Eur Biophys J (1998) 27: 446–454

ARTICLE

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Organisation and structure of microtubules and microtubule-motor protein complexes

Received: 21 January 1998 / Revised version: 2 March 1998 / Accepted: 7 March 1998

Abstract We present a short overview of the current status of work on the organisation and structure of microtubules and of microtubule-motor protein complexes. At present there is great interest in obtaining structural information that can help us to understand the movement of the kinesin family of microtubule associated molecular motors. Using electron cryomicroscopy and image reconstruction methods three dimensional maps of microtubule-motor complexes have been obtained in the presence of different nucleotides. We address a number of principles involved in different aspects of this work.

Key words Tubulin · Kinesin · Microtubule structure · Motor proteins · Microtubule-kinesin complexes

1 Introduction

Microtubules are key actors in the cytoskeleton of eukaryotic cells. Together with actin filaments they play an important role in organising the spatial distribution of organelles within the cell and they can be either extremely stable as is the case in cilia and flagella or very dynamic as in the mitotic spindle. Microtubules are ~25 nm diameter hollow tubes with walls made from tubulin heterodimers interacting head-to-tail to form protofilaments aligned lengthwise along the microtubule. The protofilaments associate laterally with a small lengthwise shift. Microtubules in eukaryotic cells usually have thirteen protofilaments. There are notable exceptions to this rule, and microtubules with from 8 to 20 or more protofilaments have been reported. For example, the touch cells of the slow-worm *C. elegans* have 15 protofilament microtubules (Savage et al. 1989) and the accessory tubules in the sperm-tail axonemes of insects can have from 13 to 20 protofilaments, see for example Lanzavecchia et al. (1996). Microtubules have both a structural and a dynamic polarity. The structural polarity is conferred by the alignment of the tubulin heterodimer along the protofilament. During in vitro assembly, microtubules show a dynamic polarity with one end, called the plus end, growing and shrinking more quickly than the other. In the cell, microtubules grow out from the microtubule organising centre (MTOC) towards the cell membrane with the plus end leading. They usually remain attached to the MTOC by their minus end. The resulting microtubule networks provide directional pathways for dynein and kinesin and, together with these motors, engage in intracellular transport, the organisation of organelles in the cytoplasm and in cell division. Motors of the dynein family move along microtubules towards the minus end whereas motors of the kinesin family usually move towards the plus end, although some of them are known to move in the opposite direction (Bloom and Endow 1995).

Many roles of microtubules depend on their relationship with motor proteins and, to understand at the molecular level how the motors use ATP hydrolysis to interact with, and move along the microtubule surface lattice, it is necessary to take into account data from many different sources. A combination of light and electron microscopy has shown that kinesins move along the direction of the microtubule protofilaments (Ray et al. 1993). Interferometry and optical trapping have been used to show that individual kinesin molecules attached to silica beads move with 8 nm steps along microtubules (Svoboda et al. 1993). Study of the ATPase cycle indicates that kinesin's two motor domains release sequentially during movement along microtubules and that kinesin is attached for most of the cycle time (Hackney 1995). The structure of the tubulin dimer and the organisation of the microtubule surface lattice are important factors in the movement of the motor proteins. In this context, electron cryomicroscopy of samples embedded in vitreous ice has given detailed information on the organisation of the microtubule surface lattice and has been used to localise the interaction sites of the kinesin motor domains. The atomic resolution structures of four motor domains and one dimer of kinesin superfamily proteins have been solved by X-ray crystallography

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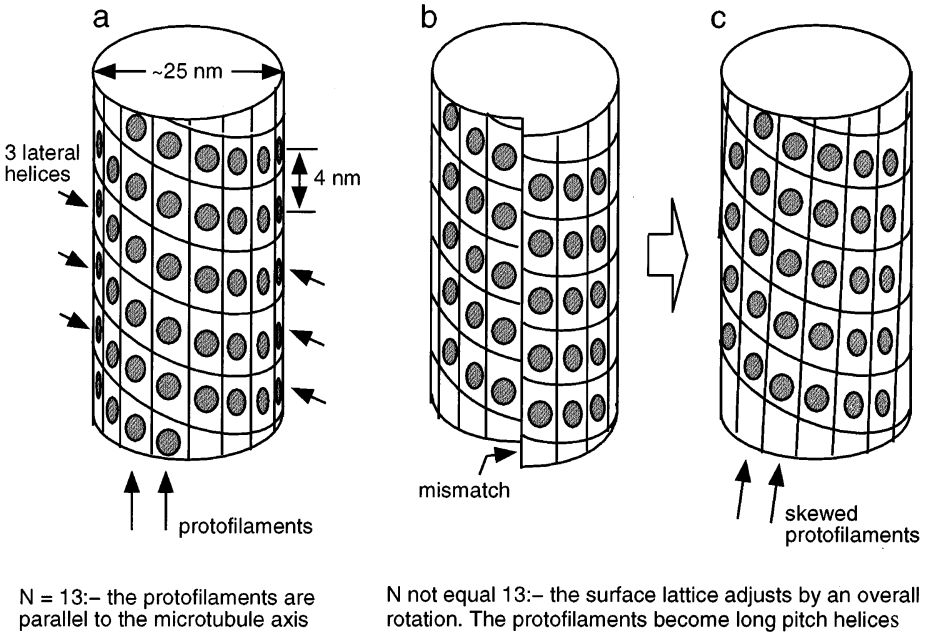

