Specification of germ cell fate in mice

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An early fundamental event during development is the segregation of germ cells from somatic cells. In many organisms, this is accomplished by the inheritance of preformed germ plasm, which apparently imposes transcriptional repression to prevent somatic cell fate. However, in mammals, pluripotent epiblast cells acquire germ cell fate in response to signalling molecules. We have used single cell analysis to study how epiblast cells acquire germ cell competence and undergo specification. Germ cell competent cells express *Fragilis* and initially progress towards a somatic mesodermal fate. However, a subset of these cells, the future primordial germ cells (PGCs), then shows rapid upregulation of *Fragilis* with concomitant transcriptional repression of a number of genes, including *Hox* and *Smad* genes. This repression may be a key event associated with germ cell specification. Furthermore, PGCs express *Stella* and other genes, such as *Oct-4* that are associated with pluripotency. While these molecules are also detected in mature oocytes as maternally inherited factors, their early role is to regulate development and maintain pluripotency, and they do not serve the role of classical germline determinants.

Keywords: germ cells; germ cell specification; transcriptional repression; totipotency

1. INTRODUCTION

The segregation of germ cells from somatic cells is one of the earliest cell fate decisions that occurs during development in many organisms. Whereas the somatic lineages generally contribute to the maintenance of the physiological integrity of individuals, germ cells provide an enduring link between generations through the creation of new organisms by gametic fusion. This remarkable capacity of the germ line has often been referred to as cellular or genomic totipotency. Therefore, it is only through the germ line that particular genetic information is transmitted to subsequent generations. Accordingly, all mutations that have resulted in the generation of biological diversity have been acquired and propagated through the germ line.

More than a century ago, August Weismann hypothesized the presence of preformed germ cell determinants (germ plasm) that are inherited only through the prospective germ cells to ensure the totipotency and continuity of the germ line (Weismann 1892). This has proved to be the case in some organisms including *Caenorhabditis elegans*, *Drosophila melanogaster* and *Xenopus laevis*, in which morphologically distinctive germ plasm from the oocyte is inherited by embryonic cells that go on to develop as germ cells (Eddy 1975; Wylie 1999). In these organisms, germ plasm has been demonstrated to be indispensable for germ cell formation, and some of its molecular components have been isolated (Wylie 1999). These studies have led to a concept that germ plasm might provide a powerful mechanism governing germ-cell-specific transcriptional

repression and RNA translation, which eventually prevents the cells that inherit it from acquiring somatic fates (Seydoux *et al.* 1996; Seydoux & Dunn 1997; Van Doren *et al.* 1998; Batchelder *et al.* 1999; Seydoux & Strome 1999).

However, unlike the organisms described above, mammals including the mouse (*Mus musculus*) seem to have no preformed germ cell determinants (Eddy 1975; Wylie 1999). Instead, in these organisms, a population of pluripotent cells with seemingly equivalent potential originates from a zygote, and it is from these cells that both germ cells and somatic cells are eventually derived (Gardner & Rossant 1979; Gardner *et al.* 1985; Lawson *et al.* 1991). Therefore, germ cell formation in mammals involves epigenesis and not preformation, and is thus a regulative event, in which certain environmental influences play a crucial role. However, scarce molecular information on how germ cells are formed has hampered our further understanding on how nascent germ cells are related to zygotes or early blastomeres and on how genomic potential or 'totipotency' is propagated through the germ line, allowing subsequent development in the next generation. To address these questions, we have recently set out to investigate the genetic basis for germ cell determination (Saitou *et al.* 2002). Here, we describe our recent advances in gaining a molecular understanding of this process in mice, and discuss several novel insights that this knowledge provides on the origin and properties of the germ line.

