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The major serum proteins of Dipteran larvae

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Summary. The major serum proteins of Dipteran larvae present something of an enigma. In many respects they are extremely well suited for studies on gene structure and control, protein chemistry and physiology. Large amounts of these proteins are synthesized by the fat body at specific stages of larval development, much is known about their biochemistry, genetics and evolution and some information is available about the control of their synthesis. There are however doubts as to their function. This article considers what is known about these proteins in Dipterans and other insect groups and relates this information to their evolution and their possible function.

The haemolymph of both adult and larval Dipterans contains a few major protein species. In the haemolymph of adult females the major proteins are vitellogenins which have been reviewed recently[1,2]. In this article we will concentrate on the major proteins of the larval haemolymph. These proteins have been discussed under the heading of 'storage proteins' by Wyatt and Pan in a general review of insect plasma proteins[3], but here we will consider them in greater detail. We will show that these proteins are the major proteins in the haemolymph of most endopterygote larvae immediately prior to pupariation and that similar and possibly related proteins may be found in the haemolymph of exopterygotes immediately prior to a moult. We will review under a number of headings the work that has been carried out on the homologous proteins in a few dipteran species, with emphasis on our own published and unpublished work and will allude to similarities reported in other endopterygote species and to the few similarities from other work on exopterygotes.

Definition

With hindsight it is possible to see hints of the presence of the major larval serum proteins in the published work of early insect biochemists and physiologists but the first definitive work on these proteins was by Munn and Greville[4] on calliphorin and protein II from the blowfly, *Calliphora erythrocephala*. In this and in other papers with their colleagues[5,6], they established the basic biochemical, physiological, and developmental features of these proteins in the Dipterans. Perhaps the most striking feature of calliphorin and its homologues in other Dipterans is the abundance of these proteins. At the end of the larval feeding period, over 60% of the haemolymph protein is calliphorin in both *Calliphora erythrocephala*[4] and *Calliphora stygia*[7], and a similar value was found for the homologous protein of *Drosophila melanogaster*, larval serum protein 1 (LSP-1) at the end of the larval feeding stage[8]. A second less abundant protein has been found in *Calliphora* and *Drosophila* species. These proteins may or may not be homologous.

The first criterion, then, of what we call the larval serum proteins is that they are a few protein species which make up most of the haemolymph protein at the end of the larval feeding stage. Within this definition fall the proteins found in Lepidopteran larvae[9,13,16,23,40], and in the Dipterans *Phormia* and *Lucilia*[11,12].

The second most striking feature about these proteins is their temporal distribution. In most Dipterans they are only synthesized in the later larval instars. In *Calliphora*, synthesis begins in the 2nd half of instar 2[4,7], and in *Drosophila* at the beginning of the 3rd instar[8]. In other species, e.g. *Manduca sexta*, the homologous protein is present in early larval instars but increases dramatically in the last instar[13]. In all cases these proteins are found only transiently in the adult and have completely disappeared within a few days of eclosion. Indeed the decrease in their concentrations begins at pupariation and continues throughout metamorphosis.

The third important fact about these proteins is that they are synthesized by the larval fat body and, in so

far as it has been examined, by no other larval tissue[5,14,15]. In *Drosophila*, they appear to be synthesized by all parts of the larval fat body[15].

Function

Before discussing the possible functions of these proteins it is relevant to discuss their developmental profile in which we might seek clues as to this function. It is clear that these proteins are synthesized by the fat body and probably by the fat body alone, and although synthesis might occur at any time during larval life in some species, the most active phase of synthesis is in the last larval instar. Once synthesized these proteins are secreted into the haemolymph where they reach very high concentrations. Towards the end of the last larval instar they are resorbed by the fat body and can be detected as dense protein granules in fat body cells[16]. It was these protein granules, detected as long ago as the end of the last century, that provided the first evidence for these proteins. During metamorphosis they disappear and cannot be detected in adults a few days after eclosion.

There is, so far as we are aware, no unambiguous function that can be attributed to these proteins. The functions that have been considered have been inferred from the evidence presented above - notably the abundance of the proteins and their developmental profile. The conclusion most frequently drawn is that they are storage proteins synthesized during the feeding stage of the last larval instar and utilized during the non-feeding period of metamorphosis. These proteins would be to metamorphosis what the vitellogenins are to embryogenesis.

It seems to us that the term storage proteins does not go very far in clarifying the function of these proteins, for if they are storage proteins then within quite broad limits the amount is unimportant. We have constructed flies with altered gene number (see below) which produce as little as 33% and as much as 167% of the wild type LSP-1 levels and both stocks develop and appear normal under laboratory conditions. Moreover, as noted by Munn et al.[6] if they do function as storage proteins one must suppose, from their amino acid composition, that there must be another protein providing cysteine.

