

The Nematode Polyprotein Allergens/Antigens

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Interest in the nematode polyprotein allergens/antigens (NPAs) originally arose because they were often found to be immunodominant antigens of nematode parasites, and in some cases also potent allergens. Quite separately, they attracted attention as the 'ladder' proteins found close to the surface of filarial nematodes. Screening of cDNA expression libraries with antibody from infected humans or domestic animals continues to reveal more NPAs. The search for the biochemical function(s) of NPAs was originally hampered by the lack of amino acid sequence similarities between NPAs and proteins of known function, but much is now known about their biochemical activity, the highly unusual means of their biosynthesis and their gene structure. Here, Malcolm Kennedy provides an update on these intriguing proteins.

With the virtual completion of the sequencing of the genome of the nematode *Caenorhabditis elegans*, it appears that $\approx 58\%$ of the encoded proteins have no easily discernible homologues in other groups of animals¹. Of this potentially enormous number of novel protein types, only two have known biochemical activities, both being lipid-binding proteins. These are the nematode polyprotein allergens/antigens (NPAs), and an unrelated family of small lipid-binding proteins (the LBP-20 family) typified by the Ov20 protein of *Onchocerca volvulus*^{2,3}.

Type specimen: the ABA-1 allergen of *Ascaris*

The first NPA to be characterized was the ABA-1 protein of *Ascaris*, which is abundant in the pseudo-coelomic fluid of the adult worms ($\approx 40\text{--}50\%$ of the total protein), and which also appears in the secretions of all parasitic stages^{4,5}. Before it was known that it was made as a polyprotein, interest in ABA-1 had arisen because it was the target of strong IgE antibody responses in *Ascaris*-infected animals⁶⁻⁹. ABA-1 appears to be identical to Allergen A, which had been isolated from adult *A. suum* and its secretions in the 1970s^{10,11}, and is a major constituent of the allergen preparations previously used for investigating allergic reactions to *Ascaris*⁴.

An interesting feature of ABA-1 is that only a subset of infected humans react to it immunologically^{7,12,13}. In rodents, only one haplotype of the major histocompatibility complex (MHC) is associated with immune responsiveness of mice (H-2^s) and rats (RT1^u)^{6,9}. This genetic restriction of the immune repertoire is under the control of the MHC class II region in mice⁸, which will presumably also apply to humans. A similar effect is observed for immune responses to the ABA-1 homologue in *Brugia malayi* (gp15/400), although genes outside the MHC also appear to be influential¹⁴. It is

possible that such a clear manifestation of genetic control of the immune repertoire results from the fact that these proteins are small (≈ 15 kDa) and produce a more restricted range of peptides under antigen processing than do larger proteins.

Allergenicity: intrinsic or reflected?

An allergen is generally defined as an entity that is the target of IgE antibody. ABA-1-type proteins (eg. ABA-1 of *Ascaris*^{6,13} and gp15/400 of *Brugia*¹⁵) are therefore allergens. But an important question is: what does this really mean – are these proteins intrinsically or self-allergenic? That is, are they able to generate allergic-type T helper type 2 (Th2) responses on their own? No clear answer has yet emerged. For example, exposure of mice to NPAs in Freund's adjuvant elicits a Th1-type response against them, and the proteins on their own elicit an immune response with no special characteristics (Refs 14,16; J.C.McE. Paterson *et al.*, unpublished). One problem is that many studies involve the use of adjuvants that themselves bias the nature of the T-cell response – Freund's complete adjuvant, for instance, is a noted Th1 inducer. A second problem is the source of protein used for immunological testing – the parasite-derived proteins are usually difficult to obtain in pure form and bacterially derived recombinant proteins are not glycosylated or may be contaminated with bacterial products, both of which could alter the bias of the immune response^{14,17}.

Therefore, the intrinsic allergenicity of the NPAs remains to be established, and it is still possible that they become allergens only in the context of nematode infection, and the potency of ABA-1 and other NPAs as allergens might partly depend on the ability of the infections to elicit generalized Th2 responses in some way. This would require, however, that all nematode proteins would become subject to strong IgE antibody responses, which might not be the case. There is growing evidence that some allergens can be auto-allergenic. For example, work on the Der p 1 allergen¹⁸ of house dust mites and a tegumental protein of schistosomes¹⁹ demonstrates such auto-allergenicity, and appropriate experimentation might show this for NPAs. In the case of Der p 1, it appears that its proteinolytic enzyme activity contributes to its allergenicity¹⁸; might the same apply to the lipid-binding activities of the NPAs? The fact that the NPAs bind pharmacologically active lipids might relate to allergenicity, and at least one other mite allergen is a lipid-binding protein²⁰.

