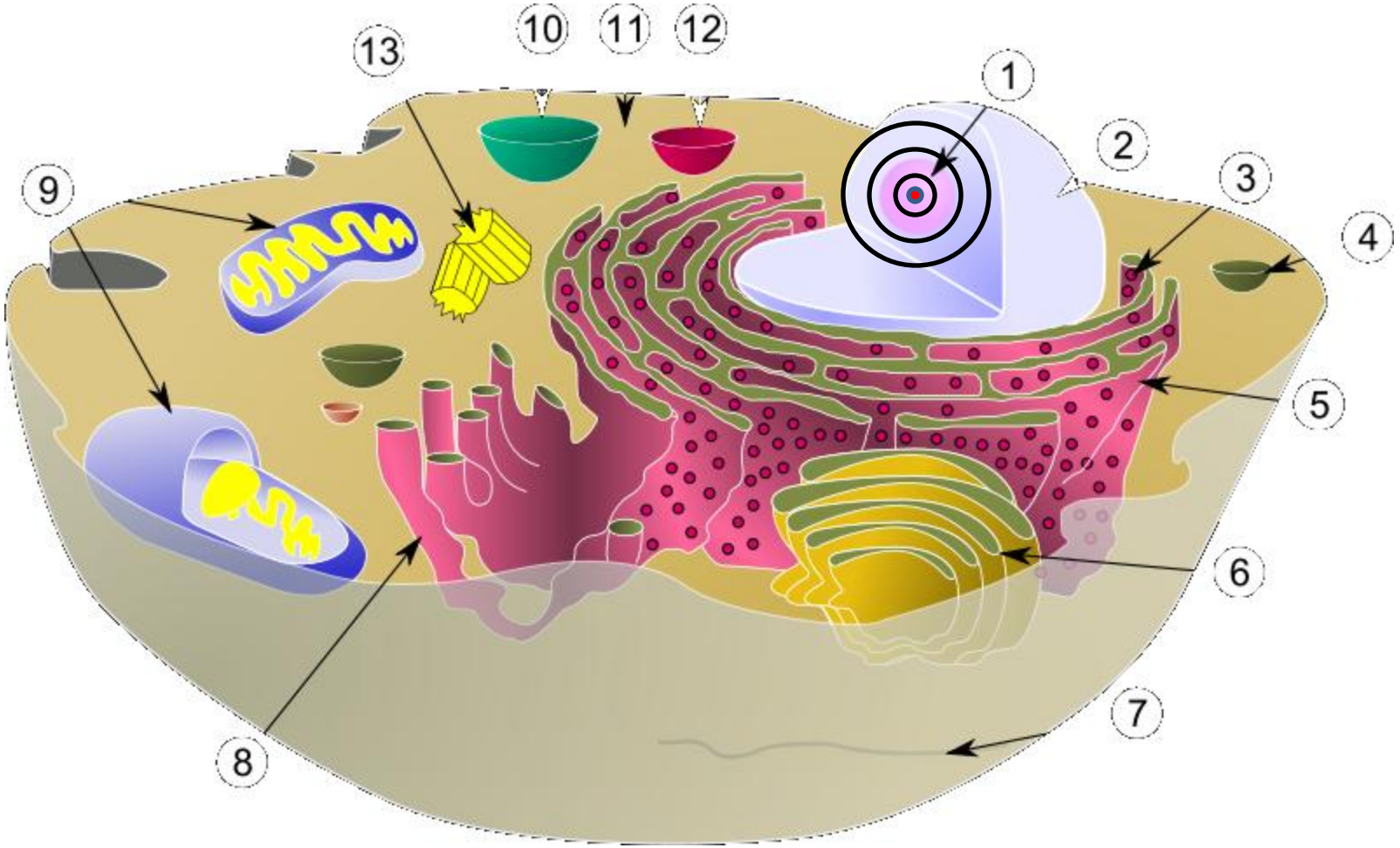
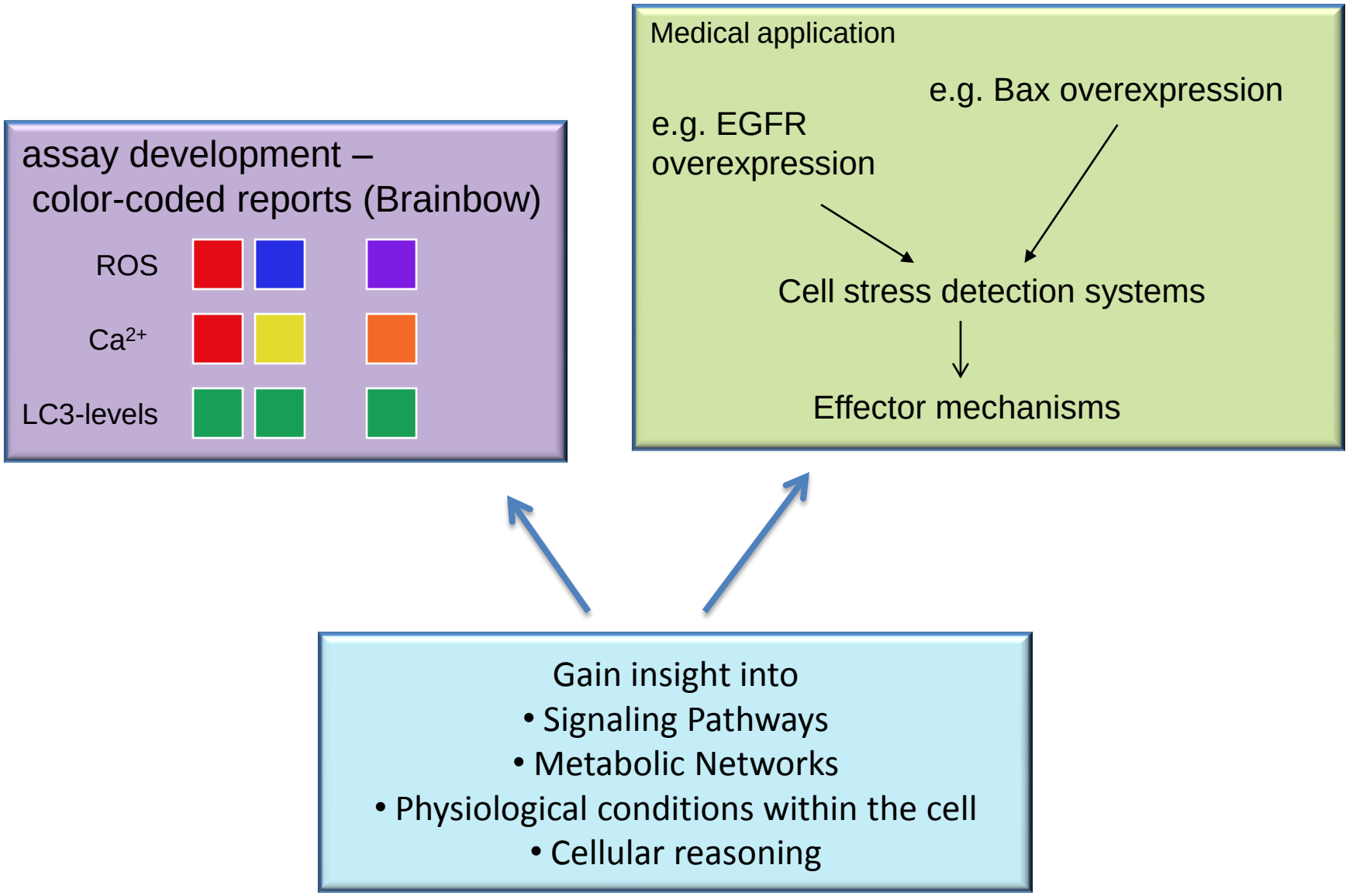
Spying on cells