There are two ways in which these proteins could disappear during metamorphosis. 1. They could be broken down into amino acids which would then be used for the synthesis of adult proteins. 2. They could be processed and no longer recognized by the techniques which normally define these proteins. This 'carryover' theory has been criticized at some length by Williams and Birt[17] who come to the conclusion that there is very little evidence for this theory. Instead they cite a corpus of evidence which suggests that a large portion of the adult protein is formed de novo from free amino acids released by the hydrolysis of larval proteins. However a specific example of a way in which these proteins could be processed and disappear would be if they were incorporated into insoluble material where they would no longer be detected by the standard techniques. One such fate would be if these were to become part of the adult cuticle.

In a recent review of the insect cuticle Andersen[18] refers to the similarity between some of the protein in the haemolymph and in the cuticle and he notes that some of the cuticular proteins are synthesized elsewhere and transported to the cuticle via the haemolymph. However for technical reasons, such as the difficulty of extraction, cuticle proteins are notoriously difficult to analyze, and from the analyses that have been carried out, it is not possible to show any relationship between these proteins and the major haemolymph proteins of the larvae.

In the cockroach *Blatella germanica*, Kunkel and Lawler[19] describe a protein called the larval specific serum protein which has a pattern of synthesis clearly related to the moulting cycle. The peak of synthesis is at a time when presumptive exo and endo cuticle synthesis would occur and the maximum concentration of this protein in haemolymph is at ecdysis. It falls off to near zero levels after ecdysis is complete. This pattern of behaviour is similar to that found in the last larval instar of the Dipterans. Notwithstanding the correlation with ecdysis there is no evidence here or from the Dipteran data to suggest either that the protein disappears in the cuticle or that it is broken down to synthesize new proteins.

In spite of the ambiguity surrounding the function of these proteins which presents a challenge to the insect physiologist, these abundant proteins have provided excellent material for the geneticist and molecular biologist to study.

Biochemistry

It can be seen from table 1 that the larval serum proteins share a number of biochemical features, including hexameric structure, similar amino acid compositions, and the tendency to dissociate at alkaline pH. However, both the number and the diversity of their subunit compositions are more variable, as indicated in table 2. These data by themselves do not offer much clue as to the function of these proteins.

If they do function merely as amino acid stores for pupal development, then there must be another source of cysteine (see above), but the high tyrosine and phenylalanine content of these proteins is consistent with the high titre of these amino acids in the insect cuticle[20]. Munn et al.[6] suggested that the dissociation of the larval serum proteins at alkaline pH may be physiologically significant since the pH rises from about 6.0 to about 7.0 at the time of pupariation. If this is true, then either *Manduca sexta* does not experience a rise in pH, or dissociation of manducin is

Table 1. Biochemical characteristics of larval serum proteins

Species	Hexameric structure at physiological pH	Dissociates at alkaline pH	High Tyr, Phe, Met, low Cys amino acid composition	Carbohydrate residues	Lipids	References
Drosophila melanogaster	Yes	Yes	Yes	Yes (N-Acetyl glucosamine LSP-2)	No	8, 21, 29
Calliphora	Yes	Yes	Yes	Yes	Yes	4, 5, 6
Lucilia	Yes	Yes	Yes	Yes	Yes	12
Hyalophora cecropia	Yes	Yes	Yes	Unknown	Unknown	16
Bombyx mori	Yes	Yes	High Met, low Cys	Unknown	Unknown	40
Manduca sexta	Yes	No	Yes	Yes (Mannose, N-acetyl glucosamine)	Yes (Phospho-lipids, 1–2 diglycerides cholesterol)	13

Table 2. Subunit composition of larval serum proteins

Species	No. of larval serum proteins	Nomenclature	No. of subunits	Subunit (mol.wt)	References
Drosophila melanogaster	2	LSP-1	3(α, β, γ)	83,000, 80,000, 77,000	8, 21, 29
		LSP-2	1	80,000	
Calliphora	2	Calliphorin	$\sim$12	83,000	4, 5, 6
		Protein II	1		
Lucilia	1	Lucilin	$\sim$12–13	83,000	12
Hyalophora cecropia	2	None	1	89,000	16
			1	85,000	
Bombyx mori	1	SP-2	1	85,000	40
Manduca sexta	2	Manducin-1	1	92,000	13
	2	Manducin-2	1	87,000	

not an absolute requirement for manducin function, since manducin does not dissociate at alkaline pH[13].

