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The early development of germ cells in chicken

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ABSTRACT Primordial germ cells (PGCs) are the founder cells for mature gametes, the vehicles by which individuals transmit genetic and epigenetic information to later generations. Since the 19th century, avian species (chickens in particular) have been widely used for germ cell research. Previous studies have used chicken PGCs for a variety of research applications, including as a model for studies focusing on germline development. Other applications of chicken PGCs, including conservation efforts for avian species and methods of producing transgenic birds, have further reinforced the importance of these cells. However, much remains to be revealed about the origin and role of PGCs during their development in the chicken. Here, we provide a comprehensive review of chicken PGCs, focusing in particular upon their initial profiles and physiological changes during development as regulated by environmental factors and/or intrinsic mechanisms. We also emphasise sex-dependent differences in PGC development after settlement within the gonads, as well as future applications for avian PGCs.

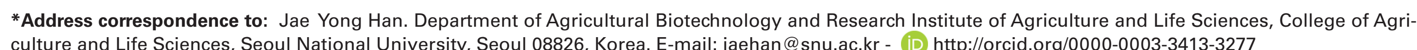
KEY WORDS: *primordial germ cell, origin, migration, specification, chicken*

Introduction

Germ cells are the only cells that can transfer the entirety of an organism's genetic information to the next generation. In many multicellular organisms, mature gametes, including sperm or eggs, originate from primordial germ cells (PGCs). PGCs occupy a small proportion of the early embryo and are clearly distinguishable from somatic cells. In the fields of developmental and evolutionary biology, gaining a comprehensive understanding of the underlying mechanisms for germ cell specification is a major goal. Germ cells are important not only for basic biological research, but also as a means of preserving genetic resources. Thus, how and when germ cells are initially originated and specified is a crucial question. There are two general models of germ cell specification: preformation and induction (Extavour and Akam, 2003). The preformation model involves germ cell determinants, which are unique cytoplasmic organelles generically termed 'germ granules' or 'germ plasm.' These organelles contain maternally inherited mRNAs, proteins, and small RNAs, and play a crucial role in germ cell specification in *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, and *Xenopus laevis* (Strome and Wood, 1983; Hay *et al.*, 1988; Ephrussi *et al.*, 1991; Tada *et al.*, 2012; Strome and Updike, 2015). On the other hand, in the induction model (which applies to taxa including *Homo sapiens* and *Mus musculus*) germ cells arise from proximal-posterior epiblasts, a process induced by bone morphogenetic protein (BMP) signalling from neighbouring extra-embryonic tissue during gastrulation (Lawson *et al.*, 1999; Tang *et al.*, 2016a).

In avian species, PGCs are initially detected as a scattered pattern in the area pellucida, the central region of Eyal-Giladi and Korchav (EGK) stage X embryos (Eyal-Giladi and Kochav, 1976; Ginsburg and Eyal-Giladi, 1987; Tsunekawa *et al.*, 2000). Between EGK stage X and Hamburger and Hamilton (HH) stage 2 (Hamburger and Hamilton, 1951), PGCs are moved passively by the overall movement of embryonic cells; they actively move to the germinal crescent at HH stage 4 (Swift, 1914; Hamburger and Hamilton, 1951; Eyal-Giladi *et al.*, 1981). Subsequently, between HH stages 9 and 12, PGCs move into blood vessels and circulate through the bloodstream (Fujimoto *et al.*, 1976; Ukeshima *et al.*, 1991), finally settling in the genital ridge (Meyer, 1964; Fujimoto *et al.*, 1976). To date, although numerous studies have been performed to elucidate the origin and specification of avian PGCs, they still remain incompletely defined. Here, we review when, where, and how these cells originate and are specified. Furthermore, we explore the current state of knowledge regarding molecular events in the development of avian PGCs and discuss their use in certain practical applications.

Abbreviations used in this paper: EGK, Eyal-Giladi and Korchav (stage); HH, Hamburger and Hamilton stage; PGC, primordial germ cell.



