Protein turnover in the cytoplasmic transport system within an insect ovary – a clue to the mechanism of microtubule-associated transport

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The movement of radioactively labelled polypeptides into the microtubule-associated transport channels in the ovaries of a hemipteran insect has been analysed using SDS-polyacrylamide slab gel electrophoresis and fluorography. The patterns of label suggest that the microtubules which pack the transport channels form a relatively static cytoskeleton while other components move independently from them along the channels. As well as illustrating the functional organisation of microtubule-associated transport in this system our studies of labelled proteins have also provided clues as to the mechanism of transport itself.

Cytoplasmic transport *(Insect ovary)* *Microtubule* *Protein turnover*

1. INTRODUCTION

In the ovarioles of certain insects the developing oocytes are connected over several millimeters to an anterior region of nutritive cells by way of cytoplasmic channels known as nutritive tubes. In *Notonecta*, which has been extensively studied, ribosomes synthesised in the nutritive cells have been shown to pass along the nutritive tubes which are packed with an enormous system of longitudinally oriented microtubules [1], but the mechanism of transport is not yet understood and it is unclear in this instance whether the microtubules play an active role in the transport [2].

One of the major complications in understanding the role, if any, of the microtubules in the production of force for transport is that it has often been assumed that components move down between the microtubules of the nutritive tubes, although this has not actually been demonstrated. It could just as easily be the case that the entire contents pass along the nutritive tube en masse. Essentially, it is not known whether material is moved through a stationary phase or carried in a moving phase, and this crucial question is addressed here.

We have previously started to characterize the protein components of the transport system within the nutritive tubes of *Notonecta* [3,4]. We considered this valuable, since if the mechanism for transport exists within the transport channel, then all its components should be resolved in this way. As one would expect from earlier ultrastructural studies, the major polypeptides of the nutritive tubes are tubulins, possibly with a number of microtubule-associated proteins (MAPs), and of the numerous other bands, some have been attributed to ribosomal proteins and some to soluble material passing from the nutritive cells along the nutritive tubes to the oocytes. Other bands remain to be characterized.

Here we set out to investigate the functional organisation of microtubule-associated transport by comparing the staining pattern of proteins within the nutritive tubes after SDS-polyacrylamide gel electrophoresis, with the appearance of newly synthesised radioactively labelled polypeptides into this pattern from the nutritive cells, as detected by fluorography. By

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doing so, we have found that the amounts of many of the polypeptides present bear little or no relation to the extent to which they are labelled in the nutritive tube, or to their levels of synthesis in the trophic region during the course of the experiments. This we interpret as indicative of the independent movement of certain compounds relative to others rather than a cohesive flow along the nutritive tubes.

Our results are discussed in relation to the different possible motile mechanisms.

2. MATERIALS AND METHODS

Females of *N. glauca* were collected from Slapton Ley in Devon. Within 48 h, each was injected with 5 μCi of a mixture of 15 tritiated amino acids (Amersham International) using a Hamilton syringe. They were kept in an aquarium at room temperature with food available for 5 days, after which the ovaries were dissected out in modified Locke's insect Ringer (fig.1). Nutritive tubes were pooled (fig.2) following microdissection from approx. 20 ovaries as described in [3] with the minor modification that the microtubule stabilizing buffer was 0.05 M Pipes, pH 6.9, 0.5 mM EGTA, 0.05 mM $MgSO_4$. Trophic regions containing nutritive cells were simply excised from the anterior ends of ovarioles. Samples were then lyophilized and prepared for electrophoresis, which was performed in SDS-polyacrylamide slab gels containing a 5–10% linear acrylamide gradient, according to Laemmli [5]. The resolved polypeptides were fixed and visualized by staining for protein with Coomassie blue [6].

