Science Progress (2009), 92(2), 113–137 doi: 10.3184/003685009X461431

Tubulin and its prokaryotic homologue FtsZ: a structural and functional comparison

NIGEL DYER

ABSTRACT

Microtubules are one of the three primary constituents of the eukaryotic cytoskeleton and are constructed from the protein tubulin. FtsZ is a close structural homologue of tubulin within prokaryotes, and plays an important structural role during cell division. This article compares what is known about the structures that these two homologues are able to form in vivo *and* in vitro *and examines the evidence that the water in the immediate vicinity of the structures, particularly in microtubules, may play an important role in their formation and stability. The article then examines evidence that this hydration layer might help our understanding of how the structures formed by tubulin and FtsZ are stabilised by associated proteins and selected cations. The article then considers recent studies of the charge distribution and dipole moments of tubulin and extends this work to include the electrostatic characteristics of FtsZ. There is then an examination of the ways in which the electrostatic similarities and differences between the two proteins might be related to the similarities and differences in the filamentary structures that they form.*

Keywords: *microtubules, tubulin, eukaryotic organisms, FtsZ*

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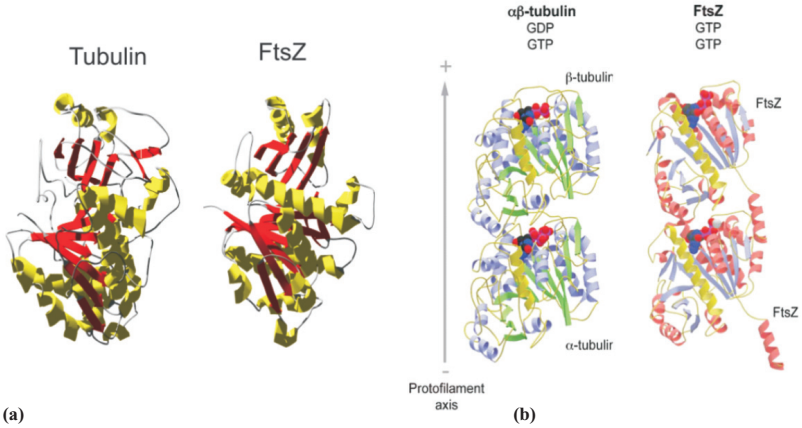

Fig. 1. *Comparison of tubulin and FtsZ.* (**a**) *Comparison of the tertiary structures of a single tubulin and FtsZ protein.* (**b**) *Comparison of the binding of tubulin and FtsZ using GTP (constituent atoms shown as red and blue spheres) to form a dimer*[1]*. The FtsZ dimer consists of two identical monomers. Further monomers are added to form a protofilament. The tubulin dimer consists of an alpha tubulin and a closely related beta tubulin polypeptide. Tubulin protofilaments are constructed of dimers stacked end to end. Reprinted, with permission, from the* Annual Review of Biochemistry, **75** ©2006 *by Annual Reviews, www.annualreviews.org*

Introduction

The cells from eukaryotic organisms, such as plants and animals and single celled yeasts, are all constructed around a scaffolding or cytoskeleton consisting of three main cytoskeletal elements: actin in the form of actin fibres, tubulin which is the core building block of microtubules and intermediate filaments. It had long been assumed that these elements were only to be found in eukaryotic cells, but recent work has shown that the much simpler prokaryotic cells contain structural homologues of all three of these cytoskeletal components. In each case, their function is both distinct from their eukaryotic cousins whilst retaining sufficient similarity to suggest some of the likely characteristics of their probable distant common ancestors[1].

The prokaryotic homologue of tubulin is FtsZ[2,3]. One indication of the close relationship between FtsZ and tubulin can be seen from their very similar protein structures (Figure 1a). Both FtsZ and tubulin form long filamentary structures by head to tail association of the individual proteins. This association is regulated by guanosine triphosphate (GTP) which is bound in the cleft between adjacent monomers (Figure 1b). FtsZ forms long protofilaments consisting of a single string of FtsZ proteins.

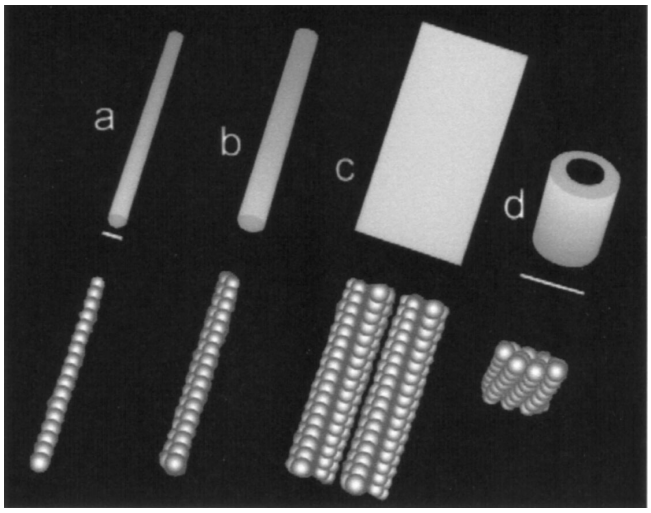

Fig. 2. *FtsZ structures formed* in vitro*: protofilament* (**a**), *Thick filament* (**b**) *Sheets* (**c**) *and tubules formed in the presence of* Ca^{2+} [4]*. Reprinted from* Journal of Molecular Biology*,* **318**, *Issue 2, Stephen G. Addinall and Barry Holland, The Tubulin Ancester, FtsZ, Draughtsman, Designer and Driving Force for Bacterial Cytokinesis, p. 18,* ©2002, *with permission from Elsevier.*

The building block of tubulin based filamentary structures is a tubulin dimer which consists of an alpha and beta tubulin polypeptide in a head to tail association. Tubulin protofilaments, consisting of many such dimers joined head to tail, do not exist in isolation but are found assembled into larger, more complex, structures. The most common of these are microtubules, which consist of typically 13 protofilaments arranged parallel to each other in the form of a tube. Microtubules form a dynamic network within the cell, and have both a structural and a transport role. In animal cells, this network normally radiates from the centrosome, which has at its heart the centriole, an enigmatic and elegant T shaped organelle that is also constructed using tubulin. The microtubule network undergoes substantial rearrangement during mitosis to form the structures which co-ordinate the separation of the replicated chromosomes to form the nuclei within the two daughter cells.

