

Functional genomics in trypanosomes

Philippe BASTIN

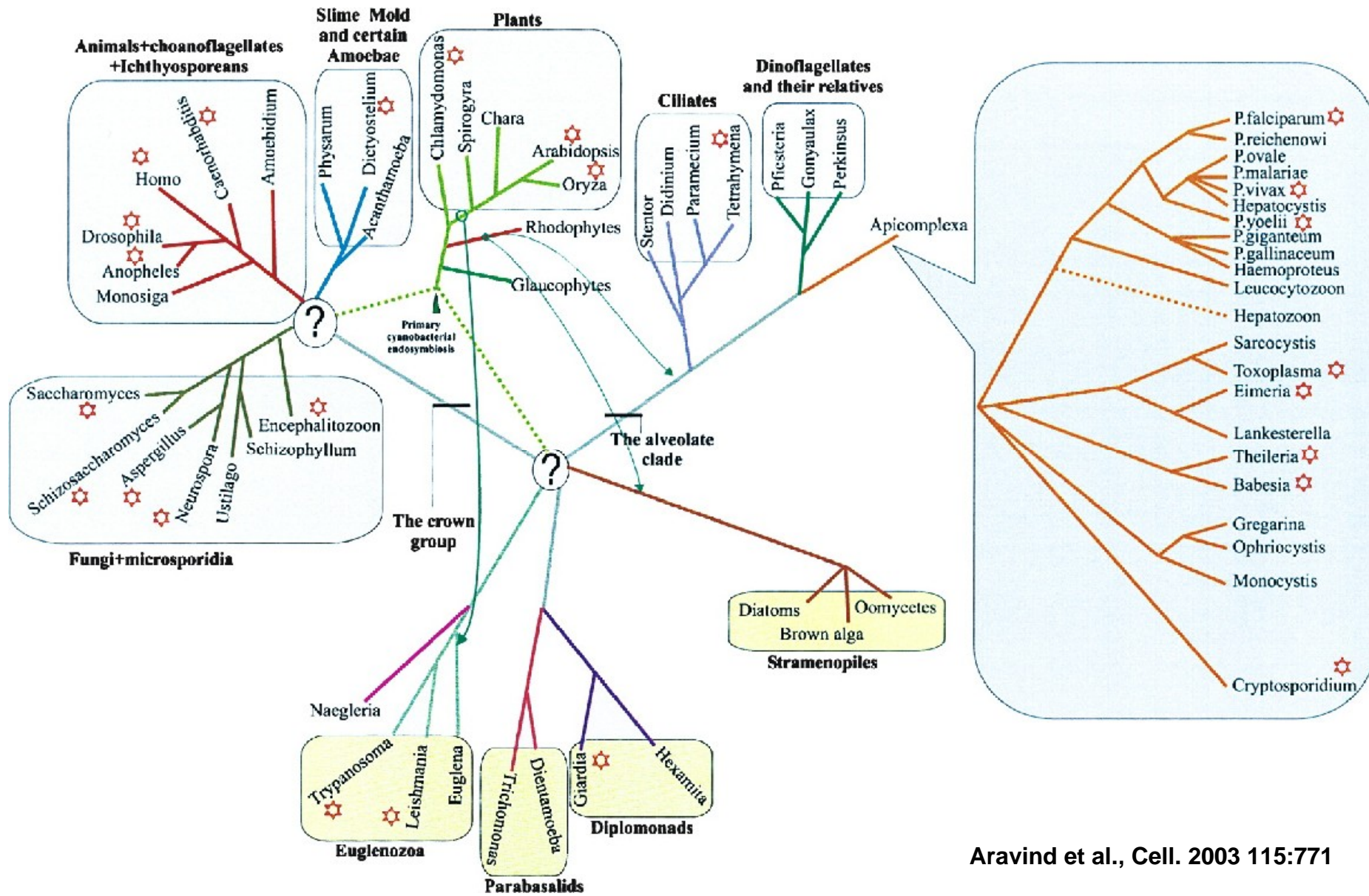
Muséum National d'Histoire Naturelle

INSERM U565 & CNRS UMR 5153 & MNHN 0503

pbastin@mnhn.fr



Sequenced Genomes



Genes identified

- 1. Already known** (case of gene families)
- 2. Novel genes:**

Search for counterparts in various genomes

Search for conserved domains

Groups of genes

And next ?

Others

**(regulatory sequences, transposons,
telomeres, centromeres etc)**

Modify gene expression

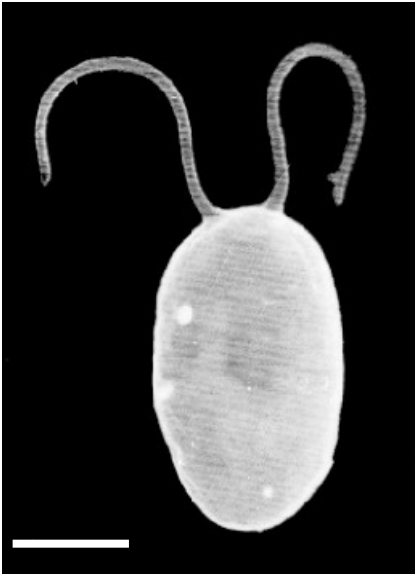
1. Reduce/delete expression

Gene knock-out/RNA interference

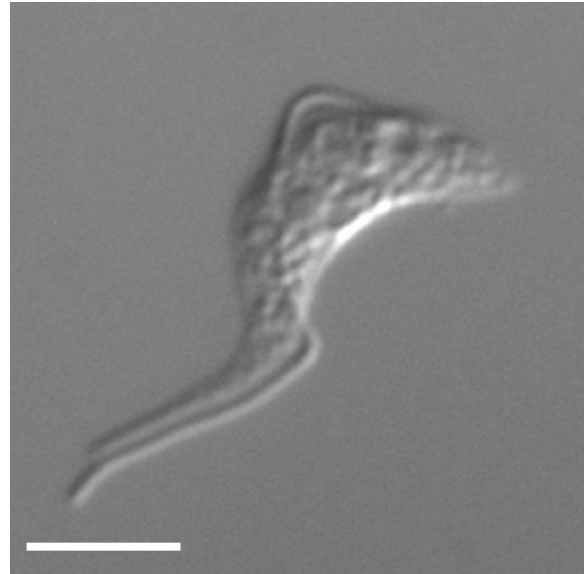
2. Over-expression

3. Dominant-negative

Cilia and flagella



Chlamydomonas
(green algae)



Trypanosoma
(kinetoplastid)

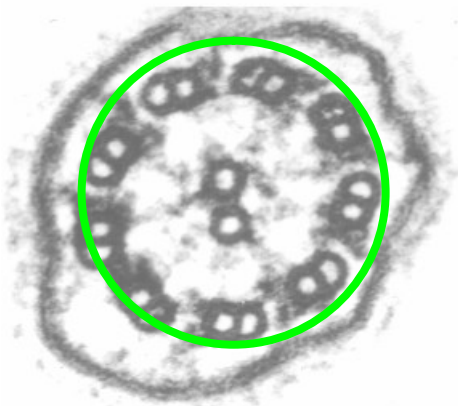


Homo
(mammal)

