

# *Trichomonas vaginalis*



A Toolbox for the study of the  
cytoskeleton

**Geneviève Bricheux, Imad Bakouche et Gérard Coffe,  
Equipe Cytosquelette , UMR CNRS 6023, Biologie des Protistes.  
Université Blaise Pascal**

# Trichomoniasis



**Non-viral STI of worldwide importance  
- 200 million cases every year**



**Clinical manifestations: asymptomatic to flagrant states .  
Unknown factors of virulence**



**Modification of vaginal ecology → predisposition to HIV  
infection and other STI.**

**Premature labor, Premature rupture, infertility, cervical  
cancer.**



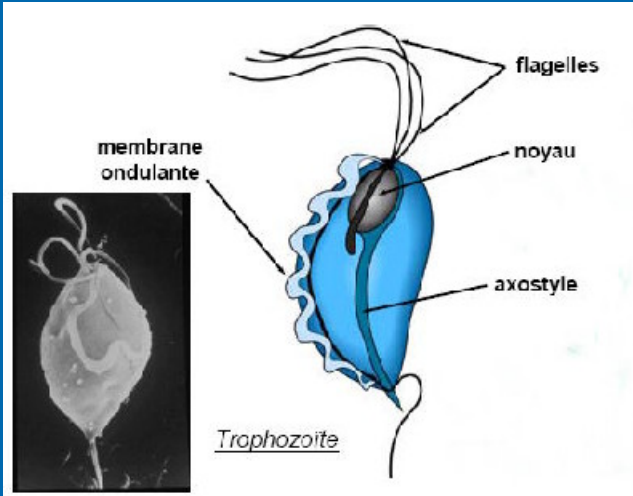
**Only one treatment: Metronidazole but increasing appearance  
of resistant strains. Side effects**



