Two Distinct Yolk Lipoprotein Complexes from Caenorhabditis elegans*

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The four yolk polypeptides of the nematode Caenorhabditis elegans are found in two types of lipoprotein particle: 12 S particles with \( M_r \) estimated at 450,000 and 8 S particles with \( M_r \) estimated at 250,000. Both types of particle contain approximately 8% phospholipids, 3% triglycerides, and 3% other lipids by mass. All four C. elegans yolk polypeptides can be found in either 12 or 8 S particles, depending upon the conditions of isolation. While the properties of the 12 and 8 S lipoprotein particles are consistent with a dimer-monomer relationship, the asymmetric distribution of the yolk polypeptides between 12 and 8 S fractions suggests that at least two different oligomeric lipoprotein complexes are present in C. elegans embryos.

In order to clarify the subunit composition of the C. elegans yolk lipoproteins, the patterns of polypeptides retained in immunoaffinity binding procedures by immunoglobulins of different antigenic specificities have been compared. When immunoaffinity binding is performed in the absence of sodium dodecyl sulfate, three C. elegans yolk proteins (yp170A, yp115, and yp88) are retained together by polyclonal immunoglobulins directed against either yp115 or yp88. A monoclonal immunoglobulin also retains these three proteins together. In contrast, a second monoclonal immunoglobulin retains only the fourth yolk protein (yp170B). Aggregate species, evidently reflecting the spontaneous formation of interchain disulfide bonds, indicate that yp170A and yp88 are physically associated, whereas yp170B self-associates in dimers. It is concluded that there are two distinct lipoprotein complexes in C. elegans: the A complex, which consists of yp170A, yp115, and yp88 and is essentially heterodimeric, and the B dimer, a simple dimer of yp170B.

Comparisons of sequence data for vitellogenin (yolk protein precursor) genes from the frog Xenopus laevis, the chicken, and the nematode Caenorhabditis elegans have revealed substantial conservation of yolk protein sequences over a large evolutionary interval (1, 2). Because yolk proteins are synthesized outside the ovary, taken up selectively by the oocyte, and sequestered within the oocyte and embryo in membrane-bounded organelles, it seems likely that conserved structural features would include those involved in receptor binding, endocytosis, and intracellular sorting. A second aspect of yolk protein structure that may explain the observed sequence conservation is suggested by the association of yolk proteins with lipids. The native yolk protein species of the vertebrates and lamprey are the lipovitellin-phosvitin complexes, oligomers of 2-fold symmetry derived from the corresponding vitellogenin dimers, and carrying approximately 15–20% lipid by weight (3–5). If the yolk proteins of C. elegans are also lipoproteins, it may be that the observed sequence conservation reflects in part the structural requirements of lipid binding and transport. The idea that lipid transport is an important function of yolk proteins is reinforced by the observation of sequence similarities between vitellogenin genes and the gene encoding human serum apolipoprotein B-100 (6).

Although much of the previous work defining the structure of yolk lipoproteins has focused on vertebrates, especially the frog, it seems that the best chance for correlating structure with biological function may now lie in the simple genetically tractable nematode. The yolk proteins of C. elegans have been characterized as isolated polypeptides (7). The genes encoding them have been cloned (8, 9) and the vit-2, vit-5 (10), and vit-6 genes have been sequenced completely. Taken together, this work reveals that a multigene family in C. elegans encodes at least three abundant primary gene products which are synthesized in intestinal cells of the adult hermaphrodite (11). Two of these proteins (yp170A and yp170B) accumulate essentially intact in oocytes. The third product is cleaved after secretion from the intestine to yield two smaller yolk proteins, yp115 and yp88 (12). This pattern of protein processing is markedly different from that observed in vertebrates, where vitellogenins are cleaved after uptake by the oocyte to yield two lipovitellins and phosvitin (13). Here we report results which indicate that two distinct lipoproteins, each similar in size and lipid content to the lipovitellin-phosvitin complexes of vertebrates and lamprey, are present in C. elegans.

EXPERIMENTAL PROCEDURES AND RESULTS§

Physical Properties of 12 and 8 S Particles—The isolation of 12 and 8 S yolk protein particles from C. elegans embryos is described in the Miniprint Supplement. All four of the previously described yolk polypeptides may be found in either 12 or 8 S fractions, depending on the conditions of isolation. In general, however, yp170A, yp115, and yp88 are found together in the 12 S fraction, whereas yp170B predominates in the 8 S fraction.

Estimates of effective particle diameter, buoyant density, endocytosis, and intracellular sorting. A second aspect of yolk protein structure that may explain the observed sequence conservation is suggested by the association of yolk proteins with lipids. The native yolk protein species of the vertebrates and lamprey are the lipovitellin-phosvitin complexes, oligomers of 2-fold symmetry derived from the corresponding vitellogenin dimers, and carrying approximately 15–20% lipid by weight (3–5). If the yolk proteins of C. elegans are also lipoproteins, it may be that the observed sequence conservation reflects in part the structural requirements of lipid binding and transport. The idea that lipid transport is an important function of yolk proteins is reinforced by the observation of sequence similarities between vitellogenin genes and the gene encoding human serum apolipoprotein B-100 (6).

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Yolk Proteins Retain Noncognate Yolk Proteins in the Absence of SDS—Although the physical properties of the 12 and 8 S yolk protein particles are consistent with a dimer-monomer relationship between 12 and 8 S particles, the buoyant density values obtained are significantly lower than the value of 1.30 g/ml conventionally taken as typical of simple proteins and are thus consistent with the presence of lipids in both types of particle. In work not shown here, 12 and 8 S particles were stained in nondenaturing gels with the lipophilic dye Sudan black, providing further evidence of direct association of lipids with the particles.

