論文の内容の要旨

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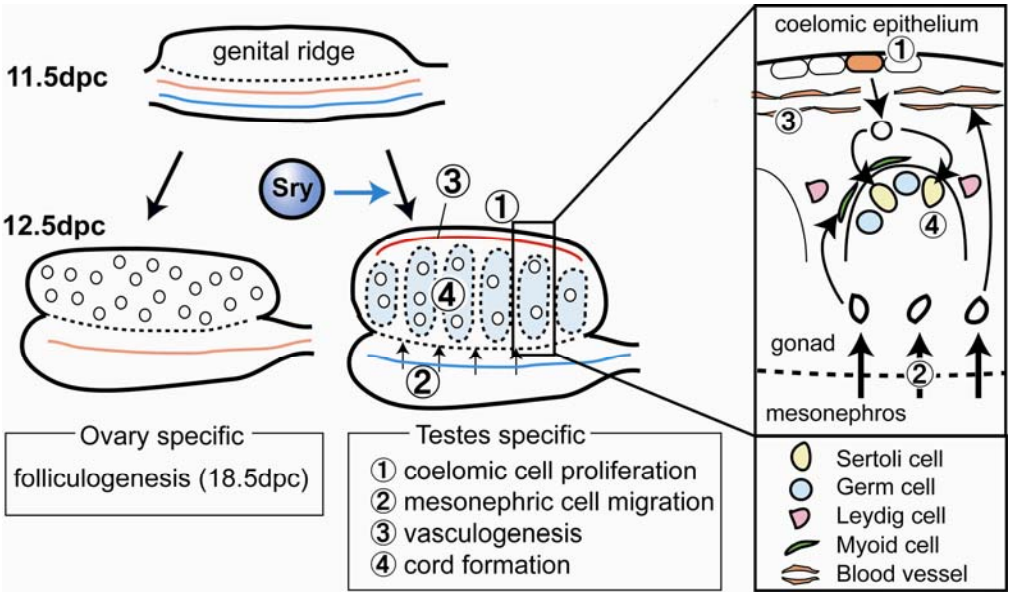
論文題目　Study of Sex Dimorphic Energy Metabolism in Mouse Gonadal Sex Differentiation
（マウス性分化期生殖腺のエネルギー代謝に関する研究）

The gonad, or genital ridge, is unique among all organs, because of its bipotential ability to differentiate into either a testis or an ovary. For this reason, the gonadal sex differentiation is a particularly interesting model that affords opportunities for comparative analyses in organogenesis. In mammals, sex differentiation of gonads, which governs the sex of individual's whole body, is genetically determined depending on the presence or absence of *Sry* (sex-determining gene on Y chromosome) encoding a putative transcription factor. The male-specific expression of *Sry* in mice is transiently activated for a short period from 11.0 days *post coitus* (dpc) to 12.0 dpc in gonadal somatic cells (pre-Seroli cells). In pre-Sertoli cells, *Sry* directly initiates the transcription of *Sox9*, a member of *Sry*-related gene family, which is also necessary and sufficient for testicular differentiation as well as *Sry*. Unlike *Sry*, however, *Sox9* continues to be expressed in pre-Sertoli cells throughout testis development, suggesting that the maintenance of sufficiently high-levels of *Sox9* expression is crucial for the subsequent testis formation. In male gonads, *Sry/Sox9* initiates several male-specific morphogenetic events such as coelomic cell proliferation, mesonephric cell migration into gonad, vasculogenesis just beneath coelomic epithelia and cord formation until 12.5 dpc. In female gonads, on the other hand, no clearly-defined morphogenesis is detected

in ovarian differentiation at this period (Fig. 1). These testis-specific morphogenetic events during sex differentiation period suggest that male gonads have a higher energy metabolism than female ones. However, the regulatory mechanisms of energy metabolism remain to be elucidated in not only gonadal sex differentiation but also general organogenesis.