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Early studies in chicken PGCs

Since the first observations of the origin of germ cells in chicken germinal epithelium by Waldeyer in 1870, chickens have been regarded as an ideal model species for germ cell research (Waldeyer, 1870). In 1880, Nussbaum claimed that PGCs are derived from a much earlier stage in embryonic formation (Nussbaum, 1880), and Swift suggested that PGCs arise from the margin of the area pellucida in the early stages of chick development, and referred to the marginal crescent-shaped region as the 'germinal crescent' (Swift, 1914). Swift also discovered that PGCs migrate via the bloodstream to all parts of the embryo and area vasculosa when blood vessels form in the mesoderm layer (Swift, 1914). Owing to the lack of reliable markers for PGCs, the process of specification for avian PGCs remained unclear for several decades. However, early researchers agreed on many points that PGCs arise from the anterior region of the embryo at the margin of the area pellucida from the primitive streak stage to the three-somite stage, then migrate to newly formed blood vessels by amoeboid movement, enter the bloodstream, and travel to the germinal epithelium (Swift, 1914; Firket, 1920; Goldsmith, 1928).

Early studies on avian PGCs focused on characterising their morphological appearance. Swift described PGCs as interspersed with hypoblast cells, and noted that they could be distinguished from neighbouring cells based on their size and the presence of many glycogen granules in the cytoplasm (Swift, 1914). Meyer found that histochemically staining of these large glycogen granules with periodic acid-Schiff (PAS) made it possible to identify PGCs at early embryonic stages (Meyer, 1960; Meyer, 1964). In late 1960s, it was proposed that PGCs originated prior to the formation of the primitive streak, at HH stage 3 and earlier (Clawson and Domm, 1969). By staining PGCs with PAS reagent at HH stages 4 to 7, Clawson and Domm found that 100–250 PGCs were already present at these stages (Clawson and Domm, 1969; Fujimoto *et al.*, 1976). In subsequent studies, Sutasurya *et al.*, found that chicken PGCs exist before the primitive streak formation by at least HH stage 4 (Sutasurya *et al.*, 1983) and Ginsburg and Eyal-Giladi suggested that chicken PGCs already be determined earlier than EGK stage X and that their further differentiation is independent of the embryo-forming process based on PAS staining results (Ginsburg and Eyal-Giladi, 1987).

Origin and specification of chicken PGCs

In 1976, Eyal-Giladi and Kochav proposed that clustered PGCs were found in chicken blastoderm at EGK stage X to HH stage 2 (Eyal-Giladi and Kochav, 1976). Subsequent chick-quail chimera studies showed that PGCs originate in the epiblast layer (Eyal-Giladi *et al.*, 1981), and that only the central region of the blastoderm can give rise to PGCs (Ginsburg and Eyal-Giladi, 1987; Ginsburg *et al.*, 1989). In order to investigate more reliable data for the origin of PGCs *in situ*, Urven *et al.*, applied the Equi merozoite antigen 1 (EMA-1), a monoclonal antibody produced against cell surface of the murine embryonic carcinoma cells, to chicken PGCs (Urven *et al.*, 1988). Like PAS, EMA-1 identified the chicken PGCs from the germinal crescent to the migratory stage and also in sexually differentiated gonad (Urven *et al.*, 1988). Another mouse embryonal carcinoma surface marker, stage-specific embryonic antigen 1 (SSEA-1), also successfully detected the PGCs at EGK stage X (Karagenc *et al.*, 1996). In more recent studies, researchers showed that chicken PGCs were efficiently isolated using SSEA-1 antibody and the isolation method allowed to examine the molecular profiles of chicken PGCs in more detail (Mozdziak *et al.*, 2006; Motono *et al.*, 2008). In particular, the studies using the antibody showed that SSEA-1-positive cells express the genes related to germ cell inductive signals, such as *Blimp1* (also known as *Prdm1*) and *Itgb1* (also known as *CD29*), which are known to be the core genes for PGC development and specification in other species (Ancelin *et al.*, 2006; Kunwar *et al.*, 2006). The results suggest that avian PGCs arise at around EGK stage X in response to inductive signals might be a well-founded conjecture.

