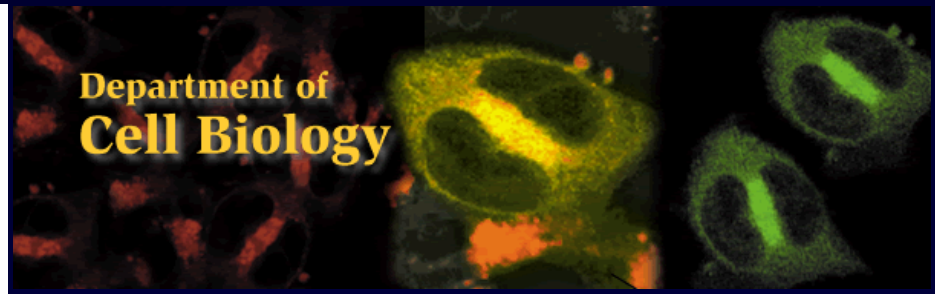

Conferința Diaspora în Cercetarea Științifică Românească
București, 17-19 Septembrie 2008

Reglarea traficului de proteine de la reticulul endoplasmic spre si prin aparatul golgi: o mai buna intelegere a mecanismelor moleculare ale bolilor neurodegenerative

Florin Manolea
University of Alberta
Canada

Golgi complex and neurodegenerative diseases

- Alzheimer disease (Banoyannis et al., 2004)
- Amyotrophic lateral sclerosis (Stieber et al., 1998)
- Creutzfeldt-Jacob disease (Sakurai et al., 2000)
- Multiple system atrophy (Sakurai et al., 2002)
- Parkinson’s disease (Fujita et al., 2006)
- Spinocerebelar ataxia type 2 (Huynh et al., 2003)
- Niemann-Pick type C (Lin et al., 2007)

Outline

- Introduction
  - Players and regulators of ER-Golgi-PM protein traffic
- Results
  - Overexpression studies
  - Knockdown studies

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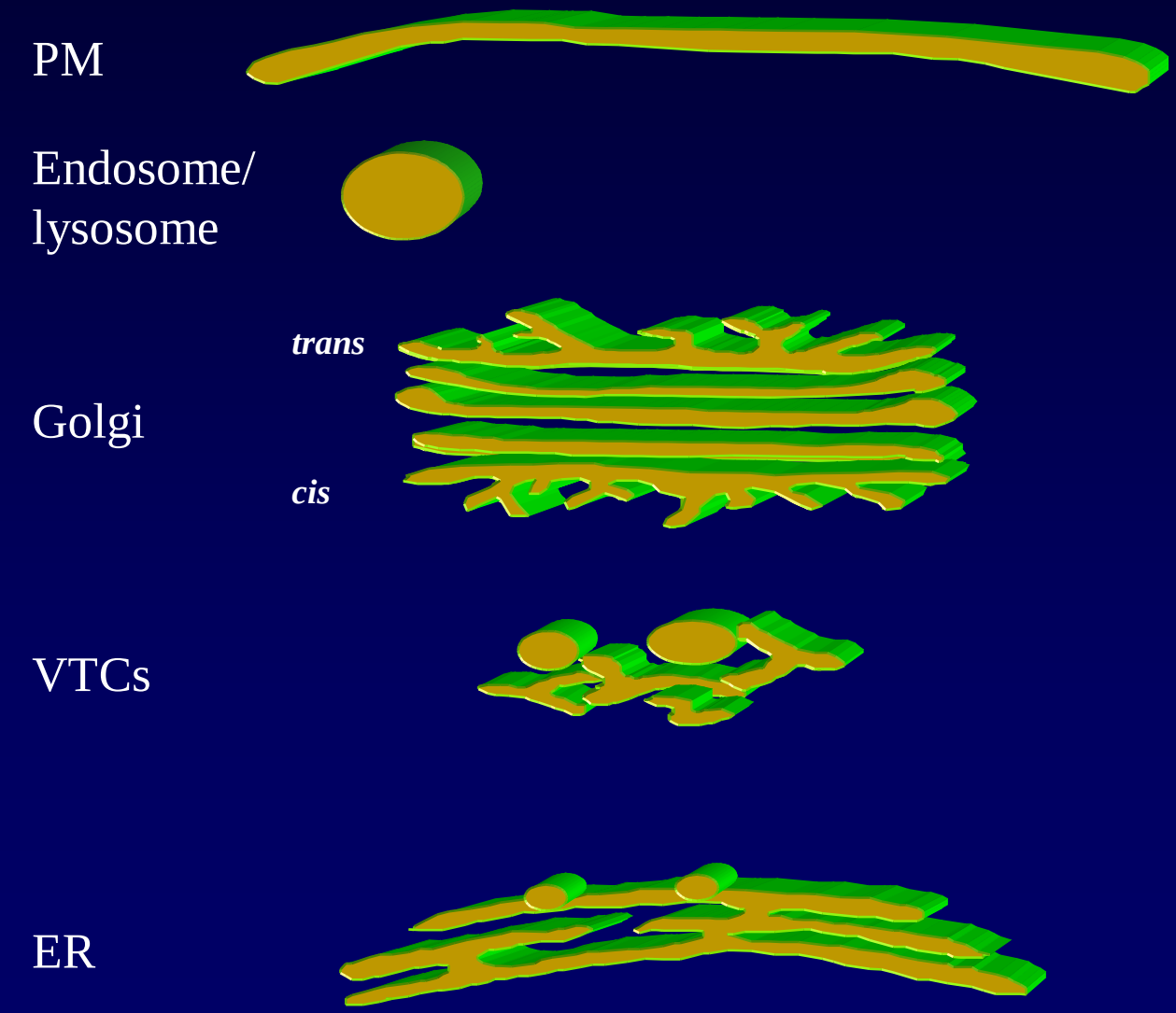
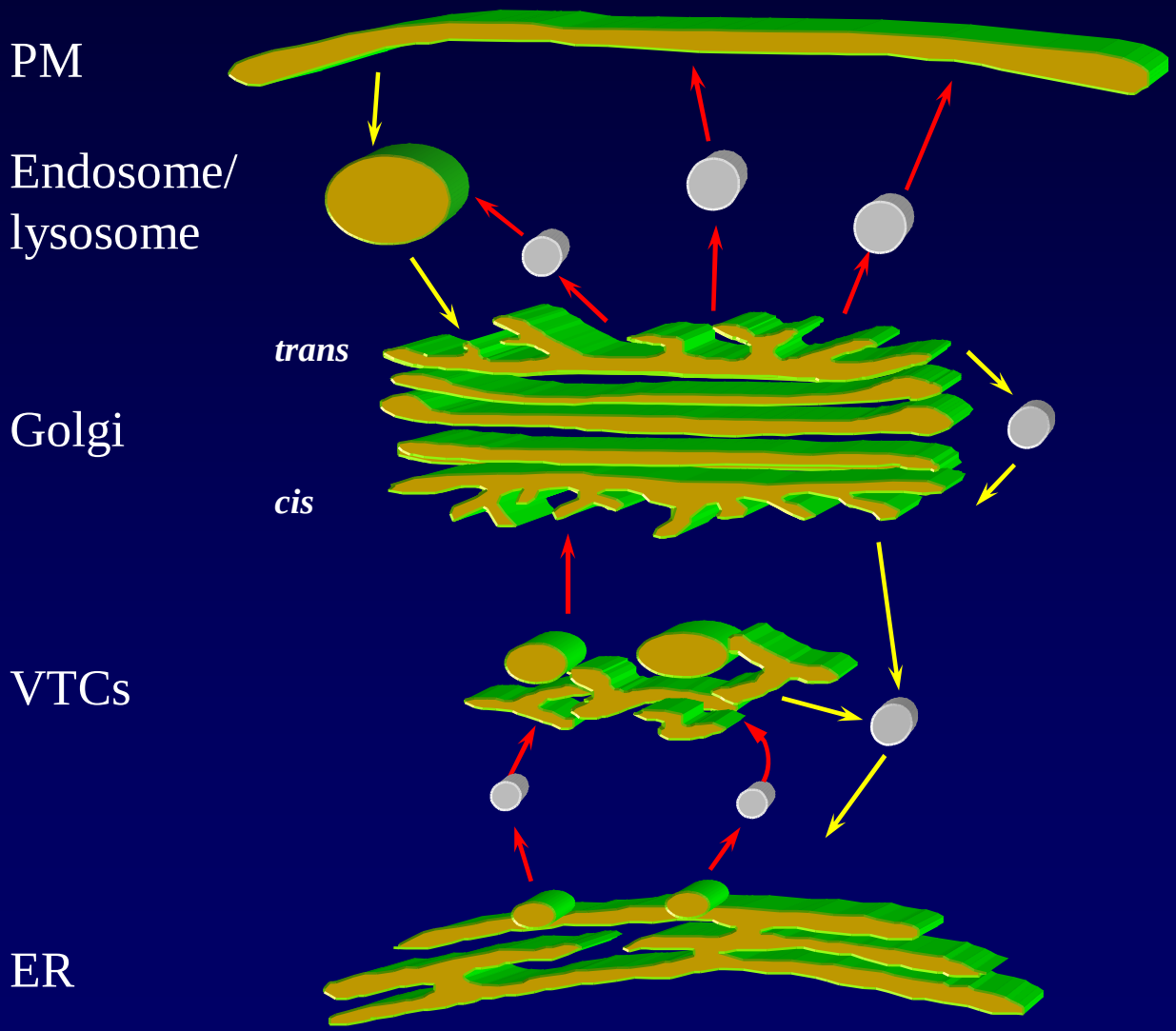
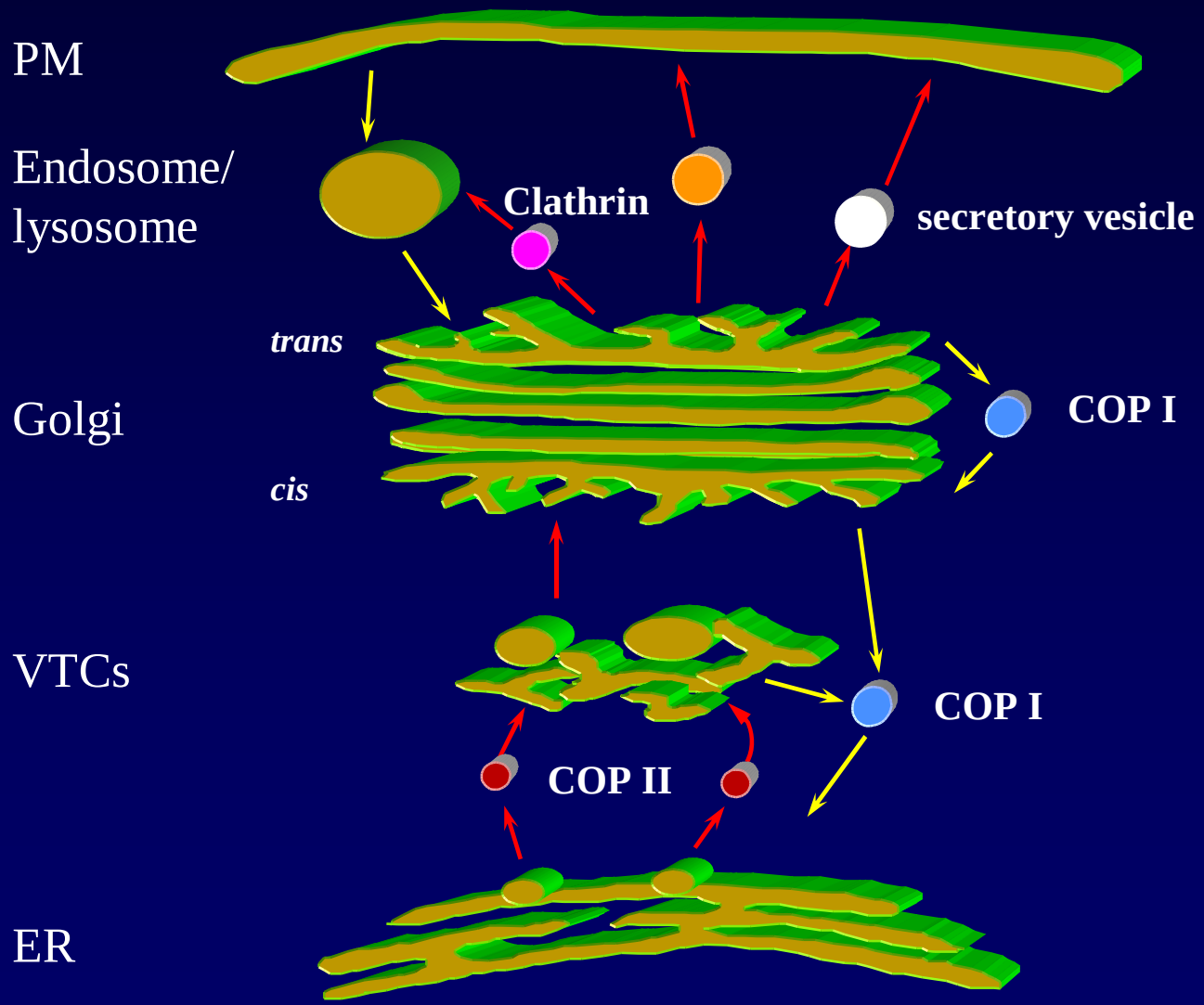
ER-Golgi-PM protein traffic