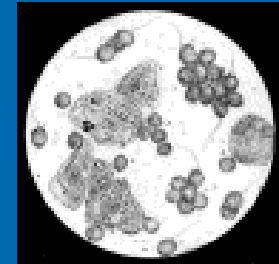
**No vaccine...**



# Trichomonas vaginalis

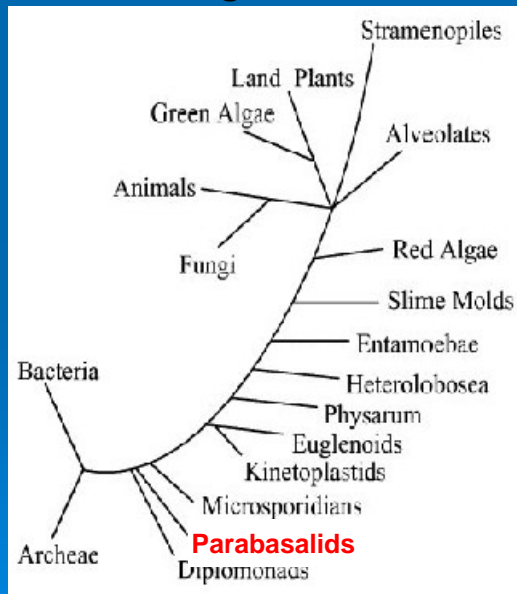


Facultative anaerobe  
Hydrogenosome

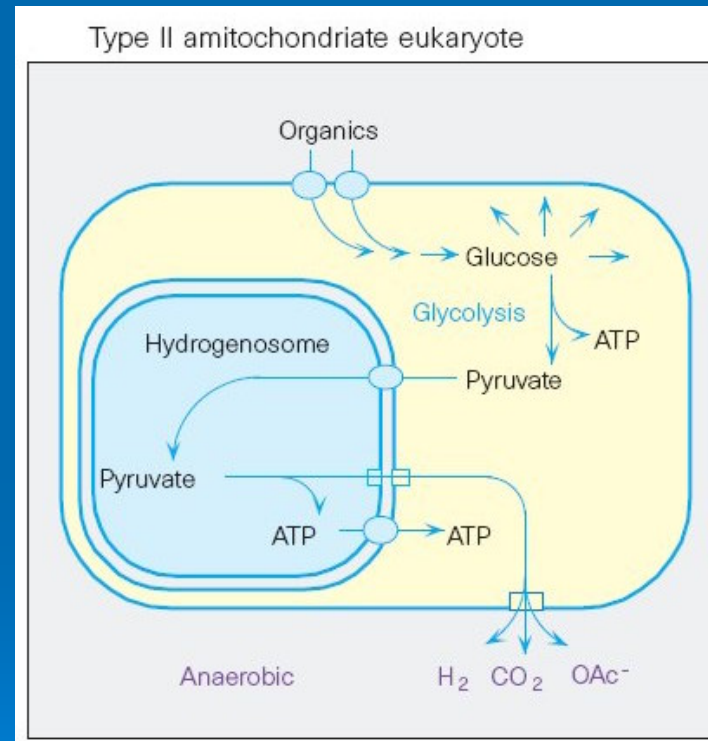


TV in 1845

Primitive eukaryotic  
organism



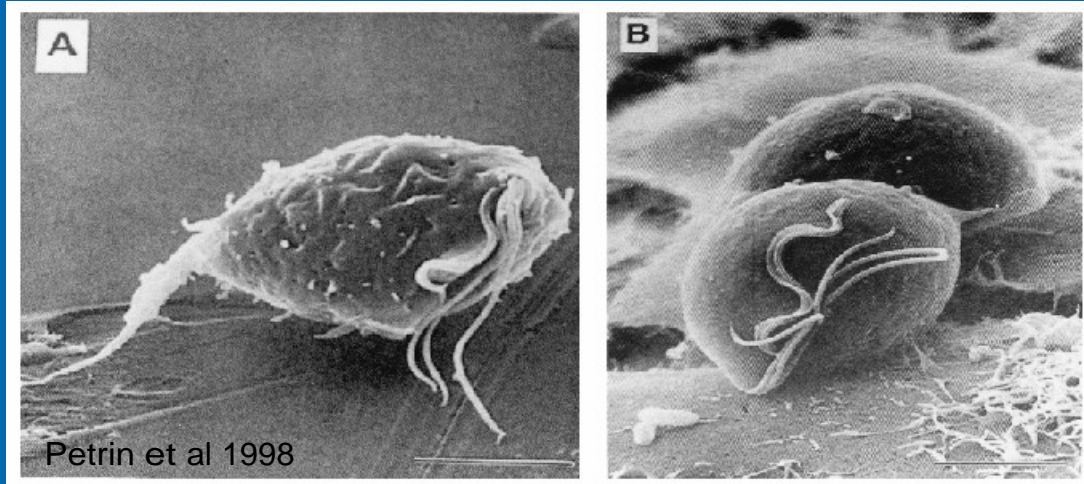
Molecular Phylogeny ssu  
rDNA (Sogin 1991)



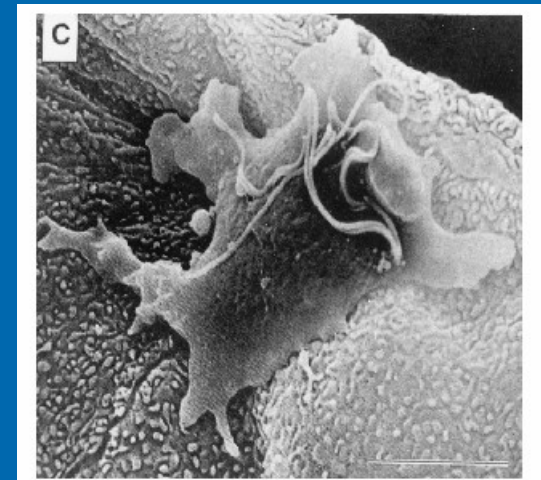
(Martin et Muller, Nature 1998)

