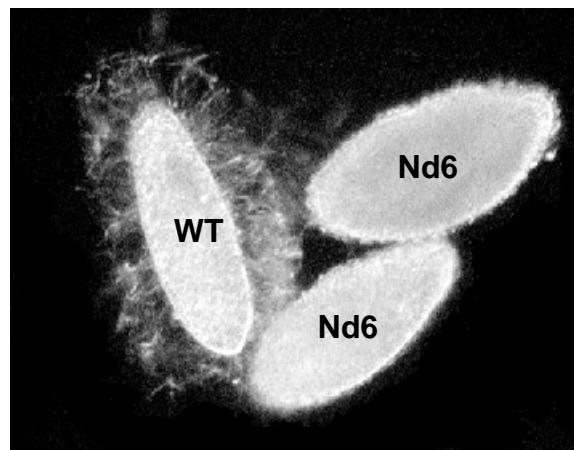
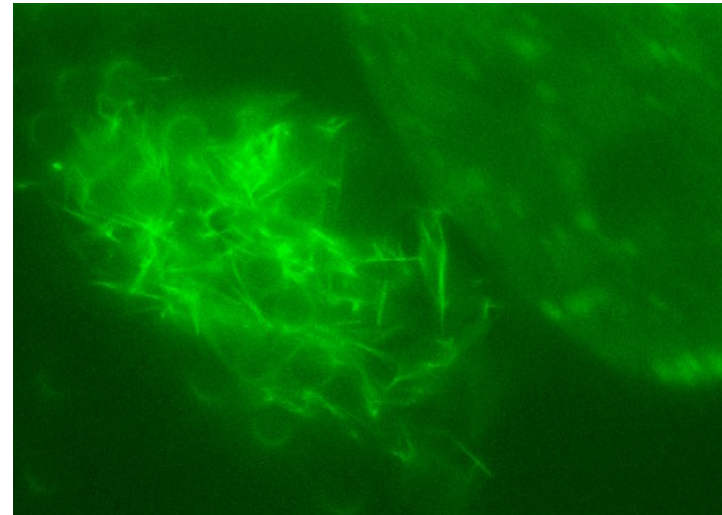
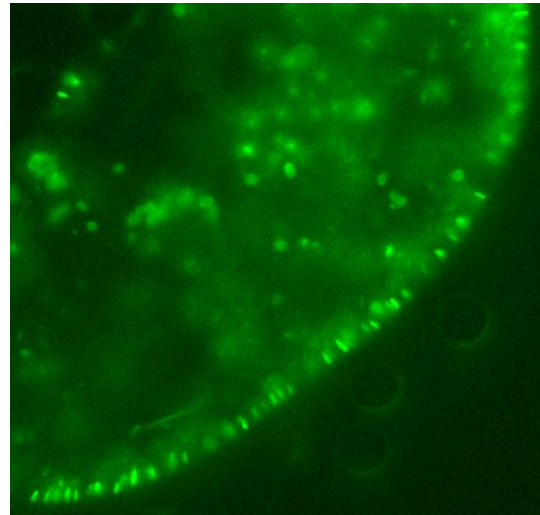


