

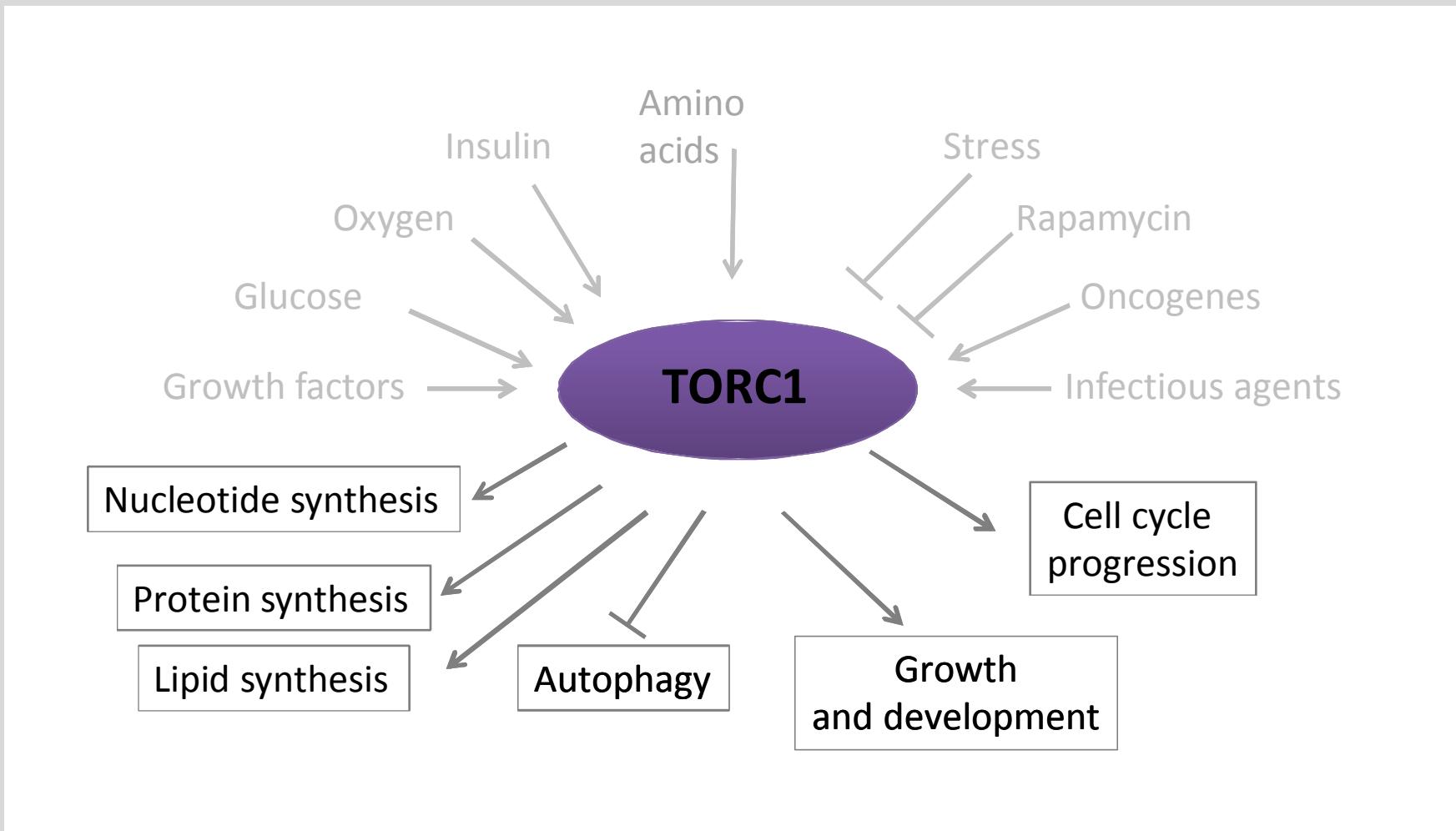
19/01/2016

Клеточный метаболизм: от молекул к макромолекулярным комплексам и органеллам

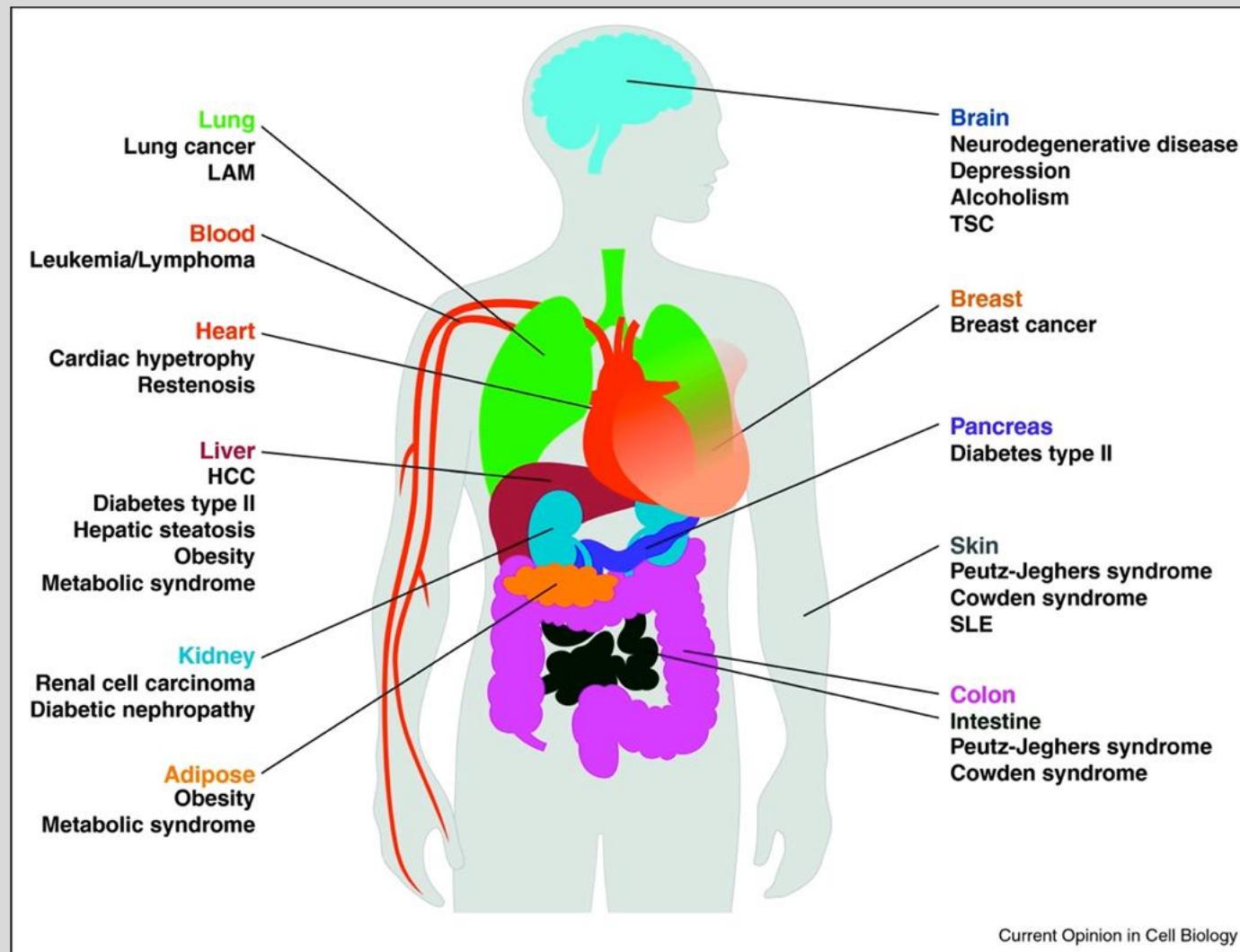
Светлана Докудовская,
UMR8126 CNRS, Institut Gustave Roussy, Villejuif



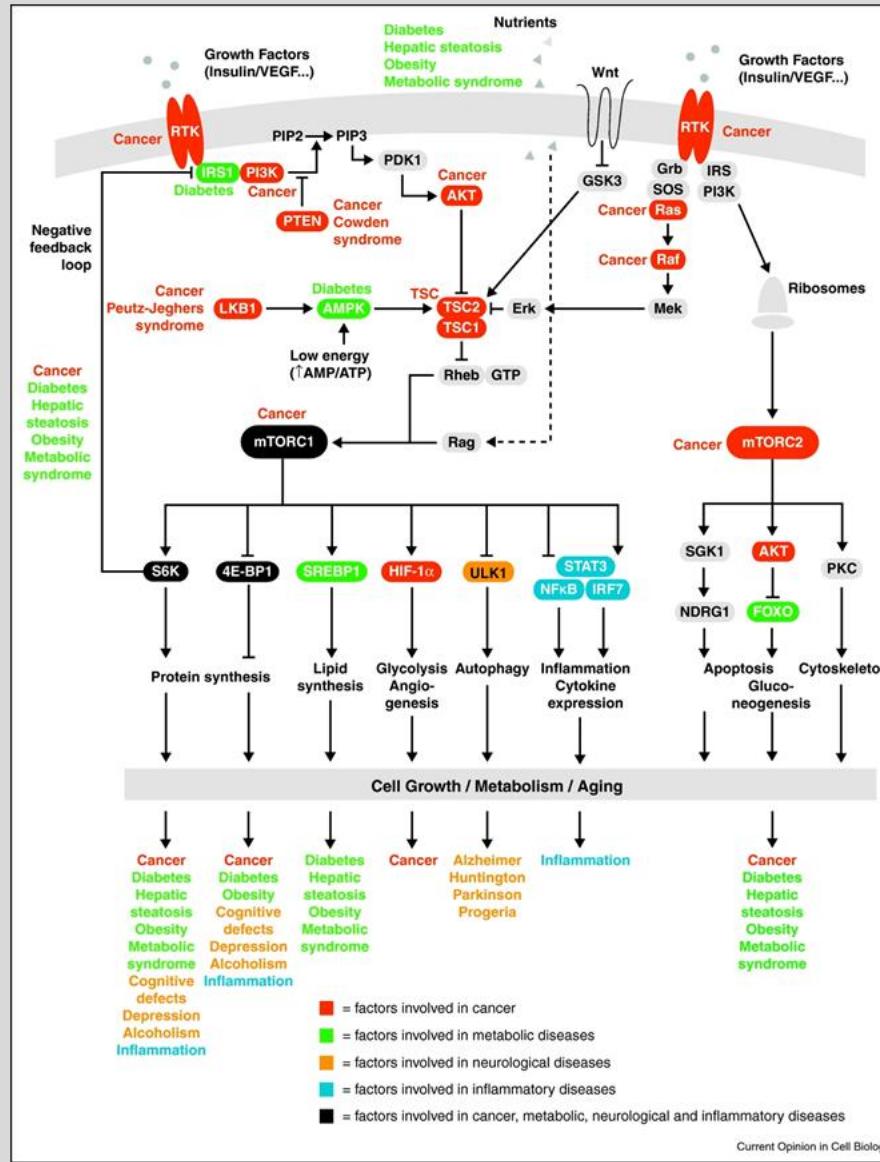
mTORC1 in the Center of the Cellular Response to Stress