It is not clear how conserved the larval serum proteins are. There is immunological cross-reaction between Dipteran larval serum proteins which is not due to lipid or carbohydrate moieties[21], indicating that some sequences have been conserved. There must be some functional constraints on the amino acid sequence to conserve the site of hexamer association, although it is not clear why hexamers themselves are important. In addition, it is likely that other sequences may be evolutionarily constrained depending on the function of the larval serum proteins, but whatever their function it is compatible with variation in mol.wt from 77,000 to 92,000 in different species, and up to 2-fold variation in the amount of any one non-phenolic amino acid. Within *Drosophila melanogaster* the α, β and γ subunits of LSP-1 share only two-thirds of their tryptic peptides[22].

Together, these facts suggest that at least some regions of the larval serum protein sequence are relatively free of evolutionary constraints, and thus that not all regions of the amino acid sequence are strictly necessary for larval serum protein function. Some of the observed biochemical similarities of these proteins may be due to the physiological constraints of their common developmental pathways, since all of these proteins are synthesized in the fat body, secreted into the haemolymph, and resorbed into the fat body where they form crystalline granules. It would be interesting to sequence the larval serum proteins to determine which if any sequences are conserved.

Analysis of the DNA and RNA coding for the larval serum proteins has lagged behind physiological, developmental and protein studies but Izumi et al.[23] isolated messenger RNA from *Bombyx* fat body and translated it in vitro in a wheat germ system, and also in the amphibian oocyte, and showed by this translation assay that bombyxin mRNA was present in large quantities in the fat body. The proteins were identified by immunoprecipitation and peptide analysis. Sekeris et al.[24] performed similar experiments with mRNA isolated from *Calliphora* fat bodies. Recently, Alonso et al.[25] showed that mRNA for calliphorin migrated at about 20S on formamide/acrylamide gels. The kinetic complexity of this RNA corresponds to a mol.wt of 2.8×10^6. Sato[15] has purified the messages for the LSP-1 and LSP-2 subunits. They also migrate at about 20S on formamide gels.

Smith et al.[26] have reported the cloning of the genes for the LSP-1 subunits. Heteroduplex mapping experiments with mRNA show that the mRNAs for the LSP-1 subunits are 2.85 kb long. S1 mapping results suggest the presence of a short intervening sequence very near the 5′ end of the coding region of all 3 LSP-1 genes, and heteroduplex mapping of the cloned LSP-1 genes shows no detectable sequence conservation outside the coding regions. These studies also show that the α and β genes share 2.5 kb of homologous sequences, β and γ share 2.0 kb, and α and γ

share only 1.7 kb of homologous sequences which results are consistent with thermal denaturation studies indicating that the greatest degree of sequence mismatch is found between the α and γ genes, and the least between the α and γ genes. Brock and Roberts (in preparation) have shown by in situ hybridization that probes prepared from each LSP-1 subunit gene cross-hybridize with both the homologous and heterologous sites, and that the ratio of grains deposited at each site is consistent with the amount of homology between the sites. The quantitative in situ hybridization data also show that in the *melanogaster* strain used in these studies, the α, β, and γ genes are present in equivalent numbers of copies.

Genetics

In order to understand the evolution of the system by a series of gene duplications and in order to understand the possible mechanisms for the control of the activity of these genes it is necessary to know their genetics. Three studies using classical genetic techniques have been carried out and two further series of experiments have localized the coding sequences on polytene chromosomes using the recently developed techniques of in situ hybridisation (see below).

The first localisation of the genes coding for the LSPs was by Hubby and Lewontin[27] in which they mapped what we now know to be the LSP-2 coding sequence of *Drosophila pseudo-obscura* on the 3rd chromosome (*melanogaster* chromosome 2R) and the LSP-1 coding sequences on chromosome 2 (*melanogaster* chromosome 3R). Our own in situ hybridisation experiments with *D. pseudo-obscura* confirm this localisation of the coding sequences.

Thomson and colleagues[12] were able to localize the genes coding for the subunits of lucilin to the 2nd chromosome of *Lucilia cuprina*. Electrophoresis of *Lucilia* haemolymph under appropriate conditions shows a number of regularly spaced major protein bands running close to the front. Immunological analysis showed that these different bands were related and moreover were related to what had been defined immunologically as lucilin. Thomson and colleagues suggest that the regular spacing of the lucilin subunits is due to 'a unitary charge change in net charge, resulting from amino acid substitutions in otherwise identical or highly similar molecules'. The electrophoretic mobility of the subunits is extremely polymorphic. In one sample of 180 larvae from a single population 90 different phenotypes were recognized. This is perhaps surprising as *L. cuprina* is thought to have come into Australia with the sheep and so cannot have been there more than a couple of hundred years. If the founding population were small then this high degree of polymorphism is indeed surprising.

