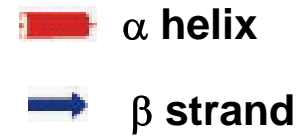
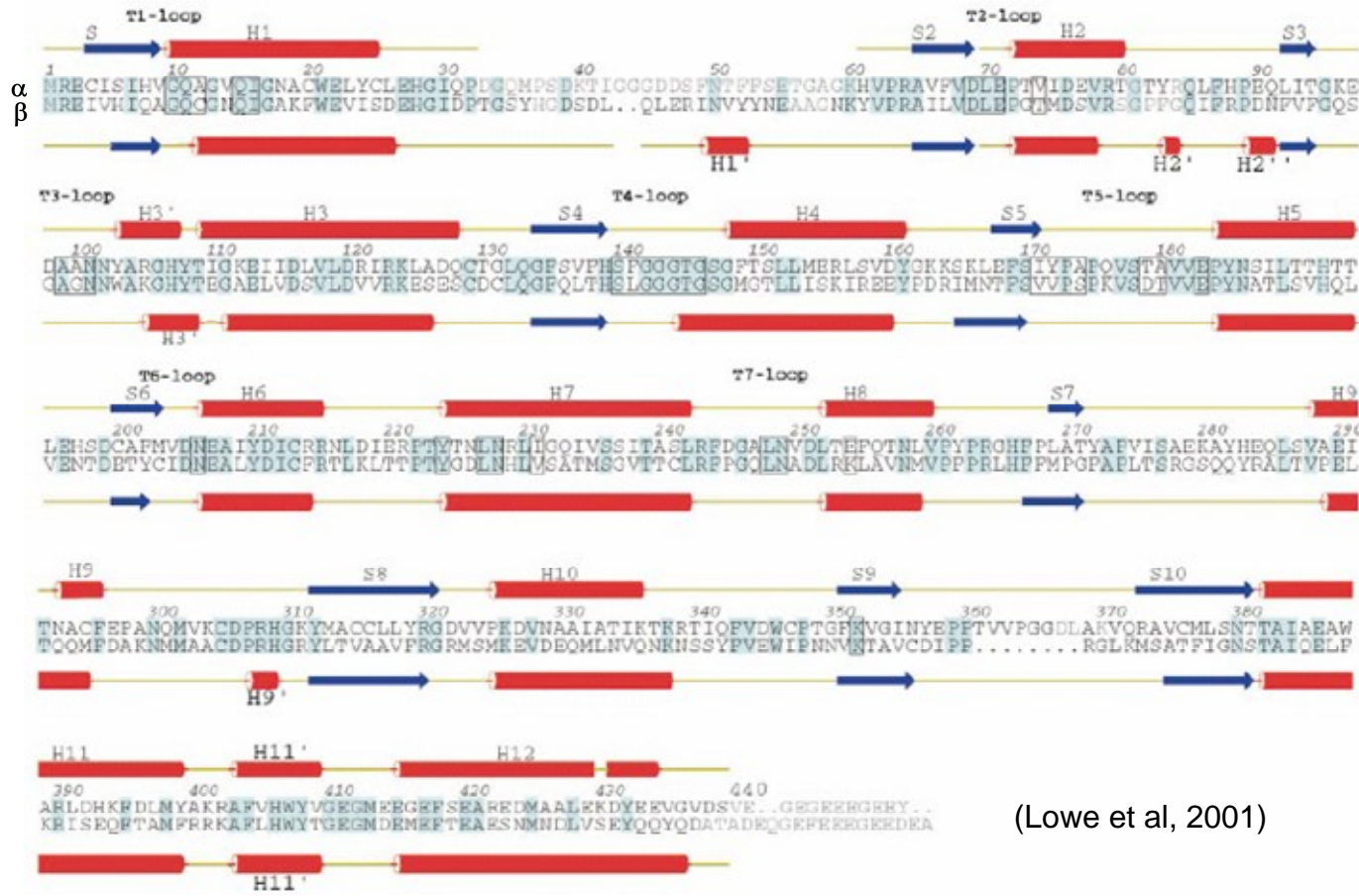
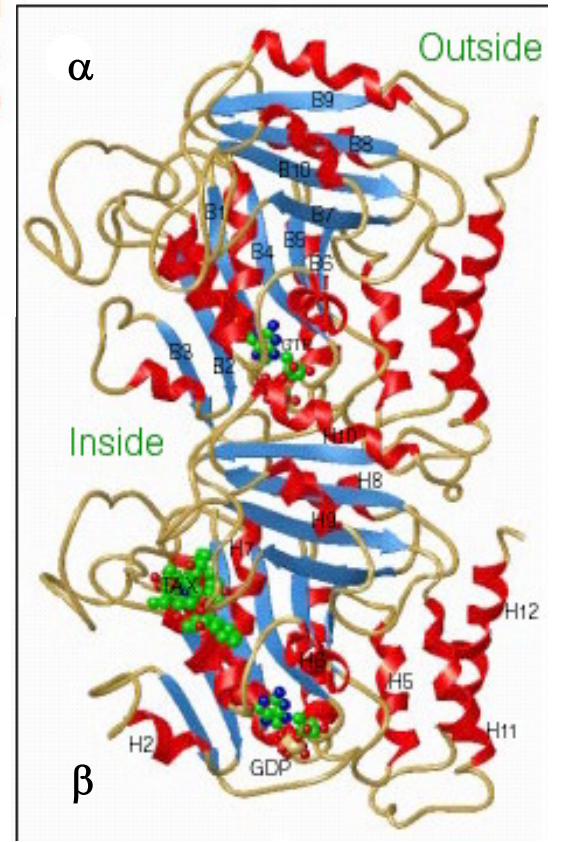


