



# Le trypanosome comme modèle d'étude des ciliopathies

**Philippe BASTIN**

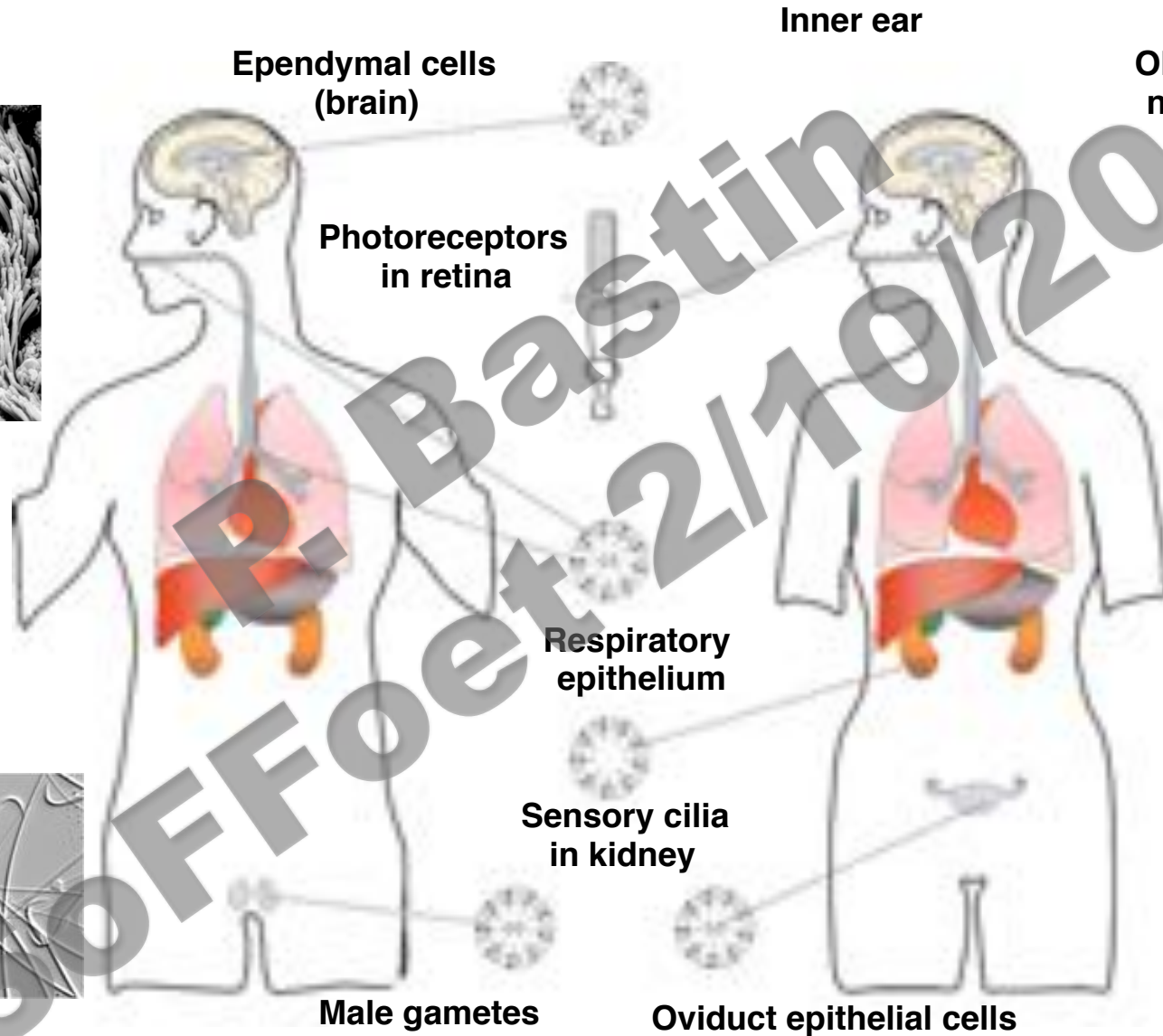
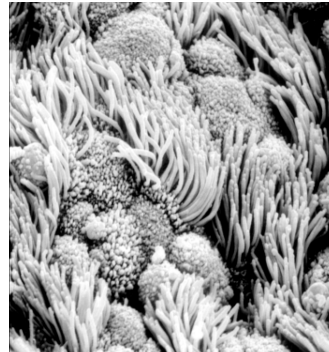
**Trypanosome Cell Biology Unit**

**Institut Pasteur & INSERM U1201**

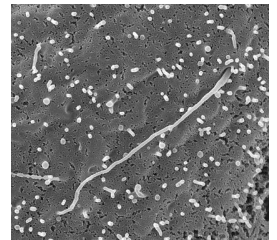
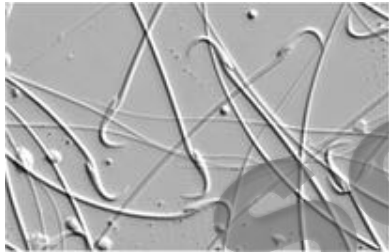
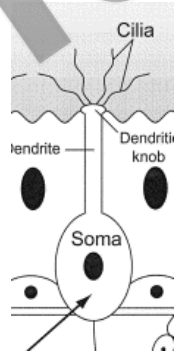
[pbastin@pasteur.fr](mailto:pbastin@pasteur.fr)

**Paris, Société Française de Fœtopathologie, 02/10/2015**

# Cilia and flagella in humans



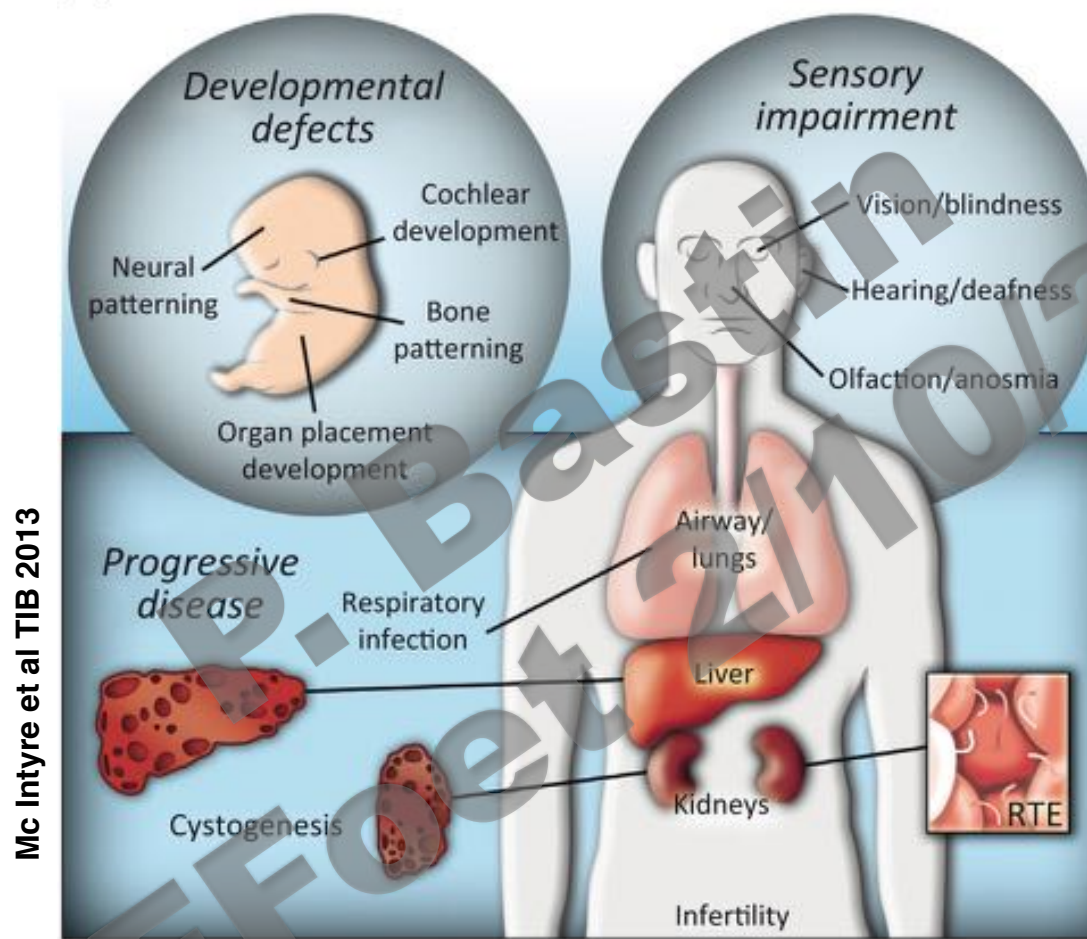
Olfactory neurons



Primary cilium

# Ciliopathies

(A)

