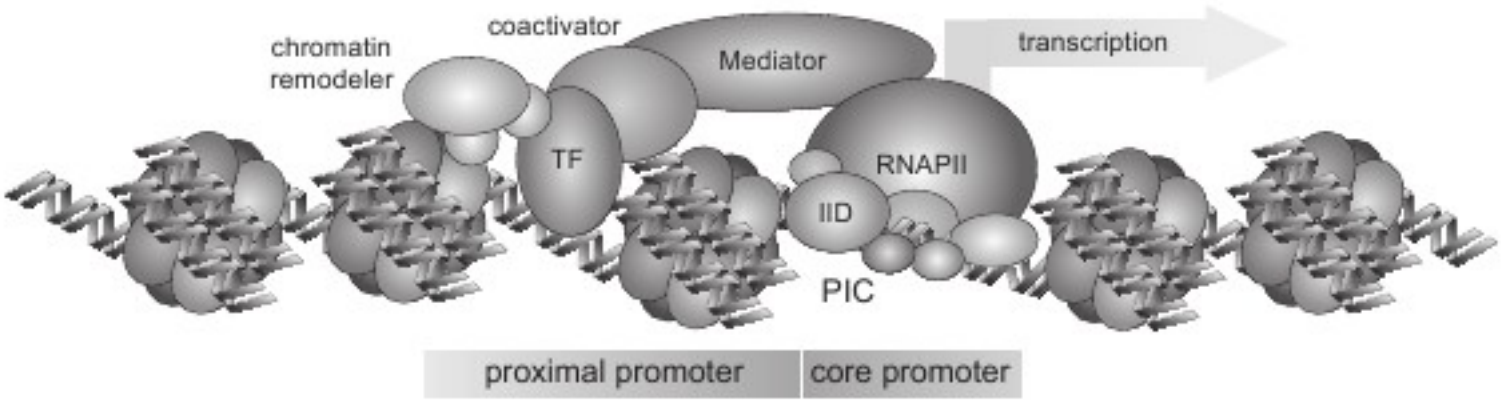
Promoters

- Constitutive promoters
- Standards
- Inducible exogenous promoters
  - TET
  - Q-mate
- Promoters of varying strength
- Pathway-sensing by promoters
- Standards for PW-Sensing promoters

Background: promoters



- Enhancers, silencers, insulators and promoters interact in eukaryotic gene expression
- Core promoter as binding site for basal transcription machinery
- Proximal promoter and enhancer bind specific transcription factors

Table 1. Summary of sequence and frequency of core promoter elements.

Core element	Position relative to TSS*	Consensus sequence**	Frequency in promoters	
			Flies	Vertebrates
TATA	approx. –31 to –26	TATAWAAR	33–43%	10–16%
Inr	–2 to +4	YYANWYY	69%	55%
DPE	+28 to +32	RGWYV	40%	48%
BRE	approx. –37 to –32	SSRCGCC	–	12–62%
MTE	+18 to +29	CSARCSSAACGS	8.5%	–

* The TSS is assigned to position +1.
** Degenerate nucleotides represented using IUPAC codes.

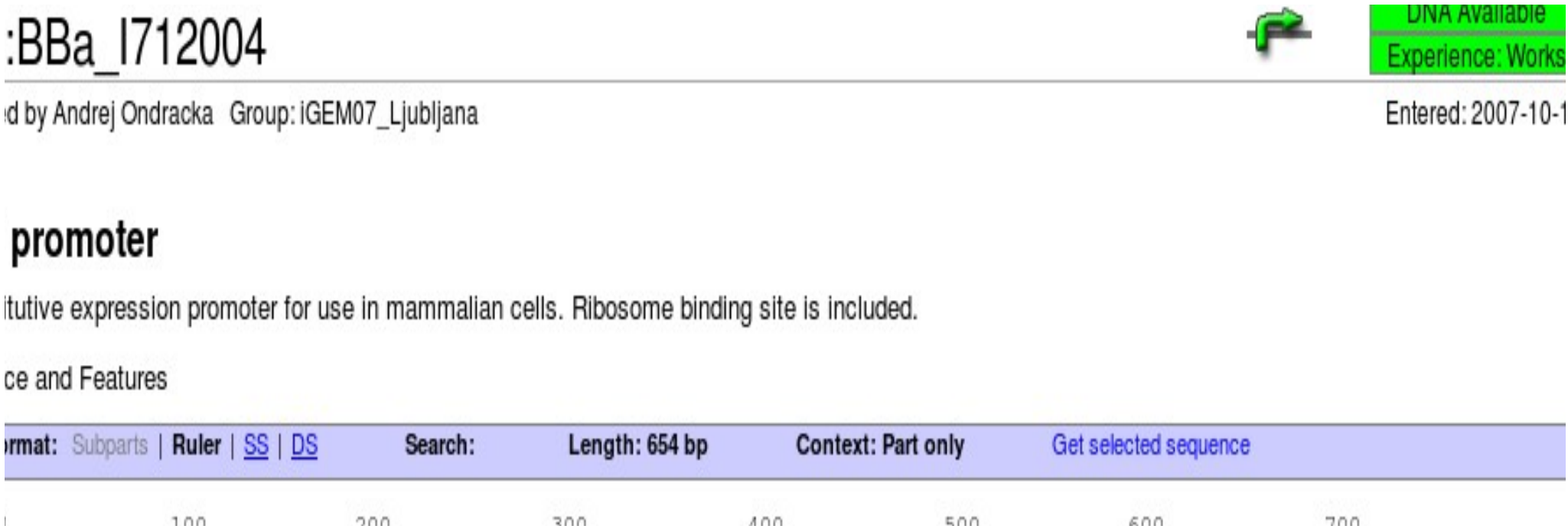
Heintzmann and Ren in Cell Mol Life Sci

Promoter resources

- http://dbtss.hgc.jp Transcriptional start sites of almost all human genes
- http://www.epd.isb-sib.ch/ Has annotated promoter sequences
- http://consite.genereg.net/ Predicts transcription factor binding
- http://partsregistry.org/Promoters/Catalog Has some eukaryotic promoters
- Pubmed: Publications describing single promoters are hard-to-find, and usually specific for a certain tissue, developmental stage, etc.