Nevertheless, the hypothesis that the germ cells are specified by maternally inherited factors, preformation mode, has been widely accepted. In 2000, chicken vasa homologue (CVH), a reliable germ cell marker, was used to identify chicken PGCs in developmental stages. Immunohistochemical analyses showed that CVH-expressing cells were detectable during early embryogenesis, starting from the first cleavage of fertilised eggs to functional oocyte (Tsunekawa *et al.*, 2000). The VASA protein is an ATP-dependent RNA helicase in the DEAD-box family, and the proteins are highly conserved among distantly related species including *C. elegans*, *D. melanogaster*, *D. rerio*, *X. laevis*, and *M. musculus*. It has known as one of the components of the germ plasm, and plays an essential role in germ cell development in diverse animal species (Hay *et al.*, 1990; Lasko and Ashburner, 1990; Gruidl *et al.*, 1996; Ikenishi and Tanaka, 1997). In the *Drosophila* polar granules, the VASA protein is observed to co-localise with spectrin and mitochondrial clouds, and the protein is also detected with the putative germ plasm of oocytes and cleavage-stage embryos in *Xenopus* (Hay *et al.*, 1988; Kloc and Etkin, 1998). Similar to *Drosophila* and *Xenopus*, VASA protein of chicken is localised in oocytes in granulofibrillar structures surrounding the mitochondrial cloud and spectrin protein-enriched structure, indicating that the CVH-containing structure is the germ plasm in the chicken. Furthermore, CVH was found in cleavage furrows and was restricted to only 6–8 cells in 300-cell-stage embryos (Tsunekawa *et al.*, 2000). Based on these findings, germ cell specification in the chicken may follow the preformation model and be specified by maternal determinants. More recently, Lee *et al.,* identified the expression of deleted in azoospermia-like (*DAZL*), another functional protein expressed in germline cells in embryos, during the intrauterine stages before oviposition (Lee *et al.*, 2016a). DAZL, which is another germline-specific RNA-binding protein, is well studied in vertebrate species for its role in meiotic progression and maintaining germ cell pluripotency (Eberhart *et al.*, 1996; Haston *et al.*, 2009). Using chicken *DAZL* as a germ plasm marker, Lee *et al.,* observed that the germ granule exhibited asymmetric localisation in oocytes, and shifted from a subcellular localisation to a diffuse form during cleavage at the point of zygotic genome activation. Furthermore, aberrant *DAZL* expression affects PGC proliferation, gene expression, and apoptosis *in vitro*, suggesting that it plays a role in germ cell integrity in chickens (Lee *et al.*, 2016a). These results further reinforce the notion that PGCs originate through maternally inherited components, and that PGCs form before the epiblast although further researches are required to

examine the specific functions of germ plasm components and clarify the mechanisms of germ cell specification in avian species.

Epigenetic regulation of chicken PGCs

Epigenetic dynamics are crucial for early embryonic development and PGCs. During embryogenesis in mouse and humans, PGCs undergo unique epigenetic reprogramming processes including genome-wide demethylation, X chromosome inactivation, histone modification, and transposon silencing regardless of somatic cells (Saitou *et al.*, 2012). In *C. elegans*, epigenetic regulations including histone modifications activating germline genes and repressing somatic genes also play a critical role in germ cell developmental programming (Strome and Updike, 2015).

In avian species, the nature of epigenetic reprogramming during germ cell development has not yet been established. However, recent studies showed that the epigenetic signature in chicken PGCs includes DNA methylation, histone modification, and post-transcriptional regulation by small RNAs (Jang *et al.*, 2013; Rengaraj *et al.*, 2014; Kress *et al.*, 2016).Through methylation, mammalian PGCs establish monoallelic expression of imprinting genes, maintain inactivated retrotransposons, inactivate one of the two X chromosomes, and suppress gene expression (Lees-Murdock and Walsh, 2008). In chicken, PGCs contain differentially methylated regions detected in male and female, which also found in the imprinting and X-linked homologous regions in mammals, suggesting that epigenetic mechanisms are evolutionarily conserved between mammals and birds (Jang *et al.*, 2013).

In addition to DNA methylation, post-translational histone modifications also specify the cellular fate of PGCs (Tang *et al.*, 2016a). Moreover, chicken PGCs exhibit trimethylation of histone H3 on lysine 9 (H3K9me3) during heterochromatin formation. Conversely, mice exhibit trimethylation of histone H3 on lysine 27 (H3K27me3) by polycomb proteins. This demonstrates that the avian-specific chromatin constitution differs from the mammalian epigenetic system (Kress *et al.*, 2016).

