# Crucial transcription factors in endoderm and embryonic gut development are expressed in gut-like structures from mouse ES cells

Rie Matsuura, Hiroshi Kogo, Takunori Ogaeri, Takashi Miwa, Masaki Kuwahara, [§]Yoshiakira Kanai, *Takumi Nakagawa, *Atsushi Kuroiwa, Toyoshi Fujimoto & Shigeko Torihashi

Dept. of Anatomy & Molecular Cell Biology, Nagoya University Graduate School of Medicine.
65 Tsurumai, Showa-ku, Nagoya 466-8550, JAPAN
[§]Dept. of Anatomy, Graduate School of Agriculture & Life Science, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, JAPAN
*Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, JAPAN

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**Abstract**

Mouse embryonic stem cells (ES cells) are pluripotent and retain the potential to form an organ similar to gut showing spontaneous contractions *in vitro*. The morphological features and their formation process using the hanging drop method to compose embryoid bodies (EBs) seem to be similar to those *in vivo*. To determine whether the same molecular mechanisms are involved in the formation process, the expressions of transcription factors regulating endoderm and gut development in the mouse embryo were examined by *in situ* hybridization and compared to those *in vivo*. The expressions of gene products were also examined by immunohistochemistry and their co-localization was analyzed with double staining. The results showed that all factors examined, i.e., *Sox17, Id2, HNF3β/Foxa2*, and *GATA4*, were expressed in both EBs and the gut-like structures. Moreover, their expression patterns were similar to those in the mouse embryo. EBs, after the hanging drop and before outgrowth, already expressed all factors co-localized with each other at the EB epithelial structures. These findings suggest that the origin of gut-like structure is determined and formed as the epithelial structure in EB during the hanging drop, and they also indicate that the *in vitro* system using mouse ES cells mimics the development *in vivo* and should prove useful in the study of molecular mechanisms for endoderm and gut development.

**Correspondence**

Dr. Shigeko Torihashi

Department of Anatomy and Molecular Cell Biology, Nagoya University Graduate School of Medicine

65 Tsurumai, Showa-ku Nagoya 466-8550, JAPAN

Phone : +81 52 744 2001

Fax: +81 52 744 2012

e-mail: storiha@med.nagoya-u.ac.jp

## Introduction

Mouse embryonic stem cells (ES cells), which are derived from the inner cell mass of blastocysts, are pluripotent. They differentiate into endoderm, mesoderm and ectoderm, and their derivatives when they spontaneously enter a program of differentiation *in vitro* [1, 2]. It was recently reported that ES cells retain the potential to develop gut-like structures composed of three germ layers [3, 4]. Embryoid bodies (EBs) made from ES cells by the hanging drop method formed a gut-like structure that mimics the gut development in the mouse embryo. Although its three-dimensional structure was cystic or dome-like rather than tubular, the epithelium, lamina propria and musculature could be identified. Furthermore, these gut-like structures showed spontaneous rhythmical contractions that are a characteristic physiological feature of the gut *in vivo* [3, 5]. The process *in vitro* was morphologically similar to the gut development *in vivo* [4], and thus appeared to be a good model system to study the differentiation of endoderm and gut organogenesis.

In the present study, to characterize the formation process of the gut-like structure *in vitro*, we investigated by *in situ* hybridization the expressions of several transcription factors crucial to endoderm formation and gut organogenesis, and compared them to patterns in the embryonic gut. A Sry-related HMG box factor, Sox17, is an early endodermal marker which is located downstream of the Nodal signaling in Zebrafish, and plays a crucial role in the differentiation of the definitive endoderm [6, 7]. Id2 exerts dominant negative transcriptional activities on β helix-loop-helix transcription factors, and controls cell differentiation and proliferation in diverse cell lineages. *Id2* mRNA is expressed in epithelium of the gastrointestinal (GI) tract during gut organogenesis, and gene product is suggested to regulate the differentiation and cell division of enterocytes [8-10]. HNF3β/Foxa2 (regulating liver-specific gene expression) is predominantly expressed in the liver, but also in the visceral and definitive endoderms, node, notochord, and floor plate during early embryogenesis [11]. A targeted mutation in *HNF3β/Foxa2* in mice results in a lack of foregut and midgut endoderm [12, 13]. *GATA4* is expressed in various mesoderm- and endoderm-derived tissues such as the embryonic heart and primitive gut [14-16]. *GATA4*-null mice showed arrested development between E7.0 and E9.5 because of severe defects in foregut formation and heart-tube fusion [17, 18]. *GATA4*-deficient mouse ES cells also showed disrupted visceral endoderm differentiation

*in vitro* [19]. None of the transcription factors to date is specific to the definitive endoderm or GI tract. Therefore, an examination of the expression pattern of these factors in the ES cell system and their co-localization may provide an answer to the question of whether this system would be an *in vitro* model of gut development at the molecular level.