Lipid Content of 12 and 8 S Particles—Thin-layer chromatography of lipid extracts, as described in the Miniprint Supplement, indicates that the principal polar lipids associated with the yolk protein particles are phosphatidylcholine and phosphatidylethanolamine. The principal neutral lipid component exhibits chromatographic mobility typical of triglycerides. Densitometry indicates that, together, phospholipids and triglycerides contribute 70–80% of the lipid mass detected by staining with Coomassie Blue. The remaining lipid mass resides in several minor components, the most abundant of which are neutral and migrate similarly to cholesterol. Table II presents summary estimates of lipid contents for 12 and 8 S particles based on the chromatographic results and phosphorus and fatty acid determinations, as described in the Miniprint Supplement. We have detected no significant difference in lipid content between 12 and 8 S particles.

Polycyclonal Igs Directed against Purified SDS-denatured Yolk Proteins Retain Nongcognate Yolk Proteins in the Absence of SDS—Although the physical properties of the 12 and 8 S yolk protein particles are consistent with a dimer-monomer relationship, the asymmetric distribution of the yolk polypeptides between 12 and 8 S fractions suggests that more than one yolk lipoprotein complex is present in C. elegans embryos. In order to detect specific associations of the yolk polypeptides, we have compared the results of immunoaffinity binding experiments conducted under different conditions. In previous work (12), polyclonal Igs arising in response to SDS-denatured and electrophoretically purified yolk polypeptides have been shown to distinguish yp115 and yp88 from each other and from the two yp170s. This specificity, however, was achieved in the presence of detergents (buffer NS, 1.0% Nonidet P-40 and 0.1% SDS), which would be expected to disrupt native lipoprotein structures. Fig. 1 compares the results of protein A-mediated immunoaffinity binding by polyclonal Igs under the original detergent conditions (NS), in the presence of 0.05% Nonidet P-40 only (buffer N), and in the absence of detergent (buffer 0) (o). Results in the presence of 0.05% Nonidet P-40 differ markedly from those obtained under the original conditions of 1.0% Nonidet P-40 and 0.1% SDS. Although the affinity matrix selectively retains the cognate yolk protein(s) with each Ig under the original high-detergent conditions, both anti-yp115 and anti-yp88 Igs retain the other of the smaller proteins in addition to the cognate species in the presence of 0.05% Nonidet P-40 only. Furthermore, both anti-yp115 and anti-yp88 Igs retain substantial amounts of material co-migrating with the yp170 doublet. The electrophoretic patterns obtained with the polyclonal Igs and unfractionated nematode homogenates are typically equivocal on the identity of the yp170 species retained by anti-yp115 and anti-yp88 Igs. However, it is clear in experiments conducted with purified 12 S yolk protein

These results contrast in one respect with those of the earlier work; here, Ig directed against yp88 consistently retains small but significant amounts of yp170A. An experiment shown in the Miniprint Supplement (Fig. 11) demonstrates that anti-yp88 Ig can bind yp170A directly, suggesting that the two proteins have antigenic features in common. In the earlier experiments, however, all three polyclonal Ig preparations displayed a high degree of specificity for the original antigen. Most probably, this variation reflects the fact that the Igs used here, whereas arising in response to the same antigen preparations as those used in the earlier experiments, are from different animals. In addition, they have been stored for several years and may have undergone selective loss of some Igs, resulting in a different effective profile of determinant recognition.
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particles (Fig. 10 of Miniprint Supplement) that the yp170 species retained by these Igs is yp170A. Furthermore, as shown in Fig. 2 below, yp170A is selectively retained with the two smaller proteins by monoclonal Ig OIC1 in experiments with unfractonated homogenates. As discussed below, the doublet character frequently observed for ypl70A retained by these Igs is apparently due to heterogeneity in the yp170A population.

The polyclonal Igs exhibit little specific affinity for the yolk proteins in the absence of detergent (Fig. 1, buffer 0). This is unsurprising, given that the Igs originated in response to SDS-denatured antigens. Evidently, many determinants exposed by SDS denaturation are inaccessible in the native lipoprotein structure.

Monoclonal Igs Recognize Yolk Protein Epitopes in the Absence of Detergent—The facilitation by Nonidet P-40 of antigen-Ig binding in the experiments of Fig. 1 suggests that the detergent can alter the structure of the native yolk lipoproteins. It was therefore important to confirm, in the absence of detergent, the results obtained with the polyclonal Igs in the presence of Nonidet P-40. Three monoclonal Igs recognizing yolk protein epitopes, isolated by other workers and generously passed on to us, provide this confirmation.

Fig. 2 shows the results of binding experiments with Igs OIC1 and PIIA3 (from S. Strome, Indiana University) and Ig M1 (from E. Hedgecock, Johns Hopkins University). Presumably because the material used to immunize animals prior to hybridoma production in these instances was not exposed to detergent, these antibodies exhibit binding activities that stand in counterpoint to those of the monoclonal Igs described above. That is, they fail to bind yolk proteins in the high-detergent SDS-containing buffer (NS) but bind the proteins efficiently either in Nonidet P-40 alone (N) or in the absence of detergent (O). Ig PIIA3 is clearly specific for yp170B, whereas Ig M1 retains material exhibiting mobility closest to that of yp170A. M1-retained material, however, typically resolves into a doublet, the two bands of which are more closely spaced than those of the yp170A/yp170B doublet evident on electrophoresis of crude homogenates. It is this doublet character of Ig-retained yp170A that complicates interpretation of some gels, such as that in Fig. 1N. In Fig. 2, however, it is clear that this doublet species is electrophoretically distinct from yp170B. It appears to reflect an element of heterogeneity in the yp170A population.