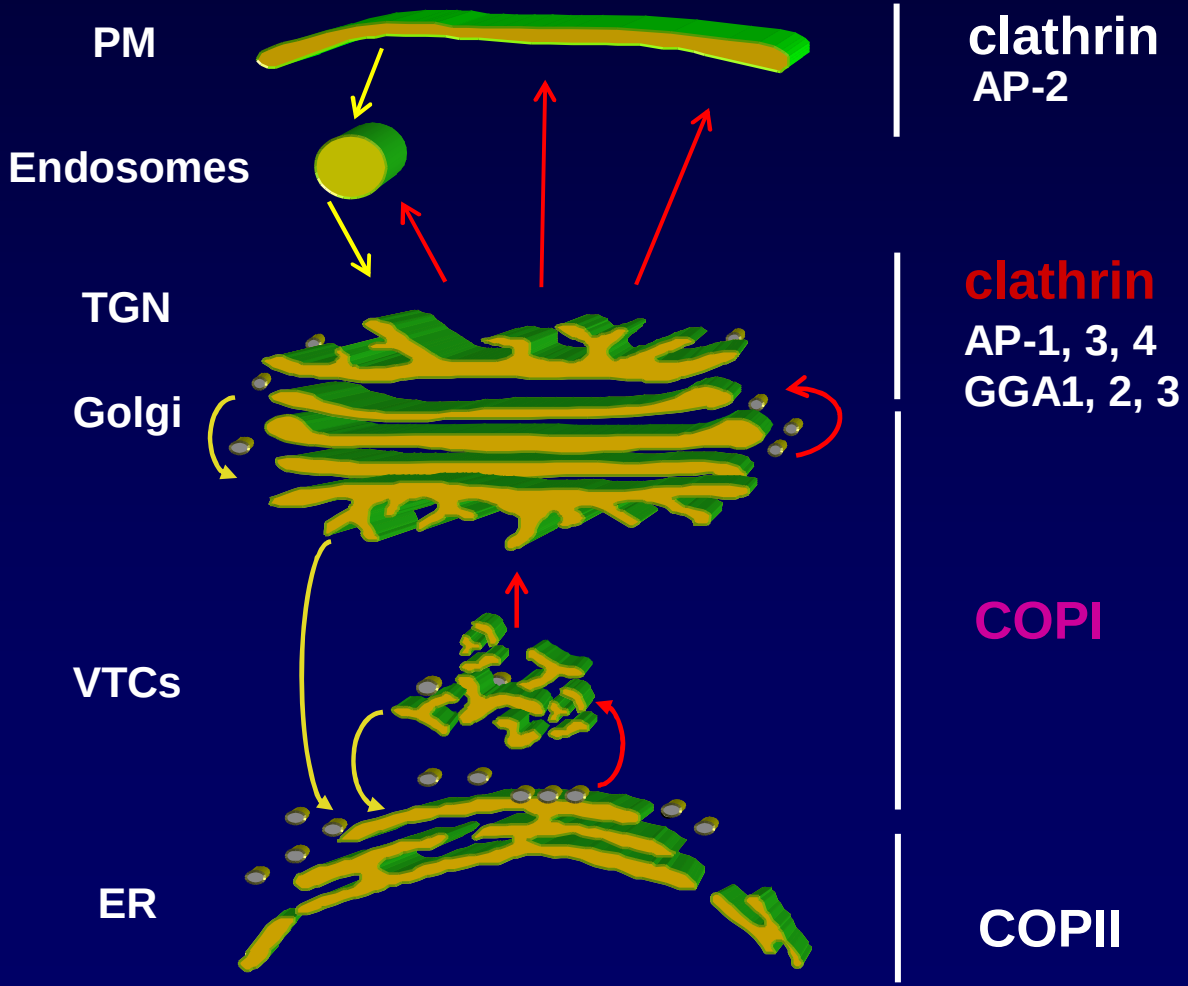
ER-Golgi-PM protein traffic is bi-directional



ER-Golgi-PM protein traffic and associated coat proteins



Coat proteins facilitate dynamic and bi-directional protein traffic in the central vacuolar system