2. EARLY DEVELOPMENT AND GERM CELL FATE DETERMINATION IN MICE

In contrast to organisms with preformed embryogenesis, the establishment of a pluripotent cell population is essential in mammals (see figure 1 for overview of early mouse

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Figure 1. Schematic representation of early mouse development and the appearance of PGCs. Mouse development starts with the fertilization of an oocyte which contains maternally inherited factors important for establishing a pluripotent cell population. Following fertilization, the second polar body is extruded into the space between the zygote and zona pelucida. Subsequently, the contents of the male and female pronuclei combine to form a reorganized diploid genome. Zygotic transcription is initiated at the two-cell stage. The first differentiated cell lineage, the trophoblast, begins to form at the eight to 16-cell stage, while cells locating on the inside form a population of pluripotent cells, the ICM, by E3.5. An embryo at this stage is called a blastocyst in which distally located ICM cells differentiate into primitive endoderm. At E4.5, the blastocyst implants and during E5.0–5.5, ICM cells cavitate to form a cup-shaped layer epithelium, the epiblast, which is proximally contacted with extraembryonic ectoderm, a derivative of trophoblast cells. The epiblast and extraembryonic ectoderm are covered by a layer of visceral endoderm. It is not until E7.25 (LS stage), shortly after the start of gastrulation, that the germ cells first become identifiable at the base of the incipient allantois. At E8.5, most germ cells start migrating towards the future genital ridges, where they undergo sexual differentiation and eventually produce either male or female gametes. Abbreviations: PB, polar body; ZP, zona pelucida; M, male; F, female; TE, trophoblast; E, endoderm; ExE, extraembryonic ectoderm; LS, late streak; PS, pre-streak; EB, early allantoic bud (Downs & Davies 1993).

development). Rather than responding to maternal factors that cause differentiation, these pluripotent cells must be maintained while extraembryonic lineages differentiate. Although the molecular process underlying this segregation of pluripotency in mammals is poorly understood, it may involve maternally inherited factors such as *Oct-4*, *Ezh2* and *Eed*, the deletion of which leads to early embryonic lethality (Shumacher *et al.* 1996; Faust *et al.* 1998; Nichols *et al.* 1998; O'Carroll *et al.* 2001). Formation of the trophoblast from the outer cells of the late eight- or 16-cell morula stage embryo is the first cell differentiation event to occur during mammalian development. These cells are necessary for implantation and for the formation of the placental tissues. Cells located on the inside of the embryo form the pluripotent ICM, which gives rise to all the cells constituting the embryo proper including germ cells (Gardner & Rossant 1979; Gardner *et al.* 1985; Lawson *et al.* 1991). Furthermore, pluripotent ES cells can be derived from ICM cells in culture (Evans & Kaufman 1981; Martin 1981). Interestingly, embryos lacking the transcriptional regulator *Oct-4* fail to form a pluripo-

tent ICM while embryos lacking the epigenetic regulator *Ezh2* cannot form ES cells, indicating that both *Oct-4* and *Ezh2* are important for the maintenance of pluripotency (Nichols *et al.* 1998; O'Carroll *et al.* 2001). In addition, it has been shown that early embryonic cells in mice undergo dynamic epigenetic reprogramming of the genome including DNA demethylation and histone tail modifications, while keeping the basic pluripotent state intact (Li 2002). After implantation, at about E5.0, ICM cells differentiate into a cup-shaped layer of epithelial cells, called epiblast, which are proximally apposed to trophoblast-derived extraembryonic ectoderm, and both of these populations are surrounded by a layer of visceral endoderm which is derived from distally located ICM cells. The epiblast cells retain the capacity to form all the embryonic cells including the germ line and extraembryonic mesoderm (Lawson *et al.* 1991). The expression of *Oct-4*, *Ezh2* and *Eed* is maintained in all the epiblast cells (Yeom *et al.* 1996; Laible *et al.* 1997; Pesce *et al.* 1998; O'Carroll *et al.* 2001; Erhardt *et al.* 2003). However, at around E6.0 shortly before gastrulation, proximal

epiblast cells begin to express mesodermal markers including *Eomesodermin*, *T(Brachyury)* and *Fgf8* (Herrmann *et al.* 1990; Herrmann 1991; Crossley & Martin 1995; Ciruna & Rossant 1999; Hancock *et al.* 1999; Russ *et al.* 2000). This suggests that the proximal epiblast cells are principally destined towards somatic cell fates.