POLYGLYCYLATION AND POLYGLUTAMYLATION OF *TETRAHYMENA* TUBULIN C TERMINI

Structural complexity,
intersubunit interactions,
involvement in ciliary matrix
and axoneme integrity



(Nogales et al, 1998)



α AND β-TUBULIN (pig brain) SEQUENCES : 40% identity

α AND β-TUBULIN 3D STRUCTURES : similarity of functional domains

- **N-terminal** : binding of nucleotide GTP or GDP
 alternance of 6 β sheets (S1-S6) with 6 α helices (H1-H6)
- **Intermediary** : β binds taxol
 3 helices (H8-H10) and β sheet (S7-S10)
- **C-terminal** : on the MT outside
 2 helices (H11-H12), but the 15 last residues are not visible

TUBULIN POST-TRANSLATIONAL MODIFICATIONS

QuickTime et un décompresseur TIFF (LZW) sont requis pour visionner cette image.

QuickTime et un décompresseur TIFF (LZW) sont requis pour visionner cette image.

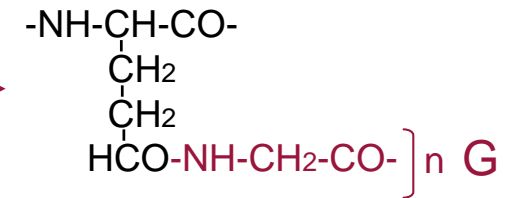
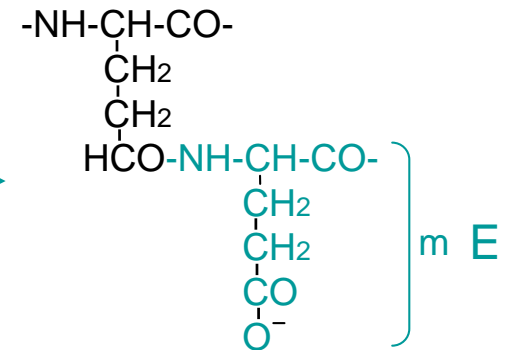
QuickTime et un décompresseur TIFF (LZW) sont requis pour visionner cette image.