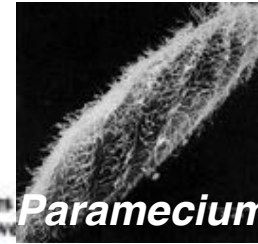


**Family of genetic diseases due to defects in ciliary genes  
(collective estimated impact 1:400)**

# Cilia across species



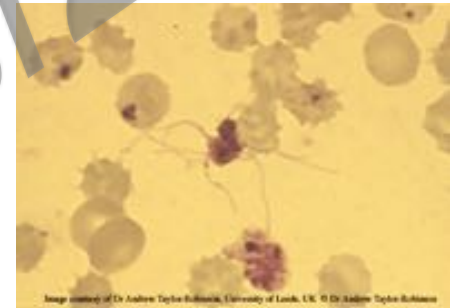
*Chlamydomonas*



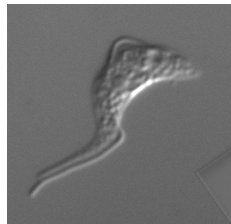
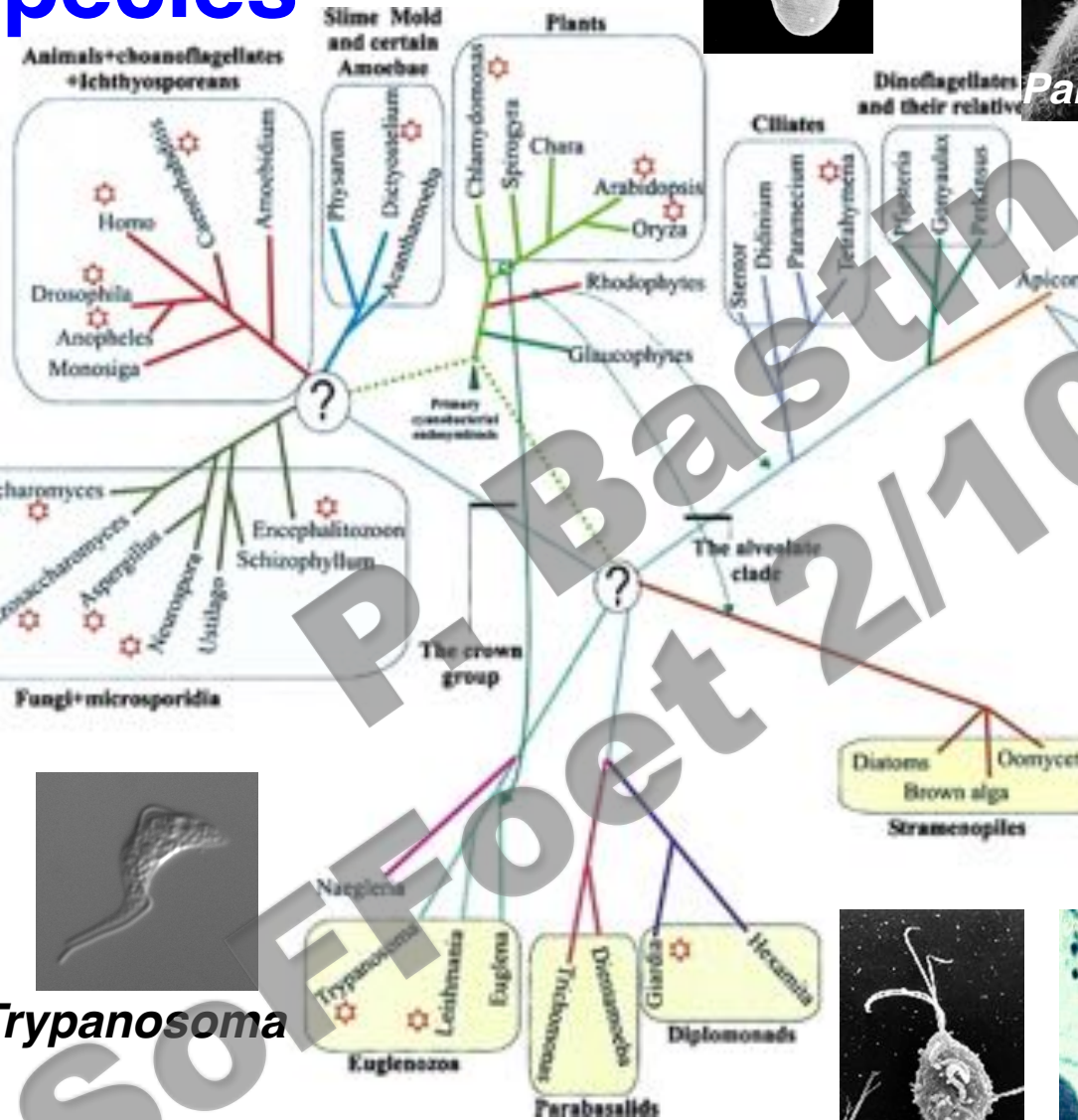
*Paramecium*



*Dinophysis*



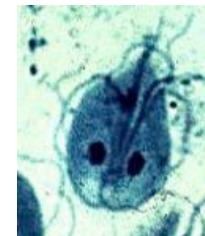
*Plasmodium*



*Trypanosoma*



*Trichomonas*



*Giardia*

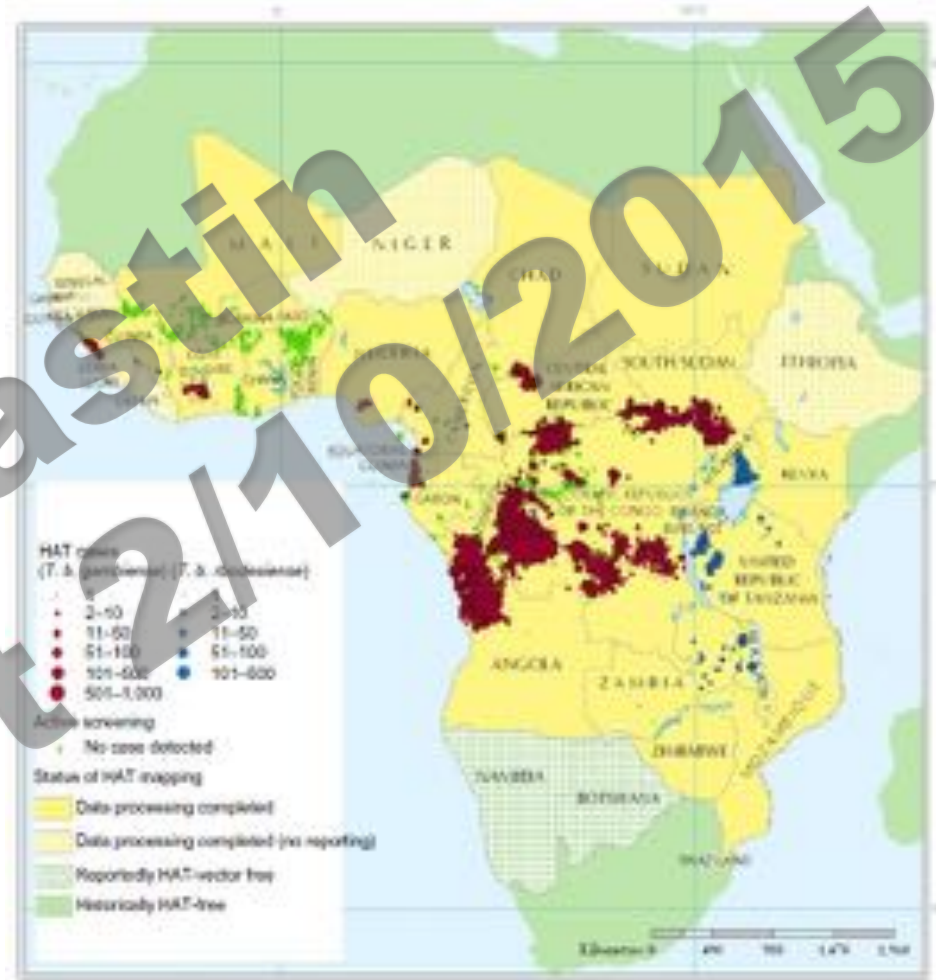
Tree from Aravind et al. (2003) Cell 115, 771

# African trypanosomiasis

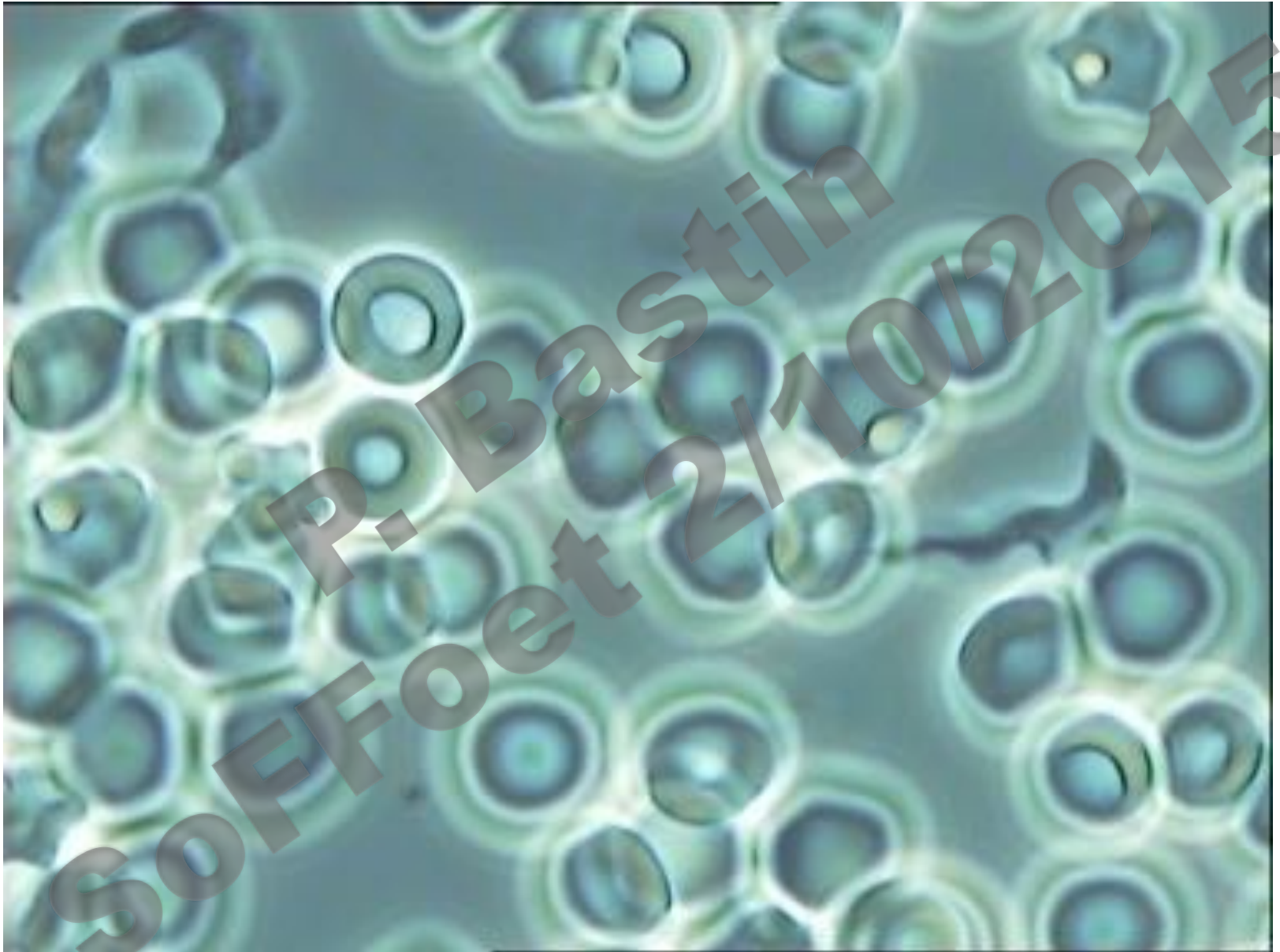
- Blood and brain infections (sleeping sickness)
- Affects human and cattle
- Fatal if non-treated
- No vaccine



