Studies on the eel sperm flagellum

I. The structure of the inner dynein arm complex

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SUMMARY

The highly motile, 9 + 0 sperm axoneme of Anguilla has inner dynein arms (IDAs) but not outer dynein arms. The in situ morphology of these IDAs is shown here to be essentially identical to the IDA morphology already seen in the axonemes of Chlamydomonas, Tetrahymena and Beroë, and in the sperm tails of echinoderms and several vertebrate species. In addition, this study demonstrates: (1) that the nexin (circumferential) links are present in Anguilla and are typical; (2) that IDA1 incorporates an archway, supported by a pillar-structure; (3) that images from thin sections and whole mounts are consistent with those from replicas of rapidly-frozen specimens; and (4) that the IDA and nexin link morphology is apparently unaffected by whether the axoneme is depleted of ATP, relaxed with ATP and vanadate, or inhibited by high ATP. An attempt has been made to reconcile the emergent morphology of the IDA complex with all earlier descriptions in the literature. From a detailed comparison of the results with published information on Chlamydomonas mutants, it is concluded that the nexin (circumferential) link is a major part of the ‘dynein regulatory complex’.

Key words: Dynein, Axoneme, Flagellum, Sperm tail

INTRODUCTION

The chemo-mechanical enzymes that power cilia and eukaryotic flagella are known as the axonemal dyneins. Each of the microtubule doublets bears two linear arrays of dyneins, the outer and inner rows of dynein arms. The relative contributions of the two rows to the overall motility is not well understood, though the removal of the outer arms (ODAs) produces a reduction in beat frequency, with little effect on waveform (Gibbons and Gibbons, 1973; Brokaw and Kamiya, 1987; Fox and Sale, 1987). The discovery that flagella exist having naturally only the inner arms (e.g. Hyams and Campbell, 1985) shows that in some situations the ODAs are dispensable and confirms that the inner dynein arms (IDAs) per se can sustain motility. The IDAs, in fact, comprise a group of several different arms, with various subdomains. There is still confusion over their topography and subunit composition (see review by Mitchell, 1994). The available analyses of IDA topography are those of Goodenough and Heuser (1985, 1989), Avolio et al. (1986), Piperno et al. (1990), Muto et al. (1991), Burgess et al. (1991b) and Mastronarde et al. (1992). Except for Avolio et al. (1986) there is agreement that the grouping has a 96 nm repeat. Otherwise much requires to be reconciled, even between accounts of the same species. It has also been unclear whether the IDAs are entirely distinct, structurally and functionally, from the radial spokes, since the spokes also occur as a set, repeat at 96 nm and show registration with the IDA subtypes. Situated partly between the IDAs and radial spokes is a junctional region, considered by Gardner et al. (1994) to be the site of some components of a ‘dynein regulatory complex’ of seven polypeptides, thought, possibly, to co-ordinate activity between the spokes and the arms.

The subject of this work is the inner dynein arm complex in the sperm flagellum of the eel, Anguilla anguilla. In Anguilla, the spermatozoon is of the 9 + 0 type (Billard and Ginsburg, 1973; Çolak and Yamamoto, 1974; Todd, 1976), a characteristic of Elopomorph fish (Mattei and Mattei, 1974). Not only are the central pair of microtubules absent but also the radial spokes and the outer row of dynein arms (Baccetti et al., 1979). The eel sperm axoneme is therefore well suited for a study of the inner arm structure. There are relatively few superimposing structures in thin sections and, in freeze-etch replicas, the inner arms are neither obscured by outer dynein arms nor by radial spokes. The aim has been to compare the morphology of these inner arms with the depictions in the literature quoted above, in the hope of recognizing and establishing a unitary pattern. The configuration of the IDA complex has also been examined in three states: without ATP; with ATP and vanadate; and in a high, inhibitory ATP concentration.