Fig. 1a–b The microtubule surface lattice can accommodate different numbers of protofilaments. In this representation the tubulin subunits are treated as 'identical' monomers. **a** In a 13 protofilament microtubule the protofilaments run parallel to the axis of the 25 nm diameter tubule. The vertical shift between adjacent protofilaments gives rise to a family of lateral helices whose pitch must match the spacing of an integer number S of monomers along a protofilament. **b** If the number of protofilaments changes there is a mismatch along the S-start lateral helices. **c** This is taken up by an overall rotation of the surface lattice. The protofilaments are now skewed with respect to the tubule axis and become long pitch 'superhelices'

(Kozielski et al. 1997; Kull et al. 1996; Sablin et al. 1996; Gulik et al. 1998; Sack et al. 1997) and the structure of the tubulin heterodimer has been solved by electron crystallography (Nogales et al. 1998). These crystal structures are of great importance but by themselves they cannot tell us how microtubules and kinesins interact. Experience with the actin/myosin system (Rayment et al. 1993), as with the decoration of viruses with monoclonal Fab fragments (Wang et al. 1992), has shown that it is possible to achieve an accurate fit of atomic resolution structures (obtained by X-ray crystallography) within the 2 to 4 nm resolution three dimensional molecular envelopes reconstructed from micrographs of vitreous ice embedded specimens. It is clear that one of the essential steps towards understanding microtubule/molecular motor systems will involve the combination of 3D structures from X-ray crystallography with those from electron cryomicroscopy, and the comparison of these results with those available from other techniques.

2 Microtubule structure and organisation

It is important to realise at the outset that the α and β subunits of the tubulin heterodimer have very similar amino acid sequences and, as is now established, three dimensional structures (Nogales et al. 1998). As a result, some properties of microtubules can be understood at the monomer level whilst in other cases subtle differences in the structure of the subunits come into play and the dimer is the important entity. Protofilaments run lengthwise along microtubules and associate laterally with a ~0.9 nm offset so that the nearest neighbour monomers describe 12 nm pitch helical pathways when followed from protofilament to protofilament around the microtubule, Fig. 1. It is convenient to classify microtubules by the two most prominent helical families that completely fill the monomer surface lattice. Thus, we use the nomenclature N : S to refer to a microtubule with a surface lattice defined by N protofilaments and S shallow-pitch, left-handed, *monomer* helices, see Fig. 1.

In vitro assembly of MAP free tubulin (PC-tubulin) usually gives a range of microtubule structures with protofilament numbers N in the range $10 \leq N \leq 16$ and values of S (the so-called start number) in the range $2 \leq S \leq 4$. Analysing the moiré patterns in microtubule images obtained by electron cryomicroscopy has been one of the main keys to understanding how microtubules can exist with a large range of protofilament numbers (Chrétien and Wade 1991; Wade and Chrétien 1993). For protofilament numbers other than 13, the S start helices will have a mismatch along a 'seam' between two protofilaments unless the surface lattice is modified in some way. Experiment shows this mismatch, Fig. 1(b), is taken up by an overall rotation of the surface lattice, Fig. 1(c), as a result of which the protofilaments are slightly skewed with respect to the axis of the microtubule and form long-pitch 'superhelices' (Wade et al. 1990). Since the initial mismatch depends on both N and S, Fig. 2(a), the pitch and hand of the protofilament helices also depend on these parameters as shown in Fig. 2(b). For N in the range 11–15, the smallest lattice rotation, and hence the lowest energy paid for distorting the bond angles between tubulin subunits along the protofilament, is found for matching S = 3; similarly, for N = 10 we expect to find S = 2 and for N = 16, S = 4. However, 'unexpected' start values have also been observed for some protofilament numbers, for example 15 : 4 (Arnal and Wade 1995). Overall, the lattice accommodation mechanism based on the monomer lattice gives an excellent description of the surface lattice organisation of microtubule polymorphs and argues in favour of quasi-identical interprotofilament contacts for both tubulin subunits. See Chrétien