In contrast to the clear specificities of Igs M1 and PIIA3 for yp170A and yp170B respectively, Fig. 2 shows that Ig OIC1 retains yp170A, yp115, and yp88 together in a pattern similar to that observed with the anti-yp115 and anti-yp88 polyclonal Igs (Fig. 1V). Because OIC1 does not retain any yolk protein species in the presence of SDS, it is not possible to determine whether it recognizes an epitope specific to a single polypeptide. By analogy with the results obtained with polyclonal Igs, however, it seems likely that the OIC1 Ig recognizes some feature of yp115 or yp88.

Disulfide Cross-linking Demonstrates the Association of yp170A with yp88 and the Self-association of yp170B in Dimers—In the electrophoretic analyses of Ig-retained material shown in Figs. 1 and 2, samples were treated with 2-mercaptoethanol before electrophoresis in order to reduce disulfide bonds to cysteine. When samples duplicating those shown in Fig. 10 are electrophoresed without prior reduction, strikingly different results are obtained, as shown in Fig. 3A. Marked changes in electrophoretic mobility are observed for yp170A, yp170B, and yp88 and are discussed below. In the present context, however, the critical result is the retention by both Ig OIC1 and Ig PIIA3 of species exceeding any of the yolk polypeptides in apparent Mr. Ig OIC1 yields a single additional band (band 4), whereas three additional bands (bands 1, 2, and 3) are present in the PIIA3-retained material. In experiments not shown here (described in the Miniprint Supplement) we have estimated the Mr of these additional nonreduced species by comparison to the mobilities of cross-linked

![Fig. 2. Immunoaffinity binding of 35S-labeled yolk proteins by monoclonal Igs. Binding procedure, elution of retained proteins, and electrophoresis are described in the Miniprint Supplement. Each panel shows electrophoresis of material retained by Ig PIIA3, Ig M1, Ig OIC1, as indicated. The left panel shows results of binding in buffer NS, the center panel shows results of binding in buffer N, and the right panel shows results of binding in buffer O (no detergent). Note that the order of Ig presentation is different in the buffer N experiment to emphasize the electrophoretic resolution of yp170A and yp170B. Gels were 5% in acrylamide. The upper third of the gels is not shown.](image-url)

![Fig. 3. A nonreducing SDS gel electrophoresis of yolk protein species retained by monoclonal Igs. The analyses duplicate those shown in Fig. 20 except that 2-mercaptoethanol was omitted from the sample buffer. The electrophoresis of reduced proteins is shown at the extreme left for comparison. Gels were 5% in acrylamide. B, re-electrophoresis of excised aggregate bands from nonreducing gel after treatment with 2-mercaptoethanol. Material retained by monoclonal Igs OIC1 and PIIA3 was electrophoresed in a nonreducing gel, with unlabelled yolk proteins in adjacent lanes as markers. After brief staining and de-staining, labeled bands 1, 2, 3, and 4 were excised from the gel and incubated for 30 min at room temperature in 0.125 M Tris-HCl, pH 6.8, 0.1% SDS, 0.1 M 2-mercaptoethanol. The gel slices were then placed in the sample wells of a second gel, overlaid with reducing sample buffer, and electrophoresed as usual. Gel was 5.5% in acrylamide.](image-url)
phosphorylase b multimers. This technique yields values of 379,000 for PIIA3-retained material (bands 1, 2, and 3 are unresolved in the gel system used for the phosphorylase b multimers) and 246,000 for OIC1-retained material (band 4).

In addition to the appearance of high-\(M_r\) bands under nonreducing conditions, the electrophoretic mobilities of \(y\)p170A, \(y\)p170B, and \(y\)p88 are markedly affected by reduction status. Unreduced \(y\)p88 migrates more rapidly than its reduced form, whereas the two unreduced \(y\)p170 polypeptides migrate more slowly than their reduced counterparts. The degree of retardation is greater for \(y\)p170B (retained by Ig PIIA3) than for \(y\)p170A (retained by Igls OIC1 and M1), such that \(y\)p170B is the slower of the two unreduced proteins, inverting the order in which they run after reduction.

The simplest explanation for the appearance of high-\(M_r\) bands under nonreducing conditions is the formation of disulfide-linked aggregates containing more than one yolk polypeptide. In order to determine the composition of bands 1-4, each band was excised from a nonreducing gel, incubated in a buffer containing 2-mercaptoethanol, and re-electrophoresed in a second gel (Fig. 3B). Reduction of band 4 yields both a \(y\)p170 species and \(y\)p88, whereas reduction of bands 1, 2, and 3 yields only a \(y\)p170 species. \(y\)p170A and \(y\)p170B are not resolved on re-electrophoresis of bands excised from nonreducing gels. However, it has been shown above (Fig. 2) that Ig OIC1 selectively retains \(y\)p170A, whereas Ig PIIA3 selectively retains \(y\)p170B. Thus, the \(y\)p170 species recovered from bands 1, 2, and 3 (retained by PIIA3) is presumably \(y\)p170B. A dimer of \(y\)p170B would have an aggregate polypeptide mass of 372,000 daltons (calculated as described in the Miniprint Supplement). This is consistent with the estimate of 379,000 given above for the \(M_r\) of the PIIA3-retained aggregate species. It seems likely, then, that bands 1, 2, and 3 all correspond to disulfide-linked \(y\)p170B dimers, but reflect the three possible combinations of reduced and unreduced \(y\)p170B monomers. Similarly, the recovery of \(y\)p88 along with a \(y\)p170 polypeptide from band 4 (retained by OIC1) indicates that band 4 consists of \(y\)p170A and \(y\)p88, linked by one or more interchain disulfide bonds. A complex of \(y\)p170A and \(y\)p88 would have a calculated mass of 268,000 daltons. This is consistent with the \(M_r\) value of 246,000 obtained for band 4. The fluorographic density of the \(y\)p88 band relative to the \(y\)p170A band in the material recovered on reduction of band 4 is 0.38, compared with a value of 0.48 predicted for a 1:1 complex as described in the Miniprint Supplement.

