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Tubulin and FtsZ form a distinct family of GTPases

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Tubulin and FtsZ share a common fold of two domains connected by a central helix. Structure-based sequence alignment shows that common residues localize on the nucleotide-binding site and a region that interacts with the nucleotide of the next tubulin subunit in the protofilament, suggesting that tubulin and FtsZ use similar contacts to form filaments. Surfaces that would make lateral interactions between protofilaments or interact with motor proteins are, however, different. The highly conserved nucleotide-binding sites of tubulin and FtsZ clearly differ from that of EF-Tu and other GTPases, while resembling the nucleotide site of glyceraldehyde-3-phosphate dehydrogenase. Thus, tubulin and FtsZ form a distinct family of GTP-hydrolyzing proteins.

Tubulin is a 50 kDa protein present in all eukaryotes where it polymerizes into microtubules[1]. The functions of microtubules, which include vesicle movement, chromosome segregation, and cell motility, involve the interaction of tubulin with a variety of microtubule associated proteins (MAPs) and motor proteins[2]. Essential to these functions are the structural polarity and dynamic behavior of microtubules. In the depolymerized form tubulin exists as a tight α,β-tubulin dimer. Each dimer binds two molecules of GTP, one at the non-exchangeable site in α-tubulin (N-site), and one at the exchangeable site in β-tubulin (E-site)[3,4]. GTP bound to β-tubulin is required for microtubule polymerization and is hydrolyzed following addition of a dimer to a microtubule end[5].

It has recently become possible to obtain structural information on tubulin at near-atomic resolution by using electron crystallography to study two-dimensional crystals[6,7]. These crystals are sheet-like tubulin polymers that form in the presence of zinc ions at low pH and are composed of antiparallel protofilaments[8,9]. The zinc-sheets require GTP, are stabilized by taxol, and can be depolymerized and polymerized with temperature in a microtubule-like fashion[7,10]. An atomic model of the tubulin dimer has recently been built into a 3.7-Å resolution map obtained by electron crystallography of zinc-induced polymers[11]. The model includes a molecule of GTP bound to α-tubulin and both GDP and taxol bound to β-tubulin. Because the structure was obtained in a polymerized state, the model contains information on the longitudinal interactions between dimers that results in the structure of the protofilament.

FtsZ is a 40 kDa protein ubiquitous in eubacteria and archaea that shares limited sequence identity with tubulin[12,13]. Mutagenesis as well as genetic experiments have shown that FtsZ (named after filamenting temperature sensitive strain Z) is essential for cell division[14,15]. Fusion constructs of FtsZ with green-fluorescent protein have shown FtsZ to form a filamentous ring at the division site[16]. The ring decreases in size dividing the cell in two halves by a process not yet understood. Purified FtsZ has been shown to bind and hydrolyze GTP[17]. FtsZ can self-assemble into rafts of long filaments that curve at their edges[18,19] and into sheets and rings[20]. The axial repeat of these filaments is around 40 Å, the same as