Analyzing and exploiting intracellular processes


assay development –
color-coded reports (Brainbow)
ROS
Ca2+
LC3-levels
Medical application
e.g. Bax overexpression
e.g. EGFR
overexpression
Cell stress detection systems
Effector mechanisms
Gain insight into
• Signaling Pathways
• Metabolic Networks
• Physiological conditions within the cell
• Cellular reasoning

Effectors
• Gene Therapy
• Stem cell differentiation

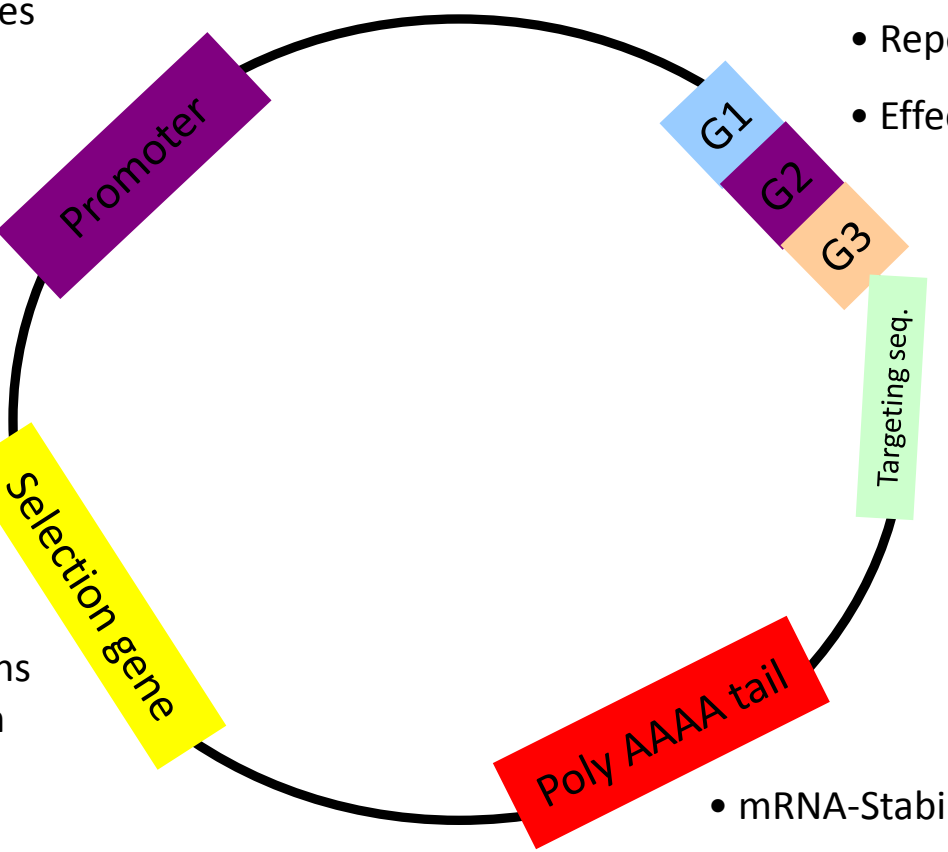
Reporters
•Brainbrow Approach
• GFP, YFP …
• adjustable reporters

Detection Tools – Inducible Promoters
• ROS, Hypoxia, Ca^{2+}…
• Key transcription factors (Signaling, Metabolism)

Framework Tools
• Special promoters
• Protein targeting
• Selection Genes
• mRNA stabilization

First eukaryotic biobrick library in the registry
First methodical approach to introduce mammalian cells to iGEM

