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Germline-specific expression of the Oct-4/green fluorescent protein (GFP) transgene in mice

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The Pic-1, Oct-1,2, Unc-86 (POU) transcription factor Oct-4 is specifically expressed in the germ cell line, and a previous study has indicated that the expression of the lacZ gene inserted into an 18 kb genomic fragment encompassing the Oct-4 gene can come close to mimicking the endogenous embryonic expression pattern of Oct-4 in transgenic mice. In the present study transgenic mice expressing green fluorescent protein (GFP) in the germ cell line were generated using the same Oct-4 genomic fragments and the expression pattern was analyzed in detail through all stages of germ cell development. The GFP expressing primordial germ cells were first detected as early as 8.0 days post-coitum (d.p.c.; early head fold stage) at the base of the allantois in living embryos. The GFP expression was thereafter found in both male and female germ cells at all developmental stages except in male germ cells after differentiating into type A spermatogonia in the postnatal testis. There was also a lower level of expression in female germ cells in the prophase of the first meiotic division. These transgenic mice therefore proved to be powerful tools for isolating living germ cells at various developmental stages to study their nature and to isolate new genes.

Key words: germ cell, green fluorescent protein, Oct-4, POU transcription factor, transgenic mouse.

Introduction

In mice, primordial germ cells (PGC) are the earliest recognizable precursors of gametes and arise outside the gonads. During embryogenesis, PGC are first detectable as alkaline phosphatase-positive cells at 7.0 days post-coitum (d.p.c.). At this time a cluster of PGC, consisting of only about 10 cells, is found within the extra-embryonic mesoderm (Chiquoine 1954; Ginsburg *et al.* 1990). Afterwards PGC increase in number to about 100 cells in the midline extra-embryonic mesoderm just posterior to the primitive streak (Ginsburg *et al.* 1990), and at 8.0 d.p.c. they leave the base of the allantois and migrate into the hindgut epithelium. Subsequently, PGC emigrate along the hindgut (9.5 d.p.c.) and the dorsal mesentery (10.5 d.p.c.), and finally enter the newly formed genital ridges (11.5 d.p.c.). During their migration and early period in the genital ridges, PGC undergo active proliferation (Tam & Snow 1981). However, at 13.5 d.p.c., both male and female germ-cell growth is arrested, and female germ cells immediately enter prophase of the first meiotic division (De Felici & McLaren 1983), while male germ cells are arrested and enter the G0 phase of the cell cycle until birth, when prospermatogonia resume mitosis (Coucouvanis & Jones 1993).

The Oct-4, a Pic-1, Oct-1,2, Unc-86 (POU) transcription factor, is the only known molecule to play an important role in the establishment and maintenance of the mammalian totipotent cell population (Nichols *et al.* 1998). Oct-4 is specifically expressed at most developmental stages of the germ cell line, and this suggests that it is also involved in the development of germ cells. Maternal Oct-4 gene product is present in the unfertilized, fully grown oocyte. The amount of gene product

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tends to decrease during the first and second cleavages of the embryo, and thereafter the embryonic transcription is up-regulated (Schöler *et al.* 1990; Palmieri *et al.* 1994). The gene product is equally distributed in each blastomere during early cleavage stages but is restricted to the inner cell mass (ICM) by the time of blastocyst formation. After implantation Oct-4 expression is confined to the epiblast. As gastrulation progresses, the expression is down-regulated in the differentiating epiblast with the appearance of mesoderm and neuroectoderm cells (Rosner *et al.* 1990). After 9.5 d.p.c., PGC are the only embryonal cell type expressing this gene (Rosner *et al.* 1990; Schöler *et al.* 1990). The gene product is present in male germ cells, from PGC throughout type A spermatogonia, while expression in female germ cells is down-regulated at around 14.5 d.p.c., but resumes in the diplotene/dictyate oocytes (Pesce *et al.* 1998).

Previous analysis of the expression of the Oct-4/lacZ transgenes indicated that the 18 kb genomic fragment