# T. vaginalis as seen in culture

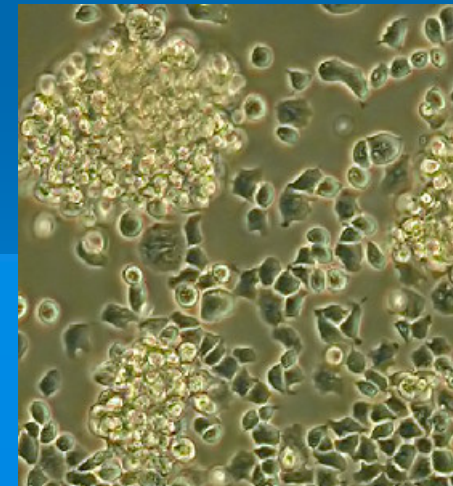
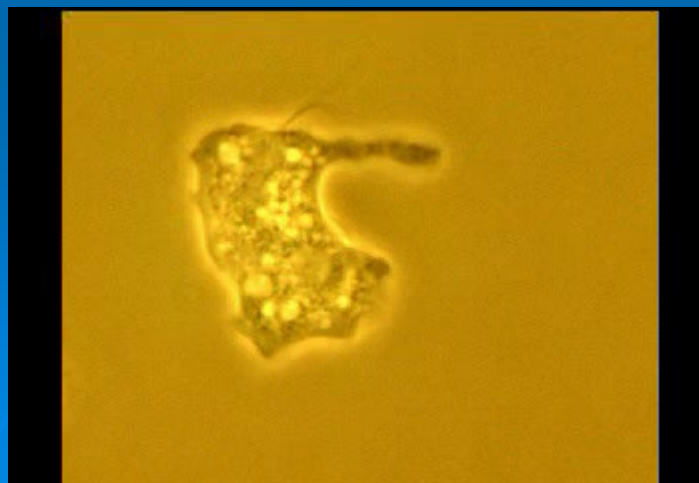
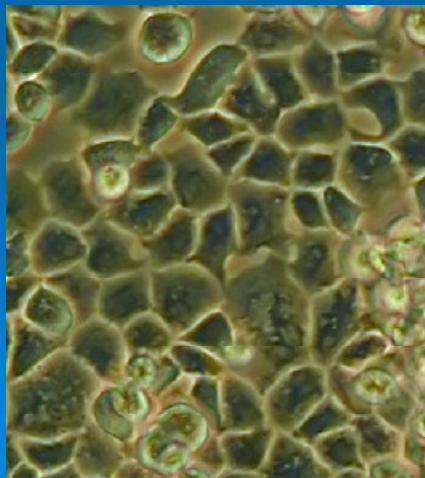
Swimming cell