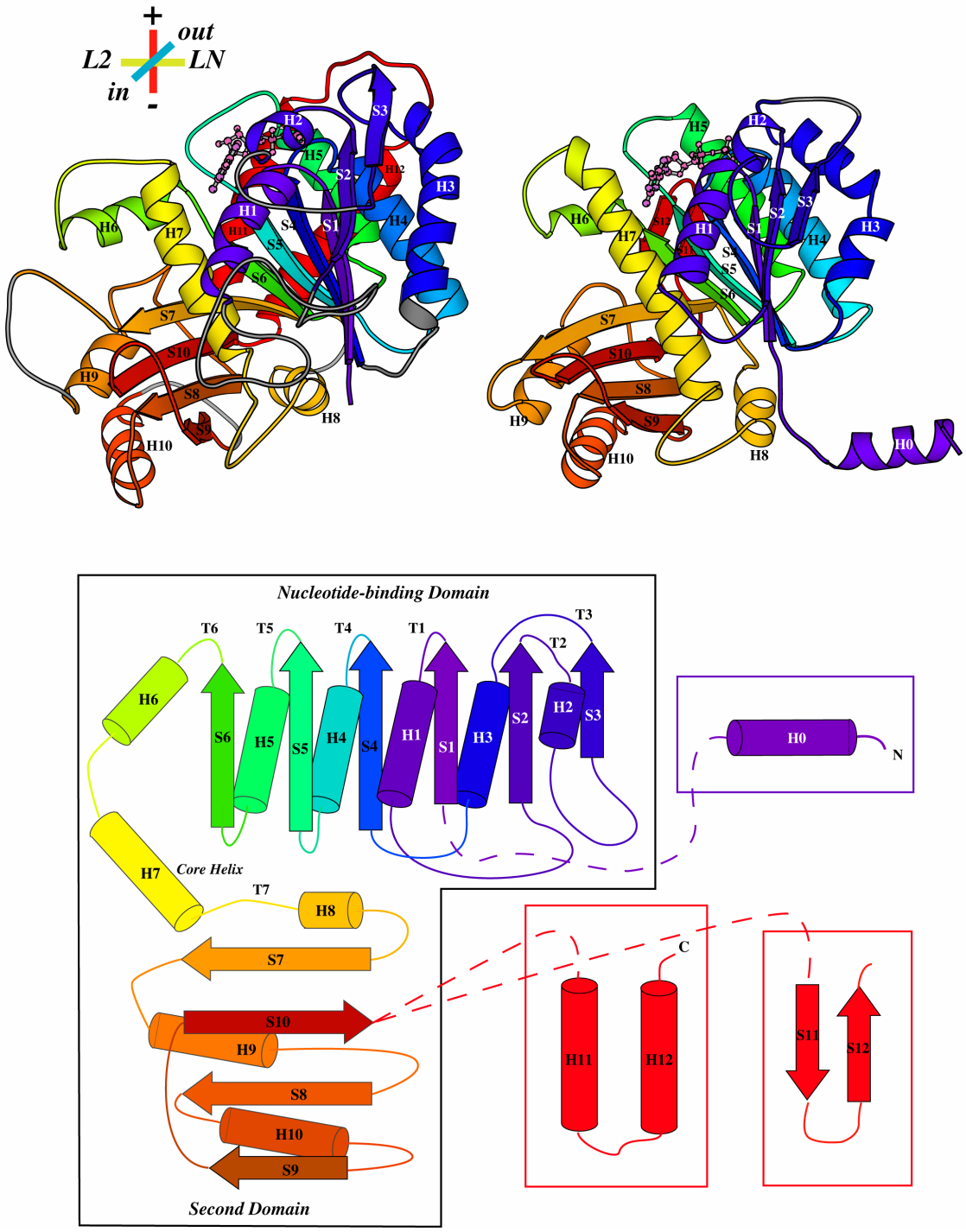

Fig. 1 - Ribbon diagrams for β-tubulin (PDB entry 1TUB) and FtsZ (PDB entry 1FSZ), both complexed with GDP, and their corresponding topology maps. The two structures are oriented with the tubulin-protofilament axis running vertically and the plus end at the top of the page (assignment of plus and minus ends and inside and outside surface from Nogales E, Downing KH, Whittaker M, & Milligan RA, personal communication). The side of the tubulin molecule facing the reader corresponds to a view from the inside of the microtubule (coordinate system: + and - correspond to the plus and minus end of a microtubule, 'in' and 'out' refer to the inside and outside surfaces of the microtubule, and 'LN' and 'L2' correspond to the sides of the tubulin monomer making lateral contacts either by the nucleotide-binding side or by the second domain). Secondary structure elements have been numbered sequentially as H1 to H10 and S1 to S10 for the common helices and strands, respectively. The loops marked as T1 to T7 are those involved in direct contact with the nucleotide and correspond to regions of high sequence homology between tubulin and FtsZ. (prepared with MOLSCRIPT[38]).

that of tubulin monomers in a protofilament. The structure of FtsZ has been solved by X-ray crystallography using crystals obtained from the FtsZ1 protein from the hyperthermophilic methanogen *Methanococcus jannaschii* [21]. The model has been refined to 2.8 Å and includes a molecule of GDP.

Limited sequence homology has also been found between tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[22], a member of the structural family of dinucleotide-binding proteins (Rossmann-fold[23]). In the absence of any structural information for tubulin, researchers have compared it to classical GTPases, a family that includes p21(ras), EF-Tu and α-transducin as their most prominent members, although homology at the sequence level is practically non-existent.

Finally, the second domain of tubulin and FtsZ is structurally related to chorismate mutase (PDB entry 1COM[24]) from *Bacillus subtilis*, although the functional significance of this finding remains enigmatic[21].

Structural homology between tubulin and FtsZ

Because of the high structural similarity between α- and β-tubulin[11], and the fact that the structures of β-tubulin and FtsZ are both bound to GDP, in this study we compare β-tubulin to FtsZ (Fig 1a and 1b, respectively). A common nomenclature has been created to refer to equivalent secondary structure elements (Fig. 1a,b and c). The common core of the two structures includes two domains, each formed by a β-sheet surrounded by helices. The nucleotide-binding domain includes helices H1 to H6 and strands S1 to S6, forming a six-stranded, parallel β-sheet (topology 3p2p1p4p5p6p) surrounded by helices on both sides. Connected by helix H7 (core helix) is a smaller, second domain which includes helices H8 to H10 and strands S7 to S10, forming a four-stranded, mainly parallel β-sheet (topology 1p4a2p3p), with two helices on one side and one on the other. Loops T1 to T7 are directly involved in nucleotide binding.

The C-terminal sections are very different for tubulin and FtsZ, being much shorter for the latter. While the tubulin structure has a pair of long helices that cross over the surface corresponding to the outside of the microtubule (H11 and H12), FtsZ has a small β-hairpin (S11 and S12). In addition, FtsZ has an extra helix, H0, at its N-terminal end sticking out of the otherwise compact structure. Generally, loops connecting strands and helices are longer for tubulin and have the effect of enlarging the width but not the height of tubulin with respect to FtsZ. In particular, there is a long insertion loop in tubulin between H1 and S2 that includes 33 residues, which is poorly defined in the density map of tubulin[11].

The common core of β-tubulin and FtsZ is superimposable with a rms deviation of 4.3 Å over all 277 Cα atoms within the common fold (Fig. 2a). Using only 178 Cα atoms in secondary-structure elements, the rms deviation is 2.4 Å, reflecting the different conformations in loop regions. The superposition of secondary-structure elements is better for the second domain (51 Cα atoms, rms 1.6 Å) compared to the nucleotide-binding domain (127 Cα atoms, rms 2.3 Å). If the two domains are aligned separately, a better fit can be obtained by rotating the second domain by 11°, with the effect of increasing the width of the molecule by opening the cleft between the sheets of the two domains in FtsZ. This difference in conformation between tubulin and FtsZ could be due to the fact that the structure of tubulin corresponds to that of a polymerized state, while that of FtsZ does not. Furthermore, in the case of the β-tubulin

