minireview

Why is there sequence similarity between insect yolk proteins and vertebrate lipases?

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Abstract The major proteins stored in the yolk of developing oocytes are thought to provide a nutritional store for utilization during embryogenesis. They seem to fall into two major families of proteins. The first are called vitellogenins and are found in frog, chicken, nematode, fish, and some insects such as the boll weevil. The other group are called yolk proteins and are found in dipteran insects such as fruitfly, housefly, fleshfly, and bluebottles. Both groups are the major proteins found in the oocyte and are female-specific proteins endocytosed from the serum or hemolymph. The yolk protein group were found to have sequence similarity to the triacylglycerol lipases and lipoprotein lipases of vertebrates, including rat, pig, and human. The yolk proteins do not have lipase activity, but the sequences conserved between yolk proteins and lipases surround the active site where there are interactions with lipids. The likely reason for the presence of this domain in the yolk proteins is to bind a steroid hormone in a storage form conjugated to lipids. This permits the storage of the hormone in an inactive form until the yolk proteins are degraded, when it can be released from its conjugate to induce developmental decisions in embryogenesis. They may also transport lipids into the oocyte for use in embryogenesis. Whilst the vitellogenin family of proteins do not share this homology with the lipases they do have similarity to the human serum protein, apolipoprotein B, which also has a role in binding lipids. These findings are discussed in relation to the evolution and functions of lipases, apolipoproteins, vitellogenins, and yolk proteins. Experiments aimed at isolating genes encoding lipases in insects and at further elucidating the function of the yolk proteins are suggested.—**Bownes, M.** Why is there sequence similarity between insect yolk proteins and vertebrate lipases? *J. Lipid Res.* 1992. **33:** 777–790.

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As the sequences of more and more proteins are determined, largely by sequencing the genes encoding them, we are able to group proteins into families with similar sequence and presumably some similarity in function. Periodically there are some rather unexpected observations on similarities between proteins that were thought to have quite different functions. Findings such as these may suggest new functions for proteins and experiments can be designed to test these ideas. Furthermore, such findings can give important clues to the evolution of proteins. This review is the result of one of these unexpected observations; namely, that there is sequence similarity between the yolk proteins of insects and vertebrate lipases. I will examine what we know about these lipases in the organisms in which they have been studied and what we know about the yolk proteins that are so similar to them over a specific domain of the protein. I will concentrate particularly on the new experiments that were undertaken as a direct result of the observed sequence similarity and how these have changed our views on the functions of the yolk proteins. I will then go on to describe what we know about the vitellogenins, another family of yolk storage proteins, and ask if they share any functional similarities, even though there is no sequence conservation between the two groups of yolk storage proteins. Finally, I will suggest some experiments that would be worth undertaking in the light of these observations.

TRIACYLGLYCEROL LIPASES AND LIPOPROTEIN LIPASES

There is a growing family of lipase genes that have been cloned and sequenced and that clearly code for related proteins. Lipoprotein lipase (LPL) is involved in the utilization of triacylglycerol-rich lipoproteins, providing free fatty acids for transport to the peripheral tissues, where they are required to provide energy. LPL may also be important in the transfer of cholesteryl esters to endothelial cells. The enzyme is synthesized in a number of tissues including adipose and heart, but is found bound to the lumen surface of capillaries. This is thought to be achieved by association with heparin sulfate and explains why the proteins have domains for interacting with heparin as well as with lipids (1, 2). To function, an apolipoprotein C-II cofactor is required. The activity of LPL is regulated by

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; PL, pancreatic lipase; YP, yolk protein.

nutrients and hormones thus allowing the organism to respond to dietary and energy requirements by either storing or utilizing triglycerides. The amino acid sequences of several lipoprotein lipases have been determined including mouse (3), human (4), bovine (1), and guinea pig (5).

Hepatic lipase whilst found mainly in the liver, where it functions to metabolize circulating lipoproteins, has been detected in other tissues including the adrenal gland and the ovary (6, 7). Its precise functions do not seem to be known though it seems likely that it can hydrolyze triglycerides in intermediate density lipoproteins and phospholipids in high density lipoproteins, thus clearing triacylglycerols and phospholipids from the circulation. The gene encoding rat hepatic lipase (HL) has been cloned and sequenced (8) and partial direct amino acid sequencing has been performed (9). Surprisingly, transcripts were only detected in the liver and not in any of the other tissues where enzyme activity had been reported. The enzyme binds tightly to heparin as does LPL, yet it clearly has a very different physiological function, and is able to function without a cofactor. Nonetheless, the two enzymes have similar substrate specificities and HL shows similarity to the LPLs in the lipid-binding region. The cDNA encoding human plasma hepatic lipase has also been isolated and sequenced and shows a high degree of sequence homology in the lipid and heparin binding regions (10).

The amino acid sequence of pig pancreatic triacylglycerol lipase has been determined (11) as has the DNA se-

