Analysis Protein-Protein Interaction by Affinity Purification

Tandem Affinity Purification (TAP)

Overview of Class

Methods to study protein-protein interactions

Methods to express proteins of interest

Examples of tandem affinity purification in different systems

Cellular processes are mainly operated by proteins

Single component:

Monomer
Dimer

Multiprotein complexes:

Spliceosome: 150-200 proteins
Proteasome: 30-40 proteins
Ribosome: 60S = 50 proteins; 40S = 30 proteins
Nuclear pore complex: 50 proteins
Many others

The Complexity of Gene Products

The total number of human genes does not differ substantially from that of nematode: the complexity may partly rely on the contextual combination of gene products

Multicomponent complexes: proteins interact with other proteins to perform particular cellular tasks

These protein assembles represent more than the sum of their parts by having a new function

Protein Purification and Identification

Protein identification: mass spectrometry (100 fmole); not a limitation

Protein purification: a limiting step;

each protein has unique properties (can be specifically purified);

can't purify thousands of proteins by thousands of different purification schemes;

A generic purification protocol is desirable for any proteins (can be used to purify protein complexes)

Methods to study (identify) protein-protein interactions

GST pull down assay

Far Western assay

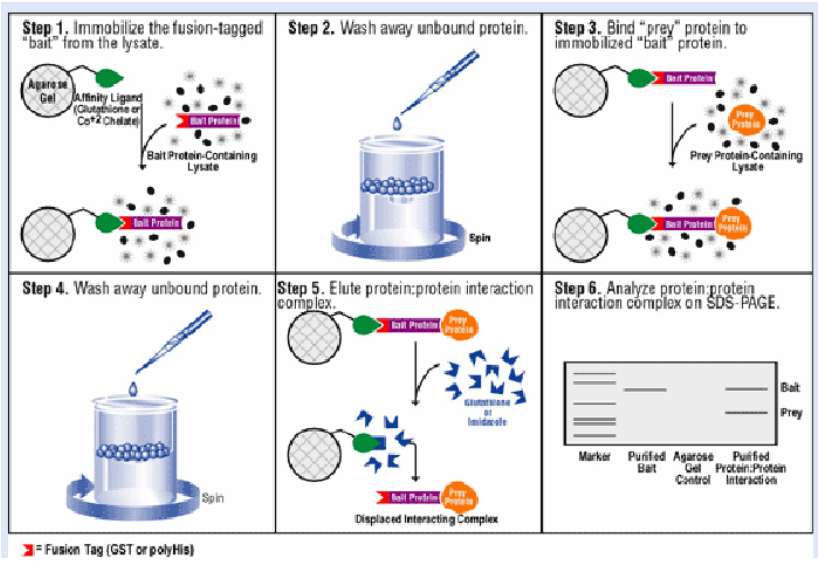
Phage display

Yeast two hybrid analysis

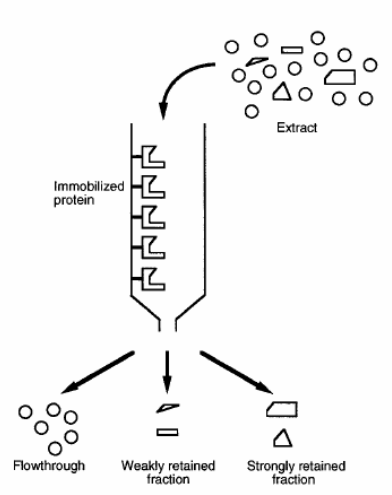
Affinity purification (immunopurification)

a. One-step affinity purification
b. Two-step affinity purification (tandem affinity purification)

