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Review

Metabolic pathways regulating the development and non-genomic heritable traits of germ cells

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Abstract. Metabolism is an important cellular process necessary not only for producing energy and building blocks for cells, but also for regulating various cell functions, including intracellular signaling, epigenomic effects, and transcription. The regulatory roles of metabolism have been extensively studied in somatic cells, including stem cells and cancer cells, but data regarding germ cells are limited. Because germ cells produce individuals of subsequent generations, understanding the role of metabolism and its regulatory functions in germ cells is important. Although limited information concerning the specific role of metabolism in germ cells is available, recent advances in related research have revealed specific metabolic states of undifferentiated germ cells in embryos as well as in germ cells undergoing oogenesis and spermatogenesis. Studies have also elucidated the functions of some metabolic pathways associated with germ cell development and the non-genomic heritable machinery of germ cells. In this review, we summarized all the available knowledge on the characteristic metabolic pathways in germ cells, focusing on their regulatory functions, while discussing the issues that need to be addressed to enhance the understanding of germ cell metabolism.
Key words: Epigenome, Germ cells, Metabolism, Oogenesis, Spermatogenesis

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Introduction

Germ cell development, from the specification of primordial germ cells (PGCs) in early embryos to gametogenesis in the testis and ovary, is a complex process involving a number of molecules and regulatory networks (Fig. 1). In addition, the critical role of germ cells in mediating the passage of healthy parental traits to offspring is regulated by precisely controlled cellular mechanisms. Considerable research in recent decades has focused on the control of germ cell development and the role of epigenetic regulation (i.e., regulation of genome functions by DNA methylation and histone modifications) in germ cell function. These studies have demonstrated that the epigenetic status of germ cells undergoes unique changes, such as DNA demethylation, in PGCs and fertilized eggs [1–5]. These changes are involved in epigenetic reprogramming, which is crucial for enabling the germ cells to acquire normal developmental potential. For instance, demethylation and subsequent allele-specific re-methylation of imprinted genes in fetal germ cells are essential for development after fertilization [1]. The importance of epigenetic regulation of specific genes in germ cell specification and their subsequent development has also been demonstrated [6–8]. In addition, epidemiological and animal model experiments have suggested that abnormal epigenetic changes in germ cells can explain the mechanisms underlying certain physiological changes in offspring induced by aberrant ancestral environmental conditions, such as excess or insufficient nutrition, aging, and exposure to chemical compounds [9–11].

Metabolism plays a crucial role in the maintenance of cellular homeostasis via the production of energy and cellular building blocks, such as nucleic acids, amino acids, and lipids. In addition to these fundamental roles, some products of metabolism control the activity of cellular components, including epigenetic factors [12], and also function as signaling molecules [13]. Several studies have revealed that epigenetic regulation of cellular status is closely associated with metabolism in cancer cells and various types of stem cells. For instance, α-ketoglutarate (α-KG), an intermediate metabolite in the tricarboxylic acid (TCA) cycle in mitochondria, activates histone demethylation enzymes and DNA demethylases, known as ten-eleven translocation methylcytosine dioxygenases (TETs), while succinate exerts opposing effects [12] (Fig. 2). Reactive oxygen species (ROS) produced as byproducts in some metabolic pathways, including the electron transport chain (ETC) in mitochondria, are also involved in the activation of cellular components, such as transcription factors [14, 15]. However, detailed information concerning the metabolic status and roles of specific metabolic pathways and metabolites in germ cells remains limited. Metabolic changes are likely to affect the epigenetic status of germ cells, which may subsequently affect the development and heritable epigenetic signatures in germ cells [16, 17]. In addition, whole-body environmental exposure can affect the metabolic status of germ cells, leading to further changes in the function of crucial molecules in these cells. In this review, we focus primarily on metabolism, as it relates to the regulatory functions of germ cells from specification in early embryos to oogenesis and spermatogenesis.

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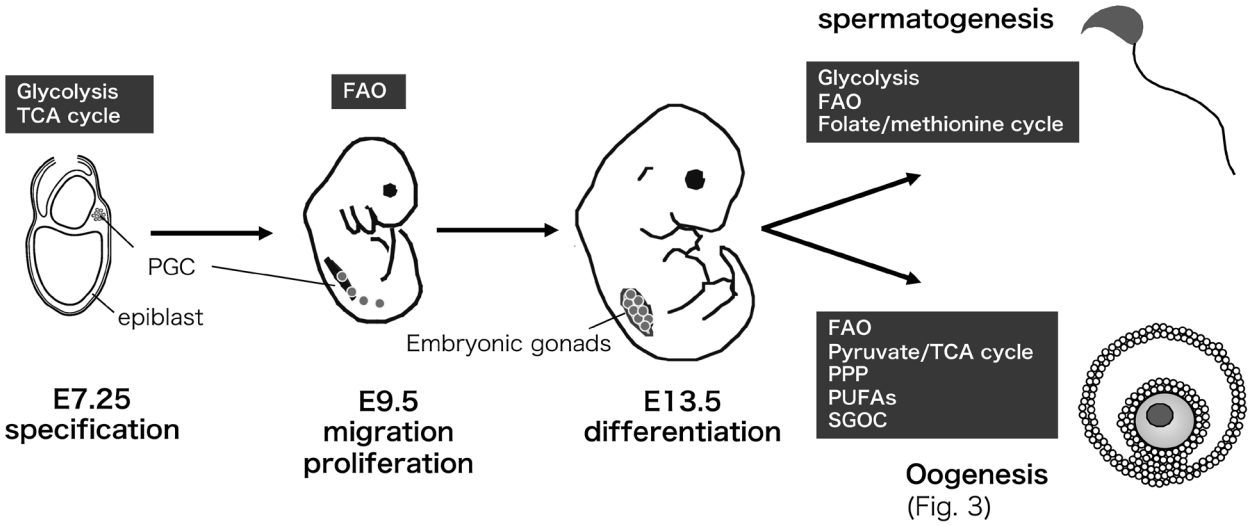

