# Structure and molecular organization of higher plant coated vesicles

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## Summary

**Suspension-cultured cells of carrot contain three populations of coated vesicles, associated with the plasma membrane (84–91 nm diameter), Golgi dictyosomes and the partially coated reticulum (61–73 nm diameter). These were observed by thin sectioning, dry-cleaving and rapid-freeze deep-etching of cells. Dissociation of clathrin coats with Tris, released triskelions that were morphologically identical with those from mammalian tissue. The triskelion arm length of carrot clathrin was greater (61 nm *versus* 44–50 nm), but packaging results in clathrin cages of pentagons and hexagons of similar size to those from mammalian cells.**

**SDS–PAGE of Tris-released triskelion preparations revealed a complex of three polypeptides of 190, 60 and 57 ($\times 10^3$) $M_r$. The $190 \times 10^3$ $M_r$ protein is the plant clathrin heavy chain, slightly larger than the mammalian heavy chain. The 60 and 57 ($\times 10^3$) $M_r$ bands showed the same sensitivities to protease treatment as mammalian light chains. Triskelion preparations containing these three proteins reassembled into polyhedral cages.**

**These results are discussed in relation to the structural organization of coated vesicles and clathrin cages in other systems.**

Key words: carrot suspension cultures, clathrin (plant), coated vesicles, electron microscopy, triskelion.

## Introduction

Plant cells, like animal cells, contain coated vesicles (Doohan & Palevitz, 1980; Newcomb, 1980; van der Valk & Fowke, 1981; Mersey *et al.* 1985; Emons & Traas, 1986). One population of these coated vesicles, outer diameter 70–100 nm, appear to be derived from coated pits formed at the plasma membrane (Tanchak *et al.* 1984; Joachim & Robinson, 1984; Hübner *et al.* 1985; Hillmer *et al.* 1986). Another population of coated vesicles, outer diameter 40–70 nm, are associated with the Golgi apparatus (Griffing & Fowke, 1985) and with the partially coated reticulum (Pesacreta & Lucas, 1985). So far no precise function can be assigned to plant coated vesicles with certainty. However, it has been suggested that they are involved in endocytosis (Tanchak *et al.* 1984; Hübner *et al.* 1985; Hillmer *et al.* 1986), exocytosis, and the transport and sorting of proteins from the Golgi to vacuoles (Griffing & Fowke, 1985).

A characteristic feature that plant coated vesicles share with their animal counterparts is a polygonal coat on the cytoplasmic face of the vesicle membrane. The major constituent of the coat is the protein clathrin (Pearse, 1975, 1976). Clathrin is released from mammalian coated vesicles as trimeric molecules, each with a characteristic three-armed structure, termed a triskelion. Each arm of a triskelion consists of two polypeptide subunits, a heavy chain ($M_r$ $180 \times 10^3$) and a light chain whose relative molecular mass depends on the tissue of origin, but is usually in the 30–40 ($\times 10^3$) range. Each tissue has two distinct types of light chain, designated LCa and LCb. In mammals LCa and LCb from brain tissue have higher apparent molecular masses (36 and 34 ($\times 10^3$)) than those from other tissues (34–30 $\times 10^3$) (Pearse, 1978; Ungewickell *et al.* 1982; Brodsky & Parham, 1983; Creutz & Harrison, 1984; Jackson *et al.* 1987).

In comparison with mammalian clathrin, there is relatively little detailed information on the molecular composition and organization of plant clathrin. Previously (Cole *et al.* 1987) we reported that coat protein could be released from coated vesicles isolated from carrot (*Daucus carota* L.) cells grown in suspension culture. The released protein was resolved by SDS–PAGE into three major polypeptide bands with relative molecular masses of 190, 60 and 57 ($\times 10^3$). By use of protein immunoblotting these bands were shown to react positively with antibodies raised against bovine brain clathrin. We tentatively designated these three

bands as the heavy chain ($190\times10^3$ $M_r$) and light chains (60 and 57 ($\times10^3$) $M_r$) of plant clathrin.

In this paper we present further information on the localization and structure of the different coated vesicle populations in cultured carrot cells. In addition we show that clathrin dissociated from isolated coated vesicles and partially purified can be visualized as triskelions that reassemble *in vitro* into cage-like structures and also that the protein subunits from isolated triskelions show differential sensitivities to proteolytic enzymes.

## Materials and methods

### *Cell culture*

The original cell line of domestic carrot (*Daucus carota* L.) was kindly supplied by Dr Hughes of Unilever Research, Sharnbrook, Bedfordshire, UK. Cells were grown as a suspension culture in Murashige & Skoog (1962) medium (M & S) (Flow Laboratories Ltd), supplemented with 2·5% (w/v) sucrose, 0·1 mg ml$^{-1}$ 2,4-dichlorophenoxyacetic acid, 0·1 mg ml$^{-1}$ zeatin and 5% (v/v) coconut milk. Cells were grown and subcultured as described by Chalmers & Coleman (1983).

### *Preparation of protoplasts*

Cells were harvested by centrifugation (250 ***g*** for 5 min) and washed with fresh M & S medium containing 0·4 M-sorbitol at pH 5·5. Washed cells were re-suspended to give 10–12% (w/v) in M & S/sorbitol to which was added 4% (w/v) cellulase (Onazuka R10) and 2% (v/v) pectinase (Sigma). Cells were then incubated for 90–120 min at 30°C with gentle shaking. The suspension was then filtered through a wad of sterile glass wool to remove debris and any cells with intact walls. The filtrate was centrifuged at 100 ***g*** for 10 min to pellet the protoplasts, which were washed twice with M & S/sorbitol and the final pellet was re-suspended in M & S/sorbitol.