Coats

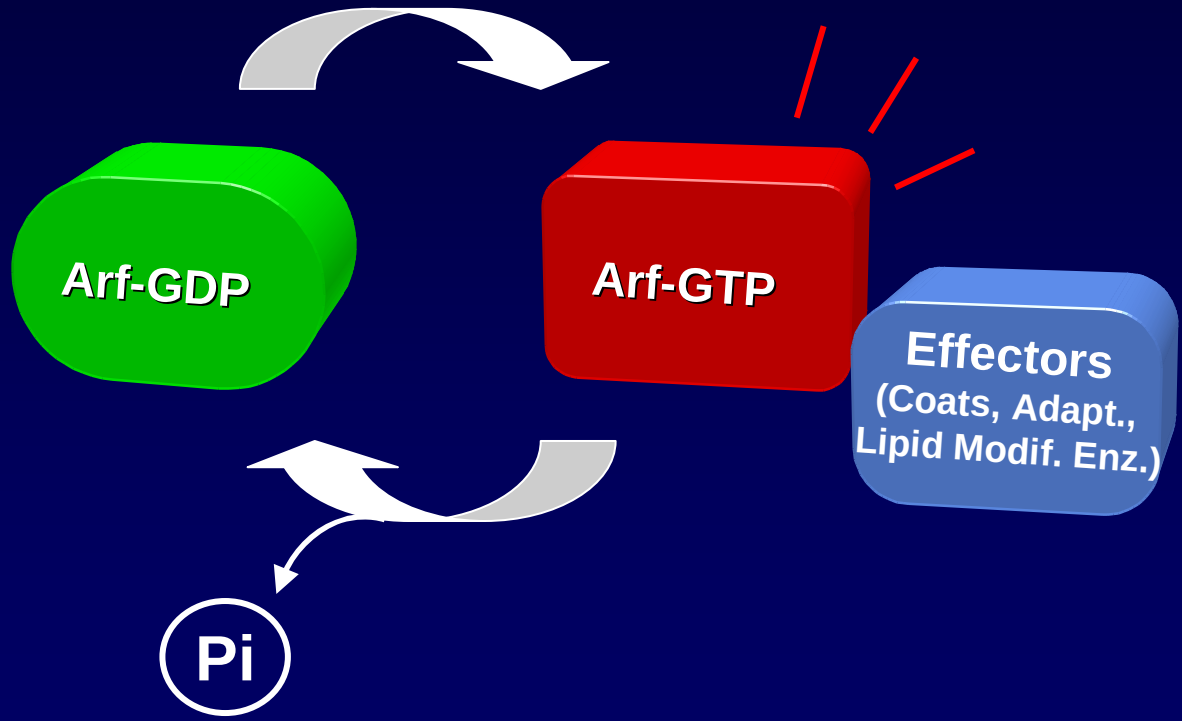
clathrin
AP-2

clathrin
AP-1, 3, 4
GGA1, 2, 3

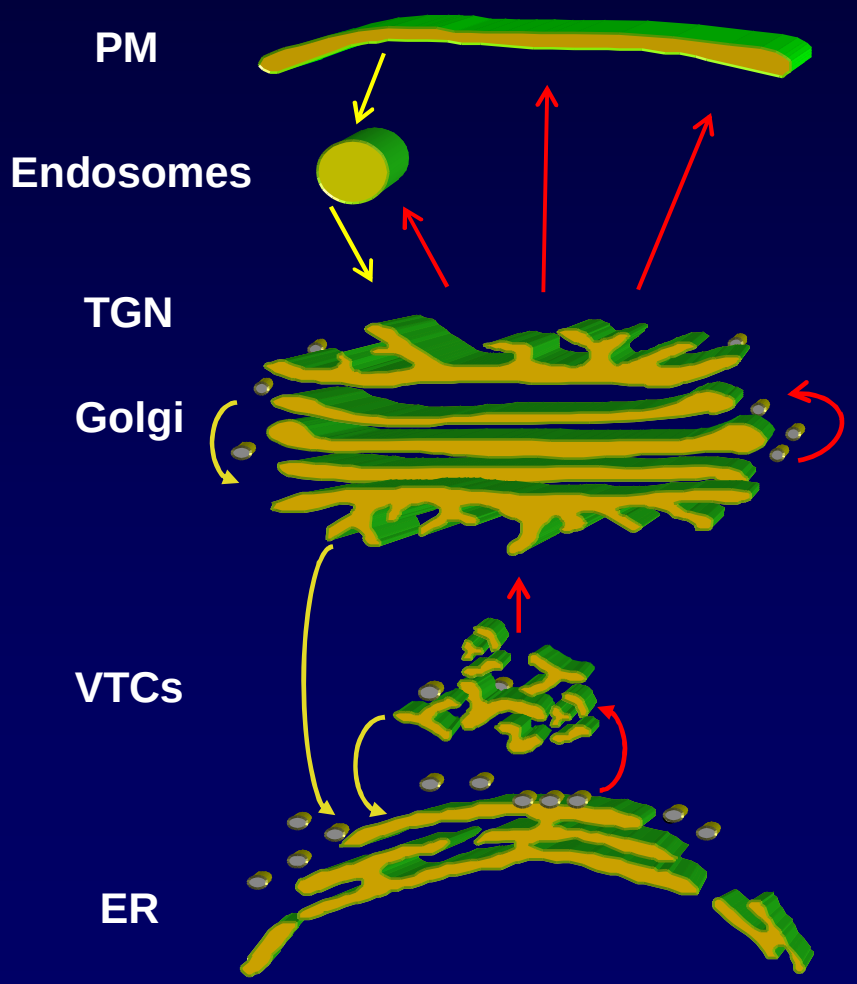
COPI

COPII

Arfs are molecular switches regulating coat assembly and lipid composition

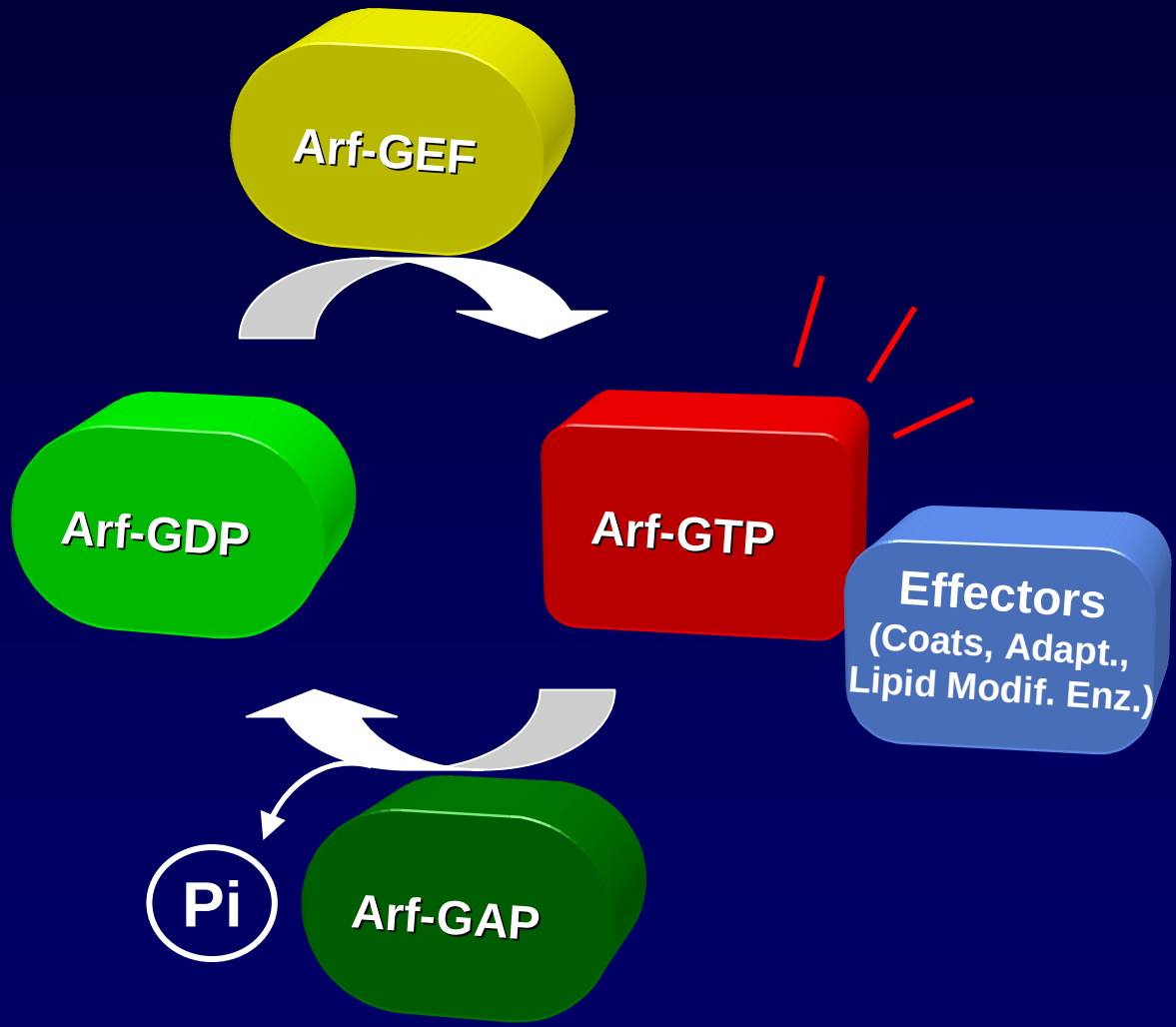


Small GTPases regulate coat recruitment

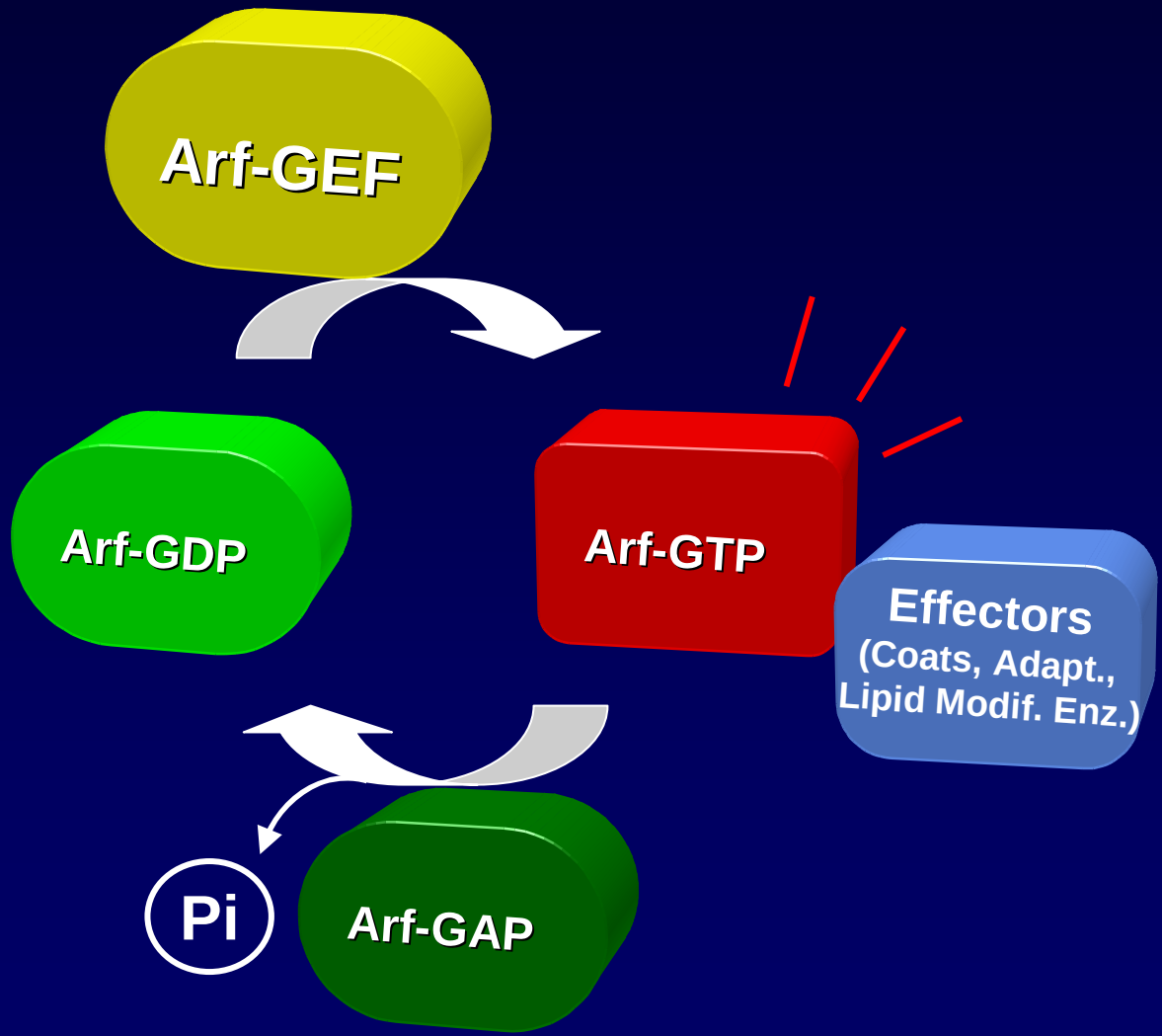


Coats	GTPases
clathrin AP-2	Arf6 Arf1
clathrin AP-1, 3, 4 GGA1, 2, 3	Arf1 Arf3, 4, 5?
COPI	Arf1 Arf4, 5?
COPII	Sar1

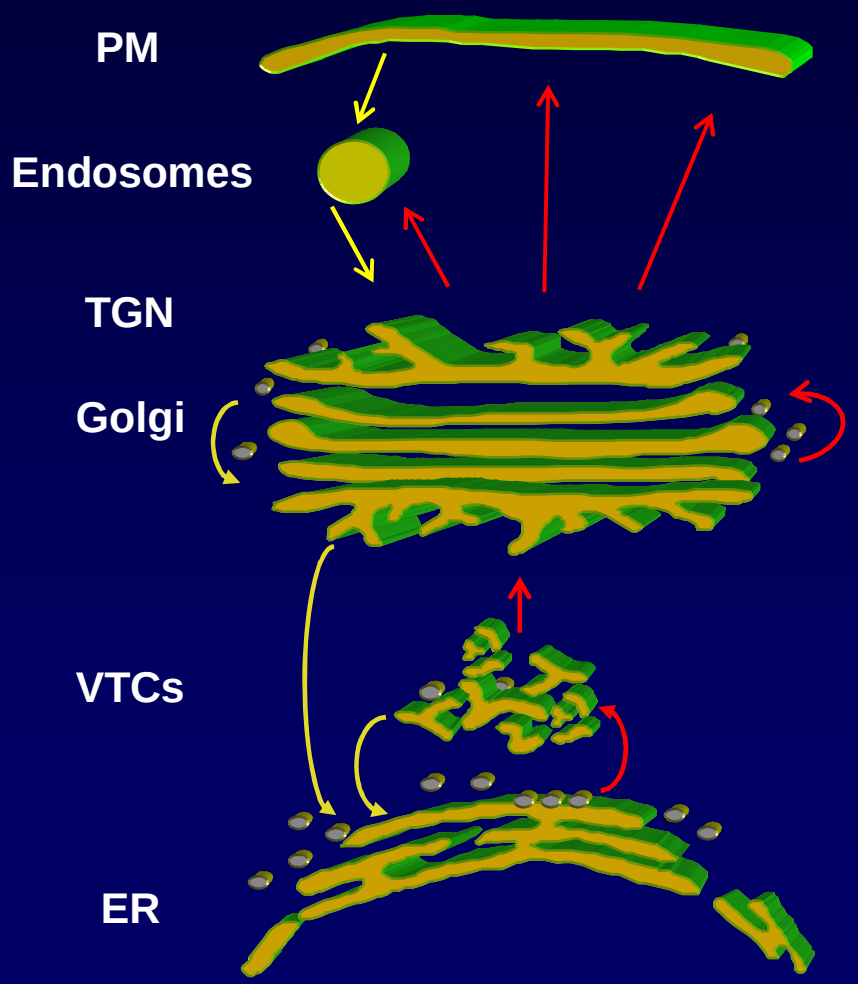
GEFs and GAPs regulate Arfs activity



GEFs activate Arfs

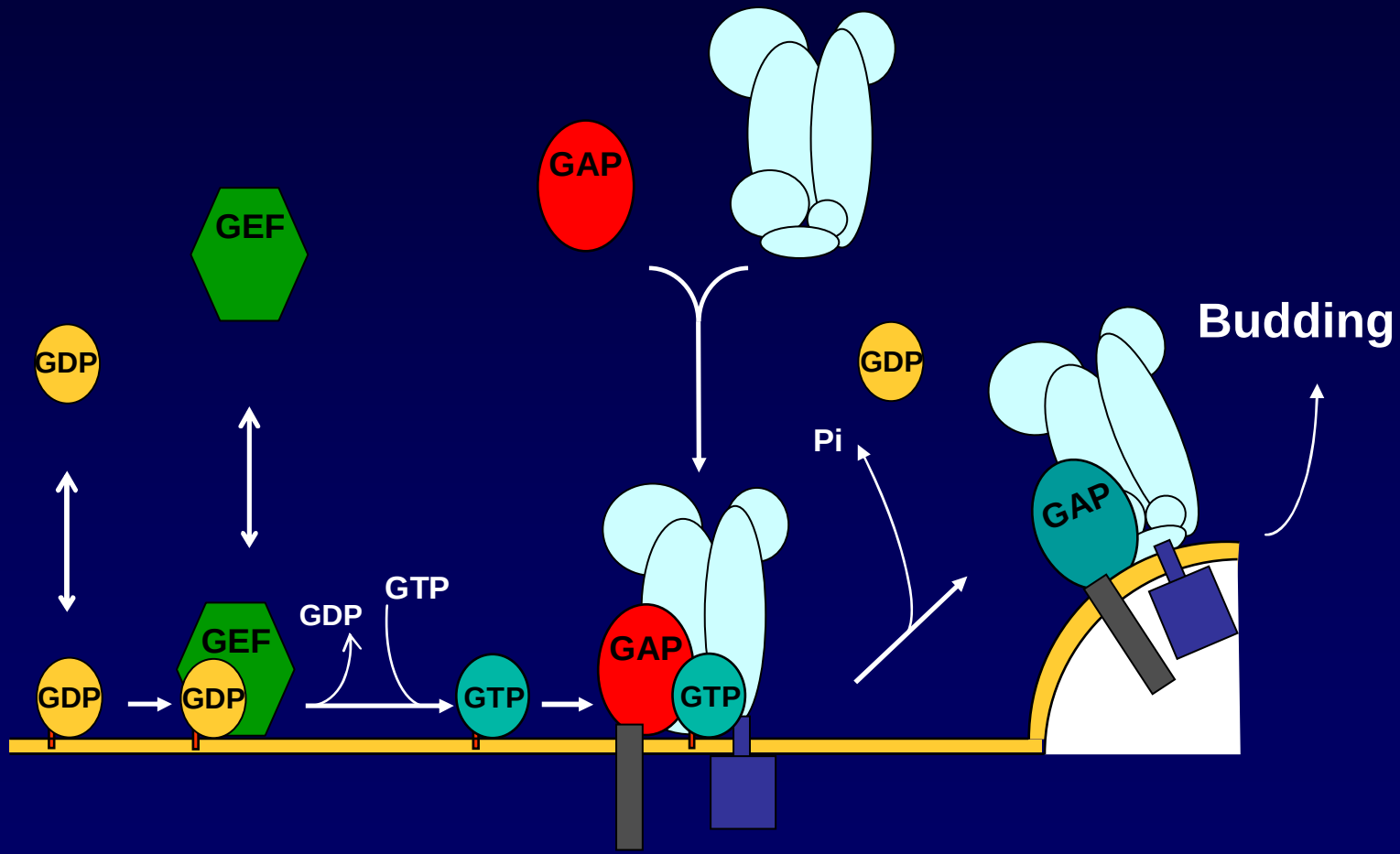


GBF1 and BIG1/2 are the only Arf-GEFs localized to Golgi complex



	Coats	GTPases	
PM / Endosomes	clathrin AP-2	Arf6 Arf1	
TGN / Golgi	clathrin AP-1, 3, 4 GGA1, 2, 3	Arf1 Arf3, 4, 5?	BIG1/2
VTCs	COPI	Arf1 Arf4, 5?	GBF1
ER	COPII	Sar1	

Arf-Guanine nucleotide exchange factors, or GEFs, initiate coat assembly on the membrane



