D107 Cloning and Characterization of cDNA Encoding 17α-Hydroxylase /17,20-lyase($P450c_{17}$) in Amphibian(*Rana dybowskii*)

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To understand the regulatory mechanism of ovarian steroidogenic pathway at molecular level in amphibian ovary, we have firstly cloned the cDNA encoding $P450c_{17}$ that mediate the conversion of progesterone to 17α-hydroxyprogesterone, ultimately to androstenedione in *R. dybowskii*. By the screening of ovarian cDNA library with RT-PCR fragment, we were isolated a 2.5kb cDNA clone encoding a single open-reading frame with 519 deduced aminoacid sequence. This sequence contain the three highly conserved domains as seen in $P450c_{17}$ of other species. Comparision of deduced aminoacid sequence of *Rana* $P450c_{17}$ with other animals showed relatively high identity with 63% in chicken, 60% in rainbow trout and 43% in human. The three different size of transcripts of $P450c_{17}$ of approximately 1.9, 2.5 and 4.0kb were detected by Northern blotting, but the genomic Southern analysis indicated a single copy gene. For functional assay, we introduced *Rana* $P450c_{17}$ expression vector into nonsteroidogenic COS-1 cells, and found that exogenous progesterone was converted into 17α-hydroxyprogesterone and androstenedione. It means that the coloned *Rana* $P450c_{17}$ has both 17α-hydroxylase and 17,20-lyase activities.

D108 IN VITRO OVULATION AND PROSTAGLANDIN SYNTHESIS BY OVARIAN FOLLICLES OF *RANA DYBOWSKII.*

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In order to assess the role of prostaglandins (PGS) in ovulation, ovarian follicles or components of follicular tissues of *R. dybowskii* obtained in hibernation period were cultured in the presence or absence of frog pituitary homogenates (FPH) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA, a protein kinase C activator), and the amount of prostaglandin F2α (PGF2α) and E2(PGE2) secreted into the medium were measured by radioimmuno assay. Ovarian follicles secreted a considerable amount of PGF2α and PGE2 during culture period without any stimulation (basal levels). Higher levels of PGE2 were secreted than that of PGF2α. FPH treatment to follicles or follicular components stimulated further the secretion of PGF2α but rather suppressed the secretion of PGE2 consistently. TPA treatment stimulated the secretion of both PGF2α and PGE2 markedly by the follicles but cAMP strongly suppressed the secretion of both PGS by the follicles. Theca/epithelium layer secreted much higher levels of both PGS than granulosa cell-enclosed oocytes in response to FPH or TPA during culture. In late hibernation (breeding period), FPH or PGF2α treatment induced oocyte ovulation but simultaneous treatment of PGE2 suppressed the ovulation effectively. Taken together, data presented here demonstrated that 1) PGF2α, not PGE2 is associated positively with ovulation, 2) protein kinase C is involved in PGS production, and 3) Theca/epithelium layer are responsible for the PGS production in *Rana* ovarian follicles.