MATERIALS AND METHODS

European eels (Anguilla anguilla L.) were obtained commercially in the UK. In an attempt to select probable males, fish of not more than 80-100 g were used. The fish were transferred to and thereafter maintained in artificial sea water at either 18°C or ambient temperature (some batches). Sexual maturation was induced by injecting the fish intramuscularly with two doses, three weeks apart, of 500 i.u. human chorionic gonadotrophin (Sigma) in Hanks’ BSS; afterwards the dosage was reduced to 250 i.u., at 2-weekly intervals (Lumare and Villani, 1973). The collection of sperm (milt), by abdominal massage,
became possible 9-10 weeks after the first injection and could be continued, at one or two weekly intervals, for several months. Injecting the fish, and collecting sperm from them, were done under MS222-induced anaesthesia (3-aminobenzoic acid ethyl ester, Sigma; 0.5 g per litre of sea water).

In order to produce samples with a high density of sperm axonemes, and to avoid having samples contaminated with solubilized DNA from the sperm nuclei, the sperm flagella were, in almost all cases, first separated from the heads by a simple mechanical procedure.

**Preparation of isolated sperm flagella**

From a pool of several sperm samples, aliquots of 0.25 ml were taken and added to 0.75 ml of a diluent/extension known to promote motility. The diluent, devised by Gibbons et al. (1985) contained 0.25 M NaCl, 1 mM MgSO$_4$, 0.1 mM EGTA, 2 mM KCl and 10 mM Tris-HCl, pH 8.3, and was supplemented with 20 mM glucose unless freeze-etching was planned. After 10 minutes to allow for suspension, each diluted sample was vortex-shaved for 10 seconds, in a 10 ml, 1 cm-wide test tube, using a Whirlimixer (Fisons) running at 2,810 rev. min$^{-1}$. The sample was then centrifuged at 1,082 g for 5 minutes in a 1.5 ml Eppendorf tube to pellet the sperm heads. The supernatants (each -0.6 ml) were removed and kept on ice. Suspended in this supernatant were separated sperm tails, with their membranes intact; they had been detached but not split or broken into segments. For rapid-freezing or chemical fixations, the flagella were spun down at 12,700 g for 5 minutes and then, for most studies, the pellets were demembranated by adding a demembranation/reativation buffer based on that of Gibbons et al. (1985) and always containing 0.22 M K acetate, 0.03 mM KCl, 0.2 mM EGTA, 1.2 mM MgSO$_4$, 1 mM dithiothreitol, 10 mM Tris-Ac (pH 8.1) and 0.04% Triton X-100.

**Electron microscopy**

The rapid-freeze, deep-etch technique, as devised by Heuser (1981) has been described in detail by Burgess et al. (1991a). Pellets of demembranated flagella were prepared in three nucleotide states: without added ATP (rigor condition); with 100 µM ATP and 1 mM MgSO$_4$; or with 2 mM ATP (substrate-inhibited). They were placed on supports of fixed/washed lung tissue and frozen by impact against a liquid helium cooled copper block. Frozen specimens were superficially fractured at -100ºC, 10$^{-2}$ Torr and etched for 60 seconds. Rotary replication involved applying carbon-platinum from either 24° (low angle) or 60° (high angle) followed by carbon from 85°. Replicas were cleaned in bleach, picked up on uncoated grids and viewed in a Philips EM300. Stereo pairs were made at ±6° tilt. The micrographs are shown as negatives (made from reversal-positives), so that the metal of the replica appears light.

For preparing thin sections, pellets of demembranated flagella were chemically fixed for 90 minutes in 2.5% glutaraldehyde in the Hepes-sucrose buffer devised by Gibbons et al. (1983) but incorporating also 0.5% tannic acid. After a rinse in buffer, osmium (1% O$_2$O$_3$H$_2$O in the same buffer for 90 minutes) and dehydration, the pellets were embedded in Epon 812. Sections of 20-50 nm thickness were mounted on uncoated or coated grids and conventionally double-stained.