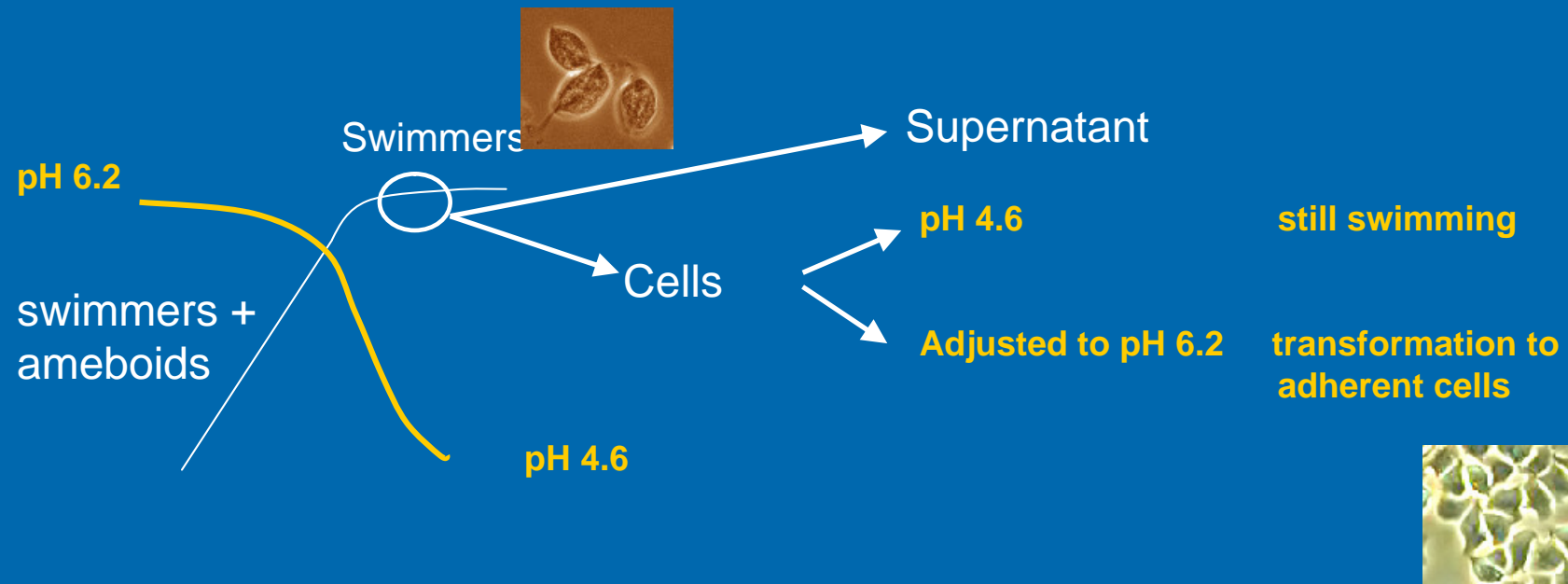
Ameboid cell



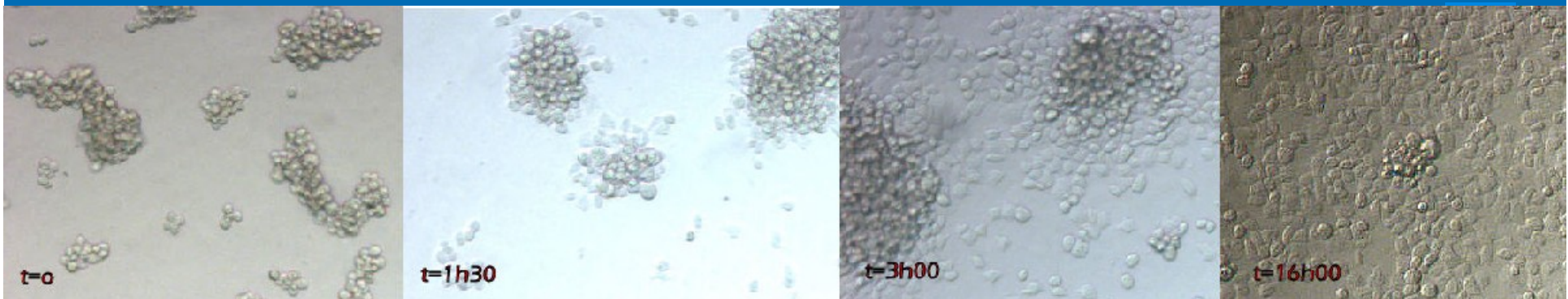
Closed relationship between pathogenicity and adhesion

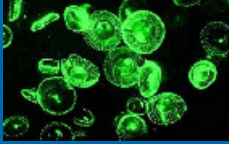


## - Adhesion versus pH



## - Adhesion versus time and cell concentration





## GFP (Green Fluorescent protein)



**GFP** (*Green Fluorescent Protein*) isolated from the jellyfish *Aequorea victoria* was found to fluoresce under excitation, requiring no substrates or coenzymes. –(27 KD)–

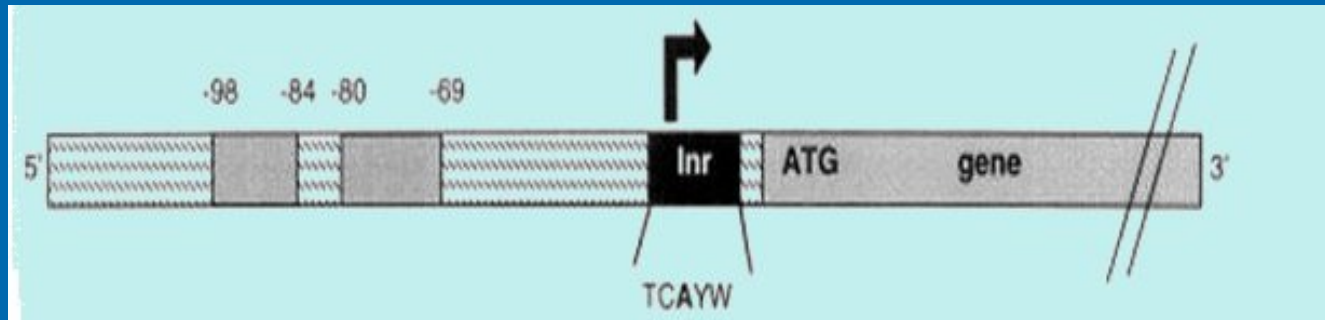
- Fluorochrome produced by auto-cyclization of Ser65-Tyr66-Gly67
- Excitation at 395 - 470nm and emission at 509 nm
- Only oxygen is required for chromophore formation
- Denatured form of GFP is not fluorescent

# Properties of the different GFP

	wtGFP	m-GFP5	EGFP	GFPmut3b
mutations	F <sup>64</sup> S <sup>65</sup> S <sup>72</sup> V <sup>163</sup> I <sup>167</sup> S <sup>175</sup>	A <sup>163</sup> T <sup>167</sup> G <sup>175</sup>	L <sup>64</sup> T <sup>65</sup> I <sup>167</sup> S <sup>175</sup>	G <sup>65</sup> A <sup>72</sup>
pH	5.5-12 mais diminution entre 4 et 5.5	?	7.0-11.5 (50% à 6.0)	6.0-7.0
T°	Bon repliement de la protéine à 30- 33°	Bon repliement de la protéine à 37°	Bon repliement de la protéine à 37°	Bon repliement de la protéine à 34°
Excitation	395 nm	395 et 473 nm	489 nm	488 nm
fluorescence	Souvent insoluble	Augmente le repliement et solubilité	X par 35 et bonne solubilité	x21
Detection limit	~1 µM	?	~30 nM ~4,000 molécules	?

