THE CLONING AND PARTIAL CHARACTERIZATION OF TWO cDNAS ENCODING THE YOLK PROTEIN VITELLOGENIN FROM THE PAINTED TURTLE, CHRYSEMYS PICTA

D. Stephen Charnock-Jones[1], Scott J. Benson[2], and Ian P. Callard[3]
[1]Department of Obstetrics and Gynaecology, University of Cambridge, England
[2]St. Cloud University, St. Cloud, MN 56301
[3]Department of Biology, Boston University, Boston, MA 02215

In oviparous vertebrates the large amount of yolk protein in the egg provides a nutrient source for the future development of the embryo. This protein is synthesized in the liver as a precursor, vitellogenin. It is secreted into the blood stream and subsequently taken up by the oocytes where it is processed into mature yolk protein. Expression of the vitellogenin gene is tightly regulated by steroids and peptide hormones (Shapiro et al. Rec Prog. Horm. Res. V. 45:29-58, 1989). Thus it offers an excellent model for the study of gene regulation, protein transport, uptake and processing, and toxicology since toxins potentially can interfere with any of these processes and subsequent embryonic development. The evolutionary history of the regulation of this gene is of interest, since it is expressed to varying degrees associated with viviparity in non-mammalian, and suppressed in eutherian mammals.

Available cDNA probes for vitellogenin from other species do not readily cross react with the vitellogenin mRNA from the painted turtle. However, this mRNA is only expressed in the liver of female turtles and from the levels of circulating protein would be expected to be quite abundant. These facts lead us to use a differential screening approach to isolate the relevant cDNA. RNA was prepared from the livers of mature male and female turtles. Due to the large amount of carbohydrate present in this tissue the RNA was washed extensively with 4M LiCl to remove this. Messenger RNA was then purified by oligo dT affinity chromatography. A cDNA library was prepared in a plasmid vector using the "Clonestruct" kit (USB). The library contained 4×10^5 clones with a 10% background of nonrecombinants. The mean insert length was 1kb and 1 in 20 clones had an insert longer than 2.5 kb. A portion of this library (10,000 clones) was plated out and screened. The probe used was ^{32}P labeled cDNA synthesized from mRNA purified from female turtle liver. The rationale for this was that the most abundant species in the probe would hybridize to give the strongest signal. Since vitellogenin was presumed to be abundant it was hoped that this cDNA would be represented among the clones with the strongest hybridization signal. A total of 16 such clones were selected and partially sequenced from each end. The DNA sequences were transferred to computers for remote analysis. These data showed that of the 16 clones 10 encoded vitellogenin. These could be further divided into two distinct classes both of which had significant homology to known vitellogenin sequences but were later shown not to cross hybridize. This is similar to

the finding in other species (Xenopus; Gallus) where multiple genes encoding vitellogenin have been identified.

We have successfully utilized differential screening to clone two vitellogenin cDNAs from the painted turtle. Further work is underway to complete the sequencing of these cDNA clones. With the probes generated we are now able to investigate the mechanism of steroid regulation of yolk protein production in C. picta and we are extending this work to other species.

We acknowledge the kind gift of reagents from United States Biochemical Corporation, Cleveland, OH. SJB was supported by a fellowship from the Pew Foundation, supported by NIH 1RO1RRO6633 to IPC.