Disulfide Cross-linking Occurs Spontaneously upon Depletion of an Endogenous Reducing Agent—In contrast to \(y\)p88, which occurs only in its unreduced form, both reduced and unreduced forms of the \(y\)p170 polypeptides are present in nematode homogenates, with the proportion of reduced to unreduced varying from one preparation to another. Most typically, the reduced forms predominate, and the disulfide-linked aggregate species of bands 1-4 are faint or undetectable. Both the reduced status of the \(y\)p170 polypeptides and the absence of the cross-linked aggregates, however, appear to depend on the presence of an endogenous reducing agent of low molecular weight. Fig. 4 shows that if the concentration of this endogenous agent is decreased by dilution or dialysis, the \(y\)p170 polypeptides shift to their unreduced mobilities, and the amount of material accounted for by the cross-linked aggregates increases.

The Predominant Native Yolk Protein Species Are 12 S Particles—The disulfide-linked aggregates of \(y\)p170B evident in Fig. 3 indicate that at least some of the \(y\)p170B in a nematode homogenate is present in dimers, the mass of which corresponds to that of the 12 S lipoprotein particles described above. However, although it is clear in Fig. 9A (Miniprint Supplement) that \(y\)p170B can be a component of the 12 S fraction, most of the \(y\)p170B is found in the 8 S fraction upon sucrose gradient sedimentation, presumably corresponding to monomeric \(y\)p170B. In order to assess the distribution of yolk proteins between the monomer and dimer populations in preparations that have not been subjected to density gradient sedimentation, we have resolved \(38^{S}\)-labeled whole animal homogenates by nondenaturing gel electrophoresis, as shown in Fig. 5.

Because the yolk proteins are among the most intensively labeled products in such homogenates, and because much of the labeled material remains at the origin in the absence of denaturants, the distribution of yolk protein species in the gel is evident in spite of the application of a crude sample. Fig. 5A shows that, in a freshly prepared homogenate, 12 S particles (dimers) are the predominant yolk protein particles. Densitometry indicates that the fluorographic intensity of the
dimer band in Fig. 5A is approximately four times that of the 8 S (monomer) region. It is also clear from the results in Fig. 5, B and C, that, on storage of the homogenate, particularly on freezing and thawing, 17 S dimers are progressively broken down to 8 S monomers.

**Conclusions**

More Than One Yolk Lipoprotein in *C. elegans*—A simple complex of all four *C. elegans* yolk polypeptides would have an aggregate mass of approximately 560,000 daltons, exclusive of associated lipid (calculated as described in the Miniprint Supplement). Clearly, the properties of the 12 S particles described here are inconsistent with a single yolk lipoprotein in the nematode. Furthermore, the asymmetric distribution of the *C. elegans* yolk polypeptides between 12 and 8 S fractions, with ypl70B predominating in 8 S and the other three proteins predominating in 12 S, suggests that more than one type of dimeric particle is present in the nematode.

A Single Lipoprotein Complex Contains ypl70A, yp115, and yp88—The retention of several yolk proteins by polyclonal Igs specific under other conditions for single polypeptides (Fig. 1) has two possible explanations. On one hand, the noncognate proteins may be physically associated with the cognate species and hence retained indirectly by the Ig. This explanation postulates a single lipoprotein complex containing ypl70A, yp115, and yp88. A single complex of ypl70A, yp115, and yp88 is also consistent with the retention of these three proteins together by monoclonal Ig OIC1 (Fig. 2).

Alternatively, it could be argued that, at lower detergent concentration and in the absence of SDS, conformational changes may occur to some proteins survive whereas they are denatured at high detergent concentrations or by SDS. In fact, a portion of the ypl70A retained by Ig OIC1 and anti-yp88 Ig may be attributable to direct binding of ypl70A by these Igs, as shown in Fig. 11 (Miniprint Supplement). In Figs. 1 (main text) and 10 (Miniprint Supplement), however, it is clear that anti-yp115 Ig, which does not bind ypl70A directly, nevertheless retains ypl70A in addition to yp115 and yp88. This is most readily explained if all three proteins are present in a single particle. Furthermore, because the polyclonal Igs were elicited in response to SDS-denatured proteins, it seems unlikely that Igs recognizing native folded structures would be abundant in these preparations. Yet the yield of retained proteins is similar in buffers NS and N (Fig. 1), suggesting that similar effective titers of Igs are present in both cases and arguing against the conformational determinant model.