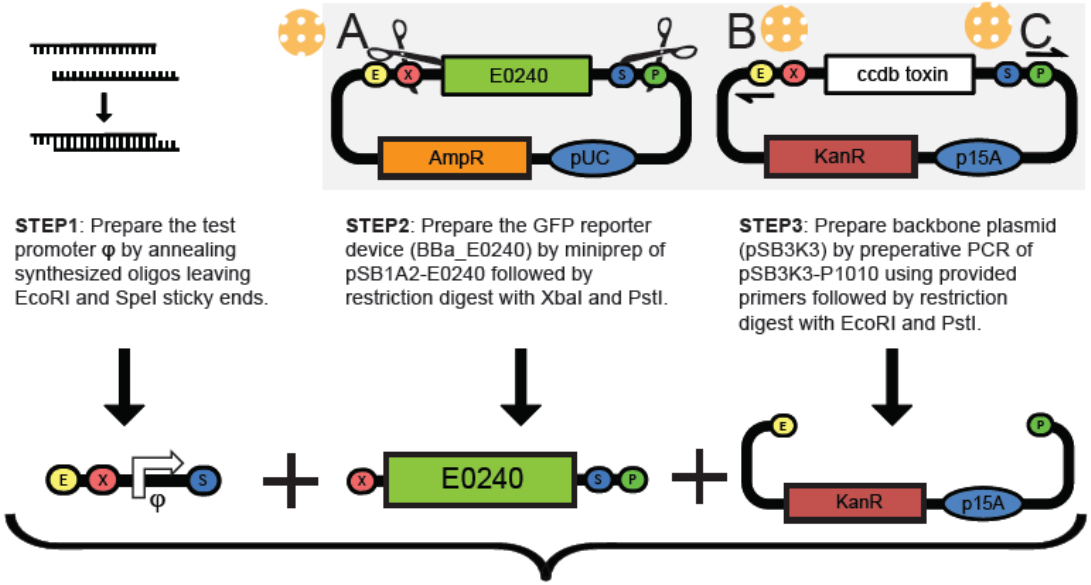
Constitutive promoters

- CMV promoter is a strong constitutive promoter
- Is a biobrick already:



- Alternative: Ubc (Ubiquitin C) promoter from Princeton 2008

Standards



STEP1: Prepare the test promoter φ by annealing synthesized oligos leaving EcoRI and SpeI sticky ends.

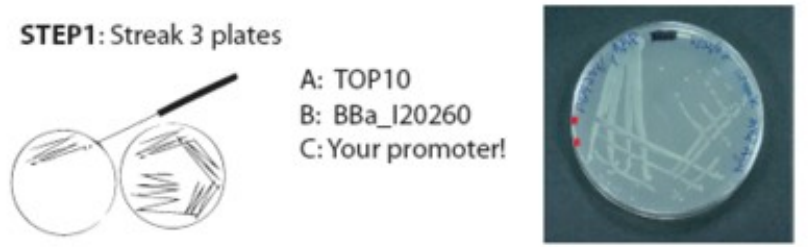
STEP2: Prepare the GFP reporter device (BBa_E0240) by miniprep of pSB1A2-E0240 followed by restriction digest with XbaI and PstI.

STEP3: Prepare backbone plasmid (pSB3K3) by preperative PCR of pSB3K3-P1010 using provided primers followed by restriction digest with EcoRI and PstI.

STEP4: Combine the test promoter φ, GFP reporter device, and backbone plasmid in a 3-way ligation to build the promoter test construct.

STEP5: Transform the promoter test construct into TOP10 cells. Select for transformants on Kanamycin plates.

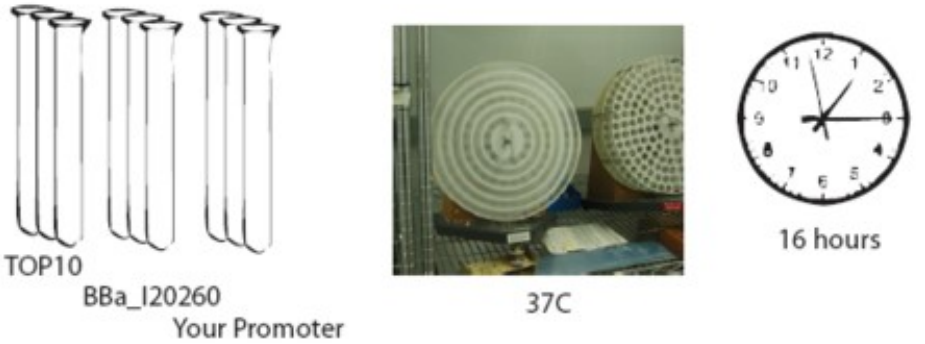
STEP6: Measure the activity of the test promoter φ relative to the activity of the reference standard promoter (BBa_J23101). Report promoter φ activity in relative units of Standard Promoter Units (SPUs).