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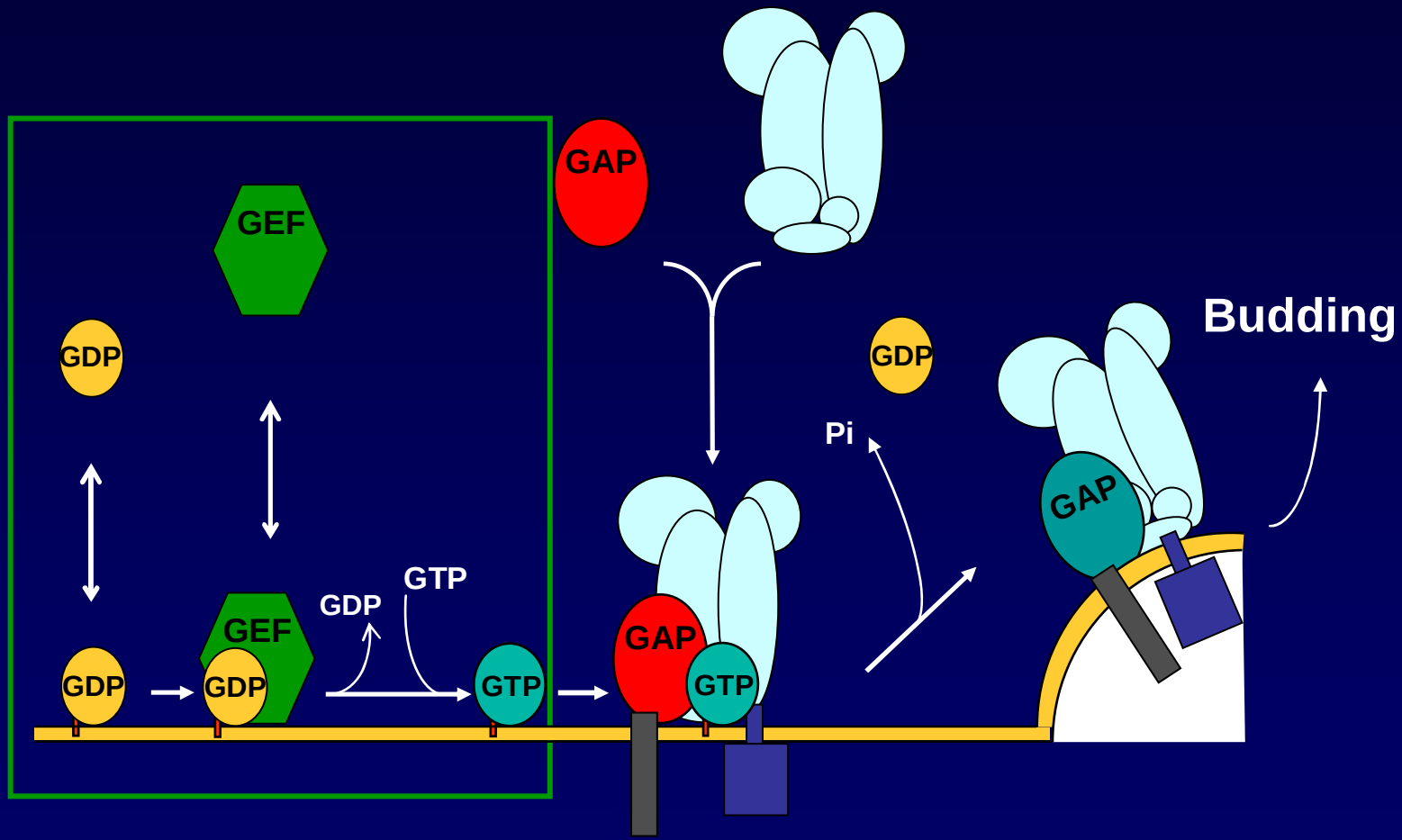
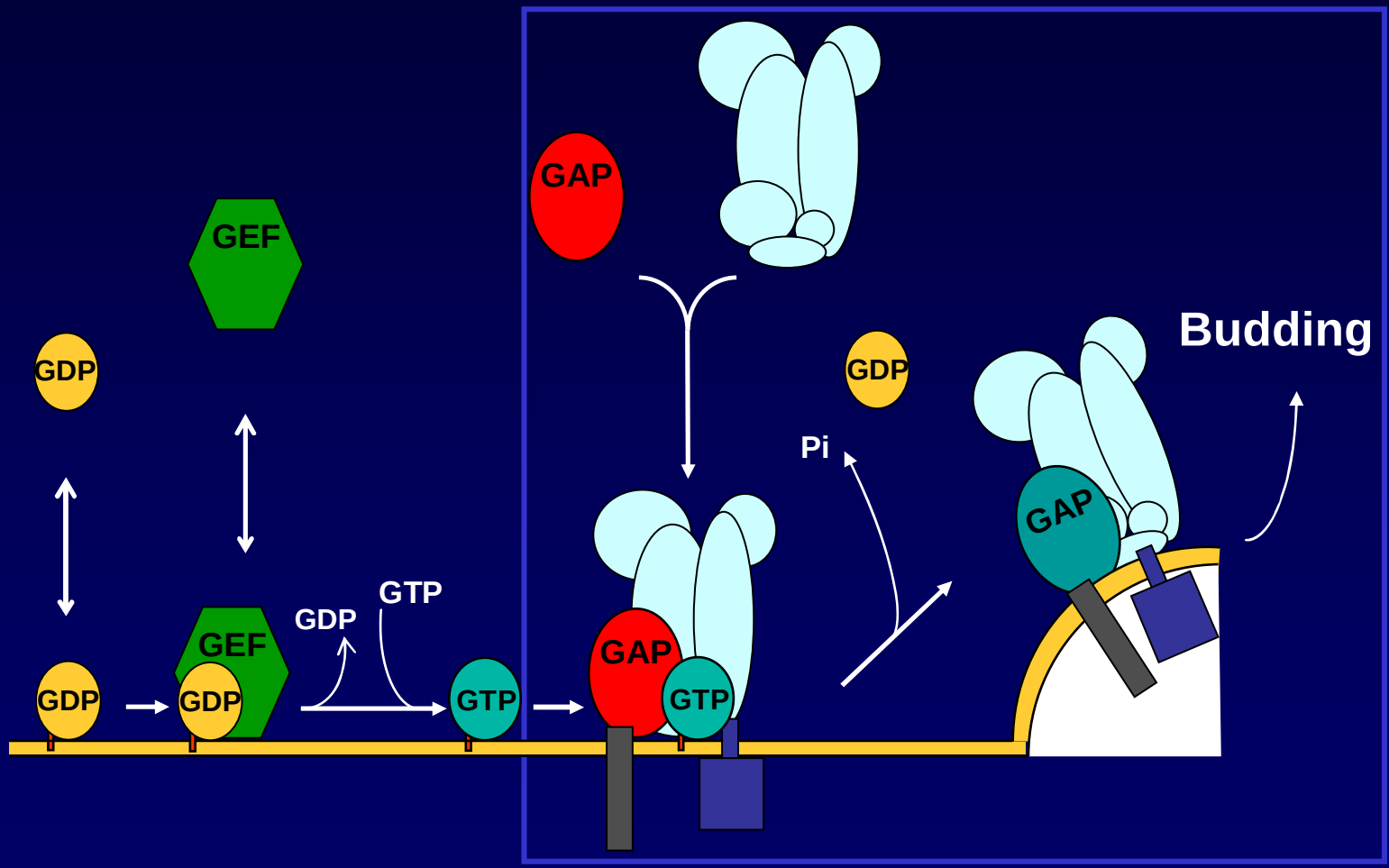
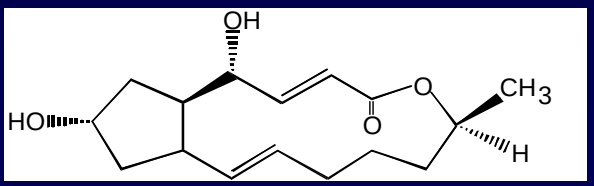
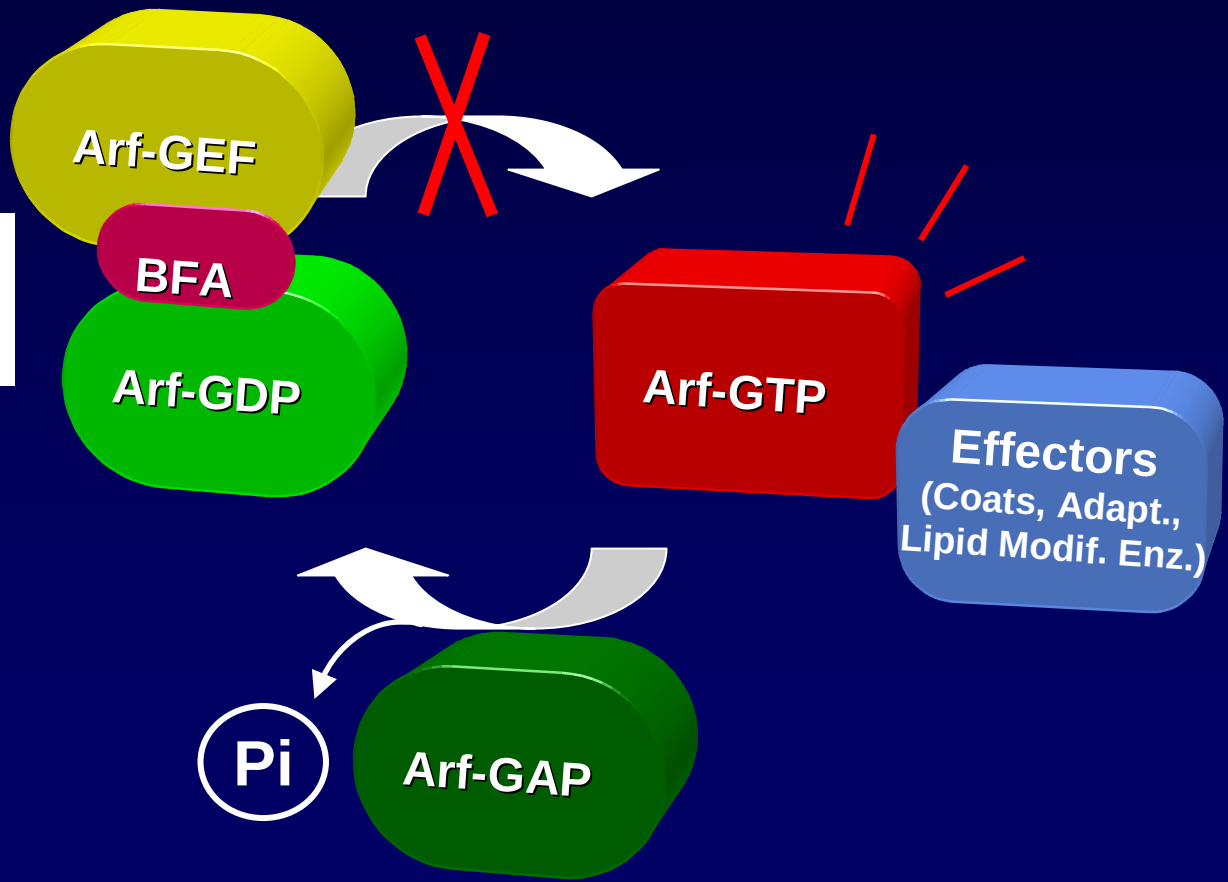


GTPase activating proteins, or GAPs, participate in cargo selection and coat formation

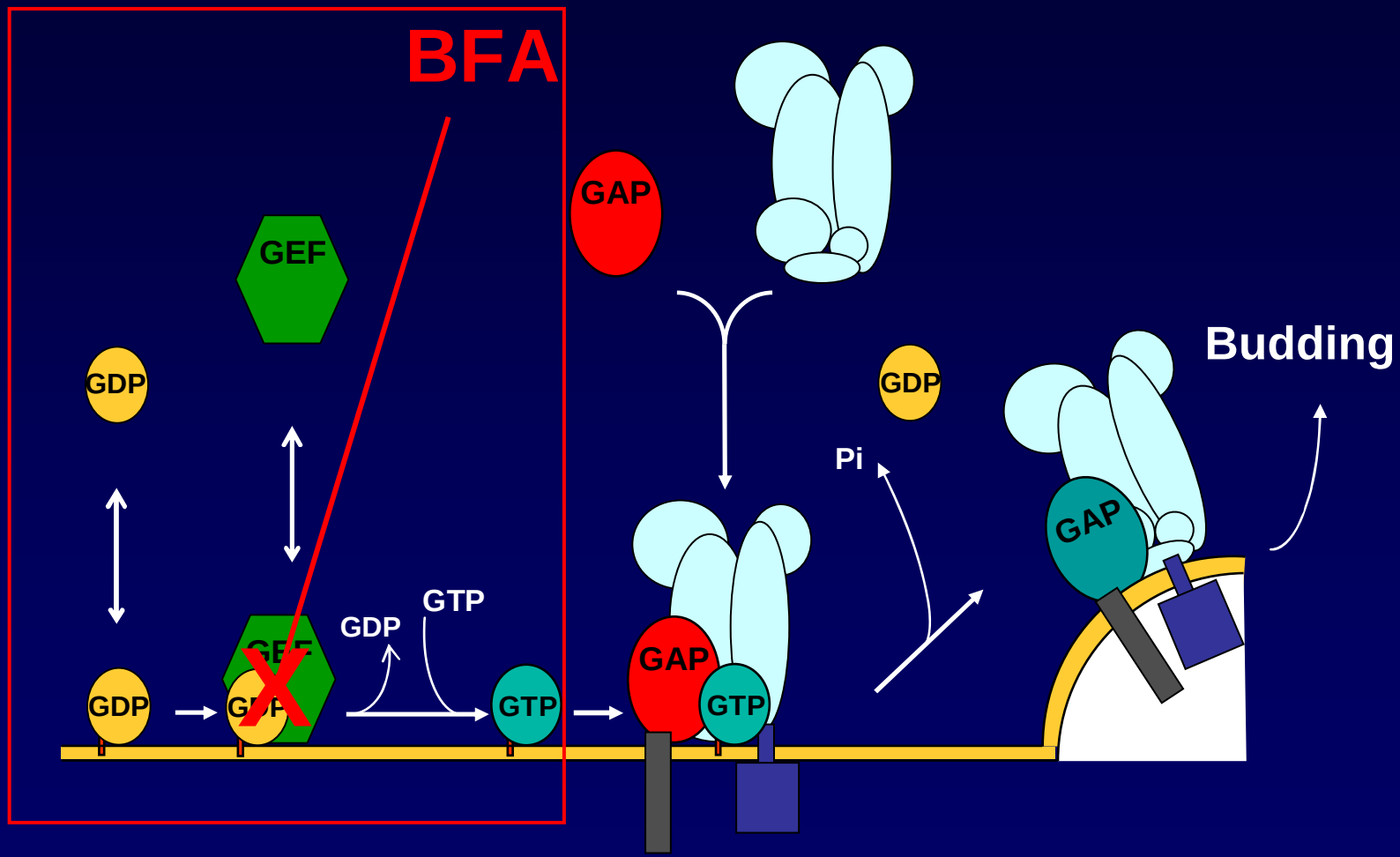


Brefeldin A blocks Arf-GEF activity and prevents following Arf activity





BFA blocks Arf activation and coat assembly on Golgi membranes



Previous studies proved:

- ✓ **GBF1 and BIGs belong to two different sub-families.**
- ✓ **Both GBF1 and BIGs displays BFA sensitivity *in vivo.***
- ✓ **GBF1, not BIG1, has a preference for Arf5 *in vitro*.**
- ✓ **BIG2 is specific for Arf1 and Arf3.**
- ✓ **GBF1 and BIGs function as dimers:**
  - ➢ **GBF1 most probably forms homodimers**
  - ➢ **BIG1 and BIG2 have different function but they also must have a common function (75% of them form hetero-dimers)**

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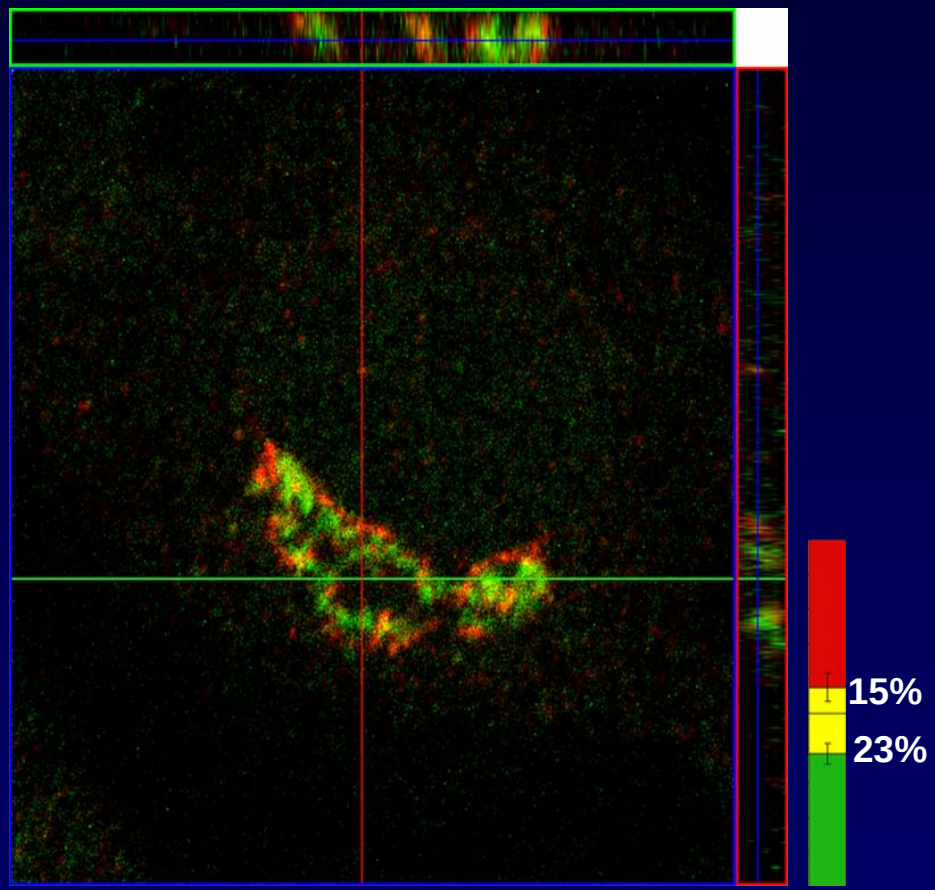
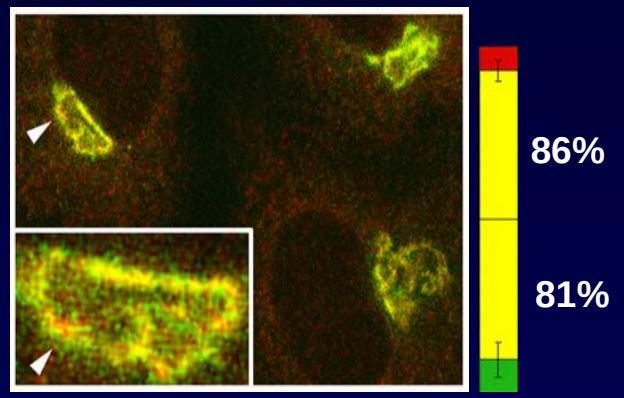
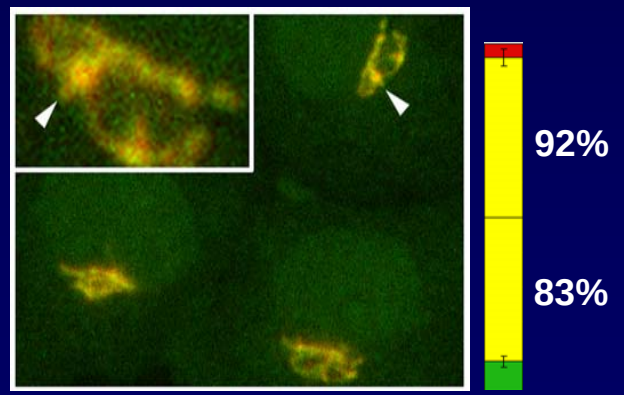
GBF1 and BIG1 localize to *cis*- and *trans*-compartments of Golgi complex, respectively



GBF1 / BIG1



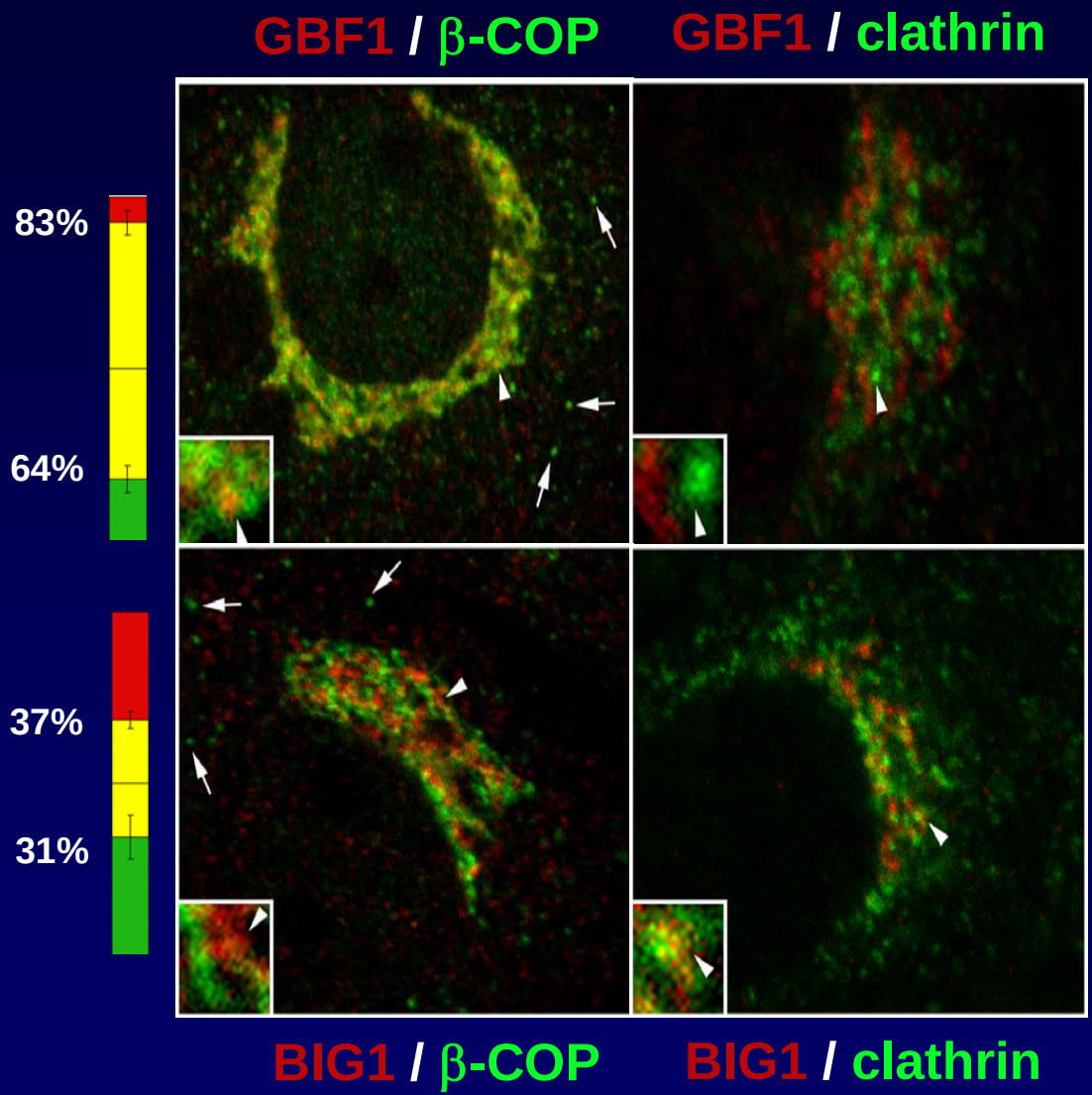
GBF1 / p115



TGN38 / BIG1

Zhao X. et al., Mol Biol Cell, 2002

GBF1 and BIG1 overlap with different coat proteins



Zhao X. et al., Mol Biol Cell, 2002

Hypothesis:

GBF1 and BIGs have different functions because they have distinct subcellular localizations and colocalize with different coat proteins

Method:

Examine relative impact of **overexpression** and **knockdown** of the two GEF families on COPI recruitment and maintenance of the Golgi complex