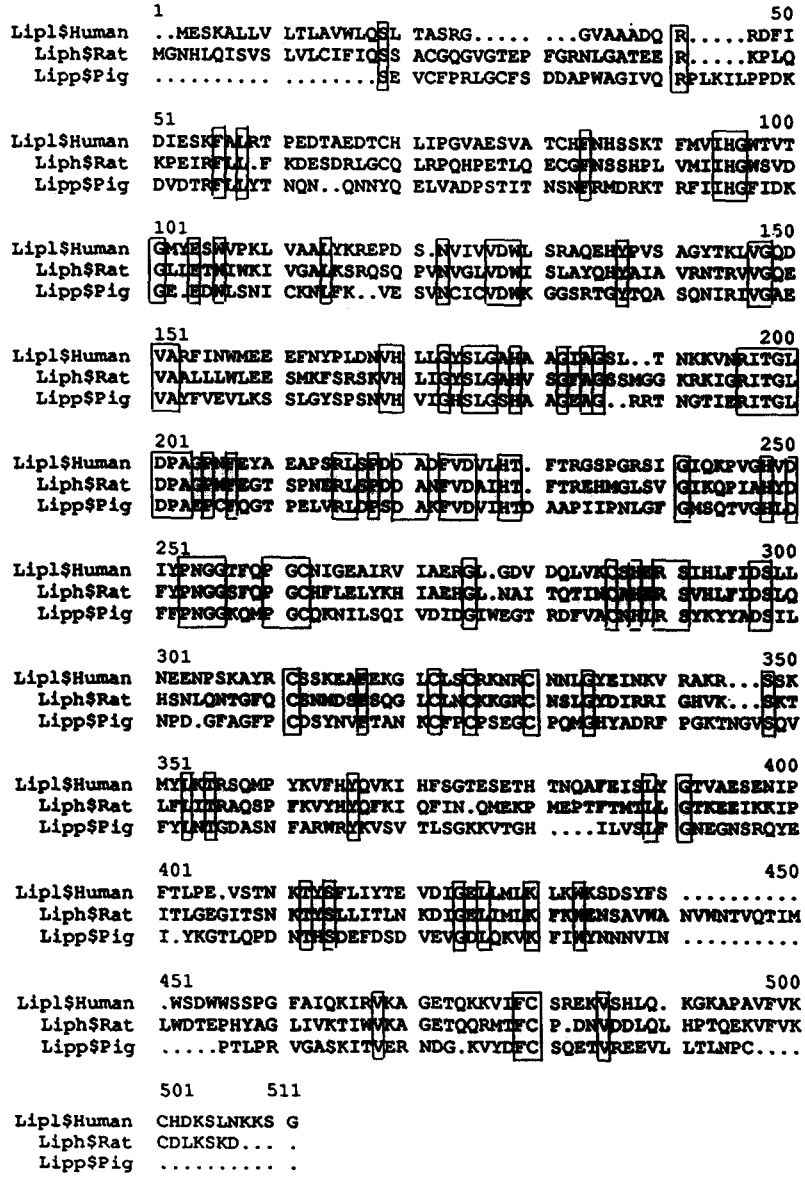


Fig. 1. Sequence comparison of three vertebrate lipases. Alignment of human lipoprotein lipase, rat hepatic lipase, and pig pancreatic lipase. Numbers refer to amino acid position. Dots are inserted to optimize the alignments. Boxed amino acids are conserved between all three lipases.

quence of the canine enzyme (12, 13). Pancreatic lipase (PL) is synthesized in the pancreas and secreted into the intestinal lumen with other digestive enzymes where it acts upon dietary triglycerides, so that glycerol and fatty acids can be transported through the intestinal wall (14). It requires the cofactor, colipase, to function. The enzymes synthesized by the pancreas must adapt to dietary intake and are likely to be regulated by secretin (15). The sequence of these proteins shows that the proteins are members of the same family as the LPLs and HLs. The PL does not, however, contain a heparin-binding site and is known not to bind heparin.

The region conserved between LPL, HL, and PL does not extend over the whole molecule, but is located in a central domain. The degree of conservation at the carboxyl terminus and amino terminus is quite high between LPL and HL, but they share no homology in these regions with the PLs. This probably reflects the difference in location and function of the two groups of enzymes, as the LPLs and HLs are bound to glycosaminoglycans at the capillary endothelium, whilst the pancreatic enzymes are secreted into a lumen to function. A comparison of the structural features of various lipases has been made by Persson et al. (16). A number of amino acid sequences representing members of all three types of lipase is shown in **Fig. 1.**

DIPTERAN YOLK PROTEINS

The yolk proteins of *Drosophila melanogaster* form a small gene family. There are three single copy genes each encoding a polypeptide (17). The three yolk proteins (YP1, 2, and 3) are synthesized in the fat body (18) and transported through the hemolymph to the ovary where they are selectively taken up by receptor-mediated endocytosis

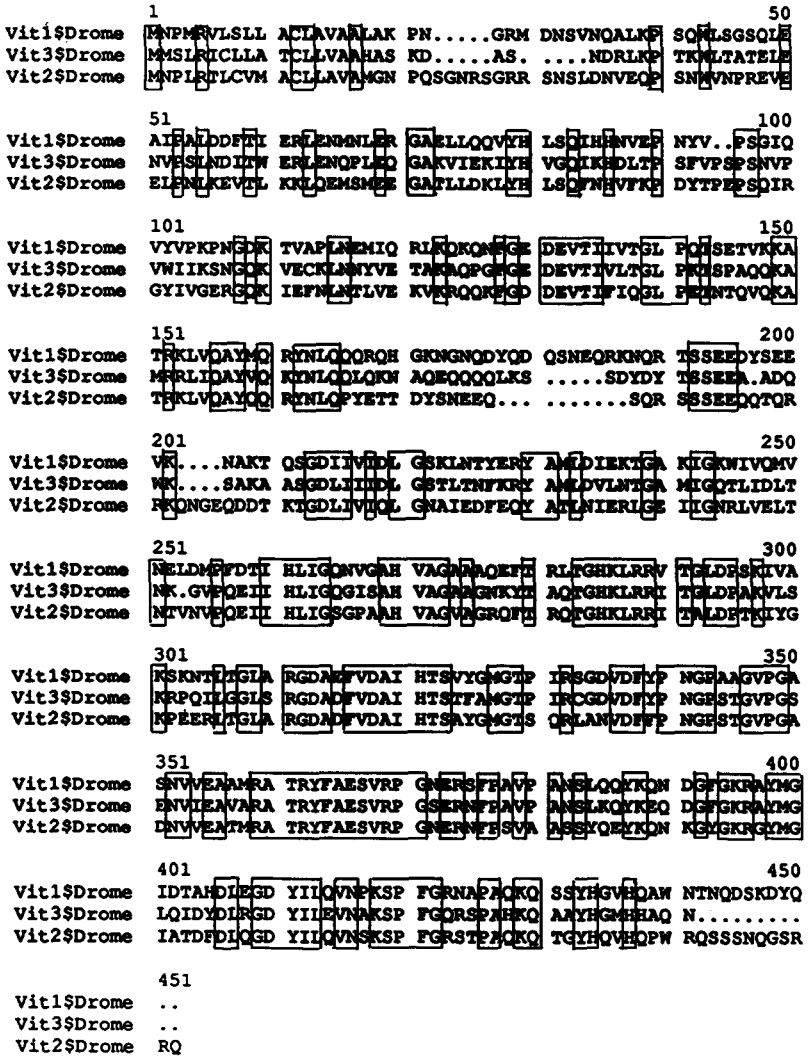


Fig. 2. Sequence comparison of three *Drosophila* yolk proteins. Comparison of sequences of *Drosophila melanogaster* yolk proteins 1, 2, and 3. Unfortunately, they are in the database as vitellogenins 1, 2, and 3. Numbers refer to amino acid position. Dots are inserted to optimize the alignment. Boxed amino acids are conserved in all three proteins.