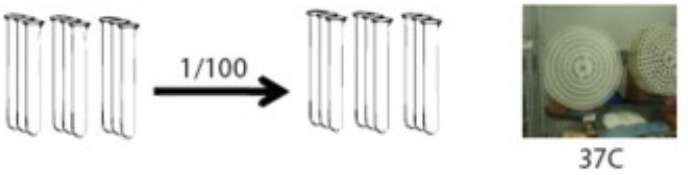
STEP1: Streak 3 plates

A: TOP10
B: BBa_I20260
C: Your promoter!

STEP 2: Pick 3 colonies from each plate to start overnight cultures in Supplemented M9 Media at 37 C (9 tubes)



STEP 3: Dilute 1/100 into fresh, pre-warmed media incubate at 37C (9 tubes)



STEP 4: After 3 hours measure GFP and OD

STEP 5: After another half hour measure GFP and OD again

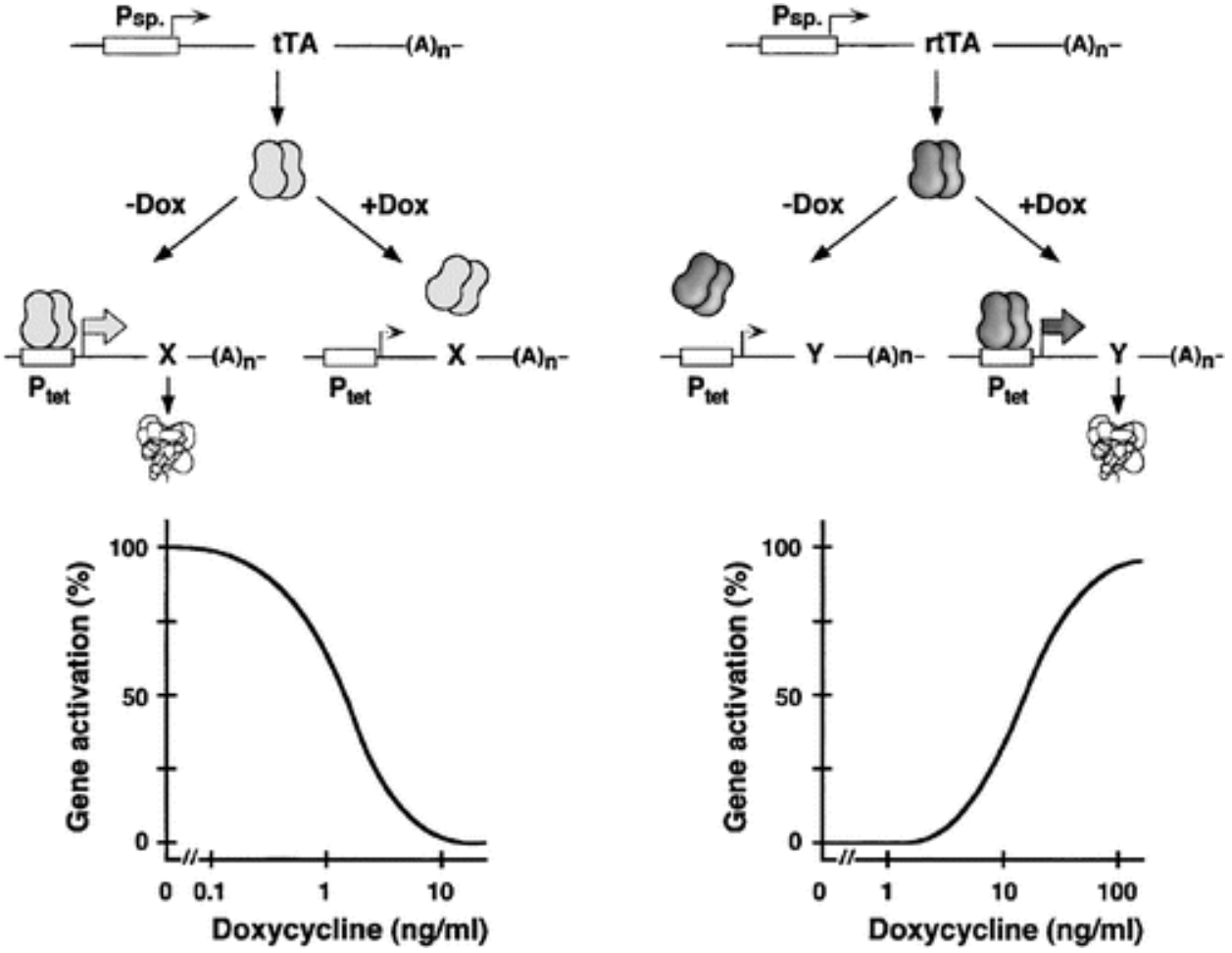
http://partsregistry.org/Measurement/RPU/Learn

Standards

- Needs to be modified
- Mammalian expression vector containing GFP, selection marker (eukaryotic + prokaryotic), origin of replication
- Use Ljubljana CMV promoter as standard?