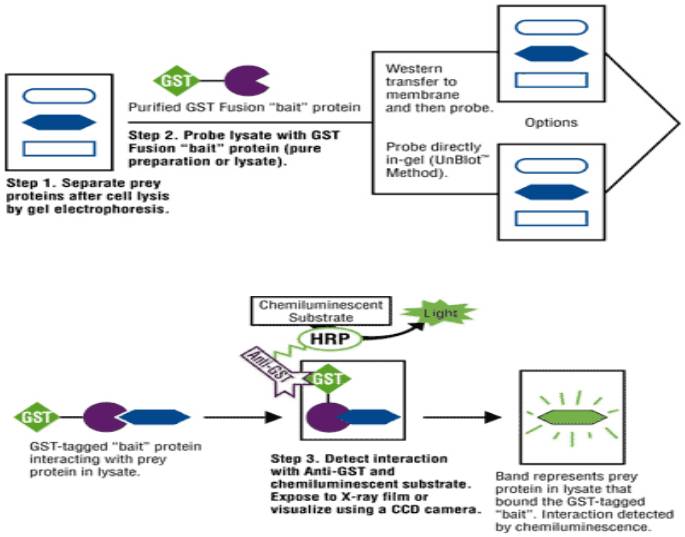
GST pull down assay



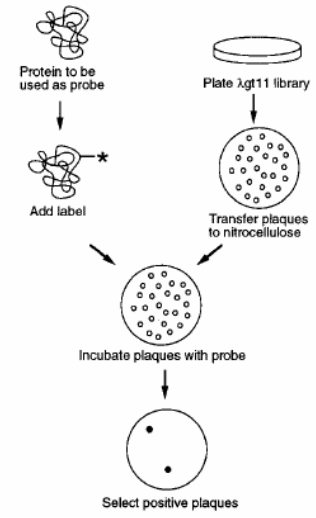
Protein Affinity Chromatography



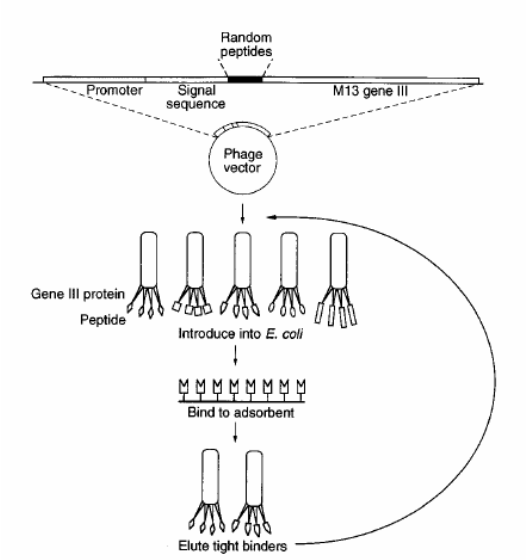
Far-Western Analysis



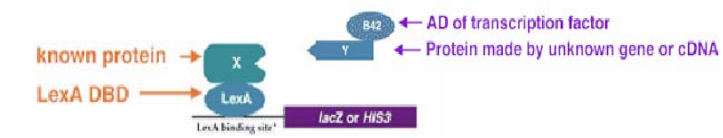
Expression Library Screen

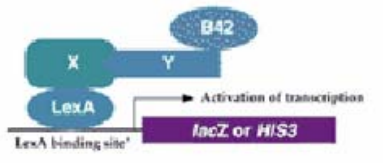


Phage Display



Yeast Two Hybrid Analysis



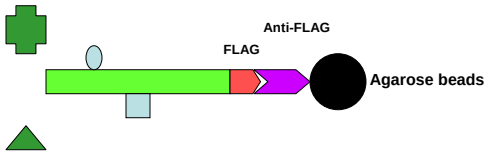


Blue or

Growth in His$^-$ medium

Affinity purification (immunopurification)