Fig. 1. Germ cell development and metabolic pathways with regulatory functions in mice. TCA, tricarboxylic acid; FAO, fatty acid oxidation; PPP, pentose phosphate pathway; PUFAs, polyunsaturated fatty acids; SGOC, serine-glycine-one carbon pathway.

Regulation of PGC Specification and Subsequent Development by Glycolysis and Mitochondrial Metabolism-related Pathways

In early post-implantation mouse embryos, PGCs are derived from cells in the proximal region of the epiblast, which consists of pluripotent stem cells. A small cluster of PGCs first emerge in the extra-embryonic mesoderm at the posterior end of the primitive streak on embryonic day 7.25 (E7.25) [18, 19] (Fig. 1). After specification, PGCs proliferate and migrate through the hindgut endoderm by E7.75, the mesentery by E9.5, and finally colonize the genital ridges, future ovary or testis, by E10.5 [19]. PGC specification can be reproduced in culture [20], in which embryonic stem cells (ESCs) are first induced to differentiate into epiblast-like cells (EpiLCs), which are further induced to differentiate into PGC-like cells (PGCLCs) by specific cytokines.

The activity in both glycolysis and oxidative phosphorylation (OXPHOS) in PGCLCs, which are equivalent to E9.5 PGCs, is as high as that in ESCs [21, 22], suggesting the importance of these metabolic pathways in PGC specification and early development. Inhibition of glycolysis or OXPHOS in culture consistently results in impaired PGCLC specification in EpiLCs. In the case of OXPHOS inhibition, cell viability was likely diminished due to depletion of the energy supply. Inhibition of glycolysis by 2-deoxyglucose (2DG), by comparison, does not appear to significantly affect the overall cell growth, but gene expression during PGC specification is compromised. For instance, expression of both the PGC marker, B-lymphocyte-induced maturation protein 1 (*Blimp1*), and the mesoderm marker, homeobox B1 (*Hoxb1*), were shown to be downregulated by 2DG [21]. Because metabolic pathways, such as the pentose-phosphate pathway (PPP) for nucleotide synthesis, hexosamine biosynthetic pathway (HBP) for *O*-linked β-N-acetylglucosamine modification of proteins, and Ser-Gly one carbon (SGOC) pathway involved in the methylation of DNA and histones, are branched from the glycolytic pathway (Fig. 2), the downstream machinery of some of these pathways likely plays a role in PGC specification.

With regard to mitochondrial metabolism, the addition of α-KG enhances PGCLC specification from EpiLCs in culture, while preventing EpiLCs from establishing high histone H3 lysine (K) 9 dimethylation (me2) and low H3K27 trimethylation (me3) levels, thus compromising the developmental competence of EpiLCs for PGC specification [23]. As α-KG is necessary for the enzymatic activity of histone demethylases [12] (Fig. 2), excess α-KG in EpiLCs may cause abnormally low H3K9me2 levels, resulting in increased H3K27me levels. Global changes in epigenetic modifications, including decreased H3K9me2 and increased H3K27me3 [24–26] levels, occur during PGC specification from epiblasts, suggesting that the establishment of specific histone modifications in epiblasts and the subsequent changes are important for PGC specification. Inhibition of glutaminolysis, which produces α-KG from glutamine (Fig. 2), represses PGCLC specification and can be rescued by the addition of α-KG [27]. These results further confirm the importance of α-KG in PGC specification.