Inducible exogenous promoters

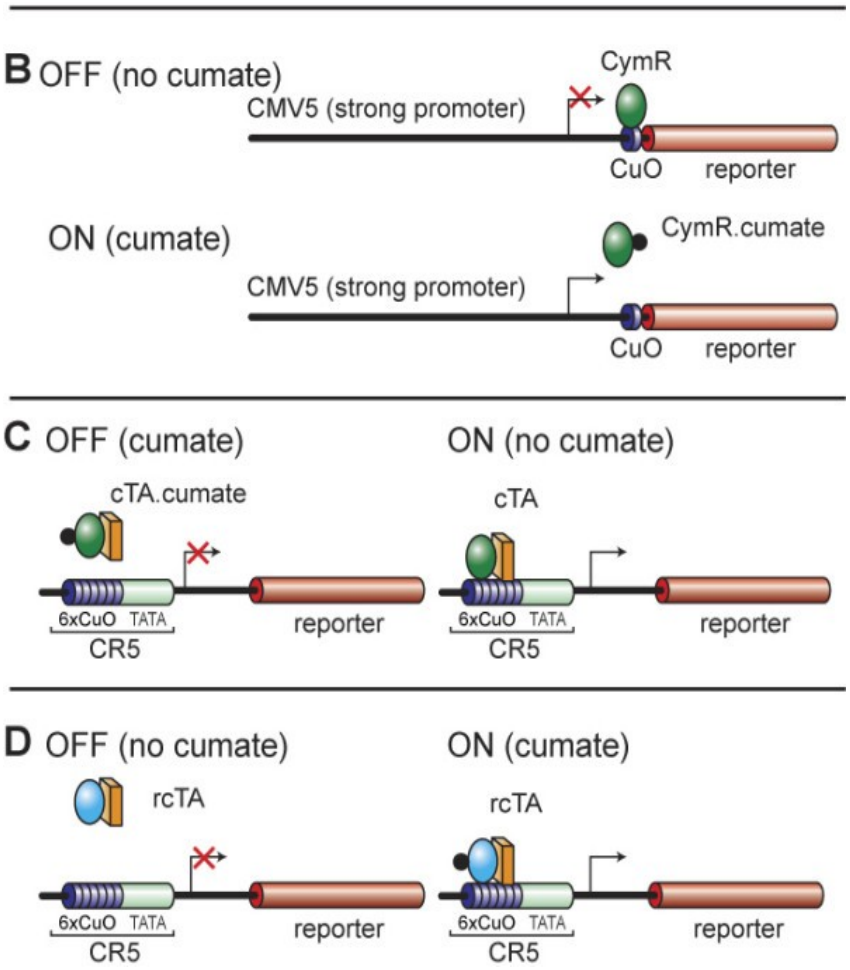
- TET: Minimal promoter plus TetO-repeats
- VP16-activation domain fused to TetR



Annual Review of Genetics
Vol. 36: 153-173

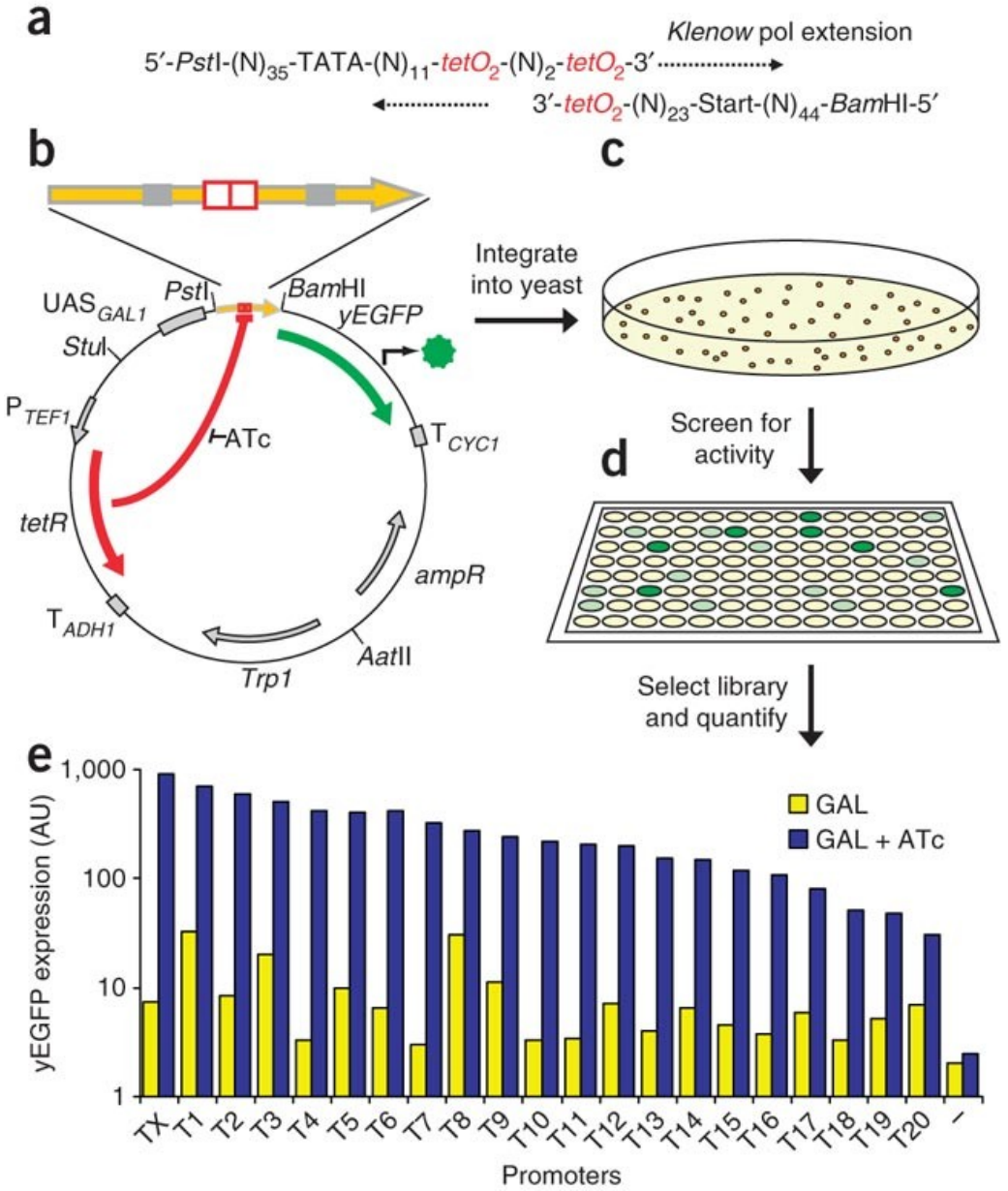
Inducible exogenous promoters

- Cumate-regulated system analogous to the Tet system
- Inducible promoters have many advantages besides being inducible:
- ***No intereference***
- *De-activation configuration can also be used for signal integration*
- *Widely used, commonly accepted*
- *A lot of varieties exist*
- *Commercially available, e.g.* *http://krackeler.com/products/fid/2755*