Framework Tools



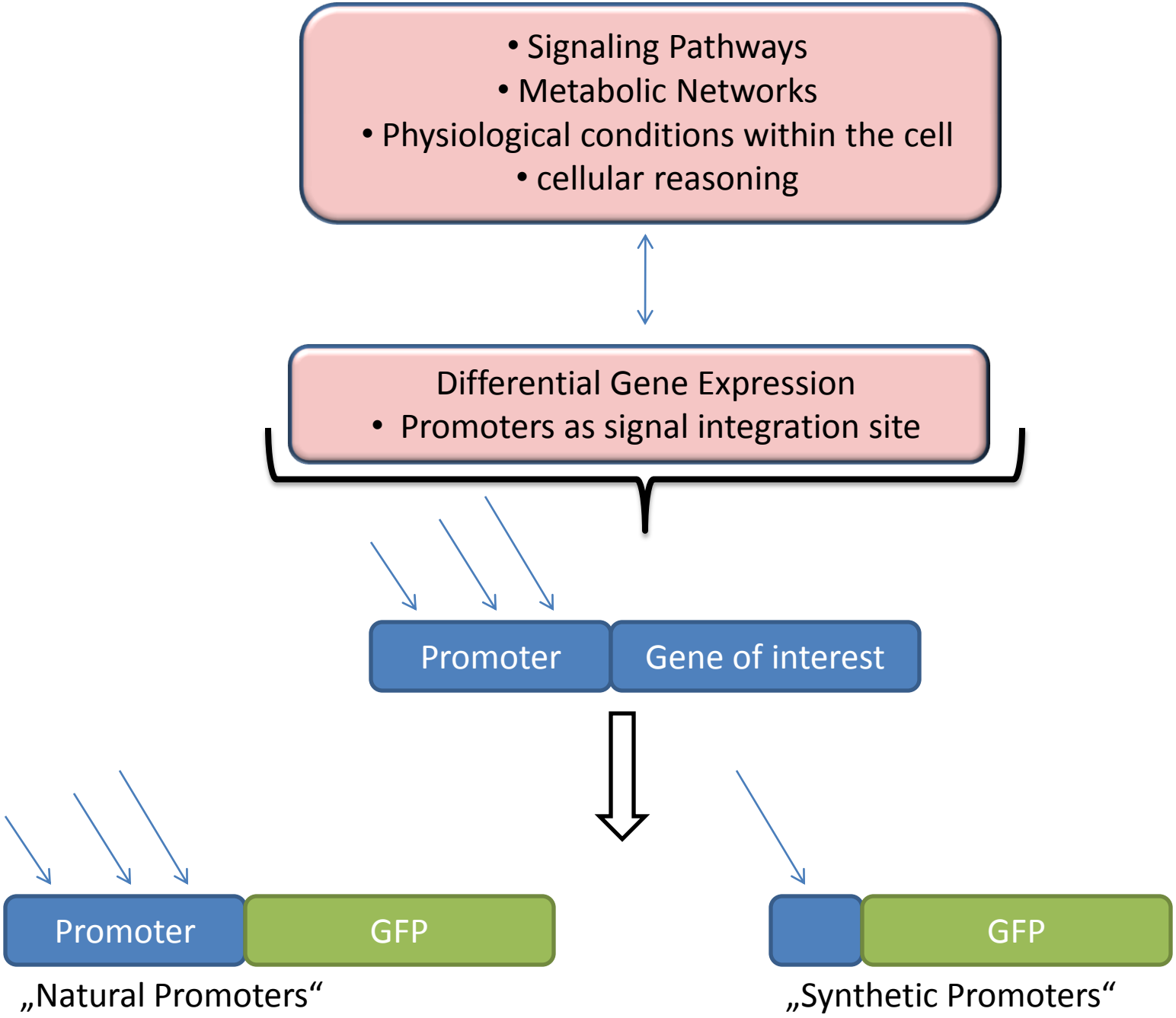
- Different efficiencies
- Induction by exogenous agents

- Reporters
- Effectors

- Organelle-specific

- Tetracycline
- Streptogramins
- Coumermycin

- mRNA-Stabilization


• Signaling Pathways
• Metabolic Networks
• Physiological conditions within the cell
• cellular reasoning
Differential Gene Expression
• Promoters as signal integration site
Promoter
Gene of interest
Promoter
GFP
GFP
„Natural Promoters“
„Synthetic Promoters“

Promoters as Detectors

Natural Promoters

- Flanking upstream-sequence of Genes with differential expression under clearly defined circumstances

Advantage

- The complex signal integration function of the natural promoter is preserved

Disadvantage

- Many pathways are too complex for this approach
- Monitoring of specific TFs is not possible