and stored in yolk granules for utilization during embryogenesis (19). The follicle cells surrounding each developing oocyte in the ovary also synthesize the YPs and these are transported directly to the oocyte membrane (18, 20, 21). The expression of the genes is regulated by the sex-determination hierarchy, as they are proteins found only in females (22). Their levels of expression are also controlled by the hormones, juvenile hormone (23) and ecdysone (24), and the nutritional status of the fly (25). Whilst a great deal of attention has been devoted to the regulation of expression of these genes (for review see ref. 26) very little attention has been paid to the proteins them-

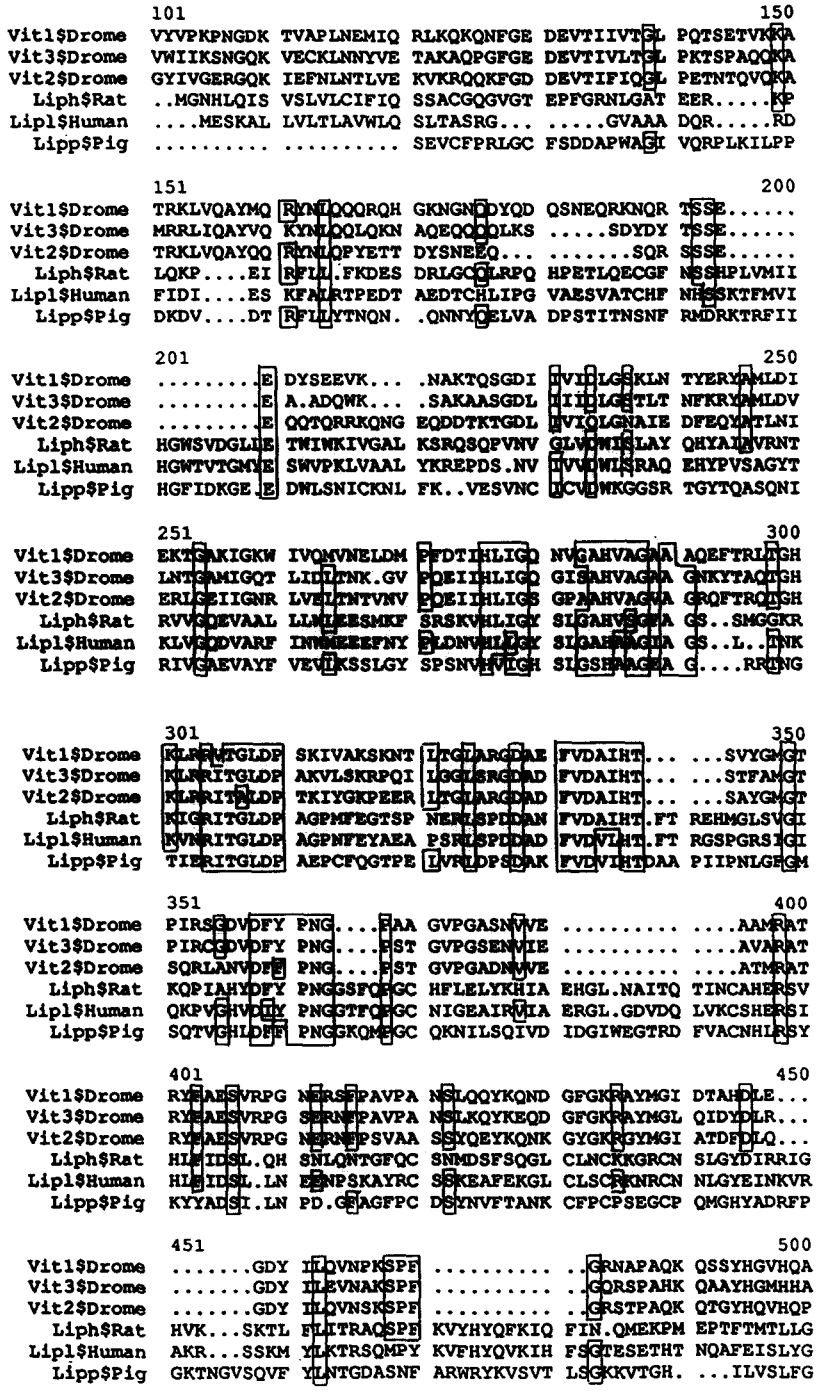


Fig. 3. Sequence comparison of yolk proteins and lipases. Alignments of the human lipoprotein lipase, rat hepatic lipase, pig pancreatic lipase, and yolk proteins 1, 2, and 3 of *Drosophila melanogaster*. Numbers represent amino acid positions. Note that the YP sequences are extended at the amino terminus and the lipase sequences are extended at the carboxyl terminus. Within the region of similarity, amino acids conserved in at least four of the six proteins are boxed, thus ensuring that this amino acid is represented in both lipases and yolk proteins.

selves, as they were just assumed to be an amino acid store for use during embryonic development. Some doubts on this simplistic view can be raised as we have recently discovered that the yolk proteins of other dipteran insects, such as the housefly and fleshfly are recognized and endocytosed by the *Drosophila* ovary (A. Martinez and M. Bownes, unpublished results). Furthermore, sequence analysis of the yolk protein genes of *Calliphora* show that the proteins have been remarkably well conserved (A. Martinez and M. Bownes, unpublished results). One would expect that a storage protein with no other function would not be well conserved, as the most important feature would be the balance of amino acids when the proteins were degraded. The sequences of the three *Drosophila* yolk proteins have been determined in several groups (27, 28) and are shown in **Fig. 2.**

The YPs are essential to the development of the embryo. If we reduce the number of copies of the *yp* genes by genetic crosses with various mutants that do not store one of the proteins in the oocyte, both the number of eggs laid by the female and the viability of those eggs is reduced (29), suggesting that the amount of yolk stored in an egg is critical for its successful development.