C. Fort



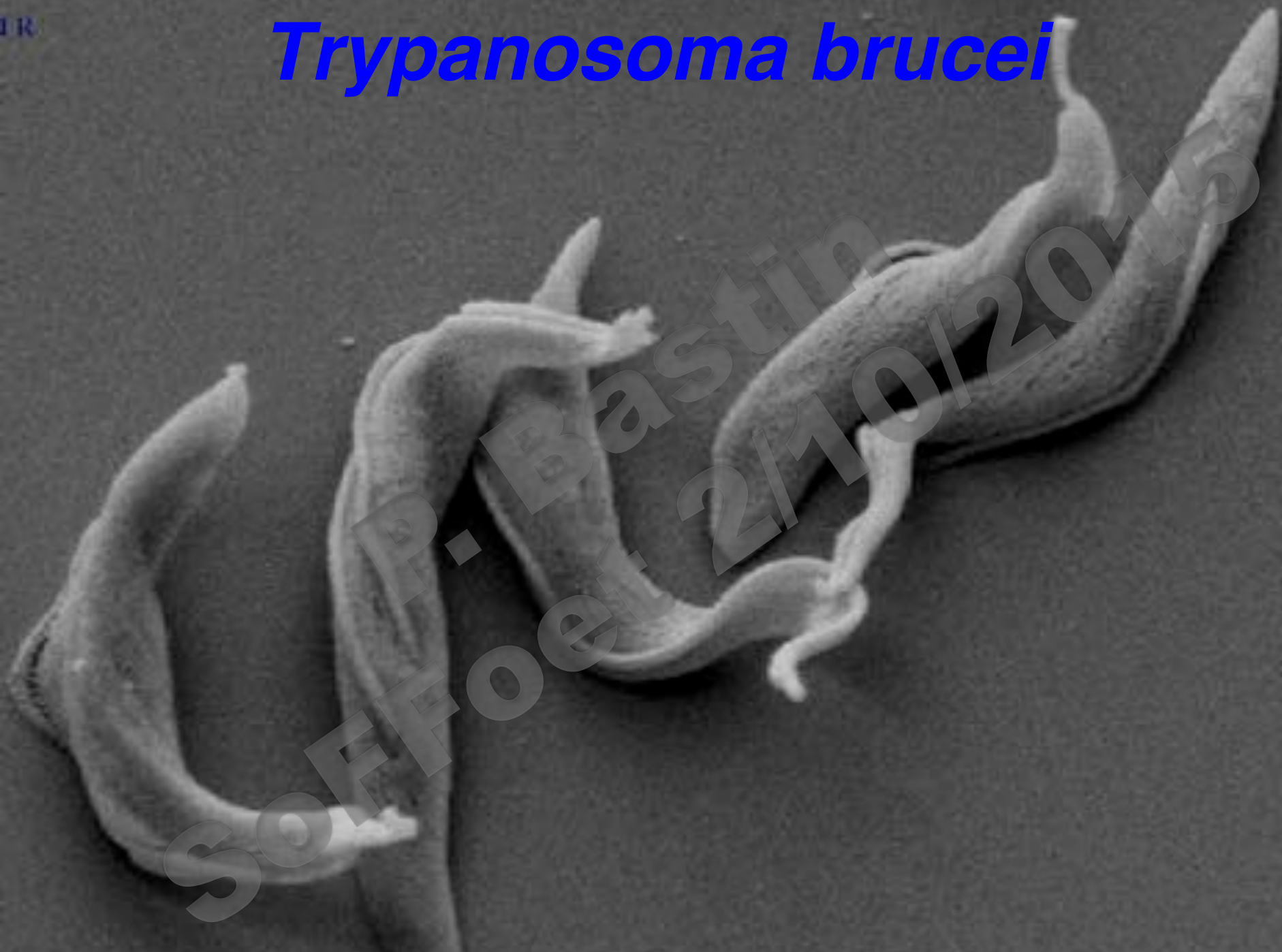
# *Trypanosoma brucei*: an extracellular parasite



Ines  
Subota

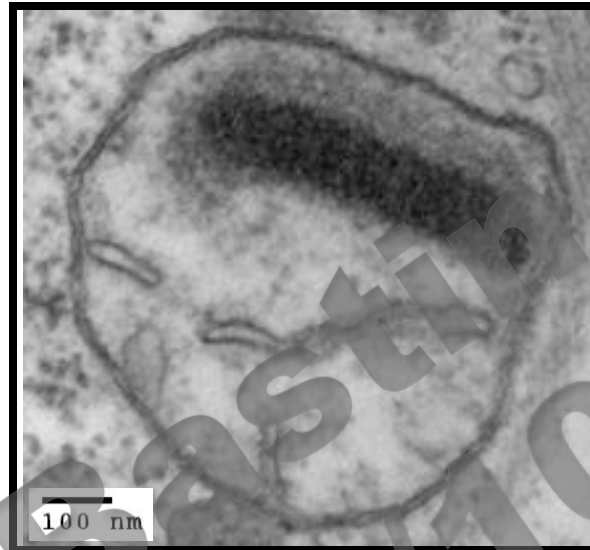
Video slowed down 2x

# *Trypanosoma brucei*



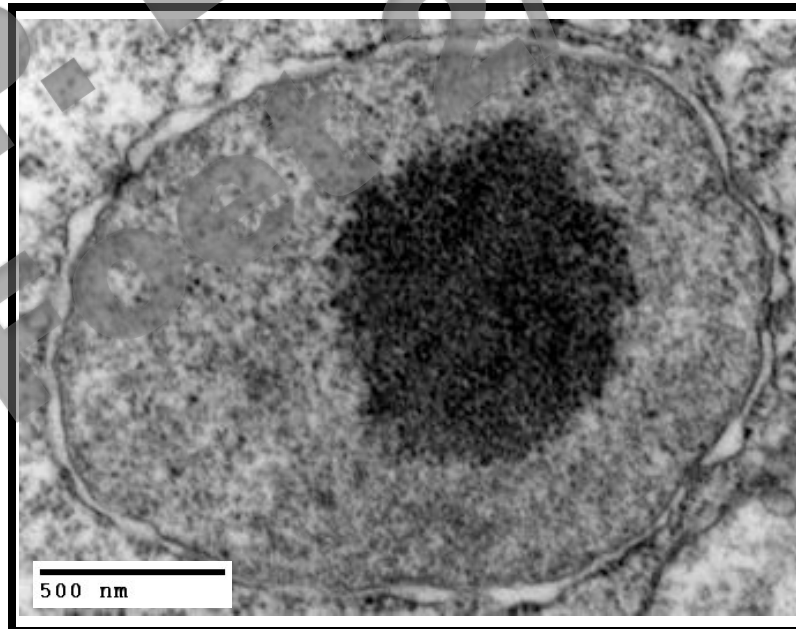
SOFFO et al. 2015

# The trypanosome as a cell



**Kinetoplast**  
(mitochondrial genome)

**RNA editing**  
**Linked to basal body**

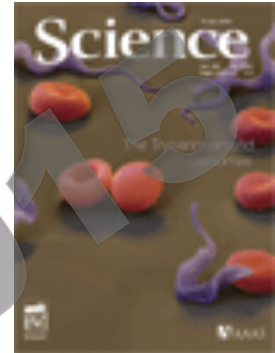


**Nucleus**  
**25 Mb**  
**9,000 genes**

**Mono-allelic expression**  
**Polycistronic transcription**  
**No intron**



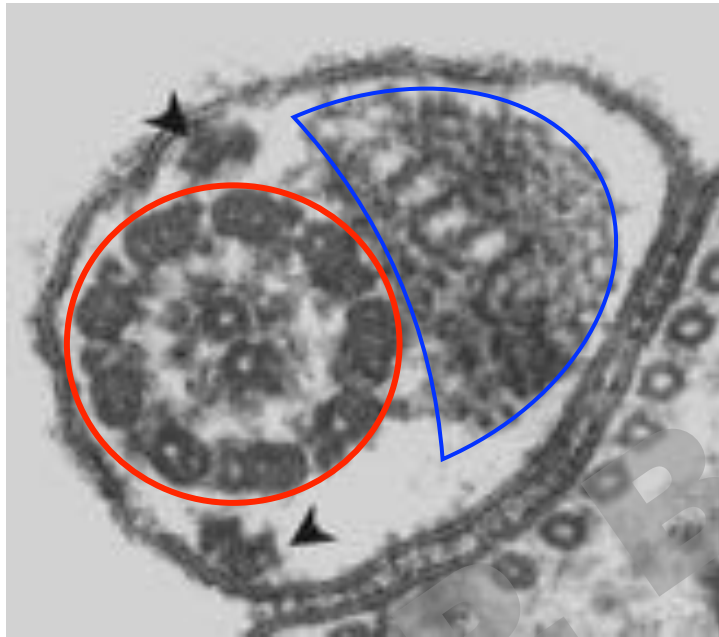
# Practical advantages



- **Flagellated organism easy to grow in culture (biochemistry)**
- **Complete genome available (4 strains)**
- **Straight forward molecular biology**
- **Reverse genetics (RNAi, KO, inducible expression systems, endogenous tagging)**
- **Imaging flagellum**
- **Strain non-pathogenic for humans**