### *Isolation of coated vesicles*

Coated vesicles were isolated from rapidly growing suspension-cultured cells 7–8 days after sub-culture. Prior to harvesting cells were placed in fresh growth medium for 12 h to maximize production of coated vesicles (Hawes, unpublished). Cells were harvested by filtration through Miracloth with the aid of gentle suction. The isolation procedure was a modification of that described by Depta & Robinson (1986) and unless otherwise stated all procedures were carried out between 0 and 4°C. Harvested cells (250–300 ***g*** fresh weight) were mixed with an equal volume of acid-washed sand and an equal volume of homogenizing medium (0·1 M-Mes, 0·5 mM-$MgCl_2$, 1 mM-EGTA, 3 mM-$NaN_3$, 1 mM-dithiothreitol and 0·005% phenylmethylsulphonyl fluoride (PMSF) at pH 6·4) and ground in a mortar with a pestle. The ground mixture was centrifuged at 3000 ***g*** for 15 min, and the supernatant was removed and retained. The pellet was re-extracted with fresh medium and the supernatants combined. The supernatant was then filtered through fine Miracloth and centrifuged at 40 000 ***g*** for 30 min. The pellet was discarded and the supernatant centrifuged at 80 000 ***g*** for 60 min. The supernatant was discarded and the pellet re-suspended in a small volume of the homogenizing medium containing protease-free ribonuclease (80 $\mu$g RNase/mg pellet protein). The suspension was incubated for 45 min at 30°C and centrifuged at 10 000 ***g*** for 40 min.

The supernatant was removed and layered onto a discontinuous gradient made up of 2 ml 5%, 4 ml 10%, 4 ml 40%, 2 ml 50% and 2 ml 60% sucrose. The gradients were centrifuged at 50 000 ***g*** for 105 min. The 10% and 40% sucrose layers were removed, diluted with 3 vol. of homogenizing buffer (minus PMSF) and centrifuged at 85 000 ***g*** for 1 h. This gave a coated vesicle pellet, which was re-suspended in a small volume of homogenizing buffer. Electron microscopy showed the pellet to be essentially coated vesicles with a small percentage of smooth membranes.

### *Dissociation of coated protein*

Coated vesicle pellets were re-suspended and incubated in 0·75 M-Tris·HCl, 25 mM-Mes/NaOH, 25 mM-EGTA, 0·12 mM-$MgCl_2$ at pH 6·5 for 12 h at 4°C. Vesicles were pelleted by centrifugation at 120 000 ***g*** for 1 h. The resulting supernatant was taken to 30% saturation with solid ammonium sulphate at 4°C. Precipitated protein was sedimented by centrifuging at 120 000 ***g*** for 30 min.

For SDS–PAGE and visualization of the triskelions the precipitated protein was redissolved in 0·5 M-Tris·HCl, 50 mM-Mes/NaOH, 0·5 mM-EGTA, 0·25 mM-$MgCl_2$, pH 6·5, and residual ammonium sulphate was removed by concentrating and reconstituting in the above buffer using Centricon-10 microconcentrators (Amicon). This procedure was repeated twice.

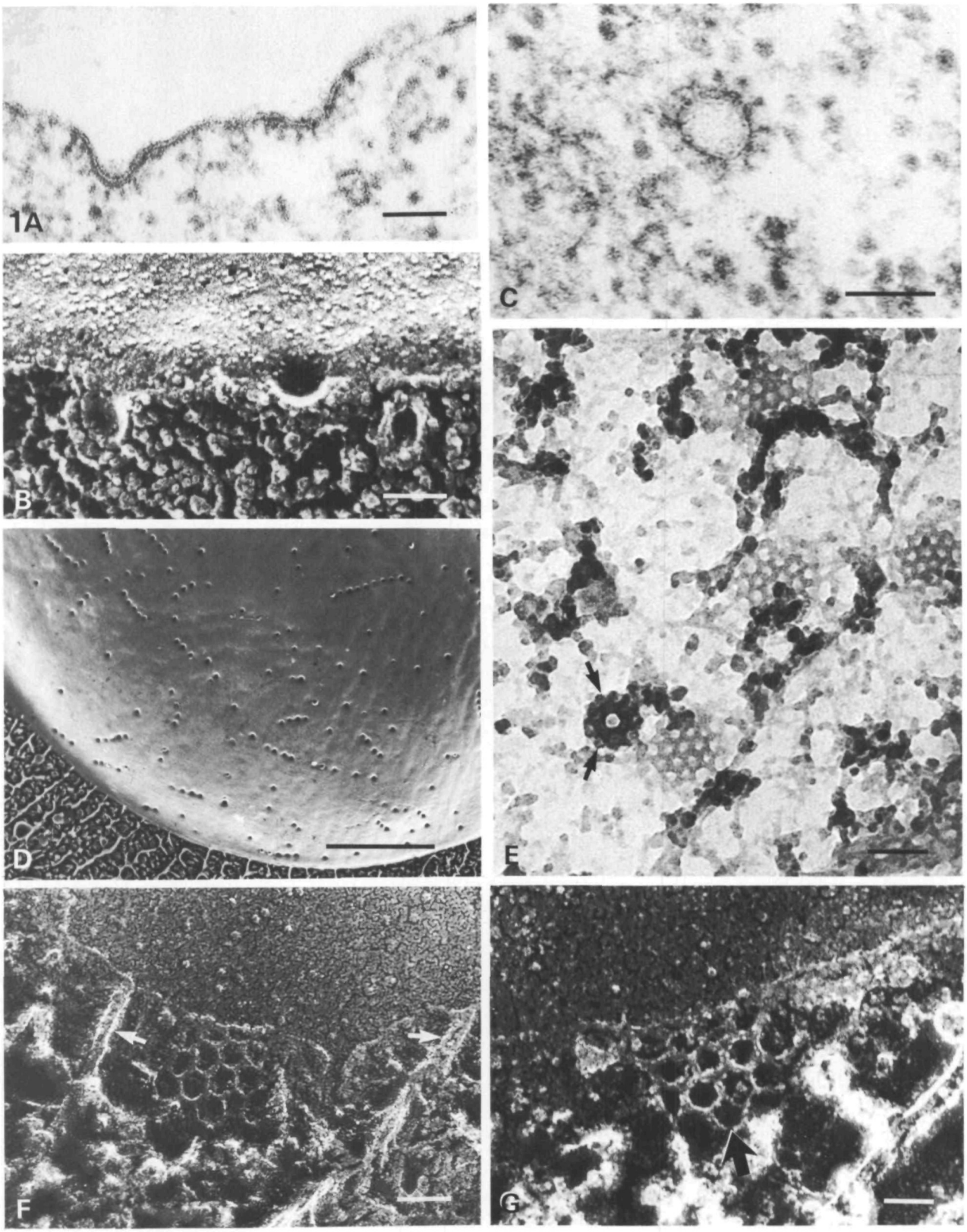
For re-assembly of clathrin cages the precipitated protein was redissolved in 20 mM-Mes, pH 6·2, 2 mM-$CaCl_2$, 1 mM-$MgCl_2$ and 1 mM-EGTA. Concentration and reconstitution was carried out as described above. Protein was finally concentrated to >30 $\mu$g ml$^{-1}$.