THE SEQUENCE SIMILARITY BETWEEN YOLK PROTEINS AND LIPASES

Database searches have shown that there is a large region of sequence similarity between the *Drosophila* yolk proteins and the pancreatic triacylglycerol lipase of the pig (30). This spanned the lipid-binding domain and the catalytic site of the enzyme. At the time of the initial search the porcine lipase was the only lipase in the database. However, very soon after we found this similarity, the sequences of many more lipases became available and many other authors have noted this sequence similarity (31–33). The alignment of the common domain from a number of lipases and the *Drosophila* yolk proteins is shown in **Fig. 3**.

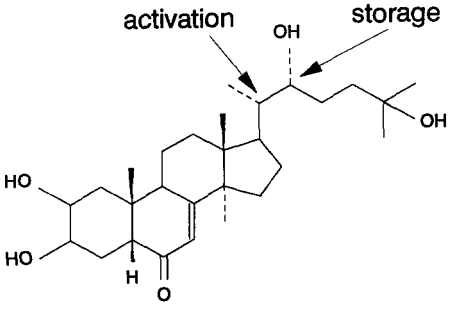
The YPs showed no lipase activity when tested, and indeed one of the key serines from the catalytic site of the enzyme is not conserved in the YPs. It is possible that enzymatic activity would not be detected with purified proteins as they may require a cofactor to function as is seen for a number of lipases. However, we have recently shown that whole ovary extracts have very low lipase activity compared to other tissues in the fly, suggesting that indeed there is no lipase enzymatic activity in yolk proteins (S. Yeaman, G. Smith, K. Rothwell, and M. Bownes, unpublished results). Very little biochemistry has been undertaken with the YPs of *Drosophila*, they are glycosylated (34), phosphorylated (35, 36), and sulfated (37), but whether or not lipids are bound to them has not been investigated.

As the insect embryo develops it needs to release a steroid hormone, 20-hydroxyecdysone, to trigger the secretion of the larval cuticle (38, 39). This hormone is normally synthesized from cholesterol, but there are no dietary sources available during embryogenesis in an enclosed egg so either the hormone or its precursors must be stored in the embryo. Evidence has suggested that the hormone is in fact stored as an inactive conjugate, and in many insects the conjugates are to fatty acids (40). The basic ecdysteroid molecule with some of the modifications that can occur is shown in **Fig. 4.** Following the time in embryogenesis when the YPs were degraded, we noticed that this approximately correlated with the first appearance of a peak of free ecdysone (41, 42). It thus seemed possible that the hormone could be conjugated to fatty acids and that these were bound to the YPs at the putative lipid-binding domain. By purifying the YPs, digesting them with protease, and releasing any bound steroid with an esterase, we were able to show that indeed the YPs do carry an inactive ecdysteroid bound to them (30). The nature of the steroid, and how and where it is bound are not yet known, but it is interesting to speculate that it is bound to the domain with sequence similarity to the vertebrate lipases.

It is also possible that other lipids and perhaps cholesterol are bound to the yolk proteins and transported into the oocyte, but this has not been investigated.

VITELLOGENINS

Most eggs that are laid contain large amounts of stored material for embryogenesis and the major storage proteins from many organisms have been characterized. These proteins are referred to as vitellogenins whilst they are circulating and vitellins once they have been processed and transported into the oocyte. The vitellogenins



Ecdysone

Fig. 4. Ecdysone. The steroid hormone ecdysone is activated by hydroxylation at carbon 20 to produce 20-hydroxyecdysone. It can be stored as a conjugate by the addition of fatty acid esters at carbon 22.