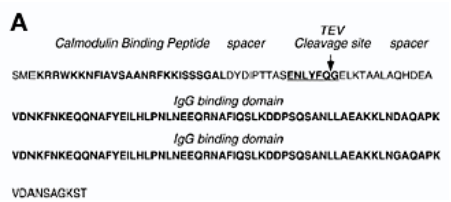
One-step affinity purification

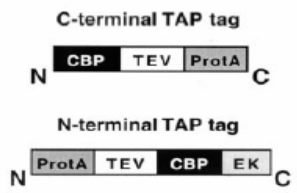


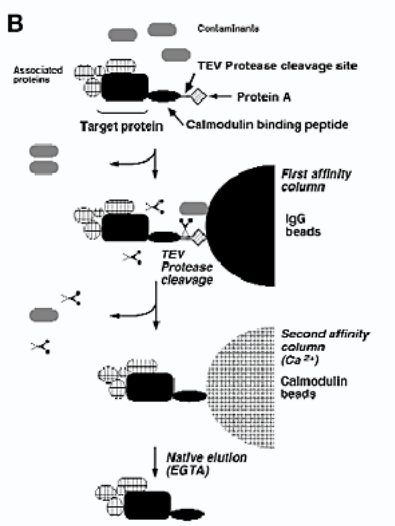
Wash and elute

Detection

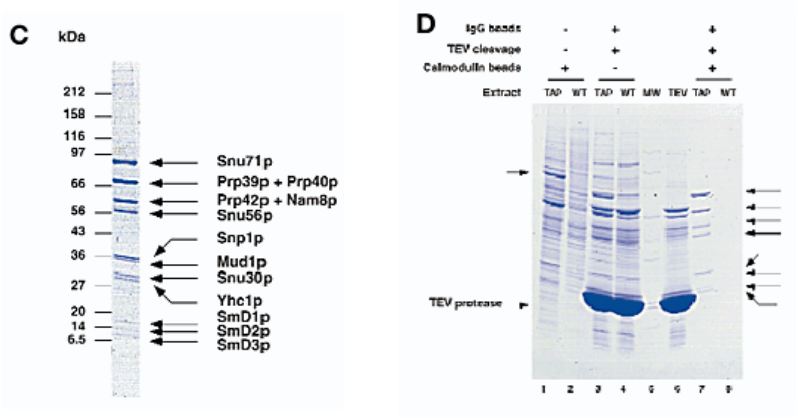
Tandem Affinity Purification (TAP) – two-step affinity purification







Two-Step Affinity Purification Is Required



Methods to express tagged proteins

Tagged protein should be expressed at or close to its natural expression levels, overexpression may lead to the assembly of non-physiological complexes

Exogenous - constitutive or inducible expression,
usually overexpression unless carefully select specific clones

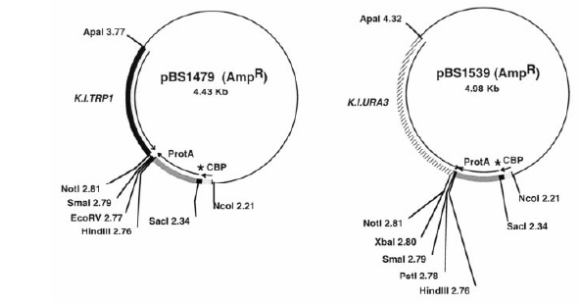
Endogenous - natural expression, homologous recombination

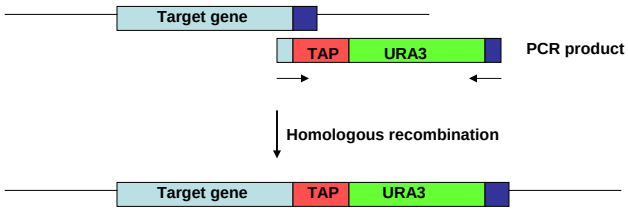
Yeast

Exogenous – the tagged protein is expressed in an extra copy driven by a heterologous promoter