While many of the details of the structures such as microtubules and centrioles that are formed by tubulin in eukaryotic cells are well established, the nature of the *in vivo* structures formed by FtsZ in prokaryotes is still unclear, although there has been some characterisation of the structures formed by FtsZ *in vitro* (Figure 2). It is still unclear to what extent these structures are representative of the structures formed by FtsZ *in vivo*.

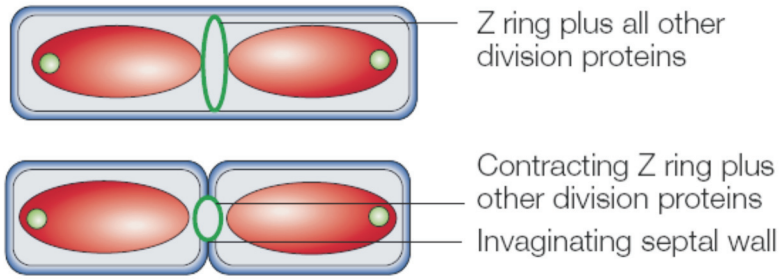

Fig. 3. *The role of the Z-ring in prokaryotic cell division*[5]*. Reprinted by permission from Macmillan Publishers Ltd:* Nature Reviews Molecular Cell Biology*, Vol. 6, Issue 11, 862–871, FtsZ and the division of prokaryotic cells and organelles, William Margolin,* ©2005.

Unlike its eukaryotic cousin, FtsZ does not appear to provide a structural role throughout the cell cycle, but instead just plays a structural role during cell division, when it forms a band, known as the Z-ring, around the inner cell wall at the location where the cell will divide (Figure 3). The Z-ring is then the foundation of the molecular machine that causes cell wall invagination and final separation of the cell into two cells or septation, as illustrated in Figure 3.

FtsZ and tubulin stability

Both FtsZ and tubulin are able to make a unique contribution to the life of the cell because they are able to form very dynamic structures which are very sensitive to the local environment within the cell. It is in this role of 'intelligent' scaffolding that eukaryotic tubulin is able to support many different dynamic processes within the cell. Some of the most critical of these processes, which would have had to have been present in the earliest of organisms, are those associated with cell division, where they both need to grow and shrink as cell division proceeds. The involvement of both FtsZ and tubulin in these processes and their extreme structural conservation across species suggest that there was a recognisable FtsZ/Tubulin homologue in primordial organisms[6].

A further possible indication of a common origin in primordial cell division is the striking similarity between the Z-ring in prokaryotes and the pre-prophase band which is constructed with microtubules on the inside of a plant cell wall at the start of mitosis and marks the plane of cell division, shown in Figure 4.

Fig. 4. *Representation of the pre-prophase band (green) constructed of microtubules which marks the future division site in a plant cell*[7]*. Reproduced with permission.* Journal of Cell Science, **115**, 1345–1354 (2002) ©2002. *The Company of Biologists Limited. Geoffrey O. Wasteneys, Microtubule organization in the green kingdom: chaos or self-order?*

Experimental data suggest that FtsZ and tubulin differ in the details of how they provide the dynamic flexibility required. In the case of tubulin, this is achieved through an almost continuous process of nucleating microtubules which then undergo periods of slow growth and catastrophic collapse, with growth and collapse primarily taking place at the opposite end from where nucleation took place, referred to as the positive end[8]. Tubulin dimers at the growing end or cap of the microtubule have bound GTP, which is hydrolysed to GDP as further tubulin dimers are added to the growing end of the microtubule, and the GDP is retained between the dimers within the microtubule. The dynamics of the microtubule are governed in part by the control of GTP hydrolysis at the cap.

The dynamics of FtsZ protofilaments are less clear, but evidence suggests that assembly is also catalysed by the presence of the GTP ligand. Unlike microtubules, hydrolysis to GDP is associated with fragmentation of the polymer, which would then be followed by a GTP induced reassembly[9]. This very dynamic process is thought to be a key factor in the way in which FtsZ provides a dynamic structural role during cell division, drawing the cell wall in, although the details of how this occurs are still unclear[10].

The role of associated proteins

There are proteins associated with both FtsZ and tubulin structures that are critical to controlling their dynamic nature. In the case of tubulin, there are broadly two classes of Microtubule Associated

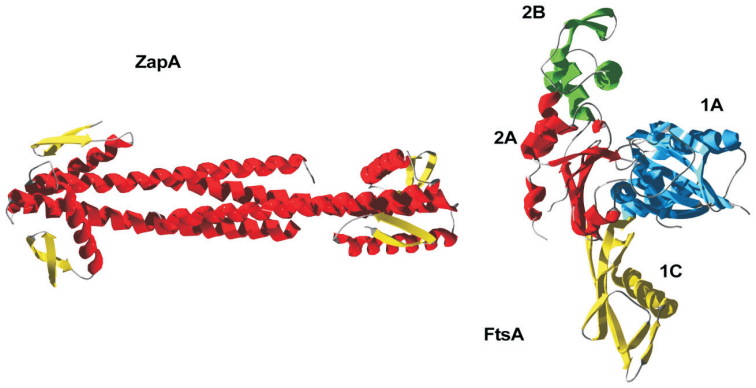

Fig. 5. *ZapA, FtsA and actin structures drawn using a cartoon representation of the protein backbone, with broader ribbons for the alpha helical segments and the parallel beta sheets, the two predominant types of secondary structure within proteins. Two ZapA monomers form dimers whose alpha helices then interlock to form tetramers. It has been suggested that the globular heads may bind to different protofilaments and consequently they are able to hold the protofilaments together*[15]*. The four domains of FtsA are labelled and highlighted in different colours to correspond to the equivalent domains in eukaryotic actin*[17]*.*

Proteins (MAPs). The first are associated with the growing ends of microtubules, such as the XMAP215/chTog family of proteins, which control the growth and collapse of the microtubules[11]. The second, such as Tau and MAP2/4 in homo sapiens, decorate the outer surface of the microtubule along its length[12].

A number of proteins are known to stabilise the FtsZ based Z-ring, including ZapA[13,14], FtsA and ZipA. However any similarity between these proteins, or between these proteins and the MAPs, is subtle at best. This difference, and in particular the differences in their structures suggests that there may be some significant differences in the way in which these proteins stabilise their associated protofilaments. An example of these structural differences can be seen in two proteins known to stabilise the Z-ring, ZapA[15] and FtsA[16], shown in Figure 5.