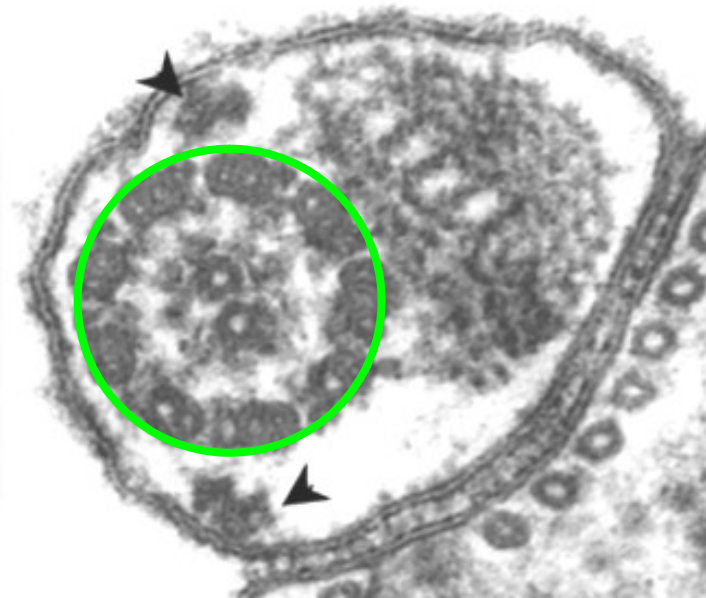
Bar = 5 μ m

Basic unit is conserved

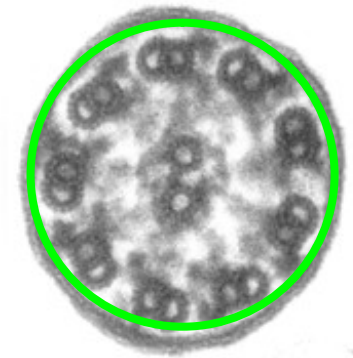
AXONEME



Chlamydomonas
(green algae)

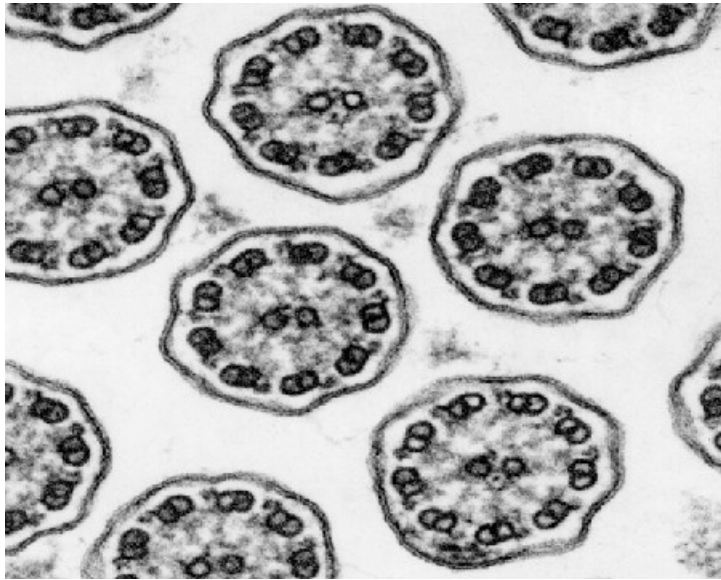


Trypanosoma
(kinetoplastid)



Homo
(mammal)

Composition of flagella

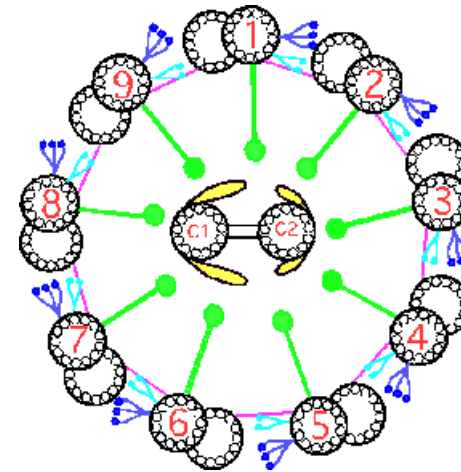


Human cilia (E. Escudier)

9 + 2 structure

250 proteins (~40 identified)

Additional structures



- Tubulins ($\alpha/\beta + \gamma$)
- Dynein arms
- Nexin links
- Radial Spokes
- Central pair projections