It is only after the onset of gastrulation at E7.25 that germ cells can first be identified as a distinctive cluster of *ca.* 45 alkaline phosphatase positive cells at the base of the incipient allantois (Chiquoine 1954; Ginsburg *et al.* 1990). Clonal analysis of E6.0–E6.5 epiblast cells revealed that the germ cell precursors reside within the most proximal part of the epiblast (Lawson & Hage 1994). However, these precursors give rise not only to germ cells, but also to somatic cells of the allantois and extraembryonic mesoderm. This important finding indicates that germ cell fate is established relatively late: the definitive segregation of germ cells from somatic lineages occurs during one or two cell cycles in cells originating from E6.5 proximal epiblast. Consistently, it was demonstrated that heterotopic transplantation of E6.5 distal epiblast cells to the proximal position led to the generation of germ cells from the transplanted cells (Tam & Zhou 1996). Conversely, when proximal epiblast cells were grafted into the distal part, they failed to differentiate into germ cells. It is therefore the proximal location of the cells rather than the influence of the preformed determinants that is essential for germ cell determination in mice. It was subsequently shown that signalling molecules from the adjacent extraembryonic ectoderm, including BMP4 and BMP8b, are required for germ cell fate allocation (Lawson *et al.* 1999; Ying *et al.* 2000). However, crucially, BMP4-deficient embryos lack an allantois as well as germ cells, suggesting the presence of (a) second signal(s) for the final segregation of germ cells (Lawson *et al.* 1999; McLaren 1999).

3. THE ORIGIN AND PROPERTIES OF FOUNDER GERM CELLS IDENTIFIED BY SINGLE-CELL TRANSCRIPT ANALYSIS

What might be the mechanism for the specification and segregation of germ cells from the neighbouring somatic cells, and what are the key intrinsic steps that lead to the founding of the germ cell lineage? Are two signals needed sequentially for germ cell formation, one for the acquisition of competence and the second for the final stages of specification? Is the transcriptional repression of genes that are essential for somatic cell fate a necessary prerequisite for germ cell formation? These important questions remain unanswered mainly because of the lack of appropriate markers to follow the process of germ cell specification. The primary marker hitherto for the founder PGCs has been the characteristic tissue non-specific alkaline phosphatase (TNAP) (Chiquoine 1954; Ginsburg *et al.* 1990). However, somatic cells also show low levels of TNAP staining. Other genes such as *Oct-4* and *Ezh2*, while being associated with germ cells, are apparently critical for pluripotent lineages and are not associated exclusively with germ cells (Yeom *et al.* 1996; Laible *et al.* 1997; Pesce *et al.* 1998).

A major difficulty in studying the genetic basis for the specification of the germ cell lineage is that the founder population of germ cells is few in number and deeply

Figure 2. The organization of a founder PGC population. (a) An LS stage (E7.25) embryo of the 129SvEv strain stained for alkaline phosphatase activity is shown. The arrowhead indicates the region with TNAP staining, which contains PGCs. Strong activity is also detected in the chorionic membrane (arrow). The epiblast has low level activity. Scale bar, 200 μ m. (b,c) Lateral and transverse semi-thin sections, respectively, of regions bearing the PGC cluster in LS stage embryos of 129SvEv strain stained by methylene blue. The black arrow indicates the incipient allantois, the grey arrow indicates the chorionic membrane, and the arrowheads indicate densely stained cells in the cluster. Scale bar, 50 μ m. (d) Electron micrograph of a densely stained cell. Note the high electron density of the cytoplasm compared with that of surrounding cells. Scale bar, 2 μ m. In (a–c) anterior is to the left and posterior to the right.

embedded in the surrounding extraembryonic mesoderm (Ginsburg *et al.* 1990; Lawson & Hage 1994). However, morphological examination revealed a cluster of densely stained cells rich in free ribosomes, which is surrounded by ordinary looking embryonic cells in the germ cell differentiating region at E7.25 (LS stage (Downs & Davies 1993; Saitou *et al.* 2002; figure 2)). These cells are likely to be founder germ cells, as this distinctive morphology is very similar to that of the germ cells at later stages (Spiegelman & Bennett 1973; Clark & Eddy 1975). This indicates that germ cells acquire characteristic morphology soon after their lineage restriction, which would reflect their underlying unique molecular properties.