### *Proteolytic digests*

Preoteolysis was carried out on Tris-released clathrin light and heavy chains by the techniques of Ungewickell *et al.* (1982). Clathrin triskelions (1 mg ml$^{-1}$ protein in 0·75 M-

**Fig. 1.** Coated pits and vesicles associated with the plasma membrane. A. Thin section of a protoplast showing two stages in the formation of a coated pit on the plasma membrane. Bar, 50 nm. B. Deep-etch replica of an unfixed, rapidly frozen and etched protoplast showing the stages of pit formation from the fracture face of the plasma membrane. Bar, 50 nm. C. Thin section of a coated vesicle showing the spiney nature of the coat in cross-section. Bar, 100 nm. D. Protoplasmic fracture face of a protoplast plasma membrane showing an uneven distribution of pits. That these are coated can be seen from the fractured protoplast in B. Bar, 2 $\mu$m. E. Dry-cleave preparation from a whole cell showing coated pits and a vesicle (arrows) associated with the protoplasmic surface of the plasma membrane. Bar, 50 nm. F,G. Deep-etch replicas demonstrating the hexagonal and pentagonal (large arrow) nature of the clathrin cage. Note also the cortical microtubules (arrows). Bar, 50 nm.

Tris·HCl, 25 mM-Mes/NaOH, pH 6·5, 0·5 mM-EGTA, 0·25 mM-$MgCl_2$) were incubated with either 0·15 mg $ml^{-1}$ trypsin for 2 h at 0°C or 0·1 $\mu$g $ml^{-1}$ elastase for 2 h at 22°C. In both cases the reaction volume was 50 $\mu$l and the reaction was stopped by adding PMSF to a final concentration of 2 mM. The reaction mixture was diluted 1:1 with SDS sample buffer and prepared for gel electrophoresis. Controls of trypsin and elastase alone were also included and run in parallel on SDS–PAGE. Electrophoresis was carried out as described by Cole *et al.* (1987).



*Electron microscopy*

*Thin sectioning*

For conventional thin-section electron microscopy, cells and protoplasts were fixed, stained and embedded in Spurr resin as described (Hawes, 1985). Thin sections were stained with lead citrate. Coated vesicles pellets were fixed for 30 min in a 2% glutaraldehyde (v/v)/1% paraformaldehyde (w/v) mixture in 0·1 M-sodium cacodylate buffer (pH 6·9), washed in buffer and stained with 1% tannic acid for 1 h. Pellets were post-fixed with 1% buffered $OsO_4$ for 10 min, washed in buffer, stained overnight in 0·5% aqueous uranyl acetate at 4°C, dehydrated in a graded water/ethanol series and embedded in Spurr resin.