BMC Biotechnology 2006, 6:43

Promoters of varying strength

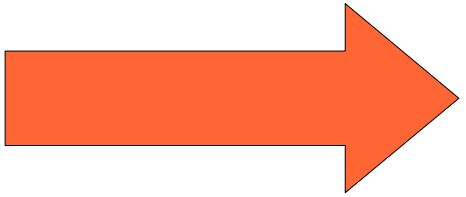


Ellis et al in Nature biotechnology

- Core elements of core promoter (here: Tet-Off system) are preserved
- Rest of **core** promoter is randomized
- Modification in DNA secondary structure alters RNA-Pol binding
- Creation of a library
- Authors were able to design **predictable** timers by this approach

Pathway sensing

- **„To see what a cell thinks“**
- Developmental pathways with cancer relevance: wnt, hedgehog, NF-kb, p53, ...
- Apoptosis and autophagy
- ROS / redox potential sensing
- *Metabolic pathways (Glucose, insulin, SREBP and/or PGC-1)*



Looking for promoters regulated *exclusively* by these pathways – exploiting negative feedback regulation or other typical target genes

Developmental pathways

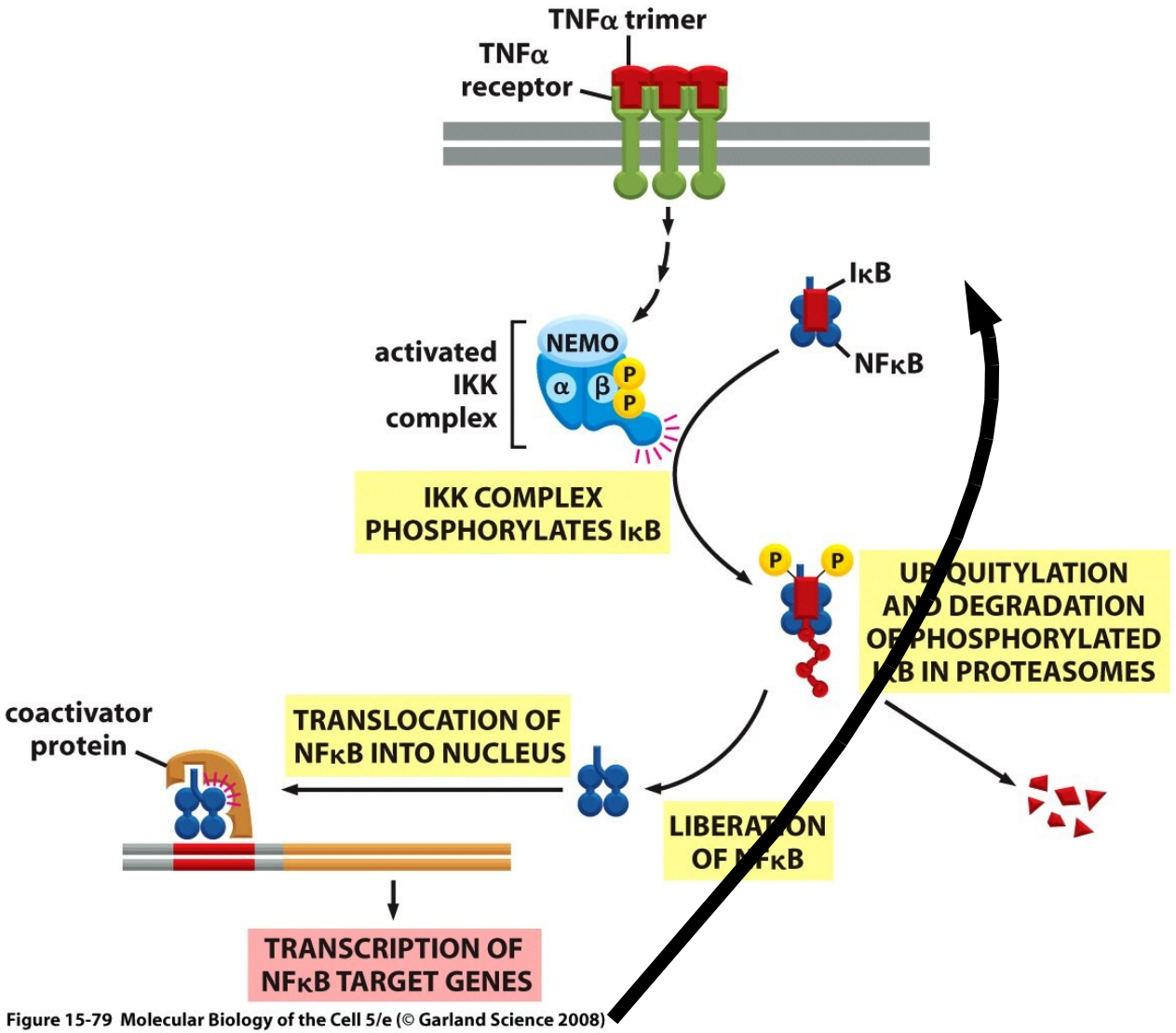


Figure 15-79 Molecular Biology of the Cell 5/e (© Garland Science 2008)

