ONTOGENIC EXPRESSION PATTERNS OF TRANSCRIPTS ENCODING EGAM1 HOMEOPROTEINS DURING MURINE ORGANOGENESIS

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ABSTRACT
Recently, we identified the structurally related homeoproteins EGAM1, EGAM1N, and EGAM1C both in preimplantation mouse embryos and mouse embryonic stem cells. When overexpressed in mouse embryonic stem cells, a series of EGAM1 homeoproteins are able to act as positive or negative regulators of differentiation and seem to also regulate the development of preimplantation embryos. Here, we investigated the ontogenic expression patterns of their transcripts during the generation of representative fetal organs. The expression levels of the Egam1 transcript were selectively higher in the adult eye among the organs examined, while Egam1n or Egam1c transcripts were expressed abundantly in the brain, eye, testis, and ovary during the fetal stages. These results, in combination with our previous observations, indicate that the respective transcripts encoding EGAM1 homeoproteins are expressed clearly not only in preimplantation mouse embryos, but also in specific embryonic organs, the extraembryonic placentae, and in adult organs, including the eye, in mice.


Keywords: Crxos1, Egam1, Egam1c, Egam1n, mouse, ontogenic expression

Introduction
In preimplantation mouse embryos, three types of cell lineages, including inner cell mass, trophectoderm, and primitive endoderm, arise (11). With the progression of embryogenesis, these cell lineages form the fetus, placenta, and yolk sac, respectively. Thus, cells constituting inner cell mass are capable of being the founders of embryonic stem (ES) cells. Assuming that preimplantation mouse embryos provide an excellent source for finding both pluripotency-maintaining and differentiation-inducing genes, we identified structurally related homeoproteins EGAM1 (GenBank accession No. AB472692, also known as CRXOS1, NM_001033638), EGAM1N (No. AB472693, CRXOS1 transcript variant 3, NM_001205274), and EGAM1C (No. AB472694, CRXOS1 transcript variant 2, NM_001145190) in preimplantation mouse embryos and also in mouse ES cells (7). These proteins are products of the Crxos1 gene (GenBank ID: 546024) as splicing or transcription variants. When overexpressed in mouse ES cells, a series of EGAM1 homeoproteins can act as positive or negative regulators of differentiation and seem to also regulate the development of preimplantation embryos (5, 7, 9). In addition, we reported recently that EGAM1C is likely to play a role in the expression of members of the placental prolactin gene family in the mouse placenta immediately before partum (8). Collectively, our recent efforts suggest that members of the EGAM1 homeoproteins, at least in part, are not only capable of playing a role in differentiation events generating cell lineages that arise in early embryogenesis, but also function in certain terminally differentiated cell types. In this study, we investigated the ontogenic expression patterns of their transcripts during the generation of representative organs.

Materials and Methods

Animals
CD-1 mice (Charles River Japan, Yokohama, Japan) were housed in an environmentally controlled facility with a 12 h light:12 h darkness cycle and allowed free access to food and water. Female mice were mated with males, and the presence of a copulatory plug was designated as embryonic day (E) 0.5 after fertilization. All animal procedures conformed to the Guidelines for the Care and Use of Laboratory Animals of Akita Prefectural University.

RNA extraction and quantitative PCR analysis
Organs isolated from fetuses (E14.5, E16.5, and E18.5) or adult mice (8 to 12 weeks old) were frozen in liquid nitrogen. Total RNA was extracted in duplicate from several frozen organs using an acid guanidinium thiocyanate–phenol–chloroform extraction method (3), as reported previously (10). In brief, contaminating DNA was degraded with RNase-free DNase I (Nippon Gene, Tokyo, Japan). DNase I-treated total RNA was purified again by acid guanidinium thiocyanate–phenol–chloroform extraction. First-strand cDNA was synthesized using reverse transcriptase (RT, ReverTra Ace, Toyobo, Osaka, Japan) and an oligo-dT20 primer in accordance with the manufacturer’s instructions. Quantitative (q) PCR was performed in duplicate using a QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany) and a CFX96 Real-Time Detection system (Bio-Rad, Hercules, CA, USA) in accordance with the manufacturer’s protocols. Hydroxymethylbilane synthase (Hmbs), a housekeeping gene, was used as a positive control to ensure the quality and quantity of cDNA. Specific primers for
Results and Discussion

Our previous study indicated qualitatively the selective expression of the respective transcripts encoding EGM1 homeoproteins in five organs, including the brain, eye, testis, ovary, and thymus, among 16 adult murine tissues, while these transcripts were undetectable in other tissues examined (7). Therefore, as shown in Fig. 1, the ontogenic expression of these transcripts was quantified by qRT-PCR in these five organs as representative examples.