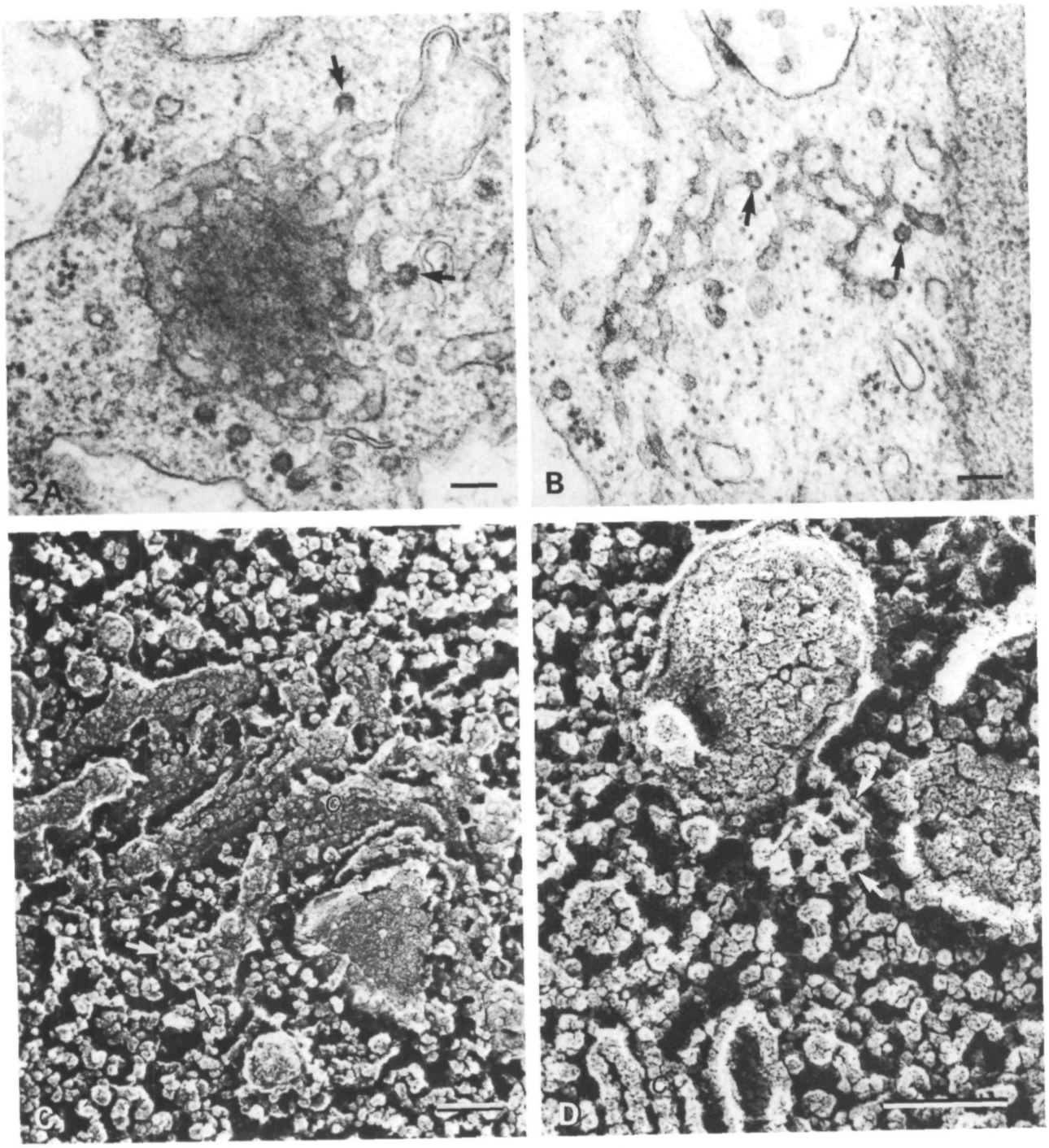



**Fig. 2.** Coated vesicles associated with the Golgi apparatus and partially coated reticulum (PCR). A. Thin section of a dictyosome cisternum in face view. Coated vesicles (arrows) appear to be budding off the peripheral reticulum. Bar, 100 nm. B. Thin section of PCR with associated coated vesicles (arrows). Bar, 100 nm. C. Deep-etch replica of a dictyosome with a clathrin-coated vesicle (arrows) budding off a cisternum (*c*). Bar, 100 nm. D. Coated vesicle (arrows) close to a Golgi stack (*c*). Bar, 100 nm.

*Dry-cleaving.* To reveal coated pits and vesicles on the PS face of the plasma membrane cells were fixed and critical-point dried on Formvar-coated grids and dry-cleaved as previously reported for carrot cells (Hawes, 1985).

*Deep-etching.* For rapid freezing and deep-etching unfixed cells and protoplasts were slammed against a helium-cooled polished copper block at a temperature of 10–12K, fractured, deep-etched, low-angle rotary shadowed with carbon/platinum and carbon replicated as described by Hawes & Martin (1986).

*Replicated triskelions.* Isolated clathrin triskelions and baskets were concentrated to 20 $\mu g\,ml^{-1}$ in a Mes buffer containing 0·1 mm-EDTA, 10 $\mu$M-dithiothreitol in 155 mM-ammonium acetate and mixed with glycerol to give a final concentration of 70% glycerol. Drops (20 $\mu l$) were sprayed onto freshly cleaved mica sheets, which were dried, rotary shadowed at an angle of 20° with carbon/platinum and coated with carbon. The carbon film was subsequently floated onto distilled water and picked up onto grids.

*Negative staining.* Reformed clathrin baskets were negatively stained with 1% phosphotungstic acid on Formvar-coated grids. All electron microscopy was carried out with a JEOL 2000 EX transmission electron microscope operating at 120 kV.

*Morphometry.* Measurements of triskelion arm lengths were made on a Kontron IPS image-processing system.

## Results

### *Structure of coated vesicles*

The cytoplasm of cultured carrot cells contains three populations of coated membranes and vesicles, associated with the plasma membrane (Fig. 1), the Golgi dictyosomes (Fig. 2A,C,D) and the partially coated reticulum (Fig. 2B).

In median section, coated vesicles derived from coated pits on the plasma membrane show the typical structure with short bristles projecting from the vesicle membrane that has been reported (Ockleford & Whyte, 1980) in both animal and plant cells (Fig. 1A,C). These coated vesicles average 84–91 nm in diameter, depending on the preparative technique used (Table 1). The abundance of coated pits on the surface of a protoplast is shown by freeze-fracture that reveals the PF face of the plasma membrane (Fig. 1D). The structure of the vesicle coat can be clearly seen in dry-cleave (Fig. 1E) and rapid-freeze/deep-etch observations of the PS face of the plasma membrane (Fig. 1B,F,G). A characteristic cage of hexagons and pentagons is seen, with dimensions as given in Table 1.

Coated vesicles associated with the Golgi dictyosomes and partially coated reticulum are smaller than those produced at the plasma membrane, averaging 61 and 73 nm in diameter, respectively (Fig. 2). It is often difficult in thin section to resolve the polygonal nature of the coat but this is occasionally revealed in deep-etch preparations of the cytoplasm where clathrin baskets can be seen attached to the periphery of the Golgi cisternae (Fig. 2A, arrows) or vesicles may be free in the cytoplasm (Fig. 2D, arrows).

**Table 1.** *Dimensions of carrot cell coated vesicles and clathrin triskelions*

| | nm |
|---|---|
| Plasma membrane coated pit diameter (dry cleave prep.) | 103·6 ± 2·92 |
| Plasma membrane CV diameter (dry cleave prep.) | 84·46 ± 2·28 |
| Plasma membrane CV diameter (thin section) | 91·03 ± 2·84 |
| Golgi CV diameter (thin section) | 61·44 ± 2·84 |
| Partially coated reticulum CV diameter (thin section) | 72·88 ± 1·97 |
| Isolated CV diameter (thin section) | 85·42 ± 1·24 |
| Reconstituted clathrin basket diameter (negative stain) | 79·12 ± 2·64 |
| Polygon centre-to-centre: | |
| Dry cleave coated pits | 20·94 ± 0·25 |
| Deep-etch coated pits | 27·45 ± 0·42 |
| Isolated vesicles | 20·69 ± 0·34 |
| Length of polygon edge (deep-etch) | 15·97 ± 0·26 |
| Length of triskelion arm plus terminal domain | 61·52 ± 0·99 |
| Length of triskelion arm minus terminal domain | 51·87 ± 0·99 |
| Average length of terminal domain | 9·65 |

CV, coated vesicle.