α

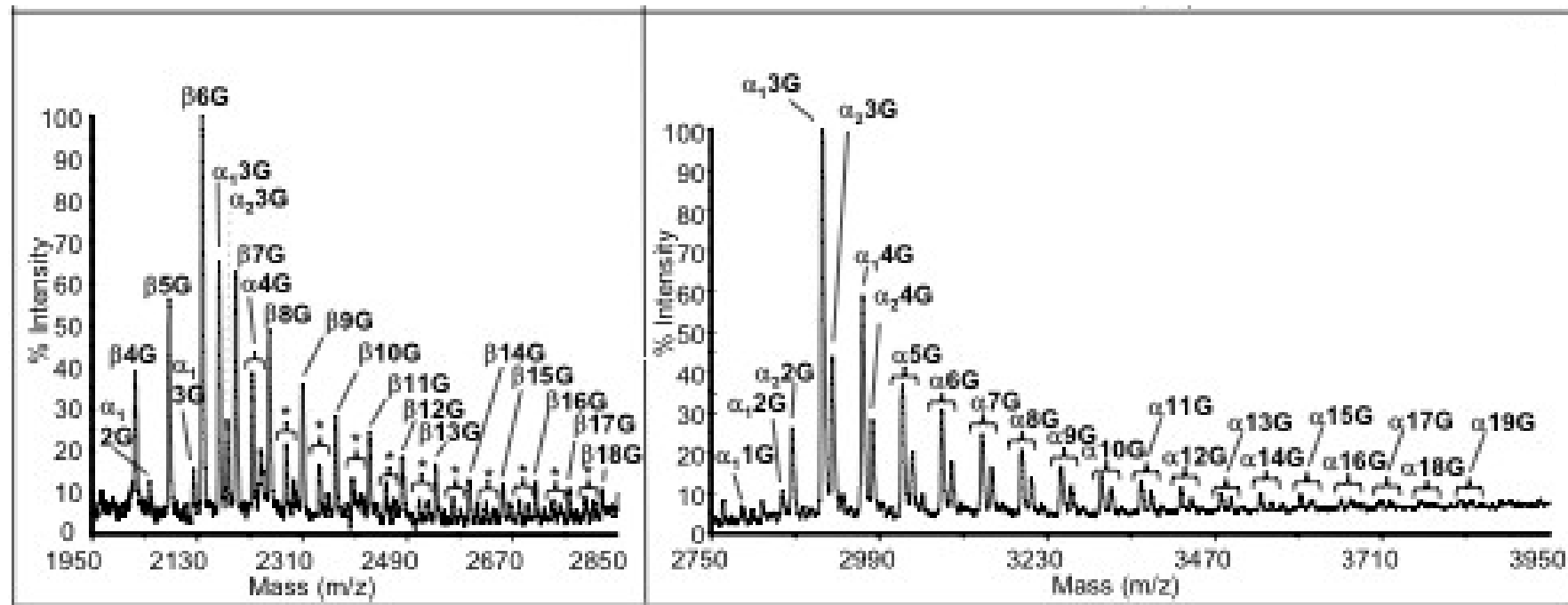
POLYMODIFICATIONS



α, β



MASS SPECTROMETRY ANALYSIS OF *PARAMECIUM* AXONEMAL α AND β -TUBULIN C-TERMINAL PEPTIDES



β -tubulin
4-32 Gly

α -tubulin
1-34 Gly

STRUCTURE OF *PARAMECIUM* AXONEMAL β -TUBULIN C-TERMINAL TAIL

- Immunochemistry with mAbs to tubulin mono and polyglycylated sites
- Fragmentation by mass spectrometry

-
- **4 glycylation sites** on C-terminal glutamates
 - **Polyglycine chains** of **variable lengths**



(adapted from Vinh et al, 1999)

-
- **Huge heterogeneity of the tubulin C terminus**
 - **Negatively charged bulky C-terminal domain**
Bearing **hydrophobic** chains (**G**) surrounded by **acidic** residues

