced after Exonuclease III/S1 nuclease nested deletions.

Genomic cloning and genotyping

ROSA5 genomic DNA was partially digested by MboI and 15- to 23-kb fragments were cloned at the BamHI site of pDashl (Stratagene). Following in vitro packaging, the phage library was screened with a neo probe. None of the five clones isolated, spanning ~20 kb of the insertion locus, hybridized to the TEF-1 cDNA, suggesting that the provirus has inserted into a large intron. For genotyping, a 250-bp PstI genomic DNA fragment 0.7 kb downstream of the proviral insertion site was used. After digestion of genomic DNA with SacI, the probe detects a 3.6-kb band from wild-type allele and a 4.0-kb band from the mutant allele.

RT-PCR

To amplify fragments of the TEF-1 cDNA and the TEF-1/β-geo fusion cDNA, total embryo RNA isolated from E9.5 embryos was mixed with 10 pmoles of either a lacZ reverse primer 5'-ATGCGCTAGCTGAATTTACGACGC-3' or a TEF-1 5' reverse primer 5'-GGAAAGGTGGGGCGTGAAAG-3' and denatured by heating at 70°C for 3 min. RT reactions were performed at 37°C for 60 min with 200 units of Moloney murine leukemia virus reverse transcriptase in 20 μl of 1× PCR buffer plus 0.5 mM of each dNTP, 10 mM DTT, and 10 units of RNasin (Promega). After the reverse transcriptase reaction, the volume of each reaction was increased to 100 μl by adding 80 μl of 1× PCR buffer containing 10 pmoles of a TEF-1 5' forward primer 5'-CTGGAGTAACGCGGGGC-3' and 5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). Forty cycles of PCR was performed with the following temperature profile: denaturation at 94°C for 1 min, annealing at 64°C for 1 min and extending at 72°C for 1.5 min.

To detect 3' exons of the TEF-1 cDNA, RT-PCR was performed essentially as above except that [1] 100 pmoles of random hexamers were used in the reverse transcriptase reaction; and [2] forward primer 5'-AAGGCCGGCAGCGACACAC-3' and reverse 5'-TCTAACCACACAGGAAAC-3' TEF-1 3' oligonucleotides were employed.

For cTnC RT-PCR, forward 5'-AATGGATGACATCTAC-AAA-3' and reverse 5'-TGGCTTTCAATGCTACCT-3' oligonucleotides were used in a PCR reaction consisting of 40 cycles of denaturation at 93°C for 30 sec, annealing at 54°C for 40 sec, and extension at 65°C for 40 sec. Amplification with this set of primers generates a 410-bp cTnC cDNA fragment.

Protein isolation and Western blot analysis

Protein was isolated from E10.5 embryos by homogenization in Laemmli buffer followed by boiling for 5 min. Forty micrograms of protein was loaded per lane and separated by SDS-PAGE. After blotting to nitrocellulose, Western blot analysis was performed with the following antibodies: MF-20 recognizing all myosin isoforms, mAb 13-11 specific for cTnT, and T1-4 recognizing both cTnI and skeletal troponin I (Saggin et al. 1989).

Acknowledgments

We thank Pierre Chambon for human TEF-1 cDNA, Jeffrey Leiden for troponin C cDNA, Simonetta Ausoni, Sarah Larkin, and Charles Ordahl for antibodies to troponin I, troponin T, and myosin, and for helpful suggestions, Liz Caldwell and Judy Groombridge from Fred Hutchinson Cancer Research Center Shared Resources for electron microscopy, Paul Goodwin and Tim Knight for image analysis. We thank Elizabeth Thomas for care of the animals and our colleagues for critical reading of the manuscripts. Z.C. was supported by Fellowship DRG-1231 from the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation. This research was supported by grant HD24875 from the National Institute of Child Health and Human Development to P.S.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 USC section 1734 solely to indicate this fact.

References

Andrianopoulos, A. and W.E. Timberlake. 1994. The Aspergillus nidulans abaA gene encodes a transcriptional activator that acts as a genetic switch to control development. Mol. Cell. Biol. 14: 2503–2515.

Blatt, C. and M.L. DePamphilis. 1993. Striking homology between mouse and human transcription enhancer factor-1 (TEF-1). Nucleic Acids Res. 21: 747–748.