Outline

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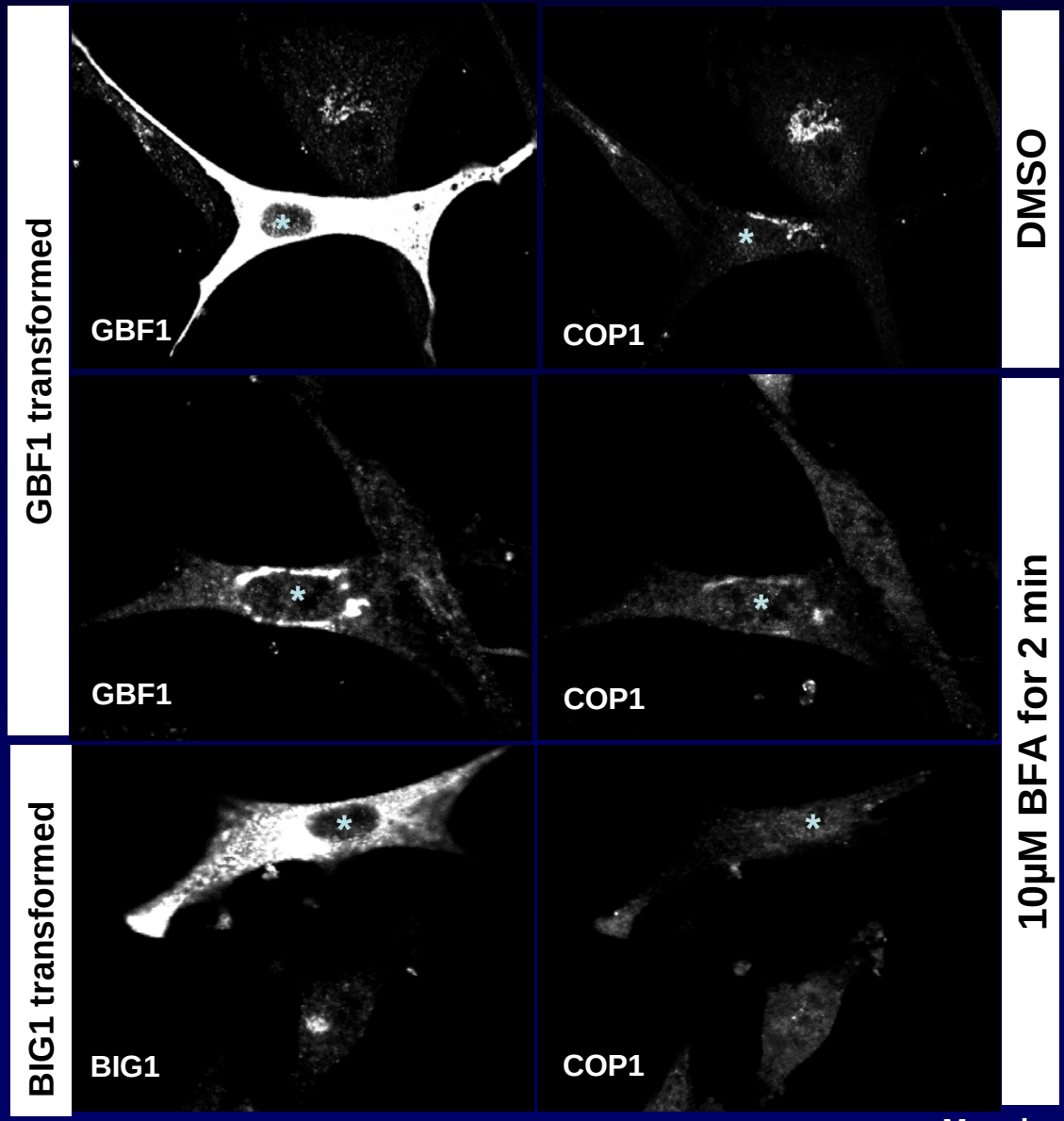
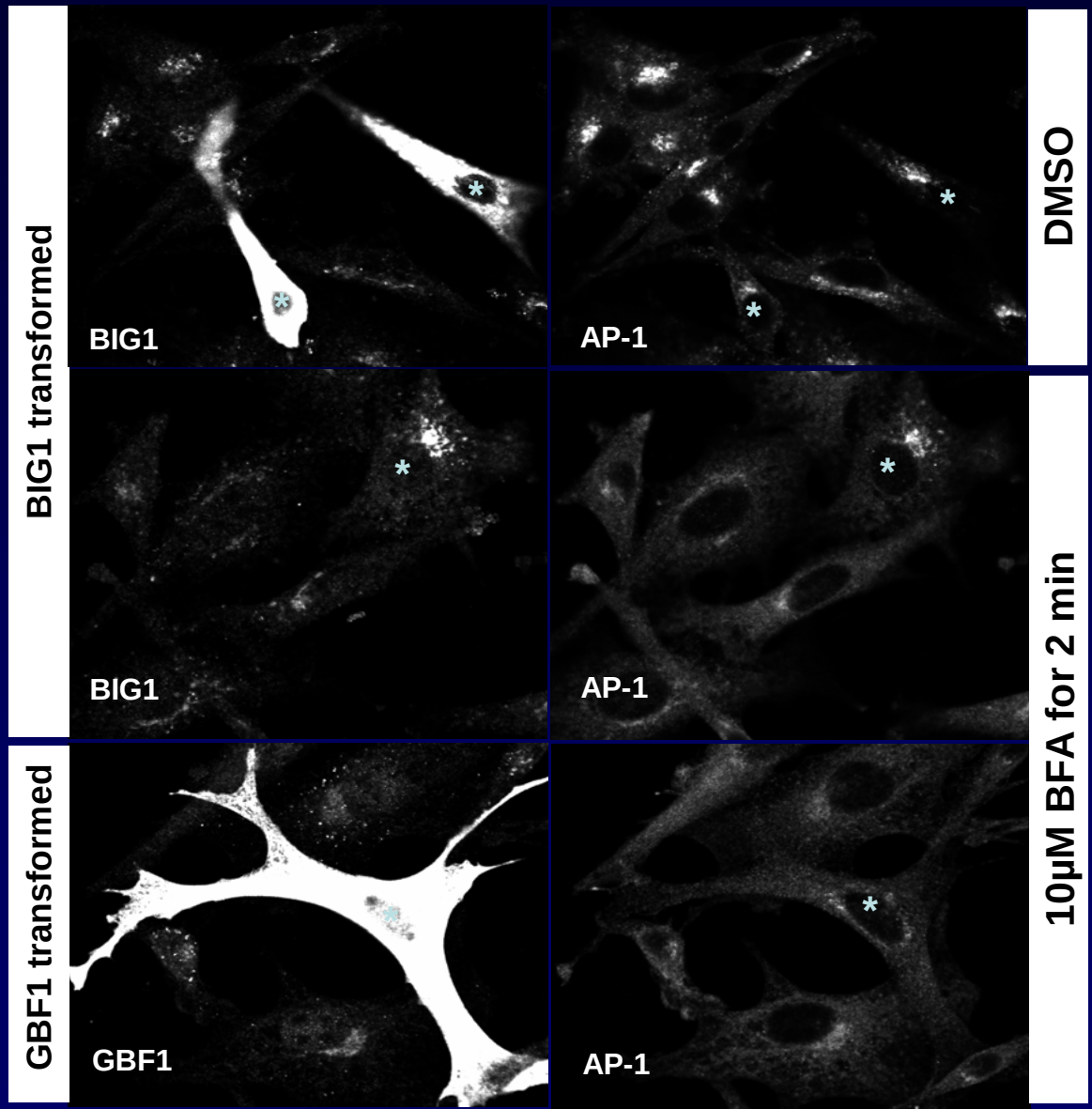
GBF1 overexpression protects COP1



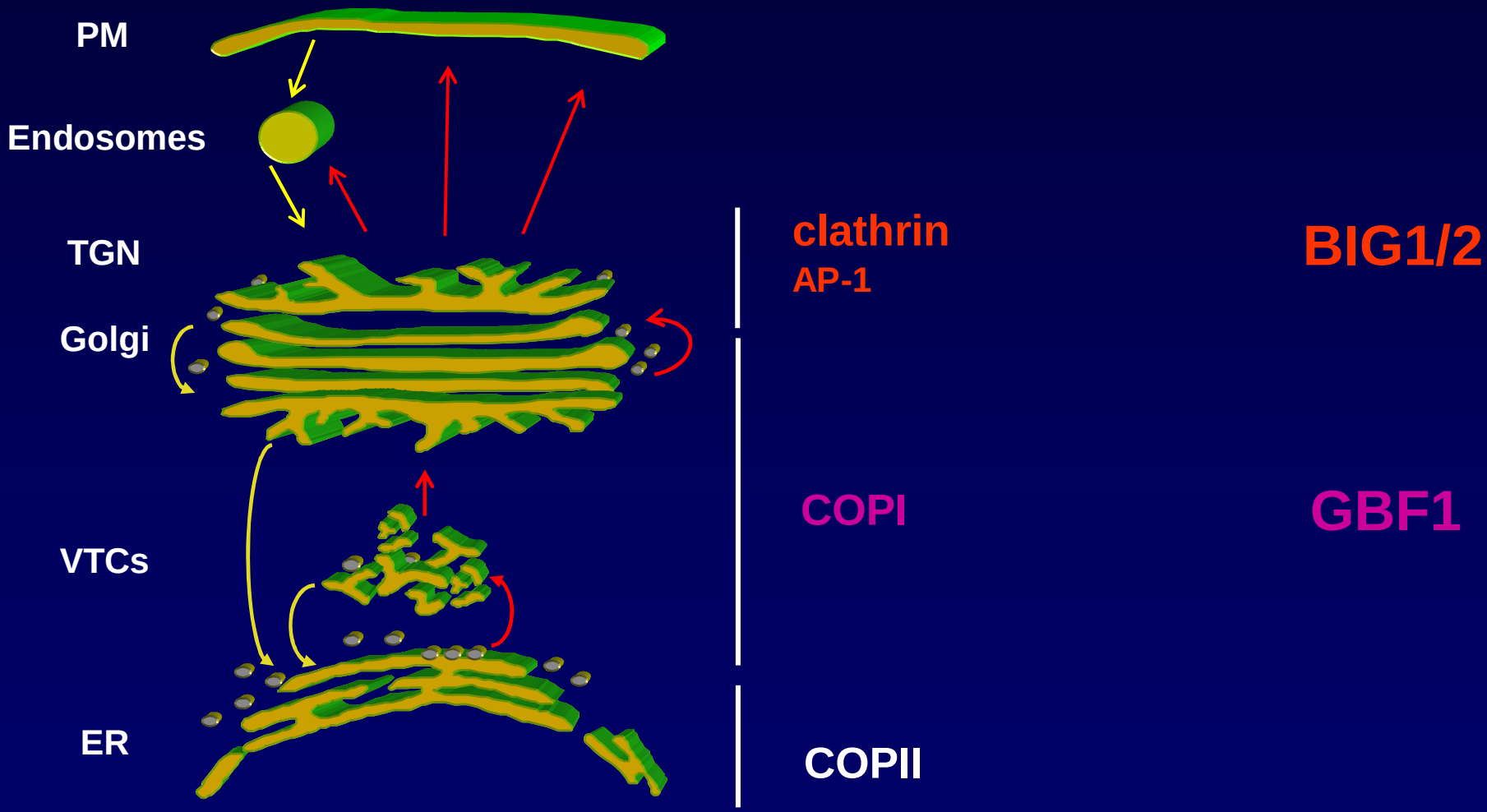
Manolea . et al., Mol Biol Cell, 2008

BIG1 overexpression protects AP-1



Manolea . et al., Mol Biol Cell, 2008

GBF1 and BIG1 relate to COP1 and clathrin, respectively



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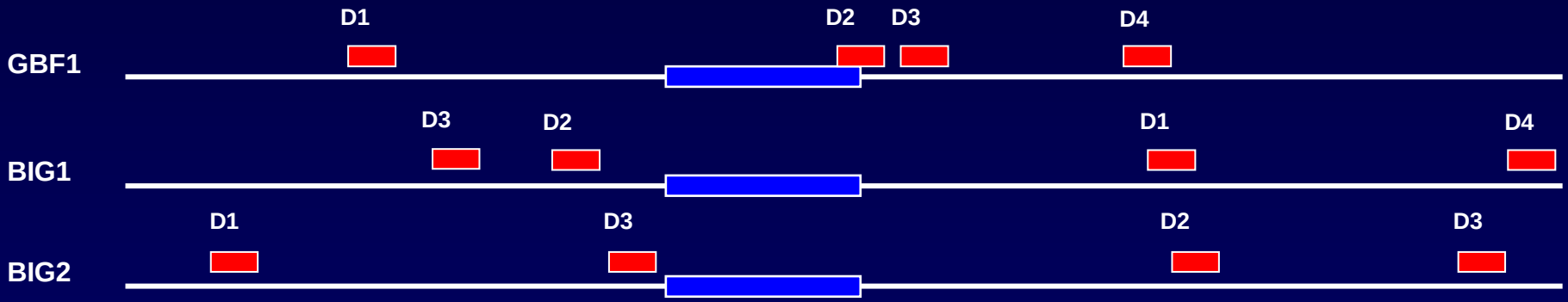
Outline