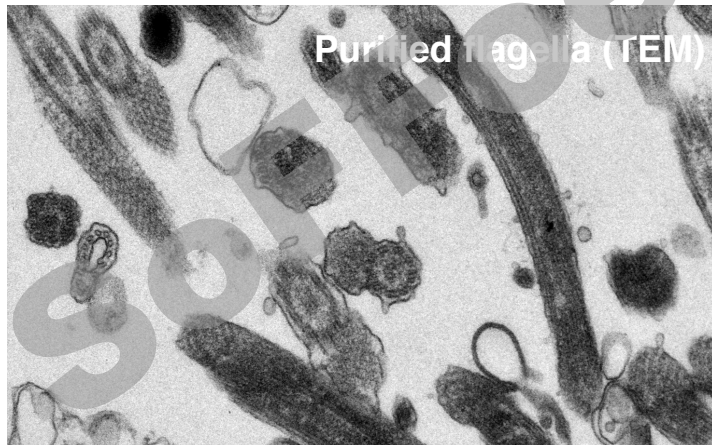
# Structural & molecular conservation

*T. brucei*



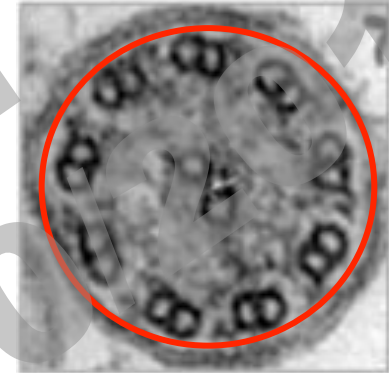
Bastin et al MI2000

**9+2 axoneme** with a **Paraflagellar Rod (PFR)**



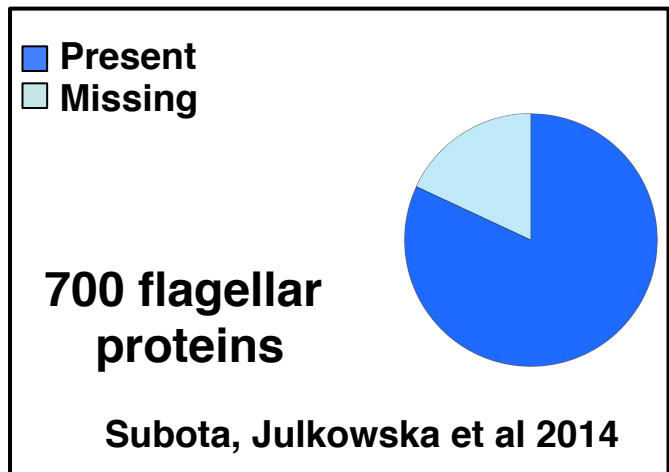
Purified flagella (TEM)

Human cilia

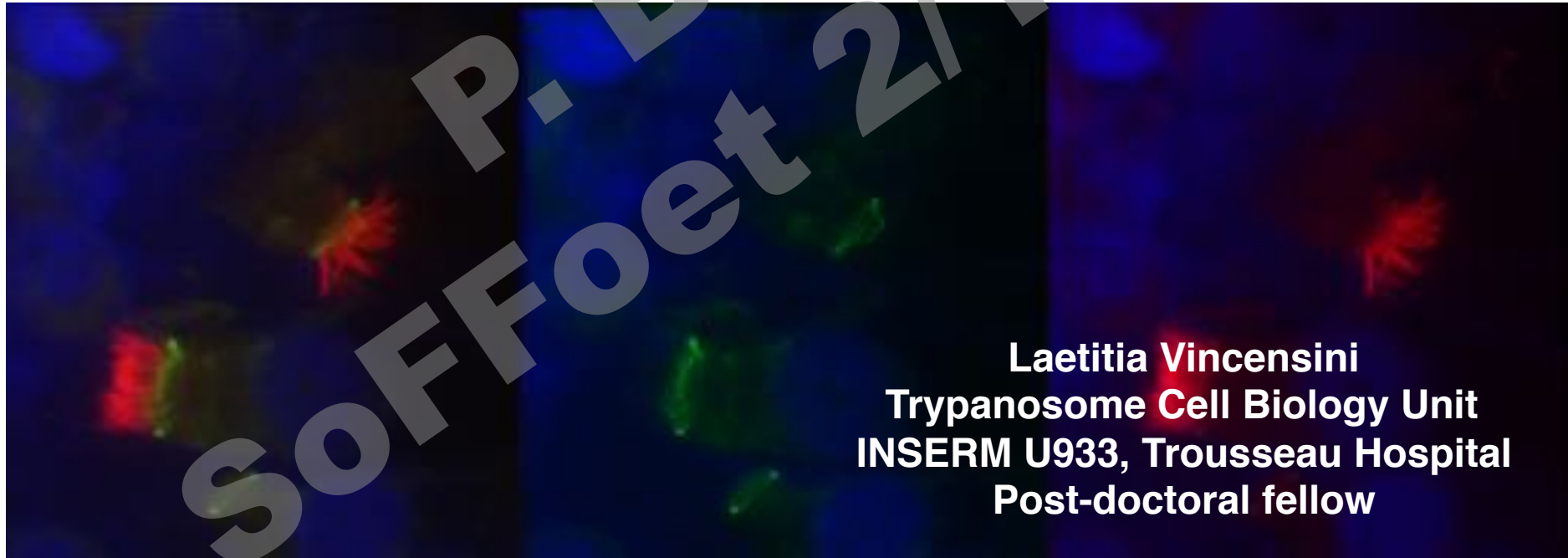


E. Escudier

**9+2 axoneme**

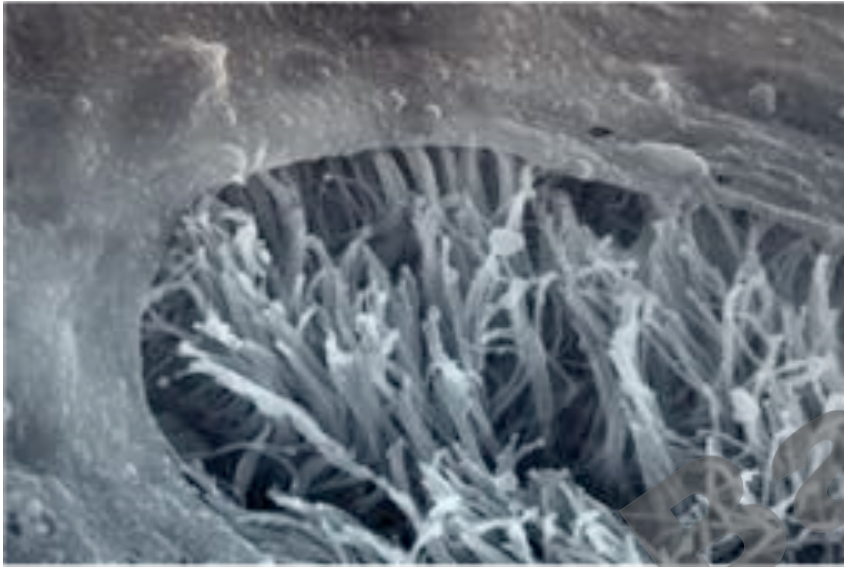


# Linking trypanosomes and human genetic diseases: dynein arm assembly



Laetitia Vincensini  
Trypanosome Cell Biology Unit  
INSERM U933, Trousseau Hospital  
Post-doctoral fellow

# Primary ciliary dyskinesia (PCD)



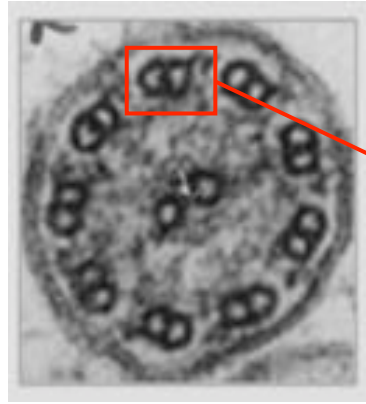
Respiratory epithelium

- Cilia with beating defect
- Respiratory tract infections
- Sterility
- *situs inversus*