Mitochondrial metabolism-related pathways also play a role in the subsequent developmental stages of fetal mouse germ cells. Metabolic flux analyses revealed that OXPHOS activity is higher in PGCs at E11.5 than the PGCLCs corresponding to PGCs at E9.5 [21], which gradually decreases by E18.5, but OXPHOS activity in germ cells remains significantly higher than that in gonadal somatic cells at E18.5 [28]. The evolutionary conservation of active mitochondrial metabolism in PGCs is supported by the observation that the mitochondrial copy number in a cell gradually increases from the migrating stage of human PGCs in embryos to the primordial follicle stage in adults [29]. In OXPHOS, carbohydrate as well as other substrates, such as fatty acids and amino acids, are utilized, and mitochondrial fuel flux analyses revealed that OXPHOS in female germ cells at E13.5 exhibits greater dependence on fatty acids and glucose than glutamine [30]. Treatment with etomoxir, an inhibitor of carnitine palmitoyltransferase 1 (CPT1), the key rate-limiting enzyme in fatty acid oxidation (FAO) (Figs. 2 and 4A), consistently results in a significant decrease in the number of PGCs and number of cells in the S-phase of the cell cycle in cultured E11.5 ovaries, indicating its importance in the proliferation of female PGCs [31] (Fig. 1). Such inhibition also results in increased expression levels of Ca^{2+}/CamKII/5'-adenosine monophosphate–activated protein kinase (AMPK), phosphorylated AMPK, phosphorylated p53, and cyclin-dependent kinase inhibitor 1 (p21) in PGCs. These results suggest that activation of the p53 pathway by this FAO inhibitor

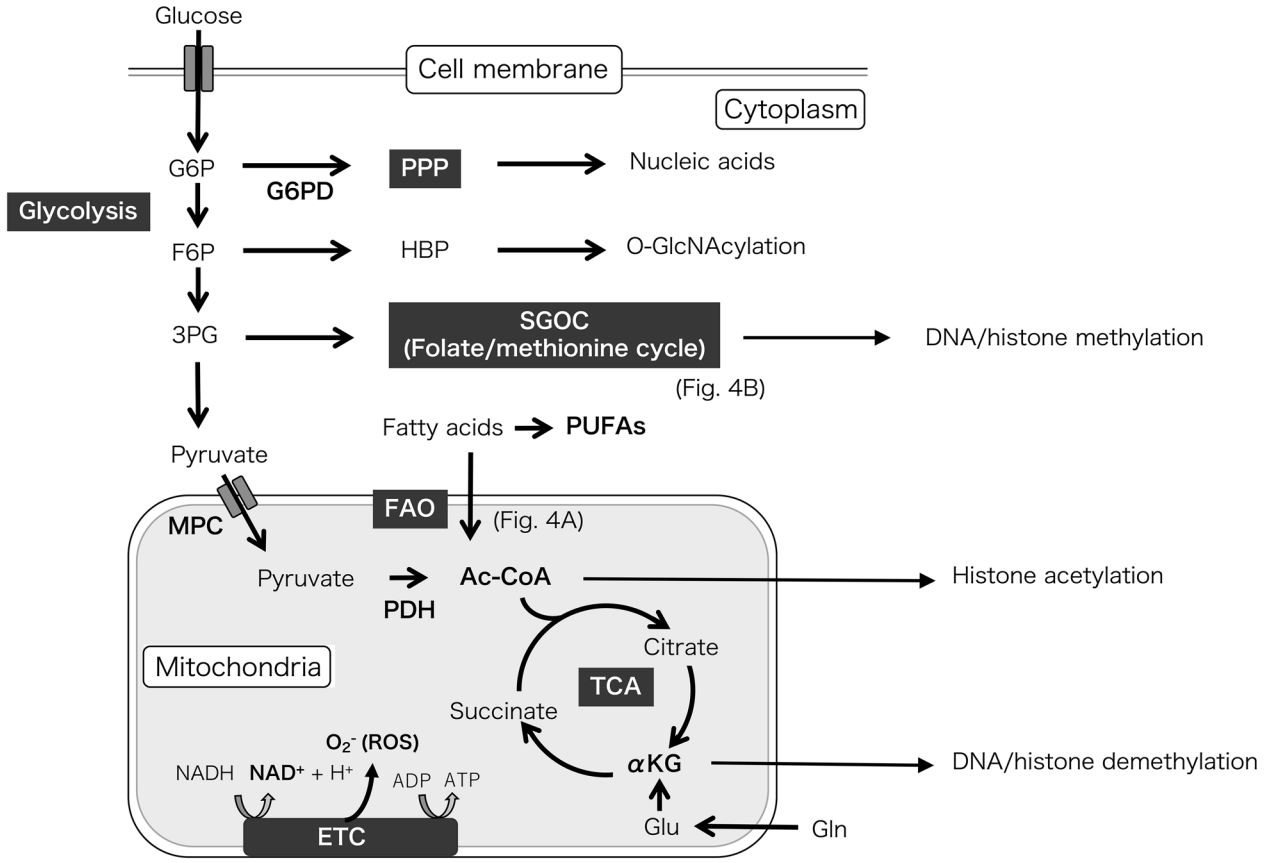

Fig. 2. Energy metabolism-related pathways with regulatory functions in germ cells. Metabolic pathways and metabolic enzymes/metabolites are highlighted by outlined characters with a gray background and bold characters, respectively. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; 3PG, 3-phosphoglycerate; FAO, fatty acid oxidation; PUFAs, polyunsaturated fatty acids; TCA, tricarboxylic acid; Ac-CoA, acetyl CoA; αKG, α-ketoglutarate; NAD(H), nicotinamide adenine dinucleotide; ROS, reactive oxygen species; G6PD, glucose-6-phosphate dehydrogenase; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; PPP, pentose phosphate pathway; HBP, hexosamine biosynthetic pathway; SGOC, serine-glycine-one carbon pathway; ETC, electron transport chain.

diminishes the proliferation of PGCs [31], although the mechanism of AMPK upregulation associated with FAO inhibition remains unclear.