### *Isolation of coated vesicles and release of the clathrin coat*

Isolated coated vesicles exhibit the same morphology as vesicles derived from the plasma membrane (Fig. 3A,B), with both the polygonal coat and the vesicle membrane revealed in thin section. Measurements of median cross-sections of these isolated vesicles showed them to average 85 nm in diameter, slightly smaller than plasma membrane coated vesicles when measured in sections (Table 1).

Following release of the vesicle coat by Tris, the supernatant protein fraction precipitating at 30% ammonium sulphate was redissolved, concentrated, sprayed onto mica sheets and replicated. Characteristic clathrin triskelions were observed, each arm averaging 61·5 nm in length with a globular terminal domain of around 9·6 nm (Fig. 3C–H, Table 1). As with animal clathrin triskelions, each arm has a characteristic kink about 20 nm along its length. Negative staining of the Tris-released pellet failed to reveal any coated vesicles, but areas of uncoated membrane were abundant (Fig. 4A). When the ammonium sulphate-precipitated protein was redissolved and concentrated in a Tris-free buffer, the clathrin trimers reassociated into cages that could be observed by negative staining (Fig. 4C,D).

These cages were also sprayed onto mica and rotary shadowed. Although in many cases the baskets disassembled into clathrin trimers some trimer aggregates with polygonal appearance were observed (Fig. 4B).

*Electrophoresis*

Electrophoresis of isolated coated vesicles revealed major bands at 190, 60 and 57 ($\times 10^3$) $M_r$ plus many other minor bands (Fig. 5A). Tris treatment of the isolated vesicles released the clathrin triskelions and when run on gels they were seen to be composed of the 190, 60 and 57 ($\times 10^3$) $M_r$ proteins (Fig. 5B). Elastase treatment completely degraded the 60 and 57 ($\times 10^3$) $M_r$ proteins whilst largely leaving the $190 \times 10^3$ $M_r$ band intact (Fig. 6B). Trypsin treatment however, resulted in the complete loss of the original three protein bands and the appearance of four major fragments at 120, 105, 49 and 32 ($\times 10^3$) $M_r$ (Fig. 7).

## Discussion

Our results show that in cells from suspension cultures of carrot there are at least three locations where coated pits or vesicles are seen. These are the plasma membrane, Golgi apparatus and partially coated reticulum (PCR). It has recently been demonstrated that coated pits on the plasma membrane of plant cells and protoplasts can be endocytotic and will take up cationized ferritin (Tanchak *et al.* 1984; Joachim & Robinson, 1984), heavy-metal salts (Hübner *et al.* 1985) and gold-labelled concanavalin A (Hillmer *et al.* 1986). Our unpublished results also indicate that carrot protoplasts will endocytose cationic ferritin *via* coated pits. The structure of these coated pits and vesicles is the same as previously reported for plant cells (van der Valk & Fowke, 1981; Emons & Traas, 1986) and similar to those found in animal systems (Ockleford &