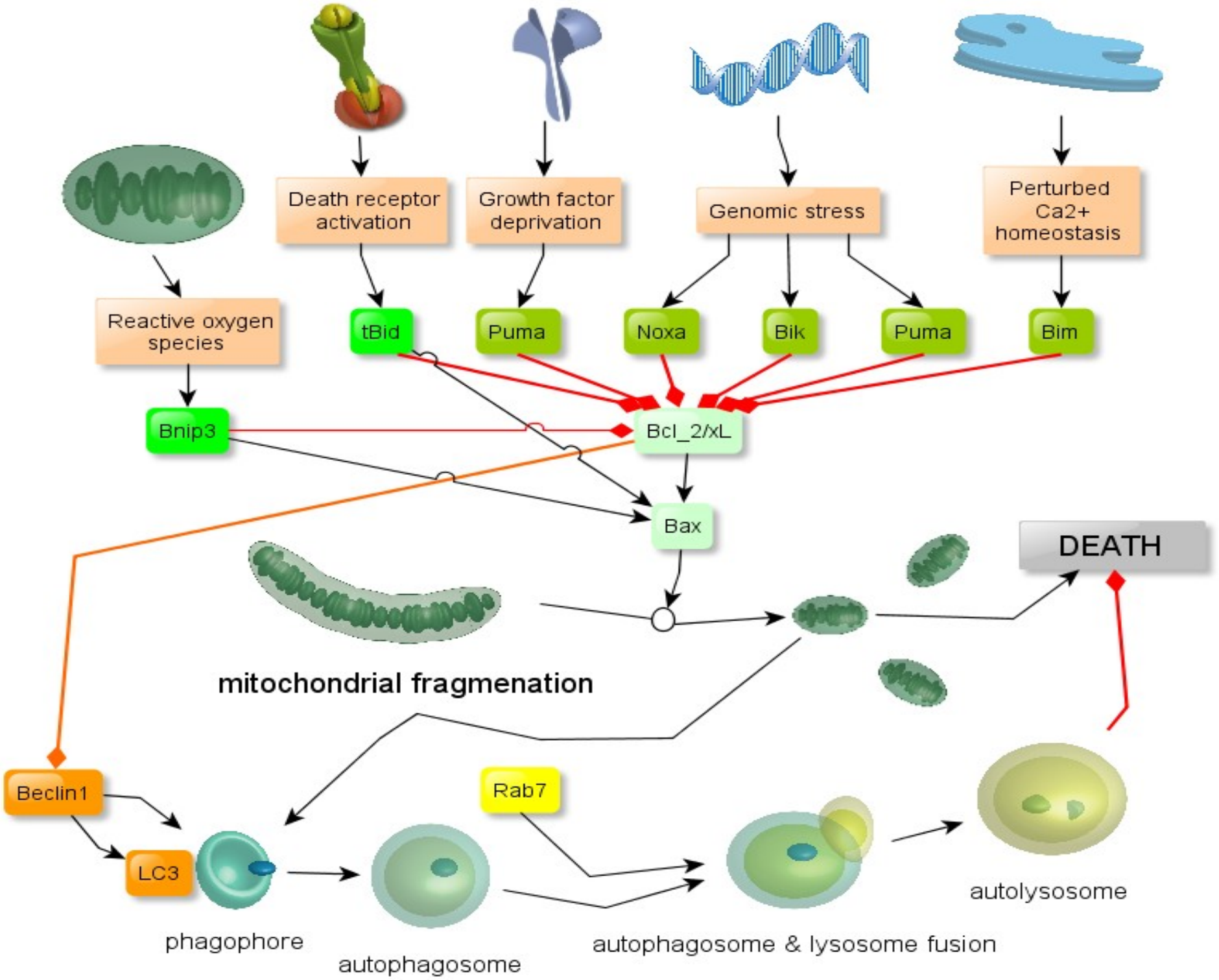
NF-κβ

IκBα promoter

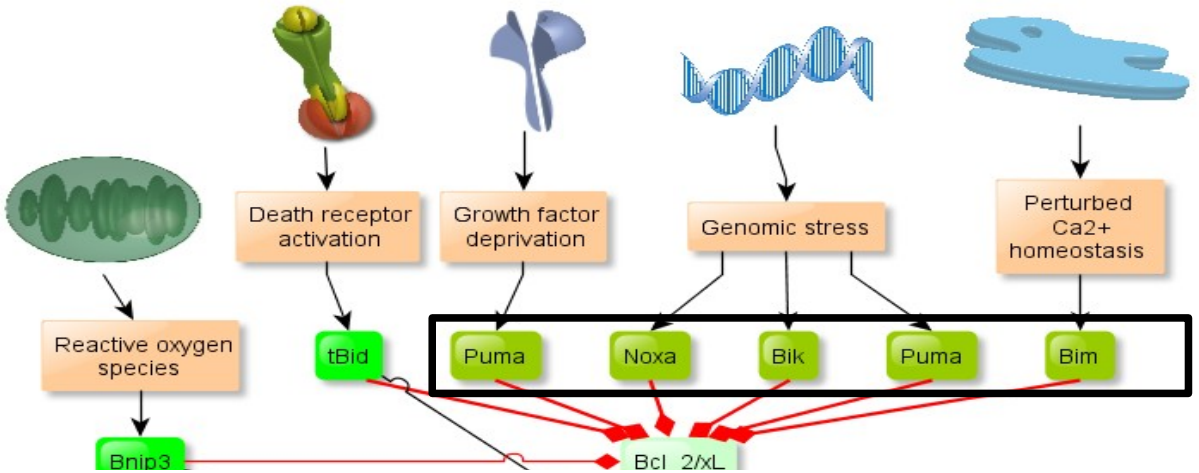
In
http://www.epd.isb-sib.ch/

Developmental pathways

- ***Hedgehog: PTC1 promoter*** *(patched). Described in dbtss.hgc.jp*
- ***p53:***
  - ***p21 promoter*** *(described in PubMED ID: 16434701, PubMED ID 18719376 and others)*
  - ***PUMA promoter*** *(cloning in PubMED ID 18811981)*
- ***Wnt:*** *conductin / axin-2 as a negative feedback regulator (PubMED ID 11809809 even has a gene-mouse expressing lacZ under the conductin promoter)*