We set out to establish a system to identify key factors that determine germ cell fate and to understand the distinctive properties that the germ cells acquire at the molecular level (Saitou *et al.* 2002). We dissected out an embryonic fragment consisting of about 300 cells from the region containing the founder germ cells at E7.5 (EB stage) and dissociated it into single cells. These cells are morphologically indistinguishable under a dissection

microscope, and we therefore picked single cells randomly and amplified a representative cDNA population from each one. Stepwise selection of nearly 100 single cell cDNAs eventually identified *BMP4* negative, *TNAP* strongly positive, *Hoxb1* negative cells as the founder germ cells. Precise comparison of single germ cell cDNAs with single somatic cell cDNAs that share common ancestry revealed two novel genes associated with germ cell differentiation and molecular properties that distinguish germ cells from somatic cells.

The first gene, *Fragilis*, encodes a novel member of the IFN inducible transmembrane protein family of genes that, in humans, is predicted to be involved in homotypic cell adhesion and cell cycle elongation (Friedman *et al.* 1984; Evans *et al.* 1990, 1993; Deblandre *et al.* 1995). *Fragilis* has two tandemly repeated ISREs (Reid *et al.* 1989) within its promoter region. It is conserved at least among vertebrates, and other members of this family also appear to be associated with germ cell specification (Lange *et al.* 2003) (also see below). The second gene, *Stella*, encodes a novel nuclear-cytoplasmic protein that may be involved in chromosomal organization and RNA metabolism. Homologues of *Stella* were identified in rat and human within syntenic chromosomal locations. Human *Stella* expressed sequence tags are detected predominantly in germ cell tumours (B. Payer, unpublished data).

By RNA *in situ* hybridization, *Fragilis* is diffusely and weakly expressed throughout the epiblast until E6.0 (figure 3*a*). Expression of *Fragilis* begins to increase at E6.25 in the cells of the proximal rim of the epiblast (figure 3*a*), which are competent for the germ cell differentiation. During gastrulation, *Fragilis* expression shifts posteriorly and becomes rapidly intensified in a region where the founder germ cells can be identified at E7.25 (figure 3*a,b,d*). Expression of *Fragilis* is not enhanced within the allantoic mesoderm. Therefore, it appears that *Fragilis* is the first gene to mark the onset of germ cell competence and show an expression pattern consistent with the eventual segregation of germ cells from allantoic precursors. Interestingly, at E7.25, *Stella* expression is induced in a subset of cells within the centre of the *Fragilis* positive cluster (figure 3*e*). These *Stella*-positive cells are considered to be the lineage-restricted germ cells and *Stella* is the first gene identified to be expressed exclusively in founder germ cells. Migrating germ cells continue to show strong and specific expression of *Stella* thereafter (figure 3*f,g*).

Molecular properties of the founder germ cells compared with surrounding somatic cells were examined using the single cell cDNAs we generated (figure 4). Consistent with *in situ* findings, only those cells that express the highest amounts of *Fragilis* showed expression of *Stella*. The *Stella*-positive founder germ cells, as well as the neighbouring somatic cells, were positive for the expression of mesodermal markers, such as *T(brachyury)* and *Fgf8*. This finding suggests that germ cell fate is imposed upon a population of mesodermally differentiating cells. However, most importantly, the *Stella*-positive germ cells were found to exhibit strong repression of all the homeobox genes examined, including *Hoxb1*, *Hoxa1*, *Evx1* and *Lim1*, which are highly expressed in the neighbouring somatic cells (Bastian & Gruss 1990; Frohman *et al.* 1990; Barnes *et al.* 1994; Saitou *et al.* 2002). As the general role of