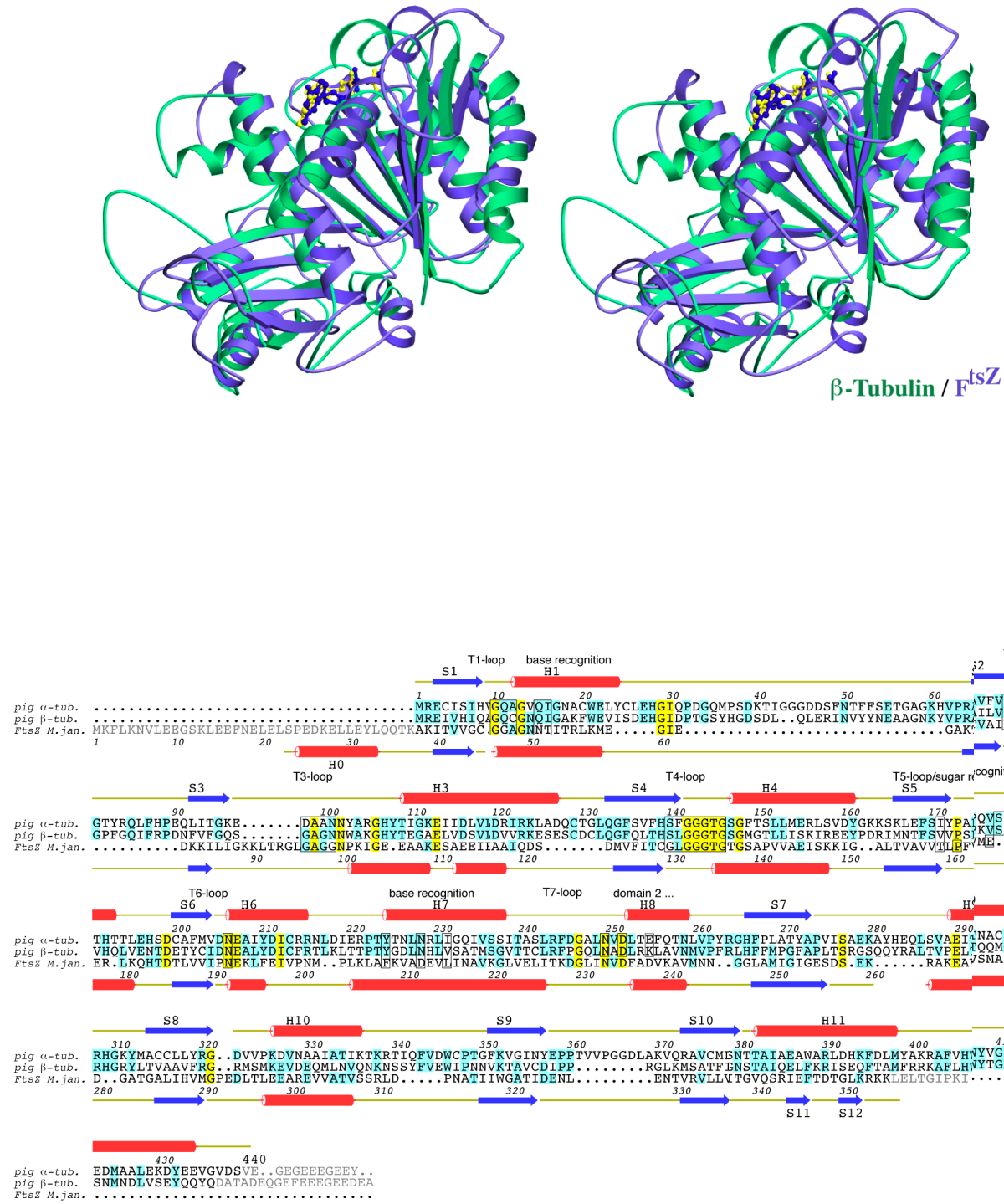

Fig. 2 - (a) Stereo view of the superposition of tubulin and FtsZ including the common core (nucleotide-binding domain, core helix, and second domain; the long insertion loop in tubulin between H1 and S2 has not been included). □-tubulin is shown in green with its nucleotide in yellow, and FtsZ is shown in blue with its nucleotide in dark blue. The orientation is the same as in Fig. 1 (figure prepared with MOLSCRIPT, 38 and RASTER3D, 39). (b) Structure-based sequence alignment of □- and □-tubulin from pig brain 40 and FtsZ1 (Methanococcus jannaschii, 41). Common residues are marked in yellow, those common to only two are marked in blue. Boxes highlight residues involved in nucleotide binding (prepared with ALSCRIPT[42]).

molecule the GDP structure is "constrained" after hydrolysis by the contacts with other subunits in the polymer. It has been proposed that this mechanism stores conformational energy in the microtubule lattice which can then be used in the depolymerization process[25]. Finally, although the model of β-tubulin was obtained with this molecule bound to taxol[11], taxol does not apreciably alter the conformation of polymerized tubulin[7].

Sequence alignment

The structure-based sequence alignment (Fig. 2b) of tubulin and FtsZ differs significantly from earlier ones prepared without structural information[13,18]. Differences include the presence of the extra helix H0 at the N-terminus of FtsZ, and the large insertion loop present in tubulin between H1 and S2. Residues common to α-tubulin, β-tubulin and FtsZ (marked yellow, Fig. 2b) constitute about 7% of the tubulin sequence. The identity among the three sequences considering only the nucleotide binding domain is 10%. Regions of high homology or identity cluster on the loops involved in nucleotide binding: T1 (with two common glycines), T2 (with the highly conservative substitutions Asp to Asn and Glu to Asp), T3 (common Ala and Asn), T6 (common Asn), and most clearly, T4 (tubulin signature motif GGGTGS/TG). Residues common to the three sequences are totally conserved for all known sequences of tubulin and FtsZ (data not shown). There is little sequence homology between tubulin and FtsZ sequences for loop T5, which in both proteins is involved in sugar recognition. This loop in tubulin is also involved in making longitudinal contacts between monomers and dimers along the protofilament.

Finally, there is an additional cluster of common residues in loop T7 connecting the core helix H7 with H8. This loop may seem unrelated to nucleotide binding, but it corresponds to a site in tubulin which is involved in longitudinal interactions between subunits[11]. This interaction brings the common Asn and Asp in T7 and other residues in H8 close to the phosphates of the nucleotide in the active site of an down-stream subunit. Conservation of this region in FtsZ strongly indicates that the same interaction occurs in FtsZ filaments. Together with the common axial repeat of 40Å for monomers in the protofilaments of tubulin and FtsZ [20], the conservation of loop T7 leads to the assumption that FtsZ forms protofilaments very similar to tubulin. Indeed, when two FtsZ molecules are aligned with tubulin, and one is displaced by 40 Å in the direction of the tubulin protofilament, the Asn and Asp in loop T7 in one FtsZ monomer come in close contact with the nucleotide on the next FtsZ monomer (data not shown). Such displacement, however, results in several clashes that may indicate a conformational change in the FtsZ molecule upon polymerization.

Although there is no detectable sequence identity or homology between tubulin and FtsZ after loop T7, the second domains of these proteins are as superimposable as their nucleotide-binding domains (Fig 2a.).