The association of ypl70A with yp88 is directly supported by the existence of a disulfide-linked complex of these two proteins (Fig. 3). Moreover, upon dilution, most of the ypl70A and yp88 present in a sample can be driven into this disulfide-linked pair (Fig. 4). Although no similarly direct evidence for the association of yp115 with ypl70A and yp88 is available, a single complex of these three proteins is consistent not only with the retention of all three proteins by Igs, but also with several observations described in the Miniprint Supplement. First, ypl70A, yp115, and yp88 co-sediment in the 12 S fraction of sucrose gradients (e.g. Fig. 7), and all three proteins appear coincidently in the 8 S fraction after exposure to detergent (Fig. 8) or on repeated sedimentation (Fig. 6B). Second, densitometry of the 12 S sample shown in Fig. 10 yields intensities (relative to ypl70A = 1.0) of 0.75 and 0.54 for yp115 and yp88, respectively. The corresponding intensities, predicted as described in the Miniprint Supplement for a complex containing one copy of each polypeptide, are 0.74 and 0.48. Finally, yp115 and yp88 co-sediment with the ypl70 polypeptides in the 8 S fraction (Fig. 8), suggesting that the two smaller yolk proteins remain associated after dissociation of the 12 S complex. Because yp115 and yp88 originate in a single primary gene product (12) with demonstrated homology to the genes encoding the ypl70 polypeptides (9), it seems likely that they constitute a single functional unit despite proteolytic cleavage. Thus, a complex of ypl70A, yp115, and yp88 may be seen to dimerize essentially heterodimeric.

One aspect of the immunofluorescence binding results appears to conflict with this interpretation. Monoclonal Ig M1, which clearly recognizes ypl70A, does not retain significant amounts of yp115 or yp88 (Fig. 2). Similarly, polyclonal Igs directed against the ypl70 polypeptides retain little or none of the smaller yolk polypeptides (Fig. 1). These results can be reconciled with the postulated complex of ypl70A, yp115, and yp88 if the material retained by these Igs is monomeric. Indeed, monomers are probably abundant in the binding reactions involving the polyclonal Igs, because these Igs bind the yolk proteins only in the presence of detergent. The dissociative effect of Nonidet P-40 is noted in the Miniprint Supplement (Fig. 8). Monoclonal Ig M1, however, retains little or no yp115 or yp88 even in the absence of detergent (Fig. 2). This result can be reconciled with the proposed complex only if the M1 Ig has a higher affinity for ypl70A as a monomer than for the same protein as part of the complex with yp115 and yp88. Monomer specificity for Ig M1 would also be consistent with the observation that, in contrast to Igs OIC1 and PIIA3, Ig M1 retains no disulfide-linked aggre gate species (Fig. 3A). Such specificity could arise if the epitope recognized by M1 is buried or masked in the structure of the ypl70A-yp115-yp88 complex. The experiment of Fig. 10 (Miniprint Supplement) shows clearly that Ig M1 does not bind ypl70A as it occurs in 12 S particles, substantiating this explanation of the results.

The Native Structure of ypl70B Is a Dimer—Fig. 5 shows that 12 S particles are the predominant yolk protein species in a fresh nematode homogenate. Taking this result with the demonstration of disulfide-linked ypl70B dimers in Fig. 3, we conclude that the native structure of ypl70B is a dimer. An absence of interaction between ypl70B and the other yolk polypeptides explains the retention of ypl70B alone by monoclonal Ig PIIA3 (Fig. 2). The selective partition of ypl70B to the 8 S monomer fraction in density gradients, then, suggests that the ypl70B dimer is more susceptible to dissociation during sedimentation than the complex of ypl70A, yp115, and yp88.

The A Complex and B Dimer—The molecular masses for the ypl70B dimer and ypl70A-yp115-yp88 complex, based on the calculated masses of the yolk polypeptides and the lipid contents determined here, are estimated to be 457,000 and 439,000 daltons, respectively. These values agree well with the estimate of M, 450,000 for 12 S particles derived from physical properties (Table I). For simplicity, we propose to refer to these two particles henceforth as the “A complex” (yp170A-yp155-yp88) and the “B dimer” (yp170B-yp170B). Fig. 6 summarizes this model in the general context of yolk lipoprotein biosynthesis and transport in *C. elegans*.

Our results also argue against the four alternative dimeric configurations of yolk polypeptides. Monoclonal Ig PIIA3, which clearly binds dimeric ypl70B, would reasonably be expected to recognize the protein in a heterodimer with ypl70A as well. The fact that PIIA3 does not retain significant amounts of ypl70A (Fig. 2) suggests that ypl70A/ypl70B heterodimers, if they exist at all, are not abundant. Similarly, the specific retention of ypl70A with yp115 and yp88 by Ig OIC1 (Fig. 2) indicates that pairing of ypl70B with the yp115/
Fig. 6. Schematic summary of yolk lipoprotein biosynthesis in *C. elegans*. The representations of monomers are intended to emphasize the structural and functional similarities of the primary gene products, not as indications of actual geometry. Lipid is not indicated. It is known that the precursor to yp115 and yp88 is secreted intact from the intestine and that cleavage of the precursor occurs independent of uptake by the oocyte. It is not known whether cleavage occurs before or after assembly of the A complex.

The composition of the 12 S fraction, although consistent with the proposed stoichiometry of the A complex, does not in itself rule out the existence of yp170A dimers and (yp115)_2(yp88)_2 heterotetramers. In the dilution experiment of Fig. 4, however, it is clear that most of the yp170A and yp88 in the homogenate can be accounted for in the disulfide-linked yp170A-yp88 species. Thus, if yp170A dimers and (yp115)_2(yp88)_2 heterotetramers are present, they must be minor components. In summary, there is no direct evidence for the existence of any of the alternative oligomeric complexes, and several observations argue that they do not constitute a major proportion of the native yolk lipoprotein population.