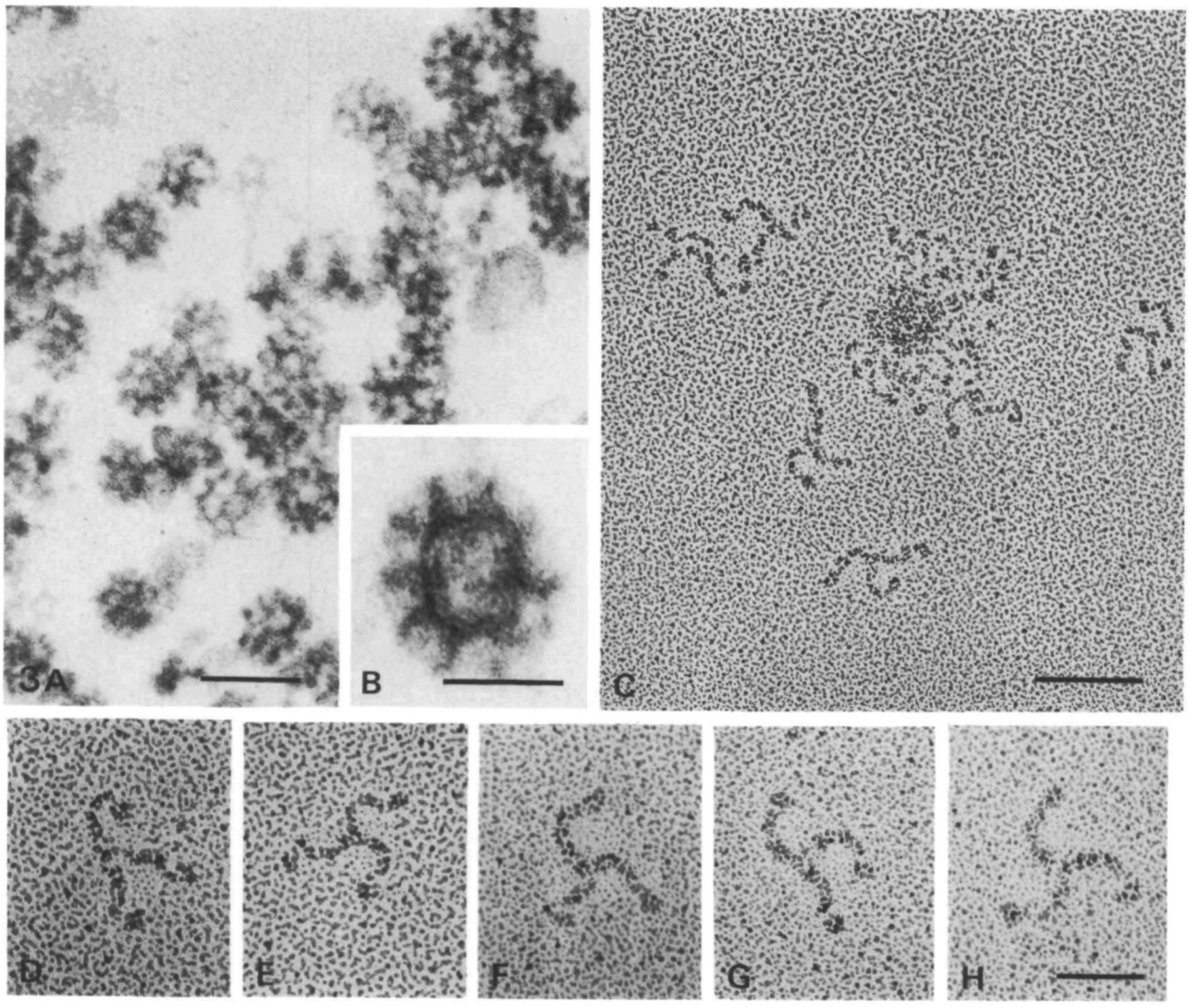



**Fig. 3.** A. Coated vesicle pellet in thin section. Bar, 100 nm. B. High magnification of an isolated vesicle demonstrating the intact unit membrane of the enclosed vesicle. Bar, 50 nm. C. Rotary-shadowed clathrin triskelions from a Tris-extracted coated-vesicle pellet as in A. Bar, 100 nm. D–H. Carrot cell triskelions. Bar, 50 nm.

Whyte, 1980; Pastan & Willingham, 1985). Dry cleaving permits observation of the PS face of the plasma membrane with its associated coated pits (Fig. 1E) and the rapid-freeze/deep-etch technique permits observations, at higher resolution, of the hexagonal and pentagonal nature of the clathrin cages, showing them to be essentially similar to those described in deep-etched fibroblasts (Heuser, 1980).

Our data show the coated vesicles associated with the Golgi cisternae (Fig. 2A) and the PCR (Fig. 2B) to be considerably smaller than those associated with the plasma membrane, again agreeing with data from other plant systems (van der Valk & Fowke, 1981; Mersey *et al.* 1985). It has been suggested that the PCR may act as the plant equivalent of the endosome (Pesacreta & Lucas, 1985) and may be involved in a vesicle shuttle with the Golgi apparatus. However, to date there is no functional evidence in support of this hypothesis, although endocytosed ferritin has been shown to be present in both Golgi cisternae and the cisternae of the PCR in soybean protoplasts (Tanchak *et al.* 1984). Our deep-etch results do, however, indicate that the Golgi-coated vesicles can have a genuine clathrin coat (Fig. 2C,D) and we assume that these vesicles and those associated with the PCR are budding-off the respective organelles.

The isolated coated vesicles have dimensions that on average are close to those associated with the plasma membrane. Since we did not attempt any further fractionation of our isolated coated vesicles, we cannot rule out the possibility that they comprised a mixture of all the cellular populations, with the plasma membrane-derived population predominant.

