Developmental expression of the putative transcription factor Egr-1 suggests that Egr-1 and c-fos are coregulated in some tissues

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Summary
We have investigated developmental expression of the gene Egr-1, which encodes a protein containing three zinc fingers. Egr-1 like c-fos is a serum inducible, early response gene, which is co-induced with c-fos in a variety of quite different situations. A single 3.7-kb RNA was detected throughout fetal mouse development, which increased in absolute levels in total fetal RNA from 9.5 to 12.5 days post coitum (p.c.). In situ hybridization to 14.5- and 17.5-day p.c. fetal tissues demonstrated Egr-1 accumulation at several specific sites. These included mesenchymal components of the developing tooth germs and salivary and nasal glands; an ectodermally derived component of the whisker pad and developing muscle, cartilage, and bone. Expression of Egr-1 in cartilage and bone showed a strikingly similar expression to previously published reports of c-fos in these tissues. High levels of Egr-1 RNA was observed at the perichondrial interface of opposing cartilaginous elements and in interstitial cells that lie in between. Bone expression was observed in membranous bone of the head, alveolar bone around the tooth germs, and at periosteal and endochondral ossification sites in the limb bones. Our data support the idea that Egr-1 and c-fos may be coregulated in vivo and together may regulate normal development of the skeleton.

Key words: Egr-1, fetal expression, bone and cartilage, coregulation, c-fos.

Introduction
The interaction between groups of cells at specific times within localized regions is necessary for the orderly development of most vertebrate structures. This is true not only for the generation of primary tissue layers, such as the mesoderm and neural ectoderm, but also for the later formation of body organs. At present, little is known of the molecules that mediate the intercellular signalling or the intracellular pathways that respond to these signals. However, increasing evidence suggests that growth factors will play a central role in these events. Several growth factors have been implicated in one of the earliest inductive events in amphibian development (for a review, see Smith, 1989). Furthermore, tissue specific expression of a number of growth factor related molecules has been reported in the developing mouse (Jakobovits, 1986; Adamson, 1987; Wilkinson et al. 1988, 1989a).

Recently, a number of genes have been reported that show rapid transcriptional activation in response to a variety of growth factors (Cochran et al. 1983; Lau and Nathans, 1985, 1987; Milbrandt, 1987; Sukhatme et al. 1987; Almendral et al. 1988). Several of these genes, which include the proto-oncogenes, c-myc, c-fos, and c-jun, share properties of transcription factors. Thus, it seems likely that some of these genes operate in vivo to regulate gene expression in response to external cues.

We have focused our attention on a growth factor inducible gene, Egr-1 (early growth response-1, Sukhatme et al. 1987; Sukhatme et al. 1988). This gene has also been referred to as NGFI-A (Milbrandt, 1987), Krox-24 (Lemaire et al. 1988) and zif/268 (Christy et al. 1988). Egr-1 was originally identified as a rapid early response gene to a variety of polypeptides including NGF (Milbrandt, 1987; Sukhatme et al. 1988), FGF and PDGF (Christy et al. 1988), and several serum proteins (Sukhatme et al. 1988; Lemaire et al. 1988). Egr-1 encodes a 533 amino acid, 57K protein containing three putative DNA-binding zinc finger sequences (Sukhatme et al. 1987; Milbrandt, 1987; Christy et al. 1988; Lemaire et al. 1988). Following mitogenic stimulation, Egr-1 shows a rapid, cycloheximide-independent, transcriptional activation with kinetics similar to those originally
described for serum induction of c-fos (Greenberg and Ziff, 1984). However, within 30 min of stimulation of quiescent cells, Egr-1 is induced to approximately ten times the level of c-fos (Sukhatme et al. 1987). Collectively, these data point to a broad role for Egr-1 in signal transduction in diverse biological processes.

In adult mouse tissues, high levels of Egr-1 RNA are restricted to heart, brain, and lung, and lower levels to kidney and spleen (Milbrandt, 1987; Sukhatme et al. 1988; Christy et al. 1988; Lemaire et al. 1988). Several cell lines, which include PC12, P19, and F9, regulate Egr-1 expression in response to differentiation in culture (Milbrandt, 1987; Sukhatme et al. 1988). However, the actual developmental profile of Egr-1 expression is unknown. We have used in situ hybridization to localize Egr-1 RNA in the developing fetus. Egr-1 accumulates at several specific sites, some of which overlap with known sites of c-fos expression. Our results point to a coregulation of Egr-1 and c-fos expression during fetal development.