In fetal germ cells, metabolic pathways for nucleotide and amino acid synthesis, including the PPP and SGOC pathways (Fig. 2), are enhanced at E13.5 [21,30], which may contribute to the hyper-transcription and hyper-translation observed in fetal germ cells [32, 33]. Hyper-transcription and hyper-translation in fetal germ cells are controlled by myelocytomatosis oncogenes, *Mycn* and *Mycl*, as well as positive transcription elongation factor b (P-TEFb), a kinase complex that promotes transcriptional elongation via phosphorylation of serine 2 in the C-terminal domain of RNA polymerase II. However, the physiological significance of hypertranscription/translation in fetal germ cells remains unclear.

ROS are generated in metabolic pathways, such as the ETC pathway in mitochondria (Fig. 2), and the cellular amount of ROS is controlled by antioxidant enzymes and molecules, such as superoxide dismutase (SOD) and glutathione. ROS damage cells by oxidizing cellular substances, but they also function as signaling molecules. For instance, ROS activate transcription factors, such as NF-E2–related factor 2 (NRF2), which regulates the expression of genes involved in anti-oxidization [15]. In *Drosophila* embryos, ROS can be detected in early PGCs during their formation, and ROS levels gradually increase during the migration of these cells into the gonads. In addition, the ROS scavenger, encoded by the *Sod1* gene, is expressed in PGCs. Knockdown of *Sod1* in PGCs results in defective PGC migration toward the embryonic gonads, with many PGCs localizing ectopically in embryos [34]. However, the mechanism by which ROS levels control PGC migration is unknown.

Roles of Mitochondrial and Lipid Metabolism, PPP, and the SGOC Pathway in Oogenesis

After colonizing the genital ridges by E10.5, female mouse germ cells enter meiosis at E13.5, with the simultaneous formation of germ cell cysts from E10.5 to E14.5 [35] (Fig. 1). Germ cell cysts then undergo cyst breakdown (CBD), during which one oocyte in each cyst survives and the others undergo programmed death after E17.5. After CBD, surviving oocytes are surrounded by a layer of pre-granulosa cells to form primordial follicles (Fig. 3). At birth, meiosis of oocytes is temporarily arrested at the diplotene stage of prophase during meiosis I. Some of these follicles develop into primary follicles with a layer of granulosa cells, according to the ovarian cycle after puberty. The primary follicles then develop into secondary follicles, with two layers of granulosa cells, which later develop into mature follicles. After oocytes reach the fully grown germinal vesicle (GV) stage, maturation-inducing hormone stimulates the resumption of meiosis, namely GV breakdown (GVBD). Oocytes proceed through the first meiotic division, start meiosis II, and are