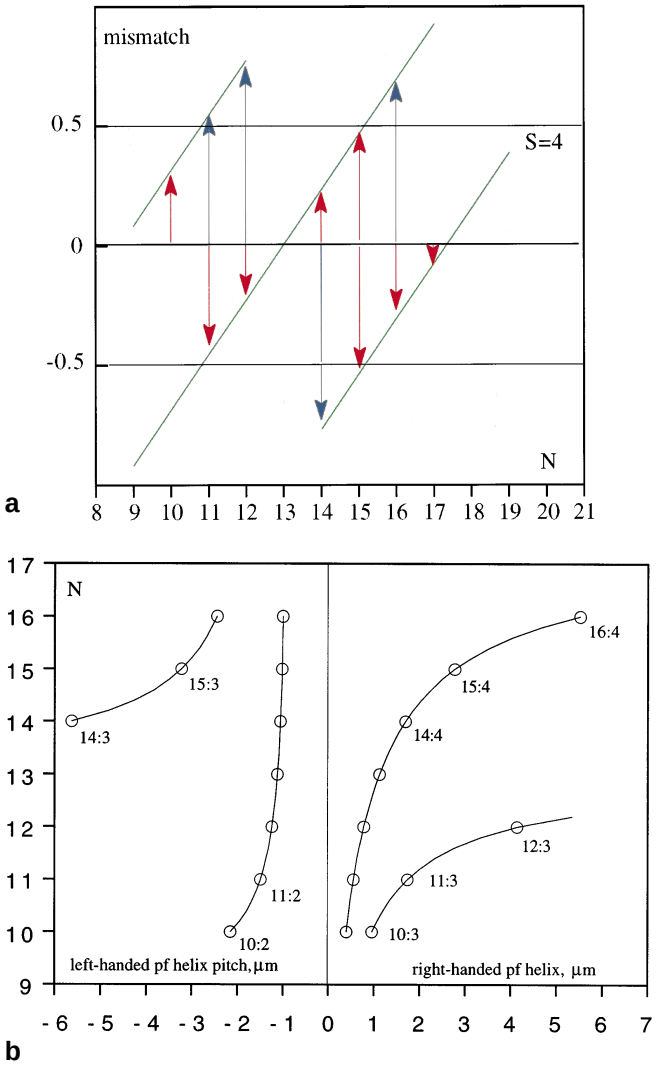

Fig. 2 **a** The surface lattice mismatch depends on the number of protofilaments *N* and on the number of lateral helices *S* (Chrétien and Wade, 1991). The mismatch is expressed in units of the monomer spacing along the protofilaments and a microtubule with *N* protofilaments is expected to have the number of lateral helices giving the smallest mismatch. **b** The pitch of the superhelical protofilaments depends on the rotation of the surface lattice required to take up the mismatch

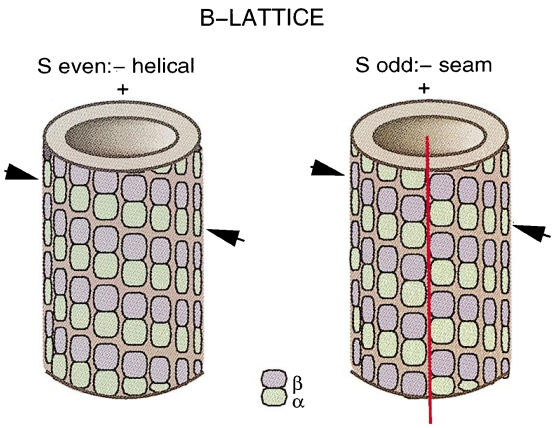

Fig. 3 Sketch of the organisation of tubulin dimers for the microtubule B-lattice. Each lateral helix contains identical α or β subunits, represented in *green* and *violet* respectively. *S* of these lateral helices are needed to cover the complete surface lattice and because tubulin is a heterodimer the surface lattice has complete helical symmetry only for even values of *S*; for odd values there is a seam

et al., this issue, for a detailed examination of some of the more subtle aspects of lattice accommodation.

In contrast, the *dimer* lattice is important when the interaction of microtubules and the motor proteins is considered. Current evidence is strongly in favour of the so-called B-lattice in which identical tubulin subunits are aligned along shallow-pitch S-start helices, Fig. 3 (Harrison et al. 1993; Hirose et al. 1995; Hoenger et al. 1995; Kikkawa et al. 1995; Song and Mandelkow 1993; Song and Mandelkow 1995; Sosa and Milligan 1996). For the B-lattice, S must be even for the surface lattice to have complete helical continuity (Amos and Klug 1974; Wade et al. 1995). Consequently the 13:3 microtubules usually found in eukaryotic cells have a packing discontinuity where the β-subunits aligned along a 3-start helix, switch after one complete turn to a set of α-subunits and so on from turn to turn. As a result, two protofilaments have neighbouring α and β subunits along the 3-start helix, this is the so-called 'seam'. It is not known whether the presence of this 'defect' has any functional role, but its existence is most likely a direct consequence of the way microtubules grow.

A longstanding problem is the relationship between the structural and dynamic polarity of microtubules. In short, which tubulin subunit is at the plus end? This is clearly of great importance in any structural investigation of motor proteins interacting with microtubules, and for understanding the directionality of kinesin related transport within the cell. Several recent electron microscope investigations have used the sheet-like extensions at microtubule ends 'decorated' with kinesin monomers as specific markers and observed in negative stain. The polarity of these images was determined by comparison with microtubules grown from fragments of sea urchin sperm flagella. Because they grow faster, microtubules on the plus end of axoneme fragments are expected to be longer than on the minus end. Initially, the results obtained in different laboratories on kinesin decorated sheets and microtubules did not agree (Song and Mandelkow 1993; Hoenger et al. 1995; Kikkawa et al. 1995; Hirose et al. 1995). After more detailed examination the question was settled and currently there is general agreement on polarity assignments (Hirose et al. 1995; Hoenger and Milligan 1996). It turns out that the visualisation of microtubules attached to only one end of an axoneme fragment cannot be taken as proof that this is the plus end because although the plus end microtubules grow faster, they also have the greater dynamic instability so they can shrink much faster than the microtubules at the negative end. Also they can easily break off during specimen preparation.