Interestingly, the expression level of Egaml transcript was extremely high (P < 0.05) in adult eyes compared with that during fetal stages or in other organs examined. In brains, testes, and ovaries obtained from adult mice, the expression levels of Egaml transcript were below the threshold level of qRT-PCR analysis. The expression levels of the Egalm transcript in brains and eyes, which are both parts of the central nervous system, were obviously higher (P < 0.05) than those in testes, ovaries, or the thymus. However, certain expression levels of the Egalm transcript were detected in fetal testes and ovaries, which are both involved in gametogenesis. Subsequently, the expression level was downregulated to almost undetectable levels in adult testes and ovaries. On the other hand, the highest expression level of the Egamc transcript at E16.5 was observed in fetal testes among all the organs examined. Meanwhile, an apparent expression of the Egamc transcript was also detected in brains, eyes, ovaries, and the thymus in fetuses. The expression of all members of transcripts for EGM1 homeoproteins was detectable in the thymus, although the levels were relatively low compared with those of other organs examined.

In adult mice, Alfano et al. (1) reported that the CrxOS transcript (substantially equivalent to Egaml) was detected in the retina. In agreement with this observation, the present study indicated a selective, temporal, and spatial expression of the Egaml transcript in adult eyes. We cannot rule out the possibility that Egaml plays a role in eye function. In fact, enforced expression of CRXOS protein in the adult retina resulted in the inhibition of expression of Crx encoding a crucial transcription factor for eye development and function (1). In contrast, a high expression level of the Egalm transcript was observed in the developing brain and eye in fetuses. Almost all of the primary amino acid sequences of the EGAM1N protein (14 kDa), which harbors a homeodomain, are shared with the EGAM1 protein (27 kDa), which contains two homeodomains, in its amino-terminal region (7). A relationship between the expression of Egalm transcript and the development of the central nervous system would also be intriguing. On the other hand, it is noteworthy that the expression levels of the transcripts encoding Egalm or Egamc were downregulated drastically between the embryonic stages to the adult stage. In mouse ES cells, the forced expression of EGAM1N or EGAM1C inhibited the differentiation or stabilized an undifferentiated state (5, 7). Further analysis should be required to clarify a role of these proteins, such as in the maintenance of germ cells in developing testes or ovaries.

![Fig. 1. Ontogenic expression patterns (qRT-PCR) of transcripts encoding EGM1 homeoproteins during murine organogenesis in CD-1 mice. The ratios of the number of cDNA copies for each transcript to that of cDNA copies for Hmbs, a housekeeping gene, are indicated as expression levels. Differences among the expression levels in fetal stages and in adults within the respective organs are indicated by solid lines. Differences among organs are indicated by dashed lines. Data are expressed as mean ± SD (n = 2). E0.5: embryonic day 0.5 after fertilization. *P < 0.05. NS: not significantly different; #: not detected.](image-url)
eyes, testes, or ovaries during the fetal stages. These results, in combination with our previous studies (5, 7, 8), indicate that the respective transcripts encoding EGAM1 homeoproteins are expressed not only in preimplantation mouse embryos but also during specific parts of embryonic organogenesis, in extraembryonic placentae, and in adult organs, including the eye. This feature regarding the expression of the Crxos1, the gene encoding all the members of the EGAM1 homeoproteins, is similar to that of Sox2 to some extent; SOX2 is a pluripotency factor (2) and indispensable for the trophectoderm formation in preimplantation embryos (6) and also indispensable for neurogenesis in fetuses (4). In subsequent experiments, exact cell types expressing the transcripts encoding EGAM1 homeoproteins should be identified and the functions of the encoded proteins should be clarified in organogenesis and cellular functions.

Conclusions
The expression level of the Egaml transcript was selectively higher in the adult eye, and Egamln or Egamlc transcripts were abundantly expressed in developing brains, eyes, testes, or ovaries during the fetal stages. In combination with our previous observations, the respective transcripts encoding EGAM1 homeoproteins are expressed clearly not only in preimplantation mouse embryos, but also in specific embryonic organs, the extraembryonic placentae, and in adult organs, including the eye, in mice.

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