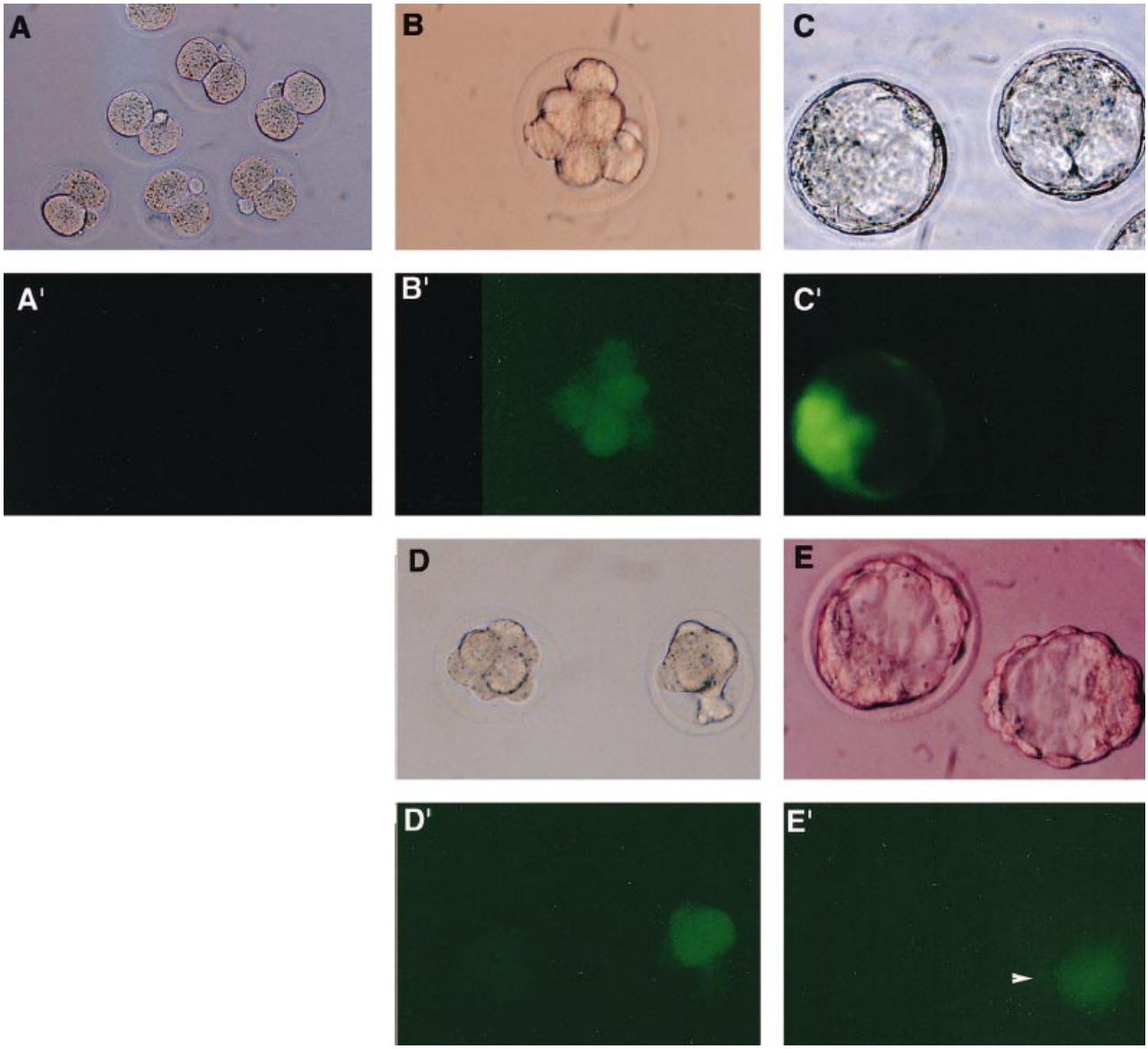


Fig. 1. Expression patterns of GOF-18/GFP and GOF-18/delta PE/GFP (green fluorescent protein) transgenes in pre-implantation mouse embryos. Two- (A′) and 8-cell (B′,D′) embryos and 3.5-day blastocysts (C′,E′) were observed under a fluorescence inverted microscope with fluorescein isothiocyanate (FITC) filter. Bright field images (A–E) and fluorescence images of the same samples (A′–E′) are shown. (A–C) Transgenic mouse line 11 of GOF-18/GFP. (D,E) Transgenic mouse line 36 of GOF-18/delta PE/GFP. The right embryo is transgenic and the left one is a non-transgenic litter mate in (D) and (E). Distinct fluorescence was seen in each blastomere (B′) and inner cell mass (ICM; C′) of GOF-18/GFP, while much weaker expression was detected in GOF-18/delta PE/GFP embryos (D′,E′).

(GOF-18), encompassing the Oct-4 gene and its 5′- and 3′-flanking sequences, is sufficient for reproducing the endogenous expression pattern in embryos. In addition, expression analysis of deletion constructs lacking specific *cis*-acting elements of the gene showed that transgene expression in pre-implantation embryos and PGC is driven by the distal enhancer (DE) element, while the proximal enhancer (PE) element is necessary for expression in the epiblast (Yeom *et al.* 1996). For example, expression of the construct lacking the PE element (GOF-18 delta PE) has been detected in morula, ICM, and PGC after 8.0 d.p.c., but no expression has been observed in the epiblast and its early derivatives except PGC. The activity profile of the PE element is interesting because it seems to coincide with the allocation of epiblast cells to PGC.

As described earlier, a reporter gene inserted into the Oct-4 genomic fragment seems to be useful for specifically visualizing the germ cell line in the mouse. Green fluorescent protein (GFP) derived from the bioluminescent jellyfish is the best reporter for this purpose because expression of GFP can be detected in living organisms under the fluorescence microscope without any staining procedure (Chalfie *et al.* 1994; Prasher 1995). Here we demonstrate the germ line-specific expression pattern of the Oct-4-GFP transgenes, and show that these transgenes are quite useful for identifying and isolating germ cells *in vivo* at various developmental stages.

Materials and Methods

Construction of transgenes and generation of transgenic mice

Construction of the GOF-18/GFP and GOF-18/delta PE/GFP transgenes has been described previously (Yeom *et al.* 1996), but the enhanced green fluorescent protein (EGFP) coding sequence (Clontech, CA, USA) was used in place of lacZ as the reporter gene in the current study. The inserts of the constructs were isolated and purified as described (Hogan *et al.* 1994). Fertilized eggs obtained from matings of C57Bl/6 × DBA/2 males and females were used for pronuclear injection of the DNA to generate transgenic mice. Founder mice were mated with C57Bl/6 mice to generate lines. Transgenic mice were identified by polymerase chain reaction (PCR) with specific primers for EGFP (DS15, 5′-aac cac tac ctg agc acc c. and OCGOFD43, 5′-acc tct aca aat gtg gta tg).