With respect to post-transcriptional regulation, small RNAs, including microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs), are also required for PGC and germline development in mouse, zebrafish, and fly (Hayashi *et al.*, 2008; Thomson and Lin, 2009). In the chicken, small RNAs are known to have important functions in maintaining germ cell integrity. Lee *et al.,* identified chicken PGC-specific miRNAs using microarray analysis. Especially, miR-181-3p was found to have multiple functions in chicken PGCs, including inhibiting somatic differentiation and preventing meiotic progression of PGCs by suppressing homeobox A1 and nuclear receptor subfamily 6, group A, member 1 (*NR6A1*) expression (Lee *et al.*, 2011). Recently, several germ cell-specific piRNAs, which can be classified as repetitive-element-sequence-derived or protein-coding-gene-derived based on their genomic origins, have been identified in the chicken PGCs. As in other species, piRNAs and piRNA pathway genes are required for the genome integrity of chicken PGCs (Rengaraj *et al.*, 2014). In summary, research on epigenetics in avian germ cells remains in its infancy. Future studies will elucidate the epigenetic functions in avian PGCs using the latest next-generation sequencing technology.

Migration of chicken PGCs

Cell migration plays important roles in a wide variety of biological phenomena. In developmental biology, identifying the mechanisms of cell migration is essential for understanding the formation of different tissues (Ribeiro *et al.*, 2003; Blackburn and Manley, 2004; Mammoto and Ingber, 2010; Herriges and Morrisey, 2014). During gastrulation, an embryo undergoes coordinated cell movement and is reorganised to form the three germ layers (Keller, 2005). In most vertebrates, germ cells are specified in one region of the embryo, then migrate extensively before reaching the genital ridge in the initial phase of development (Richardson and Lehmann, 2010). The phenomenal pathway of germ cells has been studied in various species including the fly, fish, mouse, and chicken (Sasado *et al.*, 2004; Richardson and Lehmann, 2010; Nakamura *et al.*, 2013; Kang *et al.*, 2015; Paksa and Raz, 2015). As one of example, in mice, PGCs are first identifiable as a cluster of cells in the proximal epiblast region. They then migrate to the extra-embryonic ectoderm (ExE) (Lawson and Hage, 1994; Hayashi *et al.*, 2007), and subsequently through the developing endodermal hindgut into the genital ridges (Richardson and Lehmann, 2010).

In chicken, PGCs undergo multiple stages of motility and proliferation. They are initially localised in the central zone of the area pellucida, settling on the expanding hypoblast from the epiblast at EGK stage X, and are then gradually translocated from this region (Ginsburg and Eyal-Giladi, 1986). PGCs first undergo passive movement, regulated by primitive streak formation, from the central zone of the area pellucida to the anterior region; next, they adhere to fibrous bands on basement membranes of the epiblast of the area pellucida (Wakely and England, 1979). Through a transplantation study using DF1 and PGC cell lines, Kang *et al.*, demonstrated that PGCs that passively reach the anterior region, are actively incorporated into the germinal crescent (Kang *et al.*, 2015). However, the process of active migration to the germinal crescent, guided by attractive and repulsive cues, remains poorly understood. Several research groups have shown that some migratory and cultured PGCs produce pseudopodia, suggesting amoeboid movement as a possible means of migration (Fujimoto *et al.*, 1976; Kuwana *et al.*, 1987; Choi *et al.*, 2010). PGCs penetrate into blood vessels by HH stages 9-10, and are most abundant at HH stage 12 (Fujimoto *et al.*, 1976; Lee *et al.*, 1978). Circulating PGCs in the blood stream reach the genital ridge and invade the thickened coelomic epithelium from HH stage 15 to HH stage 18 (Ukeshima *et al.*, 1987; Nakamura *et al.*, 2007). In 1986, Kuwana *et al.,* suggested that the coelomic epithelium may release a chemical cue that attracts PGCs to the gonads (Kuwana *et al.*, 1986). They isolated PGCs from HH stage 13 embryos, then placed the PGCs between the germinal ridge and other embryonic tissues (such as the neural tube, heart, allantois, and liver) *in vitro* and the PGCs moved only towards the germinal ridge, suggesting that they are attracted by specific signals that it emits (Kuwana *et al.*, 1986). Two decades later, Stebler *et al.,* revealed that the main molecules that guide the migration of chicken PGCs are chemokine stromal cell-derived factor 1 (SDF-1/CXCL12) and its receptor, G-protein-coupled receptor 4 (CXCR4), which is expressed by migrating PGCs (Stebler *et al.*, 2004). A more