Burglin, T.R. 1991. The TEA domain: a novel, conserved DNA-binding motif. Cell 66: 11–12.

Campbell, S., M. Inamdar, V. Rodrigues, V. Raghavan, M. Palazzo, and A. Chovnick. 1992. The scolloped gene encodes a novel, evolutionarily conserved transcription factor required for sensory organ differentiation in Drosophila. Genes & Dev. 6: 367–379.

Charron, J., B.A. Malynn, P. Fisher, V. Stewart, L. Jannotte, S.P. Gof1, E.J. Robertson, and F.W. Alt. 1992. Embryonic lethality in mice homozygous for a targeted disruption of the N-myC gene. Genes & DeV. 6: 2248–2257.

Chow, K.L. and R.J. Schwartz. 1990. A combination of closely associated positive and negative cis-acting promoter elements regulates transcription of the skeletal alpha-actin gene. Mol. Cell. Biol. 10: 528–538.

Davidson, I., J.H. Xiao, R. Rosales, A. Staub, and P. Chambon. 1988. The HeLa cell protein TEF-1 binds specifically and cooperatively to two SV40 enhancer motifs of unrelated sequence. Cell 54: 931–942.

Elledge, S.J., J.T. Mulligan, S.W. Ramer, M. Spottonswod, and R.W. Davis. 1991. Lambda YES: a multifunctional cDNA expression vector for the isolation of genes by complementation of yeast and Escherichia coli mutations. Proc. Natl. Acad. Sci. 88: 1731–1735.

Farrance, I.K.G., J.H. Mar, and C.P. Ordahl. 1992. M-CAT binding factor is related to the SV40 enhancer binding factor, TEF-1. J. Biol. Chem. 267: 17234–17240.

Flink, I.L., J.G. Edwards, J.J. Bahl, C.C. Liew, M. Sole, and E. Morkin. 1992. Characterization of a strong positive cis-acting element of the human beta-myosin heavy chain gene in fetal rat heart cells. J. Biol. Chem. 267: 9917–9924.

Friedrich, G. and P. Soriano. 1991. Promoter traps in embryonic stem cells: A genetic screen to identify and mutate developmentally regulated genes in mice. Genes & Dev. 5: 1513–1523.

Gossler, A., A.L. Joyce, J. Rossant, and W.C. Skarnes. 1989. Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. Science 244: 463–465.
TEF-1 gene disruption in mice

Gridley, T. 1991. Insertional versus targeted mutagenesis in mice. New Biol. 3: 1025–1034.

Ishiji, T., M.J. Lace, S. Parkkinen, R.D. Anderson, and T.H. Haugen. 1992. Transcriptional enhancer factor (TEF-1) and its cell-specific co-activator activate human papillomavirus-16 E6 and E7 oncogene transcription in keratinocytes and cervical carcinoma cells. EMBO J. 11: 2721–2728.

Joyner, A.L., A. Auerbach, and W.C. Skarnes. 1992. The gene trap approach in embryonic stem cells: the potential for genetic screens in mice. Ciba Found Symp. 165: 277–288.

Kariya, K., I.K. Farrance, and P.C. Simpson. 1993. Transcriptional enhancer factor-1 in cardiac myocytes interacts with an alpha 1-adrenergic- and beta-protein kinase C-inducible element in the rat beta-myosin heavy chain promoter. J. Biol. Chem. 268: 26658–26662.

Laloux, I., E. Dubois, M. Dewerchin, and E. Jacobs. 1990. TEC1, a gene involved in the activation of Ty1 and Ty1-mediated gene expression in Saccharomyces cerevisiae: cloning and molecular analysis. Mol. Cell. Biol. 10: 3541–3550.

Mar, J.H. and C.P. Ordahl. 1988. A conserved CATTCTC motif is required for skeletal muscle-specific activity of the cardiac troponin T gene promoter. Proc. Natl. Acad. Sci. 85: 6404–6408.

---. 1990. M-CAT binding factor, a novel trans-acting factor governing muscle-specific transcription. Mol. Cell. Biol. 10: 4271–4283.