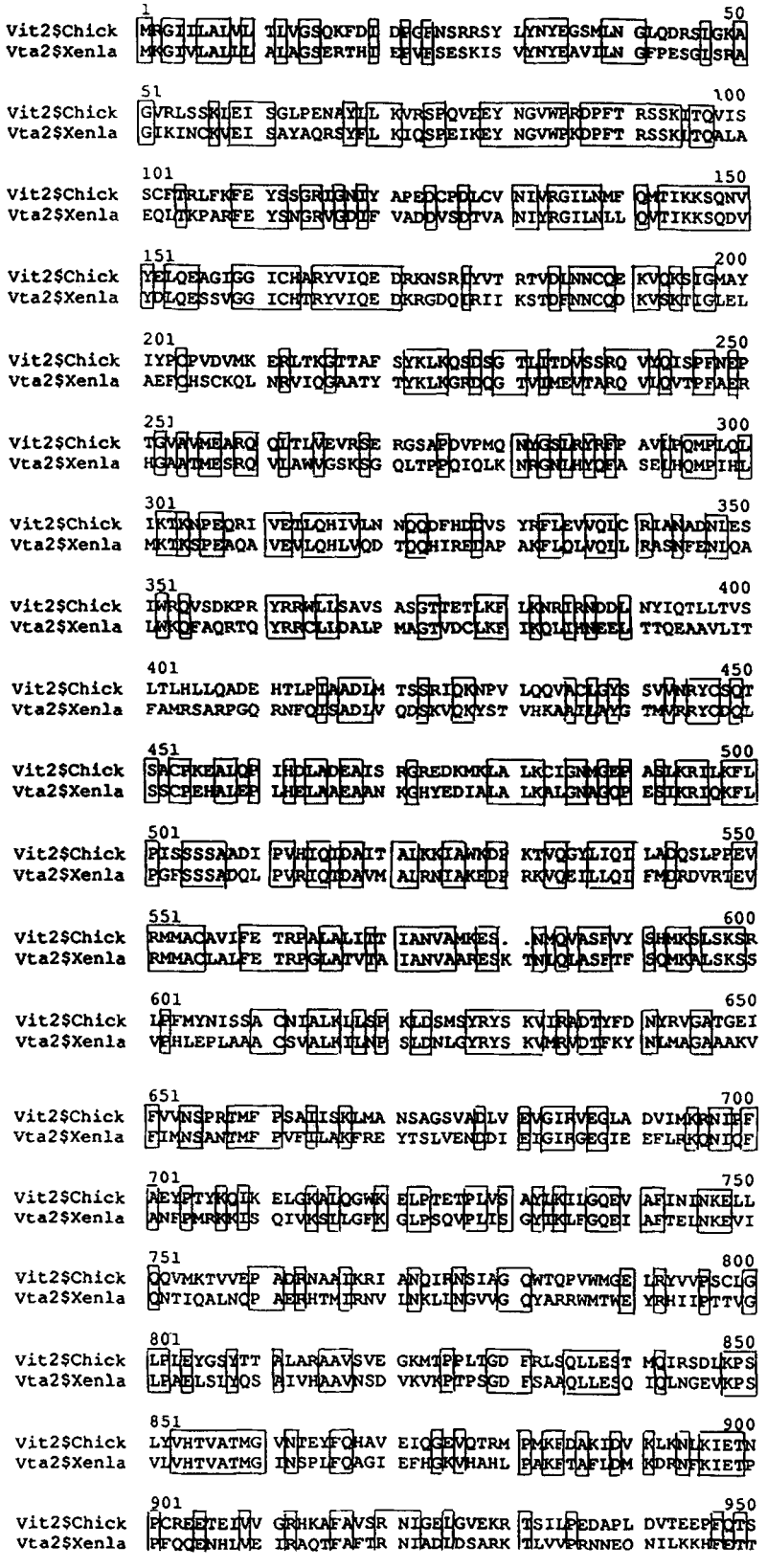
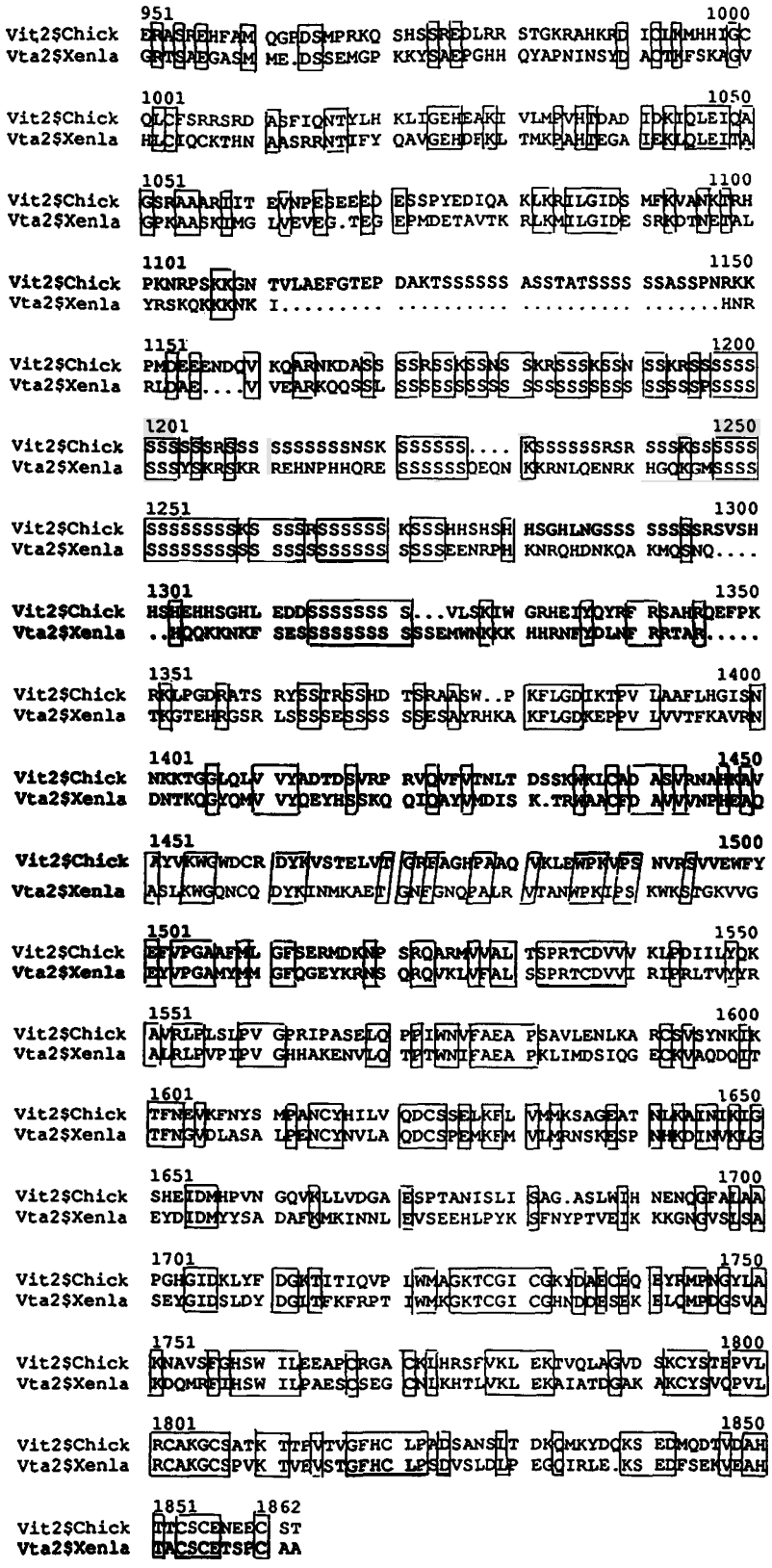


Fig. 5. Sequence comparison of chicken and frog vitellogenins. Alignment of vitellogenin 2 of *Xenopus* and chicken. Numbers represent amino acid positions. Boxed amino acids are conserved.



are synthesized in the liver of chickens, *Xenopus*, and fish, in insects they are synthesized in the fat body, and in the nematode in the intestine (43, 44). The native protein in the insects and in vertebrates is large, generally 400–500 kDa, and generally is cleaved into smaller units by the time it is assembled in the oocyte. The subunits include lipovitellins and phosvitins. For a detailed description of the proteins, their regulation, processing and all their potential functions see reference 33.

The vitellogenins and vitellins of a large number of insects, of *Xenopus, Caenorhabditis*, and chicken have been described and the genes encoding some of them have been characterized (45–48). They all share some conserved sequences, and probably all arose from the same ancestral gene (49). The *Xenopus* and chicken genes are very closely related to each other, sharing the same complex intron/exon structure. Their amino acid sequences are compared (33, 50) and are shown in **Fig. 5.** The worm and boll weevil genes are much more distantly related (49, 51, 52) though clearly part of the same family, see **Fig. 6.**

These genes, however, are not related to the yolk protein genes of *Drosophila*. This became clear as soon as we began to characterize the *Drosophila* YPs; unlike the other insects, the major egg proteins were not made as large precursors and processed before storage. In fact, we could see no difference between the stored form and the hemolymph form, thus we decided to call them yolk proteins rather than vitellogenins (53). This has not been consistently observed and the *Drosophila* proteins are often also called vitellogenins. Now that we have sequence data for both vitellogenins and yolk proteins and we know they are quite unrelated proteins (50), it seems very important to maintain this naming difference and the yolk proteins of *Drosophila* should not be called vitellogenins.