Observation of green fluorescence in early embryos and in the germ cell line

Female ICR mice mated with male founder or F1 transgenic mice were killed at appropriate stages (noon on the day of plug is 0.5 d.p.c.) to recover the pre- and post-implantation embryos. The oviduct or uterus was flushed with M2 media (Quinn *et al.* 1982) to recover ovulated MII oocytes, 2-cell embryos, 8-cell embryos, and blastocysts. Embryos at 6.0–17.5 d.p.c. were dissected, and appropriate regions containing PGC were cut out from the embryos in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine syndrome (FBS) (Hogan *et al.* 1994). Postnatal ovaries and testes were obtained from 1 to 7 days post-partum (d.p.p.), and from adult male and female mice. Green fluorescent protein was examined under a dissecting microscope (Leica MZ8, Wetzlar, Germany) or inverted or transmitted light microscopes (Leica DMIRBE or Leica DMRDX) equipped with GFP excitation sources and appropriate filters (Leica GFP fluorescence filter set). Photographs of fluorescent subjects were taken on Fuji Provia 400 film (Fuji, Tokyo, Japan) for 3–9 min of exposure time. Transgenic mice were genotyped based on the fluorescence under microscopy or on the PCR of extra-embryonic tissues.

Results

Establishing Oct-4/GFP transgenic lines

We generated six GOF-18/GFP (one male and five female) and four GOF-18/delta PE/GFP (one male and three female) founder transgenic mice. These founder mice were back-crossed with C57Bl/6 mice to establish lines. Although the expression pattern of the transgenes into each of these mice was basically the same, the level of GFP expression varied among the lines. Therefore, the data presented here focus on line 11 for GOF-18 and lines 36 and 48 for GOF-18/delta PE, because these lines exhibited higher levels of expression than the others.

GOF-18/GFP transgene expression in pre- and early post-implantation embryos

We determined whether the GOF-18/GFP transgene faithfully reproduced the endogenous expression pattern of Oct-4 in embryos and in the germ cell lineage. As shown in Fig. 1(A′–C′), 2-cell embryos did not show significant expression, but 8-cell embryos and blastocysts showed clearly visible fluorescence. In the 8-cell embryos, each blastomere showed similar fluorescence intensity (Fig. 1B′), while GFP expression was observed only in the ICM in blastocysts (Fig. 1C′).

The expression of the GOF-18/GFP transgene in post-implantation embryos at 6.0–9.0 d.p.c. appeared to be ubiquitous in the epiblast and in most of its derivative tissues (Fig. 2). Embryos, however, contained cells with stronger fluorescence in the hindgut at 8.75 d.p.c. (Fig. 2F, arrowhead) and in the dorsal mesentery at

9.0 d.p.c. (9–10 somites; Fig. 2H, arrows), which may represent PGC. In addition, transgene expression in the epiblast and its derivatives was gradually down-regulated from anterior to posterior between 8.75 and 9.0 d.p.c. (Fig. 2G). Although the down-regulation of the transgene seems to mimic that of the endogenous Oct-4 ribonucleic acid (RNA), its time course is delayed by about 12 h (Yeom *et al.* 1996).

Expression of the GOF-18/delta PE/GFP transgene in pre- and early post-implantation embryos

The expression of GOF-18/delta PE/GFP transgene in pre-implantation embryos was also examined. As in GOF-18/GFP transgenic embryos, no significant fluorescence above background was observed in 2-cell transgenic embryos (data not shown). Eight-cell transgenic embryos, however, showed low but significant expression of the transgene (Fig. 1D′, right) compared with non-transgenic embryos (Fig. 1D′, left). Similar but much weaker low-level expression than that in GOF-18/GFP embryos was also observed in the ICM (Fig. 1E′, arrowhead) and the epiblast (Fig. 3A′–C′).

At the no obvious allantoic bud (OB) stage (~7.25 d.p.c.), the posterior portion of the extra-embryonic region where PGC are located showed a slightly stronger signal than the epiblast (Fig. 3C′, arrowhead). At the early head fold (EHF) stage (~8.0 d.p.c.), a cluster of cells showing more intense fluorescence was first observed at the base of the allantois (Fig. 3D′, arrowhead), which may have been PGC. In a few hours, these GFP-expressing PGC increased in number (Fig. 3E′, arrowhead), and by 8.5 d.p.c. they moved into the hindgut wall (Fig. 3F′).