**EXPRESSION OF A PARAMECIUM
PROTEIN IN TETRAHYMENA
THERMOPHILA :
ND6P INVOLVED IN EXOCYTOSIS**

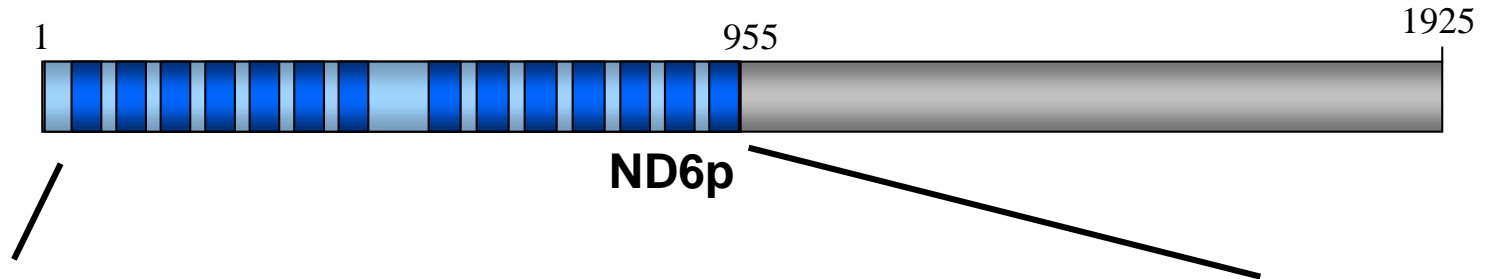
**Delphine Gogendeau, Rachel Lescasse, Anne-Marie Keller
Jean Cohen and France Koll**

**Centre de Génétique moléculaire,
Gif sur Yvette**

THE REGULATED SECRETORY PATHWAY OF TRICHOCYSTS IN PARAMECIUM



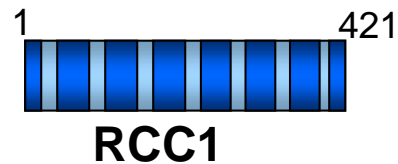
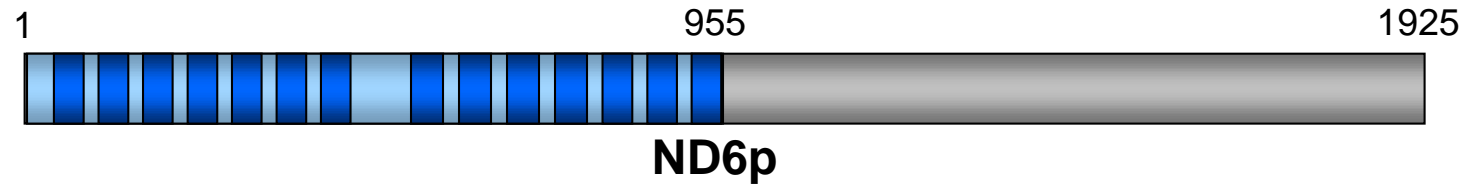
THE ND6p PROTEIN



ND6p

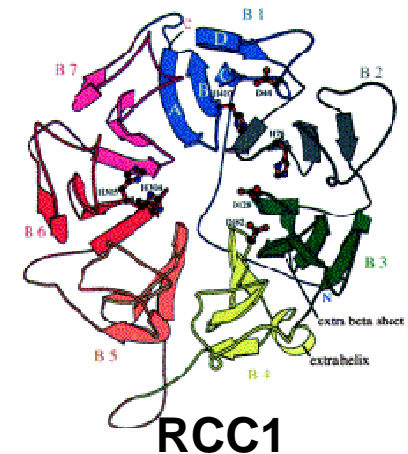
I	15	-	FARLAAG-VNHCLAIQSE-----	GD(9) LWGWGSNEFQAAGKPESIDAEIQ-----	PSPV	69
II	82	-P	LQVAG-NQHSMVLACSQD--(31aa)	-I--FSSG-SDEFGQQGRMTDKEQSQ-----	153	
III	188	-	VCQISCG-ANYTLALDIY-----	GSV--YSWG-EGATGCLGLP-----	MQVDQQR	PTRI 132
IV	239	-	MKFISAGPSHAASISEGQ-----	GSL--YTWG-VGTYGQLGHG-----	INKSSNV	PMSV 184
V	294	-	VEYVSCG-AMHTACTTTD-----	GYA--HTFG-FNKNQQLGTG-----	DFNDRWE	PVKV 338
VI	347	-	YVYAKCG-GNTTFITTLER-----	LT--FAFG-SNSKGRLGK-----	DEFGSNY	PEPQRI 193
VII	404	-	IYQLALS-YHYGLALLQS-----	GSI--ISFG-LPFK GILGRD-----	LQAELL	PKNY 448
VIII	522	-	IVDVKCG-AFHTVALTDA-----	GEV--WVWG-SNKC MQHLKDEQIEQVKTRTKFIGPFIQETIAETSY	PSQV	587
IX	598	-	VTYLATG-LEYVVVENRR-----	N--I--YSWG-KNDKGQLGLG-----	FVSDFVET	PSML 643
X	651	-	VKQVSCG-EDHTLL LDEG-----	GSL--YAVG-SPVHGKLG LG-----	PKNSVVLQTQKV	PFIF 700
XI	701	-	VAKVAVG-PHHSMAVIDK-(23aa)-V	I--YTWG-NGYGGKLG HG-----	NLDNQIYY	PKLL 769
XII	775	-	FKDVSAG-TNHSGLTIDD-----	Q--I--VWVG-VGS -YLGIAKPEDD TDDKQHLKSDKDQEAEE-----	PYYI	831
XIII	846	-	YKSLCLG-DRYNMALSQKN-----	KA--FVWG-LFDYDFQEKLNQCANAAQREKYVEVLMT--	NIVNPLCD	906
XIV	909	-	FTTVSCS-FNHAVALTENQ-----	D--VKELFSWGDGMTRLGLG-----	YEFIN	PDKE 955
			Φ-----G-----Φ	GΦ-----FG-----LG		P-F