Despite this obvious sequence difference, it is likely that YPs and vitellogenins will share some functions as they are both major storage proteins and critical for subsequent embryonic development. This is not a vertebrate/invertebrate difference, as the worm *Caenorhabditis* clearly has a vitellogenin that belongs to this family; nor is it an adaptation unique to insects as the vitellogenin from the boll weevil also belongs to the vitellogenin group (52).

APOLIPOPROTEIN B-100

Apolipoprotein B-100 is a mammalian serum protein synthesized in the liver. It is part of very low density lipoprotein, intermediate density lipoprotein, and low density lipoprotein and its role is to transport triacylglycerides and cholesterol through the body (54). Interestingly, apolipoprotein B has also been identified in avian egg yolk (55).

SIMILARITY BETWEEN THE VITELLOGENINS AND APOLIPOPROTEIN B-100

The sequence similarity between the vitellogenins and apolipoprotein B-100 was first described by Baker (56) and is shown in **Fig. 7**. The possible functional similarities between the apolipoprotein B-100 and the vitellogenins includes the fact that they enter cells by receptor-mediated endocytosis, and thus must both interact with a membrane-bound receptor. In fact there is evidence for cross-reactivity of antibodies to the receptor for chicken vitellogenin and mammalian low density lipoprotein receptors (57). The other similarity lies in the binding of lipids, as vitellogenins have been shown to have lipids bound to them. Furthermore, as we have demonstrated the binding of inactive ecdysteroids to *Drosophila* yolk proteins, other groups have also found ecdysteroids bound to vitellogenin in locusts. In the locust, ecdysteroid conjugates are bound to the vitellogenin and free hormone is released prior to the each cuticle secretion (39). Unfortunately, we do not have the sequences of these vitellogenins to see if they have domains present that suggest the presence of lipid binding sites. It is also intriguing that both vitellogenin and apolipoprotein B-100 expression is regulated by steroid hormones.

A cysteine-rich domain from von Willebrand factor has been found to share a domain of sequence similarity with vitellogenin (31, 33). At present however the functional importance of this domain is not understood.

The common theme, therefore, is that the vitellogenins, yolk proteins, lipases, and apolipoprotein B-100 all bind to lipids, and may all have some connection with interacting with molecules related to cholesterol. Maybe millions of years ago there was a common ancestral protein that diverged to give the lipid/steroid storage functions, the lipid/cholesterol transport functions, and the enzymatic functions of all the proteins I have described.

FUTURE EXPERIMENTS

Many interesting experiments in several areas that would help our understanding of the evolution of proteins and the functions of vitellogenins and yolk proteins are suggested.

Why are there two distinct groups of storage proteins? Do they perform the same functions in slightly different ways, or do they have different roles in embryonic development? To investigate this, one could look to see whether there are genes or proteins similar to the vitellogenins of other insects and vertebrates in *Drosophila*. Maybe there is vitellogenin and yolk protein present in the yolk of some organisms, but with the ratios of the two proteins varying among the species.