Some preparations were negatively stained with 2% methylamine tungstate (Agar Scientific). Glutaraldehyde (4%) was added to a suspension of demembranated flagella to give a final concentration of 1% glutaraldehyde. Then the flagella were allowed to sediment on to a coated grid, drained and negatively stained.

**Interpretation and presentation of electron micrographs**

The analysis of the images of IDAs has been based almost entirely on longitudinal views of the axoneme, whether in replicas or sections. The base-tip polarity of all such views was deduced as follows. The orientation of each piece of replica in the electron microscope was determined retrospectively from stereo-pair micrographs and the reversal-positives were then printed accordingly. Replica views of the outside of the axoneme showed very slender links contacting one of the microtubules, which, based on earlier studies, must have been the B-tubule of the doublet. This information gave the $n$, $n+1$, $n+2$... succession and therefore the base-tip polarity, since this succession is clockwise as viewed from the base. These same views included also structures recognized from earlier studies as nixin (i.e. circumferential) links; and these were always tilted basally from the A-tubule to the B-tubule. This applied in all three nucleotide-states examined. Once formally established, the direction of the tilt of the nixin link in replicas was an internal check for correct polarity. The direction of the nixin link tilt in chemically-fixed, resin-embedded flagella was shown to be the same as in cryo-fixed specimens by studying 8 sets of serial, tangential, longitudinal sections (in the order cut and viewed as if looking down on the blockface). These series are not included in the Results. Polarity was deduced from the direction of the $n$, $n+1$... sequence and knowledge of whether it represented the near or the far side of the flagellum. Then the direction of the tilt of the nixin link was evident. Subsequently, the tilt direction itself was used to give the polarity of any specimen with a region sectioned in the plane of the nixin links. Soon many polarized features of the IDA assembly became known with confidence. Often a long specimen was photographed as a montage and its polarity established at a position remote from the area of particular interest.

The problem of finding the correct topographical correspondence between the inward-facing and outward-facing surfaces of the IDAs, in replicas, was solved by a few rare views of the inward-facing surface that revealed some nixin links exposed by damage to the IDAs. Because of their complexity, these images are not presented in the paper.

The policy has been to concentrate on longitudinal rather than transverse thin sections. The latter are difficult to ‘locate’ without radial spokes as a guide and would need to be examined as a sequence of at least 5 sections, each 20 nm thick, a difficult undertaking. Producing serial longitudinal sections through the IDAs is, of course, impossible but a similar effect was achieved by selecting grazing sections where the plane of section was very slightly oblique to the dynein array such that slightly more or less of the dynein complex was included in the section in successive 96 nm repeats. Correspondences between features in successive repeats were followed by the use of dividers set to measure 96 nm from some well-seen sequence of nixin link attachments within the image.

For presentation, some of the images have been changed as follows. Sectional views passing tangentially through the near side and the far side of an axoneme become identical by mirror-reversal. Therefore, to make comparison easier, some such images have been mirror-reversed so that the distal (tipward) end of all of them, is on the right, with doublet $n$ below doublet $n+1$ (i.e. they are all made to appear views of the far side of the axoneme). Replica views of the inside of the axoneme are naturally of the far side and are presented the same way, with the distal end on the right. But replica views of the outside of the axoneme are of the near side. For consistency, these are presented also with the distal end on the right. However, for the important comparison between the inward-facing and outward-facing surfaces of the IDA complex, two of the outside views have been deliberately mirror-reversed and inverted (rotated through 180°); by this manipulation the inside and outside views become superimposable.

**Nomenclature**

The surface of the IDA complex facing inwards to the centre of the axoneme will from now onwards be referred to as the axial surface. The surface facing in the opposite direction, i.e. outwards away from the central axis, is called abaxial. At 90° to each of these surfaces is a surface facing generally towards the B-tubule: this surface is seen in en face views.
RESULTS

General structure
The absence of radial spokes and outer dynein arms (ODAs) was readily confirmed (Fig. 1). Even in intact spermatozoa, no ODAs were ever seen. Occasionally, a small density resembling the A-tubule attachment of the ODA was present (Fig. 1). Images of replicas (not shown) show this feature as a ridge, with no 24 nm repeat.