THE ND6p PROTEIN CONTAINS TWO RCC1-LIKE DOMAINS



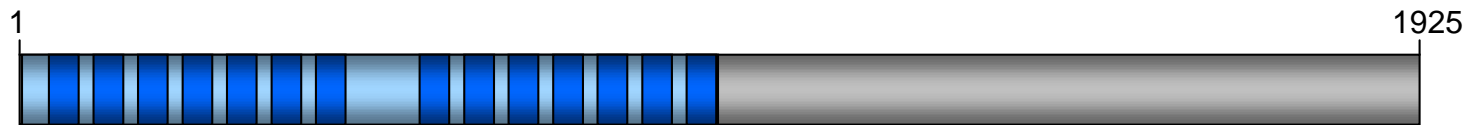
The RCC1 protein:

- Structure in β -propeller
- Conserved from yeast to human
- Involved in nucleo-cytoplasmic transport
- GTP exchange factor for the small GTPase ran
- The RCC1 domains are known to be domains of protein-protein interactions



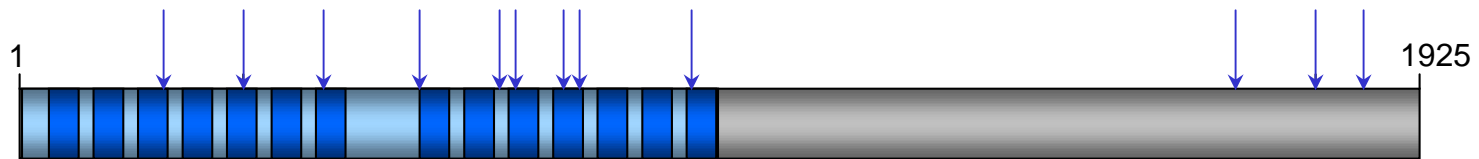
THE MUTATIONS IN ND6p ARE IN MAJORITY FOUND IN THE RCC1 DOMAINS

- Sequencing of the 12 alleles of ND6



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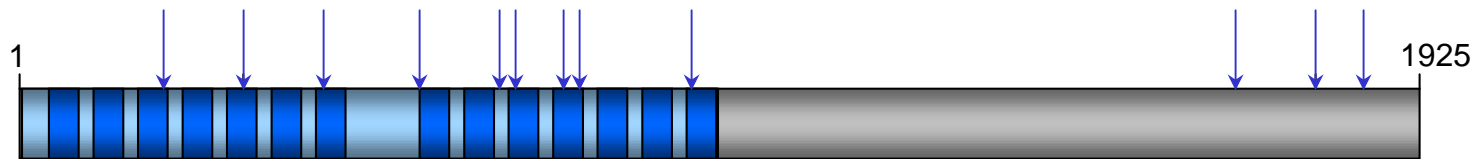
- Sequencing of the 12 alleles of ND6