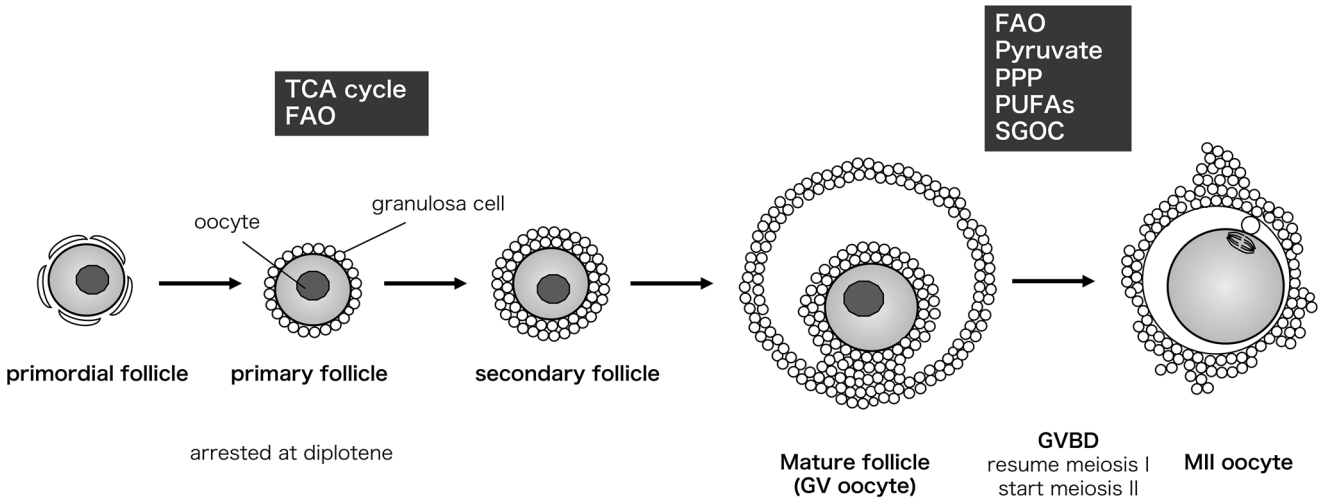

Fig. 3. Oogenesis and metabolic pathways with regulatory functions in mice. TCA, tricarboxylic acid; FAO, fatty acid oxidation; PPP, pentose phosphate pathway; PUFAs, polyunsaturated fatty acids; SGOC, serine-glycine-one carbon pathway; GVBD, germinal vesicle breakdown.

arrested again at metaphase in meiosis II (MII) until fertilization [36] (Fig. 3).

As mentioned previously, active mitochondrial metabolism depends on glucose and fatty acids at E13.5 in fetal female germ cells, and proteomic analyses showed abundant TCA cycle-related proteins in germ cells compared with gonadal somatic cells at E13.5 and E18.5 [30]. Mitochondrial copy number is increased during oogenesis [37], and pyruvate and oxygen consumption gradually increase from the primary oocyte stage to the ovulation stage [38]. Consistent with these observations, the functional importance of FAO and the TCA cycle in oogenesis in pre- and perinatal mouse ovaries has been shown [28] (Figs. 1 and 3). Inhibition of FAO at E12.5 via etomoxir in organ cultures of fetal mouse ovaries was shown to significantly decrease the proportion of secondary follicles. In addition, inhibition of pyruvate uptake by mitochondria via the mitochondrial pyruvate carrier (MPC) inhibitor UK5099 results in the repression of early folliculogenesis up to the secondary follicle stage without affecting ATP production, oocyte survival, or meiosis. At the same time, the expression of the transforming growth factor β–related genes *Gdf9* and *Bmp15*, which are crucial for folliculogenesis via the stimulation of granulosa cell development, is downregulated by UK5099, and addition of recombinant GDF9 (but not BMP15) rescued folliculogenesis. These observations suggest that insufficient *Gdf9* expression in oocytes due to UK5099 exposure results in early follicular dysgenesis. Moreover, α-KG or succinate rescued the effects of UK5099, suggesting that regulation of DNA or histone demethylating enzyme activity by these metabolites is involved in early folliculogenesis via regulation of *Gdf9* expression. The role of MPC2 *in vivo* was confirmed in germ cell-specific *Mpc2* knockout mice, in which folliculogenesis in neonatal ovaries was affected similarly to UK5099-treated cultured ovaries [28].

In oocytes, meiotic maturation is controlled by multiple metabolic pathways, including FAO and pyruvate metabolism (Fig. 3). Meiotic resumption in cumulus cell-enclosed or denuded mouse oocytes stimulated by 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), an AMPK activator, can be blocked by malonyl CoA [39], which is a negative regulator of CPT1 in FAO [40] (Fig. 4A), whereas C75, an activator of CPT1, enhances meiotic maturation [39]. These results indicate that FAO positively regulates meiotic maturation, and AMPK functions as an upstream positive regulator of FAO by blocking malonyl-CoA production. In addition, inhibition of FAO by etomoxir or FAO activation by L-carnitine during oocyte maturation impedes and enhances the subsequent development of cleavage-stage embryos, suggesting that FAO is also important in the establishment of developmental competence during oocyte maturation [41]. With regard to pyruvate metabolism, in mice with oocyte-specific knockout of *Pdha1*, which encodes pyruvate dehydrogenase E1α, the enzyme that catalyzes the conversion of pyruvate to acetyl-CoA (Fig. 2), oocytes were found to be normally ovulated and fertilized but did not complete the second meiotic division and exhibited abnormal meiotic spindles. The reduced ATP level in knockout oocytes compared with that in wild-type oocytes suggests that energy generated by mitochondrial metabolism is important for meiotic maturation [42].

Another study demonstrated that mouse oocyte maturation is accompanied by characteristic changes in particular metabolic pathways [43]. During oocyte maturation, PPP activity is enhanced, and knockdown (KD) of the rate-limiting enzyme, glucose-6-phosphate dehydrogenase (G6PD), in GV oocytes decreased the rate of meiotic maturation of oocytes and caused developmental arrest after fertilization [43] (Fig. 2). G6PD KD also results in excessive ROS production, suggesting a tuning role for G6PD as a means of obtaining the appropriate redox balance necessary for oocyte maturation. Increased ROS production following G6PD KD may be caused, at least in part, by a reduction in PPP production of nicotinamide adenine dinucleotide phosphate (NADPH), which is involved in the generation of reduced glutathione. The same research group reported the importance of decreased levels of polyunsaturated fatty acids (PUFAs) as lipid metabolites for the resumption of meiosis [43] (Figs. 2 and 3). In cultured mouse GV oocytes, the PUFA arachidonic acid (ARA) has been shown to cause meiotic defects, including disruption of meiosis I division, spindle disorganization, and chromosome misalignment in metaphase I oocytes. ARA represses the accumulation of NFKB-activating protein (NKAP), which regulates the meiotic apparatus and may cause meiotic defects. They also showed that in GV oocytes, KD of serine hydroxymethyltransferase 2 (SHMT2), which catalyzes the conversion of serine to glycine in the SGOC pathway (Fig. 4B), does not significantly affect meiotic maturation, but SHMT2 KD compromises oocyte development to the 2-cell stage [43]. In