Promoters as Detectors

Synthetic Promoters

Promoters can be engineered through the combination of

- Basal promoters
- DNA-Binding Sites
- Enhancer Elements

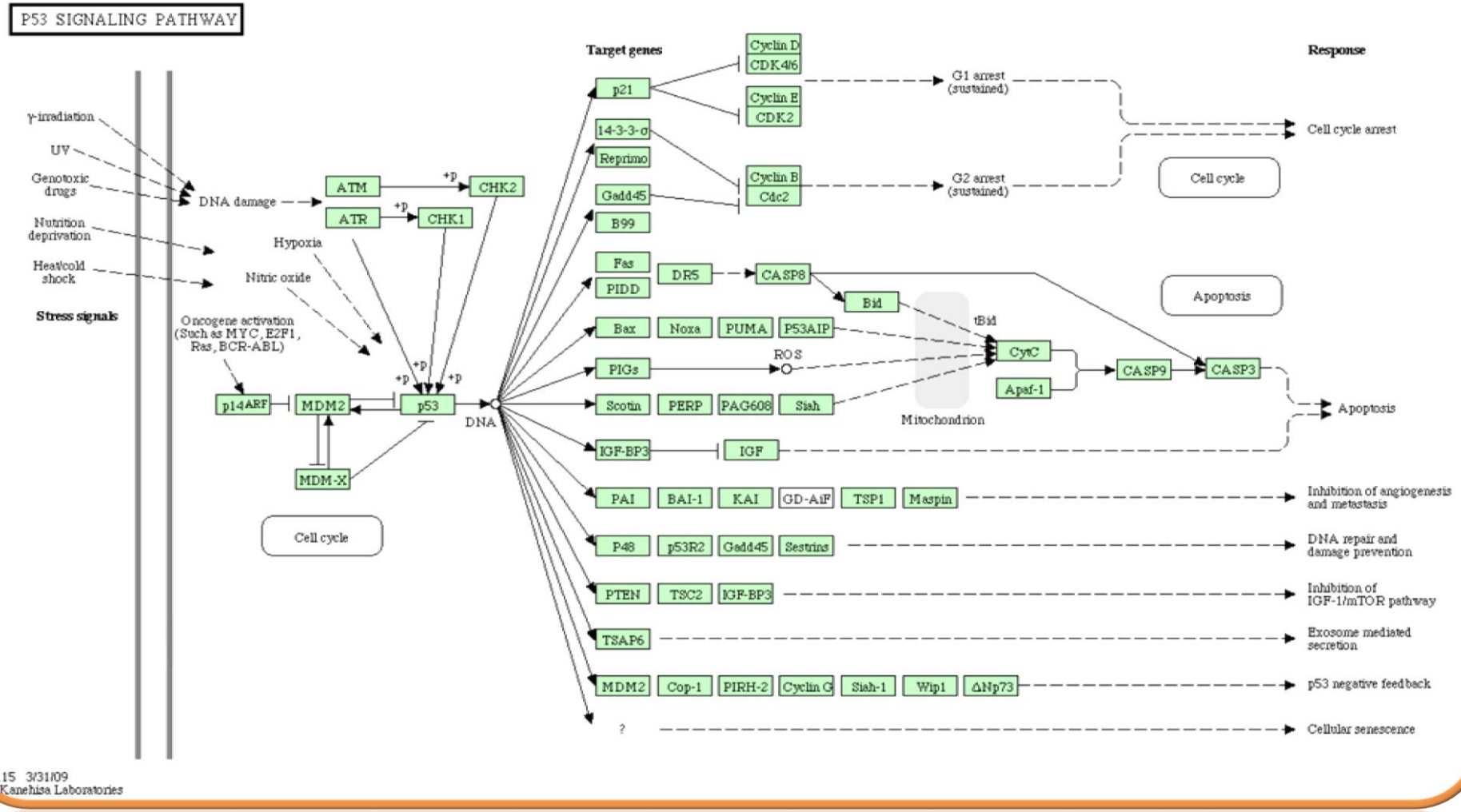
Advantages

- Analysis of specific TFs
- Allows the recombination of different binding sites to construct novel induction patterns

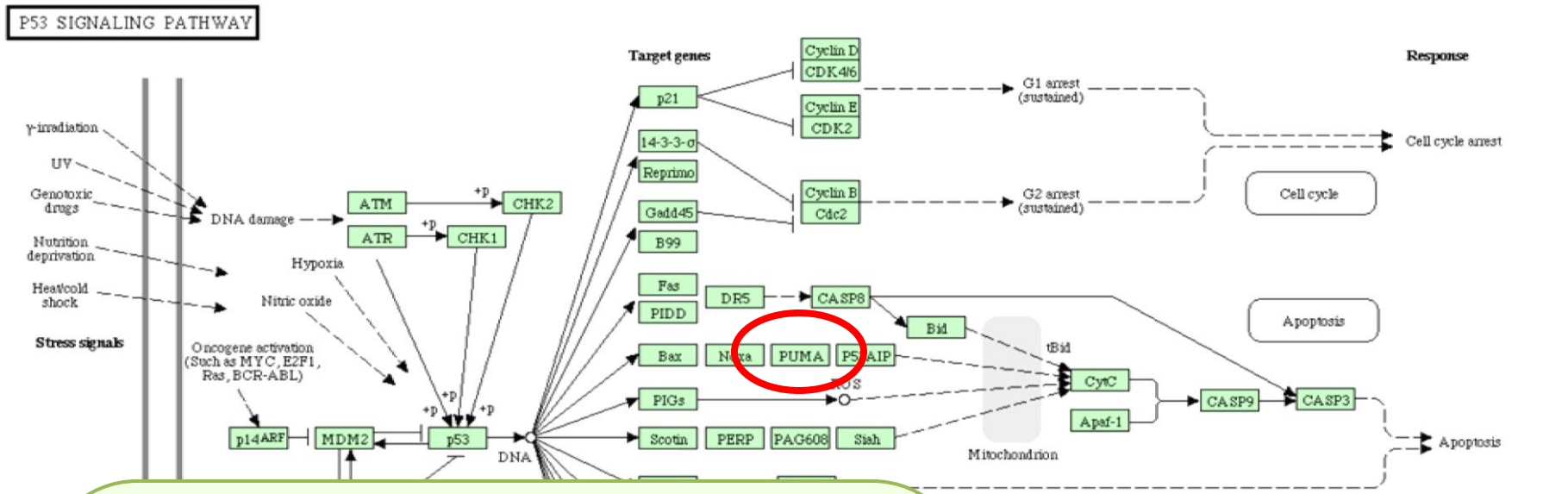
Disadvantage

- Functionality and Feasability

P53 network


P53 SIGNALING PATHWAY
γ-irradiation
UV
Genotoxic drugs
Nutrition deprivation
Heat/cold shock
Stress signals
DNA damage
ATM
ATR
CHK2
CHK1
Hypoxia
Nitric oxide
Oncogene activation (Such as MYC, E2F1, Ras, BCR-ABL)
p14ARF
MDM2
p53
MDM-X
DNA
Cell cycle
Target genes
Cyclin D
CDK4/6
Cyclin E
CDK2
p21
14-3-3-σ
Reprimo
Cyclin B
Cdc2
Gadd45
B99
G1 arrest (sustained)
G2 arrest (sustained)
Response
Cell cycle arrest
Cell cycle
Fas
PIDD
DR5
CASP8
Bid
tBid
Apoptosis
Bax
Noxa
PUMA
P53AIP
PIGs
ROS
Scotin
PERP
PAG608
Siah
CytC
Apaf-1
Mitochondrion
CASP9
CASP3
Apoptosis
IGF-BP3
IGF
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
Inhibition of angiogenesis and metastasis
P48
p53R2
Gadd45
Sestrins
DNA repair and damage prevention
PTEN
TSC2
IGF-BP3
Inhibition of IGF-1/mTOR pathway
TSAP6
Exosome mediated secretion
MDM2
Cop-1
PIRH-2
Cyclin G
Siah-1
Wip1
ΔNp73
p53 negative feedback
?
Cellular senescence
04115 3/31/09
Kanehisa Laboratories