Materials and methods

Egr-1 was cut with Mspl and Rsal to generate a 700 bp 3' non-finger-encoding region (1553 to 2254, Sukhatme et al. 1988). The 5' overhang at the Mspl cut end was filled in using the Klenow fragment of DNA polymerase (Maniatis et al. 1982) and the blunt-ended fragment ligated into the Sma1 site of pUC13. The resultant plasmid was cut with HindIII and BglIII (1959) and EcoRI and BglIII to generate 406 bp and 295 bp Egr-1 fragments, which were subsequently subcloned into the plasmid pKS (Stratagene) to generate the plasmids p4.6 and p3.6 respectively. T7 transcription of HindIII cut p4.6 and T3 transcription of SacI cut p4.6 transcribe antisense and sense Egr-1 probes respectively. T7 transcription of EcoRI cut p3.6 and T3 transcription of XbaI cut p3.6 transcribe sense and antisense Egr-1 probes respectively.

RNA blot analysis

RNA was prepared from fetal and adult samples using the lithium chloride urea procedure (Auffray and Rougeon, 1980). Five µg of total fetal or adult RNAs were fractionated by gel electrophoresis on 1.2% formaldehyde agarose gels (Maniatis et al. 1982), transferred to GeneScreen (DuPont) and u.v. crosslinked (Church and Gilbert, 1984). RNA blots were hybridized with a high specific activity, 32P-labelled, p3.6 Egr-1 DNA probe (Feinberg and Vogelstein, 1984) at 2x10⁶ cts min⁻¹ ml⁻¹ in 50% formamide, 5xDenhardt's solution, 1.0 M NaCl, 0.05 M Tris-HCl pH 7.5, 1% SDS, and 10% dextran sulfate overnight at 45°C. Filters were washed three times, 20 min each, in 2xSSC, 1% SDS at room temperature, then twice in 0.2xSSC, 1% SDS at 65°C for 1 h. Hybridization was visualized by autoradiography using Kodak AR-5 X-ray film.

In situ hybridization was performed to sections of 10.5-, 14.5-, 17.5-day p.c. fetuses. No specific hybridization was observed with either 3.6 or 4.6 sense RNA probes. For brevity these results are omitted. In contrast, specific hybridization was seen with both antisense probes. Moreover, hybridization was indistinguishable in adjacent sections hybridized with 3.6 and 4.6 antisense probes. Thus, it is clear that the hybridization is specific to antisense probes that encode different unrelated regions of the Egr-1 transcript. As identical results were obtained with 3.6 and 4.6 probes, individual results with either probe will not be distinguished in this paper.

At 10.5 days p.c., no obvious contiguous sites of Egr-1 expression were observed (data not shown). However, by 14.5 days, Egr-1 RNAs accumulate in several specific locations.

Fig. 1. Northern blot analysis of Egr-1 expression in fetal RNA. Five micrograms of RNA from 9.5- to 17.5-day fetuses were analyzed for expression of Egr-1. A single 3.7-kb RNA transcript, identical in size to the Egr-1 transcript (arrow), is present in adult brain (B) and heart (H). L, lung.
Egr-1 expression in fetal development

In situ hybridization of Egr-1 probes to 14.5- and 17.5-day p.c. fetuses revealed high levels of Egr-1 transcripts in association with cartilaginous and bony tissues. Egr-1 RNA accumulated in epiphyseal cartilage at the articular surfaces of the developing limbs and in the interstitial cells that lie in between cartilaginous elements (Fig. 2A,B). At 17.5 days p.c., this was particularly evident in the distal regions of the forelimb (Fig. 2C-F). Egr-1 expression was also observed at and between the articular surfaces of the developing head skeleton at 14.5 days and about the midline of the developing jaw (Fig. 2G,H). In the cartilage of the nasal septum, Egr-1 was only expressed at the proximal end of the median cartilage (Fig. 2I,J). In the ribs at 14.5 days, Egr-1 showed a punctate perichondrial distribution rather than a uniform labelling of all perichondrial cells (Fig. 2K,L).