It is known that a third Z-ring associated protein, ZipA, binds the FtsZ protofilaments to the cell membrane as well as providing a stabilizing role[18]. Unlike ZapA and FtsZ, it has not been possible to determine the full structure of the protein. However, it is possible to predict where there will be alpha helices and beta sheets from the sequence of amino acids, and this can be used to predict the general features of the protein's structure. The predicted secondary structure is shown in Figure 6 and indicates three distinct regions. At the N-

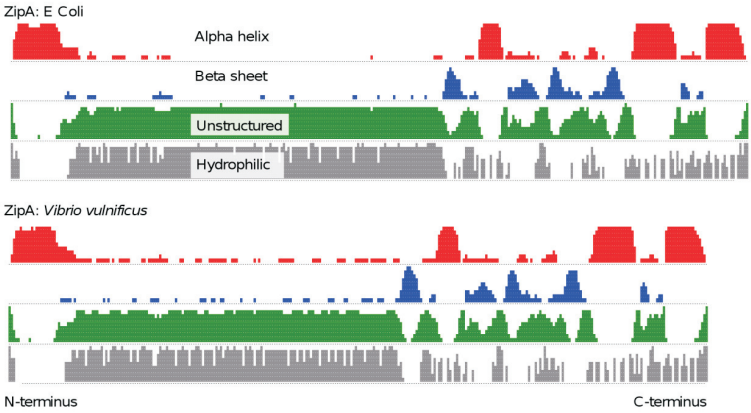

Fig. 6. *Predicted secondary structures for two different ZipA proteins, showing the probability of an alpha helix (red), beta sheet (blue) unstructured regions (green) and those regions which are hydrophilic or water loving(grey). The hydrophilic regions are likely to be in contact with the intracellular fluid. The full length of both protein sequences is shown. Structure prediction using Predictprotein:*[21].

terminus there is a hydrophobic alpha helix which is known to anchor the protein to the cell membrane[19]. At the C-terminus there is a mix of alpha helices and beta sheets which forms a globular structure which binds to FtsZ[20]. These two regions are joined by an extensive unstructured hydrophilic region. Consequently, although the full structure has not been solved for ZipA, enough is known about its probable secondary structure to be able to say that it is

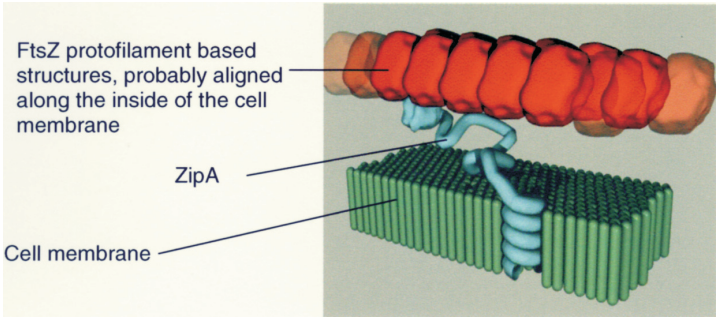

Fig. 7. *Representation of the role of ZipA in binding together the prokaryotic cell membrane and the FtsZ structures lying adjacent to the membrane drawn using Blender:*[22]*. The hydrophobic alpha helix at the N-terminus is buried in the cell membrane, and the globular domain at the C-terminus is bound to the FtsZ protofilament structure within the Z-ring*[20]*. The unstructured hydrophilic linker region connects the two.*

different from both of the other Z-ring stabilisation proteins ZapA and FtsA.

The predicted secondary structure provides a reasonable indication of the likely relationship between FtsZ, ZipA and the cell membrane, which is represented in Figure 7.

It has been noted that there are similarities between ZipA and Tau[23], in that ZipA stabilises FtsZ protofilaments and Tau stabilises microtubules and they both also have an extended hydrophilic unstructured region. It has been suggested that this region plays a role in stabilising both FtsZ protofilaments and tubulin microtubules[23]. The predicted secondary structure for Tau is shown in Figure 8.

The existence of this extended hydrophilic region indicates that the complete model for how MAPs such as Tau are able to stabilise microtubules will probably need to include an understanding of how this region interacts with the water immediately adjacent to the microtubule.

The role of water in microtubule stabilisation

One way in which the role of the hydrophilic regions in MAPs might become clearer is through examining the experimental evidence that has accumulated over many years which indicates that water plays a significant role in the function and stability of microtubules.

One of the earliest indications that the region surrounding microtubules may be a significant factor in the structure and stability of microtubules was the frequent observations that there were clear or unstained regions around microtubules within various cells when they were fixed, stained and viewed in cross section[24,25] (Figure 9). These clear regions appear to keep the microtubules separated both from each other, and from other components within the cell. Clear regions were also observed when the microtubules were assembled *in vitro* where it was shown that these clear regions disappear on the addition of Ca^{2+} ions[26] (Figure 10).

It has been suggested that the clear regions arise because the MAPs that decorate the surface of proteins create bridges that ensure that the microtubules are always a certain distance apart. This possibility was excluded when it was shown that the clear region around microtubules persisted even when they were assembled in the absence of MAPs . It was observed however that the distance between the microtubules was a function of whether the MAPs were present or not[27].

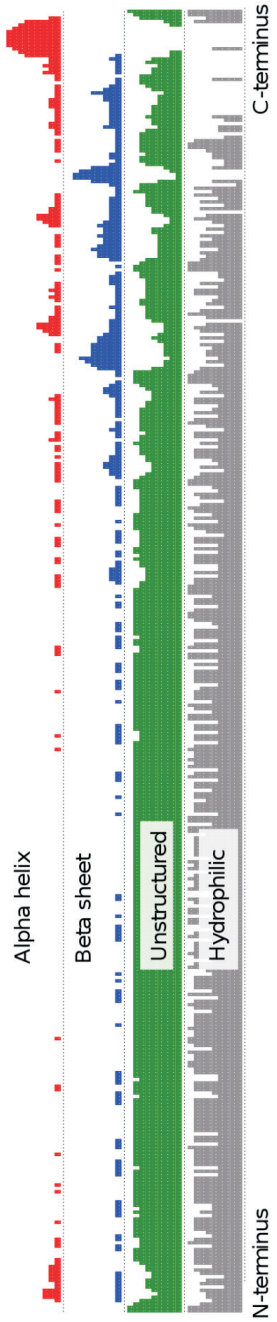

Fig. 8. *Predicted secondary structure of Tau from homo sapiens, which is known to stabilise microtubules and which, like ZipA, has an extended hydrophilic unstructured region.*