P53 network



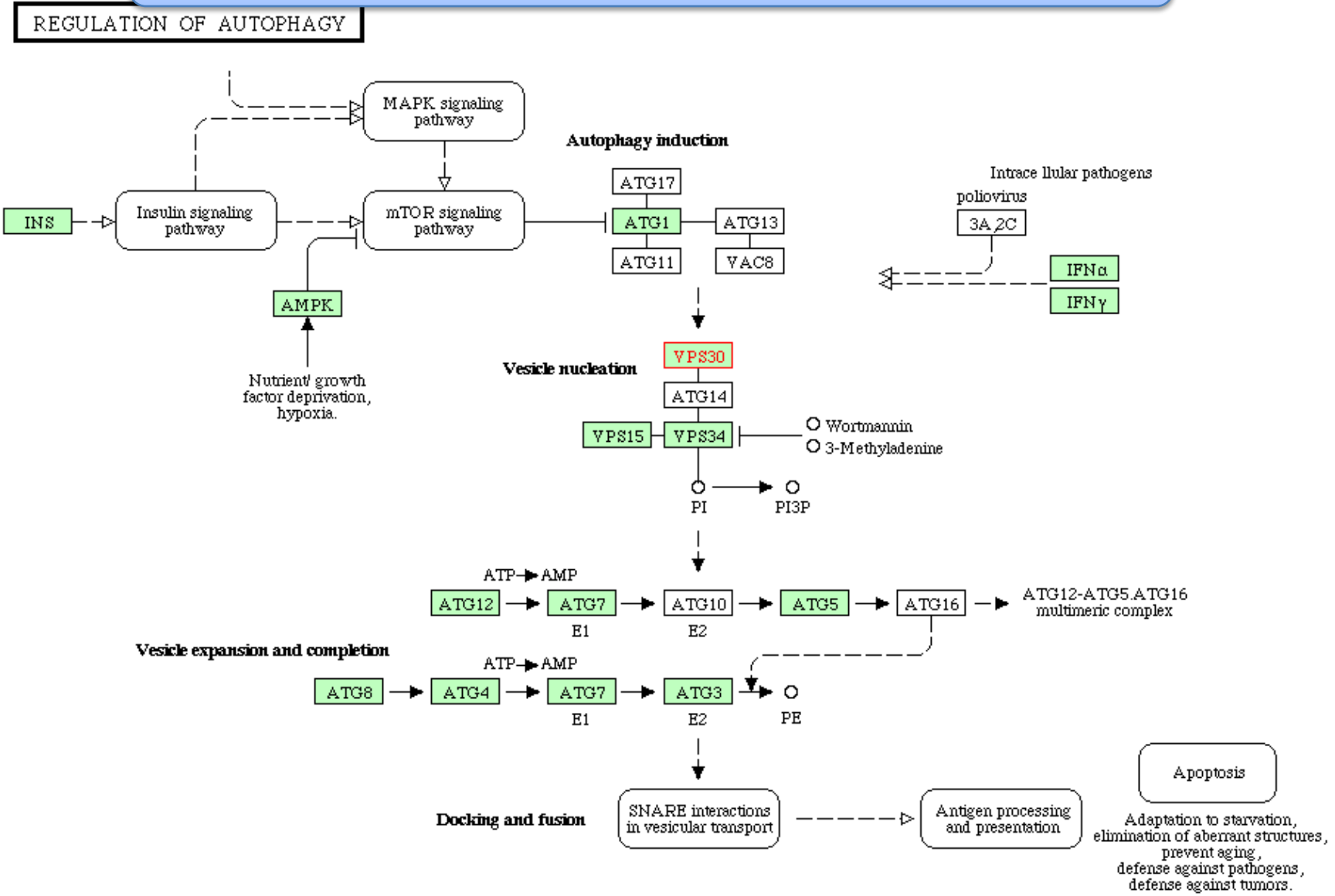
PUMA

"p53 upregulated modulator of apoptosis"

- BH3-only Bcl2 family protein
- direct binding of p53 to the PUMA promoter region
- Induces rapid apoptosis
- Essential mediater of apoptosis
- Heterodimerization with Bcl-2

Yu_J, Zhang_L et al. (2001): „PUMA induces the Rapid Apoptosis of Colorectal Cancer Cells"

autophagy network

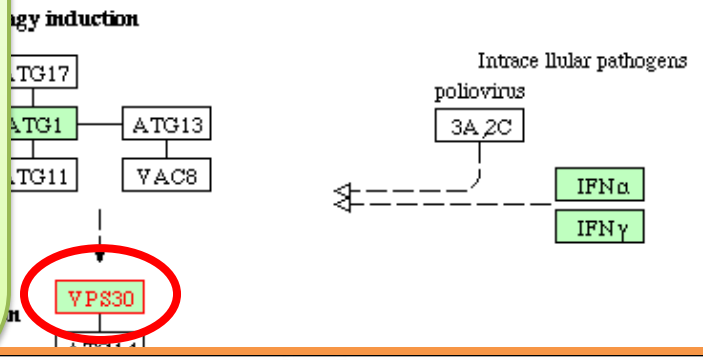


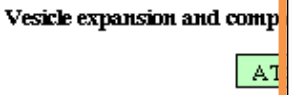
autophagy network

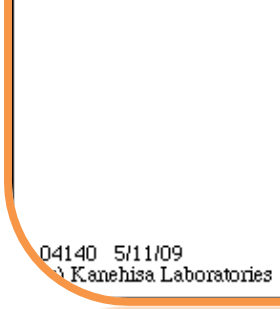
BECLIN1 (BECN1)