Endogenous - homologous recombination

Homologous recombination - C-terminal TAP





Homologous recombination - N-terminal TAP

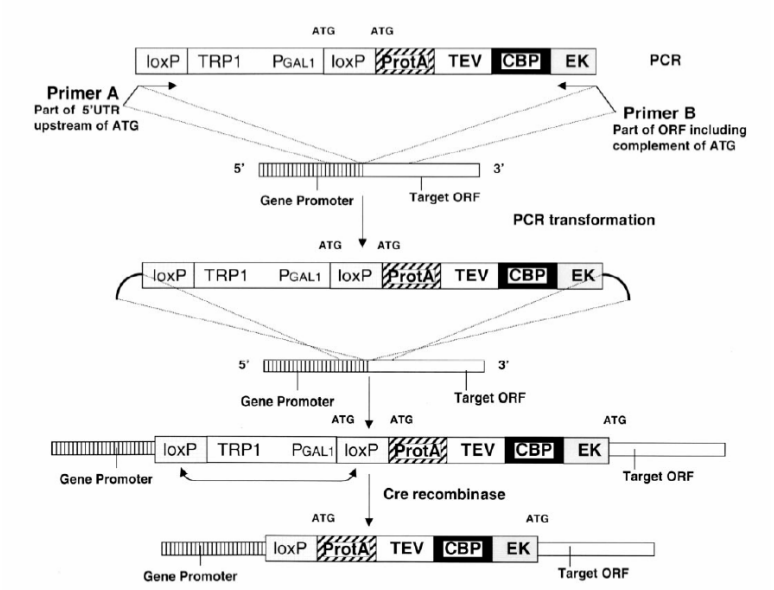


TABLE 1

Complexes Purified Using the TAP Method

Complex	Function	Protein tagged	Reference
U1 snRNP	Pre-mRNA splicing	Snu71-TAP	(7)
		Luc7p/Snu30p-TAP	(7)
		SmB-TEV-ProtA Nam8p-CBP	This article
		Nam8p-TAP	(23) and this article
U2 snRNP[a]	Pre-mRNA splicing	Lea1p-TAP	(18)
		SmB-TEV-ProtA Lea1p-CBP	(18)
"U6 snRNP"[b]	Pre-mRNA splicing	Lsm8p-TAP	(21)
CBC	Pre-mRNA splicing, nucleocytoplasmic RNA transport	Mud13p-TAP	(7)
BBP-associated	Pre-mRNA splicing, nuclear RNA retention	BBP-TAP	(22)
Mud2p-associated	Pre-mRNA splicing, nuclear RNA retention	Mud2p-TAP	(22)
SF3b	Pre-mRNA splicing	TAP-Rse1p	This article
RNases P/MRP	rRNA and tRNA processing	Pop4-TAP	This article
Dbp5p-associated	Nucleocytoplasmic mRNA transport	Dbp5p-TAP	(19)
Mex67p-associated	Nucleocytoplasmic mRNA transport	Mex67p-TAP	(20)
Mak3/10/31	Protein modification	Mak31-TAP	(7)
Lsm3p-associated	RNA degradation, pre-mRNA splicing	Lsm3p-TAP	(21)
LsmI complex	RNA degradation	Lsm3p-TAP Lsm8p-ProtA	(21)
Xrn1-associated	RNA degradation	Xrn1-TAP	(21)

[a] In this case not all the known components of the complex have been identified.
[b] Contains a mixture of U6, U4/U6, and U4/U6.U5 snRNPs; see Ref. (21).

Mammalian cells

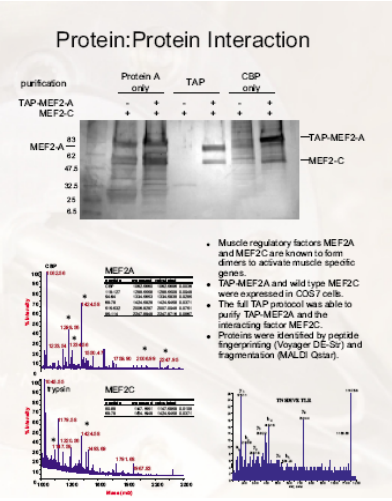
Exogenous – overexpression or close to natural expression

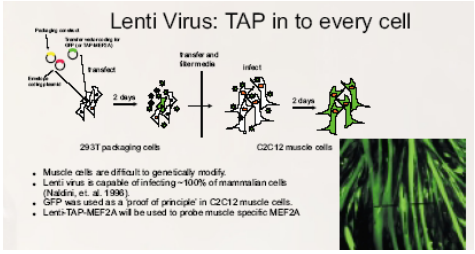
1. **Transient expression**
   Plasmid vectors
   Viral vectors

2. **Stable expression – antibiotics selection**
   Plasmid vectors
   Viral vectors