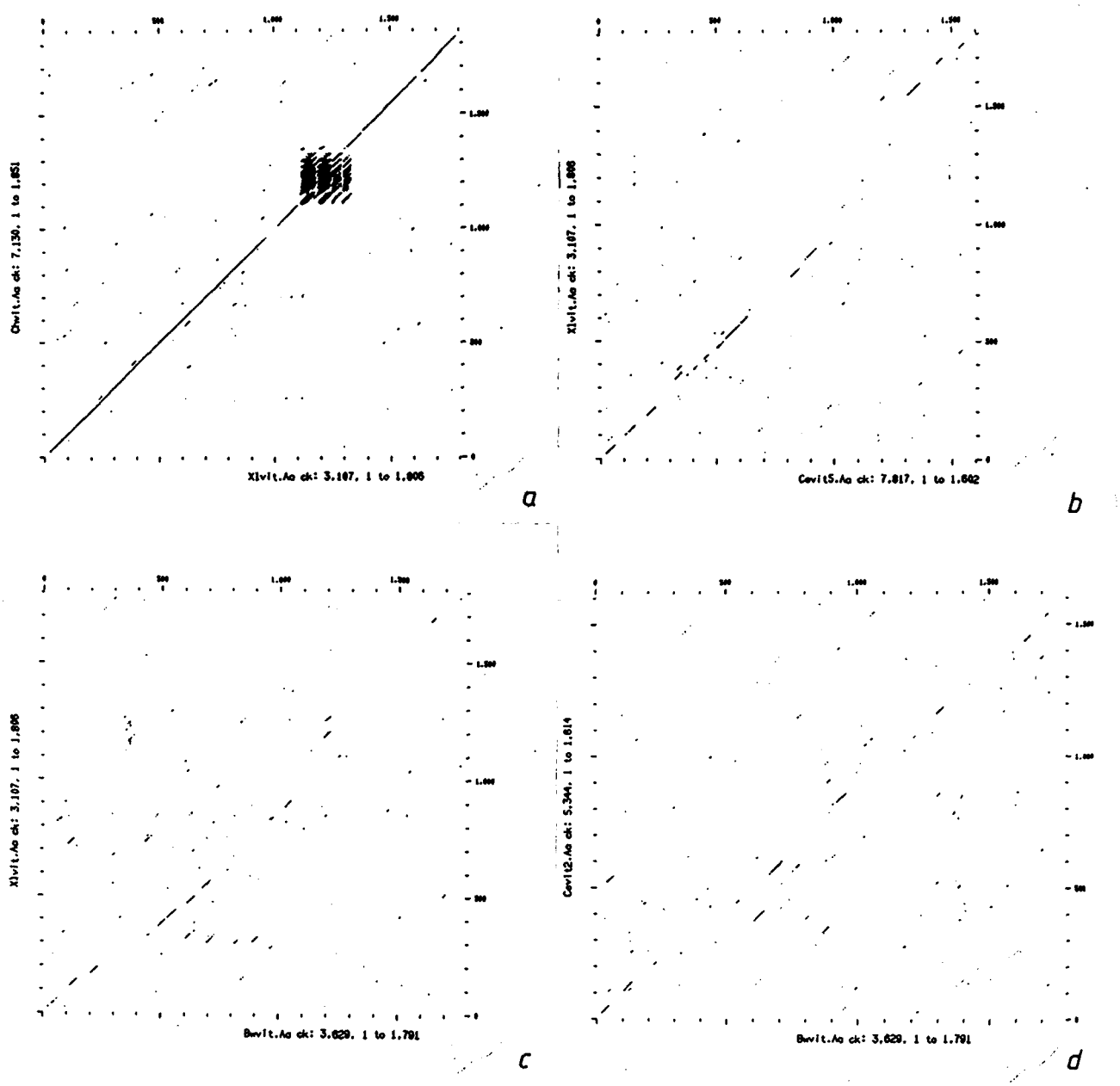


Fig. 6. Sequence comparisons of worm, boll weevil, frog, and chicken vitellogenins. Dot matrix comparison of amino acid sequences of vitellogenins of different species. a) Comparison of chicken and *Xenopus* vitellogenins. b) Comparison of *Xenopus* and *C. elegans* vitellogenins. c) Comparison of *Xenopus* and boll weevil vitellogenins. d) Comparison of *C. elegans* and boll weevil vitellogenins. Alignments and sequence similarity data were generated using the COMPARE and DOTPLOT programs from th GCG sequence analysis software package. This figure was kindly generated by P. Trewitt and K. Kumaran as the boll weevil sequence is in press and not yet in the databases.

A detailed analysis of the biochemistry of precisely what is bound to the yolk proteins of *Drosophila*, where it is bound within the protein, and at what stage in the synthesis, transport, and storage of yolk it is added is needed.

The X-ray crystal structure of a number of lipases has been determined (58, 59). The determination of the structure of the *Drosophila* yolk protein would allow a much better comparison of the overall shape and potential similarities in function of these molecules. It would certainly help to have the sequences of some of the other vitellogenins that have been characterized, especially the locust, where steroid binding has been reported.

Tests to see whether any other molecules such as cholesterol are carried into the oocyte with vertebrate vitellogenins would be of value.

One of the most exciting prospects for a better understanding of the evolution of these proteins would be to look at proteins with sequence similarities but different