Mar, J.H., P.B. Antin, T.A. Cooper, and C.P. Ordahl. 1988. Analysis of the upstream regions governing expression of the chicken cardiac troponin T gene in embryonic cardiac and skeletal muscle cells. J. Cell. Biol. 107: 573–585.

McMahon, A.P. and A. Bradley. 1990. The Wnt-1 [int-1] proto-oncogene is required for development of a large region of the mouse brain. Cell 62: 1073–1085.

M’elin, F., M. Miranda, N. Montreau, M.L. DePamphilis, and D. Blangy. 1993. Transcription enhancer factor-1 (TEF-1) DNA binding sites can specifically enhance gene expression at the beginning of mouse development. EMBO J. 12: 4657–4666.

Mirabito, P.M., T.H. Adams, and W.E. Timberlake. 1989. Interactions of three sequentially expressed genes control temporal and spatial specificity in Aspergillus nidulans. Cell 57: 859–868.

Moens, C.B., A.B. Auerbach, R.A. Conlon, A.L. Joyner, and J. Rossant. 1994. A targeted mutation reveals a role for N-myc in branching morphogenesis in the embryonic mouse lung. Genes & Dev. 6: 691–704.

Nikovits, W.J., G. Kuncio, and C.P. Ordahl. 1986. The chicken fast skeletal troponin I gene: exon organization and sequence. Nucleic Acids Res. 14: 3377–3390.

Parmacek, M.S., A.J. Vora, T. Shen, E. Barr, F. Jung, and J.M. Leiden. 1992. Identification and characterization of a cardiac-specific transcriptional regulatory element in the slow/ cardiac troponin C gene. Mol. Cell. Biol. 12: 1967–1976.

Saggin, L., L. Gorza, S. Ausoni, and S. Schiaffino. 1989. Troponin I switching in the developing heart. J. Biol. Chem. 264: 16299–16302.

Shimizu, N., E. Dizon, and R. Zak. 1992. Both muscle-specific and ubiquitous nuclear factors are required for muscle-specific expression of the myosin heavy-chain beta gene in cultured cells. Mol. Cell. Biol. 12: 619–630.

Skarnes, W.C., B.A. Auerbach, and A.L. Joyner. 1992. A gene trap approach in mouse embryonic stem cells: The lacZ reporter is activated by splicing, reflects endogenous gene expression, and is mutagenic in mice. Genes & Dev. 6: 903–918.

Stewart, A.F., S.B. Larkin, I.K. Farrance, J.H. Mar, D.E. Hall, and C.P. Ordahl. 1994. Muscle-enriched TEF-1 isoforms bind M-CAT elements from muscle-specific promoters and differentially activate transcription. J. Biol. Chem. 269: 3147–3150.

Sucov, H.M., E. Dyson, C.L. Gumeringer, J. Price, K.R. Chien, and R.M. Evans. 1994. RXRa mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. Genes & Dev. 8: 1007–1018.

Thompson, W.R., G.B. Nadal, and V. Mahdavi. 1991. A MyoD1-independent muscle enhancer controls the expression of the beta-myosin heavy chain gene in skeletal and cardiac muscle cells. J. Biol. Chem. 266: 22678–22688.

von Melchner, H. and H.E. Ruley. 1989. Identification of cellular promoters by using a retrovirus promoter trap. J. Virol. 63: 3227–3233.

---. 1992. Selective disruption of genes expressed in totipotent embryonal stem cells. Genes & Dev. 6: 919–927.

Xiao, J.H., I. Davidson, H. Matthes, J.M. Garner, and P. Chambers. 1991. Cloning, expression, and transcriptional properties of the human enhancer factor TEF-1. Cell 65: 551–568.
Transcriptional enhancer factor 1 disruption by a retroviral gene trap leads to heart defects and embryonic lethality in mice.

Z Chen, G A Friedrich and P Soriano

*Genes Dev.* 1994, 8: Access the most recent version at doi:10.1101/gad.8.19.2293

**References**

This article cites 35 articles, 24 of which can be accessed free at: [http://genesdev.cshlp.org/content/8/19/2293.full.html#ref-list-1](http://genesdev.cshlp.org/content/8/19/2293.full.html#ref-list-1)

**License**

**Email Alerting Service**

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here.](#)