recent study found that anterior vitelline veins play a key role in directly accumulating migrating PGCs, which reach the genital ridges during circulation (De Melo Bernardo *et al*., 2012). Collectively, Figure 1 represents the migration and development of chicken PGCs in early embryogenesis (Fig. 1).

Development of chicken germ cells in the sex cord

After migration into embryonic gonads and following sex differentiation, PGCs undergo mitotic/meiotic switch in female and undergo mitotic arrest after gonadal sex differentiation in male (Etches, 1995; Howarth, 1995). The decision of germ cells whether to enter meiosis or mitosis depends on signals from the environment surrounding the germ cells, especially retinoic acid (RA) signaling (Bowles *et al*., 2006; Griswold *et al*., 2012). In the mouse, RA is produced in the mesonephric kidney and diffuses into the fetal gonad. In the gonad, RA acts on germ cells and promotes the expression of a gene called stimulated by retinoic acid 8 *(Stra8)*. *Stra8* stimulates meiotic entry for germ cells by regulating the expression of meiotic markers (Bowles *et al*., 2006). In male mouse embryos, the expression of a gene called cytochrome P450 family 26 subfamily b member 1 (*Cyp26b1*), which encodes an enzyme that degrades RA, is upregulated in gonads and prevents *Stra8* expression in male germ cells (Bowles *et al*., 2006). It is also known that fibroblast growth factor 9 (FGF9), which is secreted by foetal testis cells, acts on germ cells and promotes the expression of male germ cell specific genes such as *Nanos2* (Bowles *et al*., 2010). These male germ cell specific genes inhibit meiotic entry and maintain mitotic arrest in spermatogonia.

In the chicken, at E.8 female PGCs in the germinal epithelium are more numerous than in earlier stages (Swift, 1915). This stage is the beginning of the rapid mitotic cell division of PGCs at the time of gonadal sex differentiation in the ovarian cortex (Mendez *et al*., 2005). It has been shown that *STRA8* is the driving factor for the initiation of meiosis in chicken germ cells, as it is in mammalian species (Smith *et al*., 2008). Before the initiation of meiosis, retinaldehyde dehydrogenase 2 (*RALDH2*) and *STRA8* expression begins at E.12.5 and meiotic germ cells can be observed at E15.5 (Smith *et al*., 2008). The expression of *RALDH2* and *STRA8* is restricted to the left gonadal cortex, the site where meiosis occurs (Smith *et al*., 2008). When *RALDH2* or *STRA8* expression is inhibited by short hairpin RNA (shRNA), the number of meiotic germ cells decreases. In contrast, RA treatment increases *STRA8* expression and the number of meiotic germ cells in the embryonic ovary (Yu *et al*., 2013). These results show that RA signalling is a conserved mechanism for initiating meiotic entry across vertebrates. Recently, it was also shown that polycomb repressive complex 1 (PRC1) controls the timing of meiotic entry by epigenetically regulating the expression of *STRA8*, as well as Notch ligands such as jadomycin polyketide synthase cyclase (*JAD1*) and delta-like 1 (*DLL1*), in female chick embryos (Tang *et al*., 2016b). Basic fibroblast growth factor (bFGF) signalling, which is important for PGC proliferation and survival, can suppress meiotic initiation of germ cells independently from RA signalling; furthermore, the expression of bFGF in the ovarian cortex decreases markedly throughout meiosis (Choi *et al*., 2010; He *et al*., 2012). After meiotic initiation, female germ