By careful control experiments and because the subunit pattern for an inbred strain remains constant the authors can eliminate the possibility that the multitude of subunits is an artefact of preparation. The study of band position and intensity in appropriate crosses between inbred stocks led to the most parsimonious explanation for the genetics of lucilin - that there is a series of 12 or more closely related structural genes. Once the complex band pattern had been deciphered it was possible to show that at least 2 of these coding sequences were located on chromosome 2 and to infer that the remaining loci were also on that chromosome. This suggestion was supported by a study of Kemp et al.[28] on *Calliphora vicina,* which has a similar multi-locus system coding for calliphorin, who demonstrated by in situ hybridisation a single segment of the genome coding for these polypeptides. The results from *Lucilia* and *Calliphora* suggest an ancestral gene undergoing tandem duplication to give at least twelve copies which have remained closely linked.

Although the 4 LSP genes of *Drosophila* have, like the genes coding for lucilin and calliphorin, evolved from a common ancestor the organisation of the coding sequences in the genome is very different. We mapped variants of the 4 polypeptides to 39.5 on chromosome 1 (α); 1.9 on chromosome 2L (β); -1.4 on chromosome 3L (γ)[21] and 37.0 on chromosome 3L (2)[29]. That the variant loci were likely to be the coding sequences was supported by deficiency mapping in which flies heterozygous for different deficiencies of these regions synthesized only 50% of the corresponding polypeptide which allowed us to map the genes cytogenetically to 11A7-11B9 (α), 21D2-22A1 (β), 61A1-61A6 (γ)[20], and 68E2-68E4 (2)[27]. These positions for the coding sequences were further confirmed by in situ hybridisation[25] which has also shown the dispersal of the LSP genes in all the *Drosophila* species tested (Brock and Roberts, in preparation) (see below).

These two very different strategies for organising the major serum protein genes in the *Calliphora/Lucilia* species and the *Drosophila* species poses questions about their control and evolution. In the former it is possible to envisage a relatively simple cis acting mechanism co-ordinating the expression of the lucilin genes whereas the same co-ordinate expression of LSP-1 genes would presumably require some additional trans acting elements.

The simple mechanism for the evolution of the lucilin genes would be an initial unequal cross over event (figure 1) to generate a tandem duplicate followed by normal crossing over with unequal pairing to generate a multitude of copies which subsequently diverge by the aquisition of point mutations. For the LSP-1 genes a similar initial series of events would be required to generate the duplicates followed by some dispersal mechanisms such as translocations and inversions.

This poses a paradox why should similar genes (remember lucilin and LSP-1 crossreact immunologically), under similar control have two very different organisations? What could be the selective advantage of dispersing the tandemly duplicated LSP-1 genes? One way of avoiding this paradox would be to suggest that selection favours multiple copies of these genes and that there are at least two ways of generating increased copy number. The 1st by tandem duplication as described above and the 2nd by transposable elements[30]. In the latter case a transposable element would mediate the transposition of the ancestral gene from one location to another. By appropriate crosses individuals homozygous for copies at both chromosomal locations would appear (figure 2) and would be selected for in the population. These duplicates would then diverge as before by mutation. The suggestion is that duplication of serum protein genes was at some time under powerful selection pressure and that in one insect line they were duplicated by one mechanism and in another line by another mechanism.

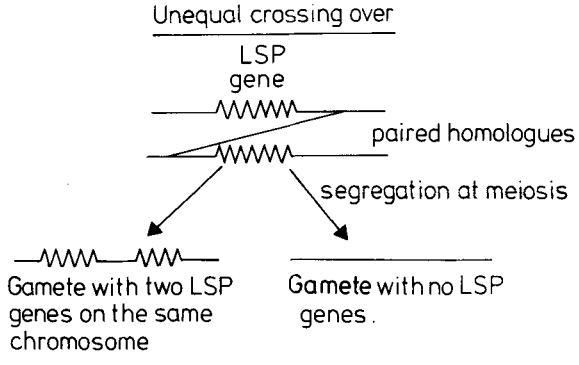

Fig. 1. Generation of gametes with 2 LSP genes by unequal crossing over. The genes are subsequently dispersed by translocations and inversions.

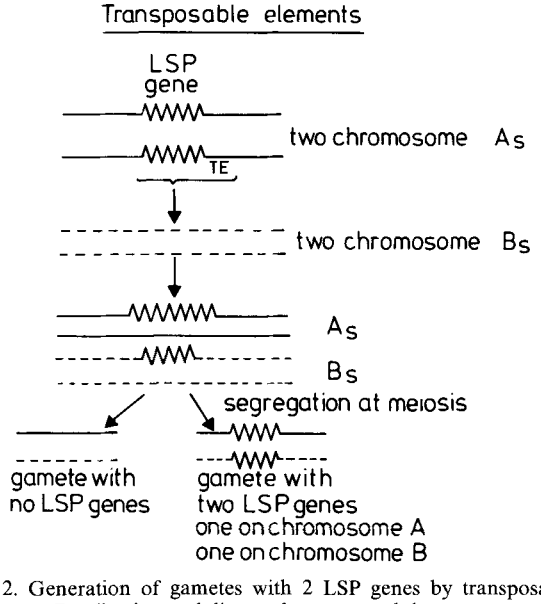

Fig. 2. Generation of gametes with 2 LSP genes by transposable elements. Duplication and dispersal are one and the same event.