A completely different and more direct approach is possible using the arrow-like moiré fringes that characterise electron micrographs of vitreous ice embedded specimens.

These have been shown to point towards the microtubule plus end for microtubules with right-handed superhelical protofilaments and towards the minus end for left-handed protofilament helices (Chrétien et al. 1996). This has the great advantage of allowing the polarity of individual microtubules to be determined directly and this method has been applied to images of microtubule-motor protein complexes used for three dimensional reconstructions (Arnal et al. 1996; Arnal and Wade 1998). Finally, the polarity can be checked out using the protofilament skew in end-on views of the final reconstruction.

Evidence in favour of β tubulin at the plus end comes mainly from work showing GTP-coated fluorescent beads to interact only with microtubule plus ends (GTP can only be exchanged on β tubulin) (Mitchison 1993) and from experiments showing an α-tubulin specific antibody to attach only to microtubule minus ends (Fan et al. 1996). Kinesin has also been shown to interact with both tubulin subunits (Larcher et al. 1996; Tucker and Goldstein 1997; Walker 1995).

3 The kinesin family of motor proteins

The kinesin superfamily of motor proteins use ATP hydrolysis to fuel movement along microtubules (Bloom and Endow 1995). For example, *Drosophila* kinesin moves at about 1 µm/sec along the protofilament direction towards the microtubule plus end with a probable step length of around 8 nm. This corresponds to the spacing between tubulin dimers along the protofilament. Kinesin remains in contact with microtubules over many steps, a characteristic called processivity (Berliner et al. 1995; Gilbert et al. 1995; Howard 1996; Hackney 1995). Another important feature is that the ATPase activity of kinesin is enhanced by up to three orders of magnitude in the presence of microtubules.

Since kinesin was first isolated in the mid 1980s many other proteins have been discovered with similar sequences in a region of some 340 amino-acids, called the motor domain, that includes the ATP binding site, and the microtubule binding regions. There are now upwards of 90 members of the kinesin superfamily listed in sequence data bases. These proteins are omnipresent among eukaryotes where, in partnership with microtubules, they are involved in cell division, in intracellular transport and in the organisation of the cytoplasm. There now appear to be a whole range of structural variants so that the superfamily can be divided into sub-groups of kinesin-like proteins (KLPs) that may be monomers, hetero- or homo-dimers, trimers, tetramers (Cole and Scholey 1995). In their 'standard' form these proteins are elongated heterotetrameric proteins with two heavy and two light polypeptide chains with molecular weights typically in the ranges ~110–130 kDa and ~60–80 kDa respectively. The heavy chains are organised into three distinct regions: the motor domain, a rod-like region and a globular tail (Scholey et al. 1989; Hirokawa et al. 1989). The motor domain is usually situated at the amino-terminal end of the heavy chain and is followed by a region predicted to be α-helical and that has a 7-residue repeat (abcdefg) with a high likelihood of having a hydrophobic residue in positions a and d. Such sequences are predicted to favour dimerisation by forming α-helical coiled-coils. The globular tail, in partnership with the light chain, is thought to be involved in the cargo specificity.

It is difficult to obtain large amounts of pure motor proteins from natural sources (for example, brain tissue) and almost all recent work has used bacterial overexpression to obtain truncated proteins corresponding to specific regions of the heavy chain. This has allowed large amounts of protein to be obtained for a range of biophysical studies. The crystal structure of the motor domain some 340 amino-acids in length has been obtained for human kinesin (Kull et al. 1996) and for *Drosophila* ncd (Sablin et al. 1996), a minus end directed member of the kinesin family that has the motor domain at the carboxyl terminus. Very recently the structures of rat kinesin (Sack et al. 1997), of Kar3 (Gulik et al. 1998) and the rat kinesin dimer (Kozielski et al. 1997) have also been solved. In view of the lack of sequence homology and of the large size difference compared to myosin it was a surprise to find that the kinesin and ncd structures show a strong structural similarity to the central core of the myosin motor domain, a seven stranded β-sheet with three α-helices on either side. This led to suggestions that some elements of the tertiary structure of myosin may have structural and functional analogues in the kinesins. For example, the coiled-coil α-helices that were absent in the recombinant proteins used to obtain the first kinesin and ncd crystal structures were tentatively positioned similarly to myosin's long α-helical lever (Vale 1996). Such predictions are clearly provisional as witnessed by the recent crystal structures of rat kinesin that shows the neck region α-helix in a position and orientation different from that of myosin (Kozielski et al. 1997; Sack et al. 1997). Also, myosin S1 is about three times bigger than the kinesin motor domain largely due to two long 'inserts' that include the actin binding regions leading to the proposal that the equivalent, shorter, inserts in kinesin contain the microtubule interaction regions (Kull et al. 1996). In kinesin these regions run from amino acids 138 to 173 (strands β4, β5 and loop L8) and from 272 to 280 (loop L12 connecting helices α4 and α5).