- Complex diagnosis
- Heterogenous disease: 27 genes known, ~70% of cases

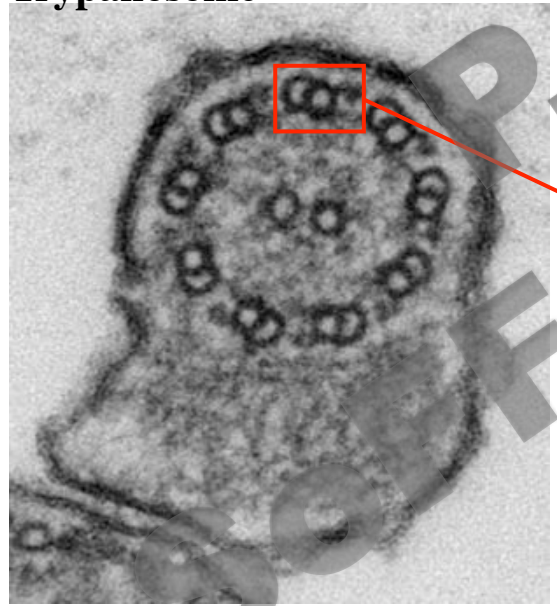
# Molecular motors: the dynein arms

Human

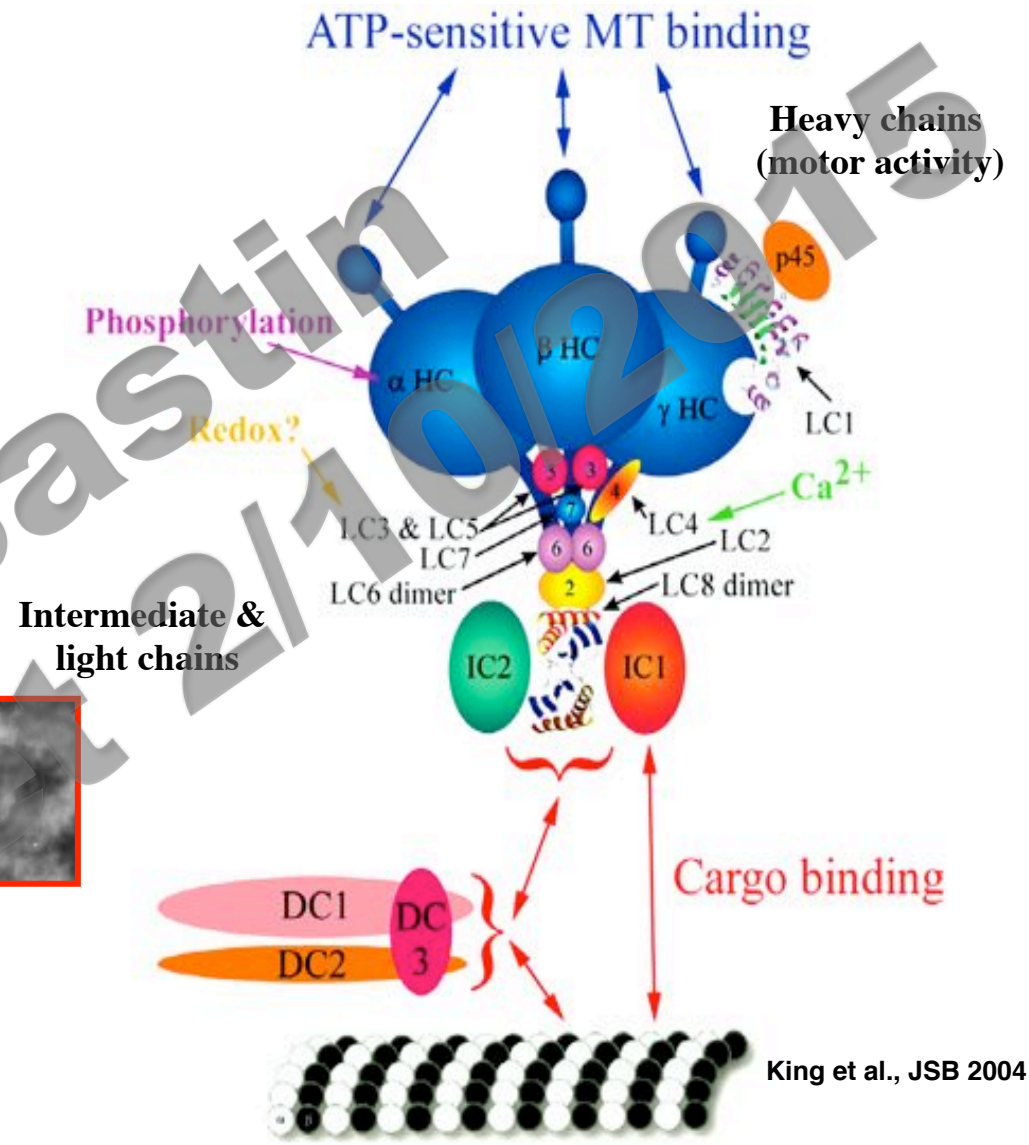


Escudier E.

Trypanosome

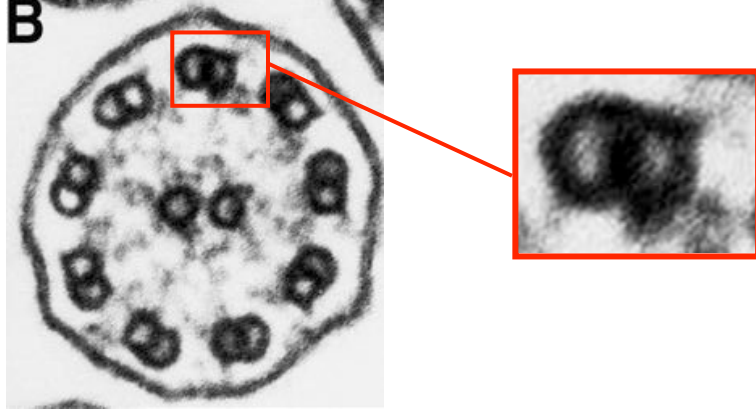


Céline LOUSSERT



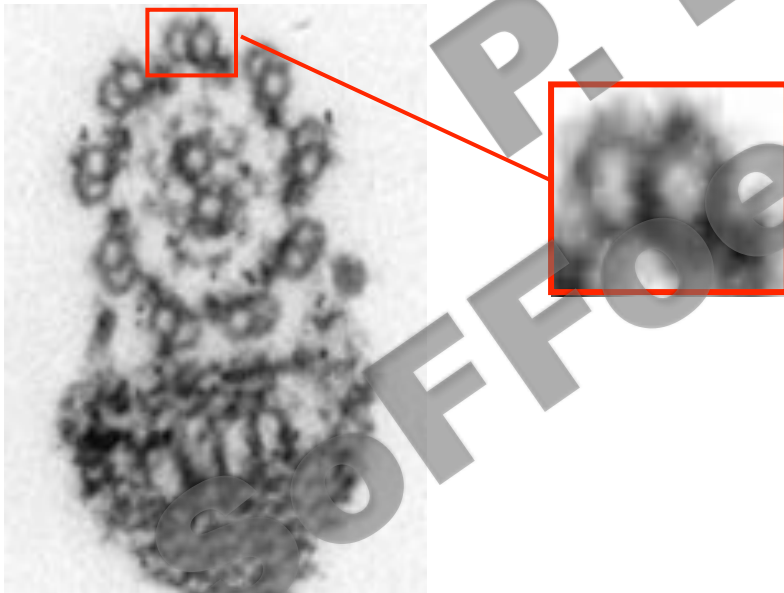
# Loss of a dynein arm component (DNAI1)

Human patient (primary ciliary dyskinesia)