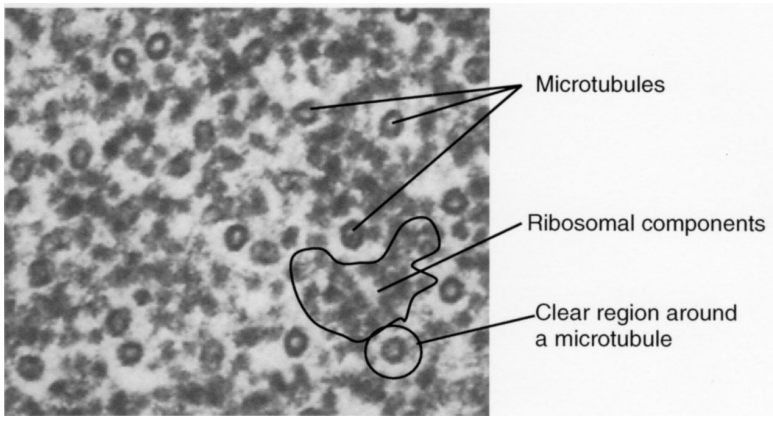

Fig. 9. *Transverse section of a nutritive tube, which passes RNA to immature eggs in insect ovaries, showing circular microtubules and ribosomal components. There is a clear region around the microtubules separating the microtubules from each other and from the ribosomal components. This indicates the absence of any significant cellular components, a conclusion supported by similar results with alternative fixing and staining techniques*[24]*. With kind permission from Springer Science + Business Media:* Cell and Tissue Research*, The nature of the clear zone around microtubules, Vol. 227, No. 3/Nov, 1982, Howard Stebbings & Cherryl Hunt.*

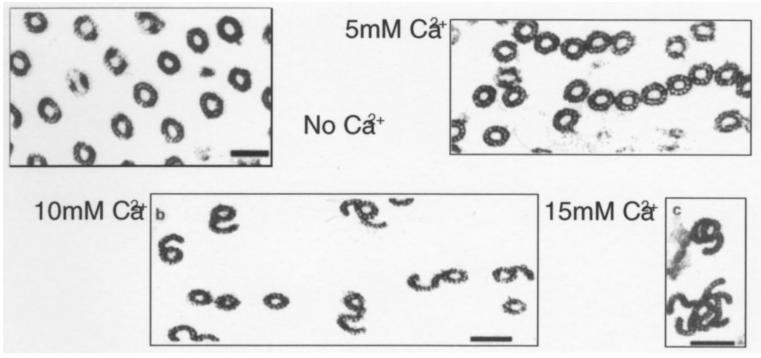

Fig. 10. *Cross sections of microtubules assembled at 37°C in the presence of increasing concentrations of* Ca^{2+}*, added subsequent to the addition of taxol, an anti-cancer drug which stabilises microtubules. With no calcium present, the microtubules remain separated from each other. Increasing calcium concentrations result in the microtubules sticking to each other, and then combining with each other to form complex aberrant structures*[26]*. Tubulin assembly in the presence of calcium ions and taxol: microtubule bundling and formation of macrotubule-ring complexes. Vater, W., Böhm, K.J. and Unger, E.* Cell Motil Cytoskeleton. ©1997; **36**(1): 76–83. *Reproduced with permission of John Wiley & Sons, Inc.*

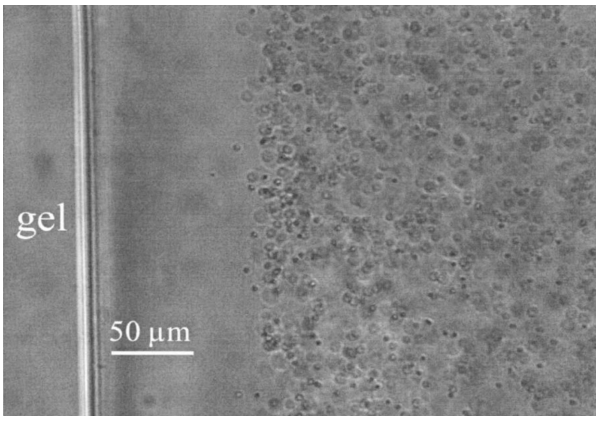

Fig. 11. *Exclusion of microspheres from the water immediately adjacent to a polyvinyl alcohol gel create a clear region similar to those seen adjacent to microtubules[28]. Reprinted figure with permission from, Zheng, J.M. and Pollack, G.H.* Phys. Rev. E Stat. Nonlin. Soft Matter Phys., 2003 Sep; **68**(3 Pt 1): ©2003 *by the American Physical Society.*

Such clear spaces have been observed around other structures within cells, which has prompted a growing interest in trying to understand their nature and significance[28]. The exact nature of the water in these regions that gives rise to these clear regions is still contentious. There has been considerable investigative work on such systems, often based on examining the similar clear regions around other materials such as polymer gels when they are immersed in water containing dissolved or suspended material (Figure 11).

NMR has been used to show that the water in the regions close to polymer gels is considerable less mobile, implying a gel-like state[28]. Earlier studies using NMR of the variation of cell water mobility through the cell cycle have shown that the cell water is less mobile when there is a greater proportion of filamentous proteins such as actin which are associated with clear cystosolic regions are present. This has been interpreted as indicating that this water is also in a gel-like state[29].

Attempts to assemble microtubules *in vitro* demonstrated that these structures are very unstable and techniques were developed for stabilising the structures so that they could be investigated. These techniques included the assembly of microtubules in glycerol concentrations greater than 1 M[30,31]. An investigation of the basis for this stabilisation[32] suggested that this was not as a result of a specific glycerol interaction because other solutes, such as DMSO, polyethylene glycol, and dextran, had similar effects.

Thermodynamic considerations[32] show that stabilisation occurs because the glycerol is excluded from the hydration layer immediately surrounding the tubulin, and it is through the stabilisation of the hydration layer that the microtubules are stabilised. The exclusion is because binding between the tubulin surface and water has a lower energy that between tubulin and glycerol, so is energetically preferable and as a result the glycerol is excluded from the protein surface. The addition of glycerol also increases the chemical energies of both tubulin and glycerol, so a minimum energy is achieved if the protein surface is minimised, making the assembly into a microtubule more energetically favourable[32]. This is a specific example of the more general way in which certain solutes are able to stabilise proteins and protein complexes[33].