Death receptor activation
Growth factor deprivation
Genomic stress
Perturbed Ca2+ homeostasis
Reactive oxygen species
tBid
Puma
Noxa
Bik
Puma
Bim
Bnip3
Bcl_2/xL
Bax
DEATH
mitochondrial fragmenation
Beclin1
Rab7
LC3
phagophore
autophagosome
autophagosome & lysosome fusion
autolysosome

Apoptosis and autophagy



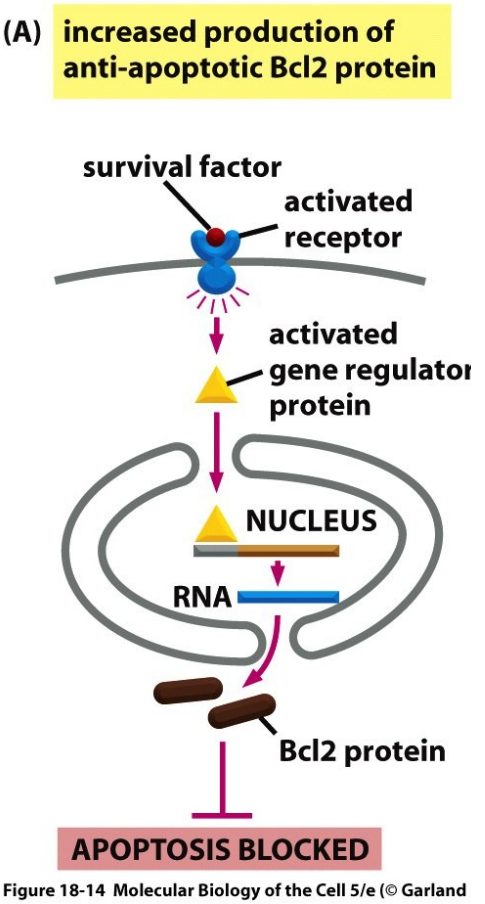


Figure 18-14 Molecular Biology of the Cell 5/e (© Garland

- ***BH3-only proteins: Transcriptional regualtion (also) decides on cell death***
- Clone promoters of some of their genes
- Bcl2 (anti-apoptosis) is also transcriptionally regulated

Bik, Noxa, Puma, Bim promoters have been cloned previously as described in PMID: 17027756, PMID: 14699081, PMID: 18811981, PMID: 18549468

Apoptosis and autophagy

- Not only transcription regulates whether Apoptosis is active
- Autophagy: constitutive, mostly non-transcriptionally regulated (I only found ATG7 as transcriptionally regulated autophagy inducer [induced during starvation, PMID: 15866887, promoter sequence in dbtss.hgc.jp])