As a result of the present study, we found that *Sox17, Id2, HNF3β/Foxa2*, and *GATA4* were expressed in EBs and the gut-like structure, and that their expression pattern was similar to that in the mouse embryo. Our findings indicate that the *in vitro* system reflects the normal development *in vivo* and is useful for the study of molecular mechanisms of endoderm and gut development.

**Experimental Procedures**

Feeder-free ES cell line G4-2 (a gift from Dr. Niwa) derived from EB3 (a sub-line originated from E14tg2a) was cultured in Dulbecco's modified Eagle's medium supplemented with 1000 U/ ml leukemia inhibitory factor (LIF), 10% fetal calf serum, 0.1 mM 2-mercaptoethanol (Sigma), 0.1 mM non-essential amino acids (GIBCO/BRL), and 1 mM sodium pyruvate (Sigma). They were then maintained in hanging drops without LIF for 6 days to develop embryoid bodies (EBs). EBs were transferred to gelatin-coated dishes attached to the substrate and began to outgrow. EBs and developing gut-like structures were used on day 2, 5, 7, 10, 14 and 21 of the outgrowth and termed EB2, 5, 7, 10, 14 and 21, respectively. EBs just before plating were also used and termed EB0. Mouse whole embryos at embryonic day (E) 8.0 and their guts at E13.0 were obtained by mating BALB/c mice. Samples were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and subjected to the whole-mount *in situ* hybridization as previously described [20].

Samples were incubated with digoxigenin (DIG)-labeled antisense riboprobes prepared using the following linearized plasmid templates and RNA polymerases: (i) *GATA4*, *Bam*HI-digested pBluescript SK phagemid λG14a [14], T7 polymerase; (ii) *Sox17*, *Hind*III-digested pBluescript SK plasmid containing the cDNA [21], T7 polymerase; (iii) *HNF3β/Foxa2*, *Bam*HI-digested pBluescript SK ($II^+$) plasmid containing the cDNA [22], T3 polymerase; (iv) *Id2*, *Hind*III-digested pBluescript SK ($II^+$) plasmid containing the cDNA [8], T3 polymerase. Sense riboprobes were used as a negative control in all cases. After hybridization, the DIG-labeled molecules were detected using NBT/BCIP (Boehringer) as a substrate for the anti-DIG antibody-coupled alkaline phosphatase. After overnight treatment with 4% PFA, some samples were processed for cryosectioning.

Co-localization of factors was examined by immunohistochemistry. EBs at EB0 were fixed with 4% PFA and then embedded in OCT compound (Sakura Finetechnical) to make frozen sections (6 μm thickness). Double staining was carried out using following antibodies; Goat anti-Sox17 (R & D; 1:200), goat anti-GATA4 (Santa Cruz sc-1237; 1:1000), rabbit anti-GATA4 (Santa Cruz sc-9053; 1:200), goat anti-HNF3β/Foxa2 (Santa Cruz sc-9187; 1:100), rabbit anti-HNF3β/Foxa2 (Upstate; 1:500), and rabbit anti-Id2 (Santa Cruz sc-489; 1:1000) antibodies. They were coupled to second antibodies

conjugated with either Alexa Fluor 594, or Alexa Fluor 488 (Molecular Probes; 1:400). Confocal images were taken by a laser confocal microscope (Zeiss LEM 5 Pascal).