The degree to which a protein will be stabilised in this way is dependent on the energetics of the interaction between its surface and the hydration shell. In the case of tubulin an additional complexity is that an isolated tubulin monomer is very negatively charged, with a typical net charge of -5 electrons based on the amino acid composition[34]. However, it is known that *in vitro* there is only an unscreened charge of approximately 0.2 electrons per monomer[34,35]. This will be because the highly negative surface of tubulin will attract positive ions, in particular intracellular potassium ions, which will neutralise the charge. While the physiological concentration of K^+ ions is in the region of 150 mM, the buffer that was originally used when examining microtubule formation *in vitro* did not contain K^+ ions[36], a practice that has largely continued since then. This is despite evidence that K^+ concentrations of up to 150 mM increased the rate of microtubule formation[37,38]. It is perhaps significant that a physiological concentration of 150 mM appears to be optimal, with the rate of microtubule formation falling off to 10% of its maximum rate at 200 mM. The sensitivity to K^+ concentration may have been a factor in the continued exclusion of K^+ ions from buffers used when investigating microtubules.

While glycerol and K^+ ions are both able to stabilise microtubules *in vitro*, it is increasingly common to use the anti-tumour drug taxol to stabilise microtubules[39]. Taxol stabilises microtubules in a very different way to glycerol or K^+ ions, binding to the inner surface of β tubulin and stabilising the binding between adjacent protofilaments as a result[40].

The picture that now emerges is that there is evidence for an extensive microtubule hydration layer which plays a critical role in determining the stability of microtubules. The presence of this layer

will be dependent on the interaction between the protein surface and the water in the cytosol. In the case of tubulin, this will be affected by the cations that are drawn to the highly negative surface of the protein, which are likely to be K^+ ions *in vitro*. The concentration of K^+ bound to the surface of the microtubule will be sensitive to the relative K^+ concentration in solution, and there is significant evidence that microtubule stability is optimal at physiological K^+ concentrations.

Additional evidence for the significance of water in the stability of microtubules is the complete absence of microtubules in seeds and dormant spores, both of which contain negligible water. This suggests that in order to survive dehydration the plant must disassemble its microtubule based structures in an orderly way rather than suffer damage when they collapse as a consequence of the disappearance of their hydration layer. The re-emergence of the microtubule network is one of the first signs of germination when the seeds are re-hydrated[41,42].

Despite the evidence that K^+ is important for microtubule stabilisation, standard microtubule assembly protocols do not include potassium in any form. This is in contrast to standard FtsZ protocols which include K^+ in the form of KCl at concentrations equivalent to those found in cells. When such buffers are used, FtsZ tends to polymerise relatively easily even when GTP is not present and it is necessary to store the monomeric FtsZ in 10% glycerol to prevent premature polymerisation[43].

The destabilisation of microtubules by calcium

A well-documented characteristic of microtubules is that they are destabilised by relatively small concentrations of Ca^{2+} cations[36,44]. In contrast Mg^{2+} cations are an essential requirement for microtubule polymerisation, with an optimal microtubule growth and stability at equal Mg^{2+} and tubulin molarities[38]. A key difference between the two cations is that Mg^{2+} plays a very specific role in binding at specific sites on the surface tubulin, stabilising both individual tubulin dimers and the binding between dimers in microtubules[45]. No such role has been identified for Ca^{2+}, which is consistent with the extremely low concentration of free Ca^{2+} ($0.1-0.2\,\mu M$) within cells, rendering it unavailable for use as an essential microtubule stabilising ligand.

In order to investigate the destabilising effect of Ca^{2+} it is necessary to select one of the techniques for stabilising microtubules. Figure 10 shows the results of a study where taxol stabilised

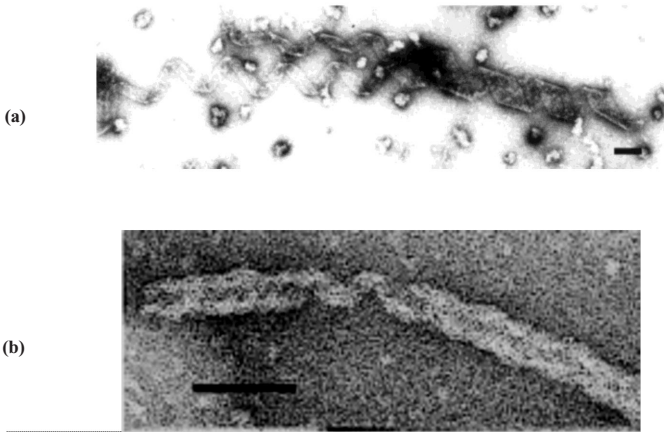

Fig. 12. *FtsZ/Tubulin spirals in the presence of Ca^{2+}/Mg^{2+} ions.* (**a**) *Spiral Tubulin structures formed at 37°C when 10 mM Ca^{2+} was added prior to the addition of taxol*[26]*. Tubulin assembly in the presence of calcium ions and taxol: microtubule bundling and formation of macrotubule-ring complexes. Vater, W., Böhm, K.J., Unger, E.* Cell Motil Cytoskeleton. ©1997; **36**(1), 76–83. *Reproduced with permission of John Wiley & Sons, Inc.* (**b**) *FtsZ spirals formed in the presence of Mg^{2+} Scale bar: 100 nm*[49]*. FtsZ has also been shown to form tubes of spirally wound FtsZ in the presence of Ca^{2+}, but no images have been published which show such a similar structure to the Tubulin spirals. Löwe, J. and Amos, L.A.* Biol. Chem., 2000 Sep–Oct; **381**(9–10), 993–999. *Helical tubes of FtsZ from Methanococcus jannaschii.*

microtubule structures in the presence of increasing concentrations of Ca^{2+} ions[26]. The increasing concentrations appear to disrupt the mechanism that ensures that a clear space exists between the microtubules, and they begin to cluster together. At higher concentrations, the tubular construction of the microtubules is disturbed and the protofilaments align in various configurations which are very different from the configurations normally found in cells. A slight change in the protocol and the addition of Ca^{2+} ions induces the formation of different non-canonical structures, such as the spirals shown in Figure 12.

These results can only be obtained in the presence of the microtubule stabilising drug taxol, and so can only be used as an indication of the changes in tubulin interactions, and not as an indication of the structures that might be seen *in vivo* in the presence of excess calcium when no taxol is present.

The simplest electrostatic model of the addition of Ca^{2+} ions is that they would be drawn to the highly negative surface of the tubulin. The effect that this would have on the hydration layer has

not been investigated, but these experimental data suggest that the hydration layer and clear zone seen around microtubules is destabilised, allowing microtubules to approach, touch each other and form larger protein aggregates.