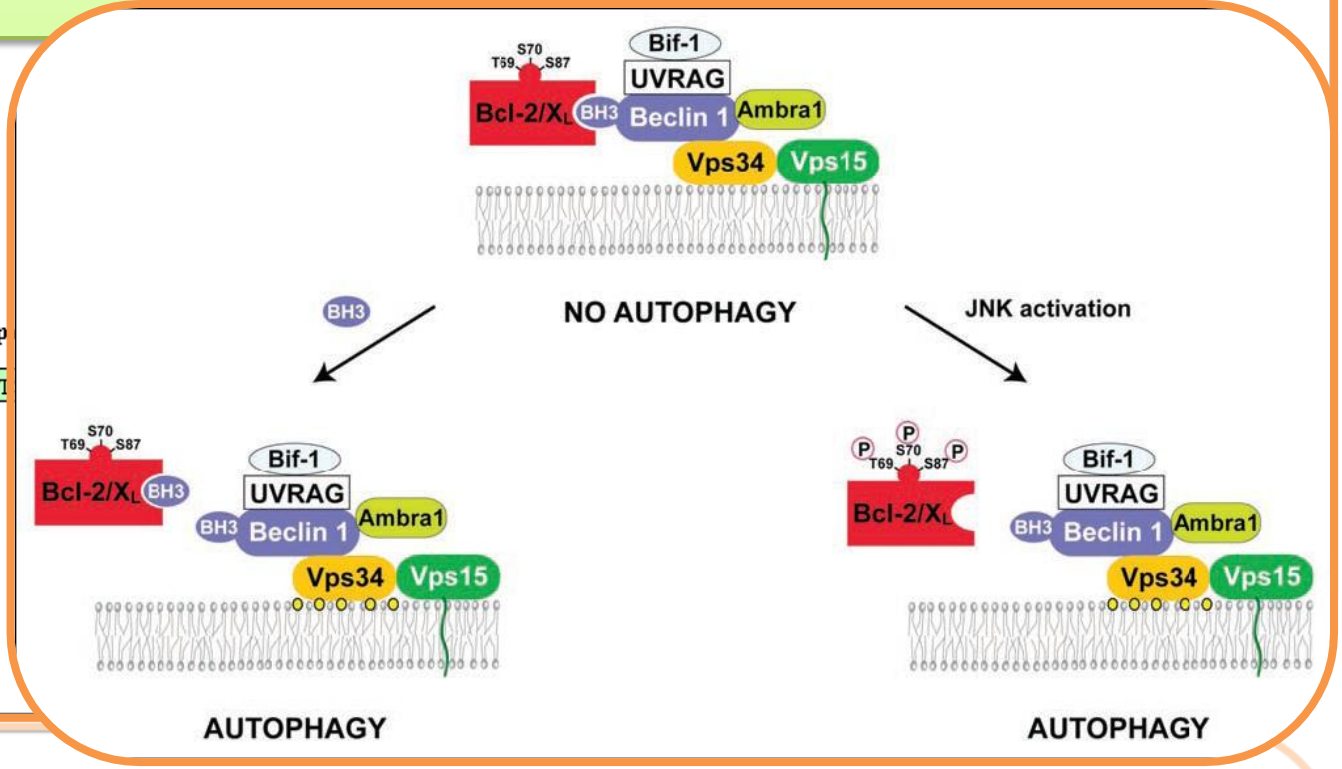
- myosin-like BCL2 interacting protein
- required for the initiation of the formation of the autophagasome
- Beclin-1 interactome for the regulation of autophagy / vesicle nucleation

Tang_H, Da_L et al. (2009): "Hepatitis B Virus X Protein Sensitizes Cells to Starvation-Induced Autophagy via Up-regulation of Beclin 1 Expression"