**DISCUSSION**

**Yolk organelles in *C. elegans***—We have observed that a particulate fraction of nematode homogenates is highly enriched for the yolk proteins. Presumably, this reflects the sequestration of the proteins in yolk organelles. Yolk organelles, variously termed yolk platelets, granules, or spheres, are conspicuous electron-dense inclusions in negatively stained sections of oocytes and embryos from many organisms. The coincidence of yolk proteins with such structures in *C. elegans* is indicated by the results of Doniaich and Hodgekin (14). In that work, a mutant exhibiting maculization of somatic tissues was found to synthesize no yolk proteins and also lacked the electron-dense inclusions in otherwise morphologically normal oocytes.

**Heterogeneity in yp170A**—In the experiments of Figs. 1 and 2, yp170A migrates as a doublet. Such heterogeneity could result from proteolysis or other modification of the yp170A polypeptide. The yp170A doublet could also reflect the activity of multiple genes. The *C. elegans* genome contains three genes (vit-3, vit-4, and vit-5) with the potential to code for yp170A (8) and recent results indicate that vit-3, as well as vit-5, is transcribed.

**Disulfide Bond Formation**—The difference in electrophoretic mobility observed between reduced and unreduced yp88 (Fig. 3A) implies that the native structure of yp88 includes at least one disulfide bond. Similarly, it is likely that the conversion of yp170A and yp170B to more slowly migrating species on dilution or dialysis (Fig. 4) reflects the formation of intramolecular disulfides. Changes in electrophoretic mobility presumably accompany the formation or reduction of disulfides because covalent cross-linkage constrains the polypeptide in a compact conformation. A smaller effective particle diameter would be expected to increase mobility relative to the reduced polypeptide, as is observed for yp88. Decreased mobility for the oxidized forms of the yp170s, on the other hand, could result if cross-linkage prevents binding of SDS to buried regions of the polypeptide. This would decrease the negative charge density of the particle relative to the reduced and fully unfolded molecule.

The facile conversion of yolk proteins from reduced to unreduced and cross-linked forms on dilution or dialysis (Fig. 4) probably accounts for the observation that Ig-retained material is enriched for the unreduced and cross-linked forms over their abundance in the crude homogenate (Fig. 2A). Conversion of Ig-bound yolk proteins to the unreduced forms could occur either during the binding reaction, in which the homogenate is substantially diluted, or during the washing steps, which would further deplete the endogenous reducing agent.

The spontaneous formation of interchain disulfides in the *C. elegans* yolk lipoproteins has proven useful in defining the subunit structures of the complexes. However, the biological significance of these interchain cross-links and of the intramolecular disulfides that apparently form on oxidation of the yp170s remains to be established. The fortuitous oxidation of cysteine sulfhydryls is not uncommon in proteins exposed to oxygen. On the other hand, cysteine residues are highly conserved between nematode and vertebrate vitellogenins (2), suggesting that disulfide bonds may be important in yolk protein structure and function. Most intriguingly, a region containing 6 conserved cysteines near the carboxyl terminus of vitellogenin exhibits striking sequence similarity to a portion of the repeated D domain of human von Willebrand factor (15). The D domain occurs twice in the von Willebrand factor propeptide, which has been shown to mediate the assembly of disulfide-linked von Willebrand multimers (16). It may be that the transport, sorting, and utilization of yolk lipoproteins require at some point the formation or rearrangement of specific disulfide bonds and that the spontaneous cross-linking observed in our preparations is a reflection of this function.

**Similarities and Contrasts between the Yolk Lipoproteins of *C. elegans* and the Lipovitellin Complexes of Vertebrates and Lamprey**—The sequence homologies detected between *C. elegans* vitellogenin genes and vitellogenin genes from *Xenopus* and chicken (1, 2) imply that elements of yolk protein structure have been conserved in the evolution of oviparous vertebrates from simple invertebrates. Our characterization of native yolk lipoprotein particles from *C. elegans* bears out this implication. Estimates of M, and sedimentation coefficient for the vertebrate lipovitellin-phosvitin complexes vary from

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4 T. Blumenthal, personal communication.
Two Distinct Yolk Lipoproteins

dimer (the B dimer) and the essentially heterodimeric A complex. Thus both nematode yolk lipoproteins exhibit the 2-fold symmetry of the vertebrate yolk lipoproteins, which are derived from dimers of the vitellogenin polypeptide such that the lipovitellin-phosvitin complex retains dimeric character despite cleavage of vitellogenin to yield the smaller lipovitellins and phosvitins.

Although the *C. elegans* yolk lipoproteins clearly resemble those of vertebrates and lamprey in their size, dimeric organization, and associated lipid, differences in polypeptide composition and processing are evident. The vertebrate and lamprey monomeric units are composed of two lipovitellins and a phosvitin. In the frog, these proteins have been shown to arise via proteolytic cleavage of vitellogenin after uptake by the oocyte (13), and it seems likely that a similar sequence of events occurs in the lamprey. In contrast, although the *C. elegans vit* genes are significantly homologous to the vertebrate vitellogenin genes, they lack the serine-rich phosvitin domain of the vertebrate vitellogenins (2). Furthermore, although the *C. elegans vit-6* gene product is cleaved to yield yp115 and yp88 (9, 12), efficient synthesis of these proteins by animals in which germ line and somatic gonadal cells have been eliminated indicates that cleavage can occur independent of uptake by the oocyte (11). Finally, the two yp170 polypeptides of *C. elegans* accumulate essentially intact in oocytes, unlike the vertebrate and lamprey vitellogenins.