## Results

### *1. Sox17*

Although the expression of *Sox17* was not observed clearly in the embryonic gut at E13.0 (Fig. 1A), it was detected in the definitive endoderm in the early headfold stage of the mouse embryo at E8.0 (Fig. 1A inset). On the other hand, EBs and the gut-like structures from ES cells showed the *Sox17* expression from EB0 (Fig. 2). EB at EB0 probably corresponds to mouse embryo from E5.0 to E7.5 (egg-cylinder stage) as has been suggested [23]. Most of EBs were ovoid in shape and composed of a small head and a large body. The expression of *Sox17* was detected mainly in the small head region of EB, including the epithelial structure (Fig. 2A and E). The expression was confirmed by immunohistochemistry at EB0. Protein Sox17 was also detected mainly in the small head region (Fig. 7A and D). After plating, EBs attached to the dish and began to outgrowth. At about EB4, some cell clusters composed of mesenchymal cells and epithelial structures became recognizable in EBs. These clusters developed and formed mature gut-like structures at about EB14 [4]. Prospective gut-like structures at EB5 showed strong *Sox17* expression (Fig. 2C); however, after EB7 its intensity decreased (Fig. 2D and F) and almost disappeared at EB14 when organogenesis was complete (data not shown). The expression pattern is summarized in figure 6.

### *2. Id2*

In the embryo, *Id2* was observed in the epithelium throughout the gastrointestinal (GI) tract at E13.0, and an intense expression was observed in the gut epithelium (Fig. 1B and inset). Id2 protein was also detected in neonates by immunohistochemistry (data not shown). Therefore, *Id2* is expressed in the epithelium throughout the gut from the early embryonic stage to the neonate. As for the EBs and gut-like structures, *Id2* was expressed in the epithelium from EB0 to EB14 (Fig. 3), and was first detected in the small head region and/or in the region between the small head and the large body of EB at EB0 (Fig. 3A), where the epithelial structure was formed and showed the *Id2* expression (Fig. 3E). Immunohistochemistry for Id2 also demonstrated its expression in the same region of EB

at EB0 (Fig. 7E and K). Subsequently, the positive area expanded; the labeling intensity was heterogeneous, but future gut-like structures after EB4 showed a definitive expression (Fig. 3 B-D). Most gut-like structures including premature state were *Id2* positive in all the stages examined, and its expression was detected on the epithelium (Fig. 3F). In figure 6 the expression pattern is summarized and compared to that of the embryonic gut.

*3. HNF3β/Foxa2*

In the mouse GI tract at E13.0, *HNF3β/Foxa2* was observed in the anterior region of the GI tract, i.e., the esophagus, stomach and anterior part of the small intestine (Fig. 1C). It was also detected in the cecum, and its expression was seen in the epithelium in the sections (data not shown). We observed the expression of *HNF3β/Foxa2* in EBs from EB0 in the epithelial structure of the small head region (Fig. 4A and E). Protein HNF3β/Foxa2 was also detected in the same area of EB by immunohistochemistry (Fig. 7H and J). After outgrowth, mRNA of *HNF3β/Foxa2* appeared clearly in the epithelium of the developing gut-like structures (Fig. 4C and F), but the expression became heterogeneous after EB7 (Fig. 4D) and was lost in most gut-like structures at EB14. The expression pattern of *HNF3β/Foxa2* in EBs and gut-like structures are summarized in figure 6.

*4. GATA4*

*GATA4* was expressed in the stomach of the mouse embryo at E13.0. Its expression was restricted to the epithelium of the distal part corresponding to the corps and pylorus (Fig. 1D, E). The proximal region of the gut also expressed *GATA4*, the expression of which decreased toward the colon. *GATA4* was also expressed in the small head region in the EB at EB0 (Fig. 5A and E); immunohistochemistry for GATA4 confirmed its expression in EB at that stage (Fig. 7B and G). After outgrowth, beating cardiac muscles showed a strong *GATA4* expression in addition to developing gut-like structures (Fig. 5B and F). We sometimes observed that EBs with well-differentiated cardiac muscles formed more gut-like structures. After EB5, the expression became heterogeneous (Fig. 5C, D). Some premature structures showed an intense expression, whereas others had weak or negligible expressions. This heterogeneity of expression continued to EB14, as summarized in figure 6.

5. Double labeling of factors in EB with immunohistochemistry

All factors examined were expressed from EB0. In EBs, transcription factors were prominent in the small head regions where immunoreactivities of the same probes were detected (Fig. 7). Thus, analysis by double staining suggested the origin of the gut-like structures in EBs. Although each factor showed its own particular expression pattern (as shown in figure 7), double-positive epithelial structures in EBs were identified in all cases. These results demonstrated that transcription factors were co-localized in EB epithelial structures.