Particularly high levels of Egr-1 RNA were found in several areas undergoing bone formation including the peristeal regions of the developing long bones (Fig. 3A,B) and in membranous and alveolar bone-forming regions in the head (Fig. 3C,D).

At 14.5 days p.c., Egr-1 expression was detected in the mesenchymal component of the developing tooth (Fig. 3E,F). Teeth develop following a complex series of interactions between the ectodermal cells of the jaw epithelium and the underlying neural crest-derived mesenchyme cells (Lumsden, 1987). The ectodermal component forms the enamel and the mesenchymal component, the dentine and pulp. At 14.5 days p.c., the mesenchyme of the incisors and first molar express high levels of Egr-1 (Fig. 3E,F). However, by 17.5 days, Egr-1 expression decreased, and the expression, now limited to the developing pulp, was patchy (data not shown).

Egr-1 expression in striated muscle and tendons

Egr-1 expression was seen in all areas in which striated muscle develops. However, in contrast to sections hybridized with a muscle-specific actin probe (data not shown), Egr-1 was not uniformly expressed throughout the developing muscle, but rather showed a patchy distribution. This is clear in sections through the muscles projecting to the developing limb and bones (Fig. 4A,B) and in the tongue, which is composed of a branching array of striated muscle fibers (Fig. 4C,D). Egr-1 expression was seen in both the central muscle and in the connective tissue sheath and developing tendons (Fig. 4A–D).

Egr-1 expression in the developing whiskers

The first hair follicles to develop in the mouse are the whisker pads, which provide the vibrissae. As with other hairs, these develop from an epithelial–mesenchymal interaction between the basal layer of the skin and underlying mesenchymal cells (Davidson and Hardy, 1952). Egr-1 RNA was detected in ectodermally derived cells of the inner root sheath in developing whisker follicles from 14.5 days p.c. (Fig. 5A–D). No

Fig. 2. Cartilage-associated expression of Egr-1.
(A,B) Egr-1 expression at the articular surfaces of the 14.5-day p.c. shoulder joint. Egr-1 RNA accumulates in the perichondrial cartilage of the scapula (s) and humerus (h), as well as in the interstitial cells that lie between these elements. (C,D) Egr-1 expression in the distal forelimb at 17.5 days p.c. Expression is restricted to the regions at which cartilaginous elements abut (arrowheads). (E,F) High-power view of the carpals boxed in C. Egr-1 expression is seen in interstitial cells between the carpals and in the perichondrium. (G,H) Egr-1 expression at the surface of opposing cartilaginous elements (arrowheads) in the developing jaw at 14.5 days p.c. Expression elsewhere is also seen in cells either side of the midline of the jaw (arrows). The neural tube at this time does not express Egr-1. (I,J) Expression of Egr-1 at the proximal end of the medial nasal cartilage (c) at 14.5 days p.c. No expression is seen in other areas of cartilage or in the olfactory epithelium (arrowheads). (K,L) Egr-1 expression in perichondrial cells of the ribs (arrows) at 14.5 days p.c. A,C,E,G,I,K – bright field illumination; B,D,F,H,J,L – dark field illumination.
expression was seen in outer root sheath cells, which are also ectodermally derived, in mesenchymal cells, which form the dermal papilla, or in the cortex of the hair (Fig. 5C–D). In the hair follicles of the skin, Egr-1 expression was not detected up to 17.5 days p.c.

Egr-1 expression in developing salivary and nasal glands
In addition to Egr-1 expression in the above-mentioned areas, Egr-1 RNA was also detected in developing glandular structures. In the nose, several sites of Egr-1 expression were observed in association with developing nasal glands (Fig. 6A,B). Expression was also seen in association with salivary glands at 14.5 days p.c. (Fig. 6C,D). In both cases, Egr-1 was not expressed in the epithelial component of the gland, but rather in the underlying mesenchymal component.