FITC 488 nm

# Promoter structure for Trichomonas



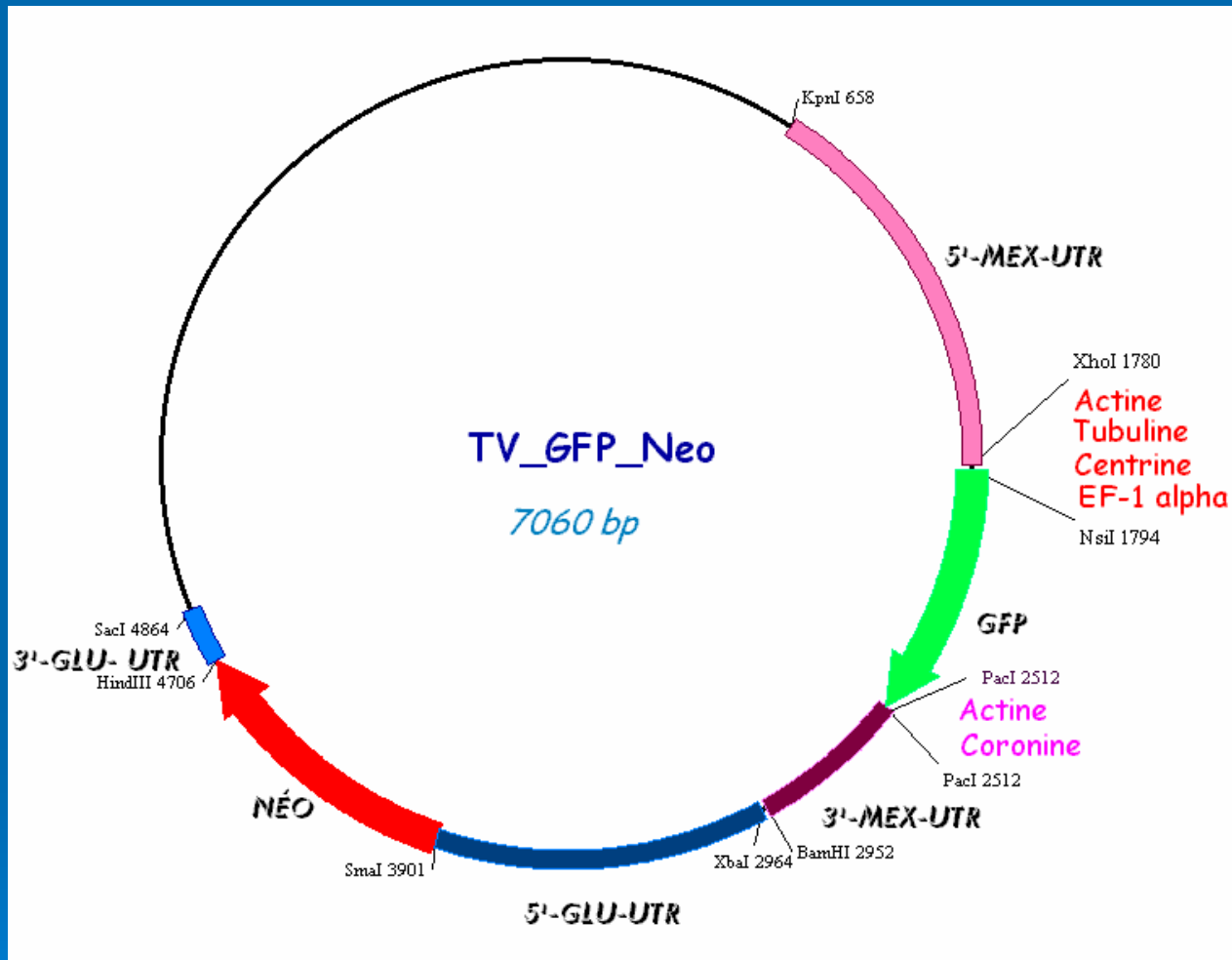
Vanacova S et al Int J Parasitol 33 (2003)