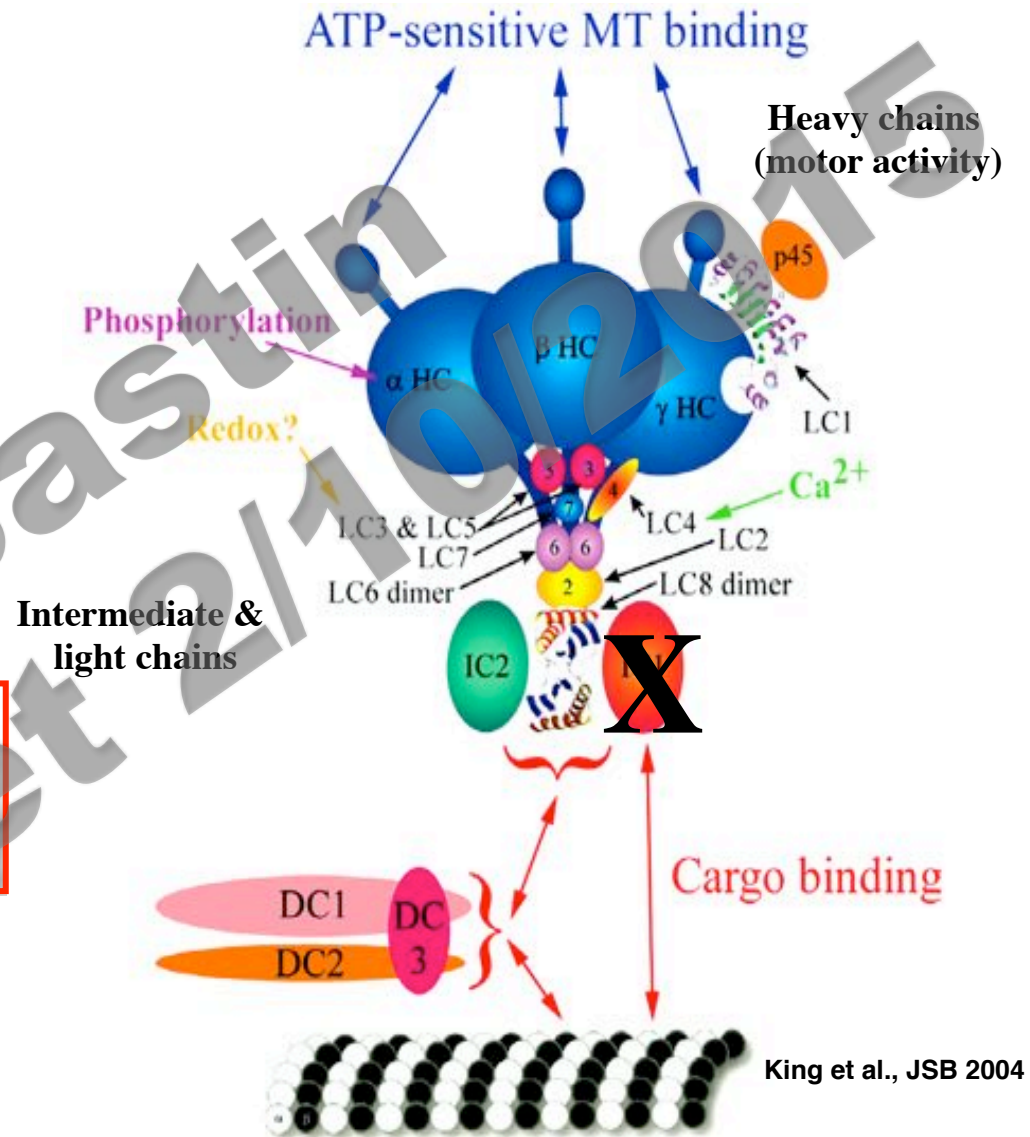


Pennarun et al. *Am J Hum Genet* 1999

Trypanosome *DNAI1<sup>RNAi</sup>* mutant

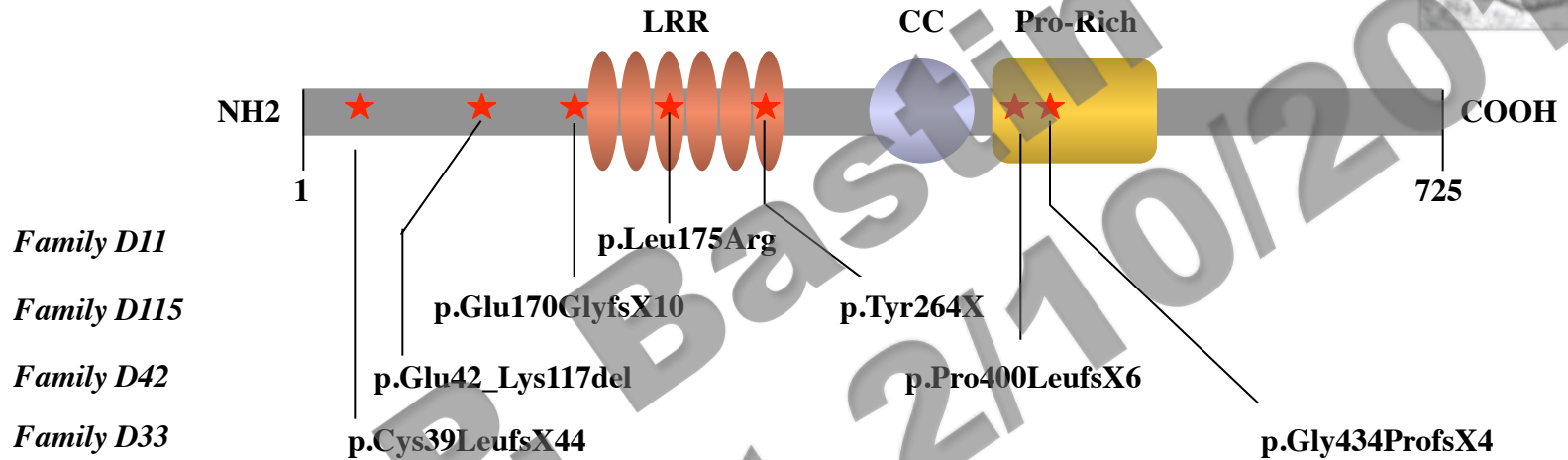
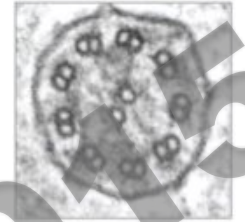


Branche et al. *J Cell Sci* 2006



# ODA7, a new gene involved in primary ciliary dyskinesia (Inserm U933, Hôpital Trousseau, S. Amselem)

- Protein rich in Leucine rich repeats – protein protein interactions
- Gene sequenced in 42 patients : Mutations found in 4 families



Laetitia Vincensini

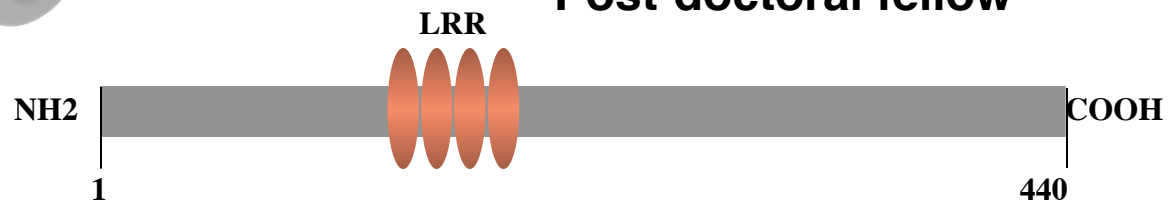
Trypanosome Cell Biology Unit

INSERM U933, Trousseau Hospital

Post-doctoral fellow

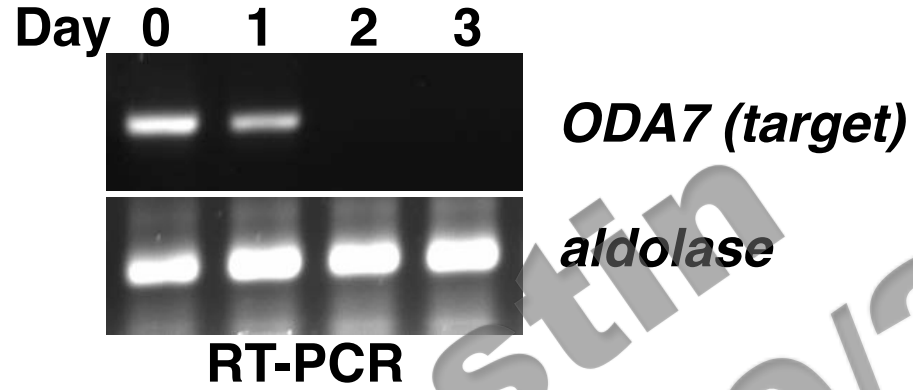
**A gene conserved in trypanosomes**

- 36% identity, 53% similarity
- 440aa, 50kDa



→ Functional investigations in *T. brucei*

# Functional study of ODA7 in trypanosomes



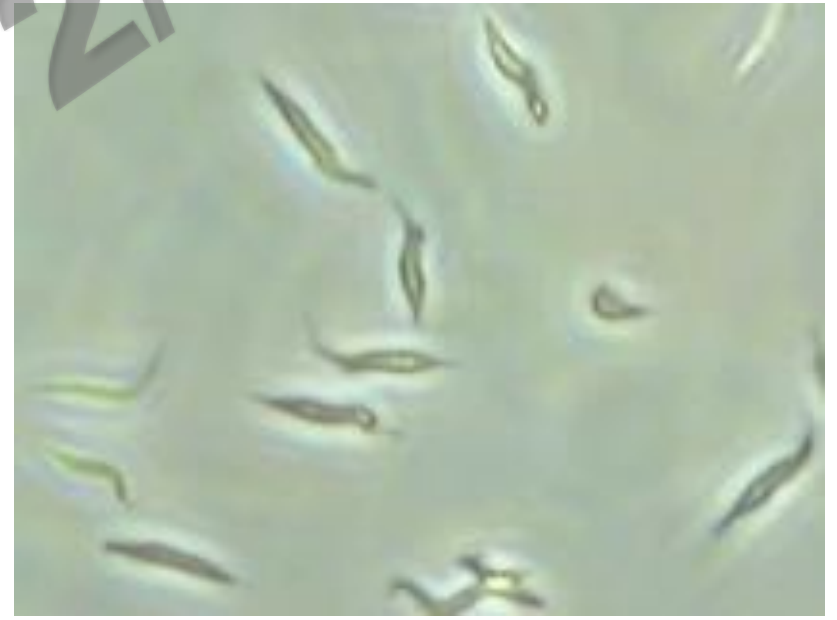
Tetracycline-inducible expression of dsRNA