Electrostatic potential and hydrophobicity

Interactions of charged surfaces have been proposed to play an important role in the interaction between microtubules and motor protein[s26]. No large patches of negatively charged residues can be found on the surfaces of the present models of tubulin (Fig. 3a, b) or FtsZ (Fig. 3d, e). However, the highly acidic C-

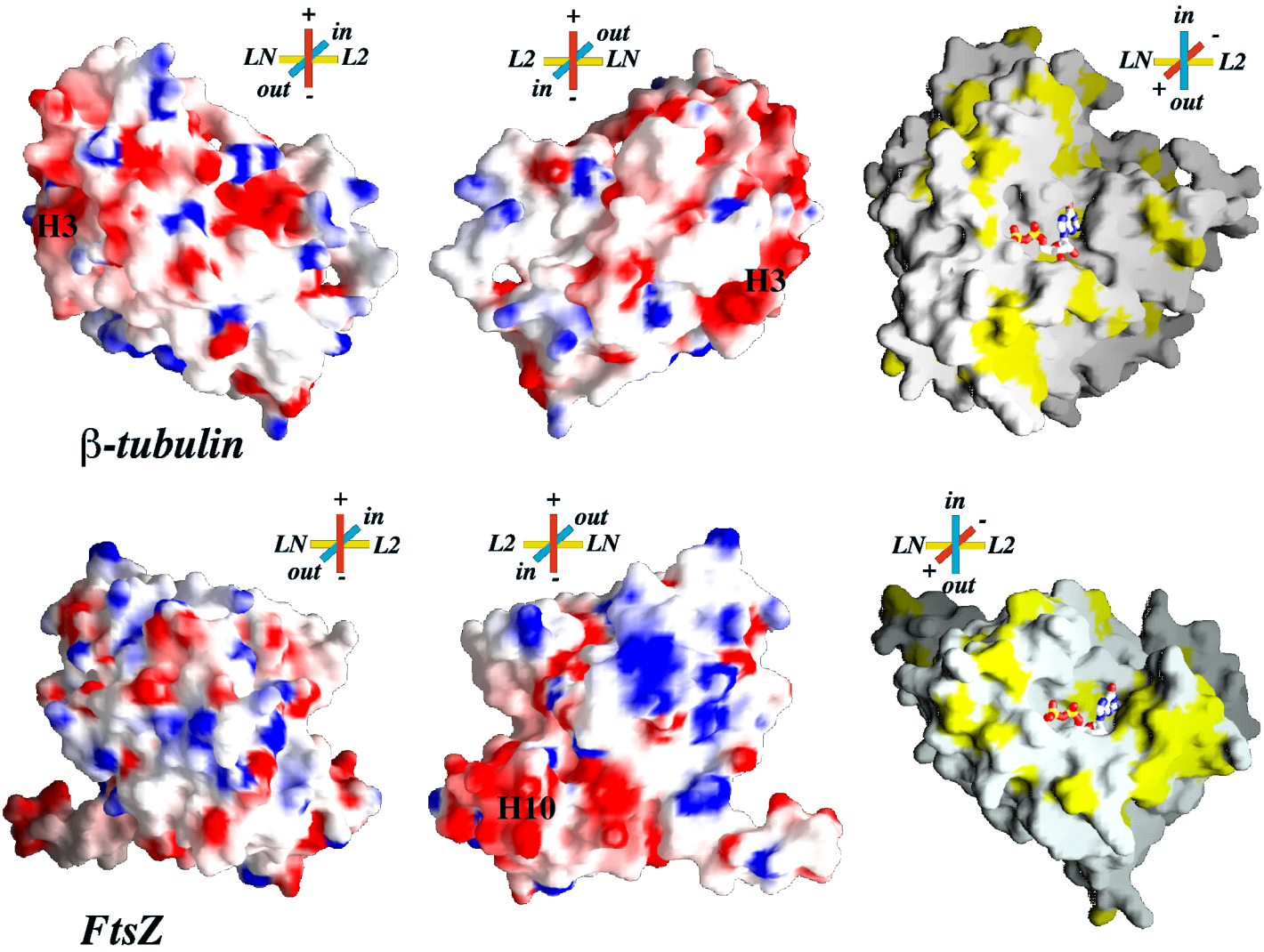

Fig. 3 - Surface maps for β-tubulin and FtsZ showing their electrostatic potential and hydrophobic regions. (a, b) Electrostatic potential in the outside and inside surfaces of b-tubulin, respectively; (d, e) corresponding surfaces for FtsZ (the orientation in (b) and (e) is the same as in Fig. 1 and 2a), coloured from -5kT/e, red to 5kT/e, blue. (c) Surface of b-tubulin containing the nucleotide binding pocket and involved in longitudinal interactions between tubulin dimers, and (f) the corresponding surface in FtsZ. Hydrophobic areas are displayed in yellow. (prepared with GRASP[43])

terminal tails of both tubulin (α-tub: 441-453, β-tub: 437:455) and FtsZ (residues 357-372) are disordered in the crystal structures. It is thus reasonable to expect that the charge distribution on the outside surfaces of both molecules would be altered upon inclusion of the C-terminal residues. The largest charged region in β-tubulin is an acidic region (Fig. 3a, b.) corresponding to helix H3, which is mainly involved in lateral contacts in the microtubule (Nogales E, Downing K.H., Whittaker M, & Milligan R.A, manuscript in preparation). In FtsZ a highly acidic section localizes on helix H10 in the second domain (Fig. 3e).

In both tubulin and FtsZ the nucleotide is surrounded by a polar environment (Fig 3c, 3f), except for those areas contacting the guanine base. There is a remarkable shape complementarity at the longitudinal interface between tubulin subunits (top and bottom surfaces in Fig. 3a, 3b) resulting in a surface burial of 1470 $Å^2$ per tubulin monomer, almost 10% of the total surface (areas calculated using the programs mspdb, ms, mssep, msav [27], and atmsrf, sumarea [28]). The same complementarity of the corresponding surfaces exists in FtsZ (top and bottom surfaces in Fig. 3d, 3e). FtsZ protofilaments do not appear to associate laterally in the same way as tubulin protofilaments associate in microtubules, nor do the sheets of FtsZ have the standard microtubule lattice[20]. This is not surprising as the differences between tubulin and FtsZ are large in the lateral regions of the molecules (Fig 1, 2a, 3).

