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論文内容の要旨

In nonmammlian vertebrates, ovarian follicle cells produce two different steroid hormones, estradiol-17β and maturation-inducing hormone (progestogens), in response to pituitary gonadotropins, which play important roles in two phases of oogenesis, vitellogenesis and oocyte maturation, respectively. Estradiol-17β promotes vitellogenesis in members of all nonmammalian vertebrates. On the other hand, a variety of progestogens have been shown to be effective in the initiation of meiotic maturation in fish and amphibian oocytes. These ovarian steroid hormones are known to be secreted from ovarian follicles in response to pituitary gonadotropins. A distinct shift from estradiol-17β to 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP, the maturation-inducing hormone of salmonid fishes) has been reported to occur in ovarian follicles immediately prior to oocyte maturation, and seems to be a prerequisite step for growing oocytes to enter the final stage of maturation. However, the regulatory mechanism of this steroidogenic shift is not clearly understood.

The medaka, *Oryzias latipes*, under a long photopeiod (14 hours light-10 hours dark) at 26℃, spawns daily within 1 hr of the onset of light for a number of consecutive days. Under these conditions, the sequence of events leading to spawning such as vitellogenesis, oocyte maturation, and ovulation can be timed accurately. These features make medaka an ideal model in which to investigate the regulatory mechanism of the steroidogenic shift occurring in ovarian follicles prior to oocyte maturation.

During vitellogenesis, estradiol-17β is secreted from medaka ovarian follicle cell layers and 17α, 20β-DP was identified as the most potent steroid to induce oocyte maturation. During oocyte maturation, 20β-hydroxysteroid dehydrogenase (20β-HSD) activity catalyzing the production of 17α, 20β-DP from 17α-hydroxyprogesterone is highin medaka ovarian follicle cell layers. Whereas 20β-HSD activity is kept undetectable during the vitellogenic stage. Instead, cytochrome P-450 aromataseactivity is high, leading to the production of estradiol-17β. However, the keyenzyme to switch this pathway is unknown. This investigation would require the precise information on steroidogenic pathway in medaka ovarian follicle cell layers.

In this study, changes in the steroidogenic pathway in medaka ovarian follicles during vitellogenesis and oocyte maturation were investigated *in vitro* by incubation of follicles with several radiolabeled steroid precursors, followed by thin layer chromatography (TLC) fractionation and recrystallizaion. When vitellogenic follicles collected at 18 hours before the expected time of spawning (vitellogenic follicles) were incubated with ^{3}H-labeled pregnenolone,

the major metabolites were 17α-hydroxypregenenolone, 17α-hydroxyprogesterone, and androstenedione. Incubations of vitellogenic follicles with androstenedione produced testosterone and estradiol-17β. By contrast, when maturing follicles (postvitellogenic follicles undergoing maturation) collected at 10 hours before spawning were incubated with ^{3}H-labeled pregnenolone, the major metabolites were 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, and 17α, 20β-DP; androstenedione was not detected. Neither vitellogenic and maturing follicles produced progesterone when they were incubated with ^{3}H-labeled pregnenolone, suggesting that in medaka ovarian follicles both estradiol-17β and 17α, 20β-DP are synthesized by the ^{5}Δ-steroid pathway. Thus, there is a distinct shift in the steroidogenic pathway from estradiol-17β to 17α, 20β-DP production in medaka ovarian follicles, and it is suggested that the decrease in C17, 20-lyase activity is responsible for this shift.

Next he attempted to mimic the decrease of C17, 20-lyase activity, by addition of several reagents. His experiments using PMSG (pregnant mare serum gonadotropin) demonstrated that gonadotropin is probably not directly responsible for controlling this shift. On the other hand, the phosphodiesterase inhibitor IBMX enhanced androstenedione production in incubations of vitellogenic follicles with ^{14}C-labeled progesterone. This may suggest that IBMX stimulates androstenedione production not by cAMP mediated signal transduction pathway alone, but by the involvement of other pathways affected by inhibition of phosphodiesterase activity. Calcium ionophore A23187 and the phorbol ester TPA (a protein kinase C activator) blocked the stimulatory actions of IBMX on androstenedione production. These findings suggest that multiple signalling pathways may participate in the regulation of ovarian steroidogenesis, and further emphasize the importance of calcium as a regulator of P-450c17 activity.