Endogenous - natural expression; **homologous recombination**

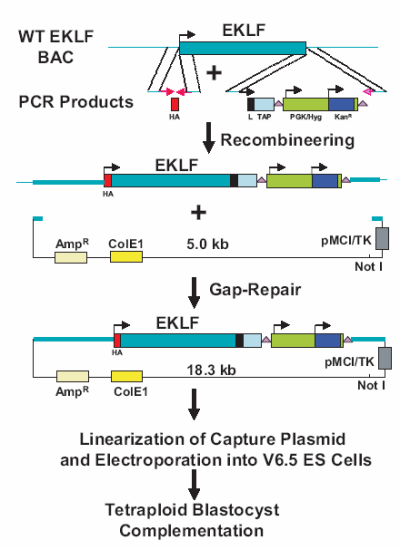
Mammalian cells - Exogenous; transient or stable

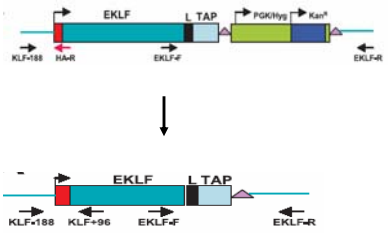




Mammalian cells

Endogenous; natural expression; homologous recombination





Example of tandem affinity purification in different systems and variations

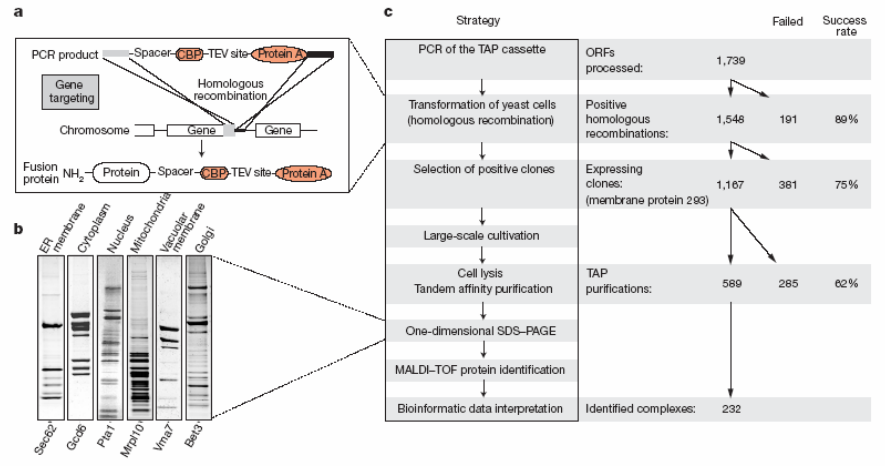
articles

Functional organization of the yeast proteome by systematic analysis of protein complexes

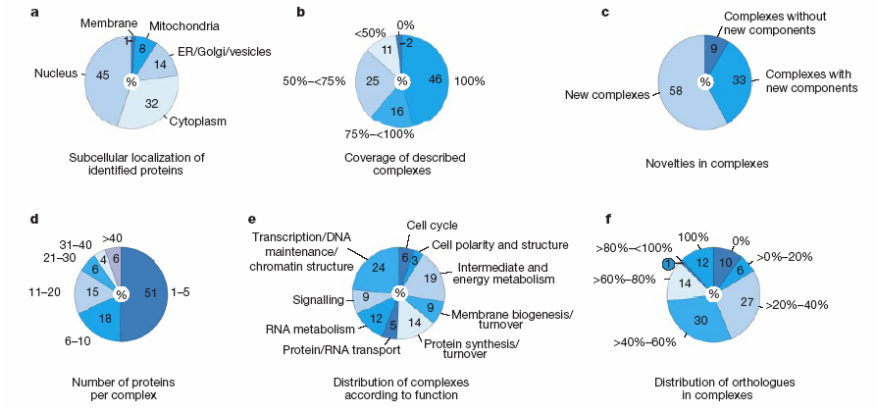
Anne-Claude Gavin*, Markus Bösche*, Roland Krause*, Paola Grandi*, Martina Marzioch*, Andreas Bauer*, Jörg Schultz*, Jens M. Rick*, Anne-Marie Michon*, Cristina-Maria Cruciat*, Marita Remor*, Christian Höfert*, Malgorzata Schelder*, Miro Brajenovic*, Heinz Ruffner*, Alejandro Merino*, Karin Klein*, Manuela Hudak*, David Dickson*, Tatjana Rudi*, Volker Gnau*, Angela Bauch*, Sonja Bastuck*, Bettina Huhse*, Christina Leutwein*, Marie-Anne Heurtier*, Richard R. Copley†, Angela Edelmann*, Erich Querfurth*, Vladimir Rybin*, Gerard Drewes*, Manfred Raida*, Tewis Bouwmeester*, Peer Bork†, Bertrand Seraphin†‡, Bernhard Kuster*, Gitte Neubauer* & Giulio Superti-Furga*†