In areas endemic for *Ascaris lumbricoides*, those people who produce relatively high levels of IgE antibody to ABA-1 are less likely to have infections with adult worms than are those producing low levels¹³. This probably applies to other allergens of *Ascaris*, and might be a general indication of the protective effect of IgE antibody against this species. Alternatively, it is possible that an IgE antibody is merely co-stimulated with the true protective response(s). For instance, individuals

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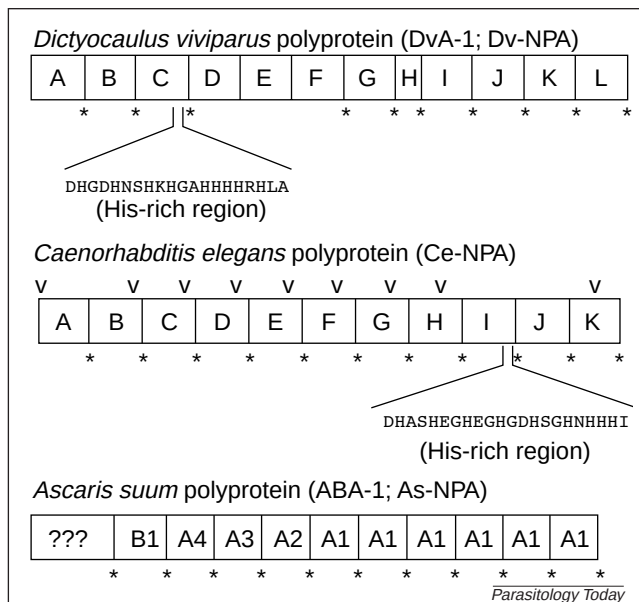


Fig. 1. Organization of the polyprotein of three species of nematode. Only the central repetitive sections of the polyproteins are illustrated, ignoring the short hydrophobic N-terminal (signal?) and C-terminal extension peptides. The full structure of the nematode polyprotein antigen/allergen (NPA) of *Dictyocaulus viviparus* is known from cDNA analysis³¹, and the *Caenorhabditis elegans* sequence derives from the genome project (cosmids VC5 and F27B10). In the case of the *Ascaris suum* NPA, the 5' region of cDNA is unresolved. For the NPAs of other species, only fragments of the central region are known. The positions of the consensus $\text{Lys/Arg-Xaa-Lys/Arg-Arg}$ cleavage sites are marked (*). In some species (eg. *D. viviparus* and *Diriofilaria immitis*) no consensus cleavage site is evident between some of the units, suggesting either that another type of enzyme is involved in processing the polyprotein at these points or that double or triple units are produced without further processing; intact multimers have been detected in extracts from whole parasites^{31,55}. After cleavage, the proteins appear to be trimmed to remove the cleavage site amino acids – mass spectrometry on ABA-1 purified from the parasite yielded a mean value of 14 643.2 (SD ± 1.4), which, within error, corresponds to the calculated mass (14 642.6) of the ABA-1A1 unit with the cleavage site amino acids (Arg-Arg-Arg-Arg) removed²¹. Unit H in *D. viviparus* is truncated in a manner not seen in any other species to date. The His-rich stretches that are found in *D. viviparus* and *C. elegans* are positioned as indicated. The positions of the small (40–396 bp) introns in the genomic DNA encoding the *C. elegans* NPA are shown (V). For complete sequences, the naming convention for the units is alphabetical from the N-terminus/5' end. The *Ascaris* NPA units were named according to their order of discovery and similarity, and should be renamed when the complete DNA sequence becomes available. In this NPA, the A1 to A4 units are grouped because of their similar amino acid sequences, which are distinctly different from the single B1 unit⁵⁶. The units within the NPA arrays of *D. viviparus* and *C. elegans* are quite different from one another, but those of ascarid species and certain filarial nematodes (eg. *Brugia malayi*) are more similar^{15,25–27,56,57}. See Table 1 for the original and suggested new nomenclatures of NPAs, the individual units within the arrays and the encoding genes. Adapted, with permission, from Ref. 58.

who appear to be naturally immune to *A. lumbricoides* tend also to have indications of more generalized inflammatory reactions, such as higher levels of C-reactive protein, eosinophil cationic protein and serum ferritin¹³. Thus, other processes might indeed be at work, although there is currently no reason to believe that these responses are more or less protective than is IgE.