Fig. 1. Schematic representation of testis-specific dynamic morphogenesis in gonadal sex differentiation in mice



In general, glucose is widely known as the major cellular energy source and forms an energy reserve as glycogen, a polymer of glucose. Previous study has shown that embryonic testis is one of glycogen-rich tissues in mouse organogenic embryos, and that glycogen accumulation predominantly occurs in the differentiating Sertoli cells within newly-formed testicular cords at 12.5 dpc (Kanai *et al.*, 1989). Moreover, the glycogen deposits in pre-Sertoli cells rapidly disappear shortly after the testicular cord formation, suggesting that the glycogen granules in pre-Sertoli cells act as an energy source in the dynamic morphogenesis of testis. However, no further information concerning the molecular mechanisms initiating glycogen accumulation and its functional significance in developing XY gonads is available at present.

In chapter 1, to reveal the mechanisms regulating glycogen accumulation in male gonads, I performed detailed histological and genetic analyses, and *in vitro* organ culture experiments. In developing XY gonads, glycogen accumulation starts to occur in pre-Sertoli cells from around 11.2 dpc in a center-to-pole pattern, similar to the spatio-temporal profile of *Sox9* expression. Glycogen accumulation was also found in XX male gonads of *Sry*-transgenic embryos, but not in XX female gonads of wildtype embryos at any developmental stages. These results imply that glycogenesis in pre-Sertoli cells is one of the earliest cellular events direct downstream of *Sry* action. Moreover, glycogen accumulation in pre-Sertoli cells was significantly inhibited by PI3K inhibitor LY294002, but not by MEK inhibitor PD98059 *in vitro*. In addition, active phospho-AKT (PI3K effector) showed a high degree of accumulation in gonadal somatic cells of genital ridges in a testis-specific manner, both *in vitro* and *in vivo*. Therefore, these findings suggest that immediately after the onset of *Sry* expression, the activation of PI3K-AKT pathway promotes testis-specific glycogen storage in pre-Sertoli cells.

In chapter 2, in order to investigate the functional significance of glycogen or high-glucose condition on gonadal sex differentiation, I performed a series of glucose-deprivation (GD) experiments *in vitro*, and conducted detailed histological and molecular analyses. My data demonstrated that, of the various somatic cell types in XY and XX gonads, pre-Sertoli cells are the most sensitive to glucose starvation. Although GD did not affect the initiation of Sox9 expression in XY genital ridges, it resulted in a markedly defective maintenance of SOX9 expression in pre-Sertoli cells, leading to the failure of testis cord formation and severely reduced expression of several extracellular matrix (ECM) components. The addition of FGF9 (SOX9-maintenance factor)/ECM gel (a mediator of FGF signal) restored SOX9 expression and the subsequent cord formation in XY genital ridges of GD. However, *Fgf9* expression was not altered in GD XY explants, and the addition of FGF9 alone did not rescue the defective SOX9 expression or cord formation in GD. These findings indicate that the establishment of SOX9 maintenance mechanism via the ECM-mediated positive feedforward pathway in pre-Sertoli cells is a metabolically active process with high-energy requirements. This further suggests the importance of the high-glucose condition assured by glycogen in the establishment of SOX9 maintenance mechanism in testis differentiation.

In summary, here I propose a novel model of molecular mechanism regulating the energy metabolism governed by *Sry* from both supply and demand aspects in pre-Sertoli cells, in gonadal sex differentiation (Fig. 2). In this model, *Sry* induces glycogenesis [energy supply] through the activation of PI3K-AKT pathway in pre-Sertoli cells. Simultaneously, *Sry* also initiates a downstream cascade requiring high-energy metabolism [energy demand], involving the establishment of SOX9 expression-maintenance mechanism through an ECM-mediated feedforward pathway. The glycogen deposits in pre-Sertoli cells induced by *Sry* are likely to act as a backup energy source for the subsequent establishment of SOX9 maintenance mechanism. Since transient glycogen accumulation is observed in various aspects of organogenesis, such cumulative glycogen may assure sustainable energy supply for the proper completion of embryogenesis. Taken together, this study not only reveals a novel role of *Sry* regulating the energy metabolism in gonadal sex differentiation but also opens up the new field of mammalian embryogenesis dealing with organogenesis and energy metabolism together.

Fig. 2. A possible model showing the regulation of energy metabolism by *Sry*