- Nine mutants are touched in the RCC1 domains

THE MUTATIONS IN ND6p ARE MAJORITARY FOUND IN THE RCC1 DOMAINS

- Sequencing of the 12 alleles of ND6



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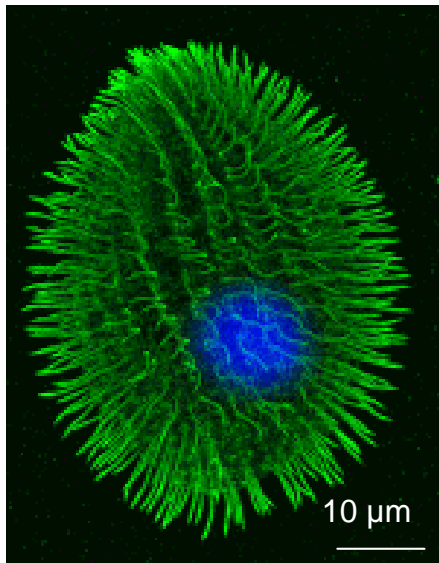


We tried to express this part of the ND6 protein

INTEREST OF PRODUCING THE RCC1 DOMAINS OF ND6p

- Raise antibodies.
- Determine the precise cellular localization of ND6p.
- Search for interacting partners.
- Analyse the composition of proteic complexes containing ND6p by non-denaturing approaches.
- Test the GEF activity.
- Obtain the 3D structure of the RCC1 domains.

AVANTAGES OF TETRAHYMENA THERMOPHILA FOR HETEROLOGOUS EXPRESSION



- *Tetrahymena* has the same divergent genetic code as *Paramecium* (TAA and TAG encode glutamine)
- Cultures with a high cellular density (10^5 - 10^6 cells per milliliter)
- Stable transformation by biolistic
- Strong, inducible promotor : pMTT

TAXOL SELECTION (Gaertig *et al*, PNAS, 1994)

Cu522 strain :



The K350M mutation in the BTU1 gene → hypersensitivity to taxol



Disruption of the BTU1 gene → loss of the taxol sensitivity

Introduction of linear DNA at the BTU1 locus

A STRONG AND INDUCIBLE PROMOTOR (Shang *et al*, PNAS, 2002)

- The metallothioneines (MTT) are small proteins fixing heavy metals and playing a role in metal homeostasis and in cadmium detoxification
- Their synthesis can be induced by heavy metals like Zinc, Cu or Cadmium.
- The pMTT promotor is thus an inducible promotor, allowing a rapid production of a large amount of mRNA.

QuickTime et un décompresseur TIFF (non compressé) sont requis pour visualiser cette image.

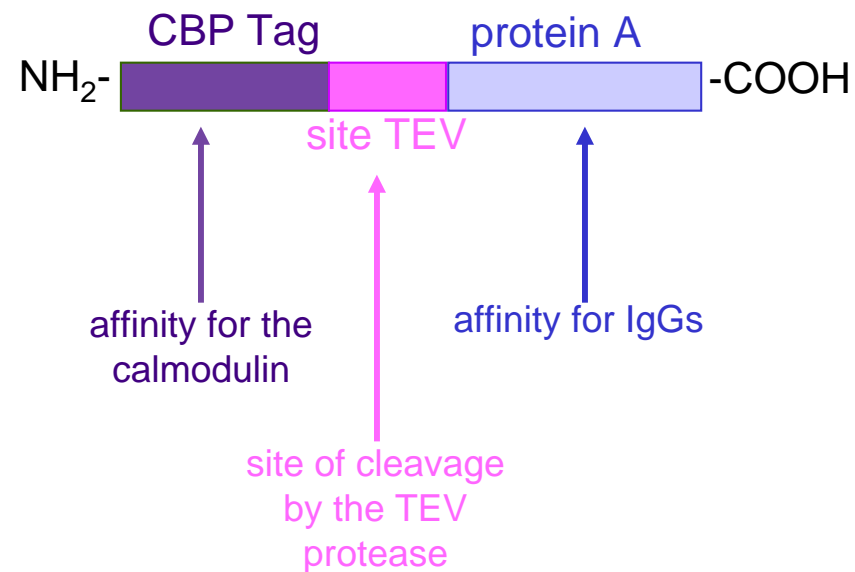
	BICH3		MTT-BICH3	
Cadmium 5µg	+	-	+	-

A STRONG AND INDUCIBLE PROMOTOR (Shang *et al*, PNAS, 2002)