Kohl & Bastin (2005), *Int. Rev. Cytol.*, in press

Identification of new flagellar genes

**Differential display
Human ciliated cells**

**Cécile Ponsard
Sandrine Middendorp
Fred Tournier
Paris VII**

**Genetics in *Drosophila*
RFX-controlled genes**

**Anne Laurençon
Raphaëlle Dubruille
Bénédicte Durand
Lyon**

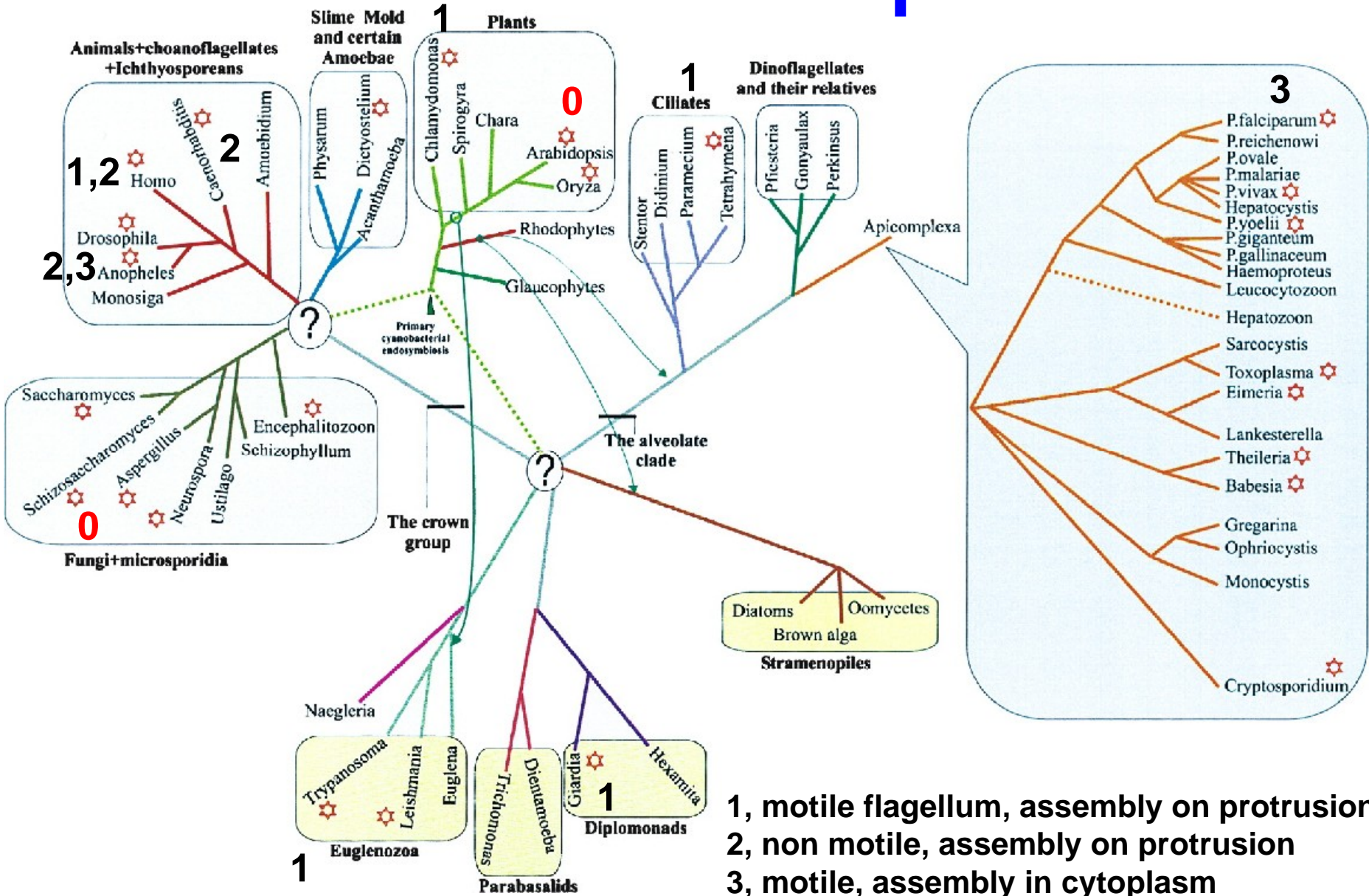
Human genetic diseases

**Anne Moore
Serge Amselem
Estelle Escudier
Créteil**

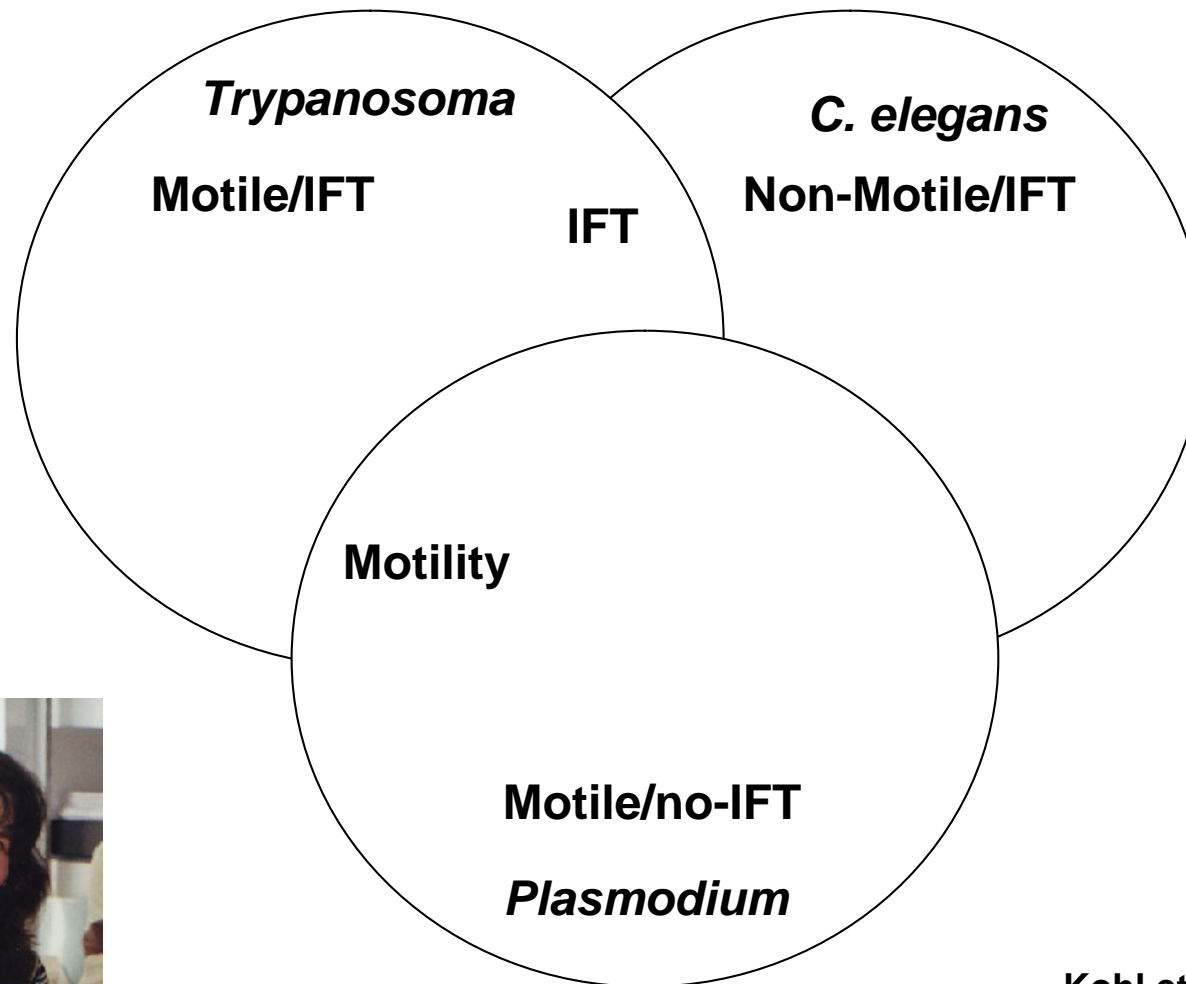
Comparative genomics

**Linda Kohl
MNHN, Paris**

Genome comparison



Gene categories



Linda Kohl

Kohl et al., 2004
Li et al., Cell 117, 541
Avidor et al., Cell 117, 527

Identification of flagellar genes

CHE2	301	Tb10.61.1560	SB	86.6	782	WD40	IFT in C.e.	IFT80 (CG9333)	I	ND	
CHE11	302	Tb10.61.2260	SB	186.0	1696	WD40	IFT in C.e	OSEG3 (CG11838)	I	ND	
OSEG4	303	Tb05.26K5.80	SB	140.6	1260	WD40,TPR	?	OSEG4/ TUBBY	I	++	
OSEG6	304	Tb11.03.0880	SB	149.0	1356	WD40	?	CG11237	I	ND	
DPCD¹	305	Tb11.01.0630	SB	23.0	203		IA dynein ?	CG13901	x	ND	
B9	306	Tb11.03.0750	SB	20.4	184	adhesion	ciliary differentiat ^o .	CG14870/CG9227	I	+	
B9bis	307	Tb10.61.2290	SB	31.8	286	adhesion	?	CG14870/CG9227	I	+	
PACRGA²	308	Tb09.211.1470	SB	35.3	312	Arm spermatogenesis		CG15120	P	++	
PACRGB^{2,3}	309	Tb03.4808.610	SB	33.7	300	Arm spermatogenesis		CG15120	P	++	
A1	310	Tb03.27F10.120	SB	52.7	477	SF-assemblin	?	CG17599	I	++	
B2	311	Tb03.5h5.700	SB	77.6	675	TPR	?	CG5142	I	++	
C3	312	Tb03.27C5.470	SB	64.8	566	TPR	?	CG4525	I	++	
BBS1	321	Tb09.211.2080	CB	67.1	609		?	CG14825	I	+	
BBS2	322	Tb06.4M18.130	CB	80.1	736	WD40,	FG-GAP?	not in Dm (but in Ce)	x	+	
BBS4	323	Tb10.6h15.3940	CB	51.8	466	TPR	Trafficking?	CG13232	P	+	
BBS5	324	Tb927.1.4140	CB	41.0	369	DM16?	?	CG1126	I	+	
BBS7	325	Tb03.28C22.950	CB	82.5	751	FG-GAP	?	not in Dm (but in Ce)	x	+	
BBS8	326	Tb11.01.4290	CB	62.6	568	TPR	?	CG13691	I	+	
MBO2	327	Tb04.1H19.60	CB	114.7	1000	CC	Motility	CG5882	M	?	
MBO2bis	328	Tb11.01.2670	CB	99.7	848	CC	Motility	CG5882	M	?	
F6 (ABC)	329	Tb11.01.8700	CB	79.1	719	ABC transp.	?	CG4225	A	NF	
G7 (SLA⁵)	330	Tb11.01.4640	CB	58.9	545	SLA	?	CG1427	A	ND	
H8	331	Tb10.26.0830	CB	52.5	486	UPF0027	?	CG9987	A	ND	