Radioactively labelled polypeptides were then

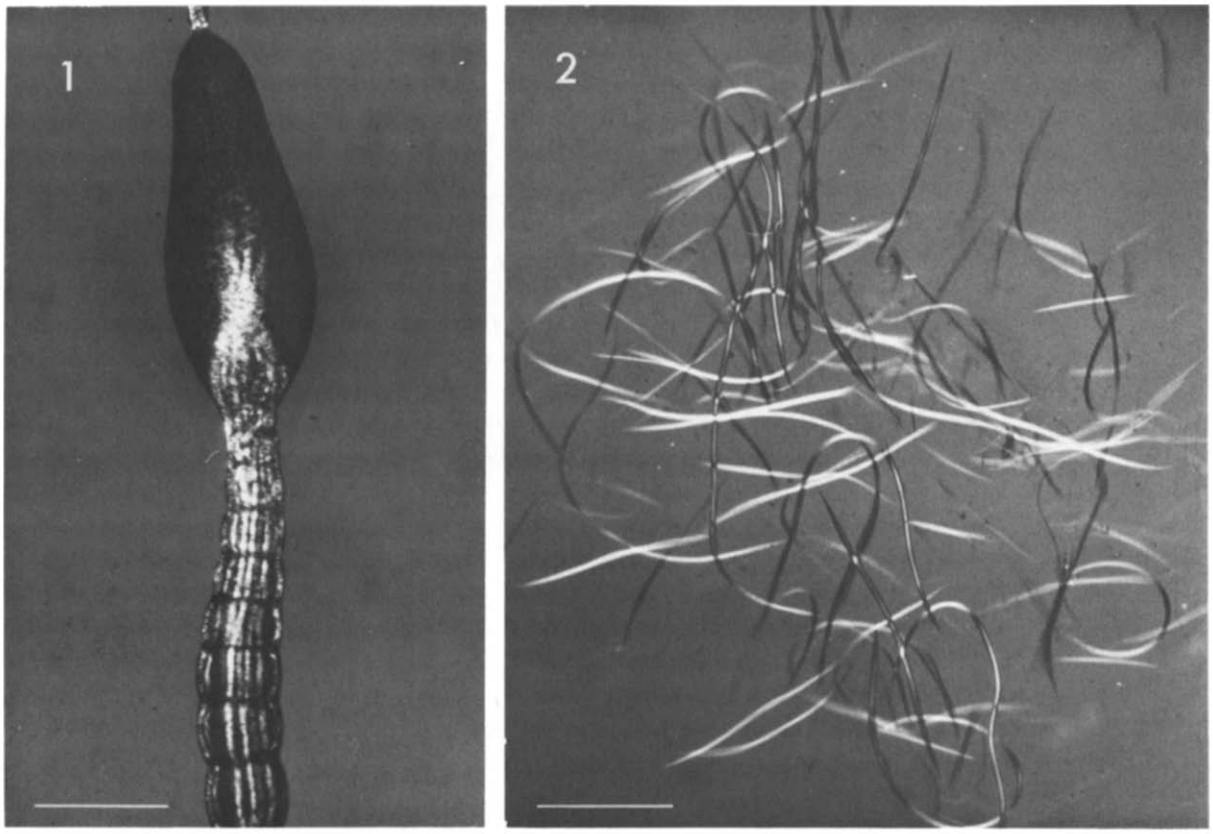


Fig.1. Ovariole of *Notonecta glauca* seen in polarized light with birefringent nutritive tubes clearly visible. Bar = 0.4 mm.

Fig.2. Pool of nutritive tubes collected from one insect, viewed in polarized light. Bar = 0.4 mm.

detected in the same gels by fluorography after the bands had been destained. For this the gels were treated with AMPLIFY (Amersham International), dried and then allowed to expose preflashed X-Omat AR-5 X-ray film (Kodak) for 12 weeks at −80°C.

