Genes and mRNAs of yolk proteins
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SUMMARY and GENERAL CONCLUSIONS

Vitellogenesis - yolk formation - in oviparous animals is a fundamental process regulated by hormones.

The major yolk proteins - in chickens the lipovitellins, phosvitin(s) and \( V(ery)D(ensity)L(ipoprotein) \)s - originate from the liver where they are formed under estrogen control. The lipovitellins and phosvitin(s) are derived from a long precursor protein, vitellogenin, which is secreted by the liver, transported by the blood to the ovary and cleaved on entry into yolk. VLDL\(_1\) is the precursor of some yolk-VLDL protein subunits; VLDL\(_{II}\) is a major subunit separately formed in the liver and deposited in the yolk unmodified. It is found as a short polypeptide of 82 amino acids.

In rooster liver, the silent vitellogenin and "near-silent" VLDL\(_{II}\) genes can be recurrently activated by estradiol to produce their female products. This activation thus forms a particular attractive system for study of gene regulation by steroid hormones. Numerous studies have shown that regulation by steroid hormones must be mediated at the DNA-RNA level. Most probably, the synthesis of (new) proteins evoked by estradiol depends on the formation, probably transcription proper, of their mRNAs, which mRNAs are then transported to cytoplasm. Ultimately it is the concentration of cytoplasmic mRNA available for translation into protein, that governs the rate of synthesis.

How transcription is regulated is still an open question. We may expect, however, that a detailed study of the mRNAs and genes coding for vitellogenin and VLDL, as well as elucidation of the complex protein-nucleic acid interactions involved, will once provide the answer. It is at this stage of study, at the shift from quantitative description to molecular characterisation, involving a shift from cytoplasm (RNA) to nucleus (DNA), where this thesis begins.

In the first chapter, after a brief summary of some of our present knowledge of gene organisation and gene expression, I outline why it is interesting to study vitellogenin and VLDL\(_{II}\) in parallel. A third protein synthesized by the liver, serum albumin, is used for comparison throughout this thesis as representative of proteins not controlled by estrogens. Chapter II deals with the properties of vitellogenin mRNA, isolated by methods minimising nucleolytic breakdown and aggregation of small (r)RNAs,
and purified to homogeneity by preparative gel electrophoresis. Vitellogenin mRNA of both chicken (Gallus domesticus) and duck (Anas platyrhynchos) contains about 7000 nucleotides and code for a polypeptide of $M_r$ 220,000 upon in vitro cell-free translation. Concomitant analysis of mRNA for serum albumin of both species showed that it is about 3000 nucleotides long and codes for preproalbumin which in chicken is somewhat shorter than in duck.

In Chapter III, I describe how purified vitellogenin mRNA is used to generate two recombinant DNAs, containing incomplete copy-DNAs, one originating from a 3'-terminal, and the other from an internal region of the mRNA. (The procedure is more extensively described by P. van den Boogaart Thesis, Groningen 1980). Both chimeric plasmids hybridised to one vitellogenin mRNA in a "double R-loop structure". Both copies are therefore proven to be derived from one RNA. A preliminary characterisation of the vitellogenin chromosomal DNA gives tentative evidence for the existence of several intervening sequences (introns) in the 3'-terminal part of the gene. In addition my results point to the occurrence of more than one vitellogenin gene per haploid genome. A complete characterisation of the vitellogenin mRNA and gene, however, is far from completed and will require the generation of more and larger cDNA clones, as well as the cloning of huge stretches of genomic DNA embracing the vitellogenin gene(s).

The second part of this thesis, starting at Chapter IV, deals with the study of the nucleic acids coding for VLDL_{II}. Chapter IV describes the isolation of VLDL_{II}mRNA, a polynucleotide of 700-750 residues coding for a pre-VLDL_{II} which has a signal peptide linked to the N-terminus of the VLDL_{II} polypeptide, by use of methods similar to those developed for vitellogenin mRNA (Chapter II). This mRNA served as template for cDNAs put into plasmid pBR322 by an insertion technique more sophisticated than that described in Chapter III. The various recombinant VLDL_{II} DNA clones were characterised by restriction enzyme mapping and electron microscopy of R-loops. I complete the characterisation of structural VLDL_{II} DNA in Chapter V by determination of the nucleotide sequence of the major part of the mRNA containing the more important regions. This sequence also establishes the amino acid sequence of pre-VLDL_{II} and thus of VLDL_{II}. A first approach to the analysis of chromosomal VLDL_{II} DNA by restriction enzyme mapping revealed that the gene is interrupted at least once by an intervening sequence having about the same length as VLDL_{II} mRNA.
In chapter VI we return to the mRNAs for VLDL\textsubscript{II}, vitellogenin and serum albumin. The initiation frequency, i.e. the degree of recognition of the mRNAs by the translational machinery \textit{in vivo} and by heterologous ribosomes \textit{in vitro} is highest for VLDL\textsubscript{II} mRNA and lowest for vitellogenin mRNA. The structural basis for this difference is, as yet, unknown. It is one of the many examples of protein-nucleic acid interactions which are very poorly understood.

Yet, I believe that qualitative studies as described in this thesis, will lead us gradually to a more complete understanding of gene regulation in eukaryotes. At any rate, I hope that for the vitellogenin study on our laboratory, this thesis has contributed in extending our knowledge; for VLDL\textsubscript{II} it may form the basis and may instigate to further study of this induction system.