I9	332	Tb05.3C6.750	CB	46.0	410		?	CG13178	I	ND	
Cofactor C	341	Tb11.01.1240	LK	34.0	308	cyclase?	Tub Chaperone	also in plants	x	ND	
Cofactor D	342	Tb08.11J15.520	LK	147.6	1343	Arm	Tub chaperone	also in plants	x	ND	
P28	343	Tb10.26.0070	LK	42.0	373		Motility	CG5987	A	?	
KLP1	344	Tb07.2F2.620	LK	96.9	891	kinesin, N-ter	?	KIF9	x	?	
KIF	345	Tb07.21H15.40	LK	113.3	1041	kinesin, N-ter	?	KIF9	x	?	
HYDIN	346	Tb06.5F5.940	LK	>500	4521		hydrocephalus	not in Dm and Ce	x	++	
LRP⁴	347	Tb03.48K5.370	LK	44.0	383	Leu-rich	dynein LC ?	CG14620	x	++	
J10	348	Tb03.30P12.250	LK	112.8	1027	WD40	MAP?	not in Dm	x	++	
K11	349	Tb11.02.5150	LK	58.6	507	?	NYD-Sp28	not in Dm/Ce	x	++	
L12	350	Tb06.30P15.410	LK	64.8	552	Leu-rich	?	Y in Dm CG13125		++	

Genes involved in flagellum assembly

DHC1b	LK	484.9	4307	DHC,P-loop	IFT	DHC2	?
DHC1bis	Tb04.5D20.75 SB/FR	427.5	4232	DHC	IFT	DHC2	?
LC8	LK			LC	Multiple		?
KIF3A	LK			CC, KHC		IFT	FLA10
IFT20	Tb06.2N9.700 SB/FR	15.4	130	DUF	IFT		I
IFT27	FR/SB			G protein		IFT ?	ARL3
IFT52	Tb10.61.1590 FR/SB	72.3	655		IFT	OSM6,NGD5	I
IFT57	Tb10.26.0670 FR/SB	46.3	413		IFT	HIPPY	I
IFT88	Tb11.55.0006 LK	89.7	800	TPR	IFT	Tg737, OSM5,NUMPB	I
	F						
IFT122	Tb10.70.1660 FR/SB	139.4	1242	WD40	IFT	DAF10	I
IFT172	Tb10.70.6920 FR/SB	196.6	1747	WD40	IFT	OSM1	I
CHE2	Tb10.61.1560 SB	86.6	782	WD40	IFT in C.e.	IFT80 (CG9333)	
CHE11	Tb10.61.2260 SB	186.0	1696	WD40	IFT in C.e.	OSEG3 (CG11838)	
OSEG4	Tb05.26K5.80 SB	140.6	1260	WD40,TPR,Zn		?	OSEG4/TUBI ++
OSEG6	Tb11.03.0880 SB	149.0	1356	WD40	?	CG11237	I
DPCD¹ 305	Tb11.01.0630 SB	23.0	203			IA dynein ?	CG13901
B9 306	Tb11.03.0750 SB	20.4	184		adhesion ?	ciliary diffentiat ^o .	x
	CG14870/CG9227	I	+				
B9bis	Tb10.61.2290 SB	31.8	286		adhesion ?	?	CG14870/CG922
PACRGA²	Tb09.211.1470 SB	35.3	312		Arm	spermatogenesis	
	CG15120 P	++					
PACRGB^{2,3}	Tb03.4808.610 SB	33.7	300		Arm	spermatogenesis	
	CG15120 P	++					
A1	Tb03.27F10.120SB	52.7	477		SF-assemblin	?	CG17599
B2	Tb03.5h5.700 SB	77.6	675	TPR	?	CG5142	I
C3	Tb03.27C5.470 SB	64.8	566		TPR	?	CG4525
ARL6	Tb08.5H5.790 ED	20.6	189	G	?	CG7735	I
RABL5	Tb11.01.8590 ED	24.4	219	G	?	not in Dm (but in Ce)	ND