These differences, in the context of the sequence homologies and structural parallels noted above, suggest that the proteolytic processing patterns and phosvitin domains of vertebrate and lamprey vitellogenins are not crucial elements in the uptake and sorting of yolk lipoproteins in *C. elegans*. It may be that specific and limited interactions between the monomeric units of a dimeric complex are important for the uptake and sorting of yolk lipoproteins.

Acknowledgments—We are particularly grateful to Tom Krick for assistance with gas chromatographic analysis of fatty acids and to Susan Strome and Ed Hedgecock for their generous provision of monoclonal antibody preparations. We thank Thomas Blumenthal for communication of results prior to publication. We thank Robert Herman, Jocelyn Shaw, Susan Strome, Gary Nelsestuen, and Bridgette Barry for helpful discussions and critical comments on the manuscript.

REFERENCES

EXPERIMENTAL PROCEDURES

Preparation and fractionation of electrophoretograms - All procedures were conducted at 0 - 4°C. For electrophoresis, potassium phosphate buffer (PH 6.9) was used, and the gel was run at 500 volts for 30 minutes. The gel was stained with Coomassie blue and destained with acetic acid.

1. Electrophoresis - Electrophoresis was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

2. Gel staining - The gel was stained with Coomassie blue and destained with acetic acid.

3. Gel electrophoresis - Electrophoresis was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

4. Gel fractionation - Gel fractionation was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

Analysis of liquid content - Lyophilized proteins were dissolved in water to a final concentration of 10 mg/mL. The proteins were then electrophoresed on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

1. Gel electrophoresis - Electrophoresis was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

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The relative proportions of proteins in liquid content were estimated using the Coomassie blue method.

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**References and Notes:**


**Supplementary Material:**

Supplementary data are available with the full text of this article. The data include:

1. Gel electrophoresis - Electrophoresis was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.

2. Gel fractionation - Gel fractionation was conducted on a 30 x 30 cm gel plate at 30 mA for 1 hour. The gel was stained with Coomassie blue and destained with acetic acid.


Quantitation of radiographic results — $^{32}P$-labeled proteins were detected by fluorography as described by Bonner and Laskey (21). Denaturing gels were loaded with $150,000 	ext{ dpm}$ of labeled protein and exposed for 3 days. In several instances, the relative proportions of $y_{170}$, $y_{170}$, and $y_{170}$ were calculated from fluorographic band densities, estimating the molar sulfur contents of the polypeptides as follows. From sequence data, $y_{170}$ is predicted to incorporate 54 sulfur-containing residues and the $y_{170} y_{170}$ precursor is predicted to contain 60. It has also been shown that $y_{170}$ originates in the carboxy-terminal portion of the precursor (21). If it is assumed that cleavage of the precursor occurs so as to yield an amino-terminal fragment of $80,000 	ext{ dtons}$ ($y_{170}$) and a carboxy-terminal fragment of $100,000 	ext{ dtons}$ ($y_{170}$), then it is predicted that $y_{170}$ contains 44 cysteines and methionines, and $y_{170}$ contains 6. Thus, the molar fluorographic densities for $y_{170}$ and $y_{170}$ relative to $y_{170}$ ($y_{170}$) are predicted to be 0.74 and 0.48, respectively.

Materials and Methods

Fractionation of embryo homogenates — The particulate fraction obtained by centrifugation of a clarified embryo homogenate at 12,000 $g$ for 15 min. was isolated for the yolk proteins. Typically, 15 to 20% of the yolk protein in an embryo homogenate is recovered in the 12,000 $g$ pellet. Yolk proteins are readily solubilized from this particulate fraction either by sonication or by extraction with the nonionic detergent NP-40. On sedimentation, the solubilized material reacts into $15$ and $125 S$ species. If the proteins are released from the prepolyosomes by sonication, the $125 S$ fraction contains most of the yolk proteins. In particular, the two major yolk proteins, $y_{170}$ and $y_{170}$, are found only in the $125 S$ fraction (Fig. 1). In contrast, when the proteins are solubilized with NP-40, the amount of material recovered at $15 S$ is increased and includes substantial amounts of $y_{170}$ and $y_{170}$ (Fig. 1).

Figure 7. Sucrose gradient sedimentation of material solubilized from a 12,000 g pellet by sonication. Ten fractions were collected and electrophoresed on a 6% polyacrylamide/3% agarose gel, stained with Comassie blue. Fractions indicated by brackets were pooled for reisololation and electrophoretic analysis (Fig. 9).

Figure 8. Sucrose gradient sedimentation of material solubilized from a 12,000 g pellet by extraction with 0.1% NP-40. Experimental details are as described in the legend to Figure 7.

The distribution of the two large yolk polypeptides $y_{170}$ and $y_{170}$, which are not resolved in the gel of Figures 7 and 8, is shown in Figure 9 for preparations solubilized by either sonication or detergent. Tertia, peak fractions from the 15 and 125 $S$ regions have been electrophoresed on a 6% gel to resolve the $y_{170}$ doublet. It is clear that while both $y_{170}$ polypeptides are present in the 125 $S$ fraction, $y_{170}$ is the major component of the 125 $S$ fraction. Significantly, amounts of $y_{170}$ sediment at 15 $S$ only after detergent treatment, consistent with the appearance of $y_{170}$ and $y_{170}$ at 15 $S$.