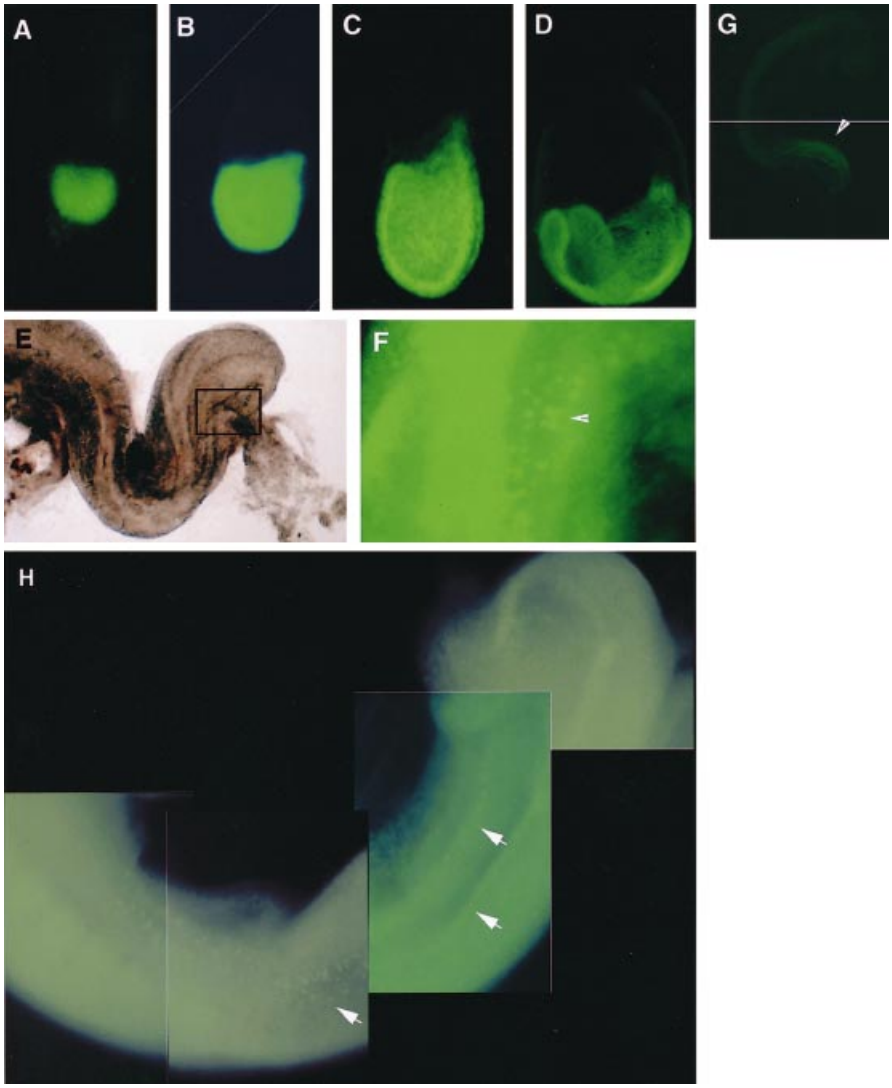


Fig. 2. Expression patterns of GOF-18/green fluorescent protein (GFP) transgenes in line 11 post-implantation embryos. Fluorescence images of the embryos at (A) 6.0, (B) 6.5 (early streak (ES) stage), (C) 7.25 (no obvious allantoic bud (OB) stage,), (D) 8.0 (early head fold (EHF) stage), (E,F) 8.5, (G,H) 9.0 days post-coitum (d.p.c.) are shown. Boxed area in (E) corresponds to (F). (G,H) Whole and posterior portion of a 9.0 d.p.c. embryo, respectively. Strong GFP expression was observed in the epiblast and its derivative tissues. Primordial germ cells (PGC) in the hindgut at 8.5 d.p.c. (arrowhead, F) and the dorsal mesenchyme at 9.0 d.p.c. (arrows, H) show stronger expression. Down-regulated expression in an anterior region from the point of the arrowhead is seen in (G). Left is anterior in all photographs except (G) in which top is anterior. Staging was according to Downs and Davies (1993).

Expression of Oct-4/GFP transgenes in PGC in migration and in the genital ridges

As for endogenous Oct-4 expression, the significant expression of both GOF-18/GFP and GOF-18/delta PE/GFP transgenes in PGC persisted during their migration and in the genital ridges, with these results being similar to those previously observed in Oct-4/lacZ transgenic mice. Figure 4(A′) shows a fluorescence image of a 9.5 d.p.c. GOF-18/GFP embryo. The PGC migrating along the dorsal mesentery toward the forming genital ridges (arrow) are positive for fluorescence, although differentiating epiblast derivatives were also expressing GFP (Fig. 2H). In contrast, in GOF-18/delta PE/GFP transgenic embryos, the expression of GFP was observed only in PGC at 9.5 and 10.5 d.p.c. (Fig. 4B′,C′, respectively). At 11.5 d.p.c., both transgenes showed the same expression for PGC in the genital ridges (data not shown). From these stages onward, the expression patterns of the two transgene constructs in the mice were identical (as described below and data not shown). Although both male and female PGC in proliferation express endogenous Oct-4, there are differential expression patterns between them after 13.5 d.p.c. Expression in female germ cells after entry into meiosis is down-regulated, while male germ cells continue to express endogenous Oct-4 even after they are mitotically arrested. In agreement with these endogenous expression patterns, 13.5 d.p.c. male and female gonads of GOF-18/GFP transgenic mice were indistinguishable in terms of GFP expression, although the female gave a spotty pattern of fluorescence (Fig. 5A′), whereas the male exhibited a wavy pattern (Fig. 5C′) reflecting testicular tubule formation. At 17.5 d.p.c., female germ cells showed low-level expression (Fig. 5B′), while GFP expression persisted in the male germ cells (Fig. 5D′,E).

Expression of Oct-4/GFP transgenes in the postnatal ovary

Expression patterns of the Oct-4/GFP transgenes in postnatal ovaries also mimic those of endogenous Oct-4 (Pesce *et al.* 1998). Both the GOF-18/GFP and GOF-18/delta PE/GFP transgenes were expressed in oocytes at the diplotene/dictyate stage onward in a similar manner. At 1 d.p.p., ovaries of the GOF-18/delta PE/GFP transgenic mice were clearly different from those of wild-type mice (Fig. 6A), and the former showed low but significant fluorescence above background in diplotene/dictyate oocytes (Fig. 6C′). The level of transgene expression in the 1 d.p.p. ovary was similar to that in the 17.5 d.p.c. ovary (Fig. 5B′). In the 7 d.p.p. ovary, the expression in primary oocytes was up-regulated, but the number of fluorescent-positive cells was