Functional studies: choice of model

Trypanosoma brucei

Flagellated organism easy to grow *in vitro* and in animals

Complete genome sequenced

Straight forward molecular biology

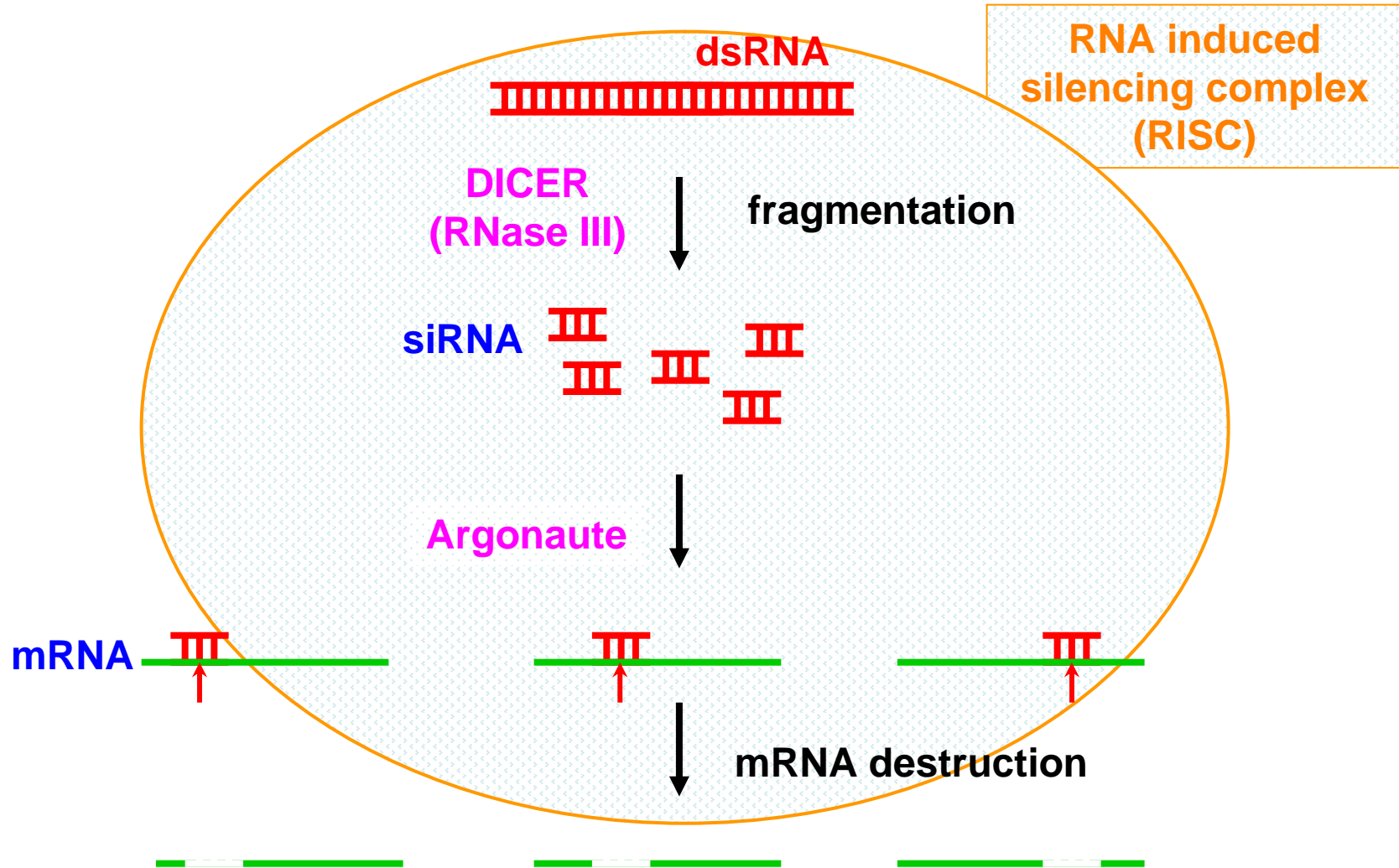
Reverse genetics available (RNAi, inducible expression systems)

Responsible for sleeping sickness

Related to *T. cruzi* and *Leishmania*

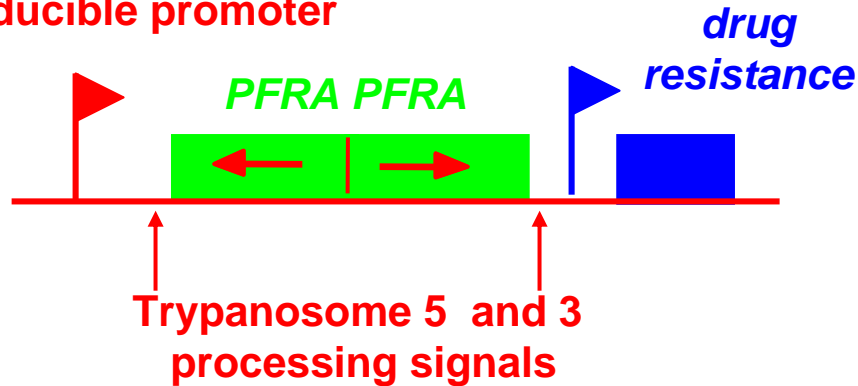
Strain non-pathogenic for humans

RNA INTERFERENCE (RNAi)



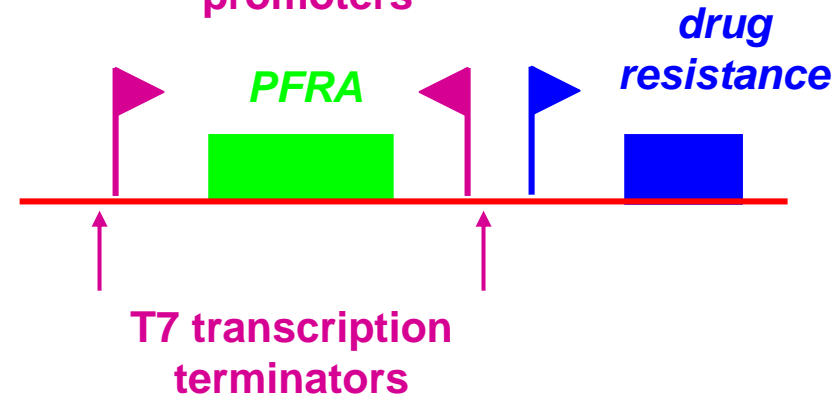
FUNCTIONAL GENOMICS BY RNAi

Trypanosome tet-inducible promoter



Bastin et al., JCS (2000) 113, 3321

T7 tet-inducible promoters



Wang et al., JBC (2000) 275, 40174



self-folding

processed/exported

difficult to clone

more efficient



annealing step

unprocessed

easy to clone

more specific

Durand-Dubief, Kohl & Bastin, MBP (2003) 129, 11-21

FUNCTIONAL GENOMICS BY RNAi



TrypanoFAN: *RNAi*t - target selection script

Efficiency/specificity

Durand-Dubief et al., MBP (2003) 129, 11-21

Exploited for target selection script

Redmond S, Vadivelu J, Field MC.
MBP (2003) 128, 115-8

<http://www.trypanofan.org/software/RNAit.html>

Functional complementation

Rusconi et al., BMC Biotechnology (2005) 5:6

This script uses [MIT Primer3](#) and [NCBI Blast](#) for the identification of primers for production of RNAi constructs, considering oligonucleotide melting temperature and PCR product size. The program is intended to prevent crosstalk between related gene products in the design of RNAi experiments.

please note: alteration of the selection criteria will affect the fragment which is blasted, and therefore may alter the result given.

paste your DNA sequence here (5'→3' ascii/fasta)

or select a file to upload (single sequences only)

blast stringency: 80% → 99%

subunit length: 20

database: *Trypanosoma brucei* *Leishmania major*
 Toxoplasma gondii *Brugia malayi*

primer melting temp: 60°C

PCR product size range: 400 bp → 600 bp

RESET SUBMIT

RNAi screen

50 genes were selected

40 already silenced (20 characterised)