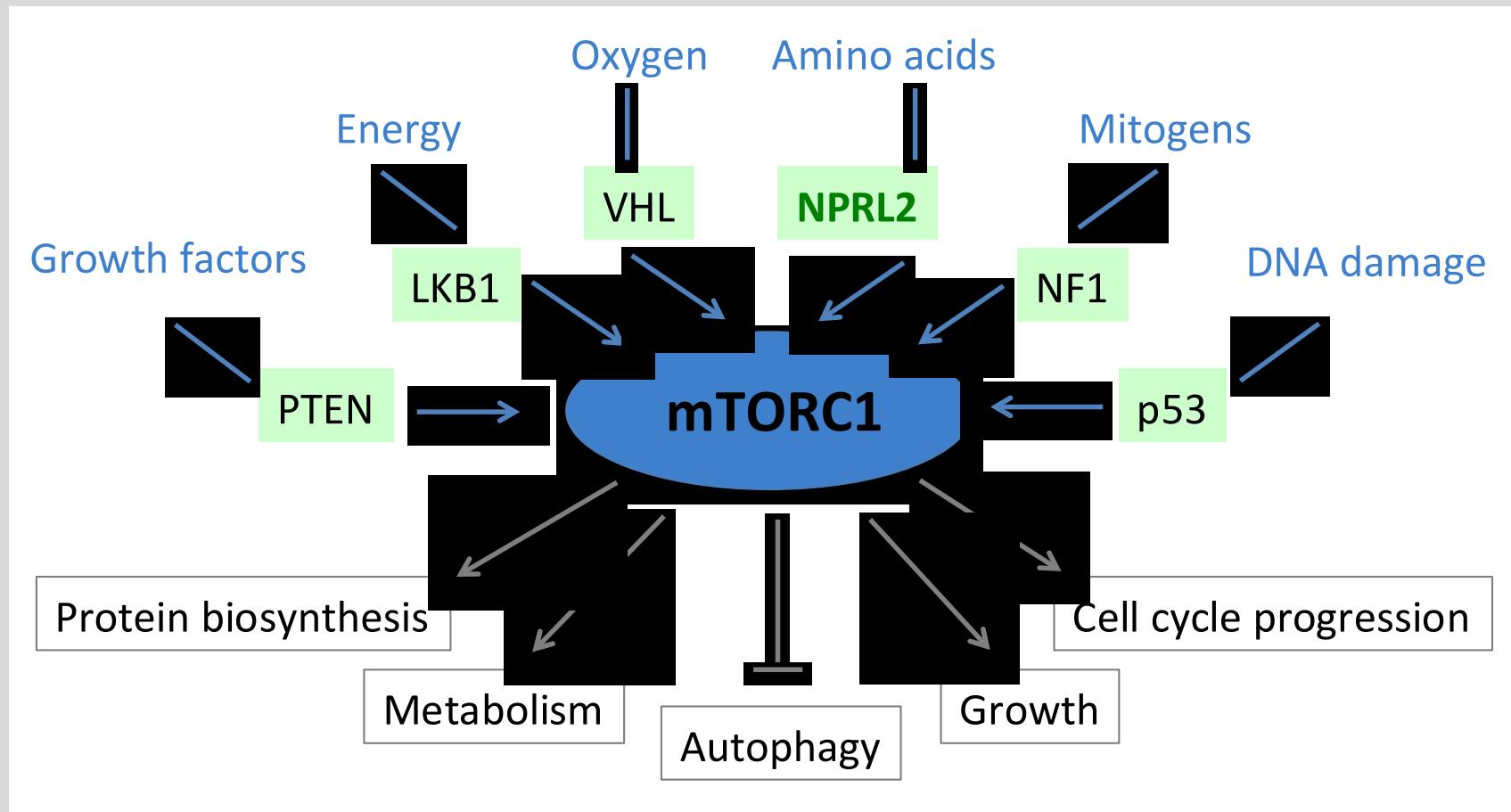
Diseases Linked to Dysregulated mTOR Signaling and Corresponding Affected Organs



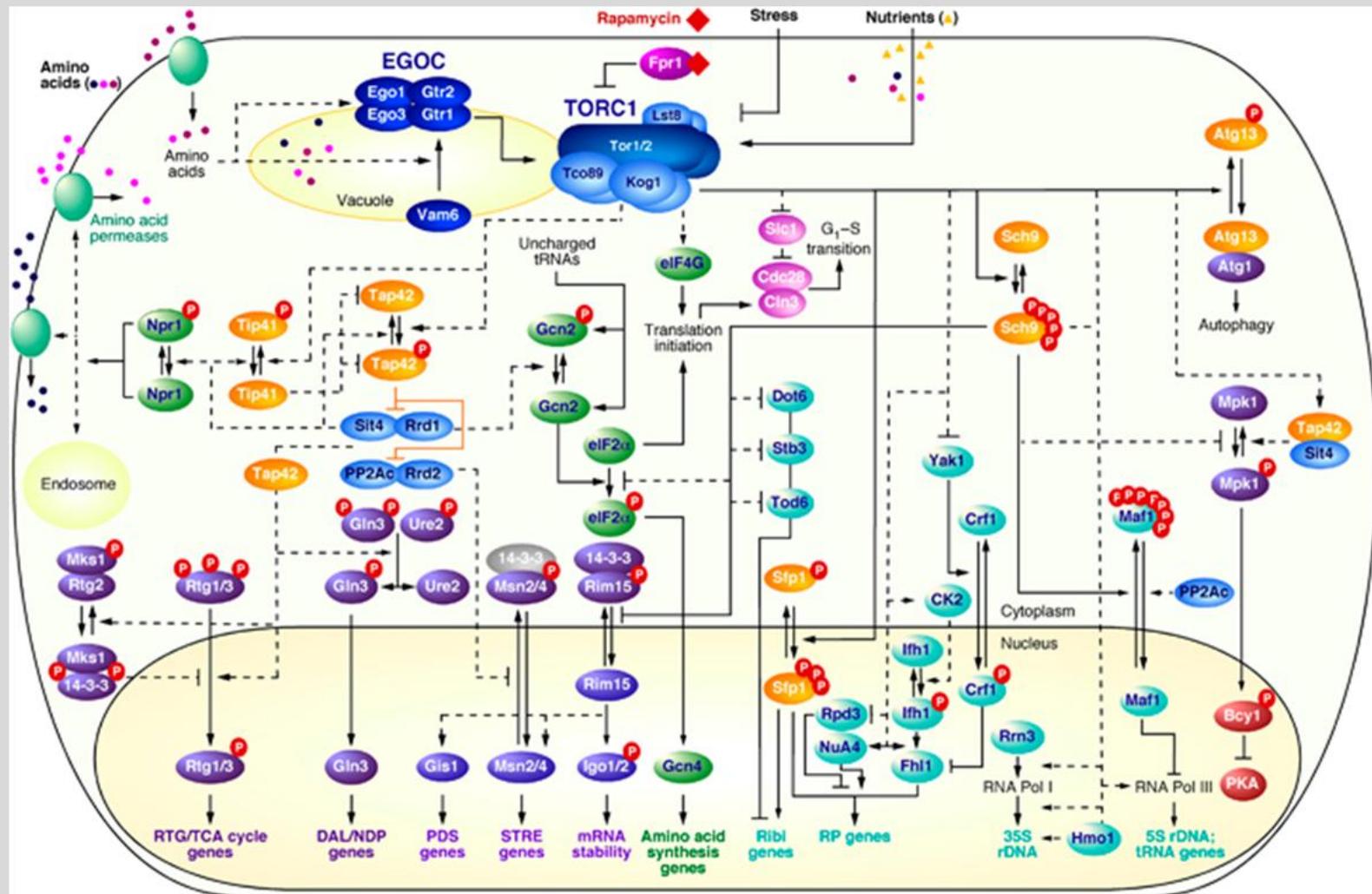
mTORC1 Signaling Pathway and Disease Implications



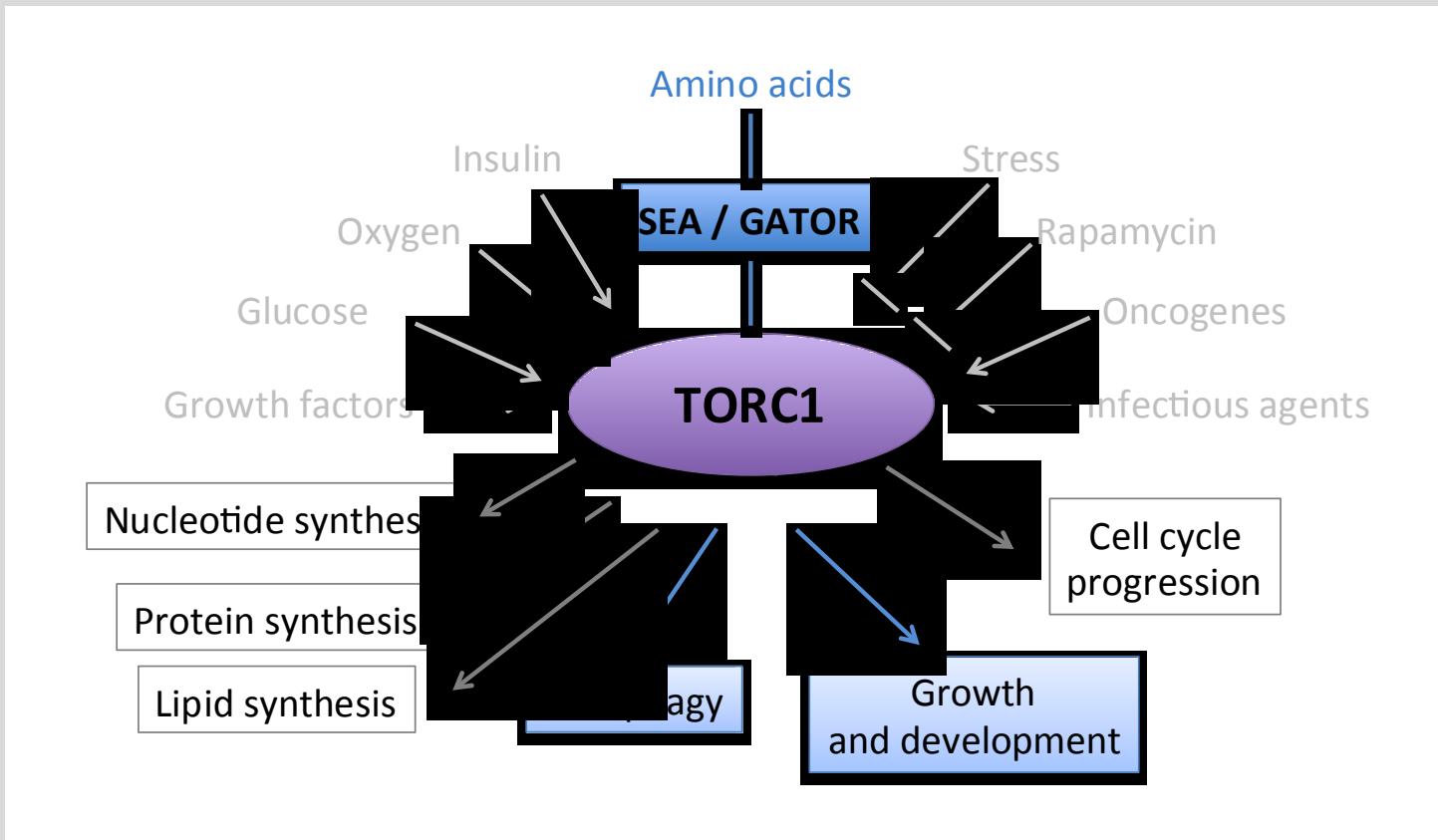
Many Upstream TORC1 Regulators are Tumor Suppressors



TORC1 Pathway – Complex Network



Amino Acid Sensing and TORC1 Pathway



Amino Acids

AA – most abundant macromolecules in the cell

Low AA – cells mobilize proteasome and autophagy to produce the stock of aa, those aa are used for the production of proteins required for survival during nutrient deprivation

Low AA, prolonged starvation – cells can also use the stock of aa for the production of other forms of energy – e.g. glucose, ketons

Minimizing translation enables aminoacids to be used as energy source.

Essential Amino Acids

Yeast can produce all amino acids *de novo*

Human – need to take essential amino acids with food

Essential	Conditionally Non-Essential	Non-Essential
Histidine	Arginine	Alanine
Isoleucine	Asparagine	Asparatate
Leucine	Glutamine	Cysteine
Methionine	Glycine	Glutamate
Phenylalanine	Proline	
Threonine	Serine	
Tryptophan	Tyrosine	
Valine		
Lysine		

(astleep))

Essential Amino Acid Mnemonic

Private Tim Hall => PVT TIM HALL

P.V.T.

- P = Phenylalanine
- V - Valine
- T - Threonine

T.I.M.

- T - Tryptophan
- I - Isoleucine
- M - Methionine

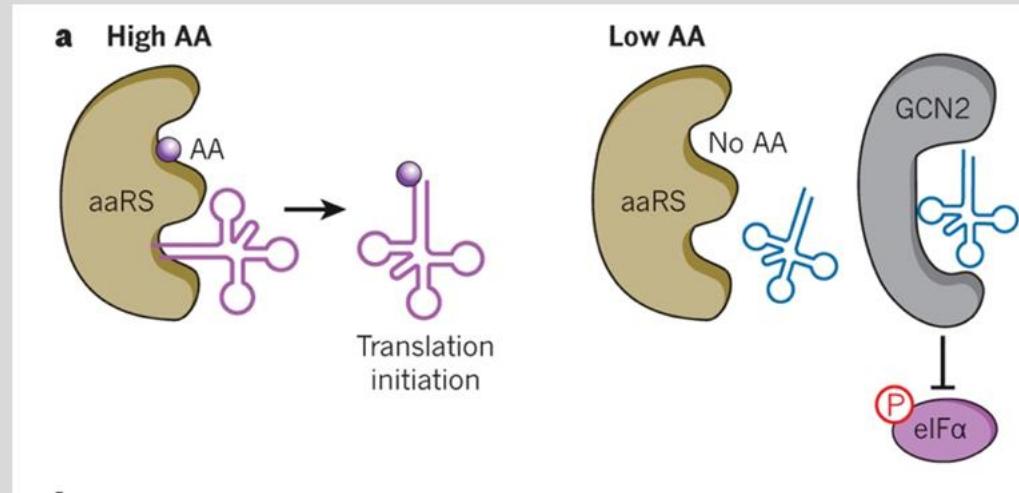


H.A.L.L.