Molecular switches

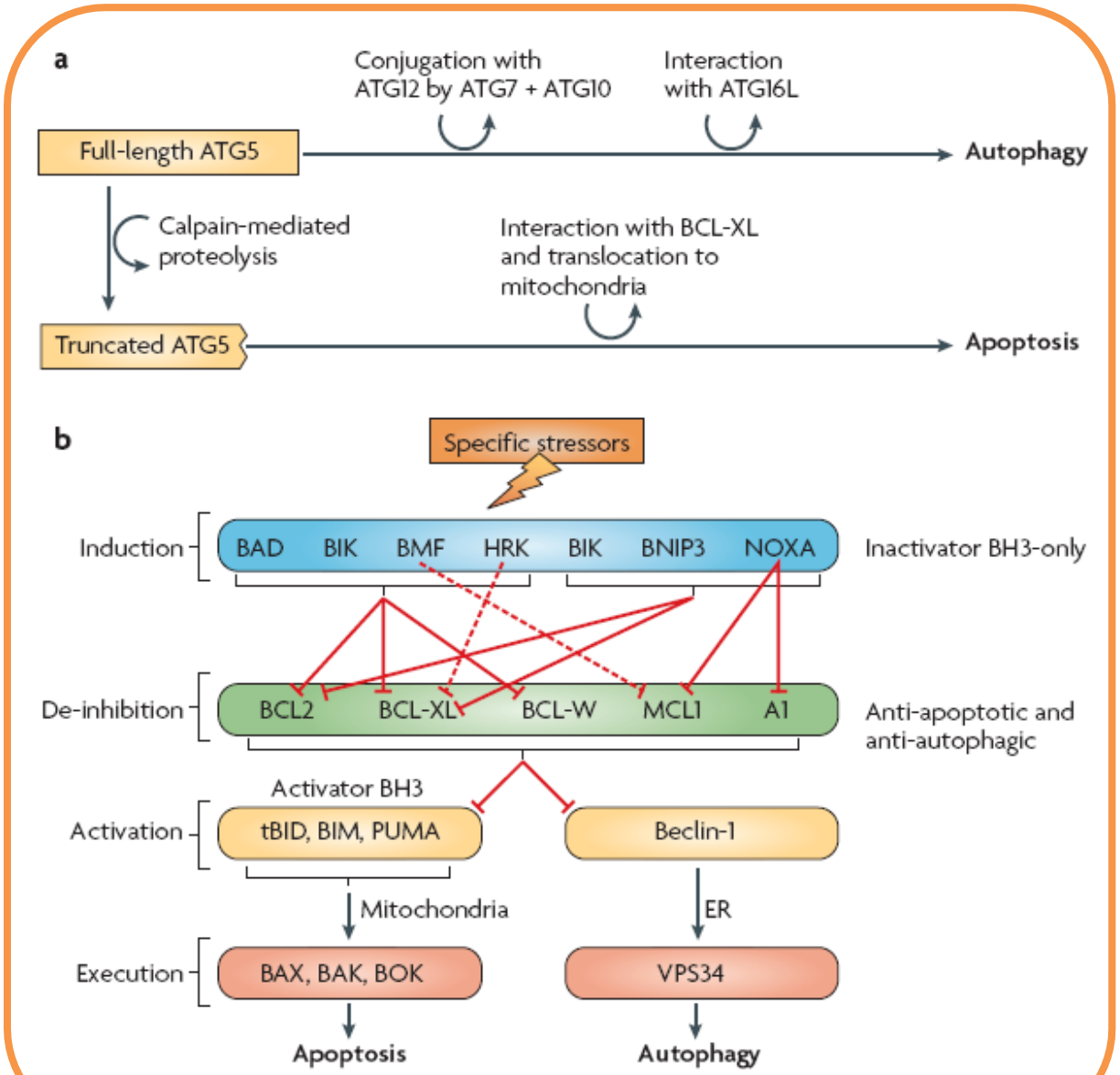


Figure 4 | **Molecular switches between apoptosis and autophagy. a** | Dual function of

Molecular switches

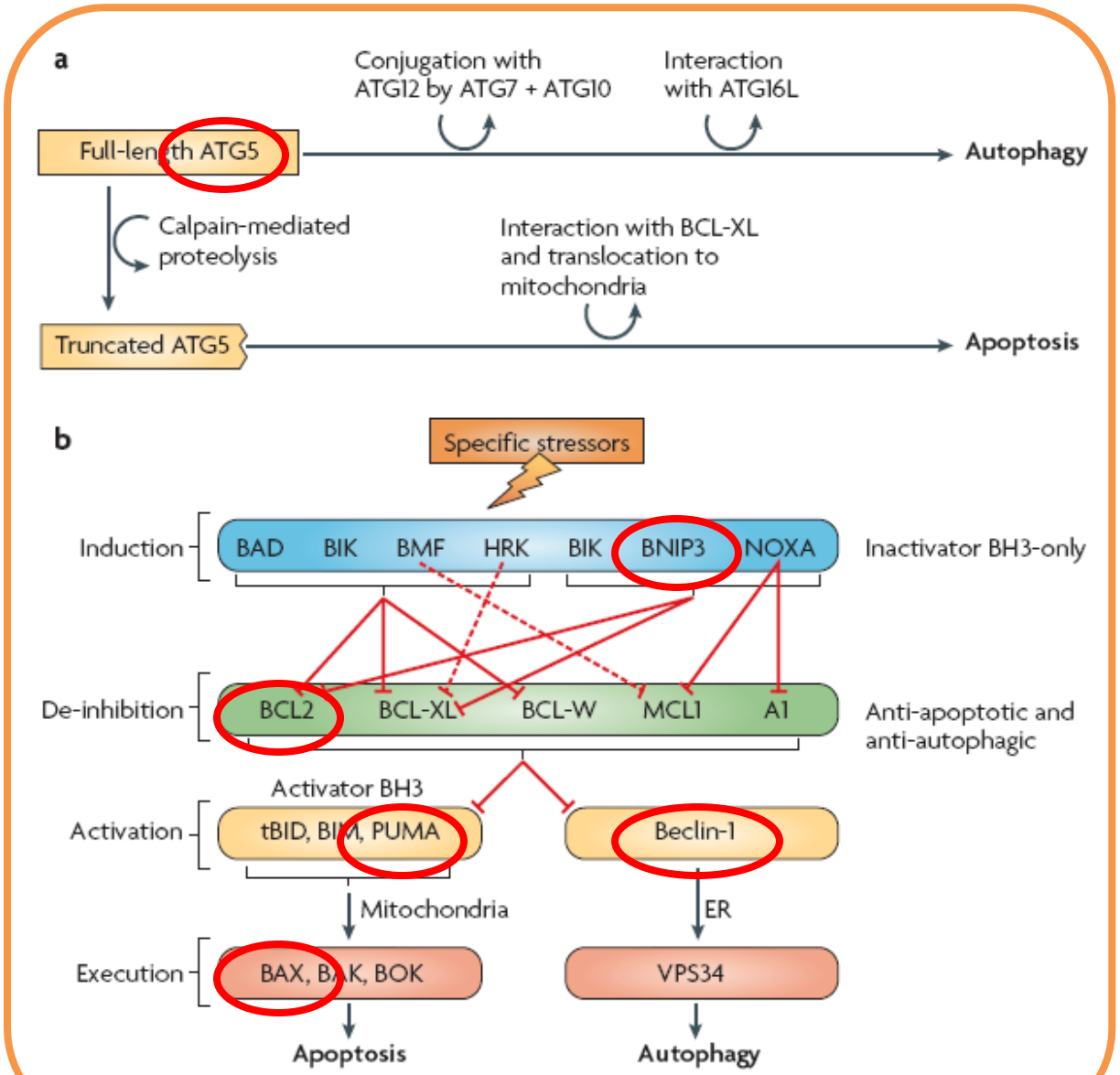
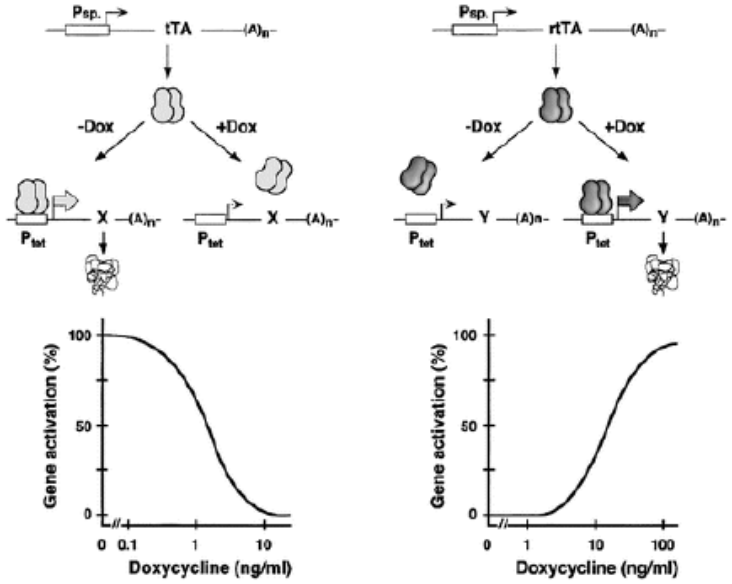
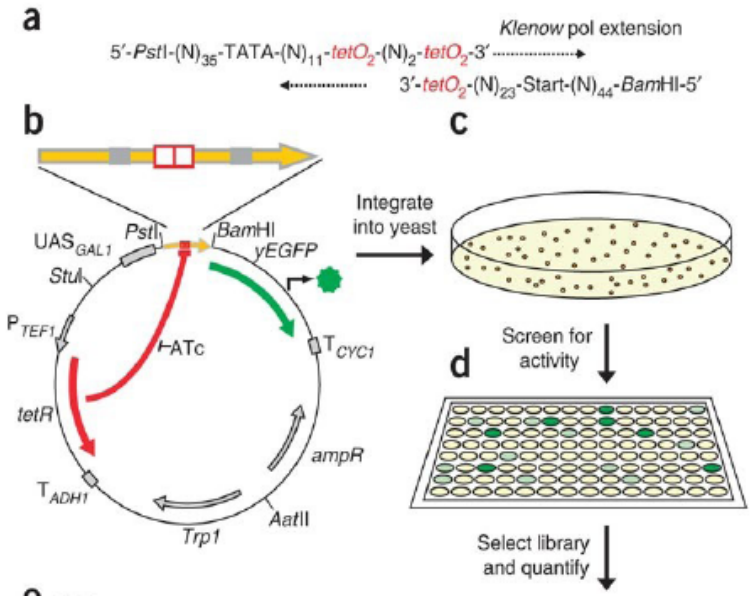
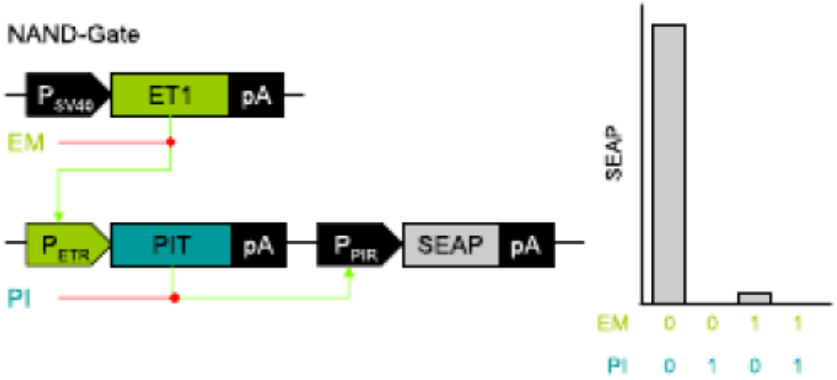