The ability of Ca^{2+} ions to promote the binding of proteins into such conglomerates mirrors the gelation effect of cations on protein complexes that is used extensively within the food products industry. In a recent article on the ability of calcium ions to aggregate whey products it was stated:

> "*Calcium-induced aggregation presumably occurs as a result of three major events, in which case (1) electrostatic interactions are diminished as a result of charge neutralization, (2) ion-specific hydrophobic interactions are induced, and (3)* Ca^{2+}*–protein bridges are formed, a process resulting in the cross-linking of adjacent anionic groups, such as glutamic and/or aspartic acid residues".*[46]

All of these mechanisms could equally apply to the means by which Ca^{2+} could promote the binding between the very anionic tubulin monomers, creating the structures seen in Figure 10 and Figure 12a.

While Ca^{2+} ions are known to destabilise microtubules, it is regarded as one of the standard ways of stabilising FtsZ polymers[47]. However it is still unclear whether the polymers so obtained are representative of the structures found within the Z-ring. Electron microscopy[48] indicates that the polymers formed in this way have a helical structure, seen more clearly when FtsZ is polymerised in the presence of Mg^{2+} cations[49] (Figure 12b). The similarities between the helical FtsZ and tubulin structures suggests that while Ca^{2+} is seen as a microtubule destabiliser and an FtsZ polymer stabiliser, there may nevertheless be similarities between the way the cation acts on these two homologues.

Charge distribution on the surface of FtsZ and tubulin

In recent years there has been a growing interest in the very distinctive electrostatic properties of the surface of tubulin, and how this might relate to the dynamic nature of tubulin structures[34,35].

Figure 13 is adapted from the work of Tuszynski *et al*., and adopts the convention they used of colouring the surface to show the positive and negative charge distribution on the surface of the

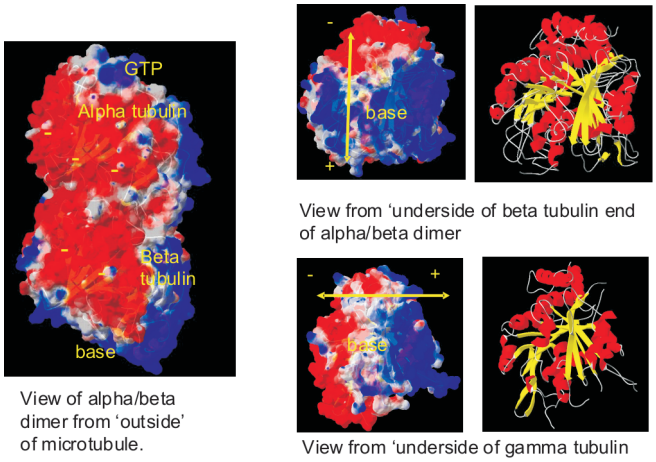

Fig. 13 *Offset electrostatic potentials for tubulin. Left and top showing net dipole of alpha/beta dimer with negative charge on outside of the microtubule. Red* $= -4.9\,K^bT/e$*, Blue* $= -0.9\,K^bT/e$*. Bottom, showing net dipole from side to side of gamma tubulin. (Kb is the Boltzmann's constant, T is temperature, and e the electronic charge.)*

protein relative to the average surface charge. This makes it possible to visualise the relative charge distribution on an extremely anionic protein such as tubulin.

Figure 13 shows the electrostatic potential for a tubulin dimer showing the $+$ve and $-$ve charged regions with respect to surface charge potential of $-2.9\,K^bT/e$. This shows that the surface of the dimer corresponding to the outer surface of the microtubule is very negatively charged. Gamma tubulin is another member of tubulin family and is used in place of alpha/beta tubulin during microtubule nucleation. Figure 13 also shows that the charge distribution for gamma tubulin is very different to that of alpha and beta tubulin, with the net dipole being at right angles to that of the tubulin dimer when viewed along the microtubule axis such that when the gamma tubulin is within a microtubule the dipole would be tangentially aligned to the circular cross section of the microtubule. This is an alternative representation of data that has previously been obtained for the variation in the orientation of dipoles for alpha, beta and gamma tubulin[34].

The difference in the orientation of the dipoles may correspond with the different roles of the different tubulin variants. It has been proposed that gamma tubulin forms a ring when it nucleates the formation of microtubules[52], and Figure 14 indicates how the

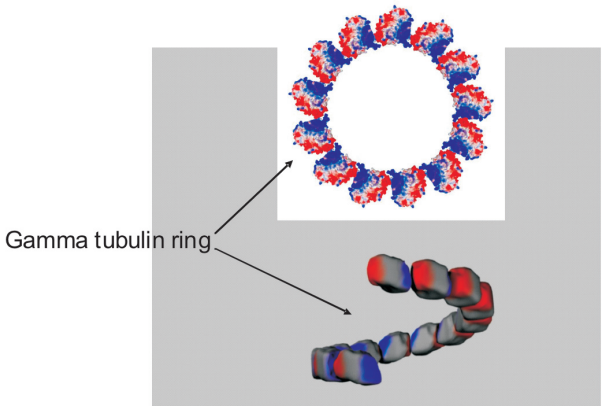

Fig. 14. *Stabilisation of gamma tubulin ring, viewed along the axis of the microtubule (top) and perpendicular to the axis (bottom), by the end to end alignment of the electrostatic dipoles, represented by red* $-ve$ *and blue* $+ve$ *regions, around the circumference of one turn of a helix.*

tangential alignment of the electrostatic dipoles within gamma tubulin would help align the gamma tubulin into such a ring structure.