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**Discussion**

*1. Sox17*

*Sox17* was originally identified as a stage-specific transcription factor during spermatogenesis [21]. In the mouse post-implantation embryo, visceral and definitive endoderms expressed *Sox17*, and its null embryos were deficient in gut endoderm. Since *Sox17*-null ES cells could hardly colonize the foregut and were completely excluded from the mid- and hindgut endoderm in chimera experiments, Sox17 protein is thought to be a critical transcriptional regulator of gut formation [24]. The expression pattern of *Sox17* changed drastically from E6.0 to E9.0, first expressed in the definitive endoderm foregut, and then moving to the posterior region [24]. In the present study we demonstrated *Sox17* on the definitive endoderm at E8.0 and its disappearance in the GI tract at E13.0. The EBs and developing gut-like structures showed a similar transient expression pattern, i.e., strong *Sox17*expression at EB5, a decrease after EB7 and its disappearance at EB14. This suggests that formation of the gut-like structure may pursue the process parallel to gut organogensis *in vivo* (Fig. 6).

*2. Id2*

The expression pattern of *Id2* was investigated in the digestive tract of mouse embryos [8]. It was already detected at E10.5 on the epithelium and was seen to persist through E16.5 by *in situ* hybridization. Although *Id2*-null mice lack both lymph nodes and Peyer's patches, they were able to develop digestive tracts without severe defects [9]. Currently, it is reported that protein Id2 works as a tumor inhibitor in the mouse GI tract

[10]. Although the function of *Id2* in gut organogenesis remains unclear, *Id2* are direct targets of the TGFβ family, including Nodal/Smad2 and BMP4 signalings in the mouse embryo and ES cells [25-27].

In the present study *Id2* was expressed in the EBs, developing and mature gut-like structures throughout all stages examined, and the pattern was similar to that in mouse embryos (Fig. 1B and 3). These results demonstrated that *Id2* is an appropriate marker of the developing gut, and suggest that the gut-like structure *in vitro* shows the same characteristics as the embryonic gut *in vivo*.

*3. HNF3β/Foxa2*

The fork head family members including HNF3β/Foxa2 are evolutionarily conserved throughout metazoans and play critical roles in gut endoderm differentiation [11, 28]. Footprint studies *in vivo* suggest that HNF3β/Foxa2 is one of the transcription factors that primarily bind to a silent gene in the endoderm to activate the local DNA region, making it more accessible to other transcription factors [28]. The expression was detected most abundantly in the entire gut of midgestation embryos (E9.5-10.5), and then decreased in newborns, though some were still expressed in adult animals. Its expression was higher in the stomach and colon than in the small intestine [29, 30].

In the present study, *HNF3β/Foxa2* is expressed in the anterior region of the embryonic GI tract at E13.0. At this stage, expression reportedly began to decrease and even became heterogeneous in the GI tract *in vivo*. Similar expression patterns were observed in the EBs and gut-like structures *in vitro*. In the early stages, mRNA of *HNF3β/Foxa2* appeared clearly in the epithelium of prospective gut-like structures (Fig. 4C), but the expression became heterogeneous after EB7 (Fig. 4D). The regional variations in *HNF3β/Foxa2* expression indicate that the gut-like structure *in vitro* contains heterogeneous portions corresponding to those in the embryonic gut (Fig. 1C and 6).

*4. GATA4*

Both *GATA4* and *HNF3β/Foxa2* are expressed in the endoderm, and *in vitro* foot-printing studies have suggested that they have a functional combination, and that

*GATA4* dominantly regulate *HNF3β/Foxa2* expression [28, 31]. *GATA4* expression in the digestive tract appears first in the foregut-midgut junction at E8.5-10.5, and weakened in the distal end of the gut; the expression is not evident in the rostral foregut, including the esophagus [16].

In the embryonic gut at E13.0, *GATA4* was expressed in the stomach and the proximal intestine as previously reported. In developing gut-like structures after EB5, *GATA4* was heterogeneously expressed (Fig. 5C, D). This heterogeneity is similar to that of the embryonic gut and again indicates that the gut-like structure reflects the normal development *in vivo* (Fig. 1D, 6).