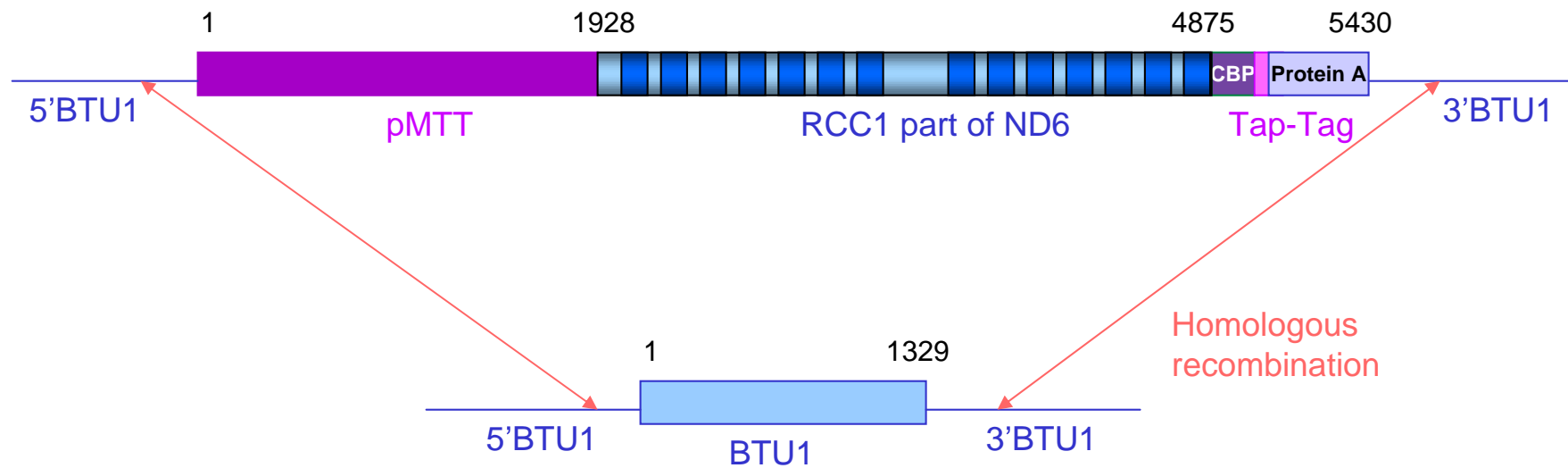
- The metallothioneines (MTT) are small proteins fixing heavy metals and playing a role in metal homeostasis and in cadmium detoxification
- Their synthesis can be induced by heavy metals like Zinc, Copper or Cadmium.
- The pMTT promotor is an inducible promotor, allowing rapid production of a large amount of mRNA.

DETECTION AND PURIFICATION OF PROTEIN OF INTEREST

TAP-tag (localization in C-terminus) Tandem Affinity Purification Tag
(Puig *et al*, Methods, 2001)