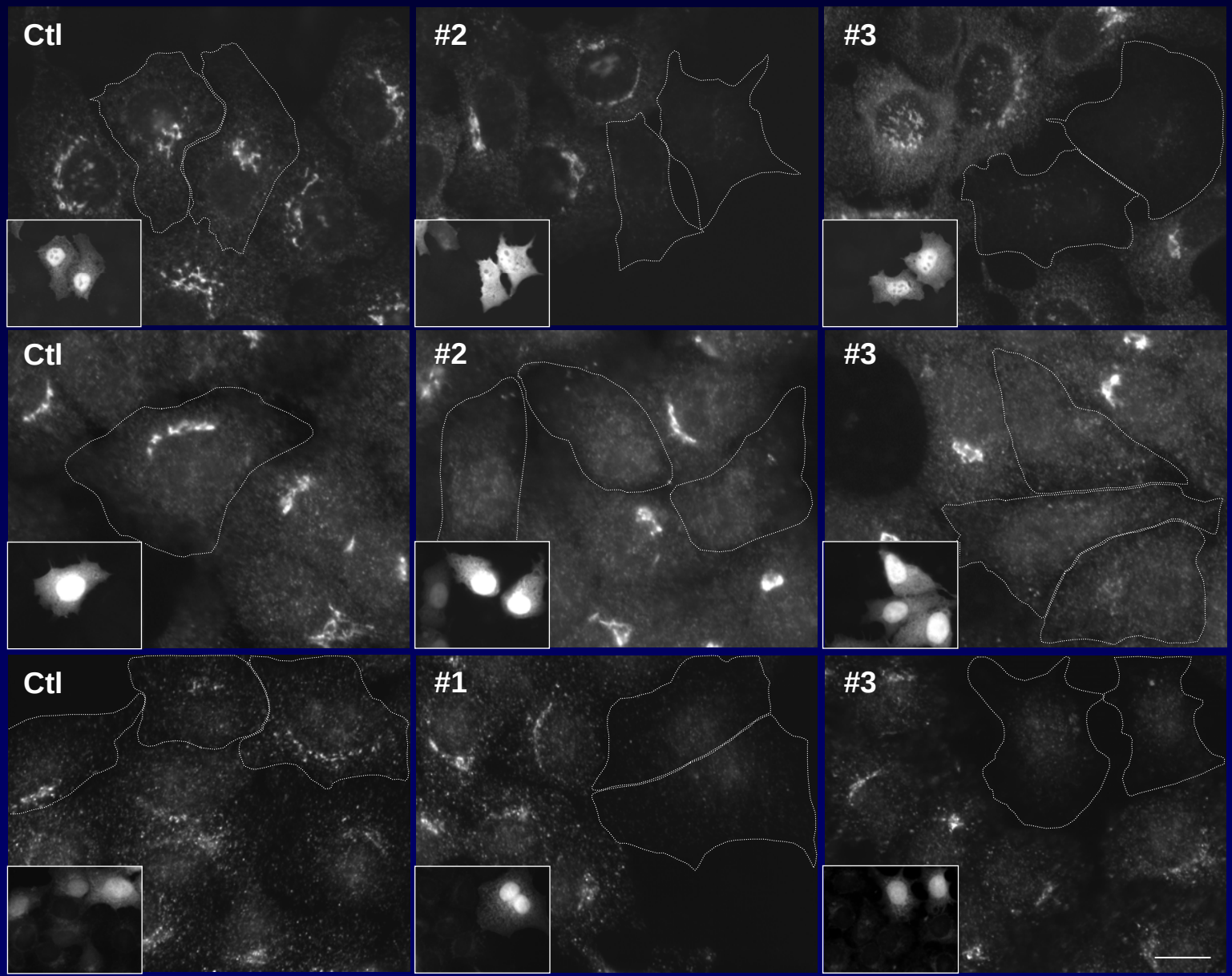
Knockdown studies

- **Identify effective target sequences**
  - **synthetic siRNAs duplexes**
  - **shRNAs produced from a pSupressor plasmid**
- **Knockdown effects on ER and Golgi morphology and anterograde protein traffic**

Identification of target sequences for RNAi-mediated knockdown

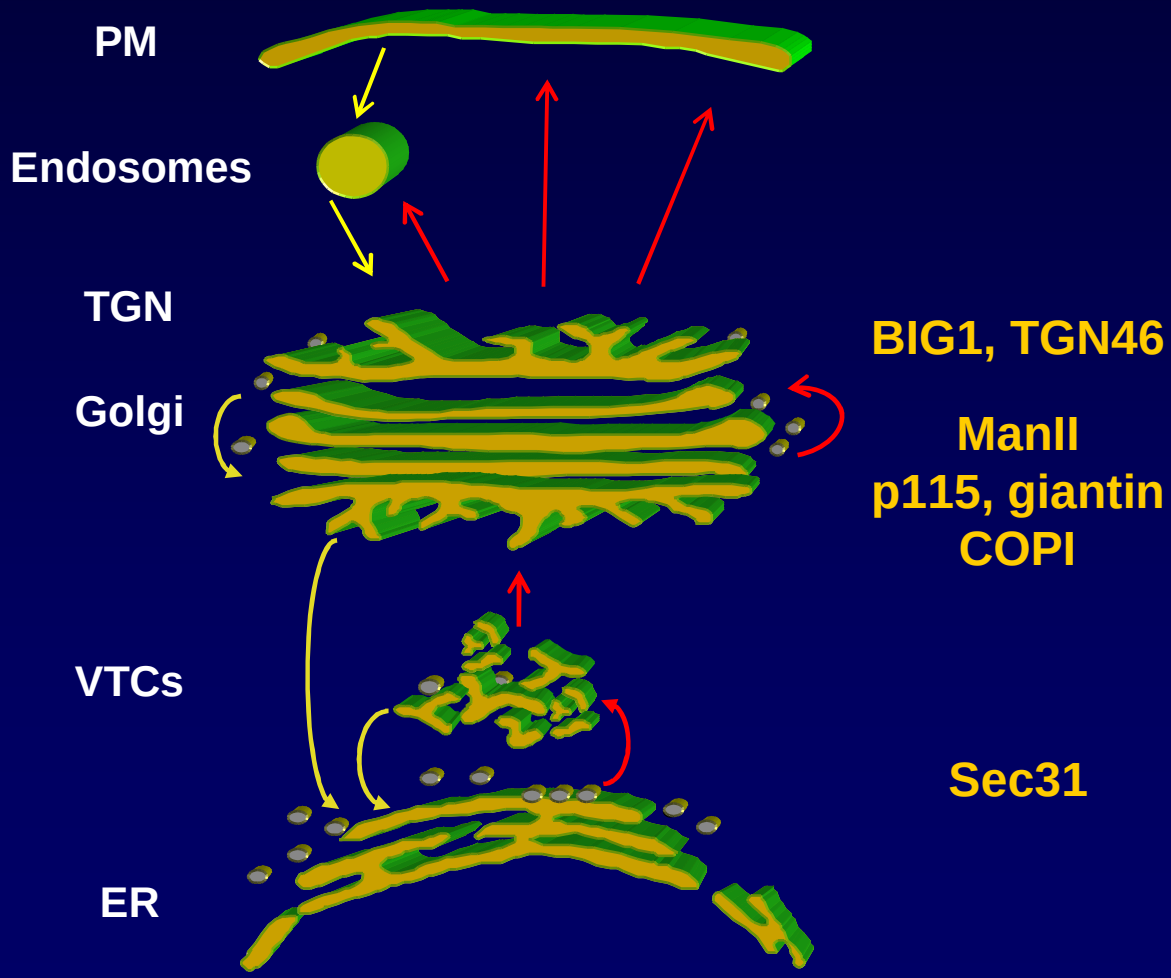


Identification of effective target sequences for knockdown of GBF1 and BIG1

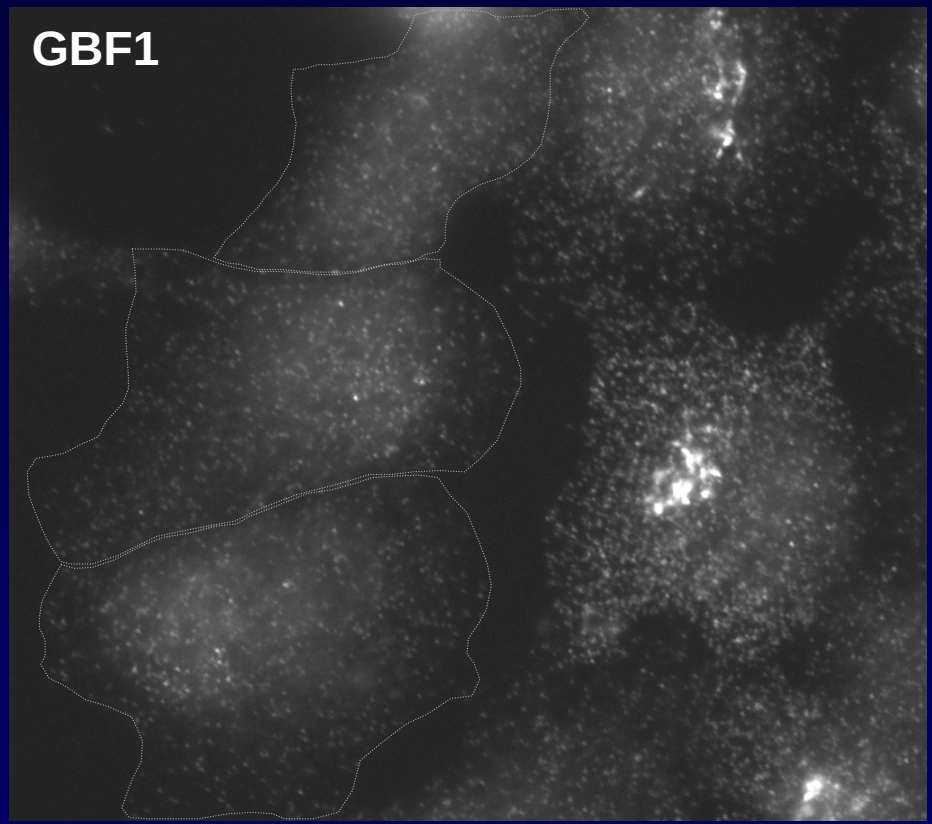


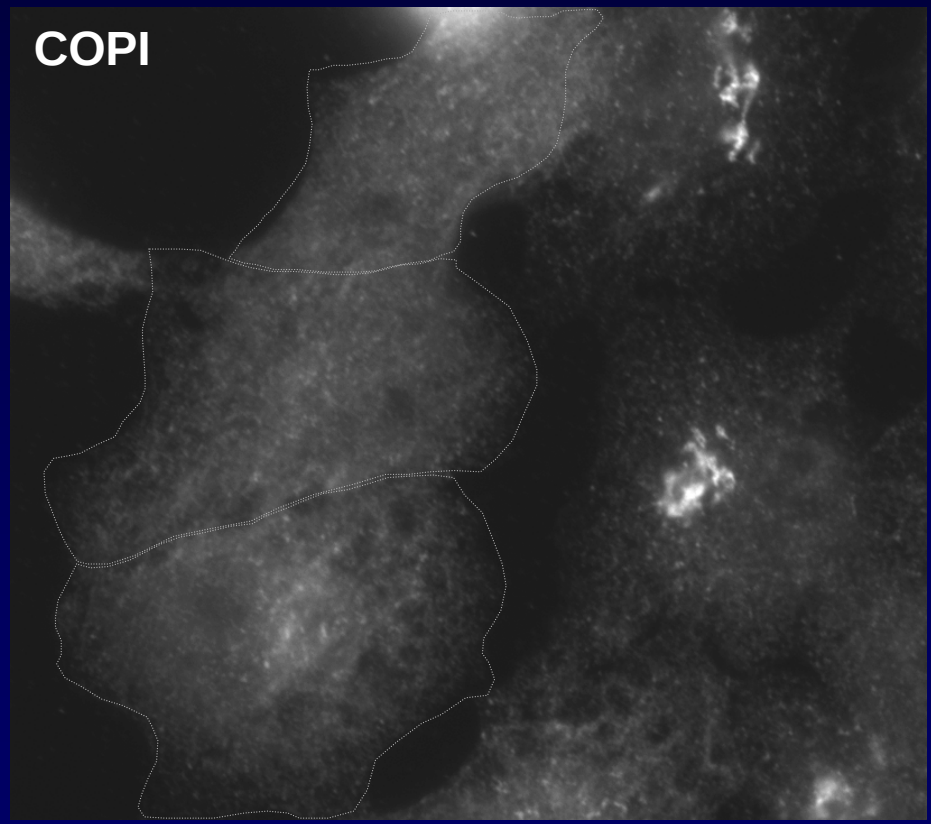
Manolea . et al., Mol Biol Cell, 2008

GBF1 knockdown and specific markers analyzed



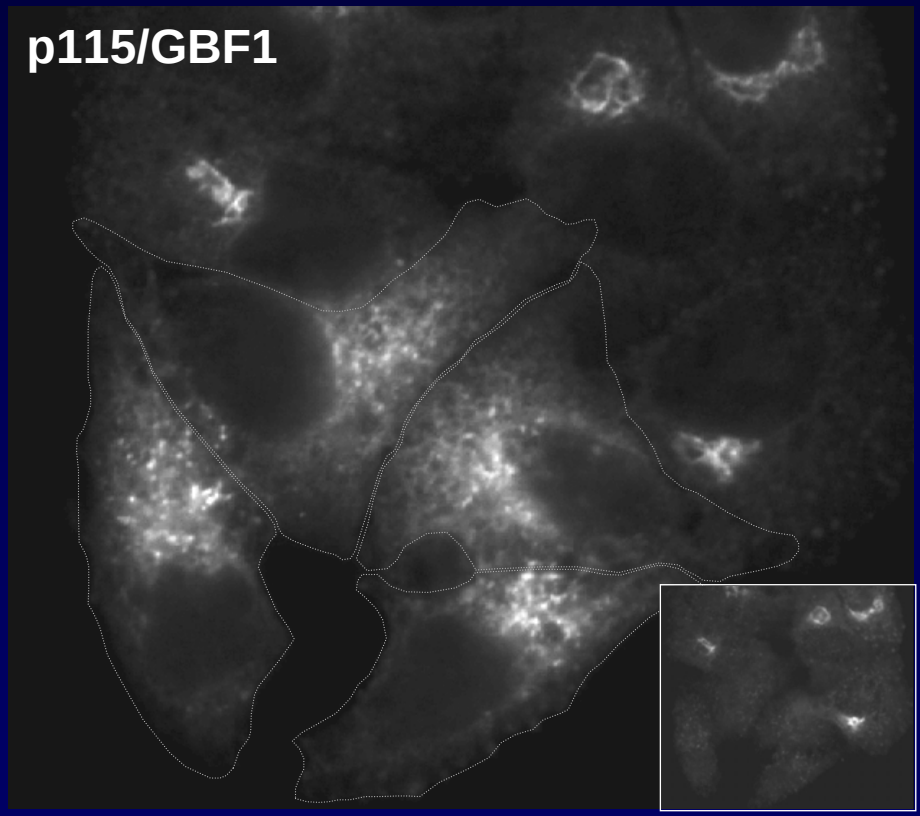
Knockdown of GBF1 redistribute the juxtanuclelar COP1 coat staining