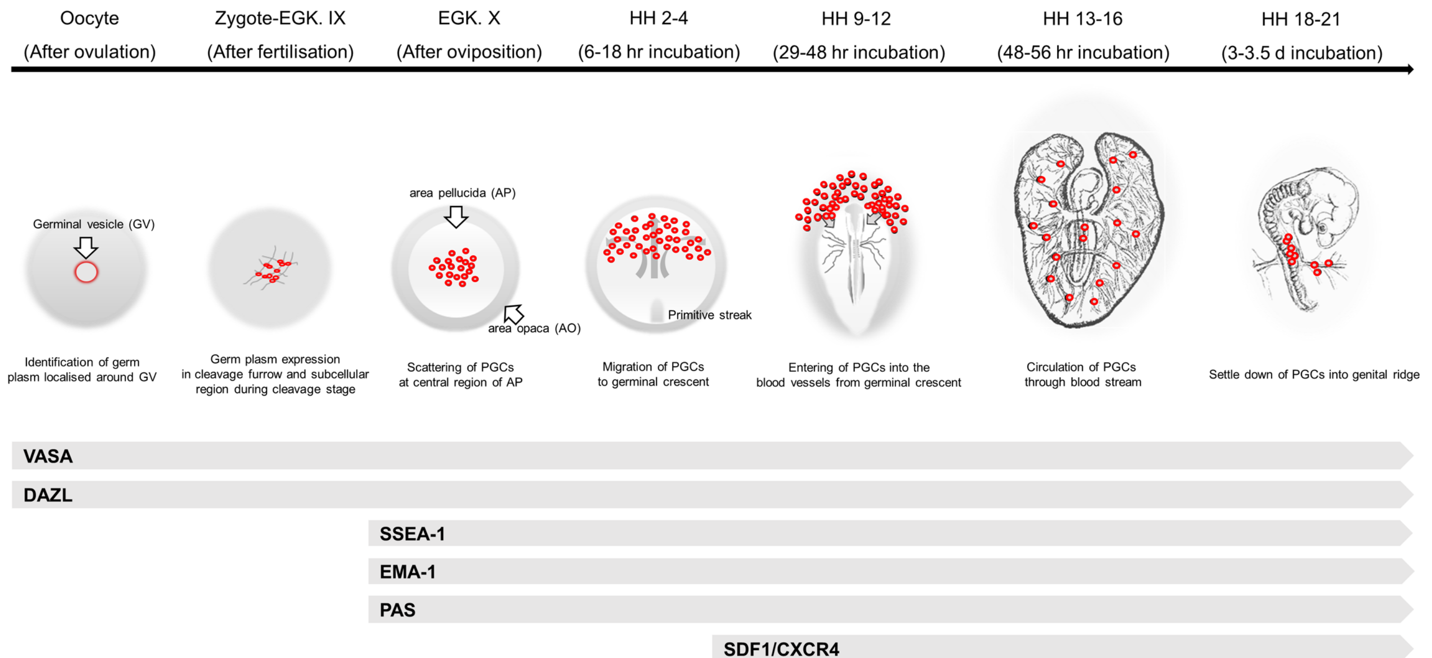

Fig. 1. Schematic representation of primordial germ cell (PGC) development and markers expression. *The germ plasms, such as VASA and DAZL, are maternally inherited and expressed in the germinal vesicle (GV) of chicken oocyte and cleavage furrow and subcellular region following cleavage stages indicating predetermined precursor PGC. At stage X (after oviposition), PGCs that express VASA and DAZL are scattered at the central zone of the area pellucida. PGCs are positive for SSEA-1 and EMA-1 antibodies, and PAS staining at Stage X. Subsequently, they move to the anterior marginal zone, following epiblast and hypoblast layer movement, and finally they are localized into the germinal crescent. And PGCs of the germinal crescent enter into extraembryonic blood vessels then circulate through the bloodstream. Finally, PGCs attracted by SDF1 interact with the chemokine receptor CXCR4 and invade the coelomic epithelium of the genital. This figure is modified from Nieuwkoop and Sutasurya (1979) and Lee et al., (2016a).*

cells undergo meiotic arrest at the diplotene stage before and after hatching (Zheng *et al.*, 2009).