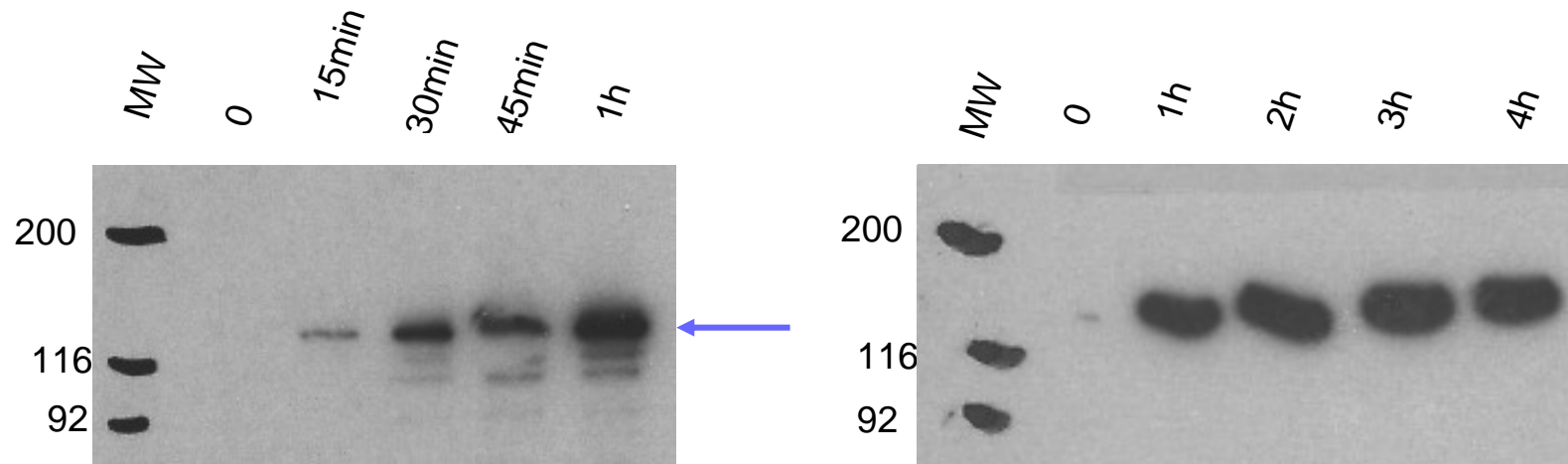


CONSTRUCTION INTRODUCED IN TETRAHYMENA



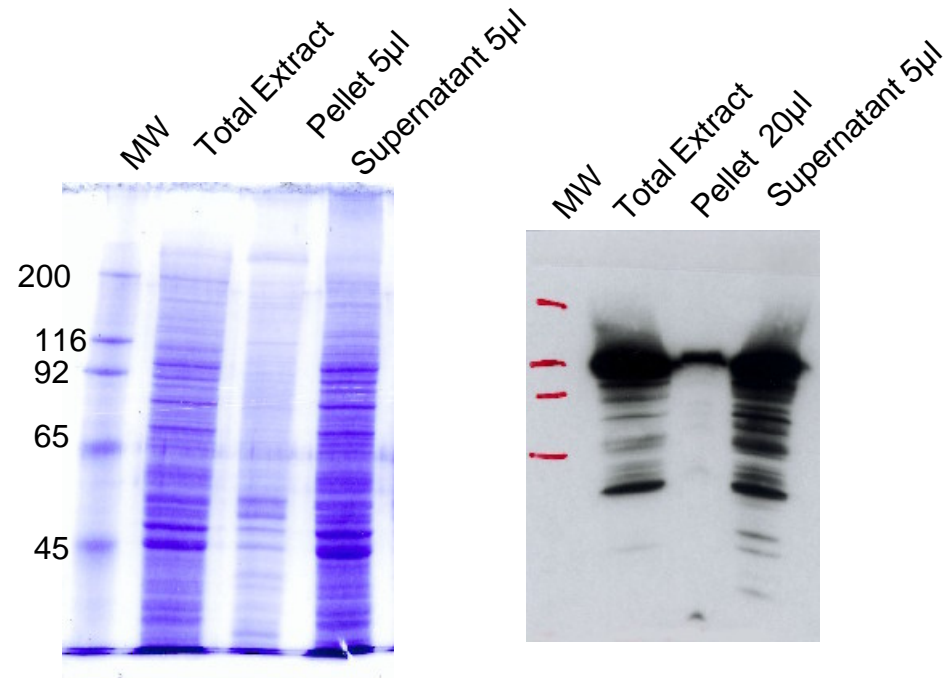
⇒ Selection of taxol resistant transformants