-Homology with the metazoan initiator core promoter (the TATA-less promoter of the murine TdT) (terminal deoxynucleotidyl transferase). Highly conserved

-Three 5'UTR tested *pAP/luc* (750 x) , *pTUB/luc* (30 000x) and actin VI

DNA regulatory sequences

# Construction of the Trichomonas plasmids



5' and 3' UTR Tubulin  
5' and 3' UTR AP 65  
5' and 3' UTR Actin VI

M-GFP5  
EGFP  
GFPmut3b

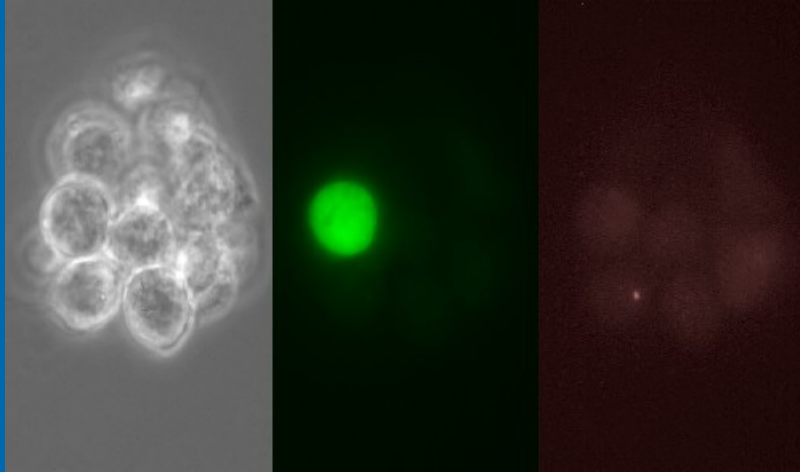
Neomycin  
Blasticidin

# Expression of GFP fused proteins in *Trichomonas vaginalis*.

Contraste de phase

GFP

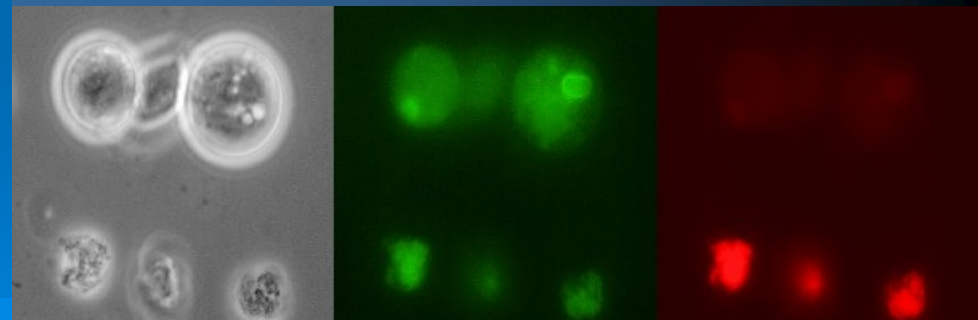
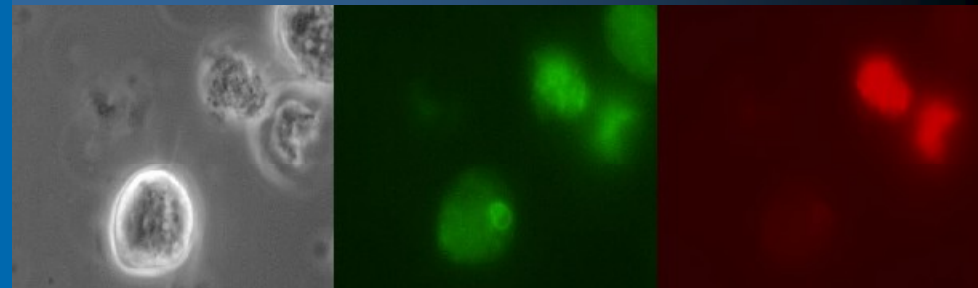
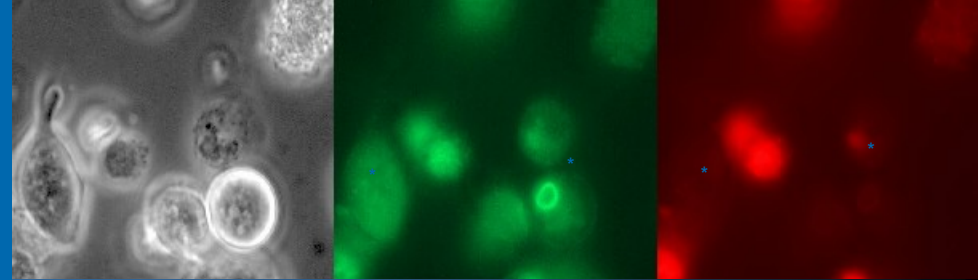
Rhodamine