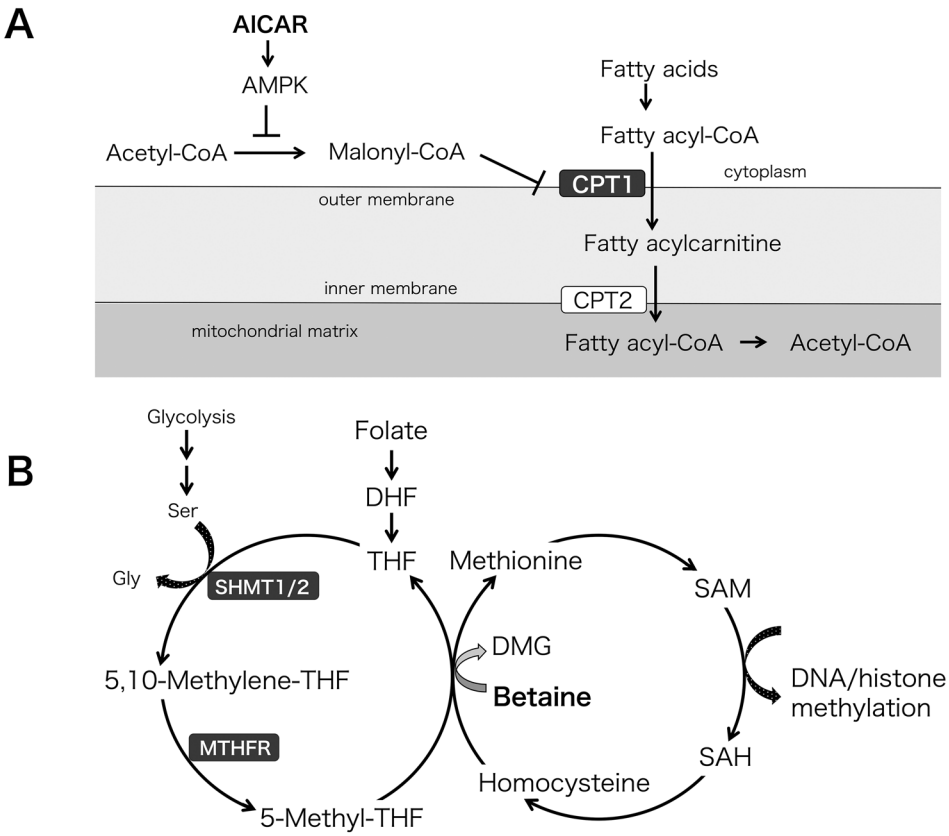

Fig. 4. Detailed schema of fatty acid oxidation (A) and folate/methionine cycle (B). Metabolic enzymes and molecules with regulatory functions in germ cells are highlighted by outlined characters with a gray background and bold characters, respectively. AMPK, AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside; CPT, carnitine palmitoyltransferase; DHF, dihydrofolate; THF, tetrahydrofolate; DMG, dimethylglycine; SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine; SHMT, serine hydroxymethyltransferase; MTHFR, methylene tetrahydrofolate reductase; SGOC, serine-glycine-one carbon pathway.

addition, levels of S-adenosyl methionine (SAM) in the methionine cycle, as well as DNA and histone methylation, decreased following *Shmt2* KD (Fig. 4B). Because SAM is necessary for methylation as a methyl group donor, the observation that repression of the SGOC pathway by *Shmt2* KD leads to diminished DNA and histone methylation is reasonable. Detailed analysis of DNA methylation in MII oocytes revealed significant hypomethylation of repetitive elements, including low copy repeats (LCRs), transposons, and short interspersed elements (SINEs), following *Shmt2* KD [43]. These results suggest that the SGOC pathway plays a crucial role in establishing the proper epigenetic status in oocytes, which is essential for early embryogenesis via control of genomic behaviors, including repression of transposable elements by DNA hypermethylation.