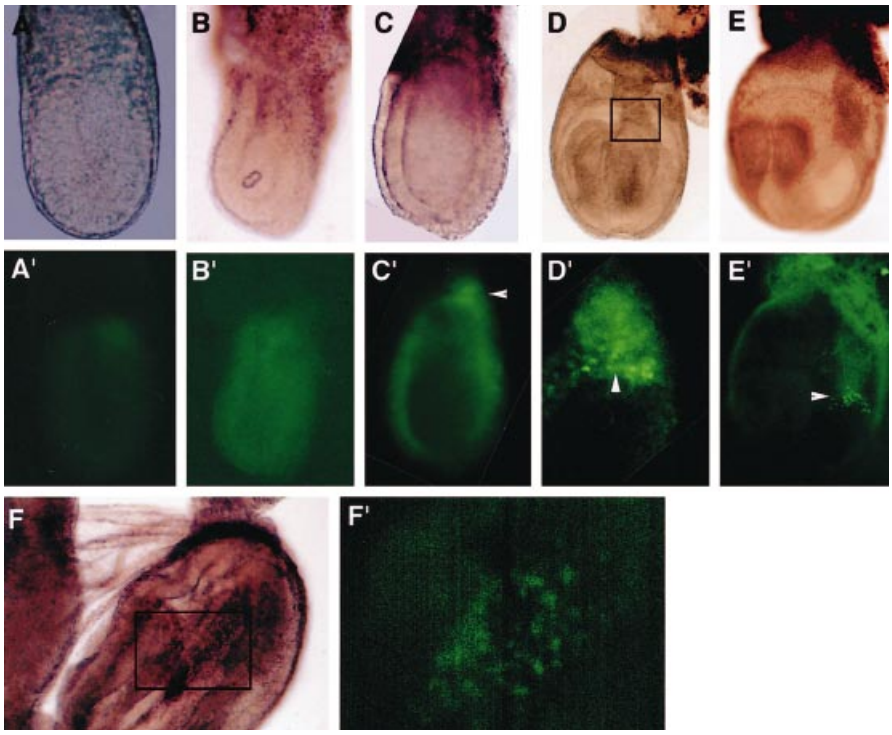


Fig. 3. Expression patterns of GOF-18/delta PE/GFP (green fluorescent protein) transgenes in line 36 post-implantation embryos. Fluorescence (A′–F′) and corresponding bright field (A–F) images are shown. (A,A′) Faint but significant expression was observed in the entire epiblast of 6.5 days post-coitum (d.p.c.), early streak embryo. (B,B′) Slightly stronger fluorescence was observed in the proximal region of the 6.75 d.p.c epiblast. (C,C′) Stronger fluorescence was seen around the posterior allantoic bud than in the rest of the epiblast in the 7.25 d.p.c. (no obvious allantoic bud (OB) stage) embryo. Arrowhead indicates the posterior end of embryos where primordial germ cells (PGC) first emerge. (D,D′) Ten or more GFP-positive PGC (arrowhead) were observed in 8.0 d.p.c. (early head fold (EHF) stage) embryo. The boxed area in (D) corresponds to (D′). (E,E′) More GFP-positive PGC (arrow) were seen in an embryo 2–3 h after the EHF stage. (F,F′) A cluster of GFP-positive PGC are found within the hindgut in the posterior part of the 8.5 d.p.c embryo. The boxed area in (F) corresponds to (F′). Left is anterior in all photographs except (D) and (D′), which show posterior views.

decreased and this caused in the ovaries localized scattering of fluorescent positive cells (Fig. 6B). These cells were primary oocytes and growing oocytes in mono- and bi-laminar follicles (Fig. 6D′). Green fluorescent protein expression was also observed in fully grown oocytes and in oocytes at the metaphase of the second meiotic division (M II) in adult ovaries (Fig. 6E′,F′). These expression patterns mimic those of endogenous Oct-4 (Palmieri *et al.* 1994; Pesce *et al.* 1998).

Expression of Oct-4/GFP transgenes in the postnatal testis

The Oct-4 protein has been shown to be expressed in mitotically arrested prospermatogonia in testes at late gestation and at birth (Pesce *et al.* 1998). This expression has also been detected in type A spermatogonia but is down-regulated in type B spermatogonia and in spermatocytes in the adult testis (Pesce *et al.* 1998). The GOF-18/GFP transgene was strongly expressed in male germ cells that were considered to be prospermatogonia in the seminiferous tubules at 17.5 d.p.c. (Fig. 5D′,E), and this expression continued until birth.

We further analyzed the expression patterns of the GOF-18/GFP and GOF-18/delta PE/GFP transgenes, which are mostly identical, in spermatogenic cells at various differentiation stages in postnatal testes. At 1 d.p.p., the expression of GOF-18/delta PE/GFP was detected in the center of the testicular tubules (Fig. 6G,I′), and the fluorescent-positive cells seemed to represent primitive type A spermatogonia, which are the progenitors of type A spermatogonial stem cells (Bellvé 1979). Although the expression persisted to 7 d.p.p. (Fig. 6H), a subset of the testicular tubules showed stronger expression than the rest (Fig. 6J). No expression was found from 10 d.p.p. onward (data not shown). The primitive type A spermatogonia may rapidly differentiate to type A spermatogonia after birth, and the latter may be negative for GFP expression.

Discussion

Oct-4/GFP transgenes are expressed in germ cells in late gestational and postnatal gonads

Oct-4 is the only known molecule specifically expressed throughout mouse germline development, and it is of great interest to determine how germline-specific expression is regulated. The regulatory regions of the Oct-4 gene have been examined, and two separate enhancer (PE and DE) elements, which are differentially regulated during embryogenesis, have been identified (Yeom *et al.* 1996). The results indicated that the DE element is necessary for gene activation in pre-implantation embryos and probably in PGC, whereas the PE element is specifically required for its expression in the epiblast. In the present study, we replaced the lacZ reporter gene with the GFP gene and basically reproduced the results previously obtained