**Summary and Conclusion**

The expression patterns of transcription factors in the EBs and gut-like structures, and those in the mouse embryo reported previously are summarized in figure 6. All factors examined are crucial for formation of the definitive endoderm and/or gut, and are expressed in the epithelium of EBs at EB0 and gut-like structures including premature state (Fig. 6 and 7). Moreover, such expression patterns were similar to those in the embryonic gut. Our results suggest that the molecular mechanisms underlying the gut development *in vivo* and the formation of gut-like structures *in vitro* are identical. Each transcription factor is expressed in a characteristic pattern at different segments of the GI tract. Therefore, their heterogeneous expression in the gut-like structure *in vitro* indicates that segments with regional specificities are reproduced (Fig. 6). Studying the onset of expression allowed us to determine the time course of differentiation during the formation of gut-like structures. EBs at EB0 are equivalent to the egg-cylinder stage (E5.0-7.5), as previously described in the case of suspension cultures [23].

Although the transcription factors examined are not specific to the definitive endoderm and/or gut, all factors were already expressed at EB0 in the small head region of EB. Further co-localization of these factors was confirmed by immunohistochemical double staining. Since they overlapped each other on the epithelial structures in EBs, the future gut-like structure was determined and formed in a particular area (the epithelial structure in the small head region of EB) during the hanging drop (Fig. 7). This assumption is supported by previous reports showing that *HNF3β/Foxa2* expression was quite low in undifferentiated ES cells but increased in EBs after three days of suspension

culture preceding the rise in transthyretin (TTR), α-Fetoprotein (AFP) and other endoderm marker gene expressions [32, 33]. EBs formed by suspension culture also expressed *GATA4* from day one in culture, and its expression increased up to day 10 [23]. These results suggest that development of the endoderm occurs in EBs even during the hanging drop period.

Our results demonstrate that the gut-like structures *in vitro* are a valid model system for the study of the mechanisms of early endoderm determination and gut organogenesis, and should provide important information needed to identify essential factors.

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**Figure legends**

**Fig. 1**

Expression of transcription factors in digestive tracts at E13.0

**A)** *Sox17* is not obviously expressed in digestive tract at E13.0, but in embryo at head-folding stage. It was detected in definitive endoderm (inset). Cross section at E8.0 (inset) showing expression located in hindgut (Hg) rather than neural plate (Np). **B)** *Id2* is expressed throughout the gut. Cross section of small intestine showing expression located in epithelium (inset). **C)** *HNF3β/Foxa2* is strongly expressed in foregut, i.e., esophagus, stomach and proximal region of small intestine, while cecum is weakly positive. **D)** *GATA4* shows unique expression pattern. The corpus to pylorus of stomach, and anterior region of small intestine express *GATA4*, whereas terminal ileum, cecum and colon do not show the expression. **E)** Heterogeneous expression of *GATA4* in the epithelium (Ep) is confirmed by a cross section of stomach cut at position indicated by a broken line in D. Small arrow, large arrow, white arrowhead, and black arrowhead indicate small intestine, colon, cardia, and cecum, respectively. Bar in A applies to B-D and indicates 500 μm. Bar in E indicates 500 μm. Bars in insets indicate 100 μm.

**Fig. 2**

Expression of *Sox17* in EB and gut-like structures

A) *Sox17* is expressed in EB at EB0, and is detected in small head region of EB (asterisk). B) At EB2, the area corresponding to small head region at EB0 retains expression (asterisk). C) Prospective gut-like structures at EB5 (arrows) have a definitive expression. D) At EB10, expression in a developing gut-like structure weakens. E) Cross section of EB at EB0 showing small head region including epithelial structure (arrow) expressing *Sox17*. F) Cross section at EB10 shown in D indicating that epithelia (arrows) in gut-like structure express *Sox17*, though expression weakens at EB10. Bars in A-D indicate 500 μm. Bars in E and F indicate 100 μm.

**Fig. 3**

Expression of *Id2* in EB and gut-like structures

A) *Id2* is expressed in EB (arrowhead) at EB0. B) Strong expression of *Id2* is seen as spots in outgrowing portion of EB at EB2. C) At EB5, prospective gut-like structures (arrows)

show clear expressions at the center, suggesting formation of epithelium. D) At EB10, almost all premature gut-like structures express *Id2* on their epithelium (arrows). E) Expression in EB at EB0 is demonstrated by a section. The clear expression is shown in the epithelium (arrows). F) Strong expression of *Id2* in developing gut-like structure at EB10 is confirmed on their epithelium (arrows) by a cross section. Bars in A and B indicate 500 μm, bar in B is applied to C and D, and bars in E and F indicate 100 μm.