The plasmalemma is attached to the axoneme along the outer face of each doublet (Fig. 1). Partial demembranation with Triton X-100 removed sleeves of membrane completely, but not from all regions of the axoneme (Figs 2 and 3). This incomplete demembranation allowed the fracture plane (in the freeze-etching process) to enter the axoneme often enough and it gave sufficient access to tannic acid (essential for giving contrast to the thin sections). Yet the structural support provided by the persisting membrane prevented wholesale splitting. Nevertheless, many axonemes did split or open longitudinally (Fig. 4), whereupon the groups of doublets adopted helical, ribbon-like forms.

The structure of the inner dynein arm complex
This is demonstrated in Figs 6-16. The author’s interpretations of fracture planes and section planes are given in Fig. 5.

Replicas of the axial surface of the IDAs show features that are already familiar. The pattern is well seen in opened-out ribbons of doublets (Fig. 6A). There is clearly a 96 nm repeat, consisting of dynein heads in three groups; these three subgroups are the IDAs 1, 2 and 3, recognized and named by Goodenough and Heuser (1985). (The term ‘head’ is used purely descriptively, without implying polypeptide composition). The interpretive tracing (Fig. 6B) establishes a style of drawing and shading to distinguish IDAs 1, 2 and 3 in that.

Figs 1-4. Thin sections (standard thickness) of partially demembranated sperm flagella. Figs 1, 3 and 4 provide 26 examples of the appearance of the inner dynein arm complex in transverse section.

Fig. 1. Transverse section of a region where the cell membrane persists, attached to each doublet. No outer dynein arms, radial spokes or central pair microtubules are present. Occasionally a small density in the place of the outer arm is seen (arrow).

Fig. 2. Tangential section showing abrupt boundary between a region of membrane (left) and a demembranated region.

Fig. 3. Transverse section, demembranated region of axoneme.

Fig. 4. Transverse section of an opened-out axoneme (ribbon of doublets), in this instance with some membrane retained. Bar, 0.1 μm, applies to Figs 1-4.

Fig. 5. Diagram (taken from the sections in Figs 3 and 4) to show the author’s interpretations of the fracture planes and section planes represented in Figs 6-15. The arrows show the direction from which the replicas were viewed. This figure is intended for reference as Figs 6-15 are inspected.
figure and in the images that follow. Figs 7 and 8 are views of axial surfaces that include IDAs seen from oblique perspectives. Fig. 8 (upper row) shows the slender B-links without fore-shortening; behind these, the nexin (circumferential) links may be seen. Fig. 9 shows that the pattern seen in replicas is visible in thin sections: three 96 nm domains are highlighted as matching most closely the views in Fig. 6. Fig. 10A shows abaxial surfaces in a replica; this image is reversed and inverted (to become Fig. 10B), so as to be superimposable on an equivalent sectional view (Fig. 11). These images are the clearest demonstration of the nexin links. Fig. 12A is another replica-view of abaxial surfaces; this image is similarly transformed (to become Fig. 12B). The heads and B links of IDA1 are well seen, though the high shadow-angle leaves the nexin links poorly differentiated from IDAs 2 and 3. Fig. 13A is a section through the abaxial region; it is superimposable on Fig. 12B and has the nexin links well contrasted. Figs 14 and 15 are en face sectional views. Fig. 14 shows the divergence between the row of nexin links and the IDA heads; the plane of section gives a view equivalent to an earlier replica view (Fig. 8, upper row of IDAs). Fig. 15 is from an exceptionally thin section: the channel running across IDA1 represents the space under the proximal side of the archway, as seen in earlier sections (Figs 9 and 13).