Figure 3. Expression of *Fragilis* (*a–d*) and *Stella* (*e–g*) in the germline-competent and lineage-restricted germ cells during gastrulation. (*a*) *Fragilis* expression in a series of embryos, representing, sequentially from left to right, stages before through to shortly after the onset of gastrulation. Before gastrulation at around E6.0, *Fragilis* is expressed diffusely throughout the epiblast. Expression of *Fragilis* intensifies in proximal epiblast cells at E6.25–E6.5 and shifts posteriorly concomitant with the onset of gastrulation. (*b*) At LS stage (E7.25), *Fragilis* expression marks the germ cell differentiation region and *Fragilis* expression looks strongest in the region's centre. (*c*) Expression of *T(Brachyury)* compared with *Fragilis* (*d*) during early stages of gastrulation. *T* expression is seen in the proximal–posterior epiblast, primitive streak mesoderm and extraembryonic mesoderm where PGCs differentiate. (*e*) Posterior view of *Stella* expression in an LS stage embryo. Compared with *Fragilis* expression shown in (*b*), *Stella* expression is apparently more restricted within a subset of cells located at the centre of the founder PGC cluster. (*f,g*) *Stella* is exclusively expressed in the migrating germ cells at E8.5 and E9.5, respectively. In (*a–d*) anterior is to the left and posterior to the right. Scale bars: (*a*) 100 μ m; (*b–e*), 200 μ m; (*f,g*) 500 μ m.

homeobox genes is to specify the regional identity of cells along the body axis or induce differentiation of cells towards specific somatic cell lineages, this suggests that

Figure 4. Schematic representation of the gene expression profile of single PGCs and neighbouring somatic cells analysed by single cell Southern blot. Expression of some of the key genes associated with germ cell differentiation in PGCs (right panel) and in surrounding somatic cells (left panel) are shown. The expression levels (see key) for each of the genes were semi-quantitatively normalized against *Gapdh* (Saitou *et al.* 2002).

the founder germ cells acquire the ability to avoid somatic specification by preventing or suppressing homeobox gene expression. This may be one of the key properties that mammalian germ cells possess which allows them to maintain or regain totipotency.

Based on these findings, we proposed a model that *Fragilis*, with its predicted ability for homotypic adhesion, might demarcate germ cell precursors from somatic cells, thereby contributing to creating a 'niche' for germ cell fate segregation. In this 'niche', those cells showing high levels of *Fragilis* expression acquire the ability to repress homeobox genes, express *Stella*, and ultimately become totipotent germ cells. These germ cells thereafter continue to express *Oct-4* and other markers which promote the maintenance of the totipotent state.

4. A MOLECULAR PATHWAY LEADING TO GERM CELL FORMATION FROM PROXIMAL EPIBLAST

As has been demonstrated previously, specification of germ cell fate is an instructive process for which BMP4 signalling from extraembryonic ectoderm is essential (Lawson *et al.* 1999). As the expression of BMP4 starts as early as E5.5 and the allantois is not formed in BMP4 deficient embryos, the role of BMP4 may be to broadly confer future extraembryonic mesoderm identity to proximal epiblast cells rather than to directly specify germ cell fate. We found that *Fragilis* expression is completely lacking in BMP4 homozygous mutants and substantially reduced in heterozygous embryos, which suggests that *Fragilis* expression is BMP4 dose dependent (Saitou *et al.* 2002). However, we do not yet know if the expression of *Fragilis* is regulated directly and exclusively by BMP4. This is first because the rapid upregulation of *Fragilis* occurs only in a subset of posteriorly locating mesodermal cells, although BMP4 is expressed widely (Lawson *et al.* 1999), and second because *Fragilis* does not seem to have clear BMP4

response sequences, while having two highly conserved ISREs at its immediate promoter region. Future genetic experiments are required to test which signalling molecules are involved in regulating the expression of *Fragilis*.