**Fig. 4**

Expression of *HNF3β/Foxa2* in EB and gut-like structures

A) *HNF3β/Foxa2* is expressed in small head regions (asterisks) of EBs at EB0. B) Expression at head region is also seen in EB2 with some forming small spot-like expressions at this stage. C) At EB5, prospective gut-like structures clearly show *HNF3β/Foxa2* mRNA (arrows). D) Some premature gut-like structures express *HNF3β/Foxa2* strongly (large arrow), but others only weakly (small arrows) at EB10. E) Cross section of EB at EB0 shows that epithelial structures (arrows) express *HNF3β/Foxa2*. F) Cross section of prospective gut-like structure at EB5 in C demonstrating *HNF3β/Foxa2* is expressed in epithelium (arrow). Bar in A indicates 500 μm and is applied to B-D. Bars in E and F indicate 100 μm.

**Fig. 5**

Expression of *GATA4* in EB and gut-like structures

A) *GATA4* mRNA is detected at small head region (asterisk) of EB at EB0. B) Expression of *GATA4* is demonstrated in many regions, including beating cardiac muscles differentiated at EB2. Asterisk indicates strong expression originating from small head region of EB at EB0. C) Prospective gut-like structures (arrow) at EB5 show *GATA4* expression. D) Expression becomes heterogeneous and at EB10 some developing gut-like structures show intensive expression (large arrow), while others show none (small arrow). E) Cross section of EB at EB0 confirms *GATA4* is expressed at epithelial structure (arrows) in small head region. F) Cross section of *GATA4* positive area at EB2 in B showing strong expression (asterisk) is demonstrated in epithelial structure. Bars in A to D indicate 500 μm. Bars in E and F indicate 100 μm.

**Fig. 6**

Time course of transcription factor expressions in EB and gut-like structures *in vitro*, and in digestive tract of mouse embryo. All factors are expressed in EBs at EB0 but, after EB5, each factor showed a different pattern. *In vivo* expression in foregut, midgut and hindgut is indicated by lines F, M and H, respectively. Before differentiation of these three regions, expressions in endoderms are shown as dotted lines. Each factor is expressed in the endoderm and presents a characteristic expression pattern in foregut, midgut and hindgut. Gradient of each line indicates the intensity of expression.

**Fig. 7**

Double staining of transcription factors in EBs at EB0 with immunohistochemistry

A) Immunohistochemistry for Sox17. B) GATA4 immunoreactivity on same section in A. C) Merged image of A and B. Although two factors exhibit particular distributions, epithelial structures (arrowheads) express both factors. D) Immunohistochemistry for Sox 17. E) Id2 immunoreactivity on same section in D. F) Merged image of D and E. Both factors are co-localized on epithelial structures (arrowheads). Sox17 immunoreactivity is located in nucleus; whereas Id2 is distributed in both cytoplasm and nucleus. G) Immunohistochemistry for GATA4. H) HNF3β/Foxa2 immunoreactivity on same section in G. I) Merged image of G and H. Epithelial structures (arrowheads) show co-localization of both factors. Their immunoreactivities are located mainly in nuclei with some distributed in cytoplasms. J) Immunohistochemistry for HNF3β/Foxa2. K) Id2 immunoreactivity on same section in J. L) Merged image of J and K revealing that epithelial structures (arrowheads) express both factors. Bar indicates 20 μm and is applied to all panels.

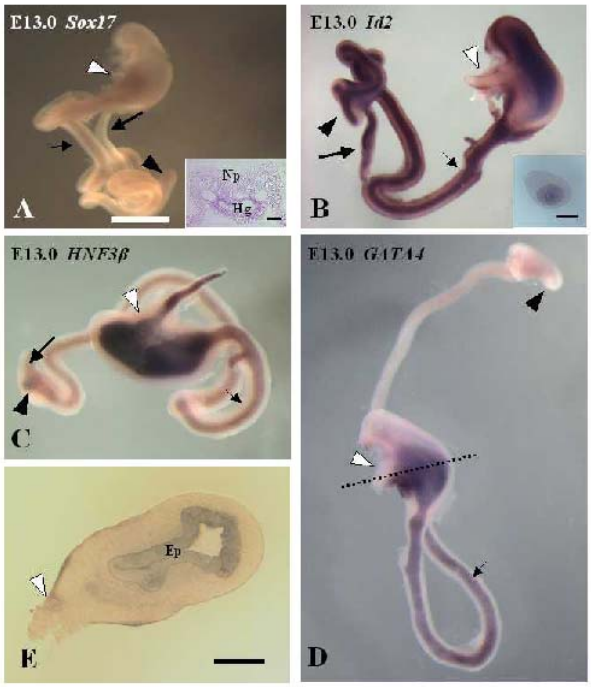
E13.0 Sox17
Ep
Hg
A
E13.0 Id2
B
E13.0 HNF3β
C
E13.0 GATA4
D
Ep
E