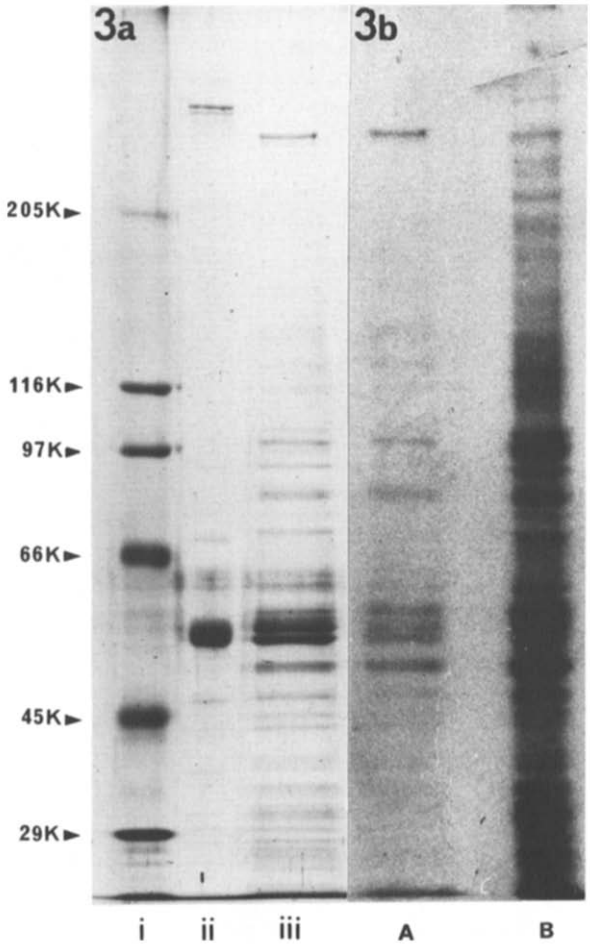
Thrice-cycled microtubule protein was prepared from porcine brain by an assembly-disassembly method [7].

3. RESULTS

Gels of nutritive tubes stained with Coomassie blue showed tubulin bands at 55 and 53 kDa based on the relative mobility of known standard proteins (fig.3a). These were by far the most prominent bands, being equivalent to approx. 50% of the total protein of the nutritive tubes. In addition, there were in the order of 15–20 bands of lower molecular mass than tubulin, the most heavily stained being one at 49 kDa, and a similar number of higher molecular mass, notably with molecular mass of 58, 65, 66, 74, 83, 91, 100 kDa and a closely migrating pair (a major and a minor species) of greater than 205 kDa.

Co-electrophoresis of nutritive tubes and brain microtubule protein in adjacent tracks on the same gel served to confirm the identity of the tubulin from the nutritive tubes (fig.3a) and also permitted a comparison to be made between the high molecular mass MAPs associated with brain tubulin and the pair of bands of slightly lower molecular mass (>205 kDa) resolved from the nutritive tube samples.

The Coomassie blue staining patterns of gels denoting the amounts of different polypeptides within the nutritive tubes show little correlation with fluorogram labelling patterns of the same gels (fig.3b). The differences are particularly conspicuous on comparison of densitometric scans of



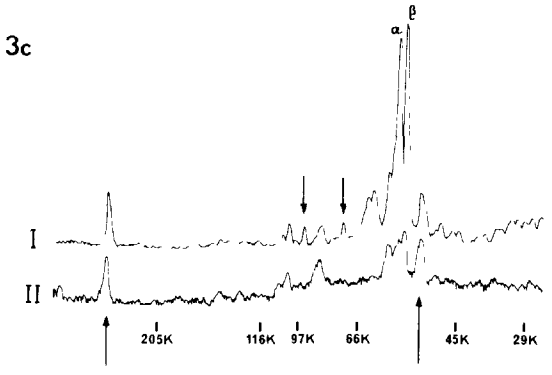


Fig.3. (a) A typical profile of nutritive tube proteins resolved by SDS-PAGE. Lanes: i, molecular mass standards; ii, reference microtubule protein purified from porcine brain showing tubulin and MAP bands; iii, nutritive tube proteins. (b) Fluorographic detection of radioactively labelled polypeptides resolved by SDS-PAGE. (A) Fluorogram of a, lane iii and (B) fluorogram of polypeptide components resolved from nutritive cells. (c) Quantitative densitometric scans of nutritive tube proteins resolved by SDS-PAGE. (I) Scan of the Coomassie blue-stained protein bands (as seen in a, lane iii) and (II) scan of fluorogram (b). α and β denote positions of respective tubulin peaks. The arrows indicate the positions of peaks referred to in the text illustrating differences between the 2 scans.