C- terminal sequences of axonemal α - and β - tubulins
From Paramecium, Tetrahymena WT and mutants

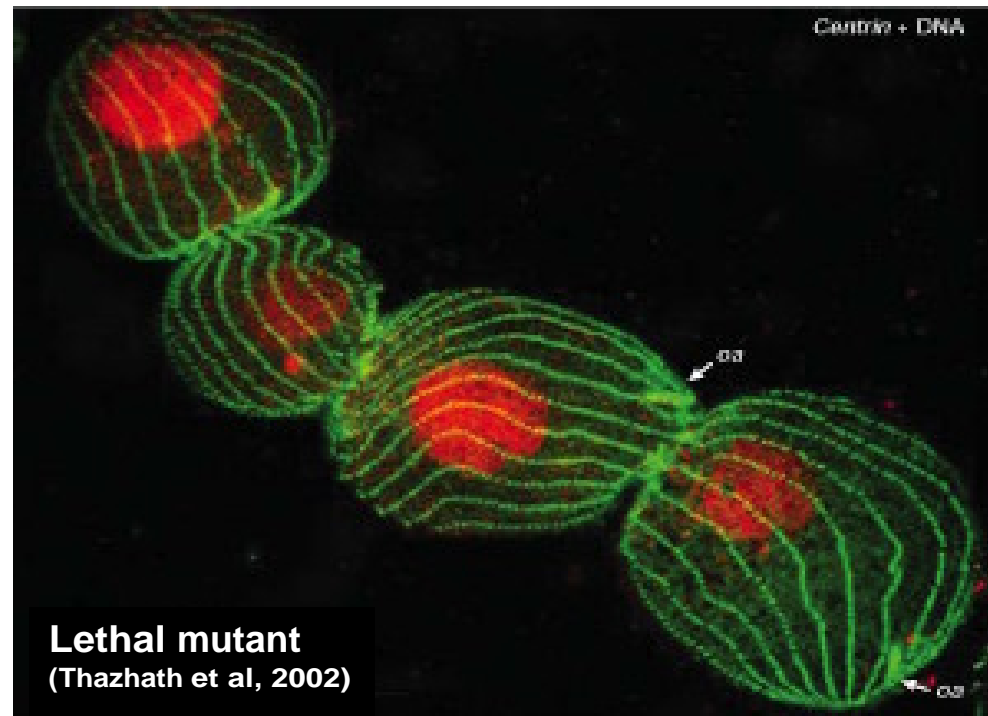
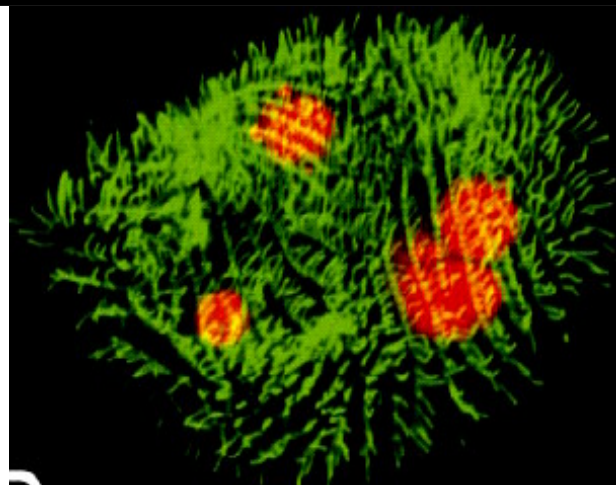
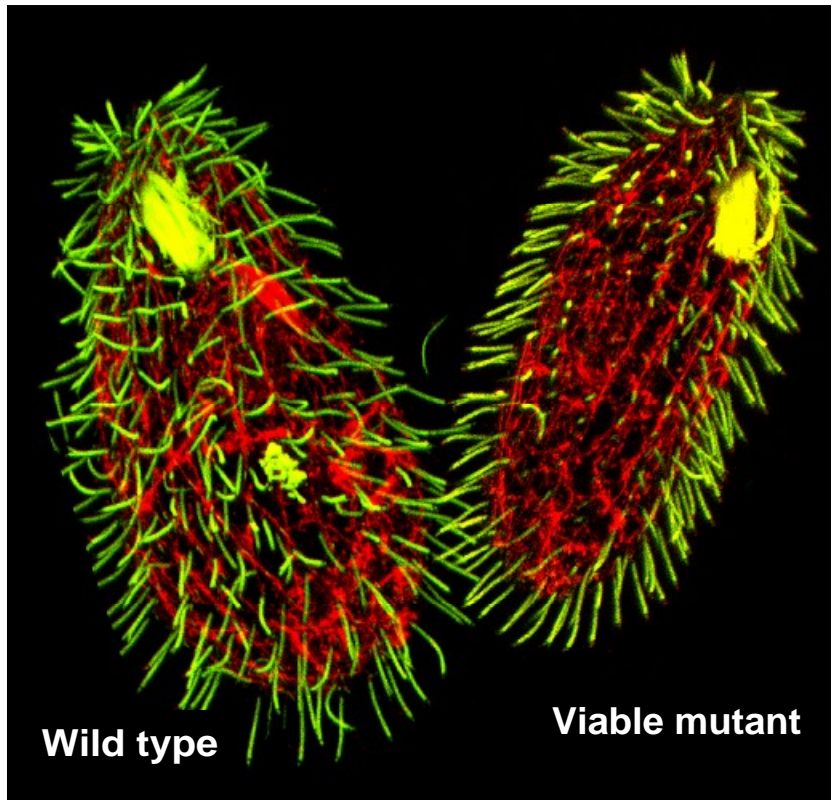
Paramecium WT	α 1	431DYEEVGIETAEGEGEEGEG
	α 2	431DYEEVGIETAEGEGEEGEA
	β	427DATAEEEGEF <u>EEEGE</u> Q
Tetrahymena WT:	α	431DYEEVGIETAEGEG <u>EEEGY</u>
	β	427DATAEEEGEF <u>EEEEGEN</u>
Tetrahymena Mutants	α	431DYEEVGIETAEGEG <u>EEEGY</u>
	β EDDD ₄₄₀	427DATAEEEGEF <u>E</u> DDD <u>GEN</u>
	α	431DYEEVGIETAEGEG <u>EEEGY</u>
	β EAAA ₄₄₀	427DATAEEEGEF <u>E</u> AAAG <u>EN</u>
α AAA ₄₄₇	431DYEEVGIETAEGEG AAAGY	
β	427DATAEEEGEF <u>EEEEGEN</u>	
α	431DYEEVGIETAEGEG <u>EEEGY</u>	
β EDDDGD ₄₄₂	427DATAEEEGEF <u>E</u> DDDG D N	