Fig. 16, from a negatively-stained whole-mount, has been contrast-reversed so that the IDAs appear dark for comparison with sectional images. The IDAs here are best seen where the adjoining B-tubule has been broken away. The 96 nm repeat is discernable throughout and the double-arched structure shown on the tracing is identified as IDA1.

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**Fig. 6.** (A) Replica of axial surface of inner dynein arms (IDAs), in a ribbon of doublets. In this and all subsequent images the distal (tipward) end of the axoneme is on the right. This specimen was prepared in the relaxed state and received high angle shadowing. The identification of the structures is given in B. All subsequent micrographs are at the same scale (i.e. x260,400, giving the 96 nm repeat as 25 nm). (B) A tracing from A: the outlines have been drawn as faithfully as possible but they are interpretations none-the-less. The different IDAs are indicated by the shading: IDA1 solid shading; IDA2 stippled shading; IDA3 open (no shading). This description follows that established by Goodenough and Heuser (1985 see text). All subsequent tracings use the same shading scheme for the 3 IDA subtypes. Bar, 0.1 μm.

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**Fig. 7.** Replica of an axonemal cylinder (rigor state) to show an oblique axial view of the IDAs (lower row). This row shows the arch-like form of IDA1 in high contrast; there is fracture damage to the A-tubule over much of this doublet. The IDAs of the upper row are poorly displayed. Bar as Fig. 6.

**Fig. 8.** Replica of axonemal cylinder (rigor state) to show an oblique axial view from the B-tubule direction (upper row). The B-links, narrow strands running perpendicularly, are seen without fore-shortening, though they may possibly be somewhat stretched. Behind the B-links of IDA2 are seen the B-tubule ends of 4 nexin links, shaded with cross-hatching in B. The lower row of IDAs shows the axial surfaces in a view similar to Fig. 6A; in this image the protruding feature of IDA3 near the A-tubule is well contrasted. Bar as Fig. 6.
Finally, the morphology of the IDA complex, as visualised in the replicas, was the same (as far as could be judged) whether the specimens were in rigor or relaxed with ATP and vanadate. A careful examination of the angulation-direction of the nexin links showed no change. The same morphology of the complex (in replicas) was also found when the axonemes were inhibited with 2 mM ATP (images not shown).

DISCUSSION

By using the sperm flagellum of *Anguilla*, electron microscopy of the inner dynein arms has been made easier; this is because there are no outer dynein arms, radial spokes or central microtubules, and because the lumen of the A-tubule is not electron-dense. The IDAs themselves appear not to have evolved special morphologies in this species. They generally conform to expectation and so, perhaps, may be taken as representative of the type. It is notable that this simplified axoneme retains the nexin link along with the IDAs. Hence, the use of the phrase ‘IDA complex’ for what is perhaps a conjoint functional unit.

Knowing the structure of the IDA complex at this level of resolution provides few, if any, clues as to how the assembly functions. Studies of changes related to the work cycle of the IDAs are required next. In this context, our failure to detect a morphological difference between the rigor and relaxed states offers no help in postulating a cycle of movement*. In contrast, comparison of the two nucleotide-states has been successful in demonstrating different functional states of the outer dynein arms (Goodenough and Heuser, 1982); this type of analysis recently culminated in the recognition of both rigor and relaxed outer arms in motile axonemes (Burgess, 1995). The third nucleotide-state examined in the present work, the inhibition of motility and sliding in 2 mM ATP, also failed to reveal any novel structural rearrangement. Nothing seems to be known about the nature of dynein inhibition by high concentrations of ATP.

*The subtle change in IDA1 seen by Goodenough and Heuser (1985), when making this same comparison, may have been missed here on account of the great susceptibility of eel sperm axonemes to damage during rapid freezing.
In the literature on the structure of the IDAs, there are differences of interpretation. In the present work, three methods have been applied: freeze-etch, ultra-thin section and negative-stain. The results have been mutually consistent and will be discussed in relation to the earlier interpretations.