Figure 15 then indicates how the helical structure formed by gamma tubulin monomers, partly stabilised by the electrostatic interaction between monomers is then able to provide a stable

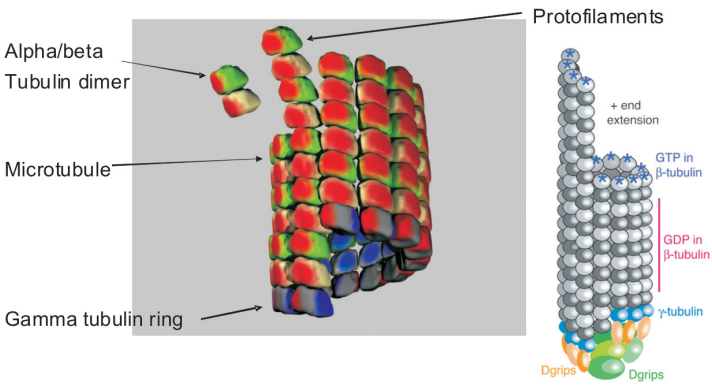

Fig. 15. *Left: Alpha/beta tubulin dimers (light grey and green) building on an initial single helical turn of gamma tubulin (dark grey), showing relative positions of* $-ve$ *(red) and* $+ve$ *(blue) charged faces of tubulin, such that the dipole is radial for alpha and beta tubulin, and tangential for gamma tubulin. Right: proposed structure of* γ *tubulin based microtubule nucleation complex*[52]. Org. Biomol. Chem., 2004, **2**, 2153–2160, doi: 10.1039/b403634d–*Reproduced by permission of the Royal Society of Chemistry.*

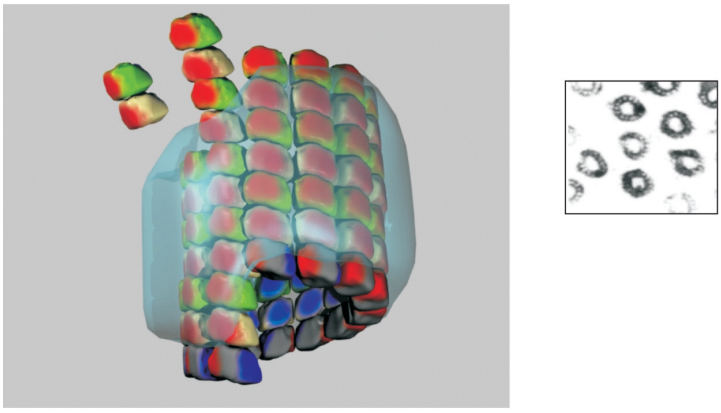

Fig. 16. *Representation of water-gel layer on the surface of the microtubule (left) showing how this could form the basis for the separation of microtubules observed* in vitro *(right). Tubulin assembly in the presence of calcium ions and taxol: microtubule bundling and formation of macrotubule-ring complexes. Vater, W., Böhm, K.J. and Unger, E.* Cell Motil Cytoskeleton. ©1997; **36**(1), 76–83. *Reproduced with permission of John Wiley & Sons, Inc.*

nucleation side for the growth of a microtubules as proposed by Amos *et al*.[52].

The alpha/beta tubulin dimers are not stabilised by electrostatic interaction in the same way as gamma tubulin, in that the dipole is neither aligned along the axis of a protofilament (such that it could help stabilise the protofilament) or tangentially to the circumference of the microtubule (where it could stabilise the alignment of protofilaments alongside each other).

While tubulin in isolation is very negatively charged, it is known that *in vivo* there is only an unscreened charge of approximately 0.2 e per monomer[31,32]. This will be because the highly negative surface of tubulin, particularly the very negative surface is on the outside of the microtubule, will attract any cations, such as K^+ or Ca^{2+} which will neutralise the charge. The accumulating evidence suggests that the attraction of the K^+ to this very negative surface may be a contributory factor to the stability of the hydration layer and clear zone around the microtubule, and consequently to the stability of the microtubule itself. This stability is compromised by the replacement of these ions with Ca^{2+} ions, represented in Figure 16. The different roles of the electrostatic dipoles in alpha/beta, and gamma tubulin would go some way to explaining the observation that these dipoles are at right angles to each other[34].

The unstructured hydrophilic tail of microtubule associated proteins such as Tau, shown in Figure 8, may have a microtubule

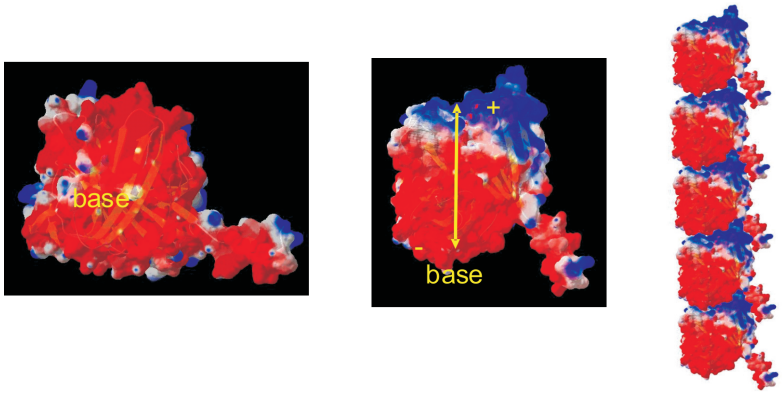

Fig. 17. *Electrostatic potential of the surface of an FtsZ monomer showing the regions that are more* $+$*ve (blue) and* $-$*ve (red) than the typical surface potential of* $-0.5\,K^bT/e$*. There is a net dipole along the axis of the protofilament formed by the end to end binding of these monomers. This suggests that the dipole may have a role in stabilising the protofilament.*

stabilising role as a result of its interaction with the extended hydration layer around the microtubule.

Figure 17 shows the surface charge of FtsZ, and indicates that its charge distribution is quite unlike that of the members of the tubulin family, with the net dipole being along the axis the protofilament formed by the monomers. This is orthogonal to the direction of the dipoles for both alpha/beta and gamma tubulin, and would be a factor that would contribute to the difference in behaviour of FtsZ compared to its eukaryotic cousins.

Such a dipole alignment would tend to stabilise FtsZ filaments, in that the head to tail association of the monomers brings together the $+$ve and $-$ve ends of the dipole. This may help explain why FtsZ has a greater tendency to form protofilaments than tubulin. It may also explain why particular techniques such as storing the monomers in 10% glycerol, need to be adopted to keep the FtsZ in its monomeric form.

The surface of FtsZ is much less negatively charged than tubulin, suggesting that interaction with cytosolic cations may have a less significant role. However, similarities between the extensive unstructured hydrophilic regions of Tau, which stabilised microtubules, and ZipA which stabilises FtsZ, suggests that water may still have a role in the stabilisation of FtsZ structures. This conclusion is supported by the evidence that substituting deuterium oxide (heavy water) for water has a significant effect on FtsZ protofilament stability[53].

Comparison of the amino acid sequences in the unstructured regions of ZipA and Tau

Figure 18 shows a comparison of the unstructured hydrophilic regions of some homologues of the ZipA FtsZ stabilising protein. Also shown is the hydrophilic region of the microtubule stabilising protein Tau and a section of the unstructured region found in the HMW gluten subunit found in wheat flour. This show the extreme variability of the unstructured region within ZipA. This suggests that the conserved aspect of the region is its general character rather than any specific amino sequences.