In male chick embryos, PGCs do not proliferate significantly after gonadal sex differentiation and undergo mitotic arrest at E8.0 and remain as pro-spermatogonia at the G1 stage of the cell cycle until hatching (Kirby and Froman, 2000). *CYP26B1* is expressed in the male gonads of chick embryos throughout development, as it is in mammalian species (Smith *et al.*, 2008). However, in female embryos, the level of *CYP26B1* decreases from E12.5 and *STRA8* expression increases (Smith *et al.*, 2008). These results indicate that *CYP26B1* degrades RA in male gonads to prevent meiotic entry of germ cell in chick embryos. Other signalling pathways that act on spermatogonia to prevent meiotic initiation and induce male germ cell-specific genes have not yet been clearly defined in the chicken.

Application of avian PGCs

Since the migratory route for avian PGCs differ from mammalian species (Niewkoop and Sutasurya, 1979), they can be isolated at various stages of embryonic development. This characteristic provides them with a huge advantage in germ cell research. Various methods have been used to isolate PGCs from embryonic tissue or blood vessels, including fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS) using PGC-specific antibodies, and density gradient centrifugation, and size-dependent isolation without using antibodies (Chang *et al.*, 1992; Ono and Machida, 1999; Zhao and Kuwana, 2003; Mozdziak *et al.*, 2005; Jung *et al.*, 2017).

PGC isolation have been attempted not only in chicken, but also in other avian species such as pheasant, quail, turkey, duck and guinea fowl, and have been produced using PGCs for the purpose of restoring endangered birds (Reynaud, 1969; Ono *et al.*, 1996; Kang *et al.*, 2008; Wernery *et al.*, 2010; Liu *et al.*, 2012; van de Lavoir *et al.*, 2012). However, historically the germline transmission rate of donor-derived progeny has been prohibitively low, owing to differences between species in traits such as reproductive cycle. PGC culture is therefore required for endangered bird restoration efforts, but the culture process has not yet been fully optimised.

Among aves, the long-term culture system of chicken PGC has been successfully established (van de Lavoir *et al.*, 2006). It has been shown that bFGF plays an essential role in the proliferation and survival of chicken PGCs via the mitogen-activated protein kinase kinase (MEK) / extracellular signal–regulated kinases (ERK) signaling pathway, and helps to maintain telomerase activity, migratory activity, and germline contribution in PGCs cultured for extended periods (Choi *et al.*, 2010; Macdonald *et al.*, 2010). More recent studies have shown that MEK1, AKT (also known as protein kinase B, PKB), and SMAD family member 3 (SMAD-3) signalling is required for PGCs to maintain germline transmission capacity, and that Wnt/β-catenin signalling is required for PGC proliferation *in vitro* (Whyte *et al.*, 2015; Lee *et al.*, 2016b). Furthermore, it has been reported that stem cell factor 2 (SCF2) derived from chickens has potent and prolonged effects on PGC proliferation via FGF2- and c-KIT-mediated growth signals, and is thus crucial for the maintenance of germ cell characteristics and germ line transmission (Daichi *et al.*, 2016).

Cultured PGCs have been utilised not only for research on germ cell signalling pathways (Choi *et al.*, 2010; Whyte *et al.*, 2015; Lee *et al.*, 2016b), but also for transgenic studies (van de Lavoir *et al.*, 2006). Transgenic poultry has the potential to be used for the production of pharmaceutical and industrial proteins in eggs (Ivarie, 2003; Sang, 2004; Han, 2009) and several successfully generated transgenic chickens as a bioreactor have been reported indeed (Zhu *et al.*, 2005; Lillico *et al.*, 2007; Cao *et al.*, 2015). Recently, genome-editing tools such as transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (CRISPR/Cas) have been adapted as promising methods for developing avian model (Park *et al.*, 2014; Dimitrov *et al.*, 2016; Oishi *et al.*, 2016; Taylor *et al.*, 2017). Applying such germline modification techniques combined with PGC culture to a variety of valuable avian species will permit expansion of this research area and have considerable benefits for the poultry industry.

Conclusion

For over 100 years, many researchers have sought to understand the origin and development of germ cells, the only cells that contain the entirety of an individual's genomic information. With struggling of researchers in avian biology, we could know basic knowledge on avian germ cells including its origin, specification, migration and differentiation. However, large portion of avian germ cell biology including germ cell origin, specification and the regulatory mechanisms for germ cell development (including epigenetic factors) are still unknown. New, cutting-edge technologies for isolation, germ cell culture system, and genome-editing will likely permit further insight into the origin and developmental fate of germ cells.

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