8 involved in flagellum formation

3 involved in flagellum motility

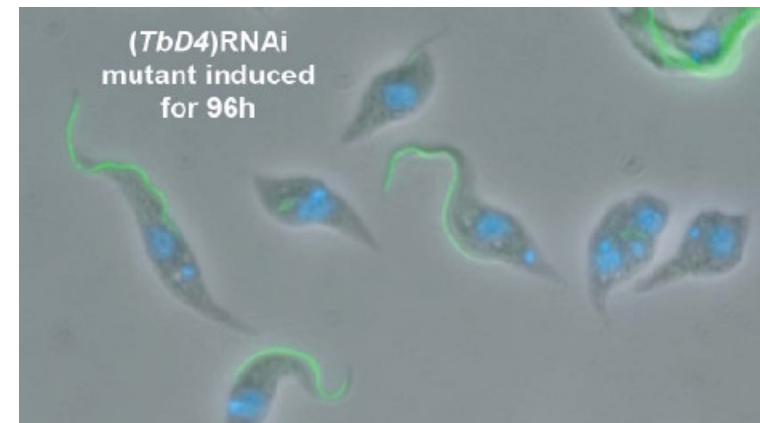
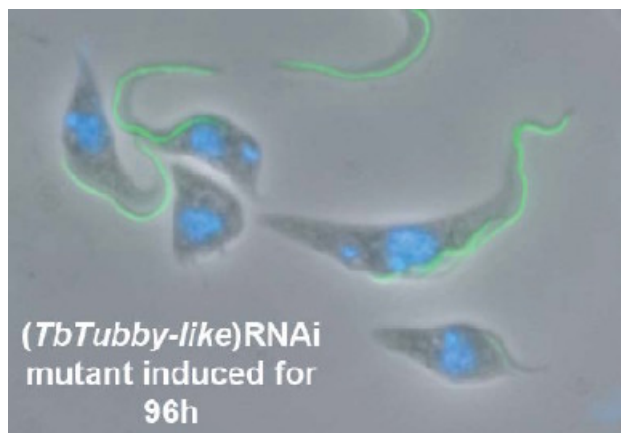
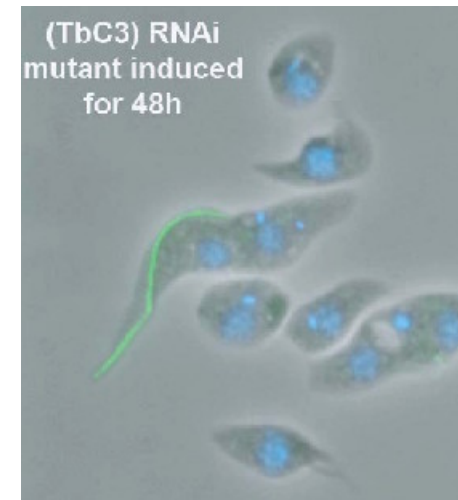
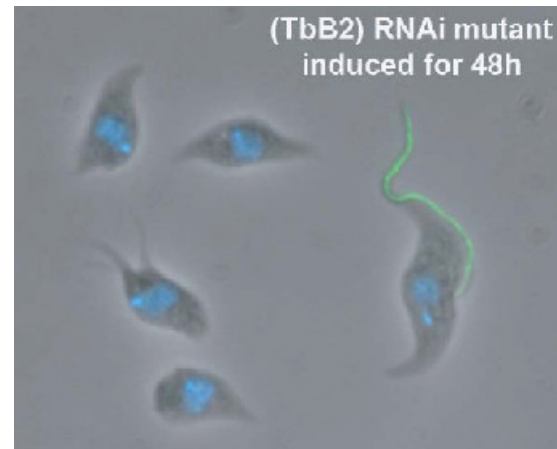
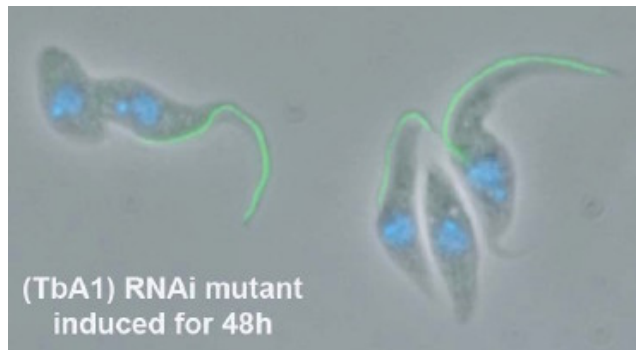
1 involved in flagellum attachment