THE EXPRESSION OF ND6p PROTEIN IS INDUCED BY CADMIUM

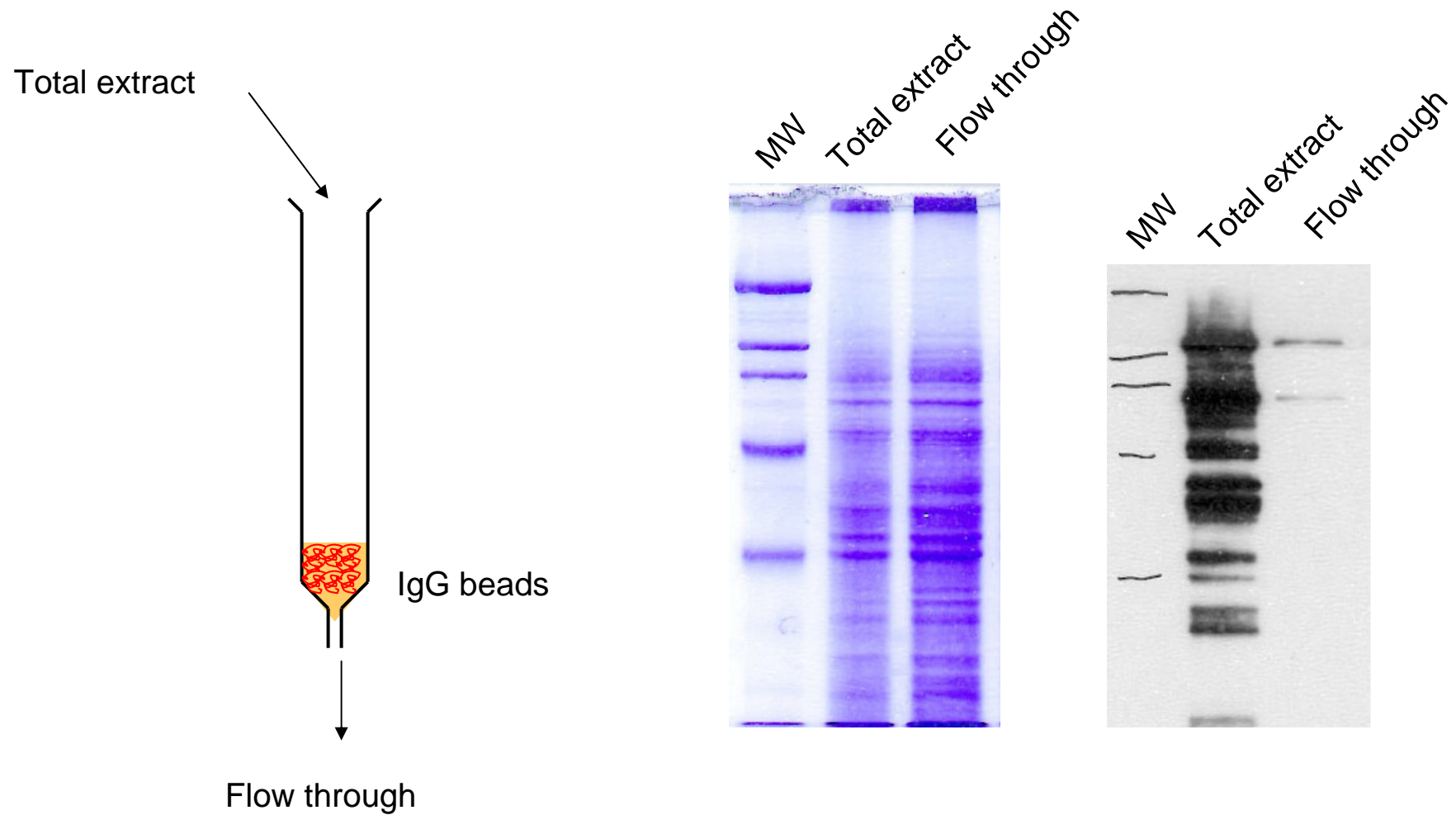


One hour of induction with $2,5\mu\text{g}/\mu\text{l}$ CdCl_2 is sufficient to have a maximal expression of our protein