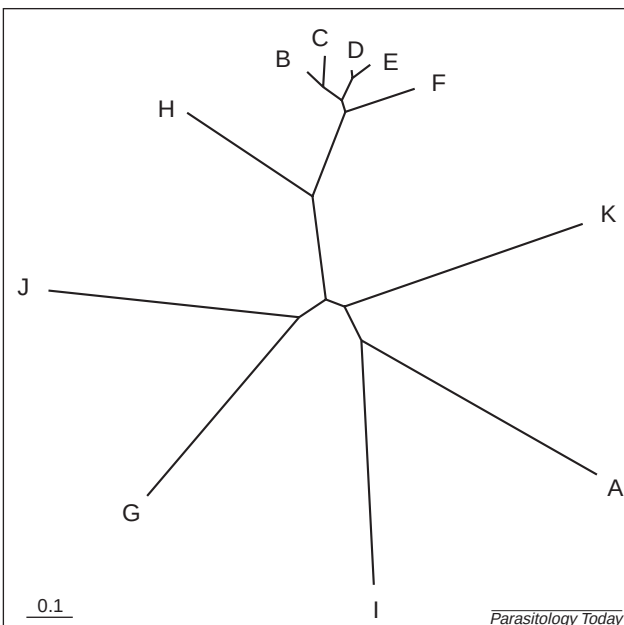


Fig. 2. Radial dendrogram to show the diversity and relationships between units in the nematode polyprotein antigen/allergen (NPA) array of *Caenorhabditis elegans*. The dendrogram was constructed using ClustalW set for the neighbourhood joining method, and used the amino acid sequences of the different units. To improve the search for relationships between the units, each was edited to remove the N-terminal sequences up to the conserved Trp (W15; Fig. 3), and beyond the second conserved Cys (C124; Fig. 3). The proteins are highly diverse in the removed regions, which compromises the search for core relationships. The units towards the N-terminus of the *C. elegans* polyprotein cluster are most similar, but the opposite is the case for the NPA array of *Dictyocaulus viviparus*³¹. Those units clustering together might represent the products of more recent amplifications, with the more divergent representing the ancestral units. For the *C. elegans* NPA, therefore, it could be postulated that the more recent duplications/amplifications were centred around the B, C, D and E units, but without involvement of the A unit. Interestingly, the most divergent units in *C. elegans* are also those not interrupted with introns – these are possibly the more ancient products of amplification, and the introns might therefore have been important in the amplification mechanism, or be relics of it. Adapted, with permission, from Ref. 58.

Location

The ABA-1 protein was originally found at high concentrations in the pseudocoelomic fluid of *Ascaris*, and similar proteins are abundant in other large ascaridids, such as adult *Toxocara canis* and *Anisakis simplex* larvae^{21,22}. NPAs appear to be secreted by several species of ascarid, although not by the larvae of *T. canis*^{23,24}. NPAs are less prominent as somatic components in other groups of nematodes, but they have attracted attention as the surface-proximal and secreted 'ladder' proteins of filarial nematodes, originally revealed by radiolabelling the surface of living *Brugia* and *Diriofilaria* worms^{25–29}. Immunoelectronmicroscopy in *Brugia* has indicated that its NPA (gp15/400) is present in the matrix of the basal laminae separating the hypodermal cord and the somatic musculature, between individual muscle blocks, in the basal lamina surrounding the oesophagus and at low levels within cells. Whether NPAs occur in the cuticle of nematodes is contentious, in that they have been detected there in *Diriofilaria immitis*²⁵ but not in *B. malayi*²⁸ or *A. suum* (I.M. Huxham and M.W. Kennedy, unpublished).

Biosynthesis

NPAs are made as large polypeptides comprising identical or similar repeated polypeptide units (Fig. 1). The polypeptide is then cleaved post-translationally at consensus cleavage sites ($\text{Lys}/\text{Arg}-\text{Xaa}-\text{Lys}/\text{Arg}-\text{Arg}$) to yield multiple copies of functional molecules of ≈ 15 kDa. Cleavage sites comprising groups of basic amino acids are commonly targeted by proteinases involved in post-translational modification of proteins³⁰. Insulin, for instance, is cleaved at a Lys–Arg and an Arg–Arg site during its processing. The processing of the NPA precursor into ≈ 15 kDa units has been followed elegantly by pulse-chase labelling of living worms, which yields a ladder appearance of bands on SDS-PAGE as the polypeptide is progressively processed²⁷. After the cleavage event, trimming might occur to remove the remainder of the cleavage target site to produce the final ≈ 15 kDa unit²¹. In some cases, however, no consensus cut sites are apparent between some units in a polypeptide (Fig. 1).

The mRNA of NPAs is large and encodes the tandemly repeated units of the polypeptide, a short N-terminal hydrophobic peptide and a short C-terminal extension peptide (Fig. 1). The precise number of repeats is known only in *Dictyocaulus viviparus* (from cDNA) and *C. elegans* (from genomic DNA), which have 12 and 11 units, respectively (Figs 2 and 3); *D. viviparus* has a single truncated unit, the like of which has not yet been found in any other species. Estimates for the number of repeat units in other species have been made from experiments with partially digested genomic DNA, and the estimates range from 10 to 50 units^{25–27}. It might be that the higher estimates will not survive the definitive sequence information emerging from the various genome sequencing projects now under way. The amino acid sequences of the individual units in an NPA can differ dramatically by as much as 82% between those in the polypeptide of a single species³¹. Many nematode mRNAs are preceded by the conserved SL1 spliced leader, or something similar, and this appears to apply to the mRNA of *D. viviparus*, but not *C. elegans* (Ref. 31; I. Johnstone, pers. commun.).