- H - Histidine
- A - Arginine*
- L - Leucine
- L - Lysine

* Only essential during (+)Nitrogen Balance

GCN2 Sensing (Intracellular)

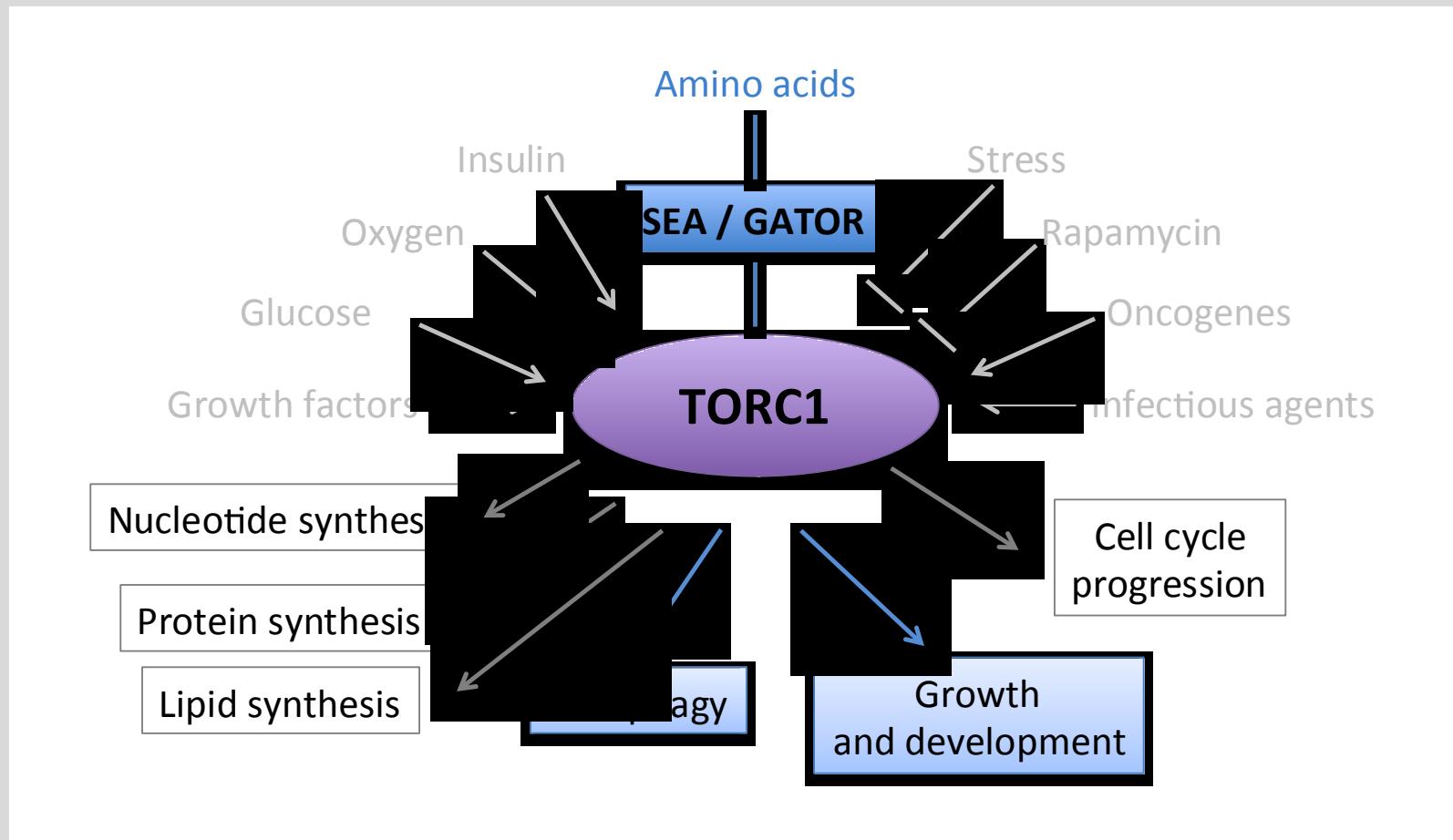


- Prokaryotes do not have GCN2, but use the same strategy of sensing of uncharged tRNA, which bind directly to ribosomes, stimulate production of odd nucleotides, which repress synthesis of rRNA and tRNA and activate aa biosynthetic genes

- Yeast: GCN2 binds uncharged tRNA (any!),
so if 19 are abundant and one is scarce it will detect it anyway
Binding triggers GCN2 homodimerization, autophosphorylation
and phosphorylation and, as a consequence, inhibition, of its only substrate – eIF2a

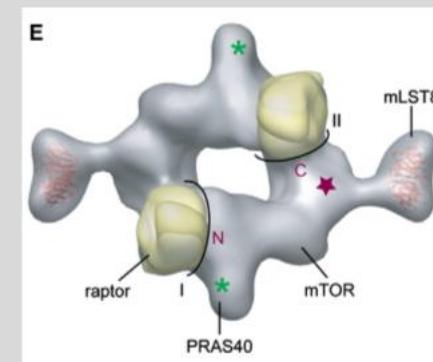
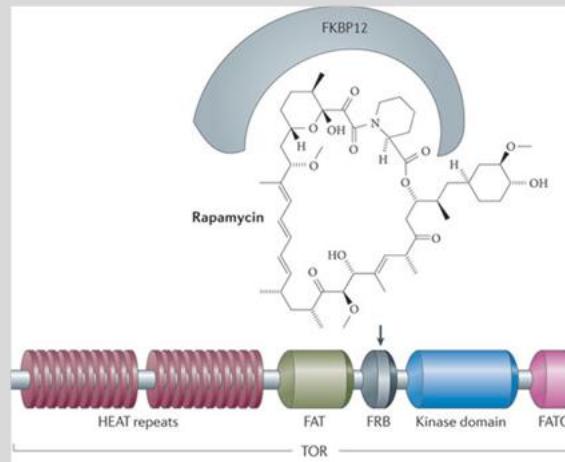
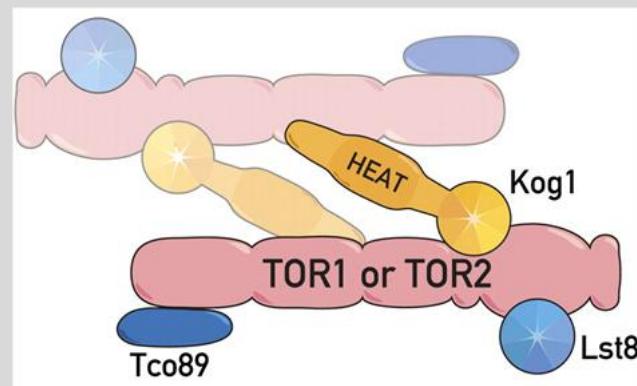
At the same time, while the majority of mRNA are repressed ,
mRNA encoding GCN4 transcription factor is derepressed and this GCN4 can now bind and activate transcription

Regulation of the TORC1 Stress Response Pathway

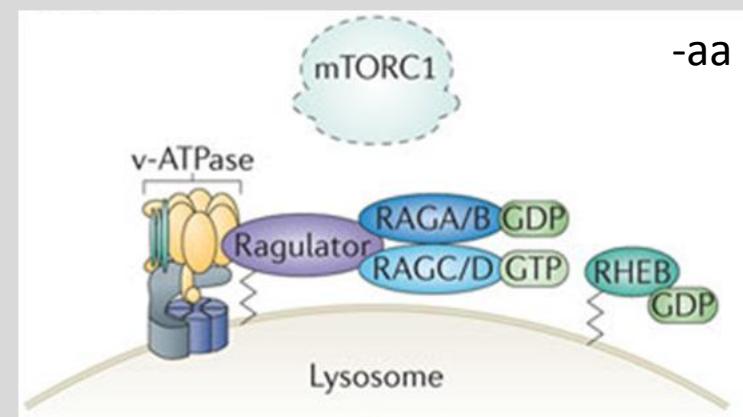
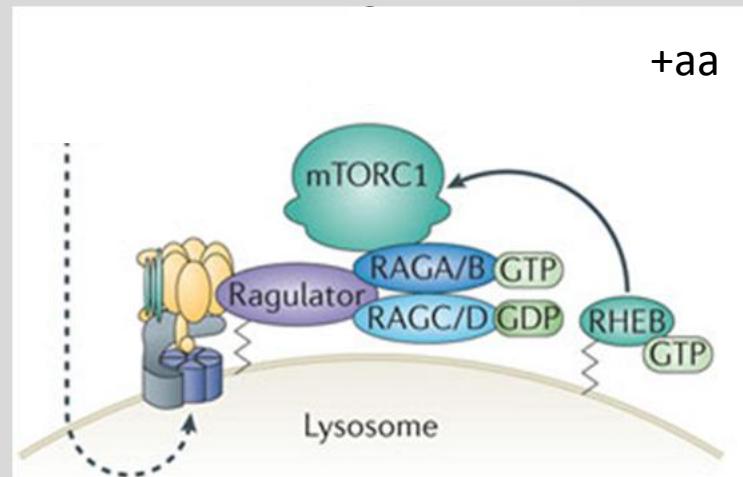
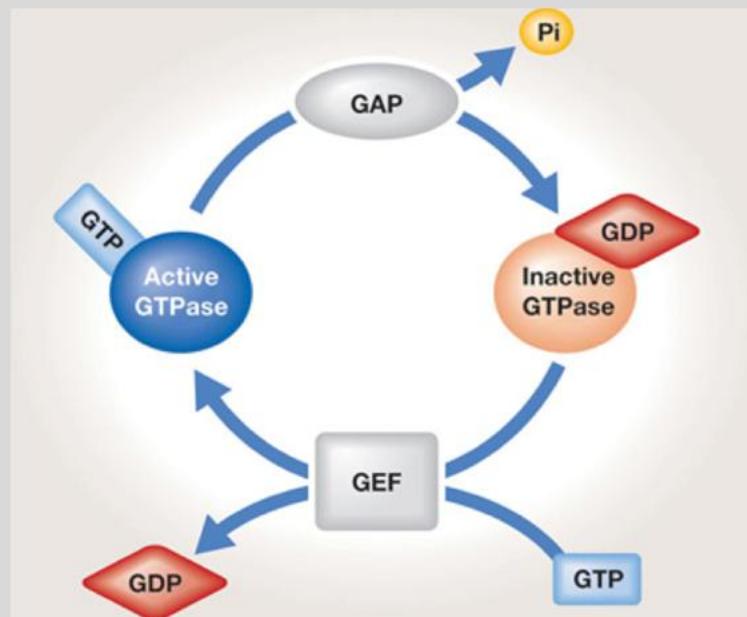