Discussion

It is clear from the results presented here that the zinc finger-protein encoding gene, Egr-1, is transcribed in several different cell types during mammalian development. These include epithelial and mesenchymal cells, which are ectodermally or mesodermally derived. Egr-1 expression at these sites does not correlate with a simple common ancestry shared by Egr-1-expressing cells. Rather, expression is presumably a requirement for the development of several unrelated tissues. Interestingly, Egr-1 is expressed in different sites at which epithelial mesenchymal interactions are important. These include
days p.c., c-fos RNA-like Egr-1 is localized in the epiphyseal perichondrium (Dony and Gruss, 1987; Sandberg et al. 1988) as well as in the interstitial cells between opposing elements of the long bones and developing hands and feet. c-fos and Egr-1 are also coexpressed in the bone-forming regions of the head (Caubet and Bernaudin, 1988) as well as in periosteal and endochondral ossification sites in the long bones (Sandberg et al. 1988). Moreover, c-fos expression also occurs in mesenchymally-derived cells of the tooth germ, albeit at a somewhat later stage than shown to express Egr-1 in the present study. Thus, in situ hybridization strongly suggests that c-fos and Egr-1 share overlapping patterns of expression in vivo.

A number of previous studies have indicated that c-fos and Egr-1 are coregulated. In a variety of different cell types, including fibroblasts, B and T lymphocytes, and epithelial cells (Sukhatme et al. 1987), Egr-1 and c-fos share similar kinetics of induction following mitogenic stimulation. Furthermore, when PC12 cells (Milbrandt, 1987; Sukhatme et al. 1988) and bone marrow cells (Ovelettes, Sukhatme and Bonventre, unpublished observations) are stimulated to differentiate with NGF and GM-CSF, respectively, c-fos and Egr-1 are induced with similar kinetics. Coexpression is also observed following renal ischemia and in the early phase of compensatory renal hypertrophy (Sukhatme, unpublished observations). Finally, in the nervous system, c-fos and Egr-1 are induced following membrane depolarization (Sukhatme et al. 1988) and seizure (Morgan et al. 1987; Sukhatme et al. 1988). In contrast, a second serum responsive gene, Egr-2/Krox-20 (Chavrier et al. 1988, 1989; Joseph et al. 1988), which shares an almost identical zinc finger domain with Egr-1 (Lemaire et al. 1988), is not induced in NGF-treated PC12 cells (Joseph et al. 1988) and shows no overlapping expression with Egr-1 and c-fos in vivo (Wilkinson et al. 1989b).

The similar kinetics of Egr-1 and c-fos induction in these systems, which in the case of serum stimulation does not require new protein synthesis (Sukhatme et al. 1987), suggests that Egr-1 and c-fos share common transcriptional regulation. Comparison of the 5' regulatory sequences indicates several similarities (Tsai-Morris et al. 1988; Christy et al. 1988). Egr-1 has six potential serum response elements (SRE, Treisman, 1986), which in c-fos are the sites at which the serum response factor binds to regulate c-fos transcription (Treisman, 1987). However, despite these similarities, induction through the serum response elements is unlikely to explain the coexpression of Egr-1 and c-fos in vivo since such elements are also present in the upstream regions of Egr-2/Krox-20 (Christy et al. 1989) as well as in α-actin genes, which show a markedly different developmental profile. More subtle similarities in the promoter elements of Egr1 and c-fos must exist to explain their coregulation in such diverse circumstances.

In the absence of a detailed knowledge of the in vivo expression of potential transcriptional regulatory molecules, it is impossible to address their normal func-
tions. In this report, we have implicated Egr-1 and c-fos together in regulating growth and differentiation of cartilage and bone. c-jun, which in association with c-fos forms parts of the API transcription complex (Franza et al. 1988; Rauscher et al. 1988a,b; Chiu et al. 1988; Sassone-Corsi et al. 1988), shows a very similar expression in the skeleton (Wilkinson et al. 1989c). Thus, a detailed picture is emerging of a number of different regulatory molecules which may function in vivo at the same sites. The observation that deregulated expression of c-fos leads to abnormal bone development (Ruther et al. 1987) suggests that misexpression of Egr-1 may invoke a similar phenotype. However, it is also clear from our study that Egr-1 and c-fos do not share completely overlapping patterns of expression, as is also the case for c-fos and c-jun (Wilkinson et al. 1989c). Thus, it is likely that these and other transcription factors will interact in different combinations to bring about appropriate regulation of gene expression at diverse sites during development.

References


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