Nucleotide-binding domain

The nucleotide binding domains of β-tubulin and FtsZ have been structurally aligned to GAPDH with bound NAD (PDB entry 1GYP, *Leishmania mexicana*)29 and to EF-Tu with bound GDP (PDB entry 1TUI, *Thermus aquaticus*)[30] (Fig. 4). GAPDH is both a representative of the family of dinucleotide-binding proteins with a classical Rossmann fold[23], and a proposed tubulin homologue based on limited sequence homology and functional similarities[22]. GAPDH has two domains, the nucleotide binding domain having the same topology as that in tubulin (Fig. 4a and 4b, respectively). Furthermore, the C-terminal helix of GAPDH (HC) aligns with the core helix (H7) in tubulin, so that the second domain of GAPDH could be viewed as being inserted between the helices equivalent to H6 and H7. EF-Tu is one of the members of the classical GTPase family. The fold of the nucleotide-binding domain of this protein is related to tubulin with different sheet-topology (2a3p1p4p5p6p) (Fig. 4c). All three proteins contain a similar nucleotide-binding domain and an unrelated second domain at the C-terminus (except for the C-terminal helix of GAPDH that is equivalent to helix H7). However orientation, fold, and function of the second domain are unrelated among the three proteins, and for EF-Tu this domain even sits on the opposite side of the nucleotide.

homology at the sequence level is practically non-existent.There is high similarity in the conformation of loops T1 to T7 of tubulin and FtsZ, with loop T5 being an exception. The structure and function of these loops have been compared with corresponding elements in GAPDH and EF-Tu (Fig. 5 and 6: (a) GAPDH, (b) FtsZ, (c) β-tubulin, and (d) EF-Tu):

Loop T1 in tubulin and FtsZ interacts both with the phosphates (β-tub: G10Q11; FtsZ:G47, G48, A49) and with the guanine base (β-tub: C12, Q15, I16; FtsZ: N51, T52). The equivalent loop in GAPDH, L1, is involved in binding the diphosphate (GAPDH: G11, R12). In EF-Tu the P-loop (G1) surrounds the

phosphates in a boat conformation (EF-Tu: H19 to T26, HVDHGKTT).

Loop T2 in tubulin and FtsZ makes interactions with the β– and γ–phosphates of the nucleotide (β-tub: D69, L70, E71; FtsZ: N70, T71, D72, Q75). The conserved Asp and Glu residues in α- and β-tubulin are homologous to the conserved Asn and Asp residues in FtsZ sequences. The equivalent loop in GAPDH, L2, makes contacts with the adenosine group of the dinucleotide (GAPDH: V37, D38, M39). G2 in EF-Tu, also known as Switch I, interacts with the β- and γ- phosphates (EF-Tu: Y47, D51) (in the GDP-bound structure shown in Fig. 5, this loop has moved away from the nucleotide). Notice that G2 runs in the opposite direction compared to tubulin/FtsZ/GAPDH due to the altered sheet topology.

Loop T3 in tubulin and FtsZ could make further interactions with the γ-phosphate (it does so in α-tubulin: GTP, not shown) and is a region of high sequence similarity (β-tub: G98, A99, G100, N101; FtsZ: G96, A97, G98, G99). L3 in GAPDH makes further contacts with the adenine base of the dinucleotide (GAPDH: A90, N91). In EF-Tu G3, also known as Switch II, contacts the magnesium ion and the γ-phosphate (EF-Tu: D81, C82, P83, G84). G3 could be seen as being in place of T2/L2 in tubulin/FtsZ/GAPDH due to the altered sheet topology in EF-Tu.

Loop T4, which is the glycine-rich tubulin signature motif, is totally conserved among all tubulins and FtsZs (GGGTGS/TG). In both proteins it is involved in main chain interactions with the α- and β-phosphates. Similarly, L4 in GAPDH covers one side of the diphosphates. Therefore the phosphates in tubulin/FtsZ and GAPDH are sandwiched between two loops, T1 and T4 for tubulin and FtsZ and L1 and L4 for GAPDH, which together play the role of the single P-loop (G1) in GTPases. The loop in EF-Tu topologically equivalent to T4 makes no contact with the nucleotide.

Loop T5 contains the most important residues involved in ribose binding in tubulin and FtsZ (β-tub:V171, S178, D179; FtsZ: T159, P161, F162, E165, R169). Except for a common proline, there is no homology in this loop between tubulin and FtsZ. The conformation of the loop is different for the two proteins, with one extra residue for tubulin. T5 in tubulin interacts also with helix H11 and with the following tubulin monomer up-stream. This may partially explain the differences with FtsZ, which lacks the C-terminal helices and is in an unpolymerized state. Interestingly, Arg169 in FtsZ sits in a position roughly comparable to that of Arg789 in the GAP:p21(ras) complex[31], and that of Arg178 in $G_{i\alpha 1}$**.** L5 in GAPDH interacts with the nicotinamide (GAPDH: S134, A135, A136). In EF-Tu the ribose points with its two hydroxyl groups in the opposite direction with respect to that in tubulin and FtsZ and is mostly exposed to solvent. The equivalent to T5 in EF-Tu is loop G4, which is involved in base binding and functionally related to H7/HC in tubulin/FtsZ/GAPDH.

Loop T6 contains a totally conserved Asn in tubulin and FtsZ with its side-chain hydrogen-bonding to the guanine base (β-tub: N206; FtsZ: N192). Loop L6 in GAPDH is hydrogen-bonding with the nicotinamide base. The topologically corresponding element in EF-Tu, G5, contains Asn136 which is in a similar position to the Asn206/192 in tubulin/FtsZ.

Core helix: In tubulin and FtsZ the contact with the base is completed by helix H7. Hydrophobic contact