FAO, Glycolysis, and the Folate Cycle as Critical Metabolic Pathways in the Regulation of Spermatogenesis

In the embryonic testis, gonocytes enter mitotic arrest at E14.5. They resume proliferation at postnatal day 5 (P5) and develop into spermatogonial stem cells (SSCs) or differentiating spermatogonia, followed by meiotic entry [44]. In fetal male germ cells, FAO plays a role in differentiation (Figs. 1 and 2). Administration of etomoxir to pregnant mice between E13.5 and E15.5 resulted in failure to maintain mitotic arrest of germ cells and reduced H3K27 acetylation (ac) in E15.5 testis compared with that in control testis [45]. FAO inhibition also causes impaired homing of spermatogonia to the basement membrane of testicular tubules and apoptosis of abnormally localized spermatogonia at P7. Because FAO produces acetyl-CoA for the TCA cycle (Fig. 2), FAO inhibition could result in deceased citrate and subsequent nuclear acetyl CoA, which may cause reduced histone acetylation and subsequent downregulation of genes essential for mitotic arrest of gonocytes in embryos and homing of spermatogonia after birth.

The maintenance of SSCs is essential for continuous spermatogenesis in the testes. Spermatogonia utilize glucose supplied from the blood as an energy source. The MYC family of transcription factors (MYC/MYCN) plays an important role in SSC self-renewal proliferation [46]. Knock-out of MYC/MYCN expression in germline stem cells (GSCs) results in not only reduced proliferation but also decreased glycolytic activity, and inhibition of glycolysis also leads to repressed proliferation. Because MYC activates the transcription of a number of glycolysis-related genes by binding to their regulatory regions [47], these results suggest that MYC/MYCN directly enhances glycolysis in GSCs and subsequently their self-renewal. In cancer cells, ATP is produced predominantly via glycolysis rather than OXPHOS, even under aerobic conditions, a phenomenon known as the Warburg effect [48]. As in cancer cells, active glycolysis may contribute to the production of cellular building blocks in GSCs, such as nucleic acids and amino acids, via metabolic pathways that branch off from glycolysis, including the PPP and SGOC pathways (Fig. 2). In addition, the pathways downstream of glycolysis may

have regulatory functions, such as the epigenetic regulation in GSCs.

Deficiency of methylene tetrahydrofolate reductase (MTHFR), an enzyme of the folate cycle in the SGOC pathway (Fig. 4B), results in impaired spermatogenesis [49]. MTHFR converts 5,10-methylene tetrahydrofolate (THF) to 5-methyl THF, which is a methyl donor for the methylation of homocysteine to methionine. *Mthfr* homozygous mutations significantly impact the survival of mice, and surviving mice exhibit defective spermatogenesis, with 80–90% of testicular tubules devoid of germ cells at 3 months of age. Betaine is a substrate of betaine-homocysteine methyltransferase and also functions as a methyl donor for the methylation of homocysteine (Fig. 4B). Providing betaine-supplemented water to *Mthfr* homozygous mice from the embryonic stage to 3 months of age results in recovery of spermatogenesis and sperm number and a marked increase in fertility [49]. These results highlight the importance of methionine production in spermatogenesis and consistently suggest the involvement of DNA hypomethylation in spermatogenic failure [50]. *Mthfr* deficiency causes DNA hypomethylation in young retrotransposons, such as LINE-1 in the sperm, suggesting that hypomethylation-associated abnormal expression of LINE-1 could result in DNA damage and consequent spermatogenesis defects [50], as in *Shmt2* KD oocytes described in the previous section [43]. In addition, clinical evidence suggests that a functional homozygous polymorphism of *MTHFR* (677C→T) in humans causes reduced male fertility [51], suggesting an evolutionarily conserved role for MTHFR in spermatogenesis. In addition to DNA methylation, changes in histone methylation, including acquisition of H3K27me3 during differentiation of spermatogonia to pachytene spermatocytes [52] further suggests that a sufficient supply of methionine via the folate cycle is necessary for establishing the proper epigenetic status required for spermatogenesis.

Metabolic Control of Heritable Traits in Germ Cells

In germ cells, metabolism plays crucial roles not only in their development, but also in the inheritance of parental traits by the offspring. During *Drosophila* oogenesis, ETC activity is maintained by insulin signaling, but mitochondrial respiration is repressed in the final stage of oogenesis by the loss of insulin signaling, and oocytes then enter cellular quiescence, namely mitochondrial respiratory quiescence (MRQ) [53]. If MRQ is prematurely induced in oocytes by artificially suppressing insulin signaling, oocyte NAD^+ levels decrease, probably due to repression of OXPHOS (Fig. 2) [54]. In the progeny of insulin signaling–inhibited oocytes, levels of the methyl group donor SAM are concomitantly reduced, intestinal carbohydrate metabolism- and lipid metabolism-related gene expression is upregulated, and H3K27me3 of some of these genes decreases. Simultaneously, carbohydrate and lipid metabolic activity is enhanced in the progeny, and these abnormalities can be rescued via a maternal diet supplemented with substrates for NAD^+ biosynthesis, such as nicotinamide mononucleotide. This study suggests that the reduced level of NAD^+ in oocytes could be a heritable factor that affects metabolism in the progeny [54]. Details regarding the mechanistic linkage between decreased NAD^+, SAM, and H3K27me3 and upregulation of intestinal metabolic genes are currently unclear, although decreased SAM could be involved in reduced H3K27me3 in the progeny. The progeny of insulin-signaling-inhibited oocytes exhibit enhanced survival to adulthood but a short life span under poor nutritional conditions, suggesting that aberrant oocyte mitochondrial metabolism positively affects the development of offspring with nutritional disadvantages at the expense of lifespan [54].