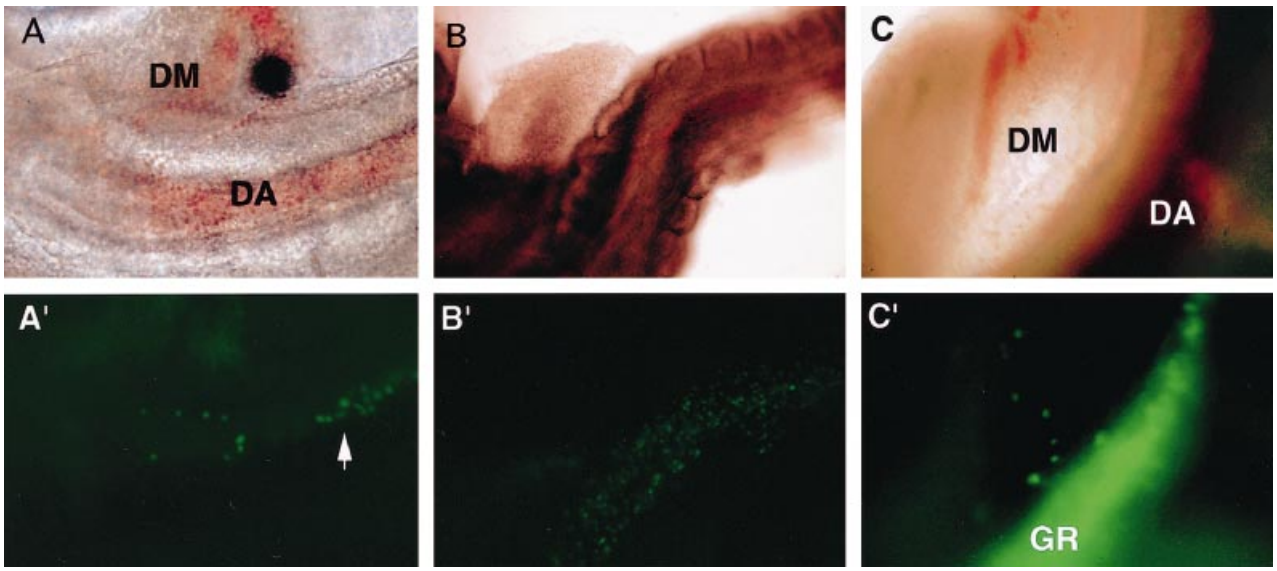


Fig. 4. Oct-4/GFP transgene expression in migratory primordial germ cells (PGC). Fluorescence (A′–C′) and corresponding bright-field (A–C) images are shown. Green fluorescence protein (GFP) positive PGC migrate along the dorsal mesenchyme (DM) toward the genital ridges (GR) in the 9.5 days post-coitum (d.p.c). GOF-18/GFP embryo (line 36, A′,B′), and in the 10.5 d.p.c. GOF-18/delta PE/GFP embryo (line 36, C′). The dorsal wall was removed in (C) and (C′). DA, dorsal aorta.

with the Oct-4/lacZ transgene construct (Yeom *et al.* 1996). The patterns of expression of the transgenes are summarized in Fig. 7. In the current study we were able to obtain additional information regarding Oct-4 gene regulation. First of all, we determined that the 18 kbp genomic fragment with or without the PE element is sufficient for expression in maturating oocytes as well as in male germ cells at late gestational and early postnatal stages (Fig. 6). The transgenes are not, however, expressed in spermatogonia that are positive for the Oct-4 protein by immunohistochemical studies (Pesce *et al.* 1998), suggesting that Oct-4 expression in spermatogonia may be regulated by other *cis*-acting elements that are not included in the GOF-18 genomic fragment.

The PE element is dispensable for basal expression of the transgene in pre-implantation embryos and in the epiblast

Previous experiments (Yeom *et al.* 1996) have shown that the GOF-18/lacZ and GOF-18/delta PE/lacZ transgenes are expressed in ICM at the same level, but that only the former construct gives significant expression in epiblasts. The present data, however, indicate that even the GOF-18/delta PE/GFP transgene shows totipotent cell-specific expression from the 8-cell embryo through the post-implantation epiblast, although the level of expression is much lower than that of GOF-18/GFP (Figs 1–3). These observations suggest that the PE element functions as a pre-implantation embryo- and epiblast-specific enhancer, but the