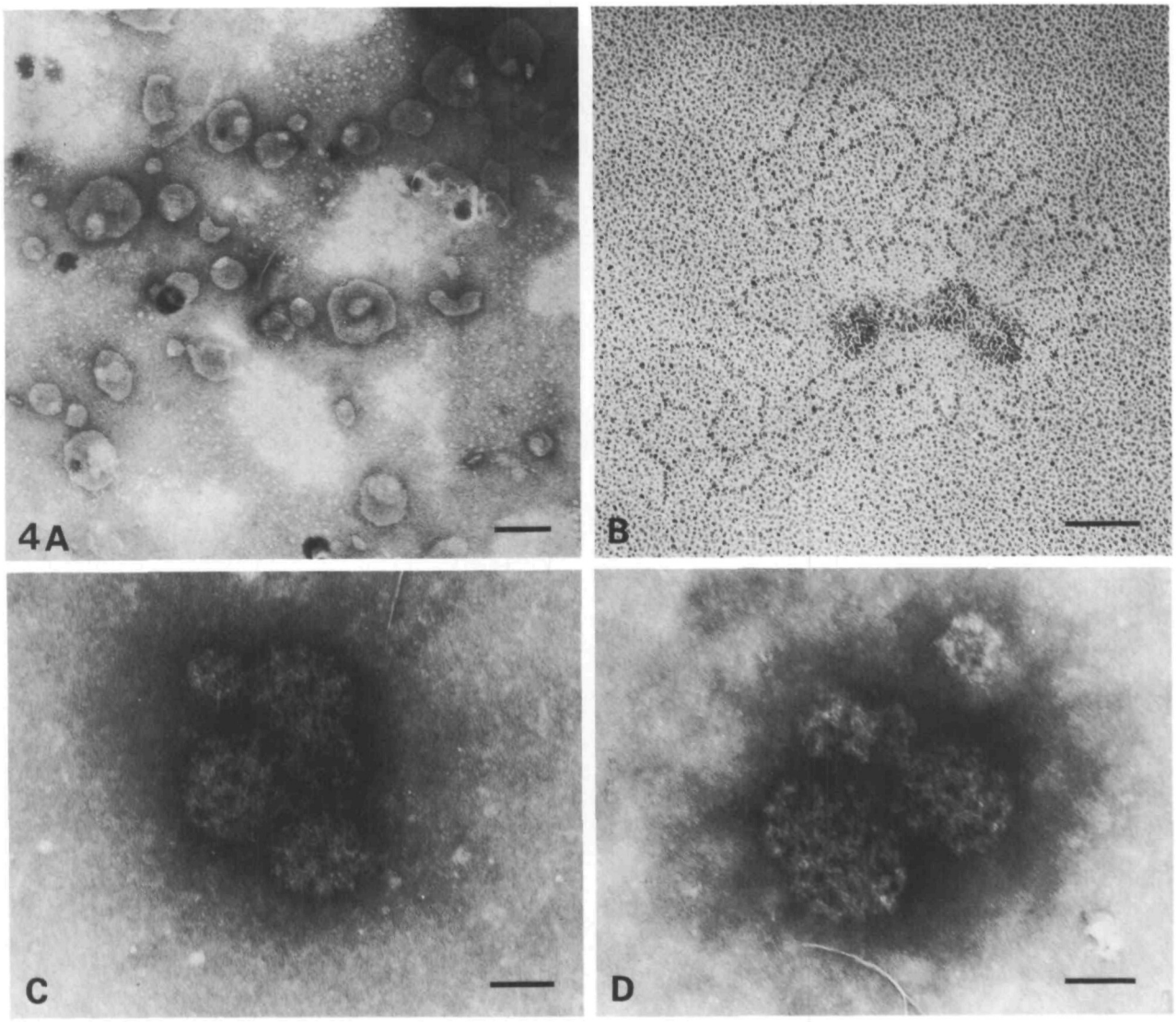



**Fig. 4.** A. Negatively stained vesicles after release of the clathrin coat by Tris. Bar, 100 nm. B. Rotary-shadowed clathrin basket re-formed after removal by Tris. Polygonal packing of the triskelions can be seen. Bar, 50 nm. C,D. Re-formed clathrin baskets negatively stained. Bar, 50 nm.

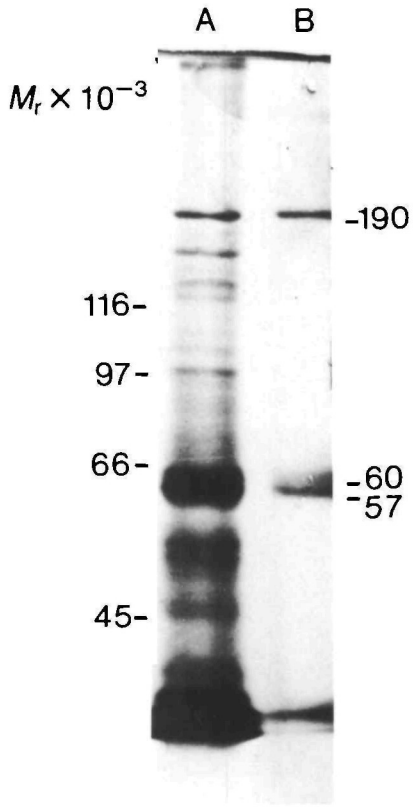



**Fig. 5.** SDS–polyacrylamide gels of carrot cell clathrin. Lane A, whole coated-vesicle pellet as in Fig. 3; lane B, clathrin release by treatment with 0·75 M-Tris. Note the dominant bands at 190, 60 and 57 ($\times 10^3$) $M_r$ in both lanes.

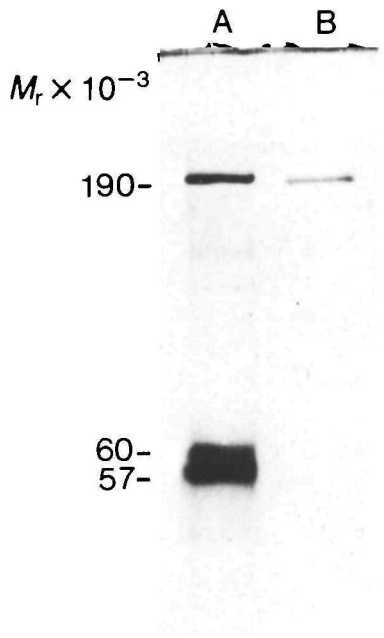



**Fig. 6.** SDS–PAGE of elastase-digested clathrin. Lane A, Tris-released clathrin; lane B, clathrin after treatment with 0·1 $\mu g\,ml^{-1}$ elastase showing complete removal of the 57 and 60 ($\times 10^3$) $M_r$ bands.

Although it is now accepted that there is a true clathrin coat surrounding plant coated vesicles, we show here for the first time that dissociation of the coat with Tris releases the characteristic triskelions. These are morphologically identical with mammalian triskelions, having three arms, each with a kink and a globular terminal domain. The major difference is in the arm length of 61 nm compared with 44–50 nm for various mammalian clathrins (Tables 1 and 2). However, the packing of the plant triskelions into cages gives hexagons and pentagons with centre-to-centre spacings and side lengths similar to those of animal clathrin cages (Heuser & Kirchhausen, 1985).

Analysis of the subunit composition of the triskelions by SDS–PAGE revealed three major polypeptides with molecular masses of 190, 60 and 57 ($\times 10^3$). Previous studies have suggested that the $190 \times 10^3$ $M_r$ polypeptide is the heavy chain (Mersey *et al.* 1985; Depta & Robinson, 1984). Recently, this was confirmed by our immunological data, which showed that this polypeptide cross-reacted with antibodies raised against bovine brain clathrin heavy chain (Cole *et al.* 1987). The clathrin heavy chain from yeast also appears to have a higher molecular mass than mammalian heavy chains, with values of $185 \times 10^3$ (Mueller & Branton, 1984) and $190 \times 10^3$ (Payne & Schekman, 1985). The higher molecular mass of the plant heavy chains compared with mammalian heavy chains is also reflected in the relative lengths of the triskelion arms (Table 2). The difference in molecular mass of $10 \times 10^3$ would represent on average 80–90 amino acid residues. If these were organized in the form of an alpha helix, then the polypeptide heavy chain would extend a further 12–13·5 nm, which corresponds closely with the difference in length of mammalian and plant triskelion arms at 11–16 nm reported here.