1 involved in flagella connexion

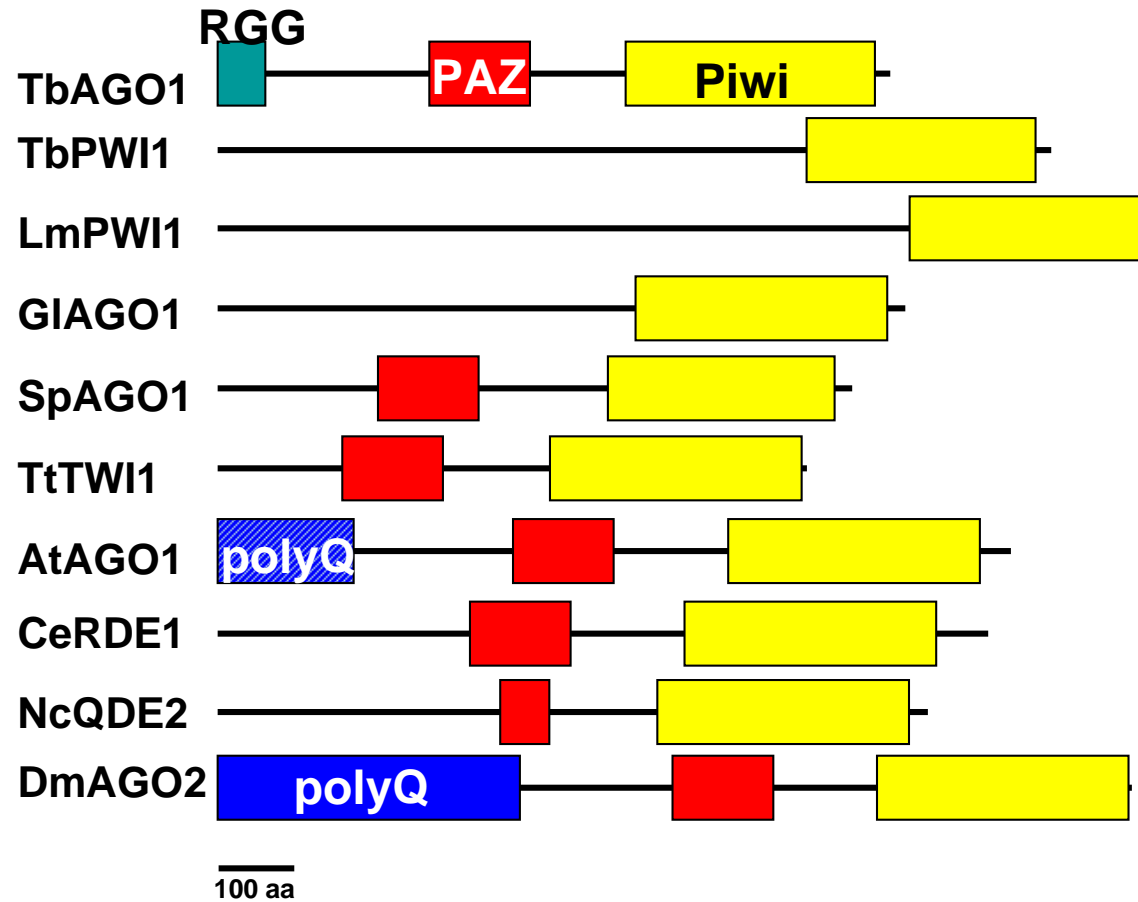
1 nuclear phenotype

Genes involved in flagellum assembly

5 genes predicted as involved in flagellum assembly on cell protrusion

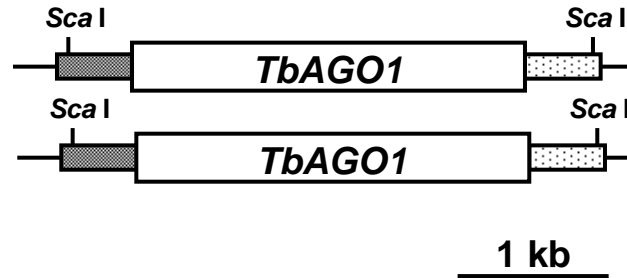


Argonaute genes (central to RNAi)

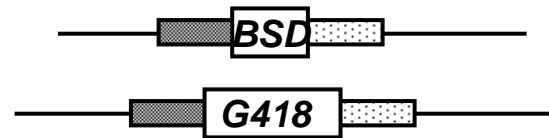


Generation of *TbAGO1*^{-/-} KO cell line

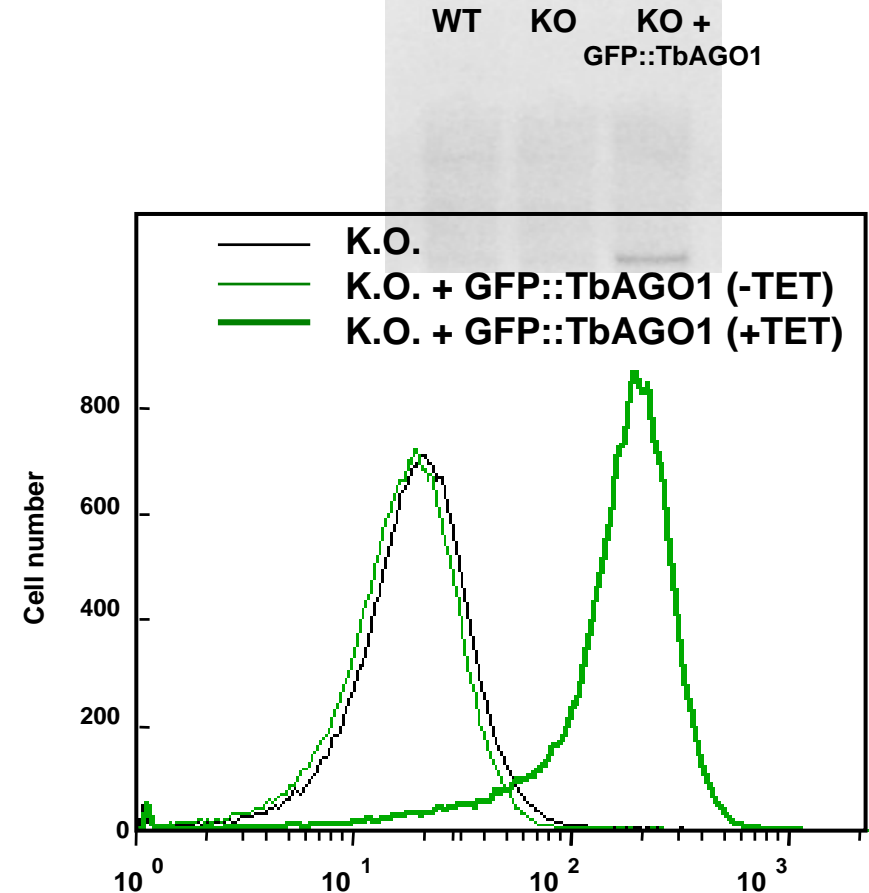
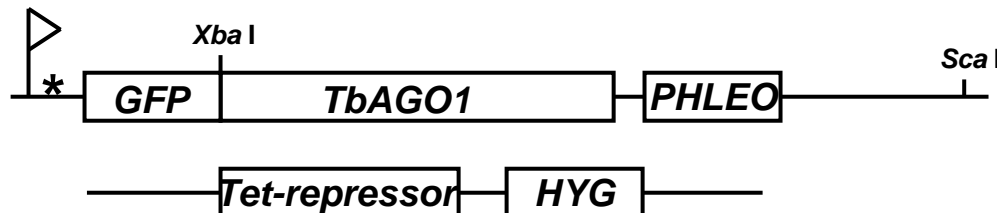
WT