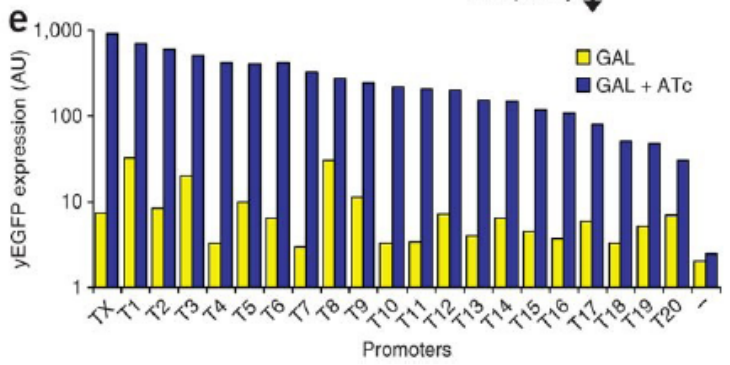
Figure 4 | **Molecular switches between apoptosis and autophagy. a** | Dual function of

Promoters as tools









Patents

Scenario	Law	BioBrick Foundation legal scheme
Using a patented invention (e.g. TET)	Research Privilege	✔
BioBricking a patented invention (e.g. TET, promoter library)	As long as no one uses it: Patent != copyright Company can restrain anyone from using it	✘
Developing something in analogy to products / patent (e.g. PW sensor)	Needs to be checked for each individual case (PW-sensors: OK)	✔
BioBricking parts from AddGene, publications	✔	✔

→ **The „Best Foundational Advances – Eukaryotic BioBrick Library“ Team should put parts into the library that are free to use for everyone**

OUTPUT: FP-based → Brainbow

- **Proteosome sensors** *(Clontech)*
- **Light sensors** *Engineering Escherichia coli to see light Nature 24 November 2005*
- **Ca++** *J Biol Chem. 1997 May 16;272(20):13270-4.*
- **Cu+/++** *J.P. Sumner et al. / Biosensors and Bioelectronics 21 (2006) 1302–1308*
- **Hg** *Richard Chapeau, Rebecca Blumberg, et al., Protein Science (2008), 17:614–622.*
- **Voltage sensors** *Journal of Neuroscience Methods 161 (2007) 32–38*
- **pH sensors** *Biophysical Journal Volume 74 March 1998 1591–1599*
- **ROS sensors**
  Maheshinie Rajapaksha et al.: Proceedings of the 3rd Annual GRASP Symposium, Wichita State University, 2007
  Journal of Bio. Chem., Vol. 279, No. 21, Issue of May 21, pp. 22284–22293, 2004

Questions?

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