D101 Localization of Progesterone Binding Sites on the Plasma Membrane of Oocyte in Amphibians, *Rana dybowskii*

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Progesterone receptor in amphibian oocyte surface was visualized by laser scanning confocal microscopy using fluorescein labeled progesterone 3-*O* -carboxymethyloxime- BSA (P-BSA-FITC). When intact follicle and denuded oocytes were cultured with P-BSA-FITC, fluorescence appeared only in the denuded oocyte surface which indicated that progesterone binds specifically to the oocyte plasma membrane. Binding of P-BSA-FITC to the oocyte was saturable and specific for progesterone. Presence of progesterone but not androstenedione caused displacement of P-BSA-FITC binding with the oocyte. Progesterone-BSA caused germinal vesicle breakdown (GVBD) of denuded oocytes in a dose dependent manner. Treatment of denuded oocytes with P-BSA resulted in a 3-fold increase in inositol triphosphate (IP_3) and a 4-fold increase in diacylglycerol (DAG) levels within 10 min and subsequent increase in protein kinase C (PKC) activity by 30 min. Thus, this study demonstrates the presence of progesterone binding sites in amphibian oocyte and its involvement in the activation of PKC pathway via the generation of membrane-mediated second messengers during oocyte maturation in amphibians.

D102 Cloning and sequencing of cDNA encoding a novel form of gonadotropin releasing hormone (amphibian GnRH) in the frog (*Rana dybowskii*)

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Gondadotropin releasing hormone (GnRH) plays an important role in the reproduction of vertebrates. cDNA encoding the novel form of gonadotropin releasing hormone (amphibian GnRH) precursor of the frog in *Rana* forebain were isolated and sequenced by reverse transcription and rapid amplification of cDNA ends (RACE). RT-PCR from forebrain, testis and muscle show the presence of GnRH transcript in forebrain and testis but not in muscle. The full length amphibian GnRH precursor cDNA consists of 521 bp, including an open reading frame of 270 bp. The 90 amino acid long *Rana dybowskii* GnRH precursor shows an identity of 79% with *Xenopus lavis* and 68% with human. Amino acid sequence of this novel decapeptide is pGlu-His-Trp-Ser-Tyr-Gly-Leu-Trp-Pro-Gly-NH2. Intrestingly, this decapeptide is defferent at (Trp^8) from those of mammalian (Arg^8) and chicken I (Gln^8) GnRH. Nucleotide sequence is same as in testis and genomic GnRH PCR product. Northern blot analysis detected a single mRNA transcript of approximately 500 bases for the amphibian GnRH precursor in the forebrain. Southern blot analysis shows a single band that strongly hybridized to a probe. This study demonstrates identification of a novel form of GnRH in amphibians.