The mix of proline and glutamine found in some members of the ZipA family is similar to that found in gluten, where it is responsible for the extensive random hydrogen bonding that occurs between gluten chains. On addition of water, gluten-gluten hydrogen bonds are replaced by gluten-water hydrogen bonds, and it is this extensive random water-gluten hydrogen bonding gives dough its sticky, elastic properties[54].

Figure 19 shows how hydrogen bonding between the ZipA linker region and water in the cytosol might provide a flexible, sticky layer that binds FtsZ to the cell membrane. When the FtsZ ring reduces in size during invagination at the later stage of prokaryotic cell division, this sticky layer could help draw the cell membrane in until it can be pinched off and the cell successfully split into two new daughter cells.

Conclusions

Tubulin and FtsZ are homologues that form filamentary structures in eukaryotes and prokaryotes respectively. Despite over 2 billion years of independent evolution since their common ancestor, there are significant similarities in their structures, and also in their use of GTP to bind individual polypeptides together in a head to tail configuration to form protofilaments. There are however significant differences in the filamentary structures that they form, and also the stability of these structures. This may be partly explained by the differences between the surface charge distribution of FtsZ and the different members of the tubulin family. In FtsZ the net dipole is oriented such that it supports protofilament formation, in gamma tubulin it supports the lateral adhesion of tubulin, and for alpha and beta tubulin it is oriented such that the outer surface of the microtubule is very negatively charged. There is some evidence that this negatively charged surface is associated with the formation

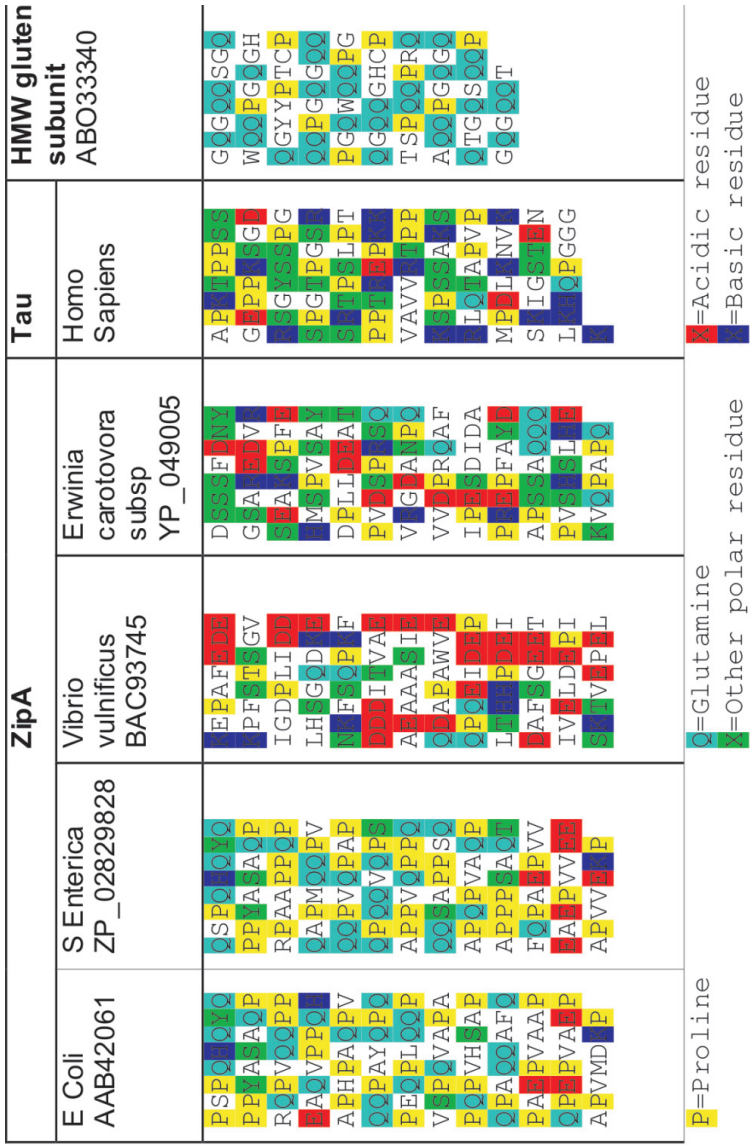

Fig. 18. *Comparison of unstructured hydrophilic regions of various ZipA homologues, Tau and the High Molecular Weight (HMW) of gluten. There is a wide variation in the nature of this region, with the hydrophilic character in some homologues primarily due to extensive amounts of glutamine making the region similar to hydrophilic gluten, while in others, acidic and basic residues are used instead, making them closer in character to the hydrophilic region in Tau.*

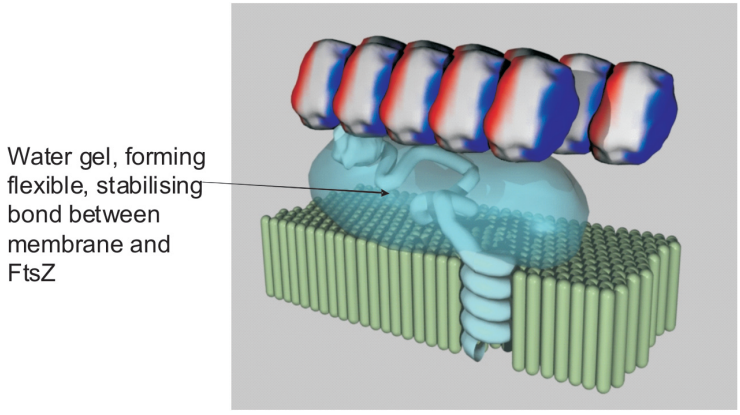

Fig. 19. *Representation of ZipA, providing flexible linker between the prokaryotic cell membrane, and the FtsZ structures, which are held together by electrostatic interactions. The hydrophilic ZipA linker region is extensively hydrogen bonded to water creating a flexible, sticky interface that binds the FtsZ structures to the cell membrane.*

of a stable and extended hydration layer on the surface of microtubules that has a significant role in microtubule stability. The ability of cations such as Ca^{2+} to destabilise microtubules may be explained in part by their interaction with this hydration layer, and the stabilising effect of some microtubule associated proteins may also be related to their interaction with this hydration layer by virtue of their extended hydrophilic regions.

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