Known glycylation sites are in red underlined and mutated sites are in blue bold.

Viable mutants analyzed :

- triple substitutions of adjacent glutamates (E) by aspartates (**D**) or alanines (**A**)
- quadruple substitution of E by **D**.

SITE-DIRECTED MUTAGENESIS OF TUBULIN GLYCYLATION SITES IN *TETRAHYMENA*

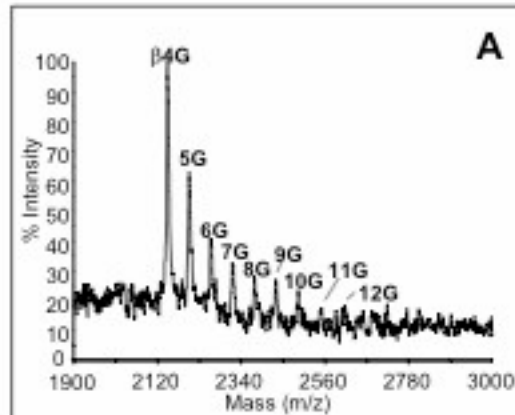


COMPARISON OF TUBULIN MODIFICATIONS IN *PARAMECIUM* ET *TETRAHYMENA*

Tetrahymena

β -tubulin

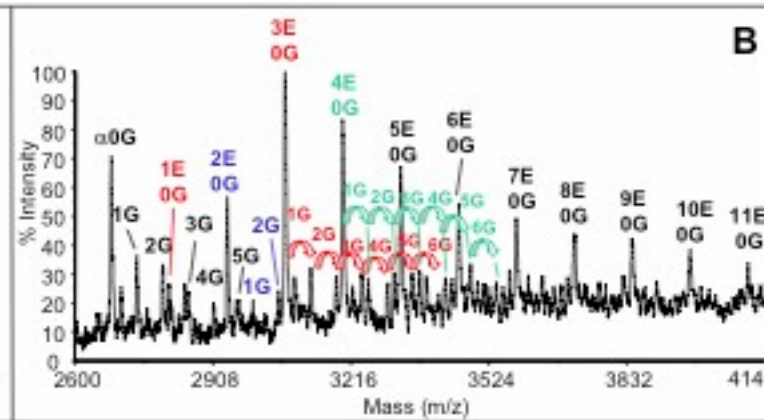
- 0-12 G
- 0-7 E



Tetrahymena

α -tubulin

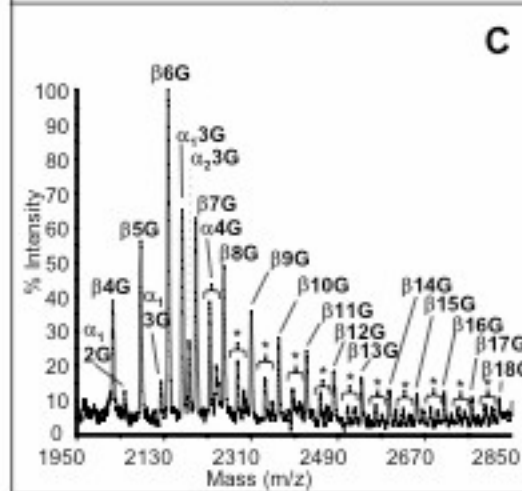
- 0-10 G
- 0-19 E



Paramecium

β -tubulin

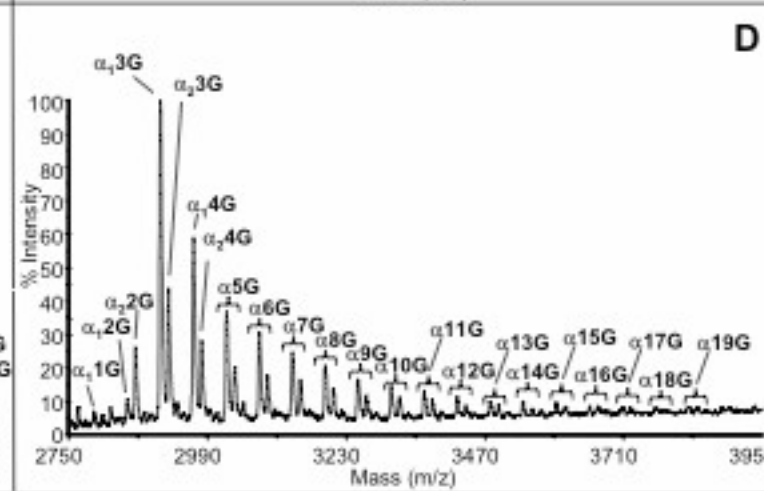
- 4-32 G
- 0 E



Paramecium

α -tubulin

- 1-34 G
- 0 E



- *Paramecium* tubulin is extensively glycylyated, faintly glutamylated (blot).
- *Tetrahymena* tubulin is glycylyated, highly glutamylated,
- α -tubulin is detyrosinated.
- Both polymodifications coexist on a same tubulin molecule in *Tetrahymena*.

EFFECTS OF GLYCYLATION SITE MUTATIONS ON POLYMODIFICATIONS OF *TETRAHYMENA* AXONEMAL TUBULIN

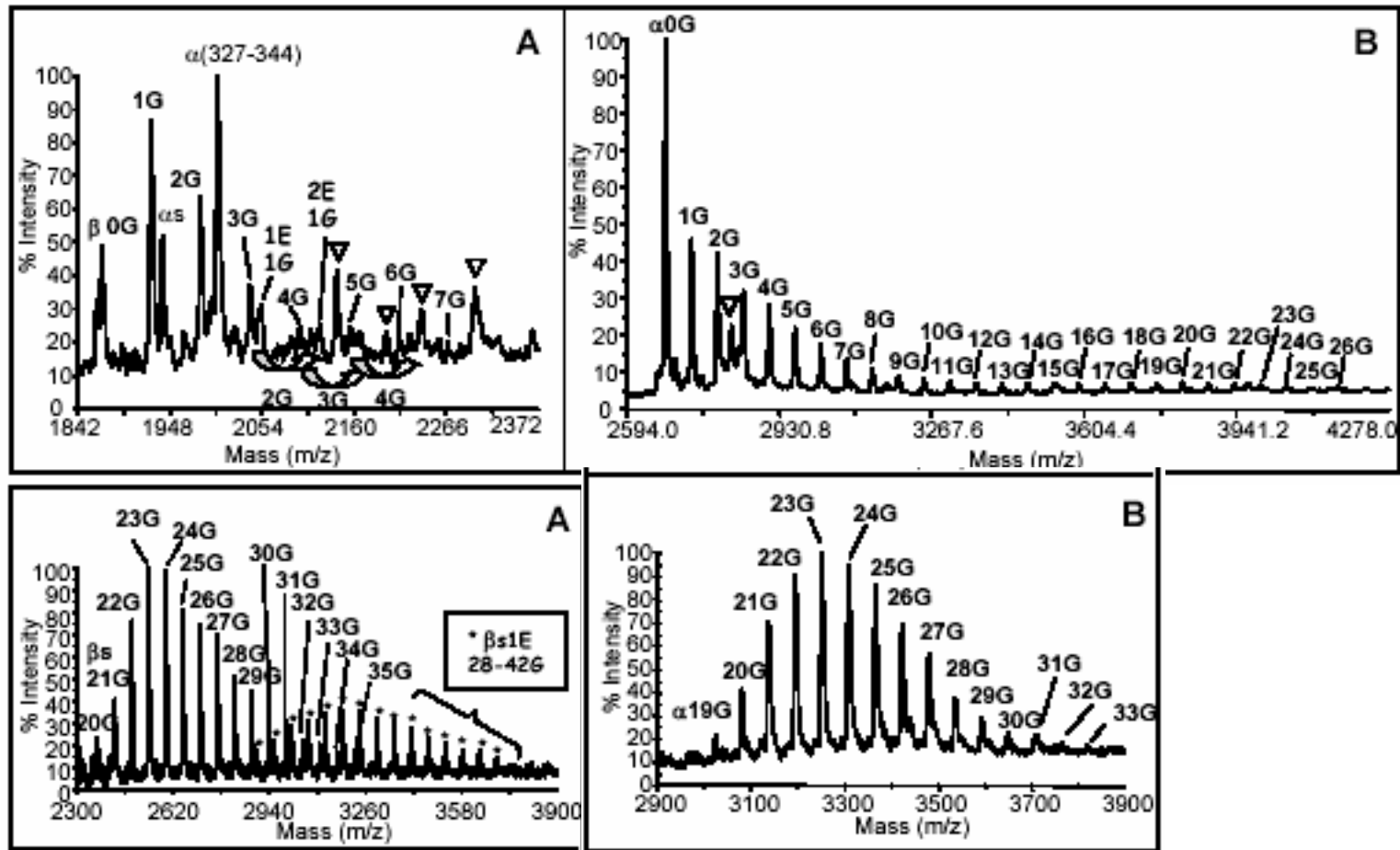
QuickTime et un
décompresseur TIFF (LZW)
sont requis pour visionner cette image.