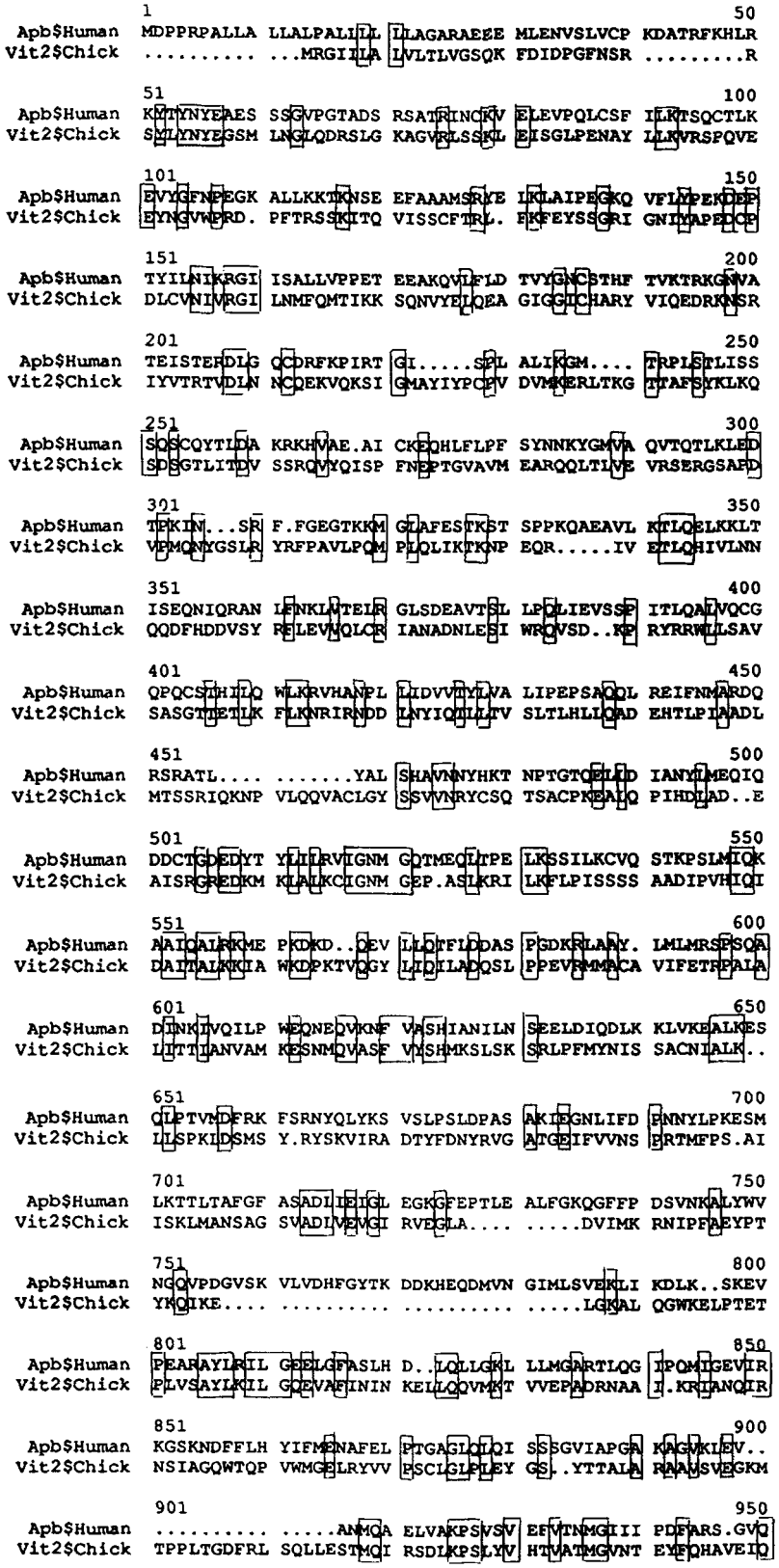
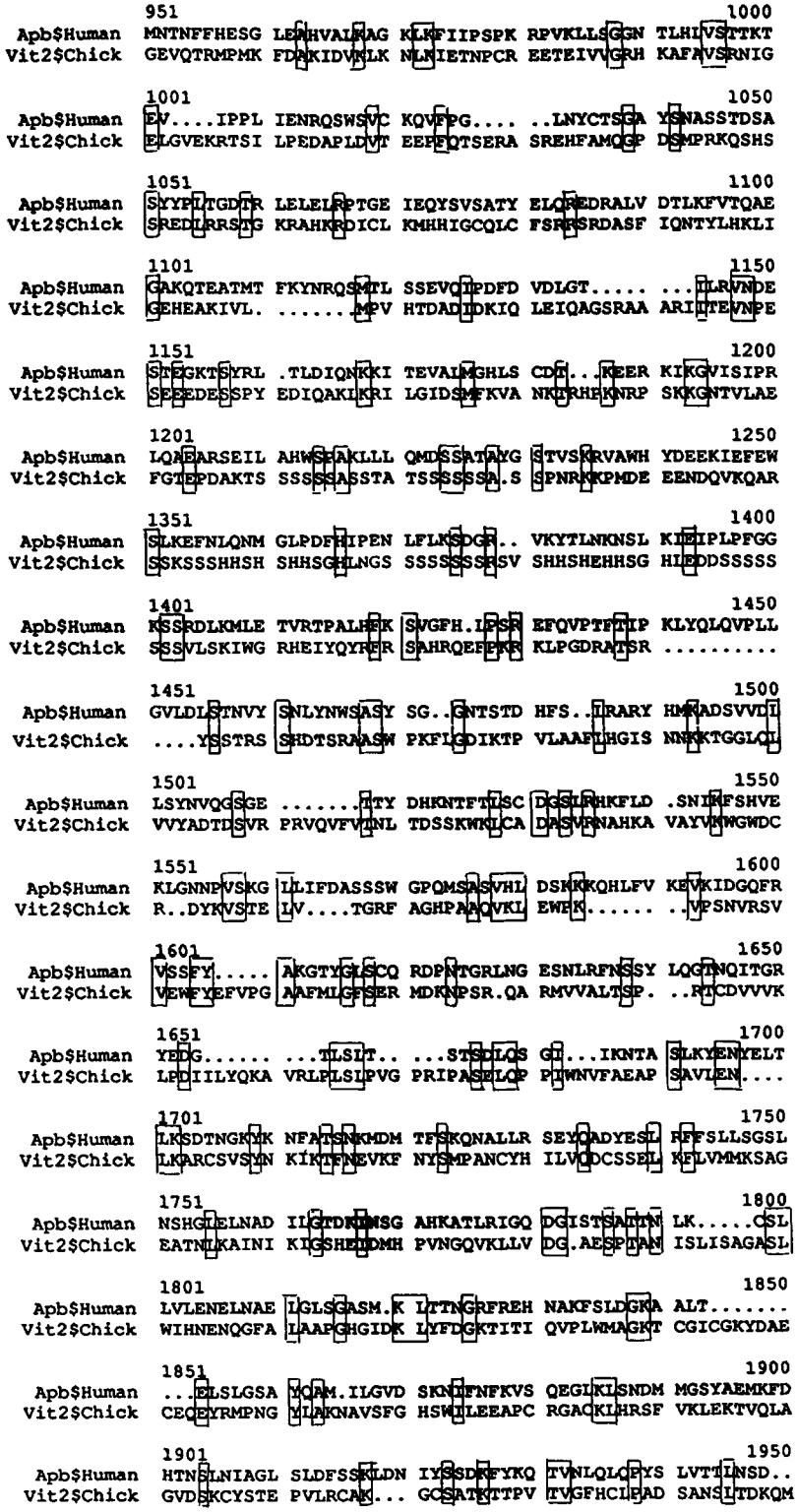


Fig. 7. Alignment of part of the apolipoprotein B-100 protein with the chicken vitellogenin 2 gene. Numbers represent amino acid position; boxed amino acids are conserved.



functions in the same organism. This can be done in two ways. First by looking at the vitellogenin/apolipoprotein B-100 similarity, and second by looking at the lipase/yolk protein similarity. At the moment we do not have details of both classes of protein in one organism.

Although largely dismissed, mammalian oocytes do contain yolk. A molecular characterization of mammalian yolk and the determination of whether or not it is related to vitellogenins will be very interesting, and may help us to understand their relationship with the mammalian serum proteins already known to be related to the vertebrate vitellogenins.

Another area that would help tremendously in understanding how these groups of proteins have evolved would be to investigate the lipases present in dipteran insects. The requirements for storage of lipids from digestion in the fat body for utilization during metamorphosis and the utilization of stored lipids for supplying energy to peripheral tissues, such as the flight muscles, must be large, and require lipases that respond to different hormones and perform slightly different functions just as in vertebrates. Lipophorin, the major lipoprotein of insect hemolymph is assembled from two subunits in response to adipokinetic hormone (60, 61), and collects diacylglycerol from the fat body and shuttles it to the recipient tissues (62, 63). There is much known about the biochemistry of the lipophorins in various insects, but only apolipophorin III, which increases the lipid carrying capacity of the lipophorin complex, has been analyzed at the molecular level (63). To my knowledge, the lipases involved in fatty acid metabolism have not been characterized in any insect. Investigation in *Drosophila* to see if proteins exist with homology to the lipoprotein lipase family that function as lipases would be most important in understanding not only lipid metabolism better but also in determining how these families of proteins might have acquired different functions during evolution.

I would like to thank Professor Steve Yeaman and Gabriele Smith for collaborating on the analysis of *Drosophila* lipases, Nian Zhang and Mike Greaves for helping to produce the figures, and Roger Gosden, Alan Shirras, and Steve Yeaman for checking the manuscript. Professor K. Kumaran and Mr. P. Trewitt kindly permitted us to use data prior to publication. Our studies on the *Drosophila* yolk proteins have been supported by the MRC and SERC, and our searches for *Drosophila* lipases were supported by the Wellcome Trust.

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