All *npa* genes of parasitic species examined to date by PCR or southern blotting analysis of genomic DNA appear to be intronless in the region encoding the repeat units²⁷. However, a large intron has been found in the region encoding the C-terminal extension peptide of the ABA-1 of *Ascaris*, beyond the repeat units (H.J. Spence, PhD thesis, University of Glasgow, 1994). The *C. elegans* gene has introns in the unit-encoding region, although they are small and do not interrupt all of the repeat units (Fig. 1). No comparable information is available yet for parasitic species, and complete

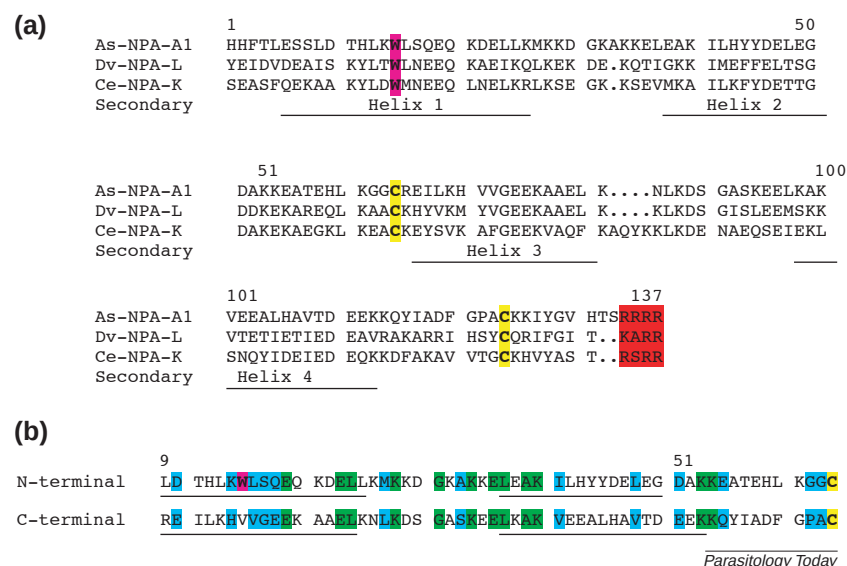


Fig. 3. Alignment of the amino acid sequences of three nematode polypeptide antigen/allergen (NPA) units to illustrate their diversity (a). Those selected are encoded by the most 3' repeat unit from the NPA array of *Ascaris suum* (As-NPA-A1, originally designated ABA-1A1), *Dictyocaulus viviparus* (Dv-NPA-L; DvA-1L) and *Caenorhabditis elegans* (Ce-NPA-K). The signature amino acids found in all NPA units known (with the single exception of the truncated unit H from *D. viviparus*) are indicated: the single tryptophan (pink) and the two cysteines (yellow). The terminal four amino acids, which conform to the $\text{Lys}/\text{Arg}-\text{Xaa}-\text{Lys}/\text{Arg}-\text{Arg}$ consensus cleavage site for processing enzymes³⁰ and are probably removed after cleavage²¹, are indicated in red. The regions predicted to form α -helices are as indicated, and no β -extended structure has been predicted or detected^{33,34}. Alignment of the N- and C-terminal halves of one unit (from *A. suum*) to illustrate the putative duplication that might have led to the modern ≈ 15 kDa units of the NPAs (b); a similar effect can be seen for other NPA units. Identical amino acids are coloured green, and those positions with similar amino acids light blue. The predicted helical sections are underlined, and the numbering corresponds to that used for the alignment in (a). Adapted, with permission, from Ref. 58.

sequencing of the NPA-encoding genomic DNA of parasitic nematodes might reveal introns, although they may be uncommon.

mRNA for the ABA-1 of *Ascaris* has been detected in gut tissue of the parasite, but in no other site, including gonads and body wall³². In *C. elegans*, the situation appears to be the same, and can be demonstrated elegantly by transformation of worms with the presumptive promoter region of the *npa* gene tagged to DNA encoding green fluorescent protein and a nuclear localization signal. Fluorescence microscopy then shows localization solely in the nuclei of gut cells (Fig. 4).

There is no unified nomenclature for NPAs and the *npa* genes, and a scheme for standardizing the nomenclature is set out in Table 1 and illustrated in Fig. 1.

Functions of NPAs

Lipid binding. The ≈ 15 kDa proteins derived from the polypeptides bind small lipids such as fatty acids and retinol (vitamin A) (Fig. 5; Table 2)^{32–36}. There appears to be a binding site for only one ligand molecule per ≈ 15 kDa NPA molecule, and this binding site appears to be the same for all types of lipid that bind^{32–36}. NPAs can therefore be classed as relatively non-specific lipid-binding proteins and probably function as extracellular carrier proteins in the pseudocoelomic fluid and connective tissues of nematodes. Our own serum albumin performs an equivalent function. Fatty acids need to be transported within specialized proteins during their passage