QuickTime et un
décompresseur TIFF (LZW)
sont requis pour visionner cette image.

Polyglutamylolation

Polyglycylation

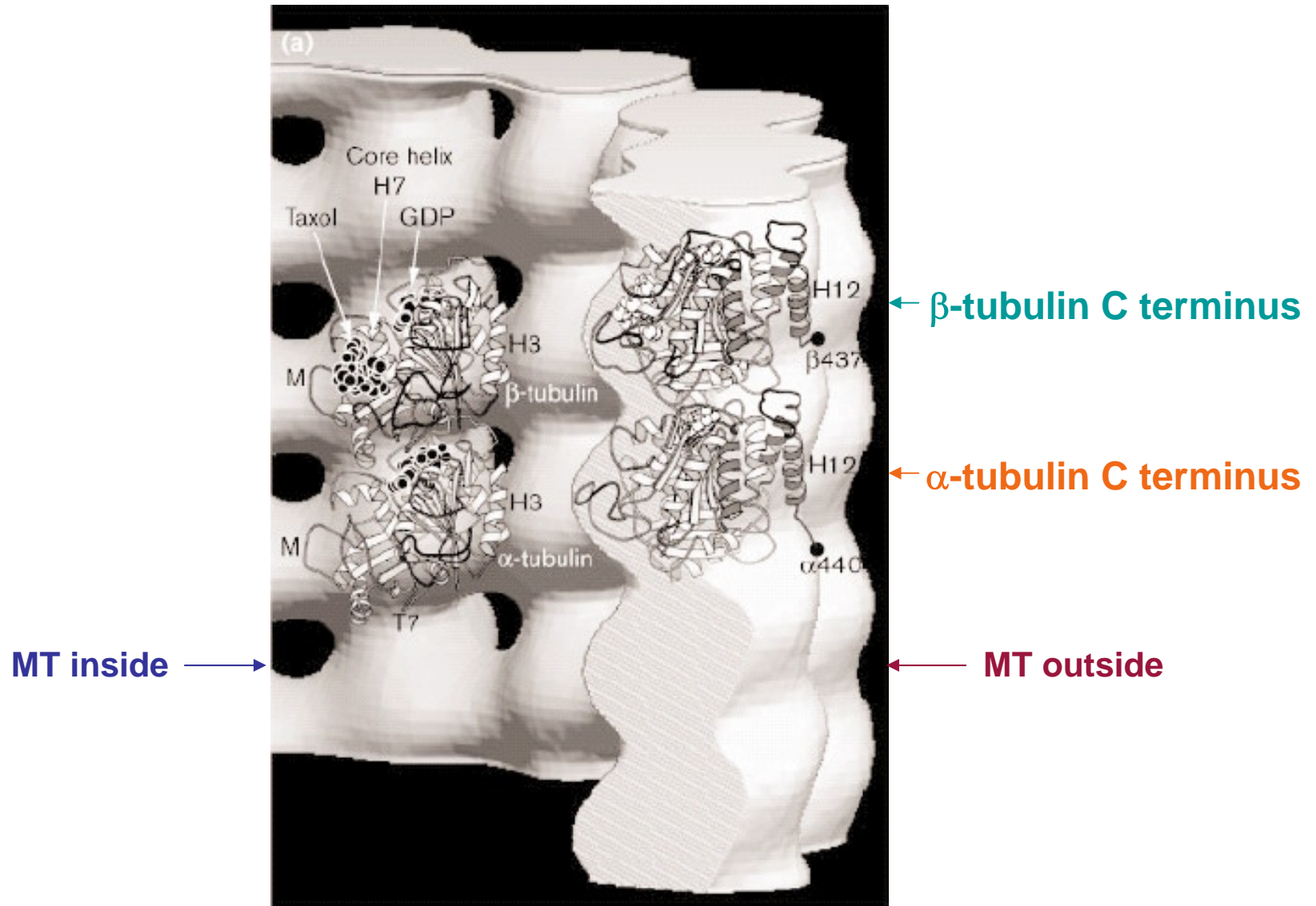
POLYMODIFICATIONS OF AXONEMAL TUBULIN IN THE *TETRAHYMENA* GLYCYLATION SITE MUTANT REDDD