4 Experimental approach

Motor dimers

Kinesin and ncd monomers complexed to microtubules, examined by electron microscopy have shown that one monomer binds per tubulin dimer and have provided the first direct evidence that in vitro assembled microtubules are organised as a B-lattice (Song and Mandelkow 1993). It is clearly important to obtain an initial structural basis for understanding the movement of these molecular motors, so although the monomer complexes have given very

informative results, similar work with the functional dimers is more likely to provide relevant information about motor movement, directionality and details of the protein-protein interactions between kinesins and microtubules.

Since overexpressed recombinant kinesin heavy chains longer than some 380 amino acids spontaneously form dimers and are viable motors (Huang et al. 1994; Hackney 1995; Young et al. 1995) these have been used in several investigations of microtubule-dimer complexes. *Drosophila* kinesin and ncd are usually used as archetype plus-end and minus-end directed motors, respectively. Both of these proteins have been well characterised (Chandra et al. 1993; Huang et al. 1994; Huang and Hackney 1994). Both binding assays and electron microscopy have indicated that, in the presence of the slowly hydrolyzable ATP analogue, AMP-PNP, these dimers interact with taxol-stabilised microtubules with stoichiometry of one motor dimer per tubulin heterodimer (Hirose et al. 1996; Arnal et al. 1996).

Microtubule stabilisation

Microtubules obtained by standard in vitro assembly from purified tubulin (PC tubulin) are rather unstable and have a strong tendency to disassemble when the pH or ionic strength varies beyond restricted limits, when they are diluted or when the temperature falls below ~30 °C. As has been done for many other biophysical applications, the structural studies use microtubules stabilised by the anti-mitotic drug taxol. Assembly with taxol gives microtubules with fewer protofilaments than the standard assembly procedure at 37 °C in the presence of excess GTP (Andreu et al. 1992; Arnal and Wade 1995). The taxol-related drug, taxotere, also has some interesting properties that make it useful as a microtubule stabilising agent (Andreu et al. 1994). In the presence of taxotere the microtubule population mainly consists of 13, 14 and 15 protofilament structures, as in the control assembly, but there is an increase in the number of 15 protofilament microtubules, significant numbers of which show a different type of lattice organisation with four starts (15:4) in addition to the standard three-start (15:3) structures. Figure 2(b) shows that for these microtubules the theoretical 'mismatch' is of similar magnitude but opposite sign for 15:3 and 15:4 microtubules. As shown in Fig. 1 this misfit is taken up by rotating the surface lattice. The rotation is anticlockwise for 15:3 microtubules giving left-handed superhelical protofilaments, and clockwise for 15:4 microtubules giving right-handed superhelical protofilaments. As imaged by electron cryomicroscopy in vitreous ice, microtubules with 15 protofilaments can easily be recognised by their width and distinctive moire patterns with segments of three fringes, blur, three fringes, blur, etc. The fringes are offset from the image centre first in one direction then in the other. The periodicity of the moire pattern is about 160 nm for the 15:4 microtubules compared to about 240 nm for the 15:3 structures. An additional check can be made once the