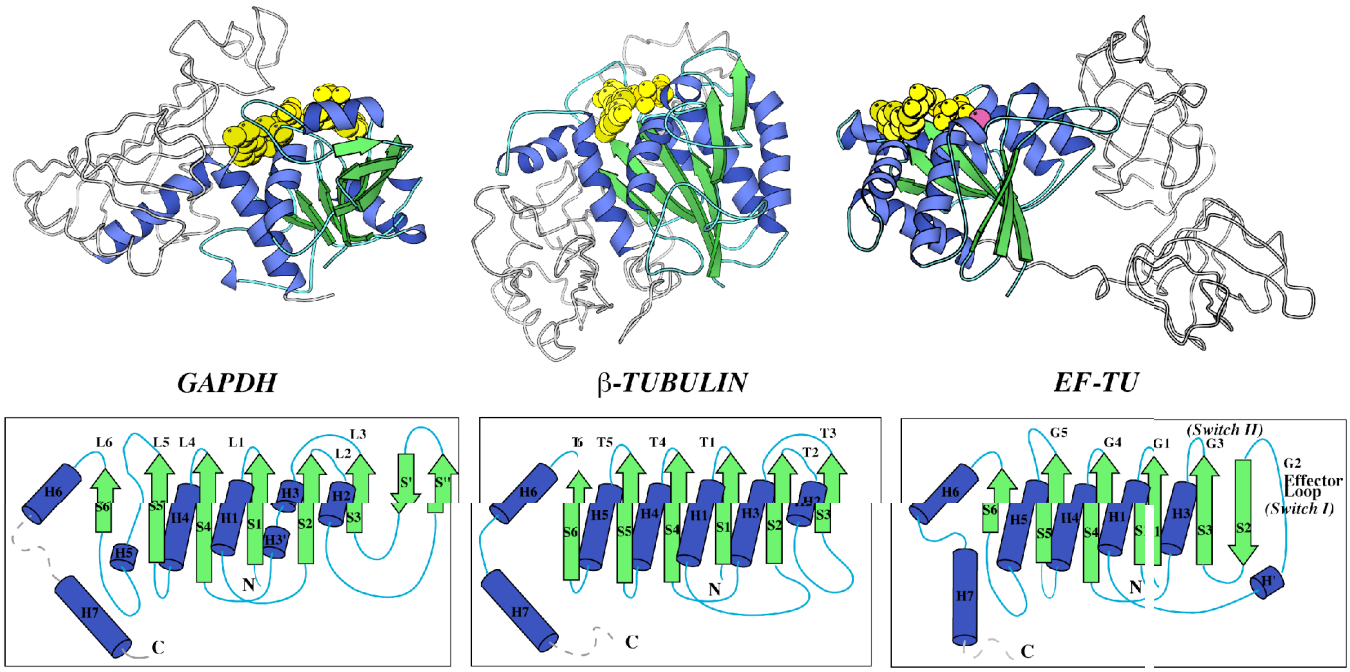

Fig. 4 - Structure and topological maps of the nucleotide-binding domains in GAPDH:NAD (a, PDB entry 1GYP), -tubulin:GDP (b, PDB entry 1TUB), and EF-Tu:GDP (c, PDB entry 1TUI). The orientation of tubulin is the same as in Fig.1 and 2a. GAPDH and EF-Tu have been manually aligned to tubulin, emphasizing the fit of the N-terminal strand and helix. Strands, helices, and nucleotide-binding loops have been numbered sequentially in the topological maps. (prepared with MOLSCRIPT[38]).

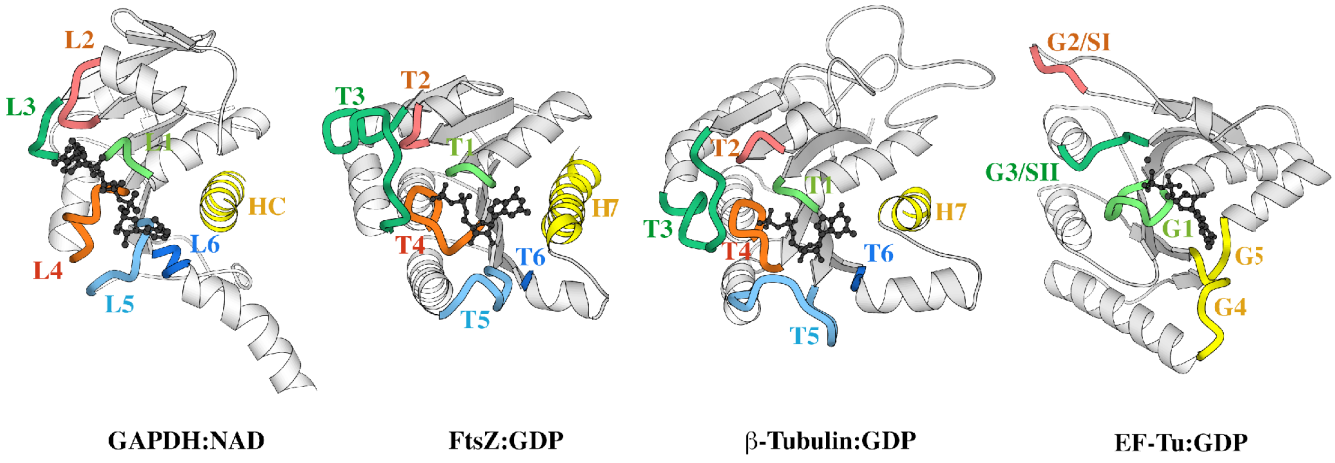

Fig. 5 - Nucleotide-binding domains of GAPDH:GDP (a, PDB entry 1GYP), FtsZ:GDP (b, PDB entry 1FSZ), b-tubulin:GDP (c, PDB entry 1TUB) and EF-Tu:GDP (d, PDB entry 1TUI). Only structural elements directly involved in nucleotide binding are drawn. The orientation for tubulin and FtsZ is the same as in fig. 3c and 3f, with the reader looking down the tubulin protofilament from the plus end (prepared with MOLSCRIPT[38]).

with the guanine is made by a conserved aromatic residue (β-tub:Y224; FtsZ:F208), while the side chain of Asn228 in tubulin and Asp212 in FtsZ further hydrogen-bond to N7 of the base. In GAPDH the side chain of Tyr339 in HC stacks against the nicotinic ring while other residues in the helix hydrogen-bond with it. In EF-Tu G4 makes hydrophobic contacts with the guanine base through the aliphatic side chain of Lys137, while the side chains of Asn136 and Asp139 hydrogen-bond with it. Further contact with the guanine base is made in EF-Tu by Leu176, Ser174 and Ala175 in G5.