the stained gels and the developed fluorograms (fig.3c). The most obvious feature is that the tubulin bands are not heavily labelled in the fluorograms, amounting to only approx. 18% of the total label. Indeed, other bands such as that at 49 kDa are more heavily labelled than those representing tubulin. Most other bands are labelled to an extent, but some, such as those at 74 and 91 kDa, show no detectable label. Strikingly, the bands at >205 kDa are some of the most heavily labelled of all. The amount of label detected in individual polypeptides from nutritive tubes appears to be unrelated to their level of synthesis in the nutritive cells as can be seen by comparing fluorograms of nutritive cells and nutritive tubes (fig.3b).

4. DISCUSSION

From this study it is quite clear that the entire contents of the nutritive tubes do not pass en masse down these channels to the oocytes. This is indicated by the fact that the pattern of radioactive label seen in fluorograms does not correspond to the pattern of stained protein bands resolved from nutritive tubes by electrophoresis, and suggests that, once synthesised, different components move at different rates into and along these transport channels.

The observation that tubulin is by far the major component of the nutritive tubes but is only relatively lightly labelled during the 5 day incorporation period, which is the time calculated for ribosomes to more than pass the whole length of the tubes [1], implies that the microtubules represent a relatively static cytoskeleton. What label is seen in the tubulin bands could be explained either by the slow but continuous elongation of the tubes, and with this, their component microtubules [8] or, alternatively, by the transport of tubulin in unpolymerized form to the oocytes which are known in certain species to accumulate tubulin.

Of the other bands, some are apparently unlabelled, while others are heavily labelled, indicative of their independent passage along the nutritive tubes. To this extent, transport along nutritive tubes is comparable to that along nerve axons which has received considerable attention [9] and where certain components move at approx. 400 mm/day while cytoskeletal elements, which of course in axons include neurofilaments, move at 0.1–10 mm/day. In fact, in axons, at least 5 different groups of polypeptides, each characterized by its rate of movement, have been demonstrated and the results presented here illustrate that a similar situation may exist with insect nutritive tubes. Certainly Mays [10] has shown at least 2 rates in the nutritive tubes of *Pyrrhocoris*.

As well as illustrating the functional organisation of microtubule-associated transport along nutritive tubes, it was considered that studies of labelled proteins might provide clues as to the mechanism of transport itself. Recently, a number of papers have described the exciting demonstrations of transport along single native microtubules isolated from squid axoplasm [11,12] and along flagellar microtubules [13] and these have provided evidence that microtubules can play an active role in cytoplasmic transport. The actual molecules responsible for generating the motive force have yet to be identified, but are clearly linked with the moving particle [13], the native microtubule or its associated proteins [11].

Since their discovery and subsequent visualization in some instances as side-arms from microtubules, MAPs have been regarded as having a possible involvement in cytoplasmic translocation [14], and MAPs have been identified structurally on microtubules and immunocytochemically, in regions of nerve axons associated with rapid axonal transport [15]. Perhaps significantly, some of the most intensely labelled bands in nutritive tubes have molecular masses comparable to MAPs and also show the same sort of quantitative ratio to tubulin as do MAPs to tubulin in microtubules from mammalian brain [16]. At present we are in the process of attempting to discover whether these polypeptides in nutritive tubes are indeed microtubule-associated, because if this is the case, the evidence presented here suggests that their association is a highly dynamic one, and their long-predicted role in cytoplasmic transport may be nearer elucidation.

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