* *Cellzome AG, Meyerhofstrasse 1, 69117 Heidelberg, Germany*
† *European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany*
‡ *CGM-CNRS, 91198 Gif sur Yvette Cedex, France*

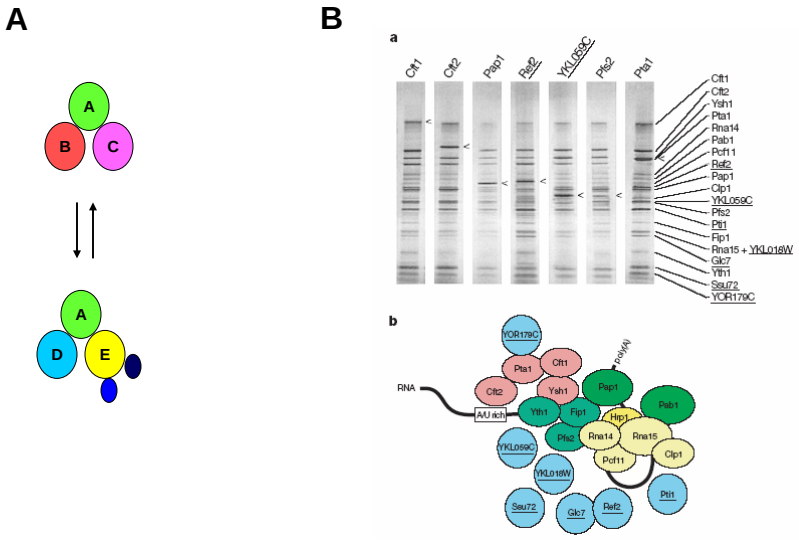
Large scale analysis of protein complexes by TAP in yeast



Statistics of proteins and complexes



Cohesive and dynamic complexes



iTAP strategy

TAP tagged orthologes from another species

Expression of the tagged protein

RNAi depletion of endogenous version of the tagged protein

TAP purification to identify protein complex

Purification of the Drosophila exosome

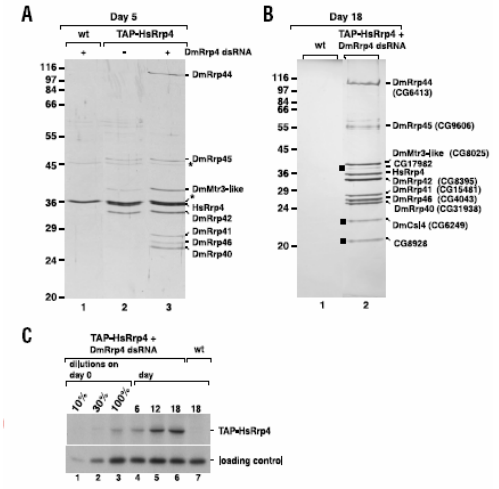
TAP-human RRP4 (exosome subunit)