The freeze-etch replica technique showed for the first time that the abaxial surfaces of the IDA complex can be superimposed upon the axial surfaces to establish the correspondence, and that the abaxial views can be compared to the sectional views as presented in the convention established by Fig. 9.

Fig. 9. A sectional view chosen for its close correspondence to the transformed image in Fig. 10B, and arranged to be in register with it, with an interpretative tracing to locate the prominent nexin links (cross-hatched). Superimposition on other images (e.g. Fig. 13) shows that the structures alternating with the nexin links must be parts of IDA1; these are glimpsed also in Fig. 10B (upper row). Bar for Figs 10 and 11 as Fig. 6.

Fig. 10. (A) Replica of the exterior of an axoneme, with abaxial surfaces of the IDA complex. The distal end is to the right. Therefore the doublets are n, n + 1, n + 2 from the top of the image to the bottom. The nexin links (arrowheads at B-tubule attachment sites) are best contrasted in oblique views (lower row); they project from doublet n obliquely and proximally towards doublet n + 1. (B) An image produced from A by mirror-reversal and inversion through 180°. By this transformation, the distal end is still to the right but doublet n is now below doublet n + 1. This means: (1) that the abaxial surfaces of the IDA complex can be superimposed upon the axial surfaces to establish the correspondence, and (2) that the abaxial views can be compared to the sectional views as presented in the convention established by Fig. 9.

Fig. 11. A sectional view chosen for its close correspondence to the transformed image in Fig. 10B, and arranged to be in register with it, with an interpretative tracing to locate the prominent nexin links (cross-hatched). Superimposition on other images (e.g. Fig. 13) shows that the structures alternating with the nexin links must be parts of IDA1; these are glimpsed also in Fig. 10B (upper row). Bar for Figs 10 and 11 as Fig. 6.

Fig. 12. Abaxial surfaces of IDAs, seen in a replica from a perpendicular viewpoint. A true image, distal end to the right. This perspective, with high-angle shadowing, leaves the nexin links poorly differentiated from IDAs 2 and 3. Nevertheless, their B-tubule attachments are indicated (arrowheads). For further detail, see B. (B) A reversed and inverted transformation of A. It may be seen more easily in this image that the features approximately mid-way between the arrowheaded nexin links are the triple-heads of IDA1, with the groups of 3 B-links projecting upwards to doublet n + 1.

Fig. 13. (A) A sectional view chosen for close correspondence to Fig. 12B and arranged to be in register. This section includes the abaxial side of the IDA complex. The nexin links are oblique and strap-like. It becomes clear that the archway of IDA1 seen in earlier instances is supported by a pillar on its abaxial side, making the arch appear double. Suggestions of this are also in Figs 9 (bottom row) and 10B. Bar for Figs 12 and 13 as Fig. 6.
the heads of the inner dynein arms as a triad-dyad-dyad sequence with a 24-36-40 nm periodicity (Goodenough and Heuser, 1985). Soon afterwards, an additional domain proximal to the triad, and the A-tubule attachment site of S3 were depicted (Goodenough and Heuser, 1989). The location of the nexin link in freeze-etch replicas was first visualized by

**Fig. 14.** A section showing the IDA complex en face, indicating the divergence between the row of nexin links projecting circumferentially to the B-tubule and the row of IDA heads projecting more inwardly than this. The divergence is seen also in the thick transverse sections (Figs 1 and 3). This sectional view corresponds to the replica view in Fig. 8A (upper row of IDAs) except that the B-links are not resolved in the section. Bar as Fig. 6.

**Fig. 15.** An extremely thin section giving an en face view of the IDA complex at a level near the A-tubule. The interpretation in B has been supported by information from outside of the area presented and from other sections in related planes. IDA1 is resolved as two masses, with a channel between them (arrowed); each proximal mass (left) is interpreted to be the proximal attachment of the archway to the A-tubule; each distal mass is the pillar of the arch, and is seen to extend abaxially (upwards in the Figure) beyond the line of attachment of the nexin links. Bar as Fig. 6.