Mutation of 3 glycylation sites in β -tubulin induces :

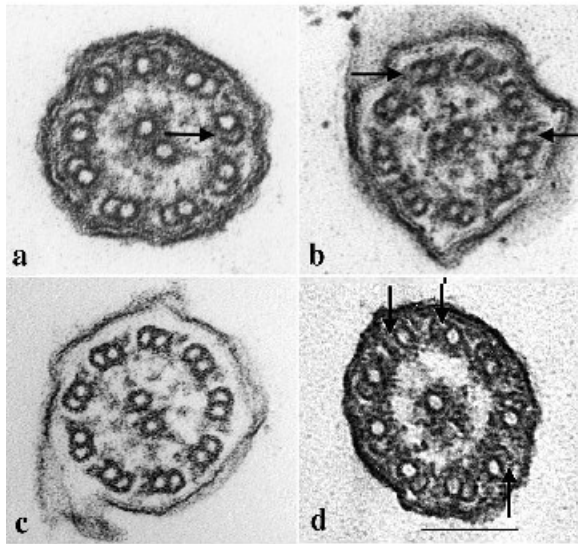
- 1) On the mutated β subunit :
 - **decrease** of the **glycylation** level of the **major isoform** : 4 → 1 ;
 - **increase** of the **maximal level of added G** : 12 → 42 ;
 - **decrease** of the level of added **E** : 7 → 2.
- 2) On the nonmutated α subunit :
 - **increase** of the **glycylation** level : 10 → 37 ;
 - **lack of detection** of **E**.

3D STRUCTURE OF $\alpha\beta$ TUBULIN DOCKED ONTO PROTOFILAMENT 3D IMAGE WITHIN A MICROTUBULE



(Amos, 2000)

ULTRASTRUCTURE OF CILIA FROM *TETRAHYMENA* WT AND TUBULIN GLYCYLATION SITE MUTANTS



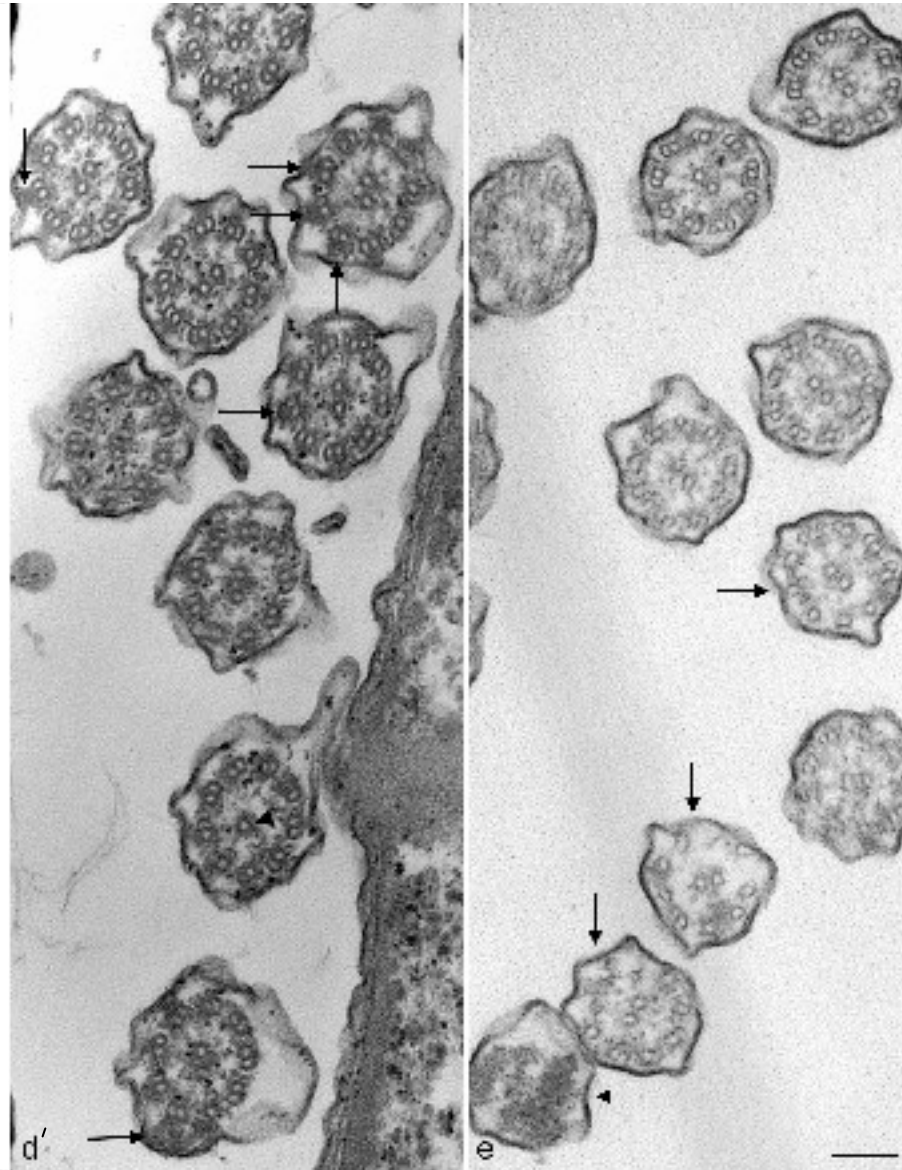
c, WT

a-b, β EDDD mutant:

→ opened B-tubules

d, β EAAA mutant:

→ opened B-tubules filled with dense material, central singlet Mt



d', β EDDDGD:



dense material between doublets and membrane



central singlet Mt

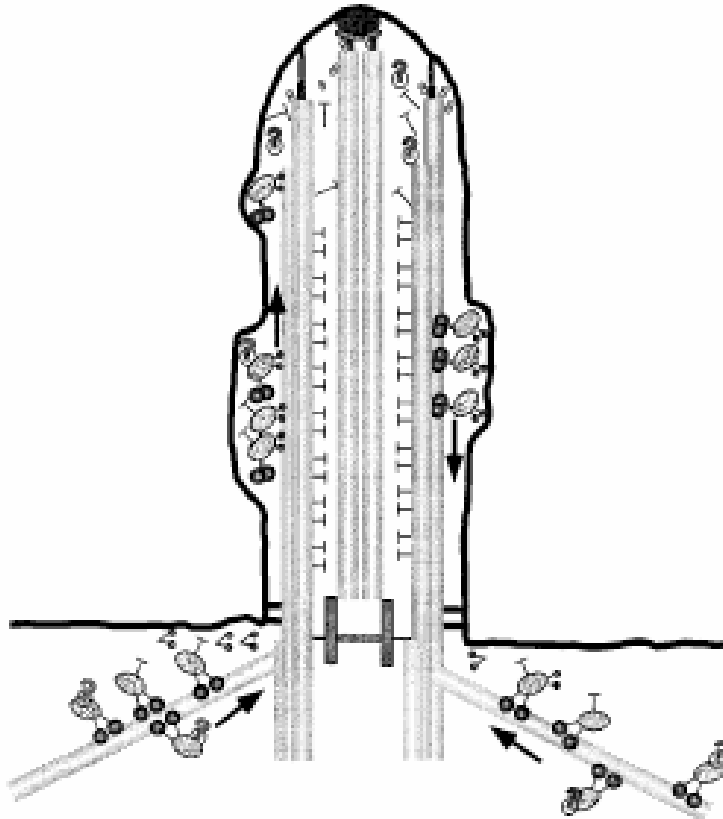
e, α AAA:



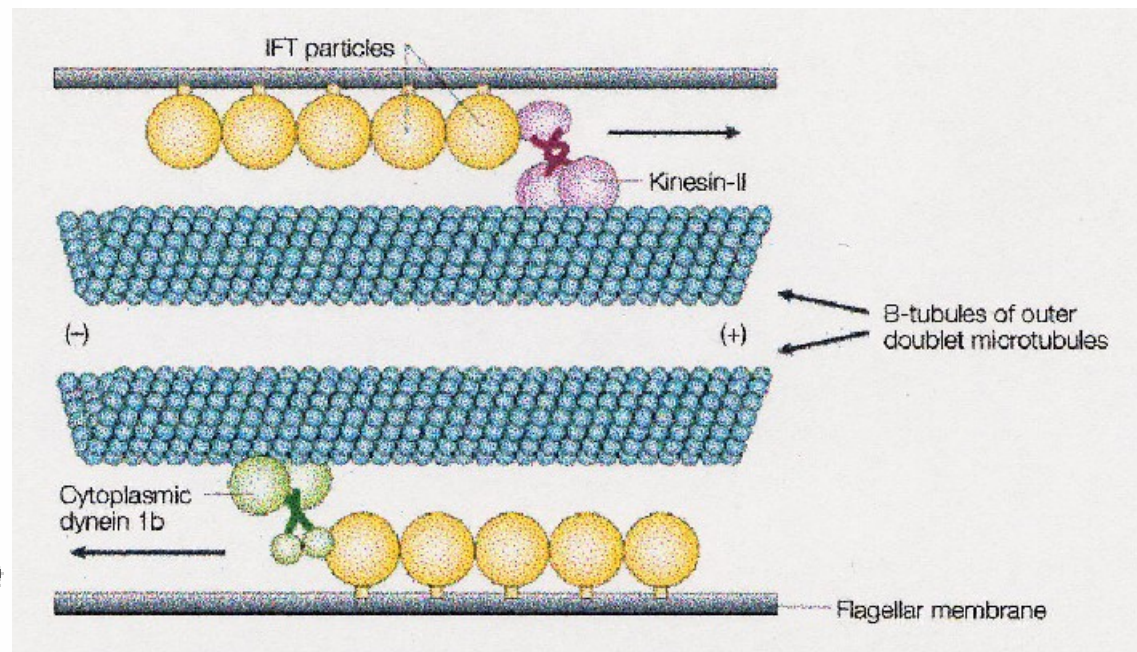
Singlet peripheral Mts

INTRAFLAGELLAR TRANSPORT (IFT)

Transport required for cilia/flagella assembly and maintenance



(Porter et al, 1999)



(Rosenbaum, Witman, 2002)

- Anterograde transport of IFT particles and their cargo (axoneme precursors, retrograde motors) from the basal body to the tip of the axoneme, by the anterograde kinesin-II motor, using **B-tubules of outer doublets** as tracks.
- Retrograde transport of IFT particles and anterograde motors by cytoplasmic dynein.

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