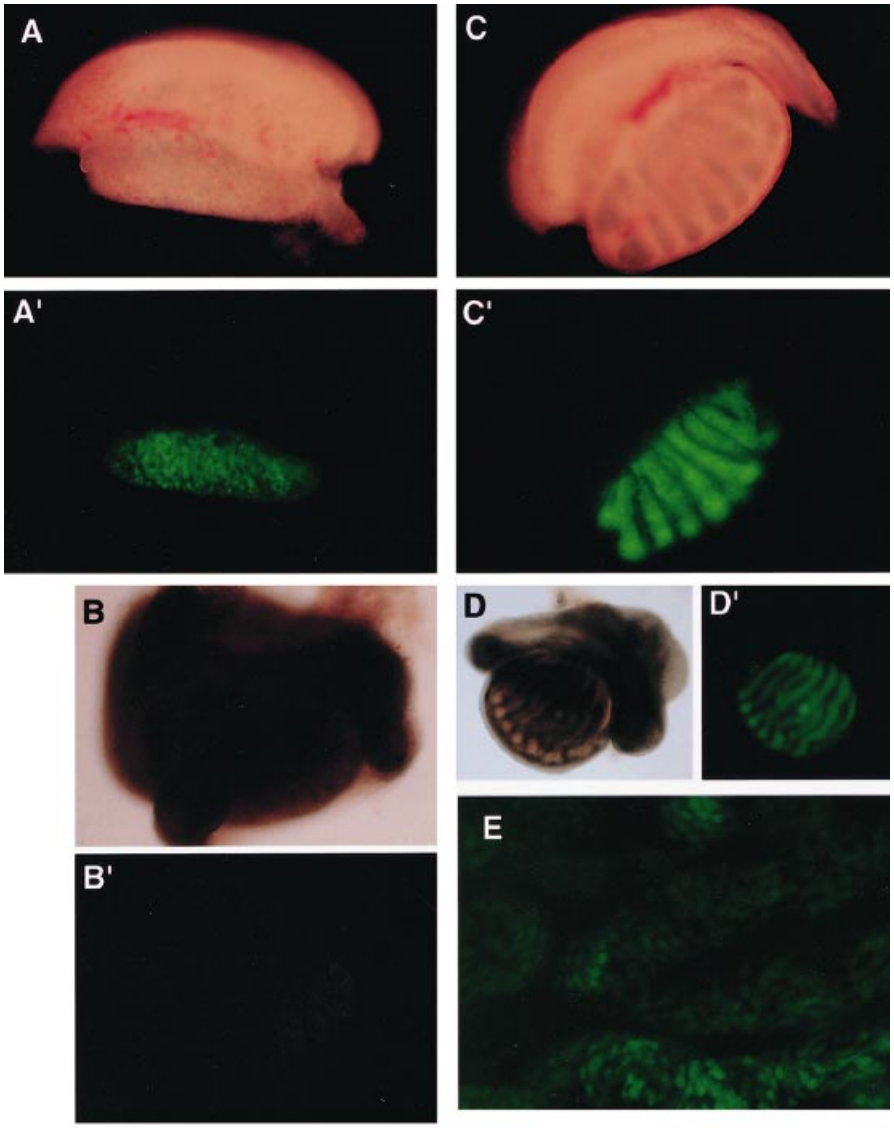
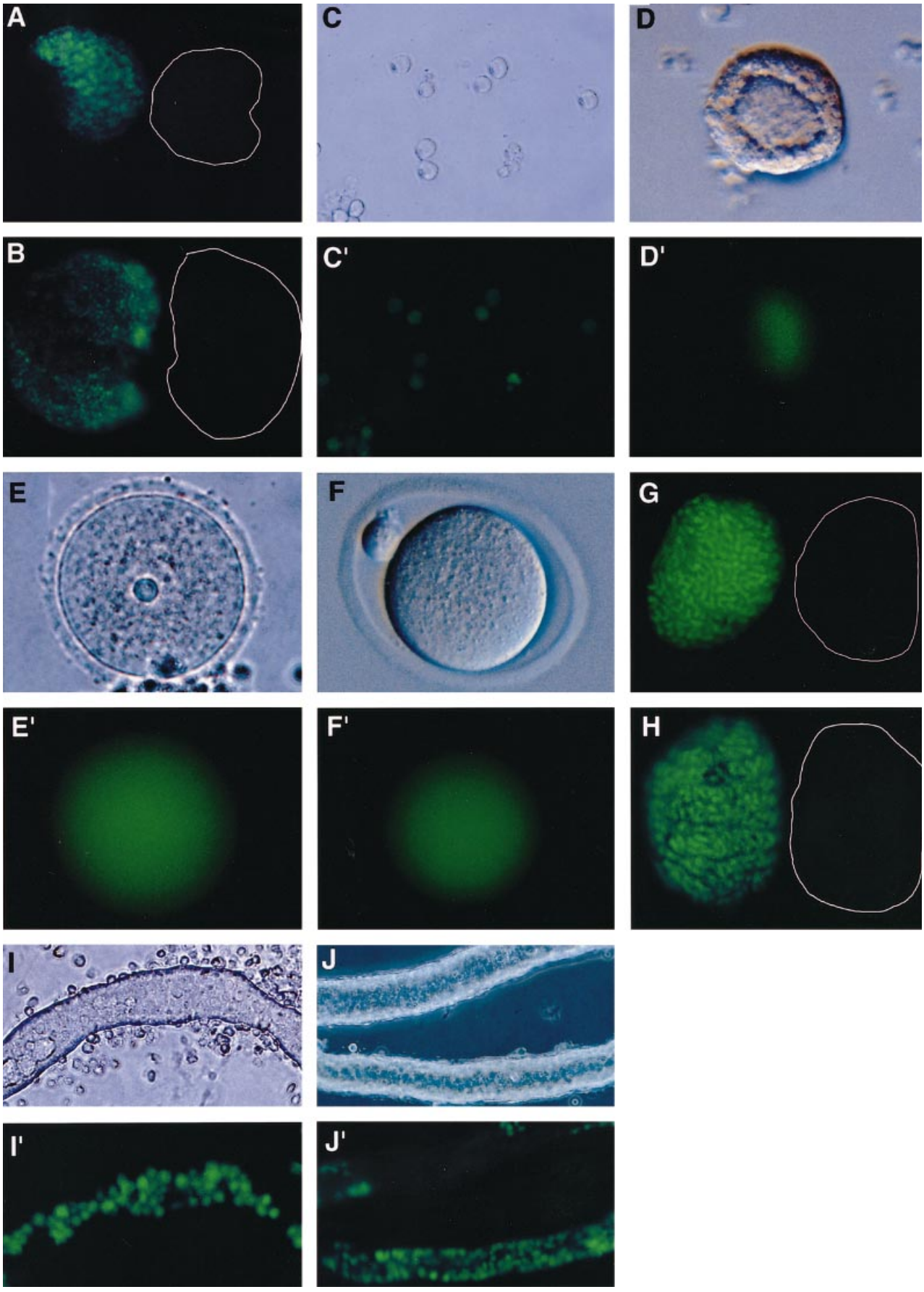


Fig. 5. GOF-18/green fluorescent protein (GFP) transgene expression in 13.5 days post-coitum (d.p.c.; A,C) and 17.5 d.p.c. (B,D) gonads (line 36). Fluorescence (A′–D′) and corresponding bright field (A–D) images are shown. (A,B) Female and (C,D) male gonads. (E) Closer fluorescent view of the 17.5 d.p.c. male gonad.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tissue specificity of the basal expression of Oct-4 may be regulated by separate elements. The inconsistent expression patterns of the previous lacZ and the present GFP transgenes may be the result of different stability and/or different sensitivity of detection of the reporter gene products. In addition, timing of the down-regulation of GOF-18/GFP transgene expression in the epiblast and its derivative tissues occurred at 9.25 d.p.c (Fig. 2), which was a half-day delay from that of GOF-18/lacZ. This may also be caused by the stability of the GFP protein (Hadjantonakis *et al.* 1998).