**Fig. 16.** A group of three doublets, fixed with glutaraldehyde and negatively stained. The contrast has been reversed, so that the IDAs appear dark, for easy comparison with the sectional views. The distal end is judged to be on the right (from the appearance of what is believed to be IDA1). The image shows evidence of repeating features every 96 nm. Further than this, there is evidence of an arched structure, frequently subdivided asymmetrically by a supporting feature (see especially under brackets). By comparison with Figs 6, 7, 9 and 13, this assembly is judged to be IDA1 and is indicated thus in the tracing. It is better displayed where the B-tubule has been torn away. Otherwise (as in the lowest row) it is partly obscured or compressed. IDAs 2 and 3 are not clearly visible. Bar as Fig. 6.
extracting dynein (Warner et al., 1985; see also Goodenough and Heuser, 1989, Figs 9-21). Essentially the same morphology and position were later confirmed for nexin links in avian and mammalian sperm flagella (Burgess et al., 1991; Bozkurt and Woolley, 1993). In respect of all these features (see Fig. 17A), the replicas of eel sperm show the same pattern and add another vertebrate class to the general picture. The main new aspect from the work on the eel is the view of the abaxial surface of the array (Fig. 17B). Most significantly, IDA1 is seen not as merely a group of three arms but as an asymmetric archway supported on a pillar, the foot of which spreads laterally beyond the ‘line’ of the nexin links, features that have been confirmed in sections. In fact this aspect of IDA1 was apparent in images of bull spermatozoa but was not remarked upon (Bozkurt and Woolley, 1993, Fig. 4).

Thin sections of IDAs were presented by Avolio et al. (1986). One very thin section of an intact axoneme, i.e. a specimen from which no attempt to extract dynein had been made, was included (see their Fig. 3a). The image is similar to Fig. 15 in the present paper, where the thinness of the section makes IDA1 appear as two densities separated by a channel. This effect probably contributed to their conclusion that four IDAs exist per 96nm (a 24 nm repeat). Piperno et al. (1990) studied thin sections of Chlamydomonas IDAs and deduced, from the comparisons of wild type with the IDA-deletion mutants pf23 and pf30, that three types of IDA occurred within each 96 nm spoke repeat. In this work it was suggested that IDA1 is composed of two heavy chains, as diagrammed subsequently by Piperno (1990).

Muto et al. (1991) also studied thin sections of Chlamydomonas flagella using a double mutant (pf 14 × oda 1) lacking both outer arms and radial spokes, material having the same advantages as eel spermatozoa. In order to apply specimen-tilt usefully, they used sections in the silver-grey range (40-50 nm). Their main conclusion was that the IDAs occur as a double row. This has immediate appeal since it conforms with the often seen cross-sectional image of one density projecting circumferentially part-way to the B-tubule and one projecting more inwardly. This is the appearance of cross-sections of eel sperm axonemes as shown in Figs 1, 3 and 4 (this paper). However, the expected good correspondence between the longitudinal sections of Muto et al. and those shown here is not at all obvious, except that one of their micrographs (their tangential section, Fig. 3D) does show a pattern similar to, for example parts of Fig. 9 (this paper). Otherwise, their tilt series suggest an axial ‘centripetal side’ row of densities recognizable as IDA heads and a more abaxial ‘centrifugal side’ row of densities. The interpretation offered here for the latter row is that it includes the nexin links and the pillar of IDA1 where it joins the A-tubule; but also, because of the silver-gray section thickness, this row of densities will probably have contained superimposed on it two parts of IDA1 (a’ and c’) where they reach to the medial surface of the A-tubule, and perhaps also the protruding structure IDA3s. Thus, in reconsidering the cross sectional appearance of the inner arm complex, there are indeed two dense projections from the A-tubule towards the B-tubule. The more axial one consists of all the IDA heads. The more abaxial density is due only to the superimposition of the pillar of IDA1 and the nexin links.