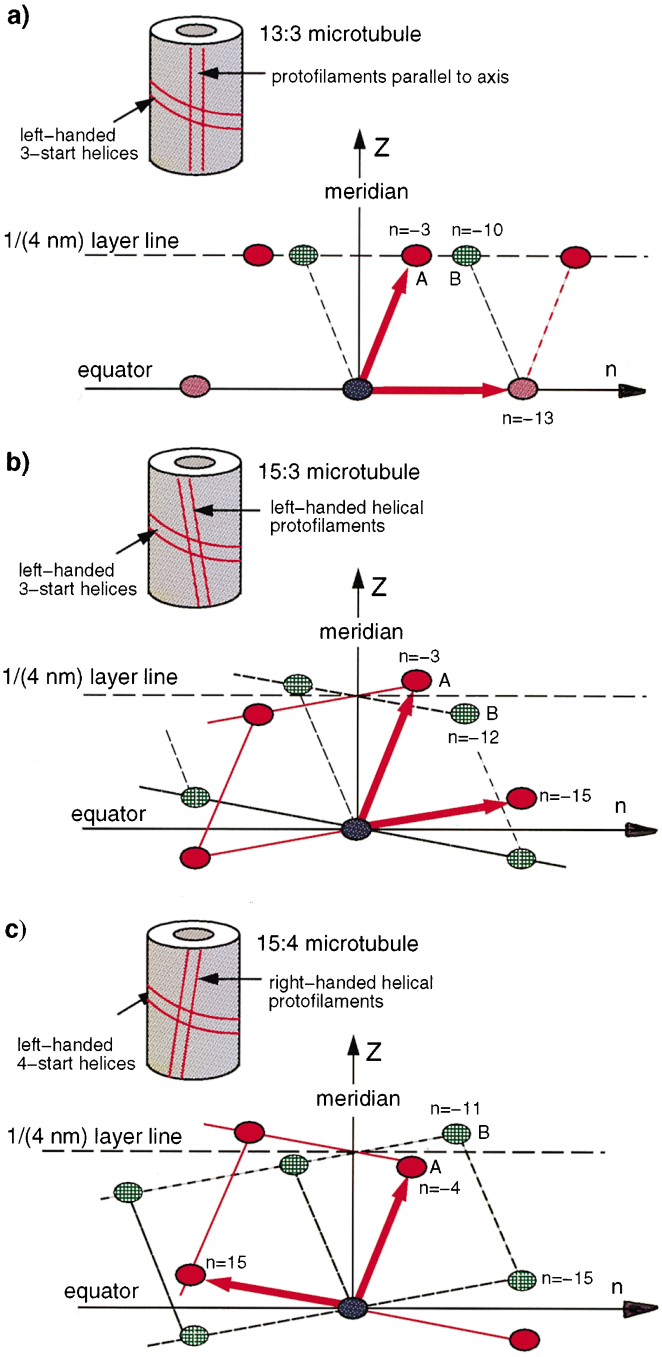

Fig. 4a–c Closely related microtubules such as 15:3 and 15:4 structures can be recognised by their distinctive diffraction patterns. The positions of peaks like *A* and *B* that are on the same layer-line (at $(4\ \text{nm})^{-1}$ in this example) for 13:3 microtubules are sensitive to the surface lattice rotations required to adjust to other protofilament numbers. **a** For 13:3 microtubules *A* and *B* lie on the same layer-line and in practice will overlap to some extent, note that peaks on the equator superpose completely. **b** For 15:3 microtubules *A* is above *B*, whilst (c) for 15:4 microtubules *A* is below *B*. Similar effects are observed on other layer-lines

image has been read into the computer, by comparing the relative positions of the two main peaks closest to the meridian on the layer-lines near to $(4\ nm)^{-1}$ in the computed diffraction pattern, as shown in Fig. 4 and discussed in more detail in the next section.

5 Obtaining three dimensional maps of microtubule-motor complexes

Helical reconstruction methods have long been a standard tool in electron microscopy (DeRosier and Moore 1970). In the case of microtubules, as we have already mentioned, to be strictly helical a B-lattice of tubulin dimers requires an even number of lateral monomer helices, i.e. S must be even. This means that in situations where the dimer lattice is important, none of the 13:3, 14:3, 15:3 microtubules found under standard in vitro assembly conditions can be used for helical reconstruction. Only the 15:4 structures, about 15% of the total microtubule population for assembly in the presence of taxotere, can be used for carrying out helical reconstructions of microtubule-kinesin complexes. In an approach that uses in vivo material, accessory microtubules from sperm tail axonemes of various insects have been used for helical reconstruction purposes since these are 4-start structures with 16 or more protofilaments (Lanzavecchia et al. 1994; Hirose et al. 1995).

To get around this restriction, a tomographic approach can be used to obtain three dimensional maps of microtubules both with and without seams (Wade et al. 1995; Metoz et al. 1996; Sosa and Milligan 1996; Sosa et al. 1997). This approach requires the microtubules to have two simple properties: they must have superhelical protofilaments of known pitch (this excludes 13 protofilament microtubules) and the tubulin heterodimer must be packed with a regular repeat along each protofilament. Under these conditions, three dimensional reconstructions can be obtained using a filtered back projection approach, provided that the image is correctly cut into sub-images, corresponding to a set of projections equivalent to a single axis tilt series, and that it provides a full set of views covering 180°. As shown in Fig. 5, such reconstructions confirm that microtubules are organised with the B-lattice and that three-start microtubules, in this case 15:3, do have a seam. In this reconstruction method, it turns out that the sub-image structure is averaged 'independently' along individual protofilaments. Although the averaging can be extended to the complete structure, it is often more convenient to use the helical reconstruction method because this gives 3D maps which are automatically averages of all the sub-units in the image. Consequently the helical reconstruction will have a better signal to noise ratio than a tomographic reconstruction from the same image.

In practice, data collection is the limiting factor for electron cryomicroscopy work with microtubule-motor dimer complexes since, for various reasons, only a small number of micrographs are suitable for further treatment. The few percent of images selected are scanned on a microdensitometer and undergo preliminary image treatment on the computer. After extracting layer-line data from computed diffraction patterns, the three dimensional reconstruction step is carried out using helical reconstruction methods (Crowther et al. 1996). From a practical point of view, the surface lattice rotation required to accommodate extra or fewer protofilaments than the canonic 13 protofilament microtubules is very helpful for extracting the layer-line data. For 13 protofilament microtubules, diffraction peaks such as A and B in Fig. 4(a) can be difficult or impossible to separate because they are on the same layer-line and often overlap. For other protofilament numbers the equivalent peaks are shifted to layer-lines above, or below the positions they would occupy for the unrotated lattice, Fig. 4 (b, c) shows the situation for 15:3 and 15:4 microtubules. Thus all the layer-line data, except on the equator, is automatically separated and can be extracted individually. Of course, images of microtubule-kinesin complexes have extra layer-lines corresponding to the 8 nm spacing of the motor proteins along the protofilaments. After a preliminary reconstruction the microtubule polarity can be determined (Chrétien et al. 1996; Arnal et al. 1996).