Loop T7 in tubulin is involved in further nucleotide-contacts (Fig 7). It contains the totally conserved common residues GxxNxD, and the N-terminal region of H8. Within this helix in FtsZ is residue D238 (*E. coli*: D212), which mutated to alanine in the *E. coli* protein completely abolishes GTP hydrolysis without significantly affecting nucleotide binding[32]. The equivalent residues in tubulin are β:K254 and α:E254. These residues come in close proximity of the γ-phosphate position in the next tubulin subunit up-stream (Fig 7). Given the high sequence homology and structural similarity in this region, we propose a similar mechanism upon polymerization of FtsZ into protofilaments. While β:K254 is by the N-site, buried within the dimer, α:E254 is by the E-site of the adjacent tubulin dimer. It is tempting to propose that FtsZ:D238 and its structurally corresponding α:E254 are residues essential for hydrolysis, while the glutamate to lysine substitution in β-tubulin makes the nucleotide in α-tubulin non-hydrolyzable. FtsZ:D238 is 100% conserved in all known FtsZ sequences as are α:E254 and β:K254 in all known sequences of α- and β-tubulin, respectively (data not shown). In tubulin the hydrolysis rate of the unpolymerized dimer is very low while it is dramatically increased upon polymerization (equal or less than 0.054 min^{-1} in the dimer[33], 12 to 21 min^{-1} in microtubules[34]). The existence of a region in tubulin that interacts with the nucleotide of the adjacent monomer strongly suggests that this region acts as an activator of hydrolysis in the tubulin-tubulin complex. This idea has recently been put forward in a review of the tubulin and FtsZ structures where the cluster GxxNxD was proposed to be act as a "synergistic" loop in the polymeri-zation/hydrolysis processes[35]. Although tubulin can be regarded as being its own GAP (GTPase Activating Protein), no clear similarity in structure or functional residues can be found between tubulin and the known rasGAP and rhoGAP[36,37]. The interactions of p21(ras) and rho with their respective GAPs involve mainly the Switch I and Switch II regions, which are structurally and topologically different in tubulin (see Fig. 4b and c). The GAP molecules use a "finger loop" and two distant helices to bind to ras and rho. In tubulin the equivalent regions are T7 and the contiguous helix H8. Finally, the essential residue in GAP function is an arginine (rasGAP:R789) supplied to the nucleotide site. FtsZ:R169 comes close to the phosphates of the nucleotide as does rasGAP:R789 but is in a different conformation and unlikely to reach the γ-phosphate. In tubulin. there is no equivalent residue to FtsZ:R169, making a critical role of this residue in nucleotide hydrolysis unlikely.

Like tubulin, actin is a nucleotide-binding protein that hydrolizes its nucleotide following self-assembly into filaments. The general structure of tubulin and actin are however very different, and the ATP-nucleotide in actin binds within a cleft in the actin monomer. There is no clear similarity in the mode of nucleotide binding between these two cytoskeletal proteins, which share no sequence similarity with each other.

In summary, the mode of nucleotide binding of EF-Tu is clearly different from that in tubulin and FtsZ.

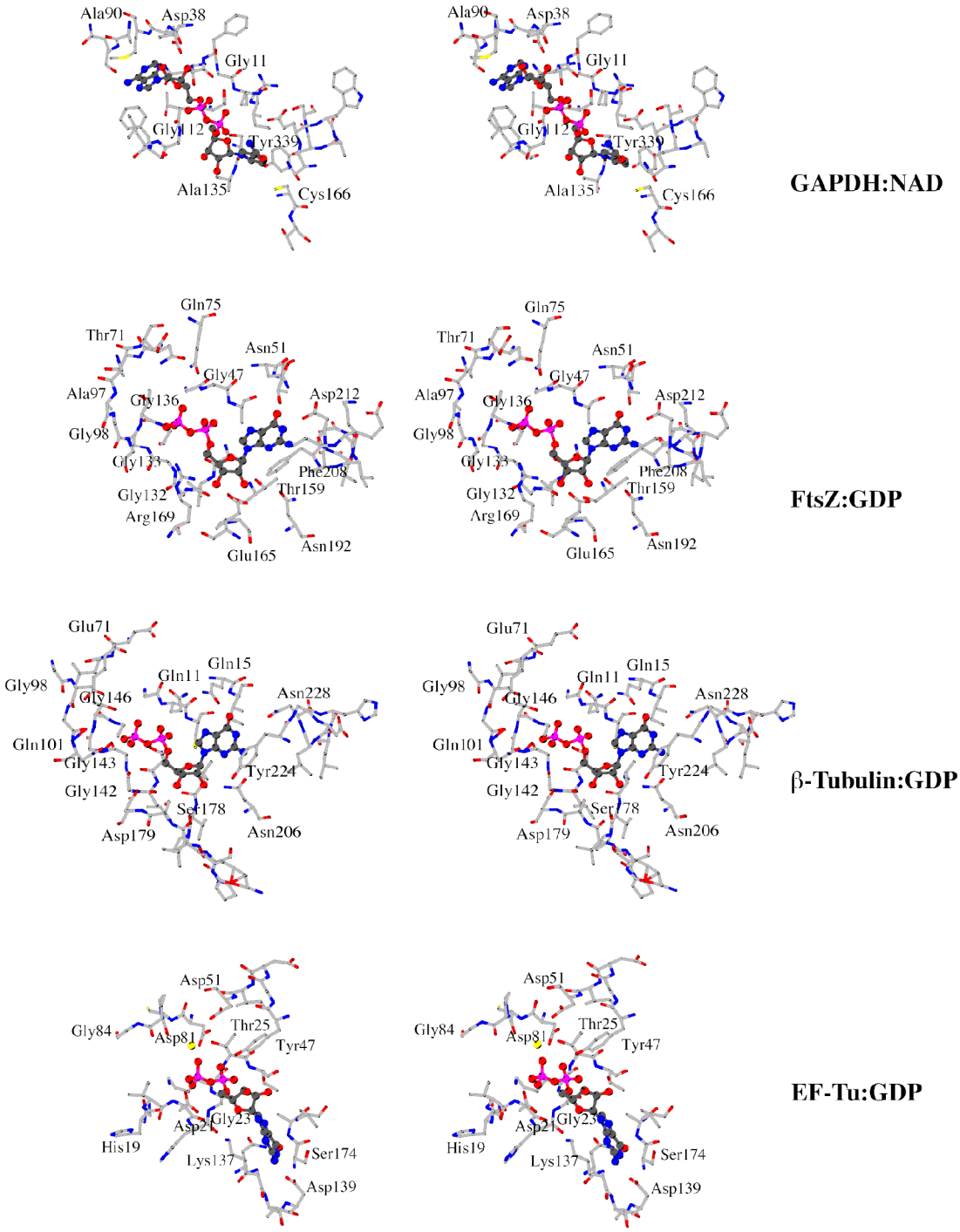

Fig.6 - Stereo views of the nucleotide-binding residues of (a) GAPDH:GDP, (b) FtsZ:GDP, (c) β-tubulin:GDP (unrefined electron crystallographic model) and (d) EF-Tu:GDP. The orientation of the molecules is the same as in Fig. 5 (prepared with MOLSCRIPT[38]).