The polytene chromosomes of Dipterans make it possible to locate the site of any gene in the genome directly. Cloned LSP-1 subunit genes were labelled by nick translation, and hybridized to polytene chromosomes prepared from different *Drosophila* species. Following hybridization, the slides are dipped in autoradiographic emulsion. After development, the deposition of silver grains can be seen at the site of hybridization which is the location of the sequence homologous to the probe. An example is shown in figure 3 using an LSP-1-β subunit as a probe. In addition to the major site of hybridization on band 21D, cross-hybridization can be observed to the α site at band 11AB on the X chromosome, and the γ site at 61A on chromosome 3L.

Evolution

The common biochemical, physiological, and developmental features of the larval serum proteins suggest that they are evolutionarily related. Antibodies prepared against calliphorin cross-react with proteins from a number of other Dipterans, including all genera of the Cyclorapha, 4 genera of the suborder Brachycera, 1 genus of the Nematocera, in addition to *Gastrophilus* and *Drosophila*[4]. We have shown that antibodies prepared against LSP-1 cross-react with calliphorin, but not with Lepidopteran or Coleopteran proteins (Wolfe and Roberts, unpublished). The common ancestor of these proteins may be very ancient indeed if the serum protein in cockroaches is homologous to the serum proteins of endopterygotes.

We have investigated the evolution of the LSP-1 subunits in more detail using immunological techniques, and electrophoresis on denaturing and non-denaturing polyacrylamide gels, in 15 *Drosophila* species. The correspondence of bands on gels with the LSP subunits was determined after blotting the proteins from the gel to nitrocellulose filters[31], reacting the blotted proteins with anti-LSP-1, anti-LSP-2, or anti LSP-1-γ antibodies, and detecting a positive reaction with (^{125}-I) labelled secondary antibody[32]. In general, the LSPs are conserved with respect to molecular weight, but not with respect to charge, as expected, since mutations which alter the net charge of a molecule are more likely to occur than mutations which increase or decrease the coding sequences of the protein. All *Drosophila* species examined possessed LSP-2 and LSP-1β-like subunits, and all species except *D. busckii* possess LSP-1γ-like subunits. However, only the *melanogaster* sibling species possess LSP-1α-like sequences, suggesting that the divergence of the LSP-1α and β genes has occurred more recently than the LSP-1-β and γ divergence. This suggestion is supported by the data mentioned above that LSP-1 β and α share about 2.5 kb of sequence as

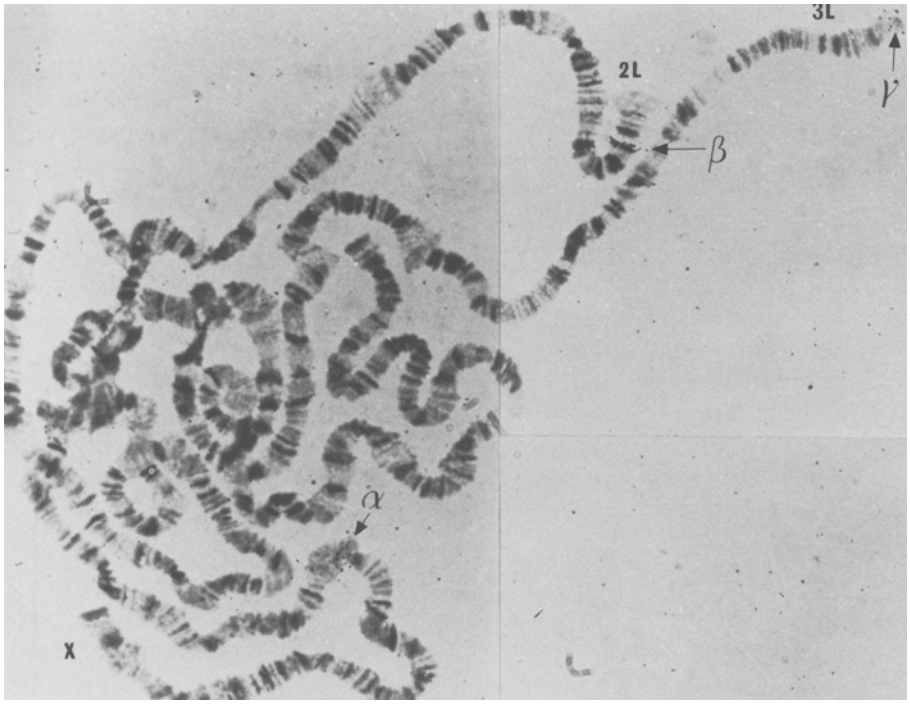

Fig. 3. In situ hybridization with cloned LSP1-β. Note crosshybridization to LSP1-α and LSP1-γ sites.