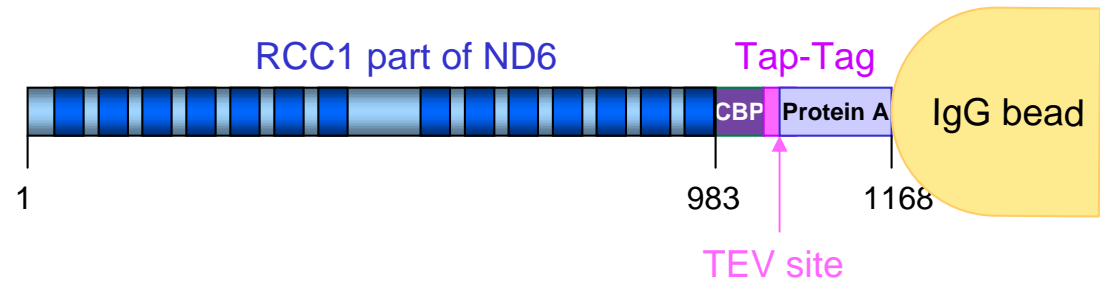
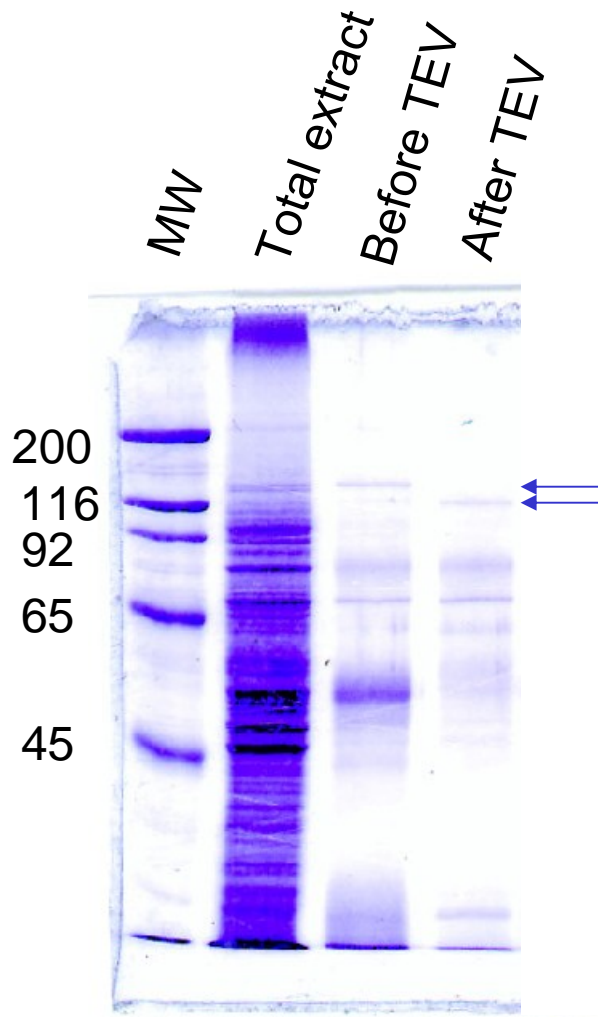
THE RCC1 PART OF ND6p IS SOLUBLE



FIXATION OF THE RCC1 DOMAIN OF ND6p ON AN IgG COLUMN



ELUTION BY THE TEV PROTEASE



- A very small quantity of protein is obtained
- The protein aggregates when we try to concentrate it

CONCLUSION

- A gene of *Paramecium* has been introduced in the *Tetrahymena* genome, at the BTU1 locus.
- The RCC1-part of ND6 is produced by the addition of Cadmium in the culture, but not “overexpressed”.
- The over-production in a heterologous system of expression may depend on the protein so *Tetrahymena* remains a possible way to produce native proteins