Conversely, we demonstrated that *Fragilis* expression can be ectopically induced in distal epiblast cells when these cells are combined with extraembryonic ectoderm *in vitro* (Saitou *et al.* 2002). However, these induced epiblast cells did not acquire *Stella* expression, although some of them became positive for both *Oct-4* and TNAP. There are two possibilities that could explain the absence of *Stella* induction. First, the culture conditions used may not be optimal to allow complete progression of induced *Fragilis* positive cells to a germ cell fate. Alternatively, these observations could suggest that other signal(s), presumably originating from extraembryonic mesoderm, may be required for the final stages of germ cell fate specification and these signals may be absent in the *in vitro* system. The latter possibility is consistent with the previous suggestions (Lawson *et al.* 1999; McLaren 1999).

To gain a more precise insight into the intrinsic events occurring during germ cell specification, we prepared single cell cDNAs from earlier germ cells at E7.25 (LS stage (Downs & Davies 1993)). These cells are considered to be developmentally *ca.* 6 h earlier than those we have analysed so far. Consistent with the *in situ* analyses, these cells already express high amounts of *Fragilis*, but have considerably lower amounts of *Stella* (M. Saitou, unpublished data). Importantly, in these earliest germ cells, expression of the homeobox gene *Evx1* was found to be as high as in neighbouring somatic cells. This observation supports the proposal that homeobox gene suppression during germ cell specification is an active process. Other interesting examples of transcriptional repression associated with germ cell specification include *Smad1* and *Smad4* (U. Lange, unpublished data), which are the transducers of BMP4 signalling. *Smad1* and *Smad4* are equally expressed

Figure 5. Schematic representation of key events involved in germ cell fate determination as revealed by single-cell transcript analysis. Relative gene expression levels for some of the genes associated with germ cell specification in nascent PGCs (upper panel) and neighbouring somatic cells (lower) during gastrulation are shown. Solid lines show the transition of gene expression levels confirmed either by single-cell transcript analysis or *in situ* hybridization. Dotted lines represent the change of gene expression predicted from previous literatures. Repression of *Hox* genes and *Smads*, and maintenance of genes linked to pluripotency such as *Oct-4* are two major events occurring during germ cell lineage-restriction period. Upregulation of *Fragilis* followed by *Stella* is concomitant with this process. Possible timings at which the first (BMP4) and a predicted second (Signal X) instructive signal(s) for PGC specification may operate are shown by green and red arrowheads, respectively. Abbreviations: PS, pre-streak; LB, late bud (Downs & Davies 1993).

in somatic cells during the LS and EB stages and in germ cells at the LS stage, but are exclusively downregulated in EB stage germ cells. This observation may suggest that germ cells need to shut off their response to BMP4 to become responsive to another signal(s) that is critical for the specification of germ cells.

Figure 5 summarizes the intrinsic events segregating germ cell fate from somatic fates. At around E7.0 (mid-streak stage) shortly after gastrulation commences, all cells locating in the proximal-posterior region express mesodermal markers such as *T (Brachyury)* and *Fgf8*. They are also strongly positive for the region-specific homeobox gene *Evx1*. These cells similarly express genes associated with pluripotency such as *Oct-4*. At E7.25 (LS stage), in a subset of cells that strongly upregulate *Fragilis* expression, repression of *Evx1* and signal transducers such as *Smad1* and 4 occurs, while expression of *Stella* is initiated. At E7.5 (EB stage), these nascent germ cells establish high expression of *Fragilis* and *Stella*, and show repression of all of the homeobox genes and *Smads*, and eventually become lineage-restricted germ cells. By contrast, in cells which do not upregulate *Fragilis* at the LS stage, expression of

homeobox genes such as *Hoxb1* and *Hoxa1* begins and expression of *Evx1* and the *Smads* is maintained. Although *Oct-4* is expressed at high levels in both somatic cells and germ cells at the time of germ cell specification, it is subsequently maintained only in germ cells which retain totipotency.