Fig. 1

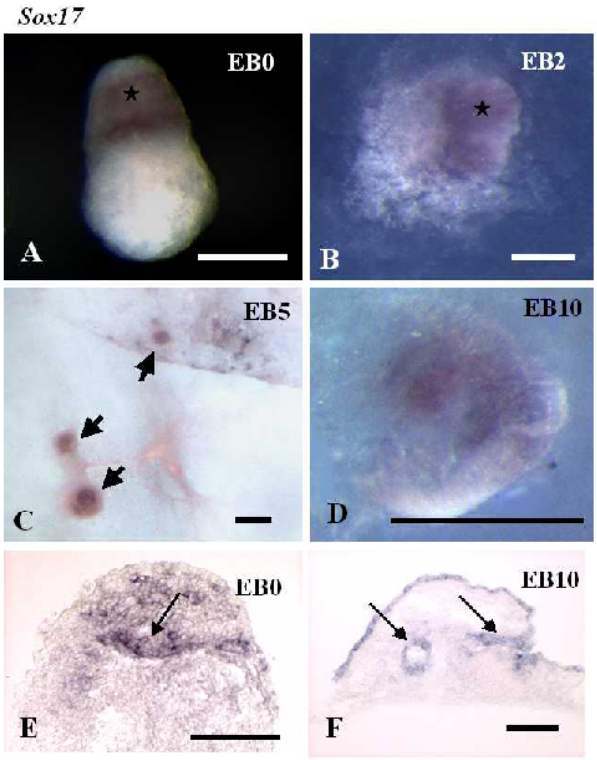
Sox17
EB0
A
EB2
B
EB5
C
EB10
D
EB0
E
EB10
F

Fig.2

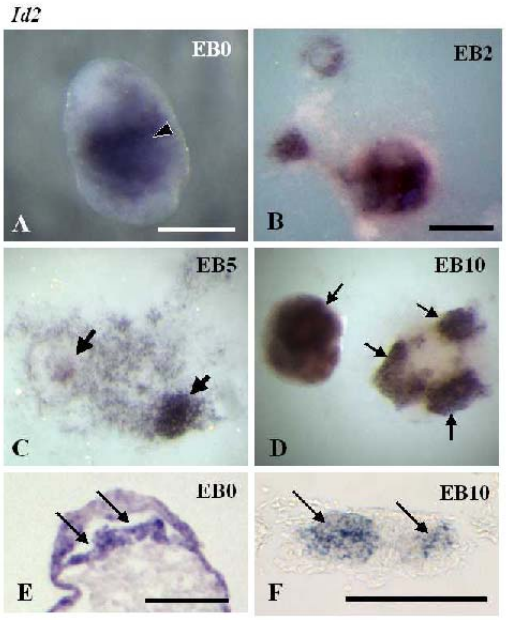
*Id2*
EB0
EB2
A
B
EB5
EB10
C
D
EB0
EB10
E
F

Fig.3

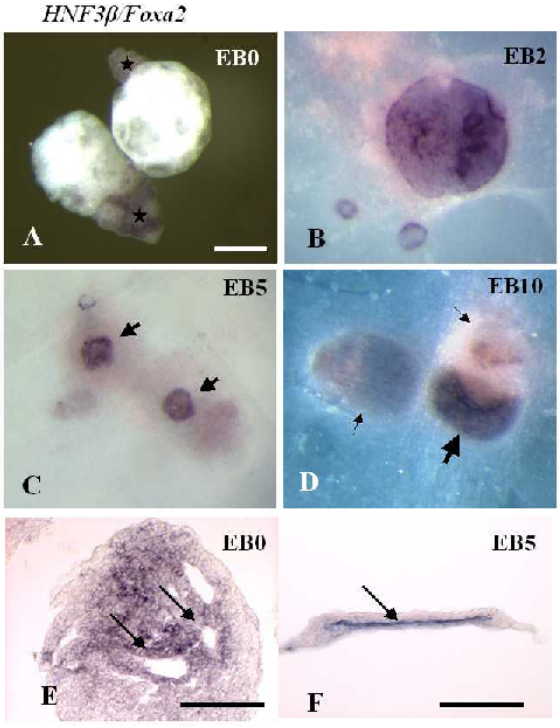
*HNF3β/Foxa2*
EB0
EB2
A
B
EB5
EB10
C
D
EB0
EB5
E
F