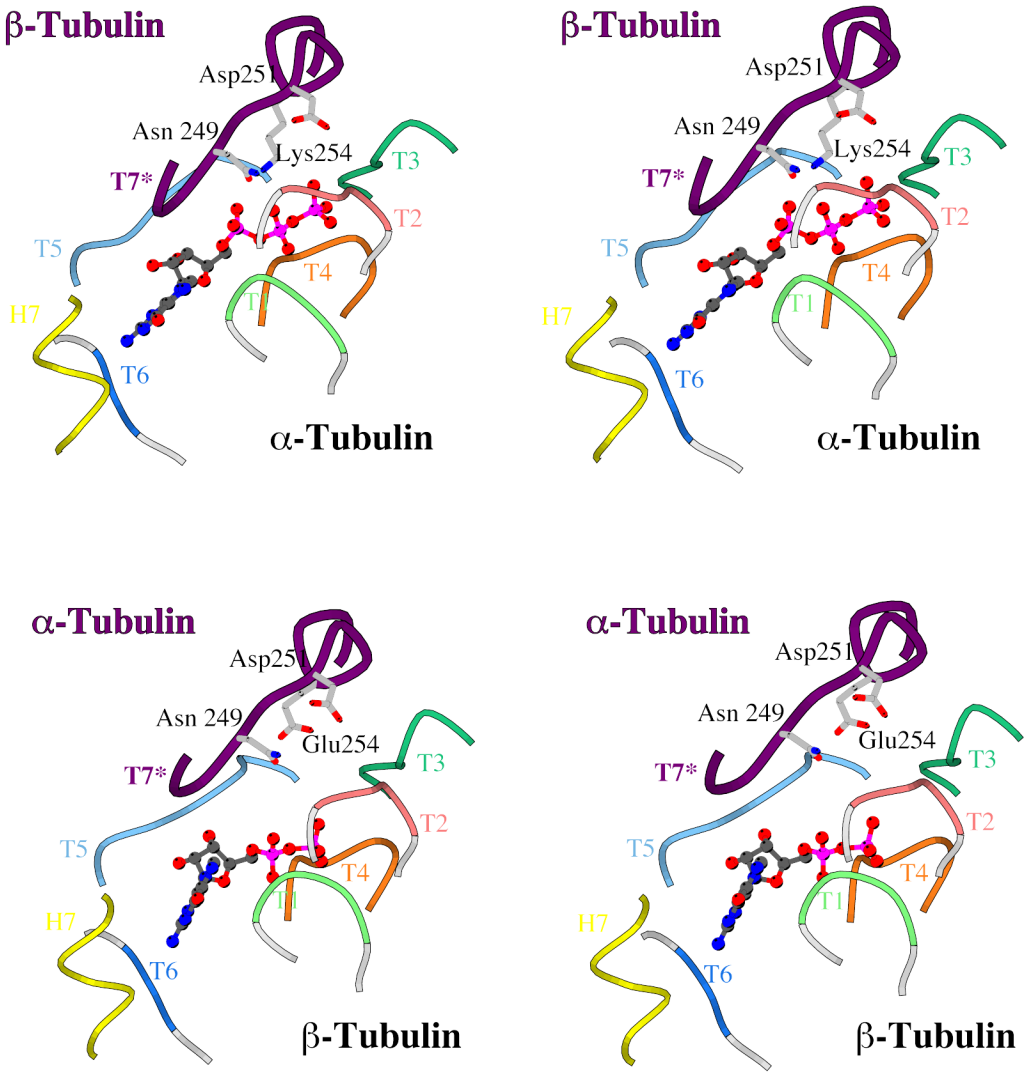

Fig7 - Stereo ribbon representation of the interaction of loop T7 at the interfaces of □- and □-tubulin. (a) Interface between dimers, (b) interface between monomers in the dimer. Residues in T7 more directly implicated in nucleotide contacts are shown. Colours for loops T1 to T6 and helix H7 are the same as those used in Fig. 5 (prepared with MOLSCRIPT[38])

The P-loop (G1) of the GTPases is replaced by two separate loops in tubulin and FtsZ: T1, a short loop topologically equivalent to the P-loop, and T4, the glycine-rich loop that constitutes the tubulin signature motif. Furthermore, the equivalents in tubulin and FtsZ of the switch I and switch II regions in EF-Tu interact with the nucleotide in a very different manner due to the partially reversed topology. Thus, the structural and functional information that exists on the effect of hydrolysis in several classical GTPases cannot be generalized to tubulin and FtsZ. There is a total of seven segments in the tubulin and FtsZ sequences involved in the binding of the nucleotide (compared to 5 in GTPases), so that the nucleotide sits at the center of a large surface, which in tubulin is involved in contacts between monomers. Position of the nucleotide at the center of the polymerization interface ensures that no nucleotide exchange would be possible in the core of the microtubule. This property is essential for the regulation of microtubule depolymerization via dynamic instability which relies on the instability of the GDP core of the microtubule. Tubulin acts as its own hydrolysis-activating protein by a mechanism that does not resemble that of classical GAPs.

Nucleotide binding in GAPDH involves exactly the same loops in the same order as in tubulin and FtsZ, leading to the conclusion that tubulin and FtsZ are more closely related to GAPDH than to EF-Tu and the classical GTPases. This is in spite of the fact that tubulin, FtsZ and EF-Tu all bind and hydrolyze GTP, while GAPDH binds a totally different nucleotide that is not hydrolyzed. The existence of an extra nucleotide-binding region that comes into place upon polymerization and is most likely directly involved in activation of GTP hydrolysis, further reinforces the concept that tubulin and FtsZ form a distinct family of GTP-hydrolysis proteins.

Acknowledgements

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