A rapid decrease in C17, 20-lyase activity appeared to be one of the critical steps in the shift of the steroidogenic pathway during oogenesis. C17, 20-lyase activity is supported by P-450c17 geneproduct. Interestingly, a single product of the cytochrome P-450c17 (P-450c17) gene has both 17α-hydroxylase and C17, 20-lyase activities in several species. The mechanism by which a single polypeptide regulates these two distinct activities differentially through oogenesis remains to be elucidated. To address this problem at the molecular level, he cloned P-450c17 cDNAs from medaka ovarian follicle cells. This study confirms that two types of cytochrome P-450c17 transcripts are present in medaka ovarian follicles. One type (P-450c17L) is similar in structure to those reported in other species. The second type (P-450c17S) is a novel type of P-450 c17cDNA which lacks exon 4. These two types of transcripts are synthesized by alternative splicing from a single copy gene. Both transcripts were abundant in

medaka gonads but absent in other tissues. Throughout the course of oogenesis, both transcripts are present during vitellogenesis and disappear at maturation. This prosperity and declin are correlated with the changes in stroidogenic activities in medaka ovarian follicle cells. Expression studies demonstrate that P-450c17L transcripts result in an enzyme with 17α-hydroxylase and C17, 20 lyase activities. Expression of P-45017S result in no detectable enzyme activity.

His preriminary experiments showed that cytochrome b_5 stimulated P-450 c17L product activities as those reported in other species. This observation in dicates that cytochrome b_5 may be involved in the steroidgenesis shift in medaka ovarian follicle cells.

審査結果の要旨

卵生脊椎動物の卵の成長と成熟は、生殖腺刺激ホルモンの作用で卵巣の濾胞細胞で合成される卵成長促進ホルモン（エストラジオール－17β）と卵成熟誘起ホルモン（多くの魚類では17α，20β－DP）により制御される。したがって、卵の成長と成熟にとってこれら2種の性ホルモンが卵巣で時期特異的に合成されることが重要であると考えられるが、現在このステロイド合成系のスウィッチ機構は不明である。本研究では、光周期の制御により卵胞の成長と成熟が調節可能なメダカを用いて、（1）卵成長期、卵成熟期におけるステロイド合成系の転換、（2）ステロイド合成系の転換に重要な役割を果たすと考えられる17α－水酸化酵素（P450c17）cDNAのクローニング及びその活性調節機構、について解析した。

卵成長（卵黄形成）期の卵胞（産卵18時間前）と卵成熟期（産卵10時間前）をアイソトープで標識した種々ステロイド前駆体と培養することにより、卵成長期にはエストラジオール－17βが、また成熟期には17α，20β－DPが生成されること、及びこれら2種のステロイドはいずれも17α－ヒドロキシプレグネノロンを経てΔ^5－経路により合成されることが明らかになった。また、このステロイド合成系のスウィッチにP450c17のもつC17、20ライエース活性の低下が重要であることを示すとともに、このライエース活性の調節に果たす種々情報伝達系の役割について検討した。

次に、2種類のメダカP450c17cDNA（P450c17L、P450c17S）をクローニングした。このうち、P450c17Lはこれまでに報告されているものとよく似た構造を持ち、P450c17Sはゲノム構造との比較からエクソン4（87個のアミノ酸）を欠くこれまでに報告のないcDNAであることを示した。また、P450c17遺伝子は一つで、二つの転写産物はオルタネティブスプライシングによって生殖腺特異的に転写されることを見出した。P450c17LはCOS－1細胞や大腸菌中で17α－ヒドロキシ活性とC17、20ライエース活性を示したが、P450c17Sはいずれの活性も示さなかった。また、P450c17Lを発現させた大腸菌の膜画分とブタのP450リダクターゼからなる再構成系中に、ブタ肝臓から精製されたチトクロームb5を添加することにより17α－ヒドロキシ活性とC17、20ライエース活性が上昇することを見出し、P450c17活性の調節にチトクロームb5が重要な役割を果たすことを明らかにした。

これらの研究成果は、脊椎動物の卵巣における性ステロイドホルモン合成機構、特に合成スウィッチ機構の研究に多大な貢献をしたことから、学位論文として充分な内容を含むものと判断された。

また、申請者により提出された学位論文の内容について口頭発表させた後、審査委員が関連事項について諮問した。さらに、申請者の関連研究分野の一般知識およびその背景となる基礎的知識についても口頭により諮問した。これらに対する申請者の応答はいずれも適切であった。また、提出された学位論文は英文で書かれており、英語の能力についても適正であると考えられた。これらの結果をもとに、審査委員会は申請者の持つ研究能力、学力、語学力は学位取得に値するものと判断した。