TORC1 is conserved

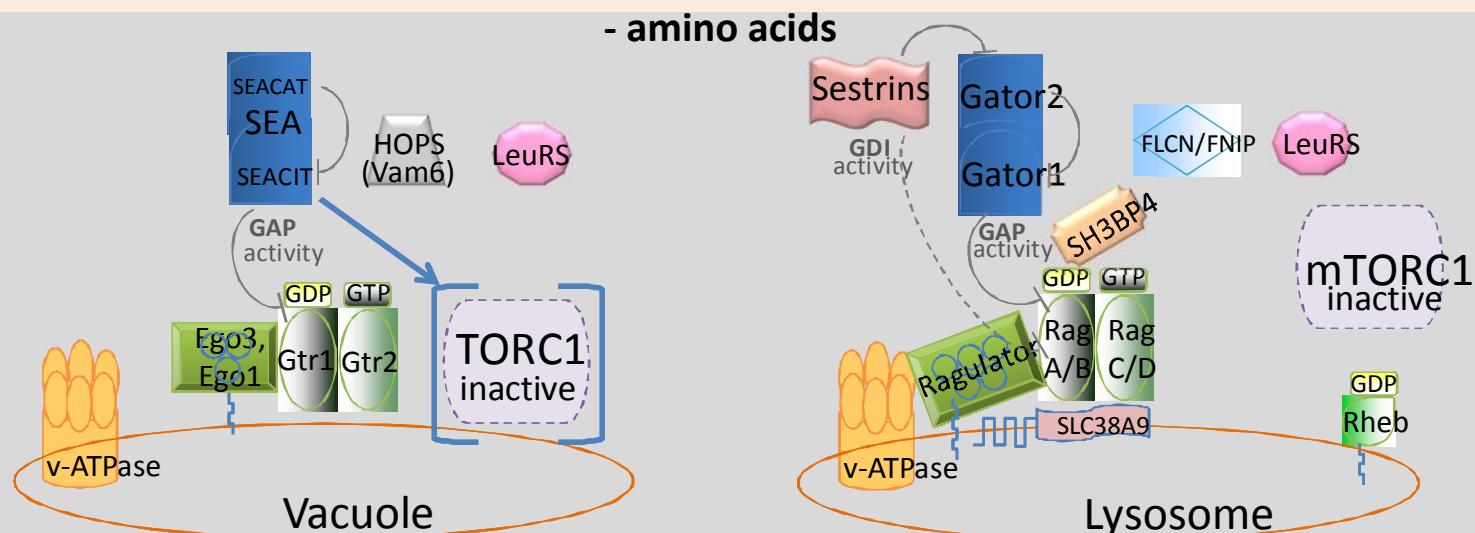
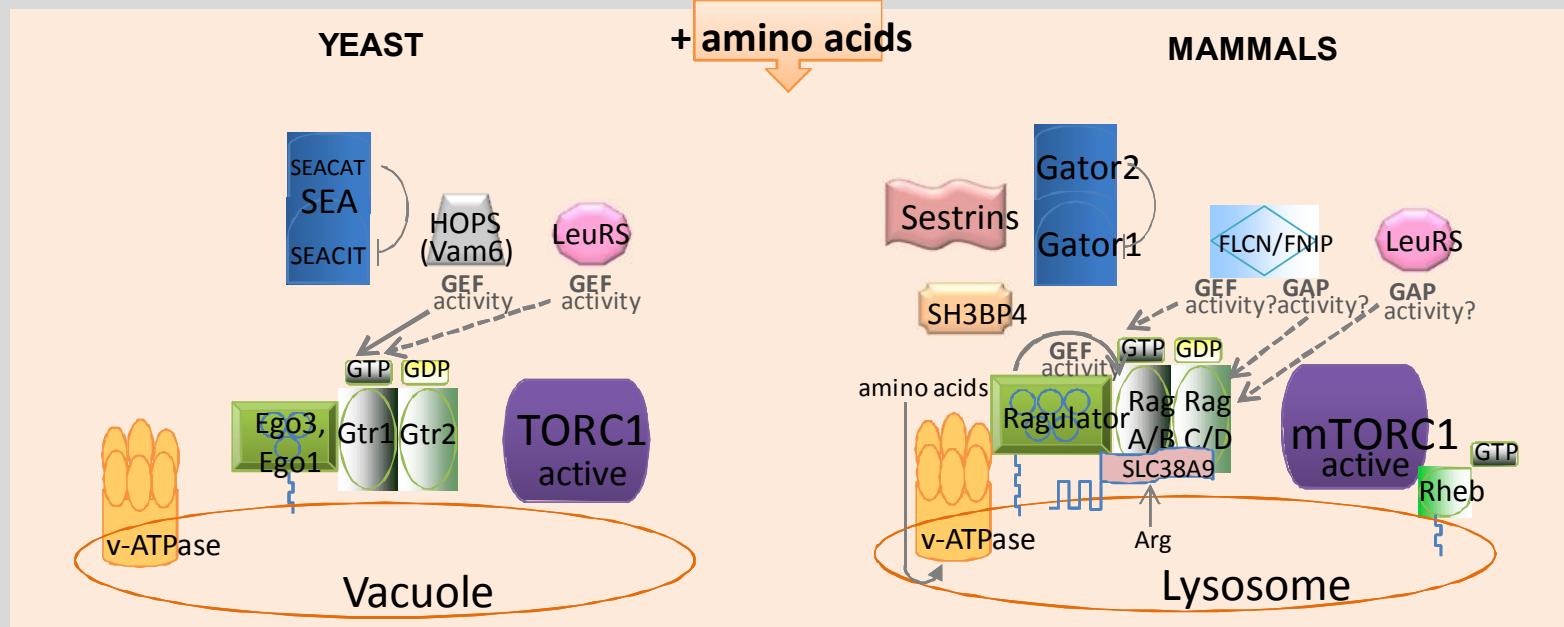
<i>S. cerevisiae</i>	<i>S. pombe</i>	<i>C. albicans</i>	<i>D. discoideum</i>	<i>C. reinhardtii</i>	<i>T. brucei</i>	<i>A. thaliana</i>	<i>C. elegans</i>	<i>D. melanogaster</i>	Mammals
TORC1 TOR1 or TOR2	TOR1 or TOR2	TOR1	TOR	TOR	TOR1	TOR	TOR	TOR	mTOR
KOG1	MIP1	-	-	-	RAPTOR	Raptor1A Raptor1B	DAF15	RAPTOR	RAPTOR
LST8	WAT1	-	-	LST8	-	-	LST8	LST8	mLST8
TCO89	TCO89	-	-	-	-	-	-	-	
-	TOC1	-	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-	PRAS40
TORC2									
TOR2	TOR1 or TOR2	TOR1	TOR	TOR	TOR2	TOR	TOR	TOR	mTOR
AVO1	SIN1	-	PiaA	-	-	-	SINH1	SIN1	mSIN1
AVO2	-	-	-	-	-	-	-	-	
AVO3	STE20	-	RIP3	-	AVO3	-	RICT1	RICTOR	RICTOR
LST8	WAT1	-	LST8	LST8	-	-	LST8	LST8	mLST8
BIT61	BIT61	-	-	-	-	-	-	-	PRR5/PRR5L



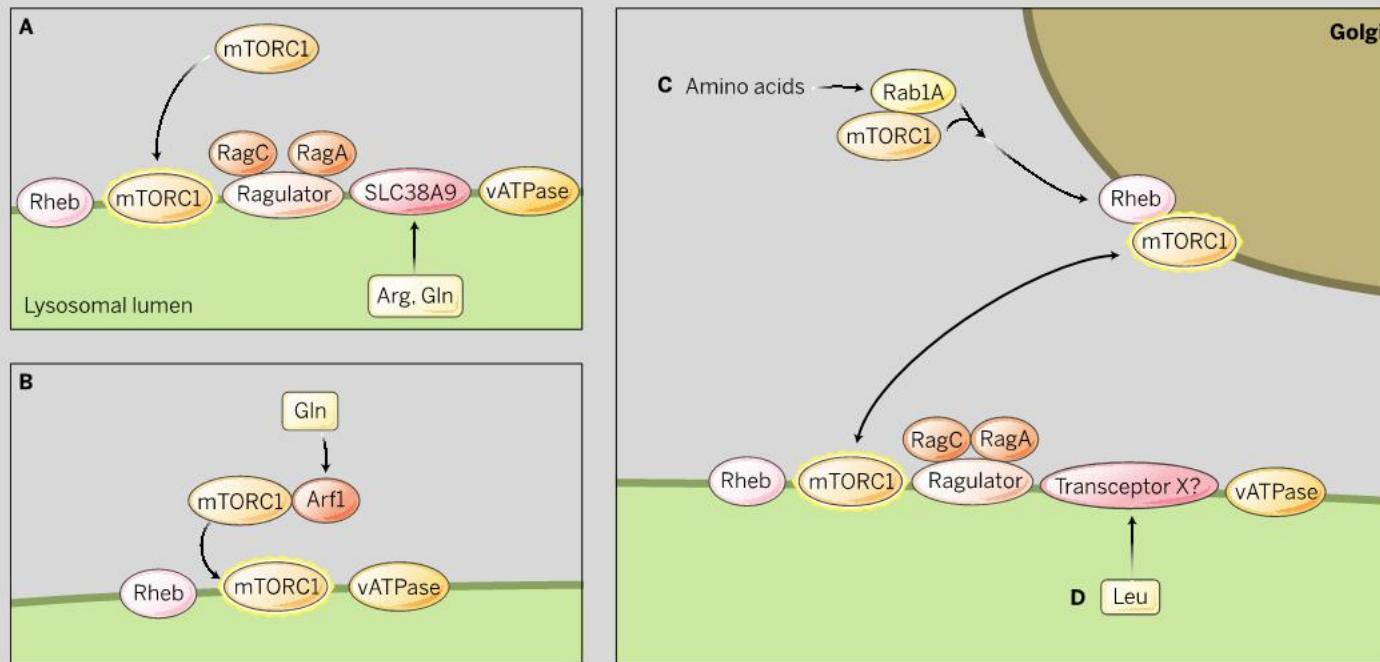
GTPases are Important in TORC1 Regulation



Amino Acid Sensing at the Vacuole / Lysosome

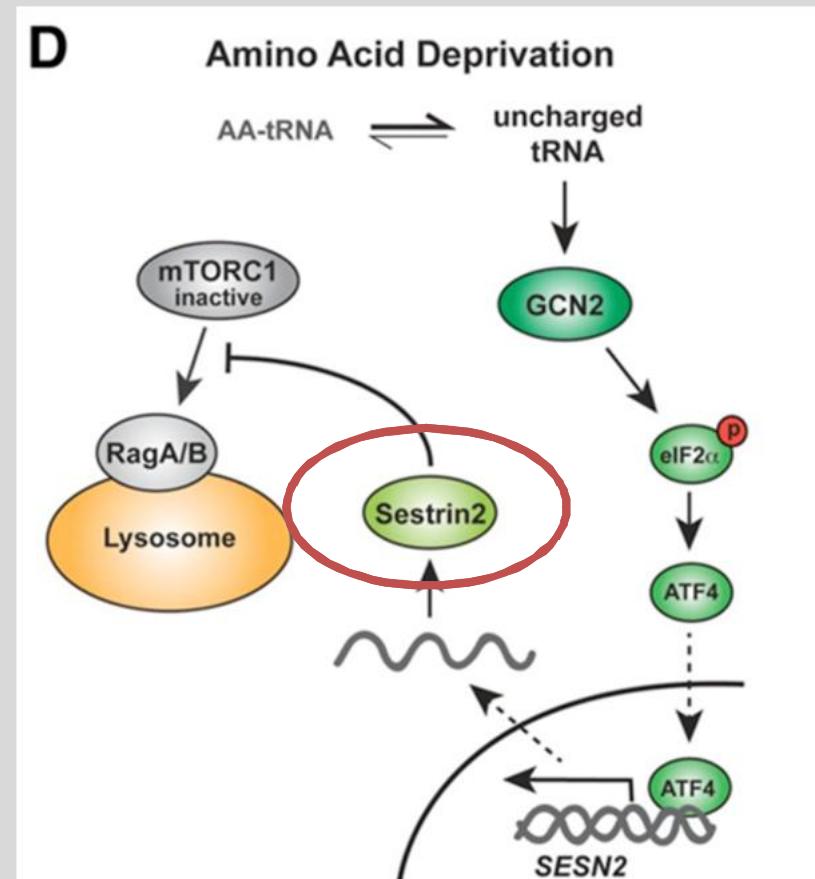
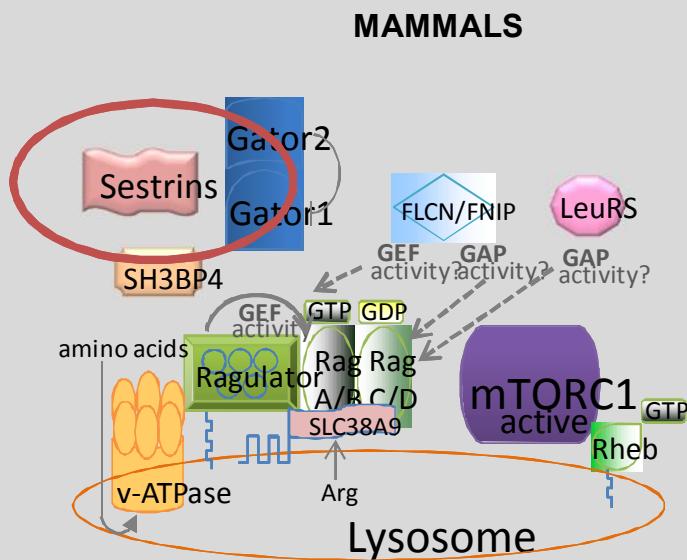