*ODA7*<sup>RNAi</sup> strain

NON  
induced

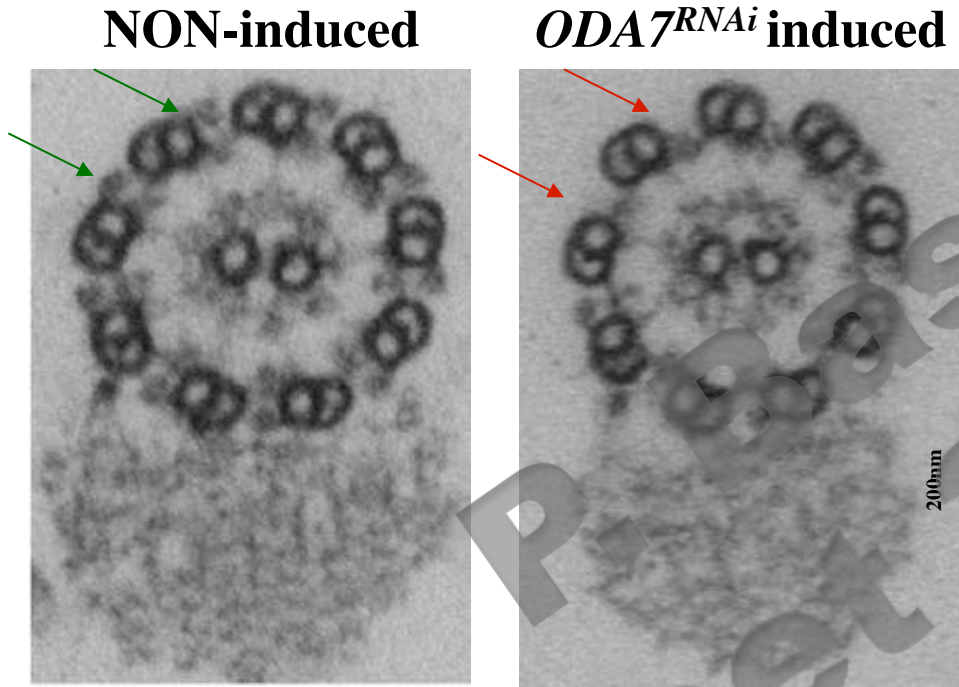


Induced

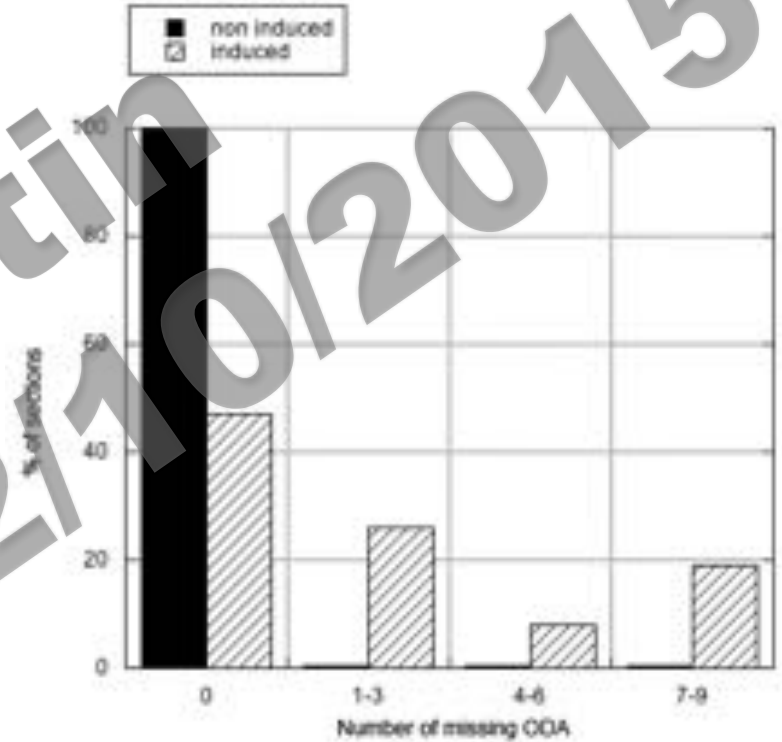




# Ultra-structural analysis: loss of outer dynein arms

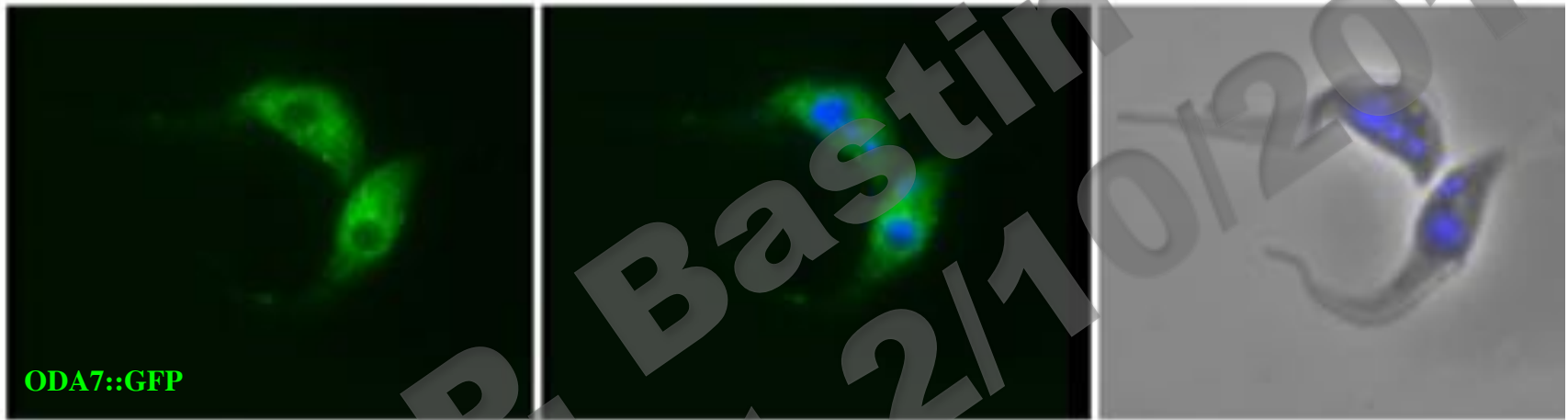


(C. Loussert)



# ODA7 sub-cellular localisation

*T. brucei* (procyclic stage)



**ODA7 is a cytoplasmic protein (not axonemal)**

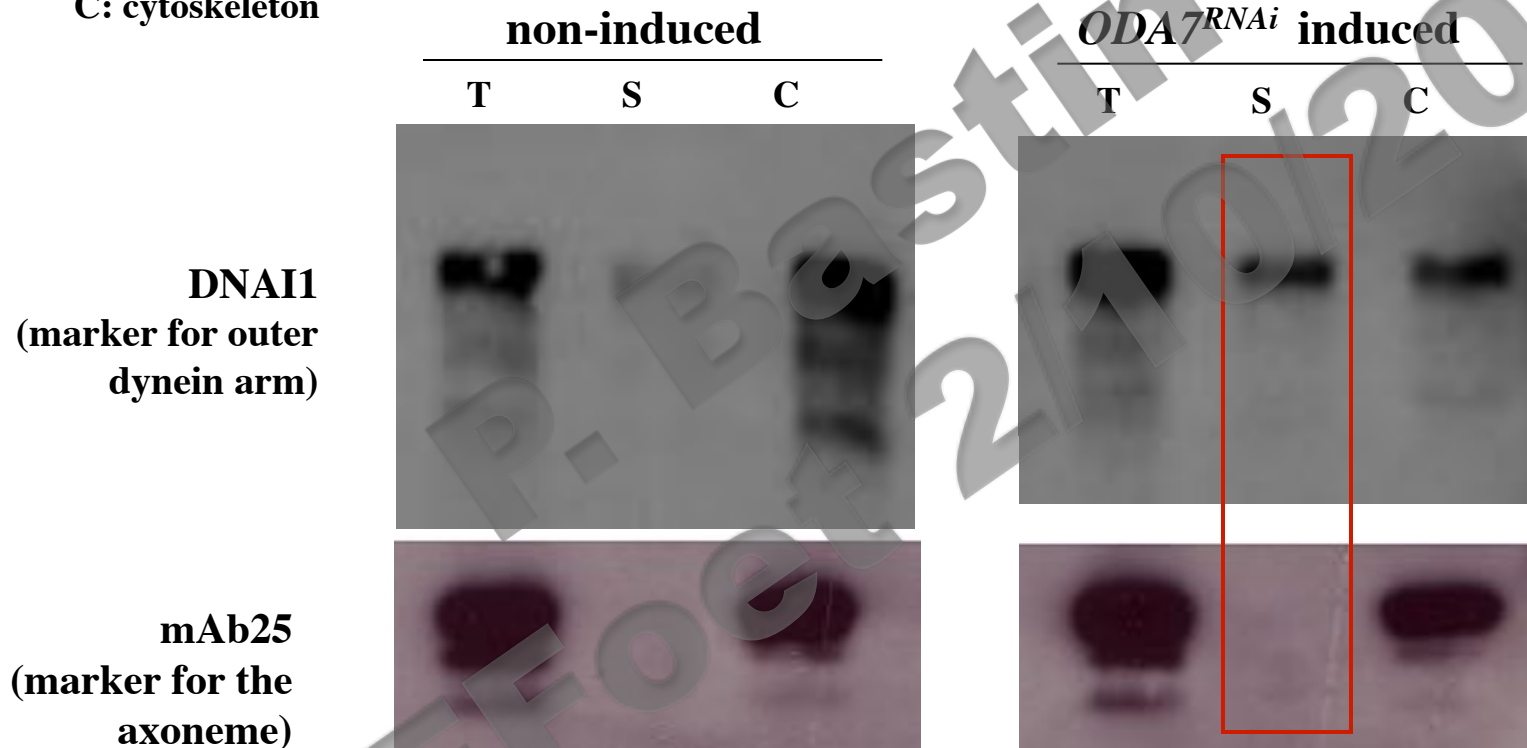
# ODA7 is required for dynein arm assembly

## Fractionation in detergent

T: total

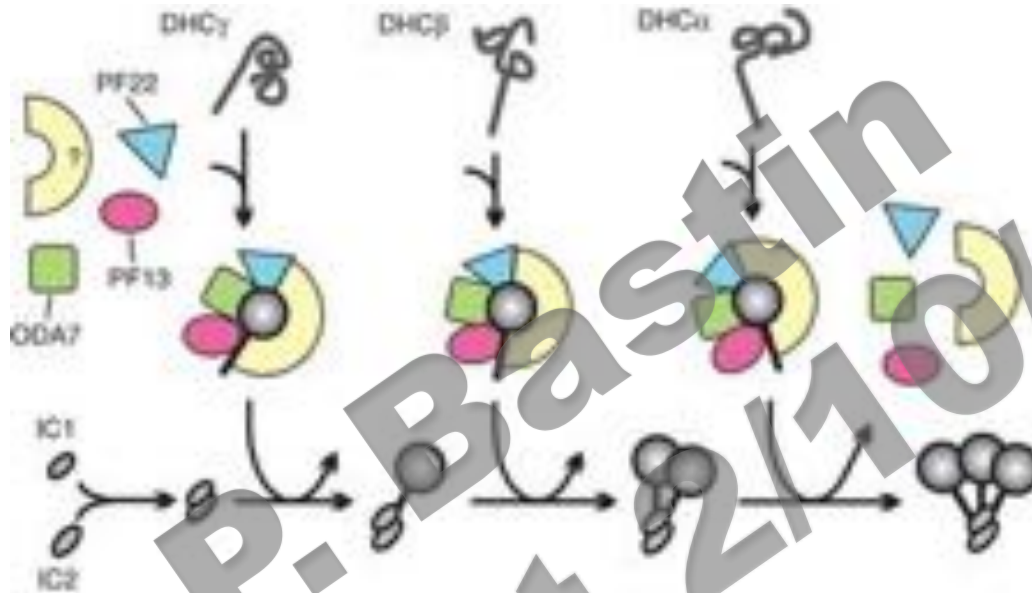
S: soluble

C: cytoskeleton



Essential for outer dynein arm assembly but not protein stability

# Dynein arm assembly takes place in the cytoplasm



SoFFoet 21/10/2015

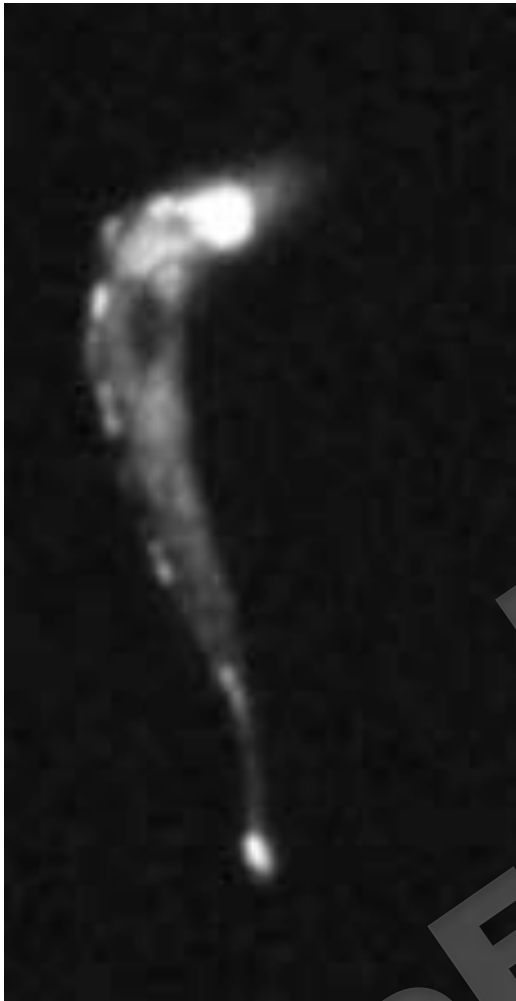
# Building the flagellum is...