determined by heteroduplex mapping, whereas β and γ share only 2.0 kb of sequence[25]. The LSP-1 β subunit appears to have duplicated and diverged in *D. pseudoobscura,* and in *D. busckii.* No *Drosophila* species has been found with more than 4 or less than 2 LSP-1 subunits. A similar variation in subunit number has been observed in the *Drosophila* yolk proteins[33].

We used in situ hybridization to determine the location of the LSP-1 subunit genes in 15 *Drosophila* species, and we found no examples where 2 different LSP-1 subunits hybridized to the same location. (Brock and Roberts, in preparation). These results suggest that dispersal of the LSP subunits after duplication is a widespread phenomenon, and as discussed above, this may reflect a different means of gene duplication in *Drosophila.* Where the banding patterns of the polytene chromosomes were sufficiently similar to establish their homology with the *D. melanogaster* chromosome arms, we could ask whether the α, β, and γ genes of LSP-1 were in the same positions rllative to their positions in *melanogaster.* In other words, we predicted that if the LSP genes had been dispersed by normal chormosomal mechanisms of inversions and translocations, then the LSP-1 subunit genes should stay in the same positions relative to their nearest neighbours in *melanogaster.* If an LSP-1 gene acquired 'new' neighbours, then a mechanism involving transposable elements might be responsible. The LSP-1 subunit genes have the same position relative to their positions in *melanogaster,* and are on the same chromosome arms, despite the fact that these species are sufficiently divergent to have accumulated up to 30 inversions relative to *melanogaster*[34]. These results suggest that if transposable elements can move LSP structural genes in the genome, then it is not a frequent event. Unfortunately, since the divergence times of the *melanogaster* sibling species is not known, frequency can only have a relative meaning. Intriguingly, the β and γ LSP-1 subunits of *D. pseudoobscura* both hybridize to chromosome 2 of this species, which is homologous to chromosome 3R in *D. melanogaster*[35]. As mentioned above the β and γ genes of LSP-1 are on chromosomes 2L and 3L respectively, in *melanogaster.* This finding suggests that at some time in the past the LSP-1 β and γ genes have moved independently of their chromosome arms in the genome, perhaps on transposable elements.

Control

One of the stimuli for a detailed study of larval serum proteins is the opportunity presented by the developmental profile and tissue specificity of synthesis for the investigation of control.

The subunits of calliphorin from *C. stygia* are synthesized under coordinate control[12], and we have shown that the subunits of LSP-1 and LSP-2 are probably coordinately synthesized (Powell et al., unpublished results). It is difficult to establish coordinate control in higher eukaryotes but our reason for suggesting the coordinate control of these proteins is the following. If we plot the concentration of the LSP-1 β chain against the concentration of the LSP-1 γ chain obtained from 3rd instar larvae of different ages we get a straight

line with a slope of 1, indicating that they are synthesized in equimolar amounts, which also intercepts at the origin showing that the initiation of the synthesis of these polypeptides occurs at the same time. Similar results were obtained for the relationship between LSP-1 and LSP-2.

An initially surprising result was obtained in a study of the relationship between LSP-1 α and either β or γ. Less α was synthesized than either of the other two. This anomaly was resolved by analysing female and male larvae separately. The ratio of α to β or γ in the female was 1:1 but in the male was 0.5:1. We have explained this by supposing that the X-linked chain gene in male larvae is not dosage compensated[36]. All other proteins in *Drosophila* which are coded for by X-linked genes and have been tested show dosage compensation, that is the amount of protein synthesized by the female and the male is the same notwithstanding the gene dose difference. We explain the absence of dosage compensation in the LSP system by suggesting a) that the LSP-1α coding sequence has only 'recently' arrived on the X chromosome (recently must be before the divergence of the *melanogaster* sibling species, see above) and has not yet aquired dosage compensation and b) that the selection pressure for the aquisition of dosage compensation is not great because the polypeptides can substitute for one another functionally.

In *Drosophila melanogaster* the LSPs and their mRNAs (Powell et al., unpublished results) are first detected after the 2nd larval ecdysis. Because we were unable to find a nuclear precursor of the mRNA at any earlier stage we believe that the initiation of synthesis is under transcriptional control.