It is not clear from our measurements whether the difference in arm length between mammalian and carrot triskelions occurs in the proximal, distal or both sections of the arm. This is further complicated by recent reports (Heuser & Kirchhausen, 1985; Kirchhausen *et al.* 1986), which show that the terminal domains of triskelions dried on mica can assume configurations varying from an open to a closed hook, with consequent variations in arm length of 44–50 nm in the mammalian system. However, from our measurements the length of the polygon edges of plant coats is the same as reported for mammalian coats (Heuser & Kirchhausen, 1985). This implies a similar

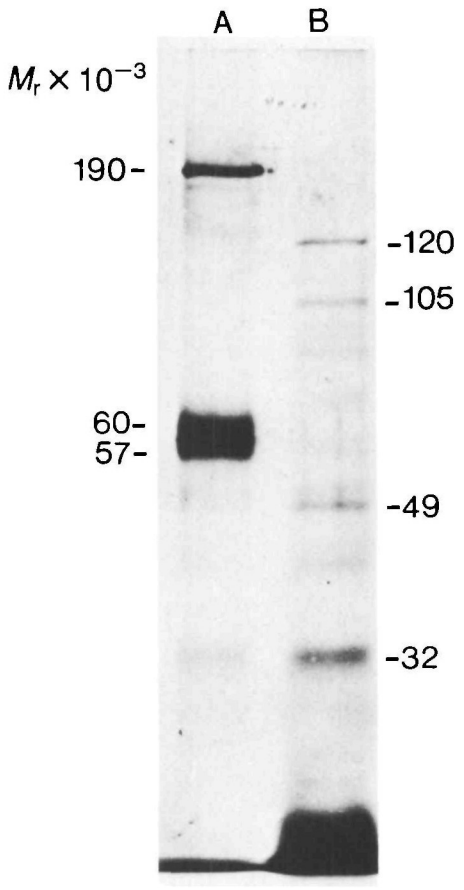



**Fig. 7.** SDS–PAGE of tryptic digests. Lane A, Tris-released clathrin; lane B, clathrin after digestion with 0·15 mg ml$^{-1}$ trypsin. There is complete removal of the 57 and 60 ($\times 10^3$) $M_r$ putative light chains and cleavage of the $190 \times 10^3$ $M_r$ heavy chain into four major bands.

**Table 2.** *Comparison between animal, yeast and carrot clathrin*

| | Animal (various authors) | Yeast (Mueller & Branton, 1984) | Yeast (Payne & Scheckman, 1985) | Carrot |
|---|---|---|---|---|
| $M_r$, heavy chains ($\times 10^3$) | 180 | 185 | 190 | 190 |
| $M_r$, light chains ($\times 10^3$) | 33<br>36 | — | 36 | 60<br>57 |
| Triskelion arm length (nm) | 44–50 | 49 ± 3·5 | — | 61 ± 0·99 |

packing of triskelions and suggests that the extra length of the plant triskelion is organized in a domain that does not affect the dimensions of the polygon.

Recently we showed that the Tris-dissociated coat protein contained two polypeptides with molecular masses of 60 and 57 ($\times 10^3$), which cross-reacted with antibodies raised against bovine brain clathrin light chains (Cole *et al.* 1986). On the basis of this data and the absence of any subunits in the 30–40 ($\times 10^3$) $M_r$ range, we suggested that the 60 and 57 ($\times 10^3$) $M_r$ polypeptides were good candidates for the plant clathrin light chains. Our proposal is further supported by the results reported here, which show that the 60 and 57 ($\times 10^3$) $M_r$ polypeptides co-fractionate with the $190 \times 10^3$ $M_r$ heavy chain on ammonium sulphate precipitation. Furthermore, these bands show the same sensitivities to proteolytic enzymes as mammalian light chains (Ungewickell *et al.* 1982). Low levels of elastase selectively degrade the 60 and 57 ($\times 10^3$) $M_r$ bands, heavy chains being less sensitive. Trypsin treatment cleaves the heavy chain into four major peptide fragments of 120, 105, 49 and 32 ($\times 10^3$) $M_r$. Since none of these tryptic fragments coincides with the proposed light chains, it seems that the latter are protein subunits and not polypeptide fragments of the $190 \times 10^3$ $M_r$ heavy chain.

Despite the evidence in favour, we cannot be absolutely certain that the 60 and 57 ($\times 10^3$) $M_r$ polypeptides are clathrin light chains. One area of concern is the apparent discrepancy in the stoichiometry of heavy to light chains in the vesicles and Tris-released preparations (Figs 5, 6). The 60 and 57 ($\times 10^3$) $M_r$ doublet appears to be in molar excess over the $190 \times 10^3$ $M_r$ band. This is inconsistent with the equimolar quantities of heavy and light chains found in other coated vesicle systems. On the other hand, if further investigations confirm the 60 and 57 ($\times 10^3$) $M_r$ bands to be clathrin light chains this would represent a significant difference between plant and mammalian light chains. It has recently been suggested that marked differences both in molecular mass and primary structure between the light chains of mammalian brain clathrin and light chains from other mammalian tissues may reflect tissue-specific functions (Brodsky *et al.* 1987; Jackson *et al.* 1987). It is therefore not inconceivable that even larger differences may occur between the plant and animal proteins.

We have shown that free plant triskelions will reassemble *in vitro* and form empty polyhedral cages. The experimental conditions used were similar to those required for the re-assembly of mammalian clathrin coats (Keen *et al.* 1979), which suggests common features in the process of self-assembly in spite of the structural differences between plant and animal clathrin reported here.

It is not yet clear whether the features of coated vesicles and clathrin described here for *D. carota* cells are applicable to higher plants in general. However, it is apparent that although the gross morphology of carrot coated vesicles is similar to that of mammalian coated vesicles, there are underlying significant differences that may have arisen in the course of evolution from a common ancestral form.

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