Table 1. The IDA complex of the Anguilla sperm flagellum related to that of the Chlamydomonas flagellum as described by Mastronarde et al. (1992) and Gardner et al. (1994)

<table>
<thead>
<tr>
<th>Anguilla IDA domain as shown in Fig. 17</th>
<th>IDA1</th>
<th>IDA2</th>
<th>IDA 3</th>
<th>The nexin link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydomonas proposed equivalent area number from Gardner et al. (1994) - their Fig. 8G</td>
<td>a′</td>
<td>a</td>
<td>s</td>
<td>a/b</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
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</table>
more abaxial density often becomes attenuated towards the B-tubule because the pillar is absent here and because the section may miss the nexin link or include only a part of it. The divergence between the row of nexin links and the row of IDA heads may be seen in Figs 8 and 14 and in the perspective drawing, Fig. 17C (this paper).

Mastronarde et al. (1992) in their study of thin sections of the IDAs of wild-type and mutant Chlamydomonas, have presented averages of en face sections that should be compatible with Fig. 15 (this paper), except that the Chlamydomonas sections are thicker (40 nm) and therefore superimpose all the domains of the IDA complex on to one plane. This method carries the risk of confusing the identities of individual domains even though a standard density pattern emerges reproducibly. A set of 10 densities or areas per 96 nm was recognized.

For comparison with Fig. 15 (this paper), the re-orientated diagram given by this research group in a later paper is more convenient (Gardner et al., 1994, Fig. 8G). The comparison has been made using a clay model of the IDA complex equivalent to Fig. 17 (this paper) with the arms mounted on glass tubes to give transparency. The proposal for equivalence is given in Table 1. All elements of this should be verifiable by applying the freeze-etch technique to the mutants. For the present, the deficits of the four mutants pf9, pf23, pf2 and ida4 may now be expressed in either notation.

Of the proposed equivalences in Table 1, the clearest and most interesting is the equating of the nexin link with area 10. This possibility was recognized tentatively by Mastronarde et al. (1992) who saw that their analysis of images from 20 nm transverse sections reinforced the idea: the strongest densities bridging to the B-tubule from the abaxial side of the IDA complex occurred between spoke S2 and (the absent) spoke S3, which is precisely the position of the bulk of the nexin link (see Fig. 17 this paper; Burgess et al., 1991; and Bozkurt and Woolley, 1993). The mutant phenotype pf2 is deficient mainly in polypeptides that constitute the ‘dynein regulatory complex’ (DRC). The loss of DRC poly peptides restores function (in some degree) to dyneins that have become inhibited as a result of radial spoke deficiencies. Together with two other DRC mutations pf3 and sup-pf-3, these 3 phenotypes have the severest reductions in DRC components and all three carry their major structural deficit in the crescentric area 10 (possibly including part of domain 9) (Gardner et al., 1994). Thus, as these workers assert, ‘it is most likely that the crescent corresponds in part to the location of the DRC’. It has been argued here that area 10 is the nexin link, which is widely thought to act in some way to restrain sliding. Therefore it seems appropriate now to propose that the nexin link is the major part of the dynein regulatory complex, and that normally it does indeed restrain sliding. Now, the only DRC poly peptides common to pf2, pf3 and sup-pf-3 are #5 and #6, which have molecular masses of 40,000 and 60,000, respectively (Piperno et al., 1994). Do these poly peptides form the nexin link? The size of the nexin link (~20 nm x 20 nm x 5 nm) suggests that a large polymeric grouping would be involved. Alternatively, a very large protein component of the DRC, additional to the 7 poly peptides may exist and await discovery. The ell spermatozoon is, of course, spoke-less but motile. Any supposed contribution of the spoke system to facilitating dynein function would have to be an effect of residual spoke components at the A-tubule would attachment sites.

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