Contraste de phase

GFP

Rhodamine

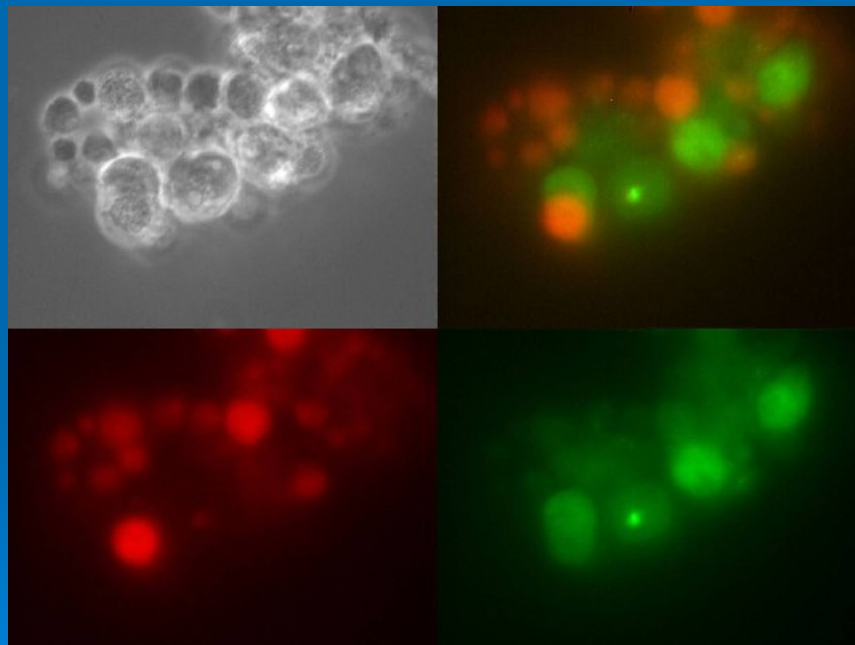
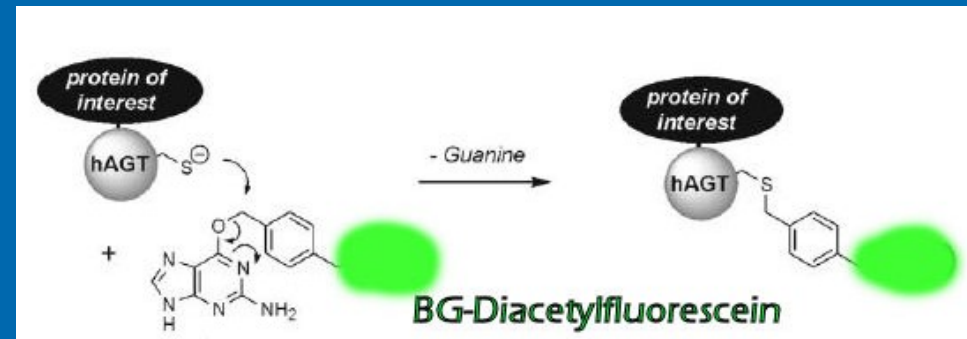


Vecteur GFP

Vecteur EF1 $\alpha$ - GFP

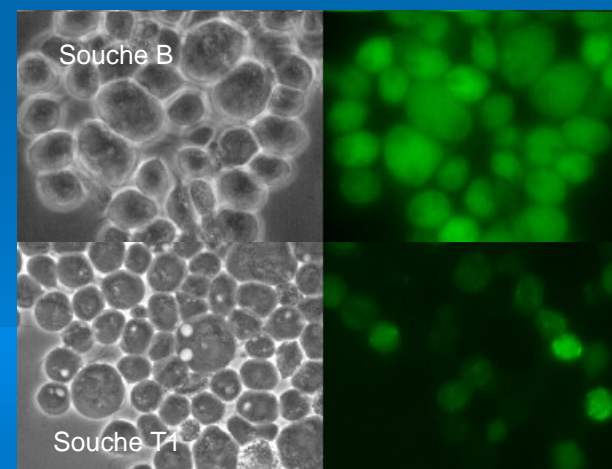
# The SNAP-Tag™ Technology

SNAP-tag is a protein sequence based on mammalian alkylguanine-DNA-alkyl transferase (AGT) and his substrate, a derivative of benzylguanines.