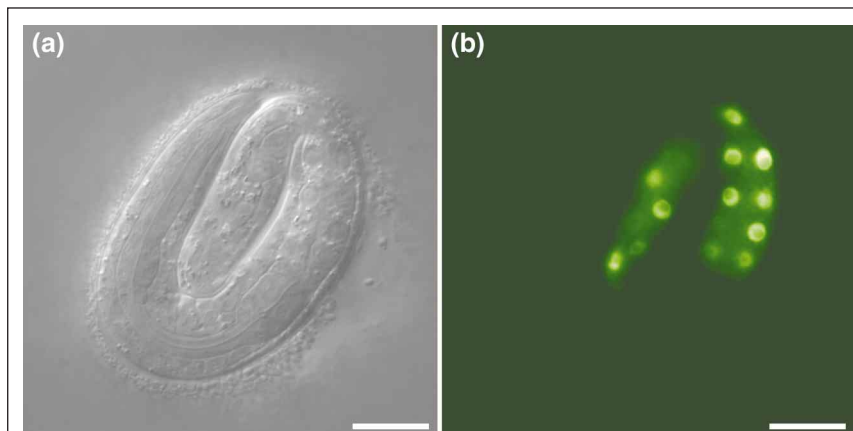


Fig. 4. Site of production of nematode polyprotein antigen/allergen (NPAs). *Caenorhabditis elegans* was transformed with a plasmid containing the putative promoter sequences of *Ce-npa-1* fused to the gene encoding green fluorescent protein (GFP). The version of GFP used is engineered to contain a nuclear localization signal, causing GFP to locate to the nucleus. The bright-field image of an egg of a transformant (a) and a fluorescence image of the same egg (b) are shown. Fluorescence is confined to the nuclei of the gut cells. Although not shown, a similar expression pattern can be seen in all later developmental stages. This suggests the normal site of expression of *Ce-npa-1* is the gut. Construction of the plasmid, transgenic strains and microscopy by Moira Watson and Iain Johnstone, Wellcome Centre for Molecular Parasitology, University of Glasgow. Scale bars = 8.3 μ m.

in body fluids or cytosol because they are membrane-damaging, insoluble and (particularly for polyunsaturated forms) sensitive to oxidation. Retinol is particularly sensitive, and decays rapidly in water unless protected. *Ascaris* might have a particular need for high

concentrations of a lipid transporter because its pseudocoelomic fluid has very high levels of fatty acids (equivalent to that of human plasma after several days of starvation and consequent mobilization of fat reserves; L.M. Fixter *et al.*, unpublished).

Therefore, it is possible that NPAs are made in the nematode gut, loaded with lipids, secreted into the pseudocoelomic fluid and thereby transported to the organ utilizing small lipids. They might transfer their cargo by direct release into cell membranes, or they might interact with a receptor, or be internalized and then released intact from the cell to continue shuttling lipids. If receptors exist, then they are most likely to be found at high density on cells with high metabolic demands, such as those of the gonads and neuromusculature. Whether or not the NPAs constitute the main vehicle for the mass transport of lipids in the pseudocoelom remains to be seen, but (aside from the vitellogenins involved in provisioning the eggs) it is not known whether nematodes have functional equivalents of the large lipoproteins of vertebrates and the lipophorins of insects.

Filarial worms might acquire part of their nutrition by absorption of metabolites across their cuticles. The

Table 1. The naming of nematode polyprotein allergen/antigen (NPA) polyproteins, units and genes^a

Organism	Name of protein	New name of polyprotein	Gene
Polyproteins			
<i>Ascaris suum</i>	ABA-I	As-NPA	<i>As-npa-1</i>
<i>Ascaris lumbricoides</i>	ABA-I	Al-NPA	<i>Al-npa-1</i>
<i>Dictyocaulus viviparus</i>	DvA-I	Dv-NPA	<i>Dv-npa-1</i>
<i>Brugia malayi</i>	gp15/400	Bm-NPA	<i>Bm-npa-1</i>
<i>Caenorhabditis elegans</i>	Ladder protein	Ce-NPA	<i>Ce-npa-1</i>
<i>Toxocara canis</i>	TBA-I	Tc-NPA	<i>Tc-npa-1</i>
<i>Loa loa</i>	LL20; 15 kDa polyprotein	Ll-NPA	<i>Ll-npa-1</i>
<i>Ascaridia galli</i>	AgFABP	Ag-NPA	<i>Ag-npa-1</i>
<i>Setaria cervi</i>	Ladder protein	Sc-NPA	<i>Sc-npa-1</i>
<i>Litomosoides carinii</i>	Ladder protein	Lc-NPA	<i>Lc-npa-1</i>
<i>Dirofilaria immitis</i>	Neutrophil chemotactic factor; Cuticular antigen; Excretory/secretory antigen	Di-NPA	<i>Di-npa-1</i>
<i>Ostertagia ostertagi</i>	Polyprotein antigen	Oo-NPA	<i>Oo-npa-1</i>
<i>Acanthocheilonema viteae</i>	Ladder protein	Av-NPA	<i>Av-npa-1</i>
<i>Strongyloides stercoralis</i>	Allergen polyprotein homologue	Ss-NPA	<i>Ss-npa-1</i>
<i>Onchocerca cervicalis</i>	Ladder protein	Oc-NPA	<i>Oc-npa-1</i>
Individual units			
<i>Dictyocaulus viviparus</i>	DvA-I unit L	Dv-NPA-L	<i>Dv-npa-l</i>
<i>Ascaris suum</i>	ABA-I unit A I	As-NPA-A I	<i>As-npa-a I</i>
<i>Ascaris suum</i>	ABA-I unit B	As-NPA-B I	<i>As-npa-b I</i>