Reisololation of 125 and 125 $S$ material recovered from gradients such as that shown in Figure 7 demonstrates that the observed sedimentation velocities are constant properties of the isolated particles (results not shown). Reisololation of 125 $S$ fractions, however, yields a significant amount of $y_{170}$ material, with $y_{170}$ and $y_{170}$ appearing in the 125 $S$ fraction. Figure 10 shows that the 125 $S$ fractions arising from reisololation of 125 $S$ material also contain substantial amounts of $y_{170}$ while the 125 $S$ region is depleted of $y_{170}$. Thus, all four yolk polypeptides may be found in either 125 or 125 $S$ fractions, depending on the conditions of isolation. The shift of material from 125 to 125 $S$ on exposure to detergent or repeated reisololation suggests that these treatments have a dissociative effect on 125 $S$ particles, yielding 125 $S$ particles.
Lipid phosphate and fatty acid contents -- Table III presents the results of analyses for phosphate and fatty acid in lipid extracts from 125 and 95 particles. The largest component of the deviatio

TABLE III

Phosphate and Fatty Acid Contents of Lipid Extracts

<table>
<thead>
<tr>
<th>Species</th>
<th>Total fatty acids (mg per g protein)</th>
<th>Phosphate (mg per g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>0.008 ± 0.001</td>
<td>351 ± 55</td>
</tr>
<tr>
<td>95</td>
<td>0.067 ± 0.004</td>
<td>145 ± 27</td>
</tr>
</tbody>
</table>

 Internacional comparison with other studies in this field shows that the values obtained for the 125 and 95 particles are consistent with the expected composition of lipid extracts.

The epitopes of yolk lipid recognized by monoclonal Ig M are not exposed in 125 particles. -- In contrast to Ig M and Ig M isolated from hemolyzed eggs, Ig M isolated from the yolk of fertilized eggs is not recognized by the monoclonal Ig M used in this study. This suggests that the epitopes recognized by Ig M are buried or masked in the 125 particles. In order to test this possibility, we have performed immunofluorescence binding experiments with labeled 125 and 215 fractions obtained by sedimentation of a labeled homogenate in a sucrose density gradient. Figure 10 shows an electrophoretic analysis of the labeled 325 fractions, and the species retained from 125 by the various Igs. It is clear that very little yolk lipid is retained by monoclonal Ig M, although yolk lipid is abundant in the 125 fraction. This result suggests strongly that the epitopes recognized by Ig M are unavailable for binding when yolk lipid is part of a 125 complex.

Polysaccharide anti-yolk Ig and monoclonal Ig 1001 can bind yolk directly. -- In Figure 11, labeled 95 particles isolated by sedimentation have been incubated with the various Igs in immunofluorescence binding reactions. As in preparations from unincubated eggs (Fig. 9), yolk 1001 is clearly present in the 95 fraction; however, it is also present in the 95 fraction. Significant amounts of yolk 1001 are also retained by the anti-yolk polysaccharide Ig and by monoclonal Ig 1001. We have noted in the main text, in discussing the results of Figure 11, that anti-yolk Ig inhibits this affinity for yolk 1001. Because the 95 fraction contains very little yolk 95 and yolk 1001, this experiment shows that these Igs can bind yolk directly.

The labeled 95 fraction of Figure 11 contains a substantial amount of yolk 950 and very little yolk 950 or yolk 1001. This contrasts with the results of Figure 9, where similar amounts of all three proteins appeared coincidently in the 95 fraction upon repeated sedimentation or treatment with detergent. Most probably, this difference reflects the fact that the 25-labeled 95 fraction was isolated from a whole nest containing homogenate while the unlabelled fractions of Figure 9 were prepared from isolated yolk. If the postulated complex of yolk 1001, yolk 1001, and yolk 1001 is the species taken up by macrophages, it would be expected that the three proteins would be present in embryos at lower equivalence. On the other hand, a small difference in biologic activity could result in a relative excess of yolk 1001 over yolk 950 and yolk 1001 in labeled preparations from whole nest homogenates. Significantly, anti-yolk 95 Ig, anti-yolk 1001 Ig, and Ig 1001 all retain substantial amounts of yolk 950 and yolk 1001 in the experiment of Figure 11, despite the fact that these proteins are far less abundant in the 95 fraction than yolk 1001. The similarity of the 950 pattern to that of the polyclonal Ig in this respect suggests that the primary epitope recognized by monoclonal Ig 1001 is located on yolk 950 or yolk 1001. It is also possible, however, that OOE recognizes a complex epitope present only in the postulated oligomeric assembly of yolk 950, yolk 1001, and yolk 1001.

Figure 10. Immunofluorescence binding of components of labeled 95 fraction. As indicated, electrophoretic patterns are of crude hemolymph, gradient-isolated 125 fraction, or material retained by monoconal Ig M preparations. Binding to polyclonal antibody Ig was performed in buffer A (0.2 M NaCl, 0.005 M sodium phosphate, pH 7.4, 0.02 M sodium azide) in presence (top) and absence (bottom) of 1% bovine serum albumin. Gel was 8% in acrylamide. The upper third of the gel is not shown.

Figure 11. Immunofluorescence binding of components of labeled 95 fraction. As indicated, electrophoretic patterns are of crude hemolymph, gradient-isolated 95 fraction, or material retained by monoclonal Ig M preparations. Binding to polyclonal antibody Ig was performed in buffer B (0.025 M NaCl, 0.001 M sodium dodecyl sulfate) in presence (top) and absence (bottom) of 1% bovine serum albumin. Gel was 8% in acrylamide. The upper third of the gel is not shown.