Initially microtubule-kinesin and microtubule-ncd complexes were imaged in the same nucleotide state, so as to check whether these two dimeric motor proteins have distinct conformations that might be related to their opposite directionality. The slowly hydrolysable analogue AMP-PNP (5′-adenylylimidodiphosphate) can be considered as non-hydrolysable over the time scale of the experiments and is used to mimic the ATP-like state (Arnal et al. 1996; Hirose et al. 1996). Recently the relationship between the structure of microtubule-kinesin complexes and the nucleotide state of the kinesin dimer has been investigated. Images of complexes in the presence of ADP, ADP-AlF_4 (to mimic the ADP-P_i state) and apyrase (to mimic the no nucleotide state) have been obtained (Arnal et al. 1998).

The three-dimensional reconstructions of microtubule-kinesin monomer complexes, Fig. 6(a), clearly show the B-lattice organisation of the microtubule and the 8 nm kinesin spacing corresponding to the tubulin dimer spacing along the protofilaments. The kinesin monomer interacts one to one with the tubulin dimer. In the case of microtubule-kinesin dimer complexes, Fig. 6(b), the kinesin dimer:tubulin dimer stoichiometry is also 1:1. The kinesin dimer has one attached head and one free head. The free head contacts the end of the attached head closest to the microtubule plus end, and, Fig. 6(b), it points sideways to the right and upwards towards the microtubule plus end, i.e., in the direction of movement of the kinesin motor. The free head appears to occupy a smaller volume than the attached head. The attached heads of the kinesin dimer have a similar volume to the kinesin monomers. Since other work has shown convincingly that the DKH 392 protein and similar constructs are indeed dimers (Huang et al. 1994; Hackney 1995; Young et al. 1995), this leads us to believe that the apparent size difference between the two heads is due to positional disorder of the free head.

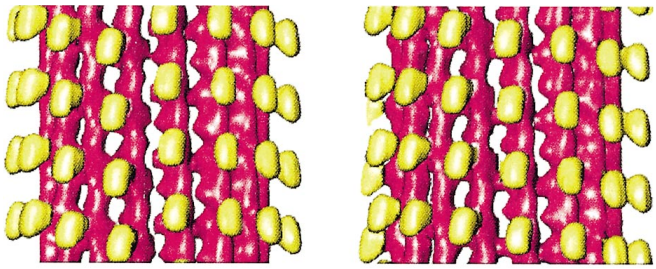

Fig. 5 Three-dimensional tomographic reconstruction of a microtubule-kinesin monomer complex. The microtubule is coloured magenta and kinesin yellow. The two side views are rotated through 180° to show the front and back of the structure. The decoration clearly shows the B-lattice organisation and the seam in this 15:3 microtubule, plus end is at the top