^a The above list of NPAs is derived from a BLAST search using the amino acid sequence of the ABA-I protein. The individual NPA (nematode polyprotein allergens/antigens) units listed are merely illustrative examples of those that have been functionally tested. The genes and polyproteins they encode are prefixed by the initials of the species, followed by the specific protein product or gene designation. Where the initials of two species are the same, then an additional letter is used to discriminate from a previously described protein/gene (for example the NPA of *Toxocara canis* is Tc-NPA, and that of *Toxocara cati* and *Teladorsagia circumcincta* would be Tct-NPA and Tcc-NPA, respectively). Genomic analysis to date has indicated that there is probably only one copy of any *npa* gene per haploid genome, but the genes are named *npa-1* in case other members of the gene family are found. The individual units of the polyproteins should be assigned letters in order of their position from the N-terminus of the protein or 5'-end of the encoding gene (Fig. 1). This is not feasible with most NPAs described to date because the full sequences are not available, so current names should be retained until full information is available.

finding that NPAs are more accessible to external radiolabelling than in other species^{27,29} possibly indicates that the proteins function to capture and transport small lipids acquired by this route.

Other functions? Within the polyprotein of *D. viviparus* is a short His-rich section in the C-terminal region of one of the units (Fig. 1). The significance of this was not appreciated until the gene for the *C. elegans* homologue was sequenced; it too encodes a short His-rich section in one of the units. The two His-rich peptides are very similar, and resemble one another more than do the main units of the respective NPAs. His-rich proteins are known to bind haem and metal ions, and synthetic peptides corresponding to the *D. viviparus* His-rich region do indeed bind metal ions (Cu^{2+} , Ni^{2+} and Zn^{2+} , but not Ca^{2+}) and haem (J. Houseley *et al.*, unpublished). Therefore, the NPA units possessing these regions might be involved in the salvage of haems released from globins and other haem-containing proteins (haems are toxic and their loss would also represent a loss of iron), as well as in the capture and transport of important metal ions. The binding (and packaging) of haem by NPAs might be as important a function for blood-feeding nematodes as it is for insects³⁷. Unfortunately, nothing is yet known of the NPAs of blood-feeding nematodes such as *Necator* and *Haemonchus*.

Structure

The small, ≈ 15 kDa lipid-binding proteins of vertebrates, insects, arachnids and flatworms belong to a family of β -sheet-rich proteins that are predominately intracellular carriers of fatty acids, retinol or retinoic acid³⁸. The NPA units are quite different in that they show no evidence of β -structure and are predicted to fall into four main stretches of α -helix (Fig. 3). Such four-helix bundle proteins include cytochrome b_{562} , myohemerythrin (a non-heme oxygen carrier of sipunculid worms), ferritin and the coat protein of some viruses. Vertebrate proteins that might be similar to NPAs are the cytoplasmic acyl coenzyme A-binding proteins, which are of a similar size and helix composition³⁹. Despite apparent similarities in lipid-binding activity and expected structures, the amino acid sequence of NPA units (both between species and within a single polyprotein) can be dramatically different, but there are invariant signature amino acid positions that are diagnostic for NPAs (Fig. 3).

NPA units form dimers, but it is not yet known whether their biochemical activity depends on the formation of a dimer, although it appears that the stability of the monomer units and the formation of the dimer might be interdependent^{32–34}. The fact that NPA units dimerize might be of some relevance to immune reactions in that a homodimer might act as an efficient crosslinker of mast cell IgE receptors because it will present duplicate epitopes.

One notable characteristic of the NPAs is that they are extraordinarily resistant to thermal denaturation; in two cases, calorimetry experiments have shown that temperatures in excess of 90°C are required for unfolding to occur. It is possible that this might contribute to the failure of cooking to destroy nematode allergens^{21,40}.

Although no details are yet known about the lipid-binding site of NPAs, it appears from fluorescence