Amino Acids Sensing – Even More Complicated

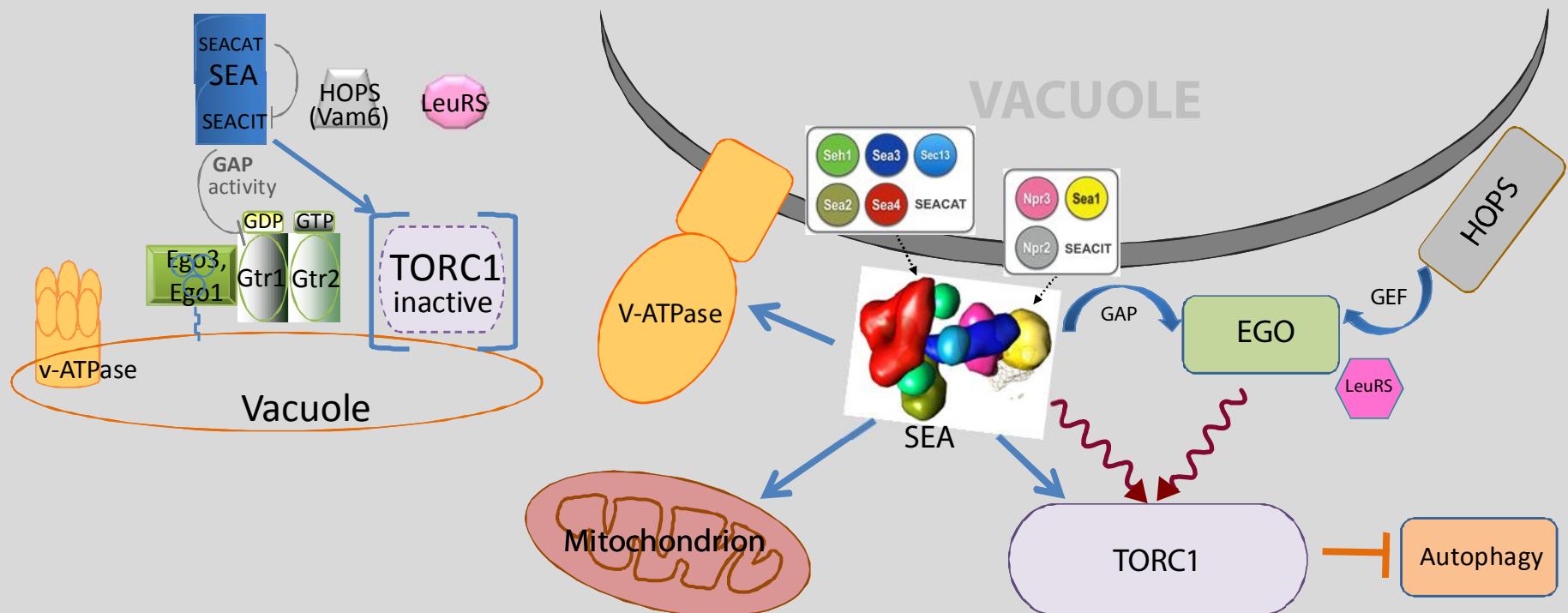


- TORC1 activation without RAGs
- TORC1 Activation at Golgi

TORC1 pathway is regulated by GCN2 during amino-acid deprivation

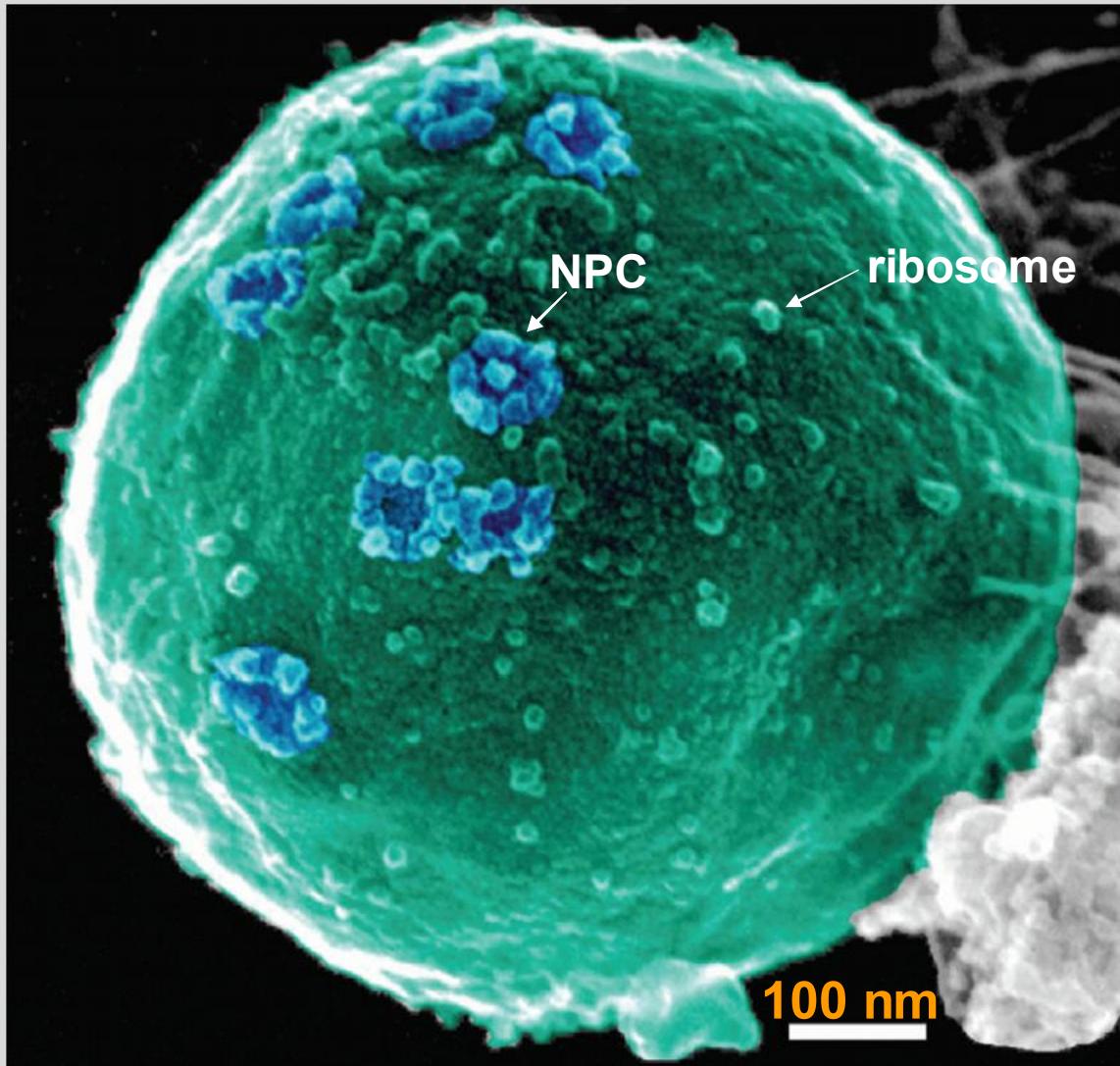


SEA Complex Function – Regulates TORC1 pathway



Algett et al., Mol.Cell Proteomics, 2014
Dokudovskaya & Rout, J.Cell Science, 2015

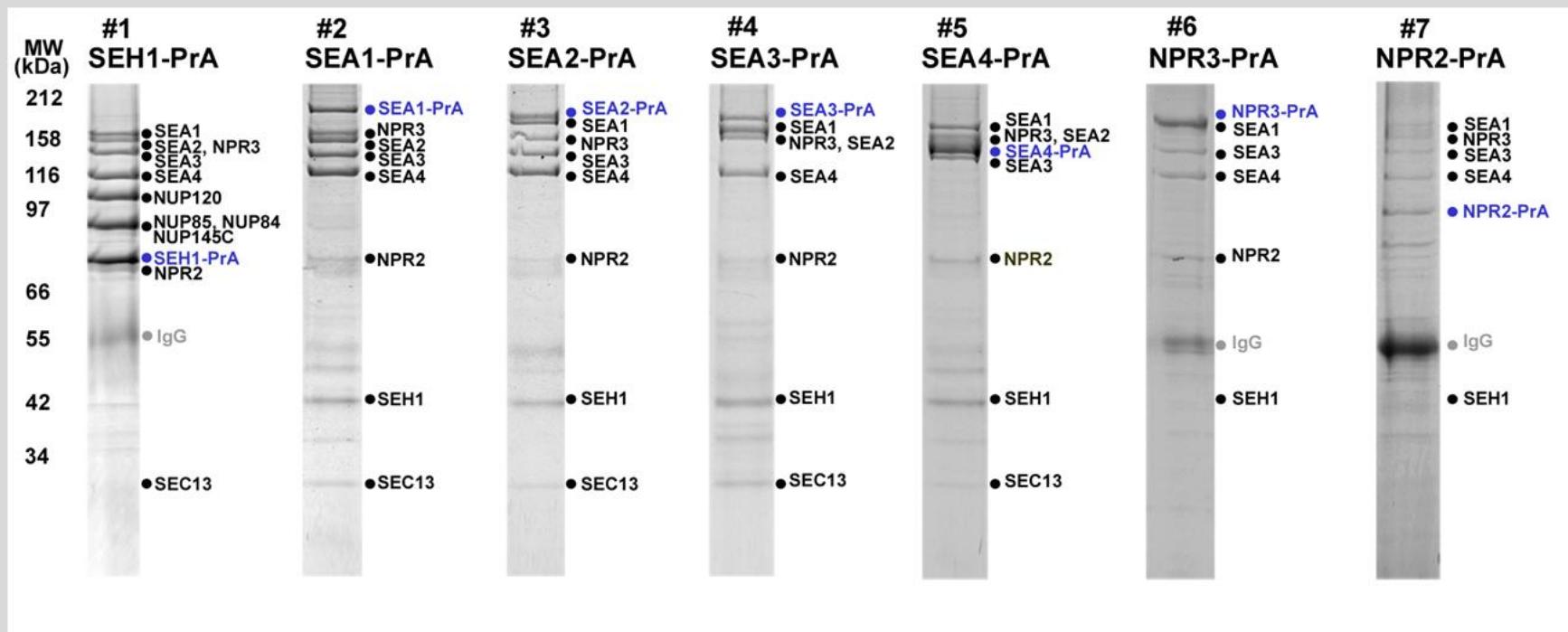
« SEA Begins Where the Land Ends » – Nuclear Pore Complex



NPC:
-50 MDa
-480 proteins
of ~30 different types

Ribosome:
-2 MDa
-80 proteins,
-3 RNAs

Identification of the SEh1 Associated Complex (SEA Complex)