Fig.4

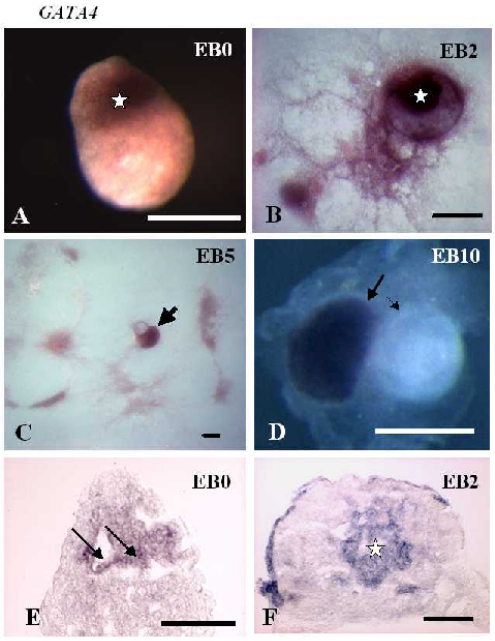


Fig.5

Expression pattern of transcription factors in ES cells and fetuses

| *in vitro* | EB0 | EB2 | EB5 | EB7 | EB10 | EB14 |
|---|---|---|---|---|---|---|
| *Sox17* | ++ | ++ | + | + | + | ± |
| *Id2* | ++ | ++ | ++ | ++ | ++ | ++ |
| *HNF3β/Foxa2* | ++ | ++ | ++ | +/− | +/− | +/− |
| *GATA4* | ++ | ++ | ++ | +/− | +/− | +/− |

| *in vivo* | E6.5 | E8.5 | E11.5 | E13.5 | E16.5 | Newborn |
|---|---|---|---|---|---|---|
| *Sox17* | F M H | | | | | |
| *Id2* | | F M H | | | | |
| *HNF3β/Foxa2* | | F M H | | | | |
| *GATA4* | | F M H | | | | |

Fig.6

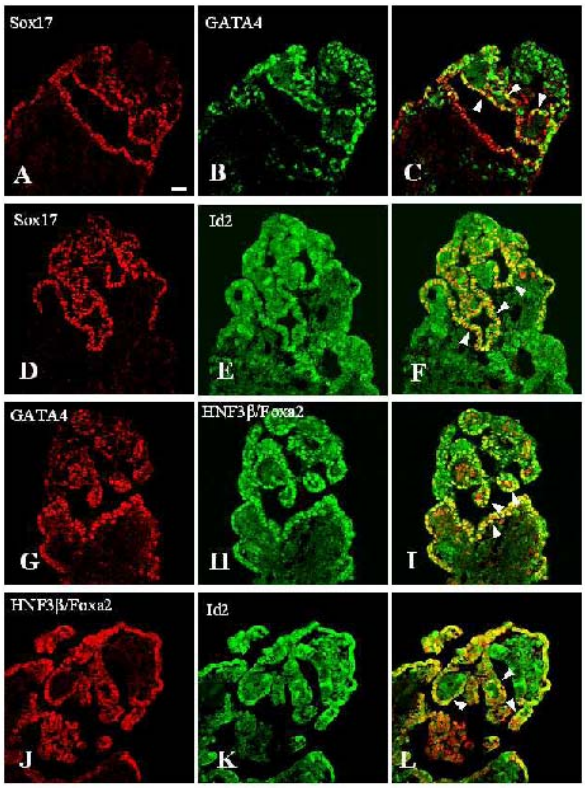
Sox17
GATA4
A
B
C
Sox17
Id2
D
E
F
GATA4
HNF3β/Foxa2
G
H
I
HNF3β/Foxa2
Id2
J
K
L

Fig. 7