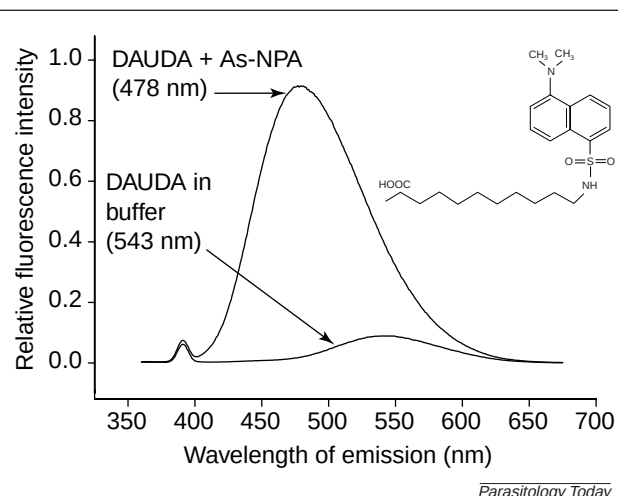


Fig. 5. Binding of fatty acids by nematode polyprotein antigen/allergen (NPAs). This is best demonstrated using the fluorescently tagged fatty acid 11-[(5-dimethylaminonaphthalene-1-sulphonyl)amino]undecanoic acid (DAUDA; structure shown), which can provide additional information on the environment of the binding site itself. The emission spectra of DAUDA in buffer alone and upon addition of ABA-I protein illustrate the dramatic increase in fluorescence intensity and the strong shift in the wavelength of peak emission towards the blue end of the visible light spectrum. The change in fluorescence emission is reversible by addition of non-fluorescent ligands, which displace the fluorescent lipid back into water, where its fluorescence is weak. Data from Ref. 32. One does not require elaborate instrumentation to see the effect – it can easily be seen by placing microcentrifuge tubes containing the reagents on a transilluminator, or even in the beam of a fluorescence microscope, and normal colour vision. The result can be obtained almost instantaneously, with no requirement for radiolabelled materials. The degree of the blue shift in fluorescence of DAUDA is much more than is seen for other fatty acid-binding proteins, such as serum albumin, so the environment of the binding site of NPAs appears to be highly unusual. The fluorescence excitation wavelength used was 345 nm, and the dissociation constant was calculated to be 8.8×10^{-8} M, which is in the expected range of affinity for reversible binding to a carrier protein. Adapted, with permission, from Ref. 58.

experiments using environment-sensitive lipid probes that the binding site of NPAs is highly apolar, much more so than are the binding sites of vertebrate fatty acid-binding proteins³³. This also means that the bound ligand is isolated from solvent water, which in turn might tell us something about the importance to nematodes of protecting the lipids carried by NPAs.

Some NPA units are glycosylated, although this is not widespread. For example, only two of the NPA units of *D. viviparus* (and one of these is an aberrant unit) and *C. elegans* have consensus glycosylation sites. The glycosylation of the gp15/400 of *Brugia* spp has, however, received attention as a potential force in controlling immune responses to the parasites, and it appears likely that glycosylated natural, or non-glycosylated recombinant, proteins are processed differently by antigen-presenting cells¹⁴.

Why polyproteins?

Polyproteins come in two main types. First, there are the polyproteins of viruses and precursors of some vertebrate hormones, which are cleaved to produce dissimilar proteins or peptides with completely different

Table 2. Ligand binding by polyprotein allergens/antigens (NPAs)^a

Binding	No binding
Oleic acid	Tocopherol (vitamin E)
Retinol	Tocopherol acetate
cis-Parinaric acid	β-Carotene
trans-Parinaric acid	Mebendazole ^c
Arachidonic acid	Albendazole ^c
Lysophosphatidic acid	Thiabendazole ^c
Lysophosphatidyl ethanolamine	Oxibendazole ^c
Lysophosphatidyl choline	Piperazine ^c
Platelet-activating factor	Tetramisole ^c
Lysoplatelet-activating factor	Pyrantel ^c
Leukotrienes B ₄ , C ₄ , D ₄ , E ₄ ^b	Diethyl carbamazepine (DEC) ^c
Bilirubin	Levamisole ^c

^a Binding was tested either by competitive displacement of a bound fluorescent ligand by a non-fluorescent compound (Fig. 5), or by detecting changes in the fluorescence of natural ligands (eg. retinol, parinaric acid). Composite data are presented from nematode-derived and recombinant NPAs^{32–35}, but mostly based on work with recombinant ABA-1 protein from *Ascaris lumbricoides*³².

^b Binding with these compounds was detectable but is difficult to quantify in the fluorescence assay.

^c These compounds are widely used anti-nematode drugs. Adapted, with permission, from Ref. 32.

functions. Second, there are the tandemly repetitive polyproteins that yield multiple copies of similar or identical proteins, which is the class to which NPAs belong. The only other examples of this class are certain structural proteins of the vertebrate epidermis, such as the filaggrins of terminally differentiating keratinocytes⁴¹. It has been suggested that this type of polyprotein might be specific to cells undergoing programmed cell death (PCD), producing specialized proteins, the functions of which are fulfilled only after the demise of the cell⁴¹. The production of polyproteins is seen as a highly efficient means of producing proteins rapidly and economically, avoiding the burden of signal sequences, etc., for every functional molecule produced. The lack of introns would contribute to this economy and will reduce the need for RNA processing machinery in a senescing cell.

NPAs show that repetitive-type polyproteins are not confined to cells undergoing PCD. In nematodes, PCD appears to occur only in somatic cells at early stages of development, so NPAs are not made by apoptotic cells. The question, then, is why are the NPAs made as polyproteins, and why are polyproteins not more common? Even in *C. elegans*, there is no sign of other types of protein being made this way.

A final distinguishing feature of the NPAs is that they are the only lipid-binding protein known to be synthesized as polyprotein precursors – even the most abundant lipid-binding protein in human blood, albumin, is made in the conventional manner.

What did NPAs arise from?