Carbohydrate metabolism and the folate cycle are also involved in the passage of heritable traits in spermatogenic cells. In *Drosophila*, paternal intervention with a sugary diet results in an obesity-like phenotype in F1 offspring, accompanied by epigenetic de-silencing of metabolic genes normally enriched in H3K9me3 and H3K27me3 in sperm and offspring embryos, and the expression of these genes is upregulated in offspring embryos [55]. Although the mechanistic link between a sugary diet and epigenetic changes has not been elucidated, metabolites in energy metabolism, including αKG and succinate, may play a role (Fig. 2).

Folate deficiency (FD) induced by low dietary folate intake throughout life affects the phenotype of offspring mice [56]. In one study, female mice were fed an FD diet before breeding with normal males as well as from pregnancy through lactation, and male pups (F1) were also fed an FD diet. F2 offspring of F1 males mated with normal females exhibited increased embryonic lethality and birth defects, including craniofacial and musculoskeletal malformations. In addition, in sperm of the F1 males, DNA methylation of genes involved in development and chronic diseases was either hyper- or hypomethylated, and H3K4me1 and H3K9me1 were decreased compared with the sperm of control males [56]. The results of this study suggest that parental FD affects offspring development via epigenetic changes in sperm. Furthermore, another study showed that postnatal paternal diet–induced FD in mice results in altered H3K4me3 of developmental genes in the sperm, which is retained in F1 embryos, and the expression of some of these genes is deregulated [57]. In addition, overexpression of Lysine demethylase 1A (KDM1A), a histone H3K4 demethylase, in spermatogenic cells was shown to exacerbate changes in H3K4me3 in sperm and developmental defects in offspring associated with paternal FD [57]. Collectively, these results indicate that proper folate cycle activity leading to an adequate methionine supply (Fig. 4B) in male germ cells is necessary to establish the normal epigenetic profiles required for normal development of the offspring as well as for spermatogenesis, as discussed in the previous section [49–51].

Concerning maternal environmental effects on offspring phenotype in mammals, caloric undernutrition or high-fat diet in pregnant mice has been shown to cause glucose intolerance and/or insulin insensitivity in descendants [58, 59]. Maternal nutritional conditions likely affect metabolism in fetal germ cells, which may cause abnormal epigenetic modifications in crucial genes. However, it is unclear how the maternal environment affects metabolism in the fetus as well as germ cells therein. Maternal nutritional substances may directly affect the fetal and/or fetal germ cells. Alternatively, it is also likely that maternal environmental substances initially affect maternal tissues, including the placenta, or be metabolized in those tissues; newly generated diffusible substances in maternal tissues can then pass through the placenta and reach the fetus. Although experimental evidence demonstrating a mechanistic connection between the maternal environment and fetal physiology is lacking, a recent study concerning the effect of maternal exercise on fetal metabolism via the placenta provides an interesting example. The study indicated that maternal exercise induces the expression of *Sod3* in mouse placenta, and SOD3 secreted from the placenta into the fetal blood induces the expression of glucose metabolism genes via 5'-AMP–activated protein kinase (MAPK)/TET signaling in the fetal liver [60]. The results showed that the placenta mediates the effects of the maternal environment on the fetus. and suggest that other maternal environmental factors, including nutritional conditions, also affect the fetus via the placenta and other maternal tissues.

Future Prospects

Studies have suggested the importance of certain metabolic pathways and metabolites in germ cell development, but research demonstrating the regulatory role of metabolism in germ cells remains limited. Comprehensive functional analyses of metabolic enzymes and metabolites may reveal the crucial metabolic steps in germ cells, which could in turn pave the way for identifying novel regulatory networks in these cells. In addition, it is necessary to characterize the molecular pathways downstream of crucial metabolic pathways as well as the upstream cues affecting the metabolic status of germ cells. Determining how metabolism in germ cells affects the inheritance of parental traits by offspring is also important, as specific metabolic pathways could be linked to the extracellular environment and epigenetic regulation in germ cells, and function as non-genomic heritable machinery. However, only a few metabolites have been suggested to play a role in the inheritance of parental traits. Studies concerning the effects of germ cell-specific metabolic enzyme deficiencies in parental germ cells on the offspring may provide important clues that will facilitate a better understanding of the missing links between germ cell metabolism and offspring phenotypes. It is also important to demonstrate the mechanistic linkage between the environmental conditions of the parents and the metabolic and epigenetic status of the parental and fetal germ cells. This area is particularly attractive because the expected results might clarify the causal relationship between parental environmental factors and changes in offspring health as well as the possible mechanisms underlying the inheritance of environmentally influenced acquired traits by offspring.

Conflict of interests: The author declares that there are no conflicts of interest.

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