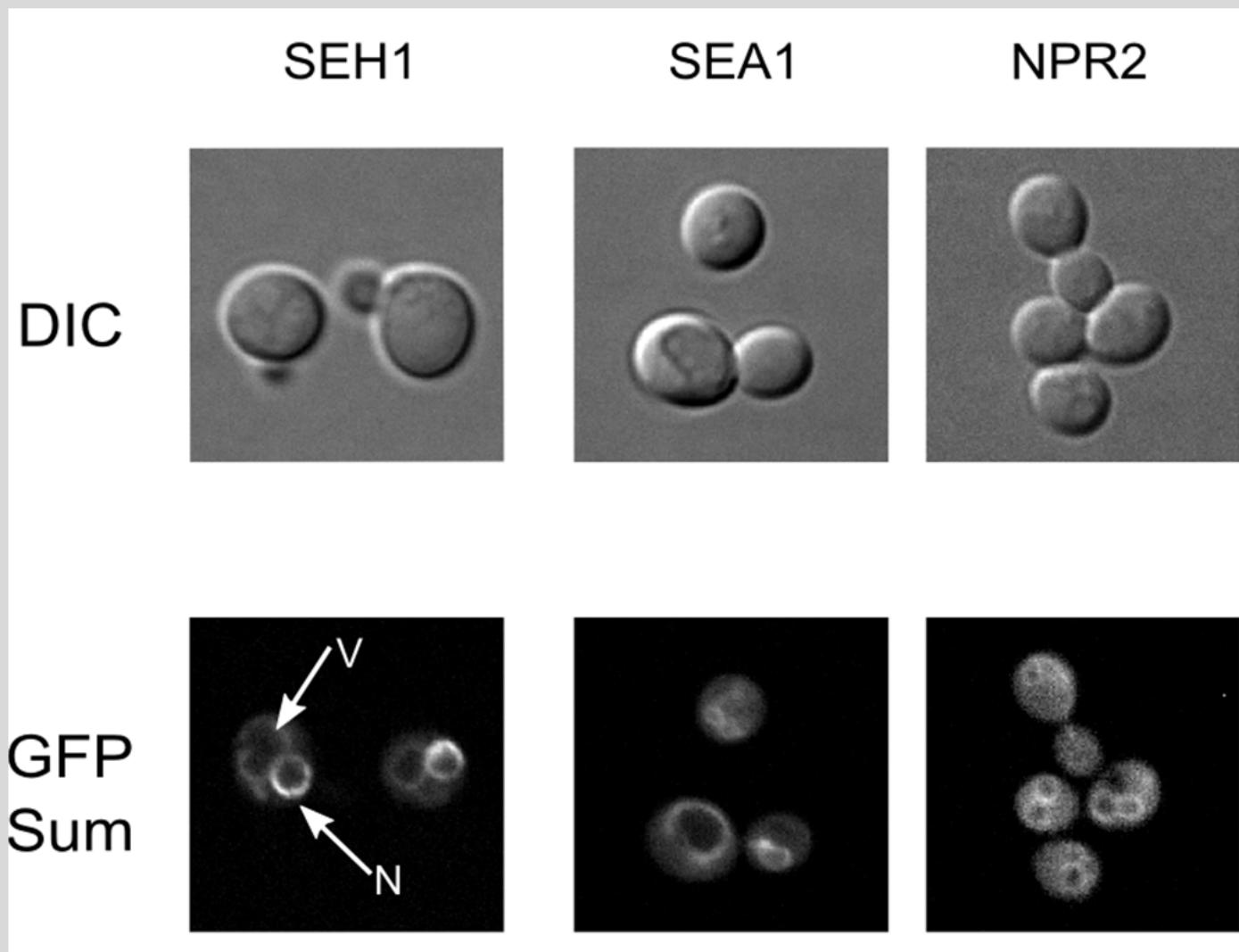


Dokudovskaya et al., Nature, 2007

Dokudovskaya et al., Mol.Cell.Proteomics, 2011

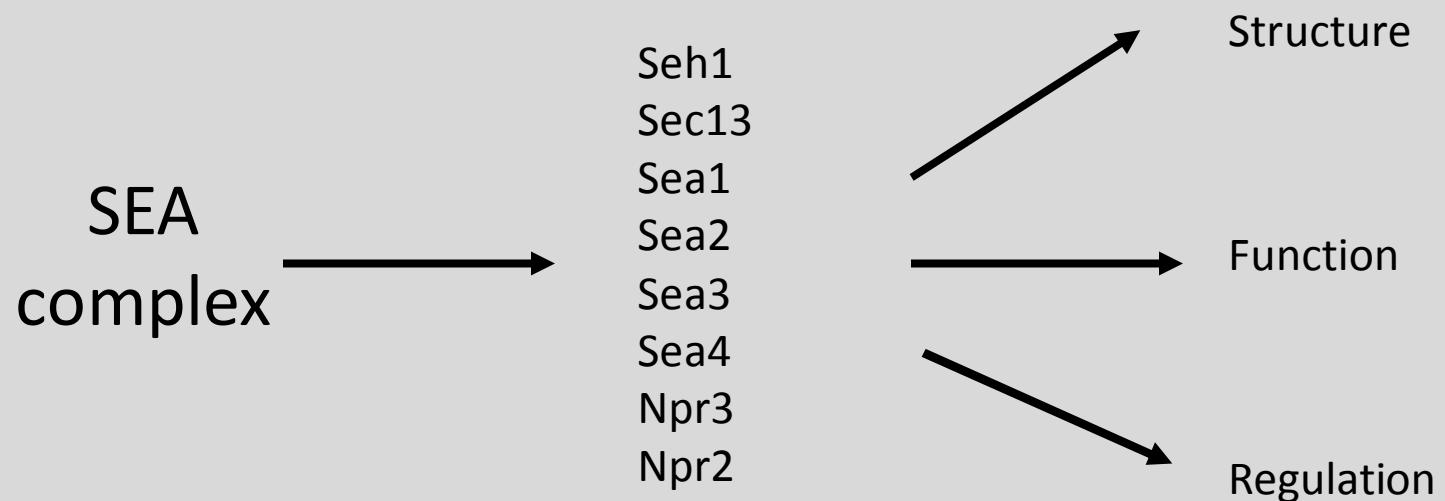
Dokudovskaya et al., Autophagy, 2011

SEA Proteins are Localized Around Vacuole Membrane



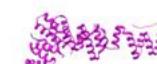
Dokudovskaya et al., Mol.Cell.Proteomics, 2011
Dokudovskaya et al., Autophagy, 2011

The SEA Complex



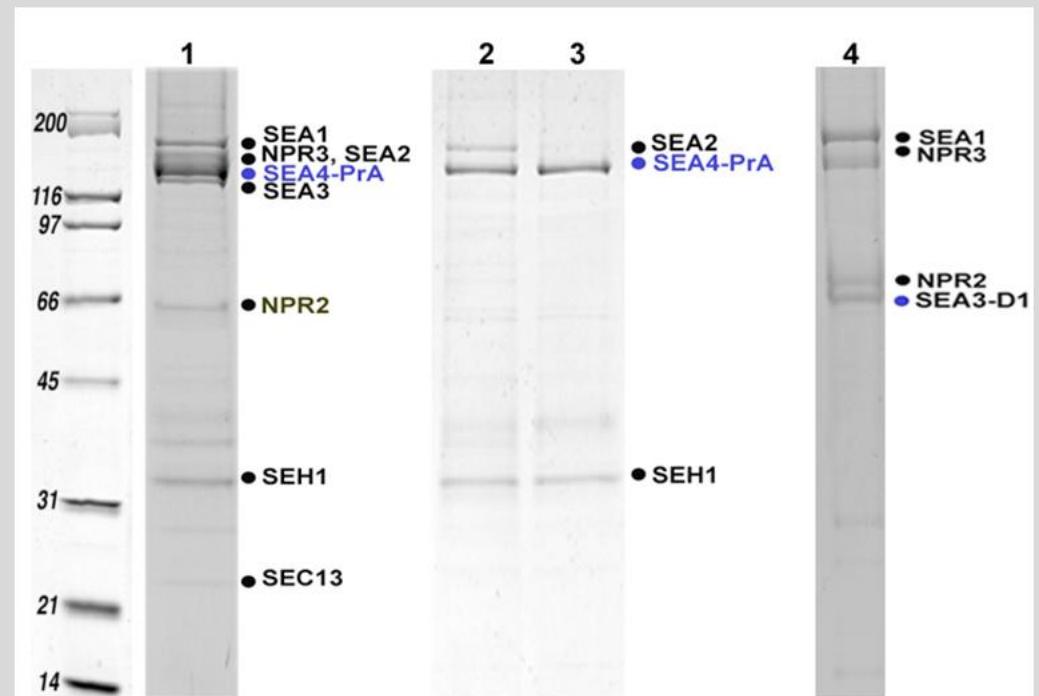
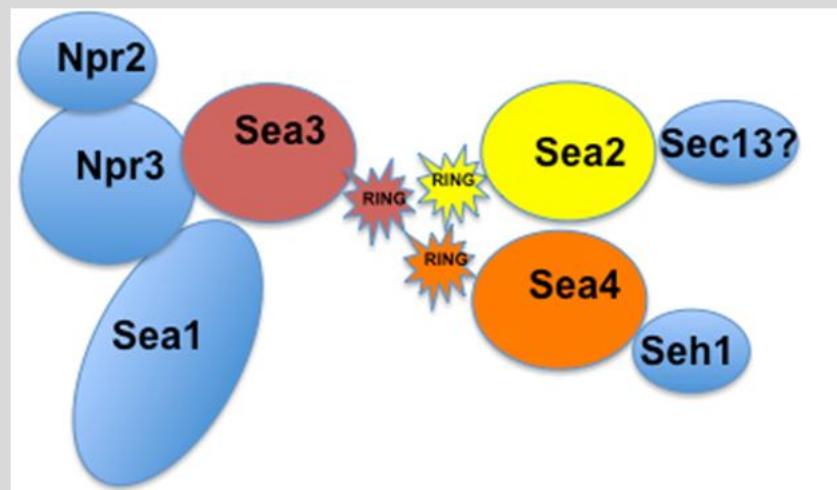
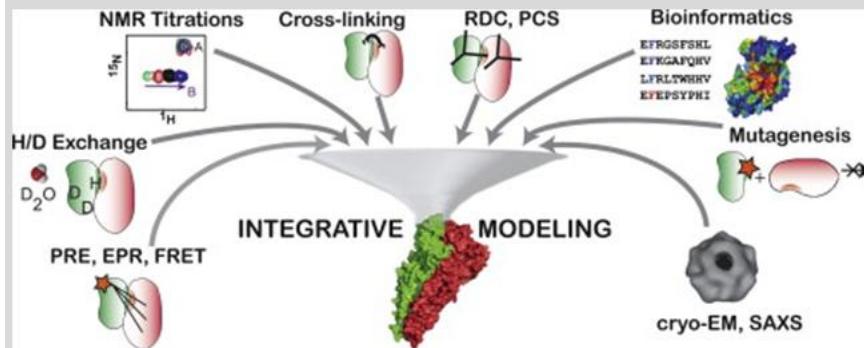
Sea proteins are ancient, non-essential, but conserved

The SEA Complex Belongs to a Superfamily of Coating Complexes Involved in Membrane Trafficking

Complex	Proteins	Structure	
COPI	α -COP		
	β -COP		
COPII	Sec31		
	Sec13		
Clathrin	Clathrin heavy chain		
NPC	Nup170, Nup157, Nup133, Nup120		
	Nup192, Nup188, Nup145C, Nup85, Nup84		
	Sec13, Seh1		
IFT	Ift40, Osm4, Wdr19, Wdr35, Ift172		
	Che2		
	Ift188		
HOPS/ CORVET	Vps3, Vps16		
	Vps8, Vps11, Vps18, Vps41		 
SEA	Sea2, Sea3		
	Sec13, Seh1		
	Sea4		 

SEA Complex Structure – Integrative Approach

I. Immunoprecipitations

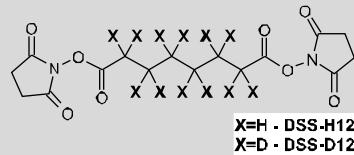


- 1: Sea4-PrA
- 2: ΔSea3, Sea4-PrA (75 mM NaCl)
- 3: ΔSea3, Sea4-PrA (300 mM NaCl)
- 4: Sea3-ΔRing-PrA

Algret et al., Mol.Cell.Proteomics, 2014

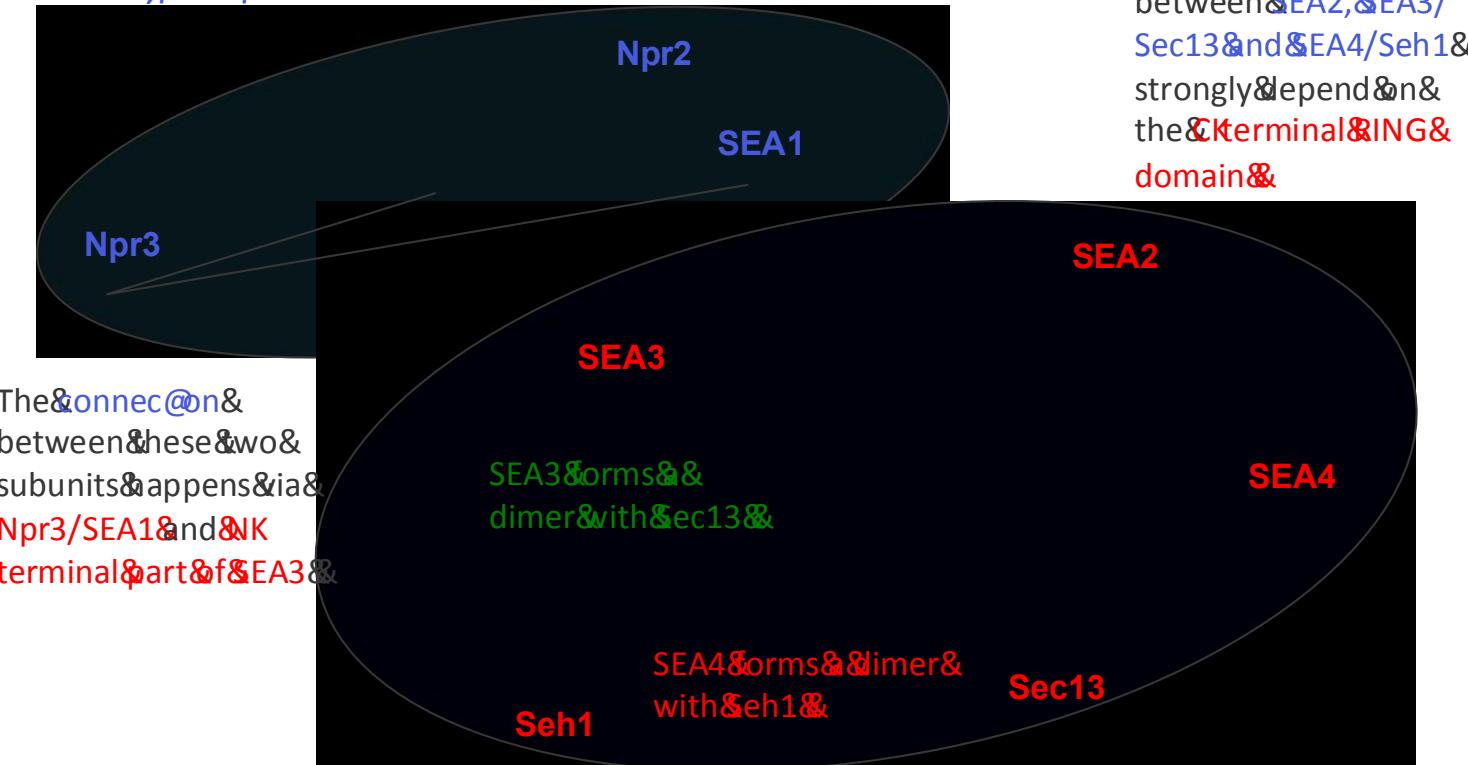
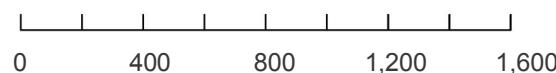
SEA Complex Interconnectivity