There are signs in the amino acid sequence of a single unit of an NPA (eg. ABA-1; Fig. 3) that the ancestral unit arose from an ancient duplication event. There is no particular reason to believe that the progenitor was itself a lipid-binding protein. However, the C-terminal half of ABA-1 can bind lipid on its own (L. McDermott, PhD thesis, University of Glasgow, 1999), so a small lipid-binding ancestor is conceivable. Very small (8–9 kDa), helix-rich, lipid-binding proteins have been described

from plants and tapeworms^{42,43}. Small extracellular proteins would be lost by the excretory systems of many organisms, so duplication or multiplication might serve to create larger entities that are above the excretory threshold – this is perhaps why albumin (also thought to have arisen through duplication) and other vertebrate blood proteins are large, or are found in plasma in complex with others [eg. retinol-binding protein (21 kDa) complexes with transthyretin]. The dimerization of NPA units will contribute to this effect and might also limit their diffusion within the nematode.

Do NPAs have a role in parasitism?

First of all, do free-living nematodes have NPAs that are similar to those of parasites? An *npa* gene has been found in *C. elegans*, and a recombinant protein derived from it has been shown to have similar biochemical activity to that of parasitic species (A. Cooper *et al.*, unpublished). Interestingly, the *C. elegans npa* gene encodes a polyprotein whose units differ dramatically in amino acid sequence, as does that of the bovine lungworm *D. viviparus*. The NPAs of filarial nematodes and *Ascaris*, however, appear to have many similar units, although there is incomplete information on these, and diversity might still await discovery. The evolution of the NPAs therefore appears to have predated parasitism, but they might nevertheless have a role in parasitism.

In addition, it must be asked whether the release of NPAs by living parasites is purposeful and not an artefact. For instance, ABA-1 appears in the culture supernatant of *Ascaris* larvae, but these larvae are undergoing moults and other developmental processes that might adventitiously release the protein; and the death and disintegration of even a small proportion of larvae could easily contaminate the culture medium. Connected to this is the finding that larvae of a related ascarid, *Toxocara canis*, do not seem to release their NPA (TBA-1) *in vitro*, and these larvae are in developmental arrest and survive better than most nematodes in culture^{23,24,44}. However, there are other cases where secretion of NPAs occurs in cultures in which the viability of the worms appears to be sound, such as with *Brugia* and *Dirofilaria*^{25,26,28}, so if NPAs have a function in survival in the host, then their value varies from species to species.

Assuming that the release of NPAs is biologically meaningful, what might their function be? Their lipid-binding activity is relatively broad and they could therefore be involved in capturing important lipids for the parasites. This would probably require the existence of a receptor for re-absorption of NPAs, but there is no information on such putative receptors at the moment. The binding propensities of NPAs potentially encompass small lipids that are very important in the regulation of local, and even systemic, tissue inflammatory and immune responses, and in tissue differentiation and repair. Examples of these important signalling lipids include polyunsaturated fatty acids such as arachidonic acid (and its metabolites – the leukotrienes, prostaglandins and thromboxanes), retinol and retinoic acid. Leukotrienes are important in allergic reactions⁴⁵, and prostaglandin D₂ has recently been shown to be potentially important in allergic asthma⁴⁶. So, if NPAs were to sequester or deliver such mediators, then the parasites could thereby

modulate local inflammatory and immune reactions or, by interfering with retinoid communications, affect tissue repair and differentiation processes⁴⁷. There is already evidence that nematodes release pharmacologically active lipids⁴⁸, as do some parasitic arthropods⁴⁹, and they may need carrier proteins to solubilize and protect these labile compounds, and perhaps even deliver them to appropriate surface receptors on host cells.

If the secretion of NPAs is important to parasites, are they therefore potential targets for drugs or vaccines? The wide range of lipids that they bind might seem to indicate that there is limited scope for the development of drugs against NPAs because interfering with their function is likely also to affect the lipid carriers of the host. However, the binding site of NPAs is highly unusual³³, so there might still be scope for developing specific drugs, although much more will need to be understood about the structure of NPAs for progress to be made. In terms of vaccination, there is little published evidence yet that NPAs are protective²⁶ although, as these authors note, successful immunization could crucially depend on the type of adjuvant used.

Other lipid-binding proteins peculiar to nematodes

Why do nematodes and vertebrates use such different proteins for apparently the same purposes – transporting lipids? Nematodes do make small, lipid-binding proteins rich in β -structure similar to our own^{50,51}, but only nematodes seem to make NPAs and LBP-20 proteins⁵². Did a common metazoan ancestor produce several types of lipid-binding protein, and nematodes retained the use of two types that were lost in other phyla, or were the NPAs and LBP-20s new inventions of the nematodes? Is there something about the physiology of nematodes that makes these proteins better suited to these organisms?

In addition to the NPAs and LBP-20s, nematodes are also known to secrete another type of lipid-binding protein – a phosphatidylethanolamine (PE)-binding protein is released by *T. canis* larvae, and possibly also by *O. volvulus*, although these have clear similarities to vertebrate PE-binding proteins^{53,54}. Fatty acid-binding proteins produced by nematodes that are like those in the cytosol of mammals are unusual in that they are secreted from the synthesizing cell and appear to be structurally modified^{50,51}. Clearly, there is still much to be understood about the role of lipid-binding proteins in nematode biology and parasitism, but a particularly interesting aspect would be their involvement in lipid-based modification of the host tissue environments they occupy.

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