Establish cells expressing the tagged protein

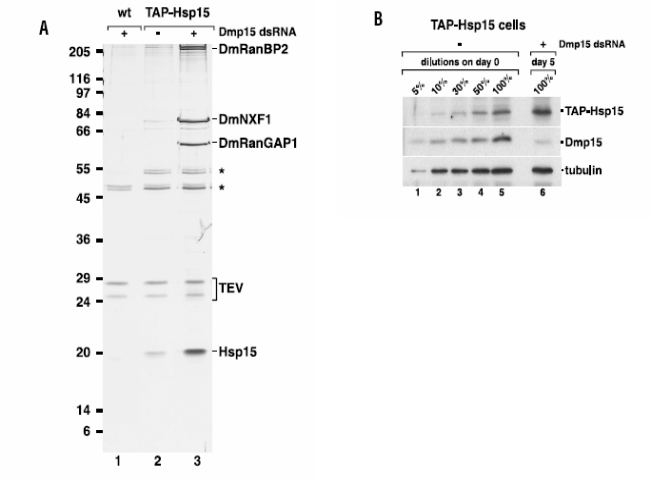
Silence the expression of DmRRP4 by RNAi

Purify the exosome complex

SDS-PAGE and mass spectrometry



Purification of a tetrameric complex involved in mRNA nuclear export



iTAP strategy

Demonstrate TAP in higher eucaryotes

Replacement of the original protein by a tagged version and RNAi

The use of native promoter to drive the expression of the tagged protein is still absent

Purification of the human exosome by the TAP method

Transfect human cells

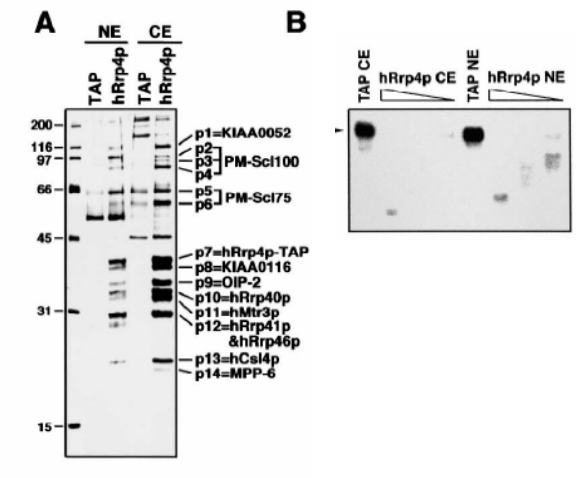
Select stable cells expressing hRRP4-TAP (exogenous)

Grow cells

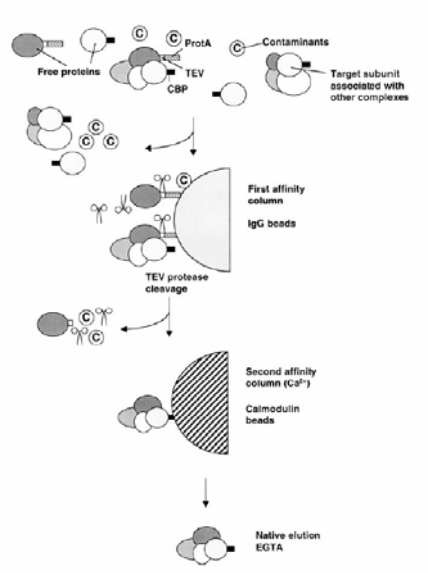
TAP purification

SDS-PAGE

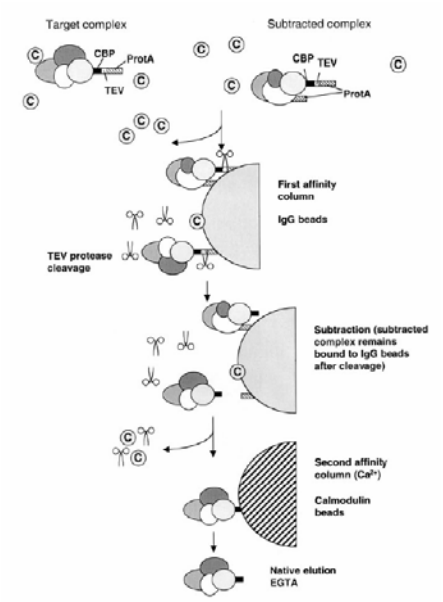
Mass spectrometry



The Split Tag



The Subtraction TAP



Nuclear Lsm complex **Cytoplasmic Lsm complex**

3 TAP

TAP 8

3 TAP
8 ProtA