KO

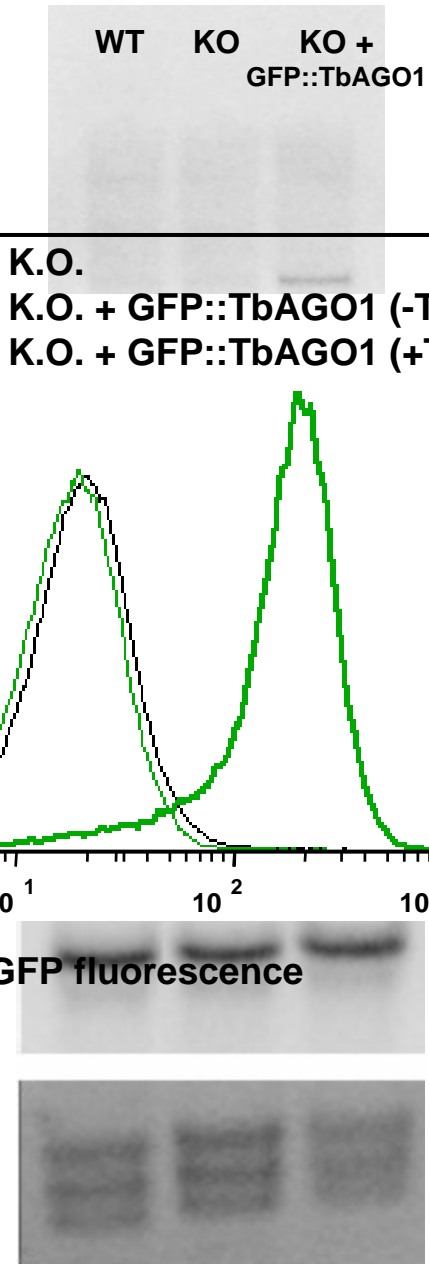


KO + GFP::*TbAGO1*



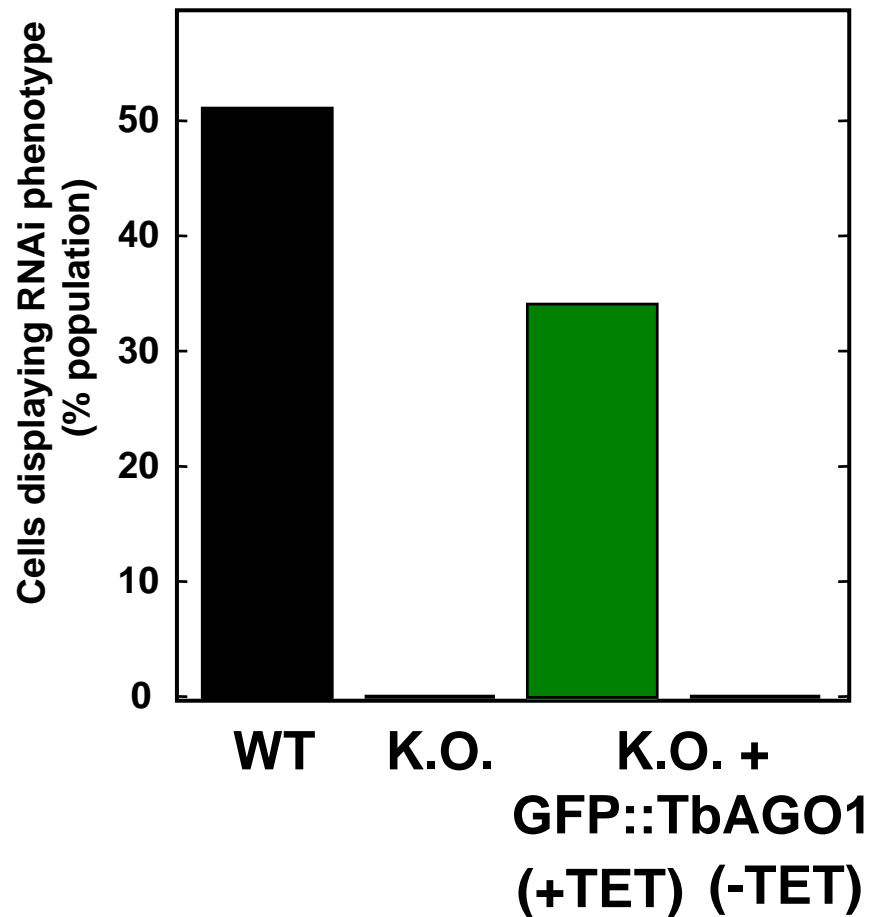
TUBGFP fluorescence

rRNA

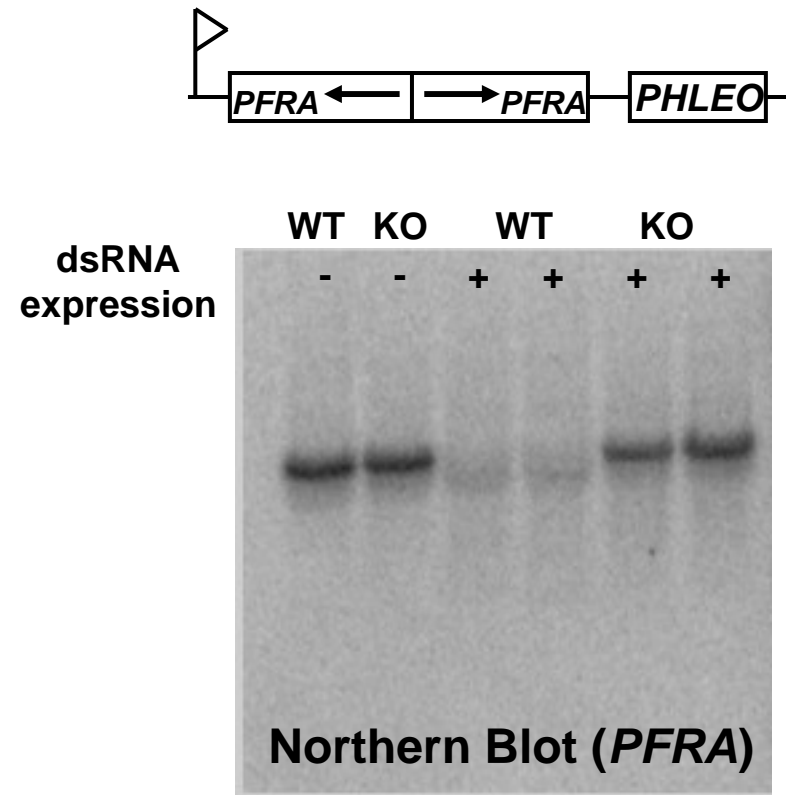


TbAGO1 is required for RNAi

Transfection of dsRNA



Expression of dsRNA



Durand-Dubief M. & Bastin P., *BMC Biology*, 1:2 2003

FUNCTIONS OF TbAGO1

- 1. TbAGO1 is essential for RNAi in *T. brucei***
- 2. The RNAi machinery appears required for proper chromosome segregation at mitosis BUT cells can compensate**
- 3. RNAi required for control of retroposon and associated pseudogene transcripts**

Conclusions

1. Power of comparative genomics
2. Efficiency of RNAi/gene KO
3. Specificity of protists

Philippe BASTIN

(Muséum National d Histoire Naturelle, Paris, Dir. C. GIOVANNANGELI & J.S. SUN)



Linda KOHL



Carole BRANCHE

**Sabrina BENGHANEM
Géraldine TOUTIRAIS
Gwénola DORE**

**Emmanuelle
DELANNOY**



**Sandra
NGWABIT**



**Mickaël
DURAND-
DUBIEF**

Mélanie BONHIVERS/Derrick ROBINSON *(Université Bordeaux II)*

Bénédicte DURAND *(Lyon)*

Serge AMSELEM / Estelle ESCUDIER *(Créteil)*

Frédéric TOURNIER *(Paris VII)*

K. GULL, P. ENGLUND, C. CLAYTON, G. CROSS

Muséum National d Histoire Naturelle (Paris)

Philippe BASTIN

Funding

ATIPE CNRS

FRM

ACI Dynamique et réactivité des assemblages biologiques

ACI Biologie du développement

GIS (Research on Rare Genetic Diseases)

ESF

Gouvernement Luxembourgeois

EMBO

CONCLUSIONS (1)

1. **TbAGO1 is essential for RNAi in *T. brucei***
2. **The RNAi machinery appears required for proper chromosome segregation at mitosis BUT cells can compensate**
3. **RNAi required for control of retroposon and associated pseudogene transcripts**