From the time of synthesis of the LSPs it is tempting to involve the rise in the ecdysone titre at the 2nd larval ecdysis in their control. We have in fact indirect evidence involving ecdysone either directly or indirectly in LSP synthesis. We described a mutant l(3)138[37] which is arrested in the early to mid 3rd instar and survives at this developmental stage for up to 30 days. These larvae do not synthesize LSPs although being at a developmental stage when LSPs are normally synthesized. If reared on medium containing ecdysone, 50% of the mutant larvae pupariate and all larvae tested synthesized LSPs. An interpretation of this result is that the mutant is defective in ecdysone synthesis but possesses sufficient maternal ecdysone to undergo both larval moults but with too low a titre at the 2nd moult to initiate the process of LSP synthesis.

The cessation of larval serum protein synthesis is a post-transcriptional event. Sekeris and Scheller[38] working on *Calliphora* have shown the presence of translatable calliphorin mRNA in the fat body of larvae which are past the age of calliphorin synthesis. We (Powell et al. unpublished results) have similar results for LSP-1 which show the presence of translatable mRNA in fat body which is not synthesizing LSP-1 in vivo. Moreover using carefully staged larvae - by analysis of salivary gland polytene chromosome puffing pattern[39] - we have shown that LSP-1 synthesis ceases before LSP-2 synthesis. LSP-1 synthesis ceases at the beginning of the wandering larval stage when the ecdysone titre begins to rise prior to pupariation and when the fat body begins to resorb the LSP-1 from the haemolymph and it may be that this increase in internal LSP-1 concentration acts as a feedback and shuts off its own synthesis.

In general however there is no feed back control of LSP-1 or LSP-2 synthesis. The amount of these proteins synthesized is dependent, within the limits tested, on the number of coding sequences present. Using a stock homozygous for a null allele of the gene[20] and heterozygous for a deficiency of the coding sequence, male larvae have only 2 of the LSP genes and synthesize only one-third of the LSP-1 synthesized by the wild type female larvae with 6 genes. At the other extreme using a heterozygous quintuplication of the β coding sequence we can increase the number of LSP-1 genes in the female larvae to 10 including 6β genes. These individuals synthesize nearly twice as much LSP-1 and 3 times as many β-chains as wild type male larvae with only 5 LSP-1 genes. Although this represents a considerable increase in the amount of protein synthesized by the fat body it does not appear to affect the levels of synthesis of 2 fat body enzymes. This absence of feedback control may be general in eukaryotes or may be the result of this particular situation where the gene products are secreted from the cells that synthesize them.

In summary, the synthesis of LSP-1 and LSP-2 is probably initiated by ecdysone at the transcriptional level and shut off post-transcriptionally by a 2nd or larger ecdysone pulse. During the early stages of synthesis the 4 polypeptide chains are synthesized coordinately.

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1 F. Engelman, Adv. Insect Physiol. *14*, 49 (1979).
2 H.H. Hagedorn and J.G. Kunkel, A. Rev. Ent. *24*, 475 (1979).
3 G.R. Wyatt and M.L. Pan, A. Rev. Biochem. *47*, 779 (1978).
4 E.A. Munn and G.D. Greville, J. Insect Physiol. *15*, 1935 (1969).
5 E.A. Munn, G.M. Price and G.D. Greville, J. Insect Physiol. *15*, 1601 (1969).
6 E.A. Munn, A. Feinstein and G.D. Greville, Biochem. J. *124*, 367 (1971).
7 J.A. Thomson, Adv. Insect Physiol. *11*, 321 (1975).