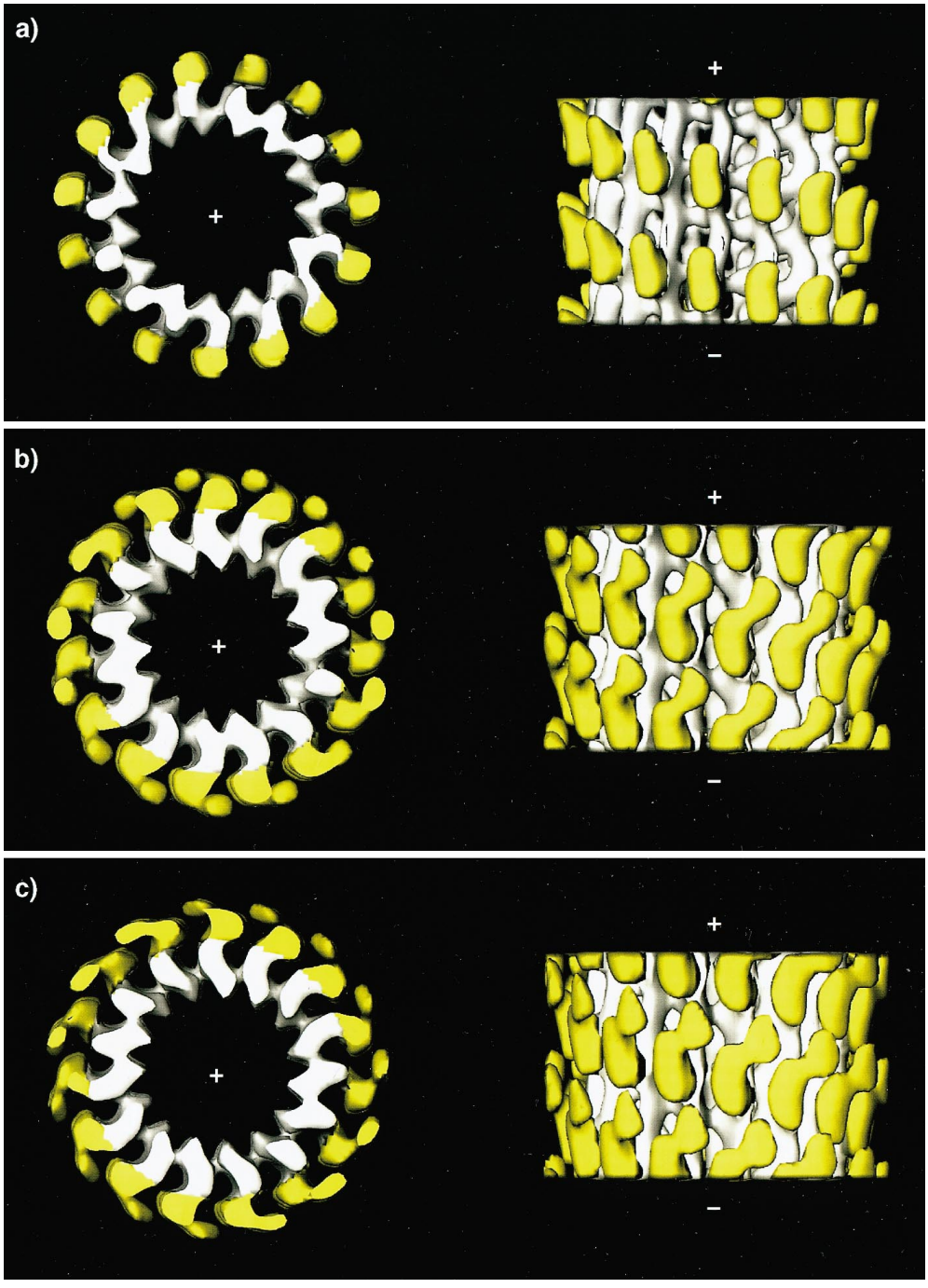

Fig. 6a–c Top and side views of three-dimensional reconstructions of microtubule-kinesin complexes. Microtubule protofilaments in white, kinesin in yellow. **a** Helical reconstruction of the kinesin monomer interacting with microtubules in the presence of AMP-PNP. **b** Microtubule-kinesin dimer complex prepared in the presence of ADP, helical reconstruction of the averaged layerlines from 13 data sets. The dimer has an attached and a free head. **c** Real space average from the same data set as for (**b**). The average includes all 13 individual three-dimensional helical reconstructions of microtubule-kinesin dimer complexes

Another useful possibility, especially in view of statistical analysis, is to carry out real space averaging of individual helical reconstructions. Because it is quite straightforward to calculate three dimensional averages and standard deviation maps this approach can be especially useful for generating tests to estimate the significance of difference maps and of small conformational changes (Milligan and Flicker 1987). It has also been used to compare the signal to noise in three dimensional reconstructions of microtubule-motor complexes (Hirose et al. 1996). Figure 6(c) shows a real space average of the microtubule-kinesin dimer complex in the presence of ADP as calculated from the same data set used to obtain the helical reconstruction average in Fig. 6(b).

6 Conclusions

Varying numbers of protofilaments can be accommodated by microtubules by a simple rotation of the surface lattice in which the local geometry of tubulin-tubulin interactions is accurately preserved. There is only a small latitude for shifts between the protofilaments and the interprotofilament binding would appear to have a similar geometry for α-α, β-β or α-β contacts. It would be interesting to interpret this apparent identity, or near identity, in the lateral binding sites in the light of the recently published atomic level structure of the tubulin heterodimer.

Concerning microtubule-motor protein complexes, the motor dimers of kinesin and ncd have been shown to have distinctly different conformations that may be related to their opposite directivity. Microtubule-kinesin dimer complexes have been imaged in the presence of AMPPNP, ADP, ADP-AlF_4, and in the presence of apyrase, a nucleotide 'scavenger'. The corresponding three dimensional reconstructions show, in all cases, one attached and one free head. The conformation of the free head is nucleotide dependent.

As higher resolution images of microtubules and microtubule-motor protein complexes are obtained, the crystallographic structures of motor proteins and of tubulin that are becoming available will be used to build high resolution models of microtubules and of microtubule-motor protein complexes. It will be possible to study in detail how protofilaments interact, the effect of stabilising agents and the conformational changes induced by GTP hydrolysis. The atomic resolution microtubule model will be combined with motor protein structures to give a detailed description of the interactions between the tubulin heterodimer and the motors, thereby making a major contribution to understanding the molecular mechanisms involved in motor protein movement.

Acknowledgements This work was supported in part by grants from l'Association pour la Recherche sur le Cancer, from the Ministère de l'Education Nationale, de l'Enseignement Supérieur, de la Recherche et de l'Insertion Professionnelle (ACC5-SV), and from the Centre Nationale de la Recherche Scientifique (Biologie Cellulaire: du Normal au Pathologique). This is publication number 534 of the Institut de Biologie Structurale Jean-Pierre Ebel.

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