Activation of the GOF18/delta PE/GFP transgene is coincident with PGC formation

The PGC are known to originate from the proximal region of the pregastrulating epiblast (Lawson & Hage 1994), and first emerge within the posterior extra-embryonic mesoderm at 7.2 d.p.c (Ginsburg *et al.* 1990). At these stages, however, neither endogenous Oct-4 nor GOF-18 transgene expression is restricted to PGC or their precursors, and these transgenes are also expressed in the surrounding cells. Our previous report indicated that the GOF-18/delta PE/lacZ transgene is not expressed in the epiblast, but that the expression is specifically detected in PGC at 8.0–8.25 d.p.c. This raises the possibility that GOF-18/delta PE is specifically expressed in PGC at earlier developmental stages, but the details of this earlier expression have not yet been reported. Therefore, we have made an effort to determine the expression of GOF-18/delta PE/GFP at these earlier time points. As described earlier, weak expression of GOF-18/delta PE/GFP in totipotent cells was detected from the 8-cell stage onward, but a sign of the up-regulation of its expression in germ cell lineage occurred at 6.75 d.p.c. when the proximal region of the epiblast gave stronger fluorescence than the rest of the epiblast (Fig. 3B′). At 7.25 d.p.c. the posterior end of the embryo, which is a portion of the future allantoic bud, emitted more intensive fluorescence than the epiblast (Fig. 3C′). Because PGC are located within this region, it is likely that PGC at this stage express the GFP transgene. At 8.0 d.p.c. the presomitic early head fold stage, the GFP expression was up-regulated within a broad region around the base of the allantois in which cells with stronger fluorescence could be recognized as bright spots of fluorescence (Fig. 3D′), and the number of these strongly positive cells increased to 30–40 in a few hours. In 8.75 d.p.c. embryos, the number of GFP-expressing cells increased further, and a cluster of PGC consisting of 60–80 cells showing bright fluorescence could be found in the hindgut wall. All these results indicate that activation of the GOF-18/delta PE/GFP transgene is coincident with PGC formation from epiblast cells and may represent a process in which epiblast cells acquire the character of PGC.

As described, the present Oct-4/GFP transgenic mice provide a good opportunity to isolate living germ

Fig. 6. GOF-18/delta PE/GFP (green fluorescent protein) transgene expression in postnatal gonads (line 48). Fluorescence (A,B,C′–F′,G,H,I′,J′) and corresponding bright field (C–F,I,J) images are shown. (A) One day post-partum (d.p.p.) and (B) 7 d.p.p. ovaries. (C) Oocytes at the diplotene/dictyate stage in the 1 d.p.p. ovary. (D) Growing oocytes with mono- or bi-laminar follicles in the 7 d.p.p. ovary. (E) Fully grown oocytes and (F) M II oocytes in the adult ovary. (G) One day post-partum and (H) 7 d.p.p. testes. (I) One day post-partum (and) 7 d.p.p. seminiferous tubules. The upper and lower tubules in (J,J′) were obtained from a testis. Left is the transgenic and right is the non-transgenic gonad in (A,B,G,H).

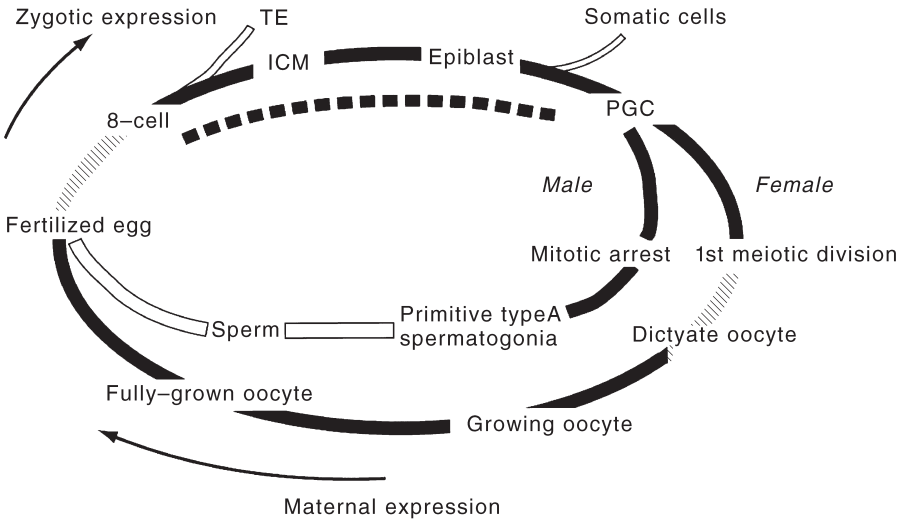

Fig. 7. A summary of Oct-4/GFP transgene expression in the totipotent cell cycle. Open lines, stages not expressing Oct-4; striped lines, transition from expressing to not expressing stages; broken line, low level expression of the GOF18/delta PE/GFP (green fluorescent protein) transgene; solid lines, expressing stages.

cells at various developmental stages without an additional staining procedure. Transgenic mice expressing lacZ under control of the tissue-non-specific alkaline phosphatase (TNAP) locus have been used to purify PGC (Abe *et al.* 1996), but the Oct-4/GFP transgenic mice are more convenient for this purpose. In addition, these mice should also be quite useful not only for the analysis of germ cell behavior *in vivo*. For example, after introducing Oct-4/GFP transgenes into various mutant mice potentially affecting PGC development, GFP expression should provide an exceptional tool for following the behavior of the mutant PGC in living embryos, in chimeras, and even after transplantation. These mice should allow the real-time observation of developing PGC in living embryos.

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