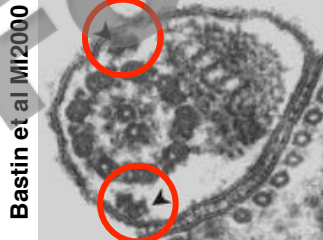
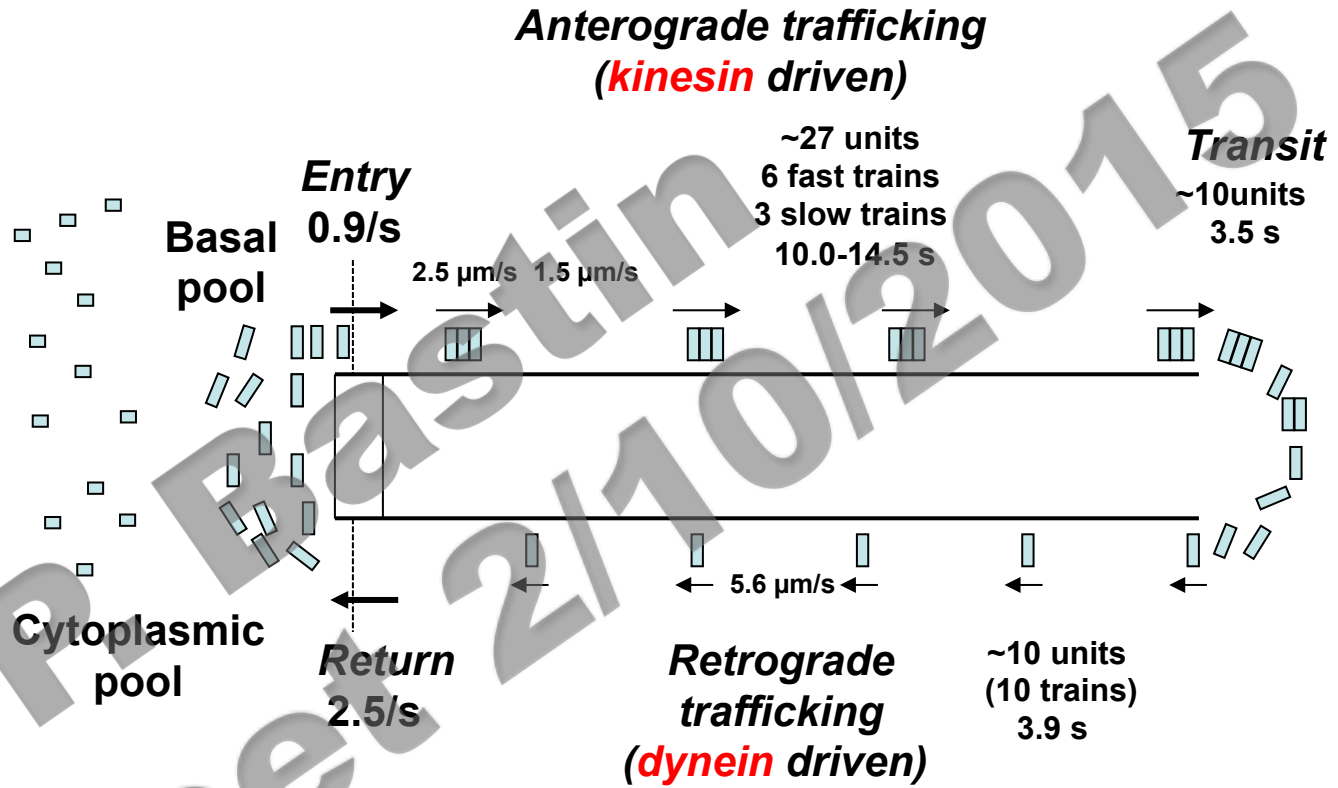
*...like building a tower*

SOFFHOET 2/10/2015

# IntraFlagellar Transport (IFT)



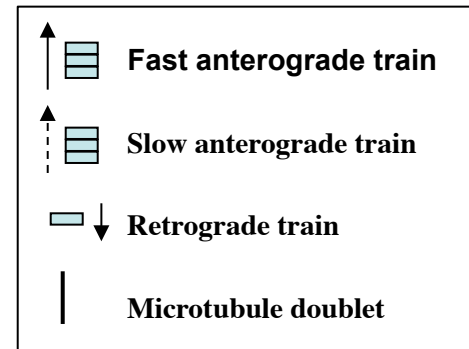
GFP::IFT52 (Shown for 10 different IFT)



Bastin et al IM2000

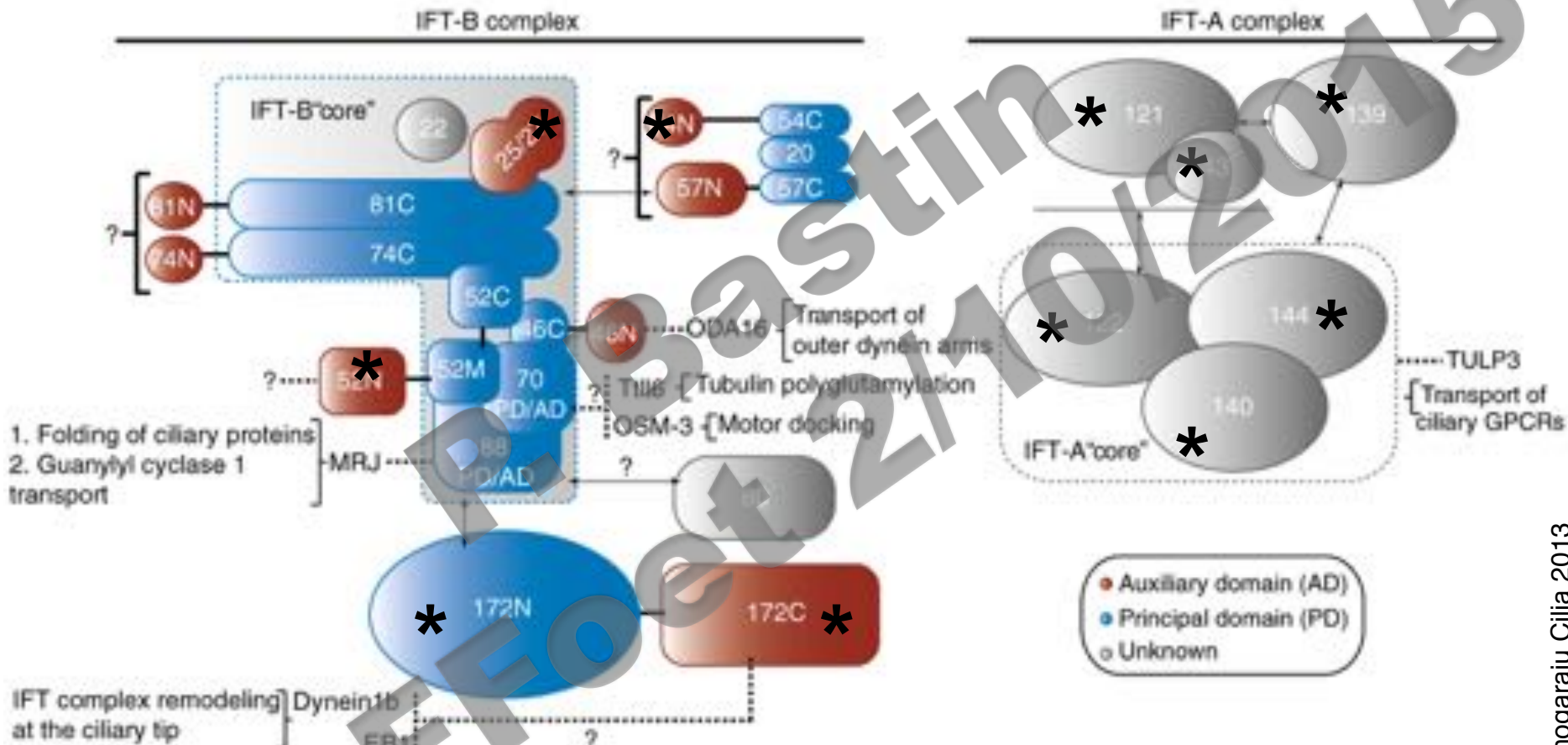


Johanna BUISSON



Nicolas CHENOUEARD  
Thibault LAGACHE  
(J. C. Olivo-Marin lab)

# IFT-B and IFT-A proteins



**IFT genes conserved in most ciliated species**

**\* Mutated in ciliopathy patients**

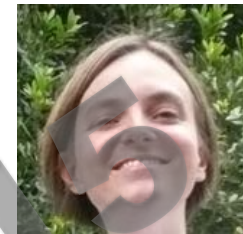
# Trypanosomes convenient models

- **Flagellar gene function**
- **Flagellar protein localisation**
- **Flagellar protein dynamics**
- **Impact of mutations in flagellar genes**
- **Basic knowledge of cilia and flagella**





**Thierry  
BLISNICK**



**Laetitia  
VINCENSINI**



**Brice  
ROTUREAU**



Plate-forme de Cytométrie en Flux  
Plate-forme d'Imagerie Dynamique  
Plate-forme de Microscopie Ultrastructurale  
Plate-forme Centre d'Immunologie Humaine



**LabEx IBEID**



Institut national  
de la santé et de la recherche médicale