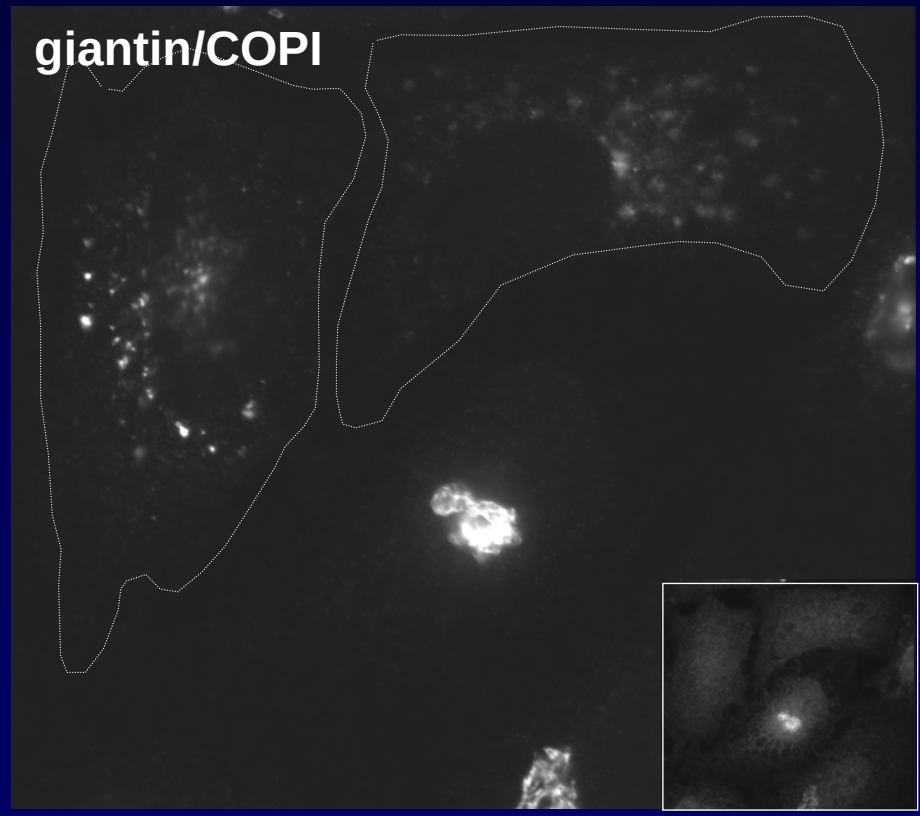




Manolea . et al., Mol Biol Cell, 2008

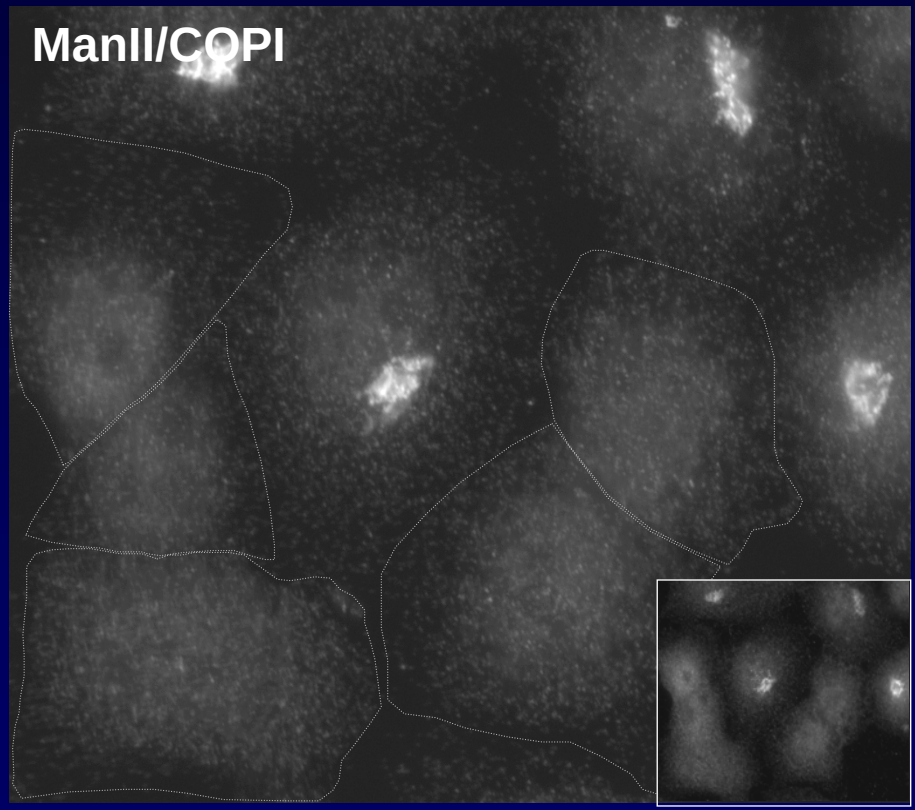
Knockdown of GBF1 redistribute the juxtanuclelar p115 staining





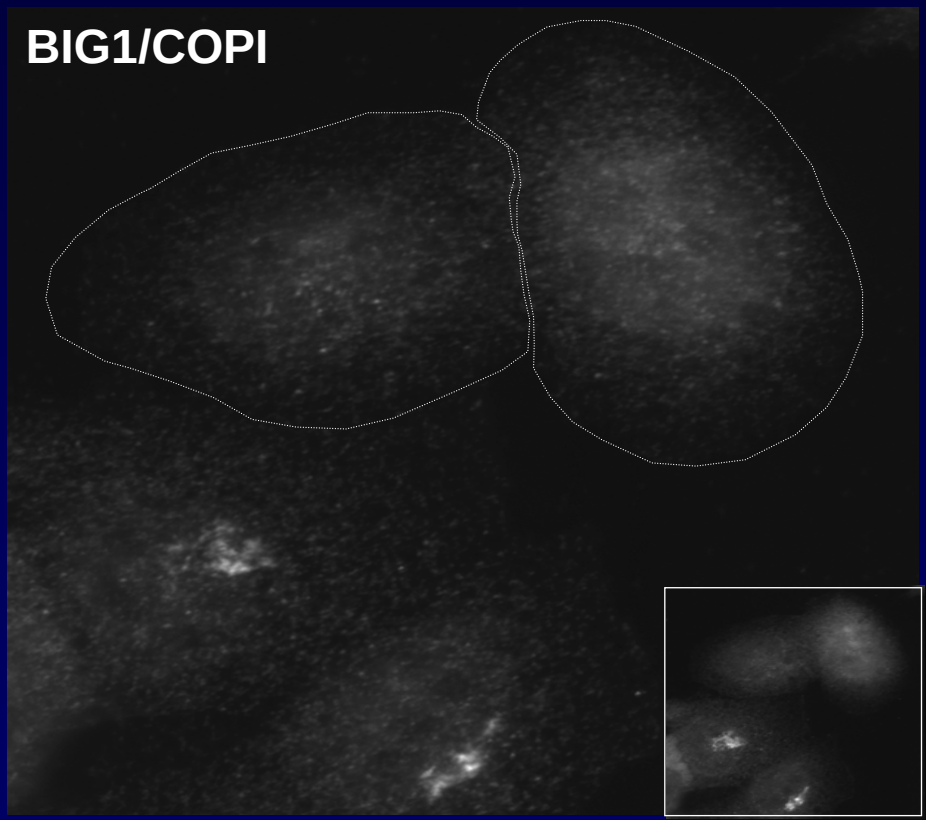
Manolea . et al., Mol Biol Cell, 2008

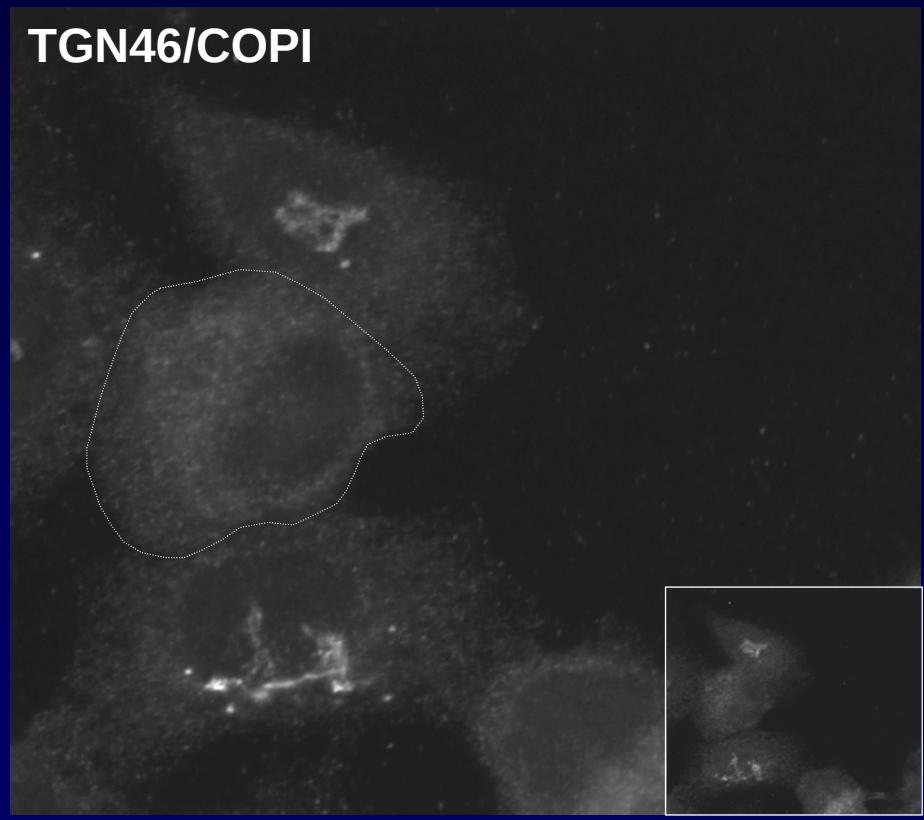
Knockdown of GBF1 redistribute the juxtanuclelar ManII staining



Manolea . et al., Mol Biol Cell, 2008

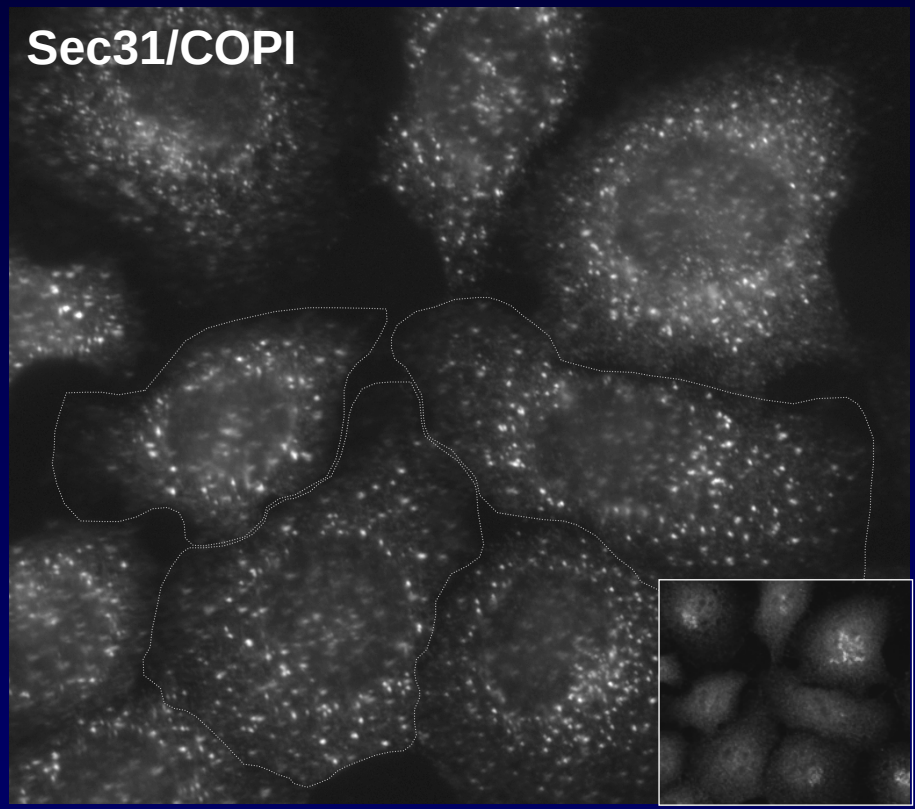
Knockdown of GBF1 redistribute the juxtanuclelar TGN38 staining





Manolea . et al., Mol Biol Cell, 2008

Knockdown of GBF1 does not affect COP2 localization



Manolea . et al., Mol Biol Cell, 2008

GBF1 is essential for maintenance of the Golgi complex but not for ERES