II. Cross-links

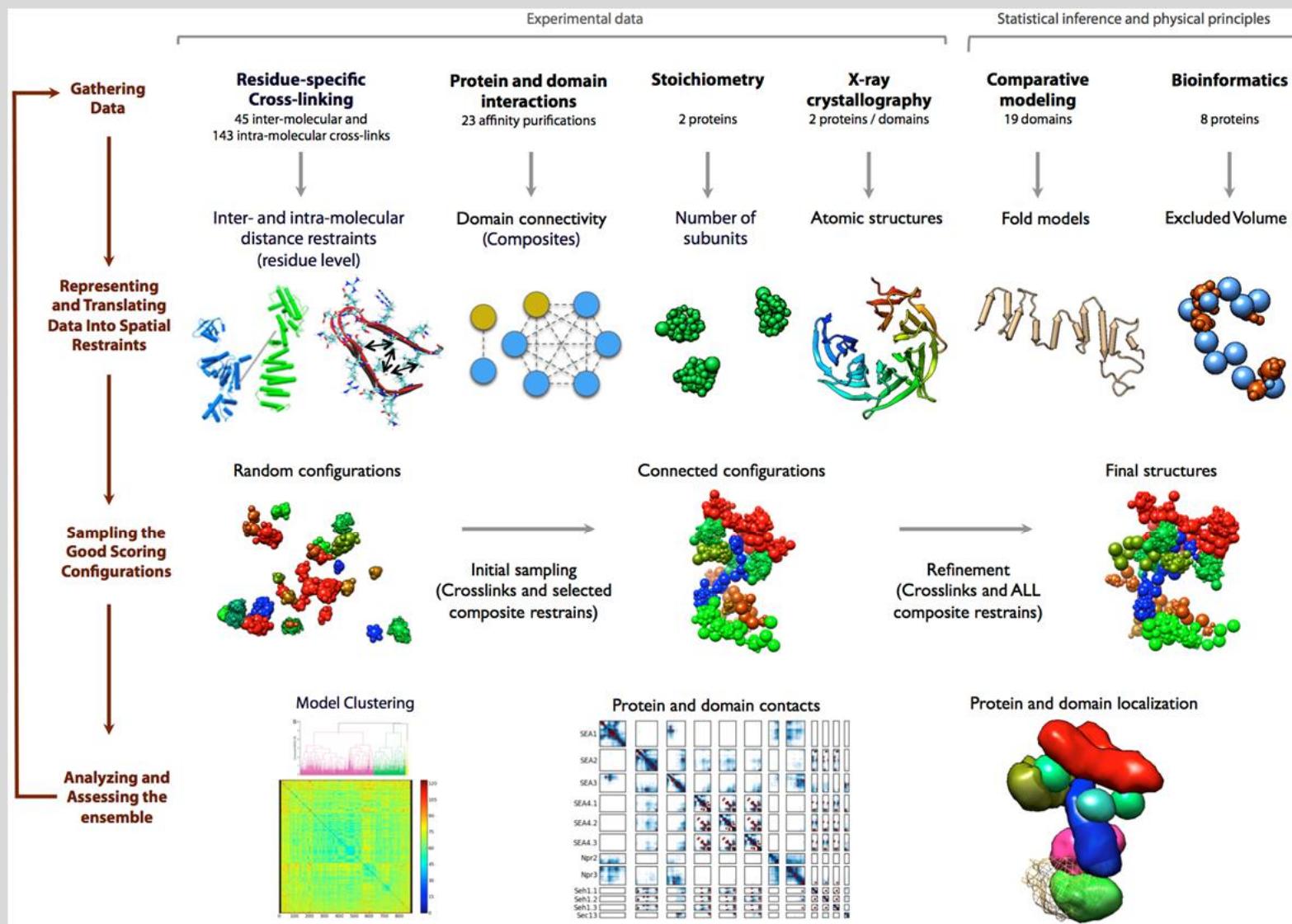


Two subunits &

1. SEA1, Npr2, Npr3
2. SEA3/Sec13, SEA4/
Seh1, SEA2

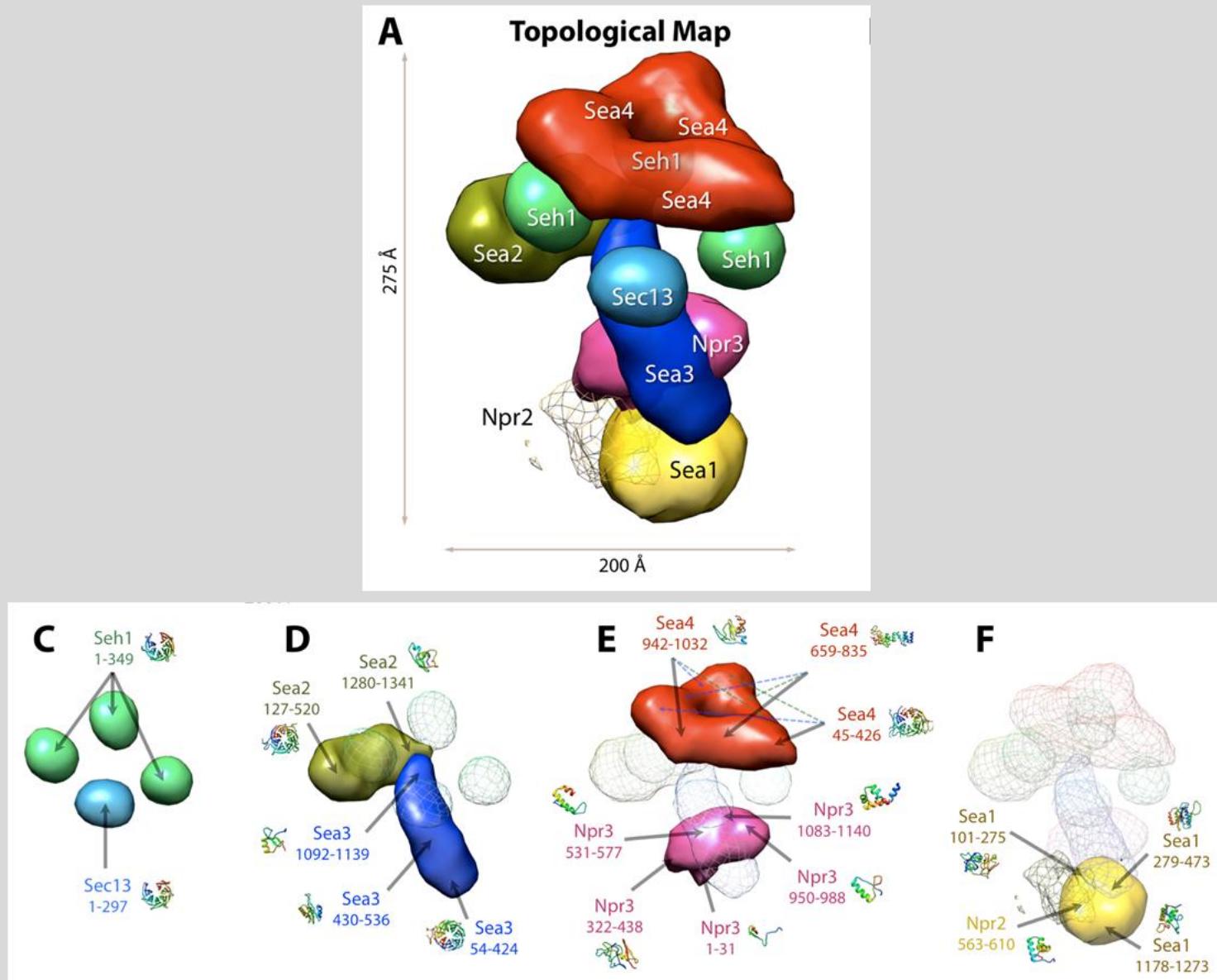


SEA Complex Structure – Integrative Approach



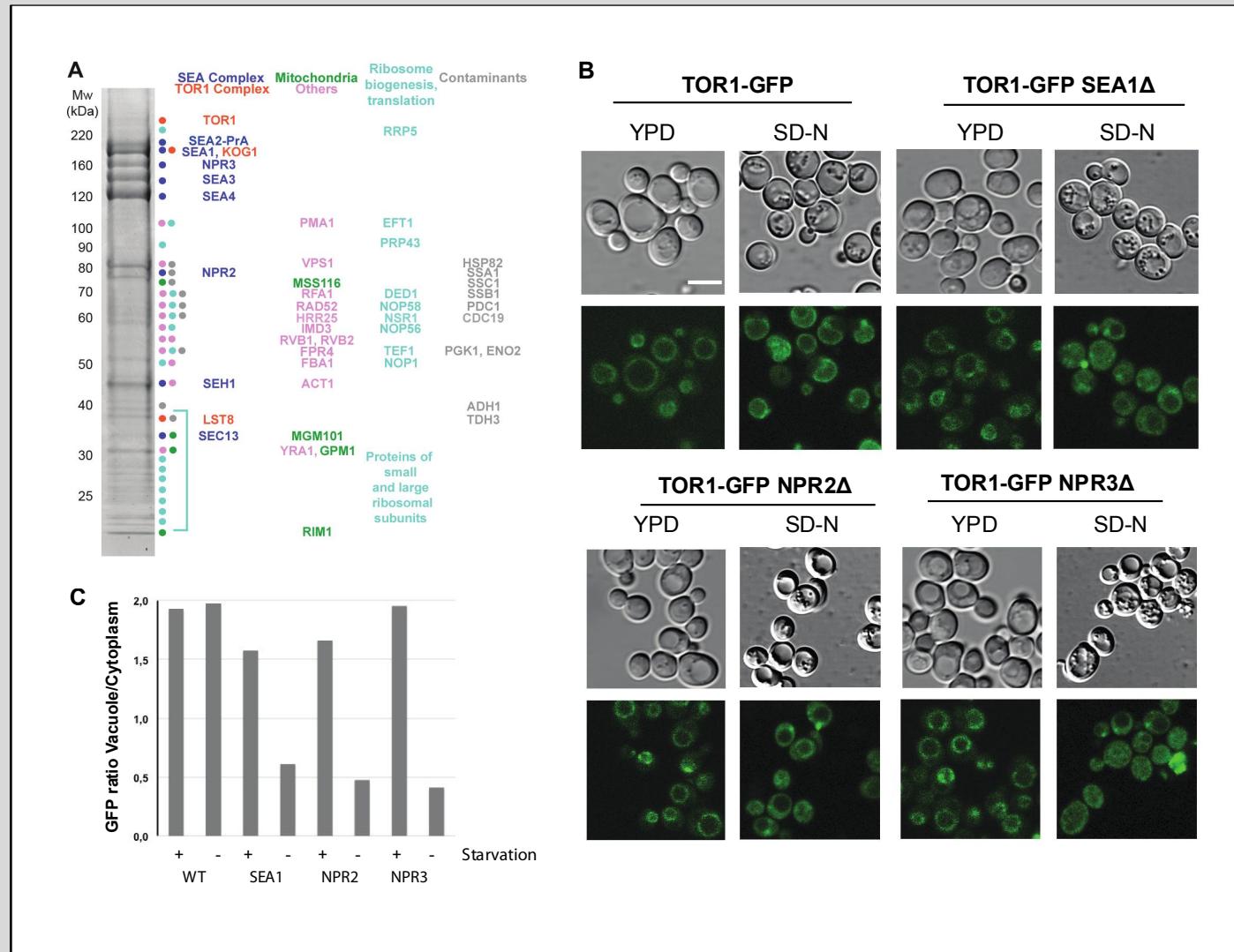
Algett et al., Mol.Cell Proteomics, 2014

SEA Complex Structure – a First Topological Map



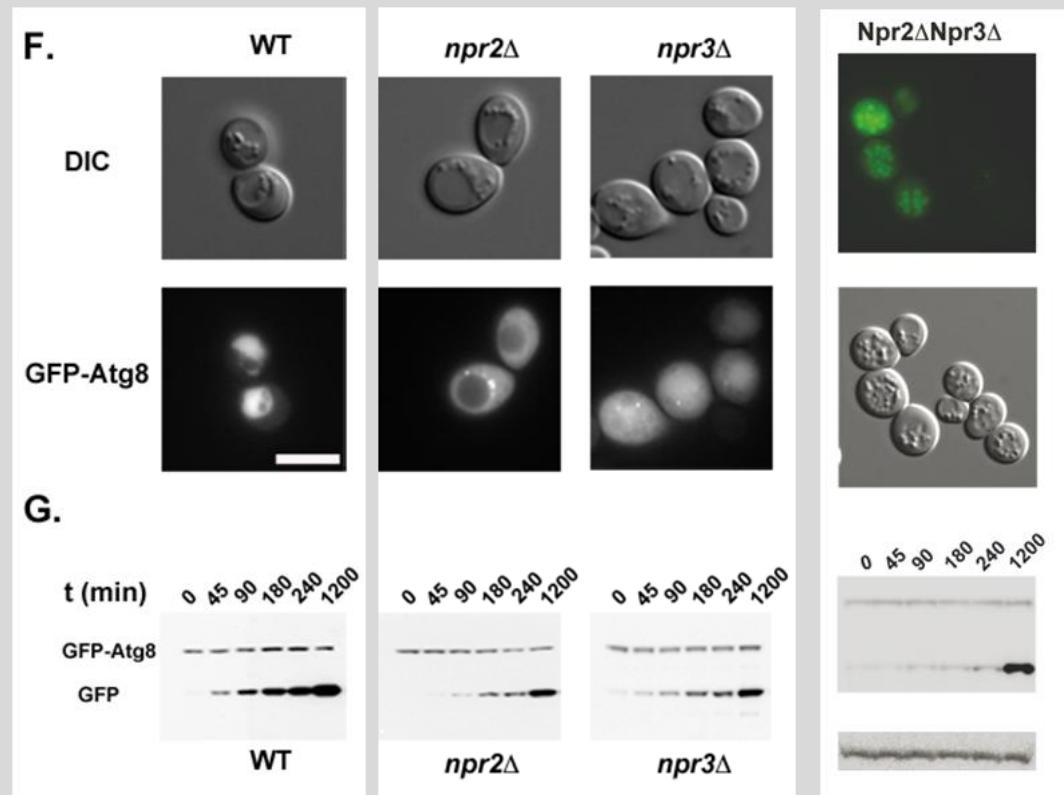
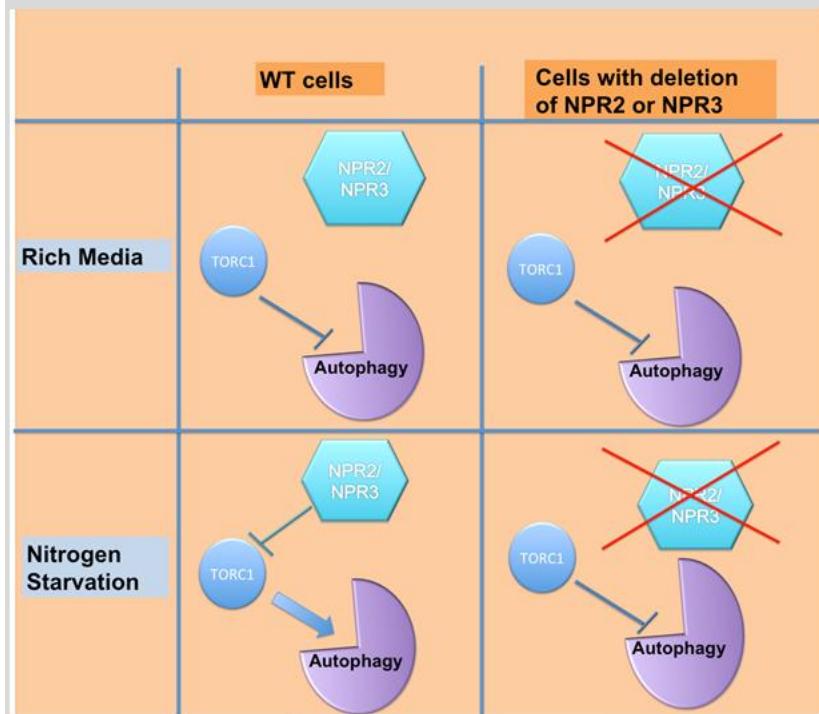
Algret et al., Mol.Cell Proteomics, 2014