5. CANDIDATES MEDIATING TRANSCRIPTIONAL REPRESSION UNIQUE TO THE GERM LINE

We have proposed that the founder germ cells acquire the ability to avoid being specified as somatic cells by preventing homeobox gene expression. Transducers of BMP4 signalling, such as *Smad 1* and 4, are also repressed in germ cells. These events might represent part of the overall germ-cell-specific repression mechanism which operates on many other genes normally expressed in the somatic cells. This proposal requires experimental verification and may involve genes responsible for transcriptional repression.

Potentially, high expression of *Fragilis*, which always precedes and is associated with *Hox* gene repression,

might play a role in this process. We found that the *Fragilis* gene family clusters in a tight group on mouse distal chromosome seven (Lange *et al.* 2003). Interestingly, as well as *Fragilis*, *Fragilis2* and *Fragilis3* are strongly upregulated in the germ cells, although they are also expressed elsewhere in gastrulating embryos. These proteins could function cooperatively to initiate repression of *Hox* genes in founder germ cells. The tight clustering and similarity of expression pattern of *Fragilis*, *Fragilis2* and *Fragilis3* raises the interesting possibility that the *Fragilis* family is co-regulated when germ cells are specified, and their expression could be mediated through conserved ISREs that are present upstream of these genes. Cytokines, including IFNs, that are capable of activating expression through ISREs (O'Shea *et al.* 2002), are thus candidates for the proposed second signal involved in germ cell specification.

The onset of *Stella* expression also correlates well with the beginning of *Hox* gene repression. The function of *Stella* in *Hox* gene repression should be examined by either gene knock-out or knock-down technology. Interestingly, apart from germ cells, expression of *Stella* is also detected during preimplantation development (Sato *et al.* 2002). *Stella* protein is maternally inherited in unfertilized oocytes, and embryonic transcription of *Stella* commences at the two-cell stage (B. Payer, unpublished data). However, expression of *Stella* ceases in early postimplantation embryos and it is not detected in the epiblast. *Stella* expression is regained only in germ cells and it is subsequently maintained in the germ cell lineage. The identification of factors that activate *Stella* expression will be crucial for understanding the mechanism of germ cell specification.

Members of the PcG proteins are known to be associated with the repression of the *Hox* genes (Orlando 2003). *Ezh2* and *Eed* are the major PcG genes expressed at this stage of development. However, they are similarly expressed both in the founder germ cells and neighbouring somatic cells (S. Erhardt, unpublished data). Despite this, it is possible that germ cell specific factors recruit these polycomb proteins to the *Hox* gene loci and repress their expression only in germ cells. Indeed, we have identified several new genes specifically associated with the founder germ cells, the function of which should be elucidated in the future.

6. CONCLUSION

Germ cell specification in mice is not a preformistic event but involves epigenesis. It is imposed upon a population of epiblast cells that have the potential to form germ cells as well as somatic cells. Two principal events necessary for the imposition of germ cell fate are the repression of homeobox genes and the continued expression of genes associated with pluripotency. Signalling molecules from neighbouring extraembryonic tissues are required to accomplish these ends. Genes, including members of the *Fragilis* family and *Stella*, are apparently responsive to these signalling cues, and therefore likely candidates for intrinsically regulating germ cell specification. Future studies will be aimed at analysing the roles of these genes, as well as identifying other molecules that are involved in this process. The epigenetic state that germ cells acquire,

in relation to other pluripotent cells, should also be investigated to understand the potential for totipotency maintained in the germ line.

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GLOSSARY

- BMP4, BMP8b: bone morphogenetic protein 4/8b
 E: embryonic day
 EB: early bud
 ES: embryonic stem
 Eed: embryonic ectoderm development
 Ezh2: enhancer of zeste 2
 ICM: inner cell mass
 IFN: interferon
 ISRE: IFN stimutable response element
 LS: late streak
 PcG: polycomb group
 PGC: primordial germ cell