Vecteur EF1a-GFP

Substrat seul sur les différentes souches de TV

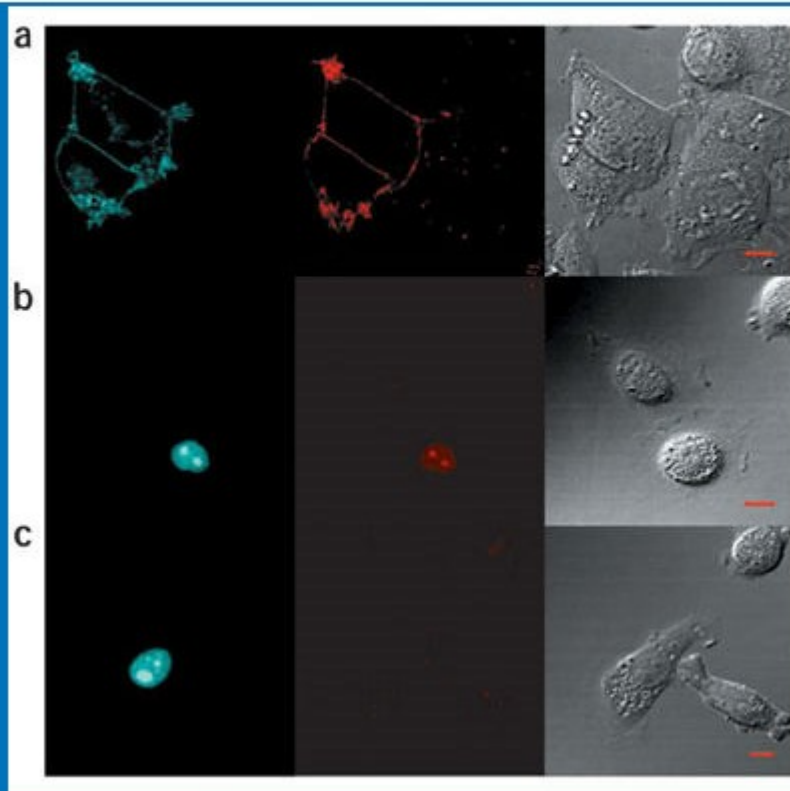


# eDHFR (Dihydrofolate réductase)/Trimethoprim A chemical tag ...

*Nature Methods* 2, 255 - 257 (2005)  
Published online: 23 March 2005; | doi:10.1038/nmeth749

## *In vivo* protein labeling with trimethoprim conjugates: a flexible chemical tag

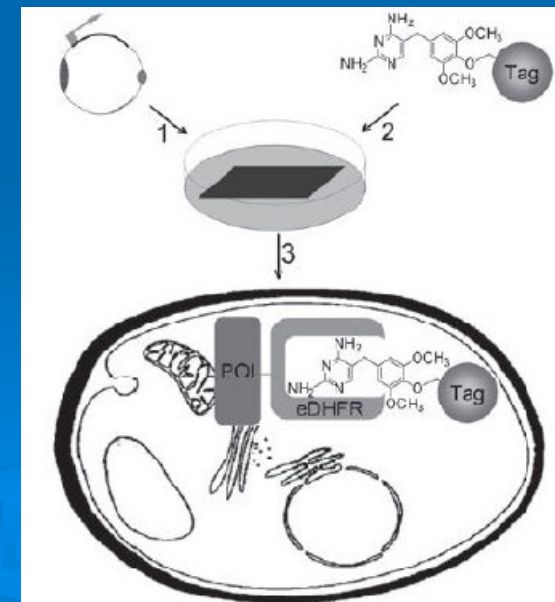
Lawrence W Miller, Yunfei Cai, Michael P Sheetz & Virginia W Cornish



**Figure 2. Selective chemical labeling of subcellularly targeted eDHFR in wild-type CHO cells.**

The living cells are transfected with DNA encoding the protein of interest fused to the eDHFR.

Upon expression of the receptor fusion, a cell permeable ligand (TMP) coupled to a detectable tag is added to the medium .



**GFP:** Few fluorescent cells  
transient expression ...

It's easier to get green rabbit than green  
Trichomonas