SEA Complex Function – Regulates TORC1 Pathway

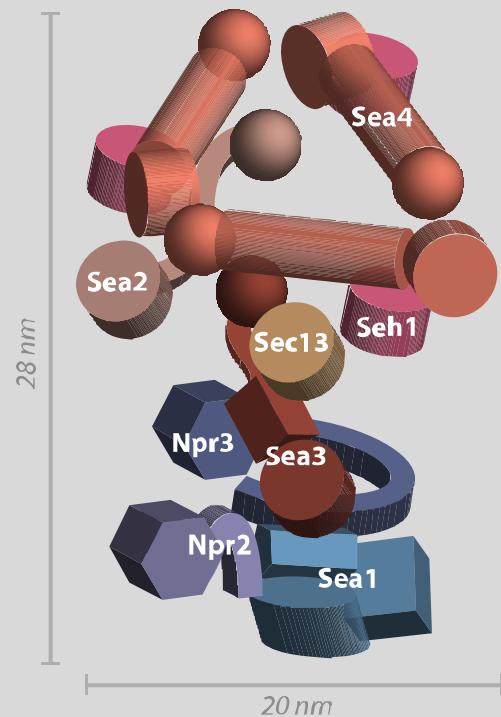


Algret et al., Mol.Cell Proteomics, 2014

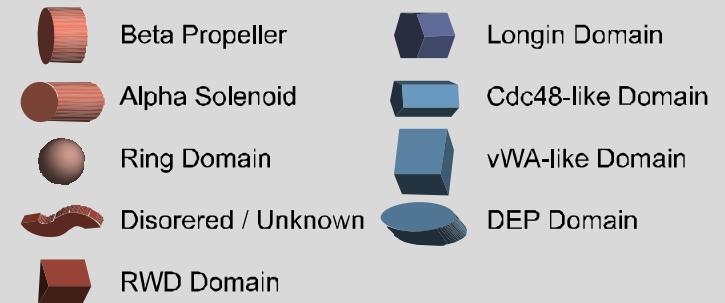
Function of the SEA Complex: Nitrogen Starvation and Autophagy



The GATOR Complex – Mammalian Homologue of the SEA Complex: Deregulation in Cancer and other Diseases



SEACAT	GATOR2
Sea4	Mios
Sea2	Wdr24
Seh1	Seh1L
Sec13	Sec13
Sea3	Wdr59
SEACIT	GATOR1
Npr3	Nprl3
Npr2	Nprl2
Sea1/lml1	Depdc5



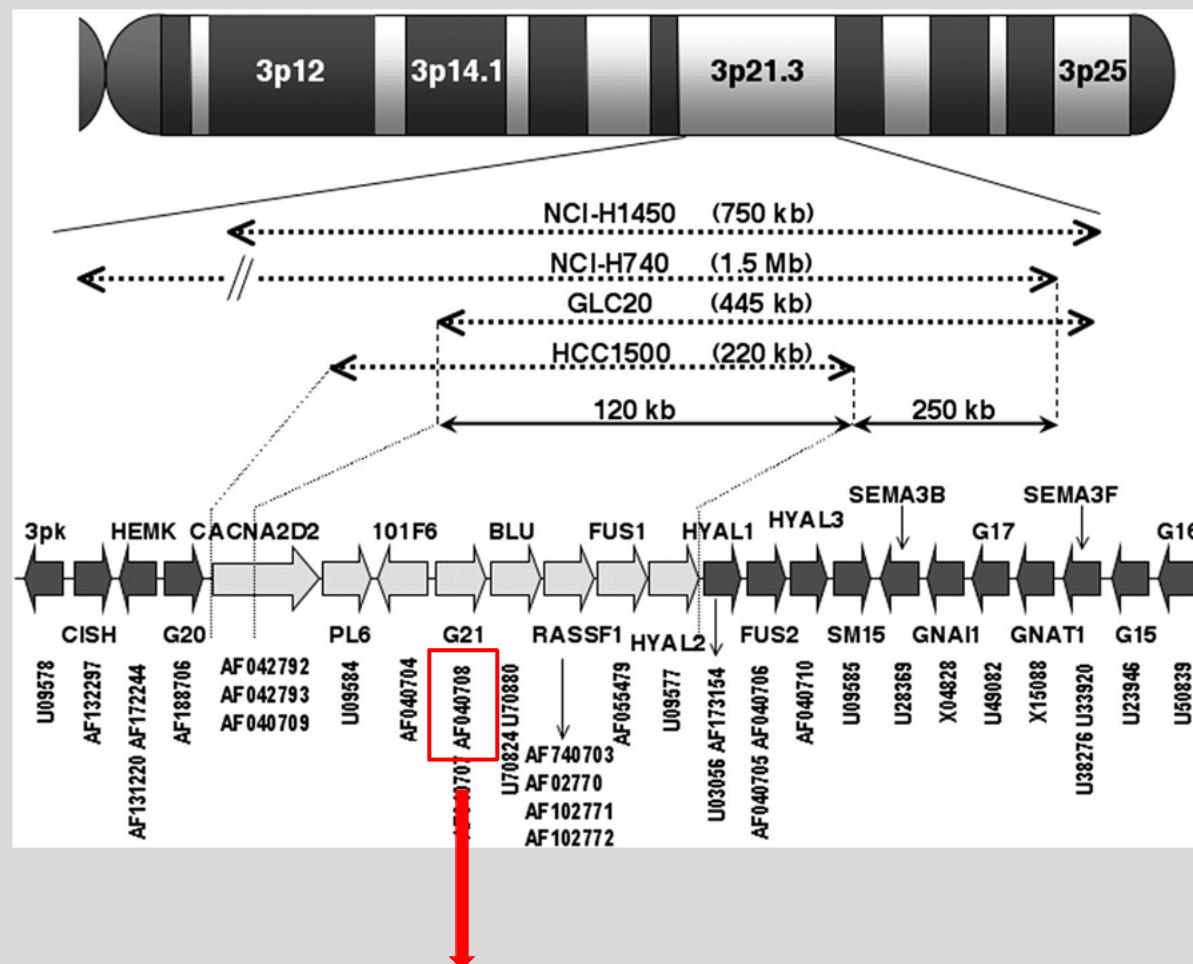
NPRL3
Cardiovascular diseases

NPRL2
Tumor suppression:
Lung cancer, ovarian cancer,
breast cancer, glioblastomas
Resistance to anticancer drugs
Doxorubicin, cisplatin

DEPDC5
Glyoblastoma, ovarian cancer
Familial focal epilepsy

Algett & Dokudovskaya, *Biopolymers and Cell*, 2012
Dokudovskaya & Rout, *J.Cell Science*, 2015

Human Chromosome Region 3p.21.3 Contains Tumor Suppressor Genes



NPRL2 / TUSC4

NPRL2 – a Novel Tumor Suppressor Important for the Success of the Cisplatin Treatment

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Mol Pharmacol 64:259–268, 2003

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Printed in U.S.A.

Anticancer Drug Resistance Induced by Disruption of the *Saccharomyces cerevisiae* NPRL2 Gene: a Novel Component Involved in Cisplatin- and Doxorubicin-Provoked Cell Kill

PAUL W. SCHENK, MARIËL BROK, ANTONIUS W. M. BOERSMA, JOURICA A. BRANDSMA, HANS DEN DULK,
HERMAN BURGER, GERRIT STOTER, JAAP BROUWER, and KEES NOOTER

Section of Experimental Chemotherapy, Department of Medical Oncology, Erasmus University Medical Center Rotterdam, Josephine Nefkens Institute, Rotterdam, the Netherlands (P.W.S., M.B., A.W.M.B., H.B., G.S., K.N.); and Medical Genetics Centre South-West Netherlands, Department of Molecular Genetics, Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, Leiden, the Netherlands (J.A.B., H.D.D., J.B.)

Research Article

The 3p21.3 Tumor Suppressor **NPRL2** Plays an Important Role in Cisplatin-Induced Resistance in Human Non-Small-Cell Lung Cancer Cells

Kentaro Ueda,¹ Hiroyuki Kawashima,¹ Shoichiro Ohtani,¹ Wu-Guo Deng,¹ Murali Ravoori,² Jim Bankson,³ Boning Gao,⁴ Luc Girard,⁴ John D. Minna,⁴ Jack A. Roth,¹ Vikas Kundra,² and Lin Ji¹

Lung Cancer News

Useful Links

Video Library

Introgen's Novel Nanoparticle Tumor Suppressor **NPRL2** Shows Promise In The Treatment Of Drug Resistant Cancers

Main Category: Lung Cancer

Also Included In: Cancer / Oncology; Clinical Trials / Drug Trials

Article Date: 21 Nov 2006 - 0:00 PDT



Equipe “Trafic membranaire, complexes macromoléculaire et cancer”

UMR8126, Institut Gustave Roussy, Villejuif

Licia Silveri - Post-doc, 2010-2013



(Subvention de Fondation pour la Recherche Médicale)

Michael Foss – Post-doc, 2011-2012

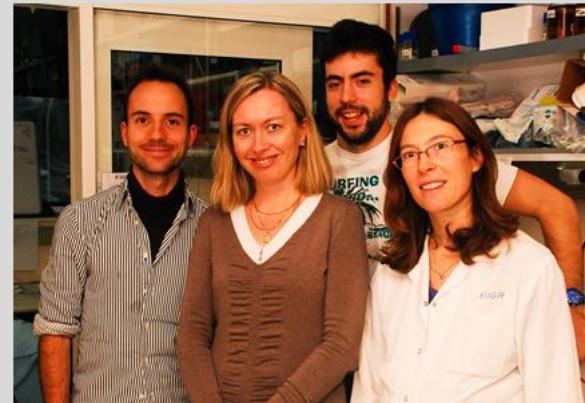


(Subvention de Fondation Gustave Roussy)

Romain Algret - PhD student, 2010-2013



(Contrats doctoraux de l'Université Paris-Sud11)



Claire Martel-Jantin – Post-doc

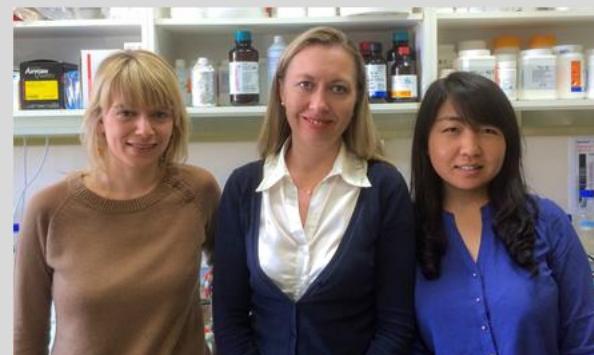


(Canceropole Ile-de-France, since 2014)

Yinxing Ma – PhD student



(Chinese Scholarship Council and l'Université Paris-Sud,
since 2013)



Staelle Makamte, Jihane Mzoughi M 1 students

Sarah Issa , Marie-Ange Calemjane,
Diana Losyeva – M2 students