8 D.B. Roberts, J. Wolfe and M.E. Akam, J. Insect Physiol. *23*, 871 (1977).
9 B.G. Loughton and A.S. West, J. Insect Physiol. *11*, 919 (1965).
10 G.M. Chippendale and B.A. Kilby, J. Insect Physiol. *15*, 905 (1969).
11 P.S. Chen and L. Levenbook, J. Insect Physiol. *12*, 1595 (1966).
12 J.A. Thomson, K.R. Radok, D.C. Shaw, M.J. Whitten, G.G. Foster and L.M. Birt, Biochem. Genet. *14*, 145 (1976).
13 S.J. Kramer, E.C. Mundall and J.H. Law, Insect Biochem. *10*, 279 (1980).
14 J.F. Kinnear, M.D. Martin and J.A. Thomson, Aust. J. biol. Sci. *24*, 275 (1971).
15 J.D. Sato, D. phil. thesis, Oxford University, Oxford 1980.
16 S. Tojo, T. Betchaku, V.J. Ziccardi and G.R. Wyatt, J. Cell Biol. *78*, 823 (1978).
17 K.L. Williams and L.M. Birt, Insect Biochem. *2*, 305 (1972).
18 S.O. Andersen, A. Rev. Ent. *24*, 29 (1979).
19 J.G. Kunkel and D.M. Lawler, Comp. Biochem. Physiol. *47B*, 697 (1974).
20 G.M. Price, Biol. Rev. *48*, 333 (1973).
21 D.B. Roberts and S.M. Evans-Roberts, Genetics *93*, 663 (1979).
22 H.W. Brock and D.B. Roberts, Eur. J. Biochem. *106*, 129 (1980).
23 S. Izumi, S. Tojo and S. Tomino, Insect Biochem. *10*, 429 (1980).
24 C.E. Sekeris, R. Perassi, J. Arnemann, A. Ullrich and K. Scheller, Insect Biochem. *7*, 529 (1977).
25 A. Alonso, C.N. Flytzanis, U. Schatzle, K. Scheller and C.E. Sekeris. Eur. J. Biochem. *94*, 601 (1979).
26 D.F. Smith, A. McLelland, B.N. White, C.F. Addison and D.M. Glover, Cell in press (1981).
27 J.L. Hubby and R.C. Lewontin, Genetics *54*, 577 (1966).
28 D.J. Kemp, J.A. Thomson, W.J. Peacock and T.J.V. Higgins, Biochem. Genet. *16*, 355 (1978).
29 M.E. Akam, D.B. Roberts and J. Wolfe, Biochem. Genet. *16*, 101 (1978).
30 N. Kleckner, Cell *11*, 11 (1977).
31 B. Bowen, J. Steinberg, U.K. Laemmli and H. Weintraub, Nucl. Acid. Res. *8*, 1 (1980).
32 H. Towbin, T. Staehlin and J. Gordon, Proc. natl Acad. Sci. USA *76*, 4350 (1979).
33 Z. Srdic, H. Beck and H. Gloor, Experientia *34*, 1572 (1978).
34 F.Lemeunier and M. Ashburner, Proc. R. Soc. B *193*, 275 (1976).
35 A.H. Sturtevant and C.C. Tan, J. Genet. *34*, 415 (1937).
36 D.B. Roberts and S.M. Evans-Roberts, Nature *280*, 691 (1979).
37 D.B. Roberts and S.M. Evans-Roberts, Molec. gen. Genet. *148*, 57 (1976).
38 C.E. Sekeris and K. Scheller, Dev. Biol. *59*, 12 (1977).
39 M. Ashburner, Chromosoma *21*, 398 (1967).
40 S. Tojo, M. Nagata and M. Kobayashi, Insect Biochem. *10*, 289 (1980).

SPECIALIA

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Desacetylscalaradial, a cytotoxic metabolite from the sponge *Cacospongia scalaris*

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Summary. From the marine sponge *Cacospongia scalaris*, scalaradial **1**, desacetylscalaradial **2**, and heteronemin **3** were isolated. Compound **2** showed potent cell growth inhibition. The stereochemistry of **3** is briefly discussed.

Sponge material (collected at Wakayama) frozen with dry ice, was immersed in dichloromethane. Reverse phase chromatography (LiChroprep RP-18/MeOH) of the dichloromethane layer, which showed mainly 2 spots (dialdehyde components **1** and **2**; $R_f = 0.20$ and 0.25, respectively) under UV light on TLC plate, resulted in a change of both spots which made them undetectable by UV light (cyclic dimethylacetal **4** and **5**; $R_f = 0.40$ and 0.47, respectively). Further chromatography of **4** and **5** resulted in separation of each into 2 epimers (**4a, 4b** and **5a, 5b,** respectively). Treatment of either **4a** or **4b** in dichloromethane with hydrochloric acid gave a dialdehyde, which proved to be scalaradial **1,** a metabolite from the sponge *C. mollior*[2,3], based on its spectra. Inferring that formation of cyclic dimethylacetals from dialdehyde was catalysed by a trace amount of acid remaining on the RP-18 support during reverse phase chromatography, we treated **1** with ion exchange resin (acid form) in methanol. As expected, cyclic dimethylacetals **4a** and **4b** were formed.

The structure of the cyclic dimethylacetal was examined first for the major products **4a** and **4b**. Interpretation of their spectra (table) suggested that the difference between them was only the orientation of the 19-MeO group. The stereochemistry of the cyclic dimethylacetal part depicted as **4a** and **4b** was deduced by ^{1}H-NMR spin decoupling[2,4]. On irradiation of the 20-H signal, the 16-H signal remained unchanged (i.e., the angle between allylic 16-H and 20-H was near 0°). Irradiation of 15-H signal caused 16-H signal to collapse to a doublet ($/J/ = 3.0$ Hz, i.e., the angle between 16-H and allylic 18-H was near 90°), and irradiation of 18-H caused 16-H to change into a triplet ($J = 3.5$ Hz). The J value of 19-H indicated β orientation of