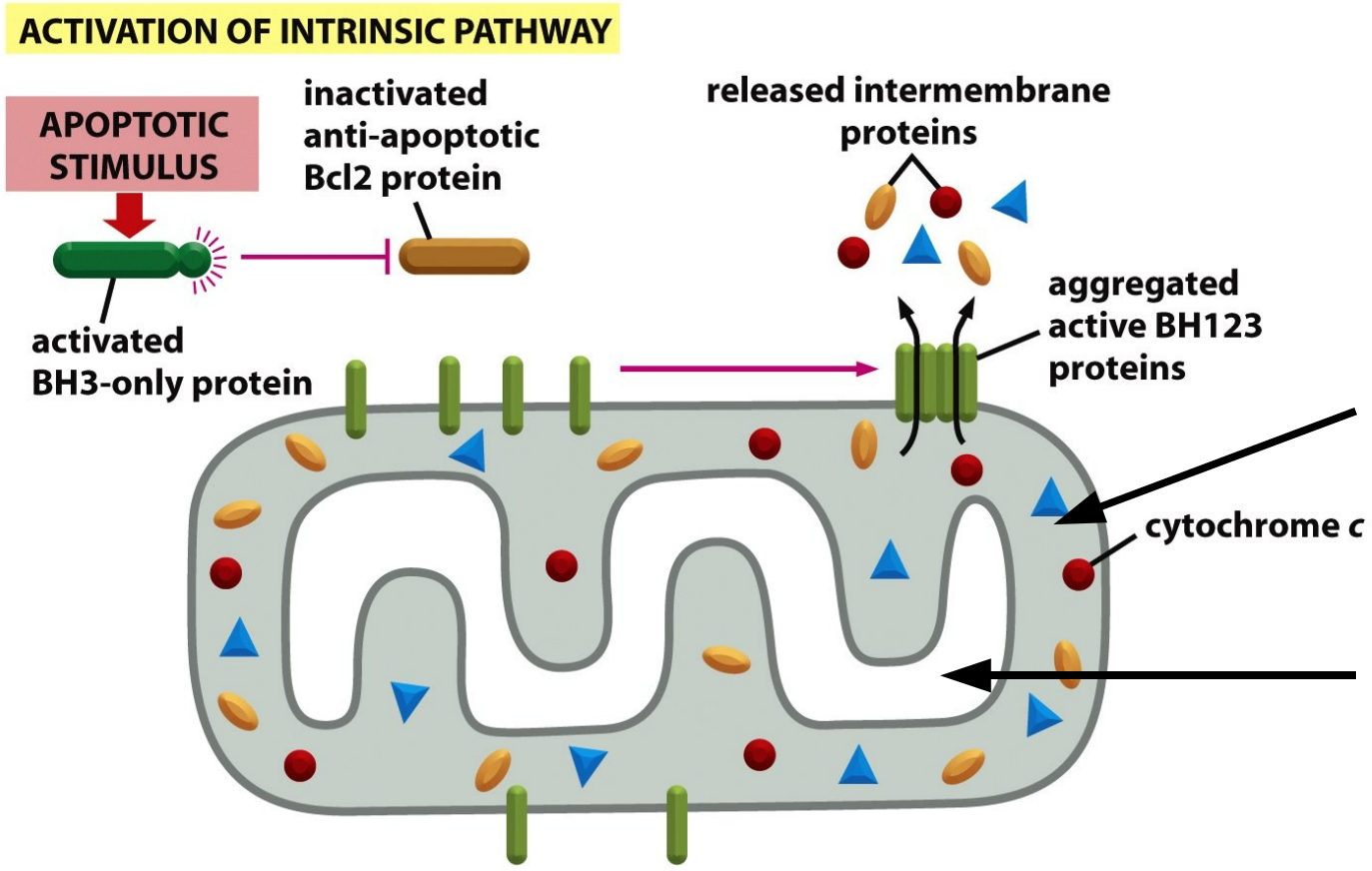


Figure 18-11b Molecular Biology of the Cell 5/e (© Garland Science 2008)

To the nucleus upon apoptosis

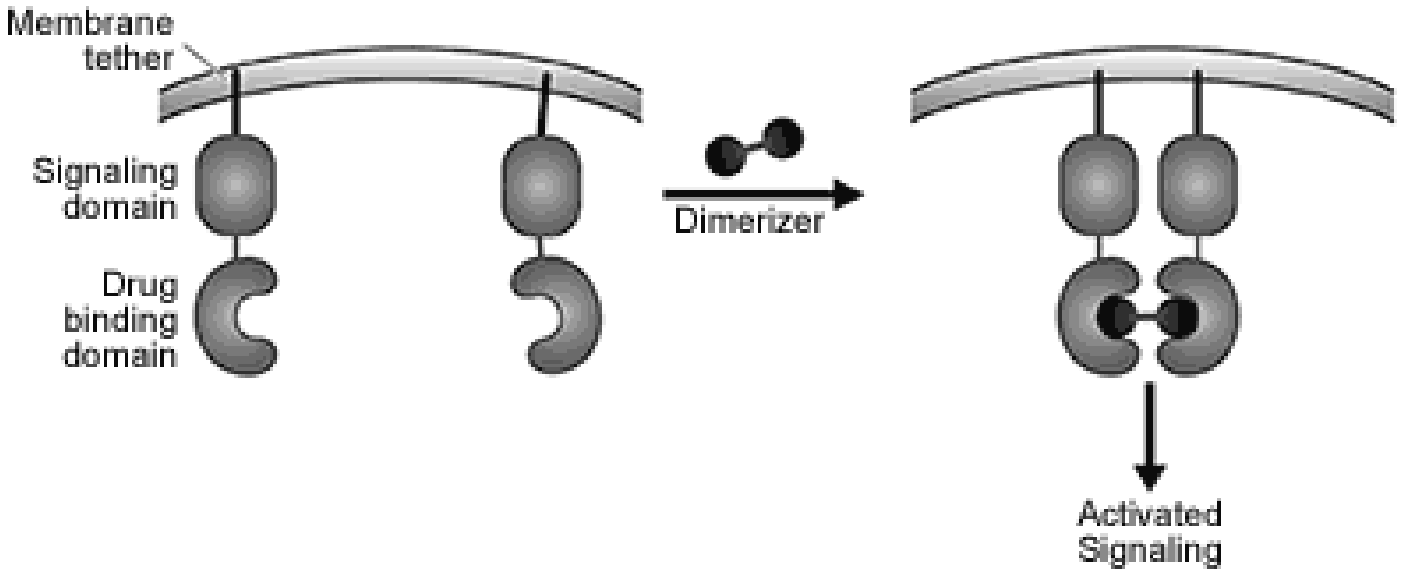
Exogenous Gene regulatory protein, targetted by addition of the MISS-motif (PubMed 19297525)

Ratiomeric pHlourin, targetted by the addition of the mitochondrial target sequence

To the lysosome upon autophagy

Standards for PW-sensing Promoters

- Need to find maximal and minimal induction of these promoters
- Switching on pathways by ARIAD ARGENT dimerization kits possible for some pathways



- Otherwise: siRNA-mediated knockout of pathway activators / inhibitors in order to sense min/max expression?
- Chemically stressing the cells?

Links and Literature

- Background: Promoters
  - Rolf Knippers: Molekluare Genetik (9th edition), Chapter 12 and 13
  - Heintzmann, N.D. and Ren, B.: The gateway to transcription: identifying, characterizing and understanding promoters in the eukaryotic genome. In: Cellular and Molecular Life Sciences 64 (2007), pp. 386-400
- Existing Biobrick promoters:
  - http://partsregistry.org/Promoters/Catalog
- Measuring promoter strength in RPU:
  - http://partsregistry.org/Measurement/RPU/Learn
- Inducible exogenous promoters:
  - Weber, W. and Fussenwegger, M.: Novel Gene Switches. In: Handbook of Experimental Pharmacology 178 (2007), pp. 73-103
- Promoters of varying strenth
  - Ellis, T. Et al: Diversity-based, model-guided construction of synthetic gene networks with predicted functions. in Nature Biotechnology (online publication 19.4.2009)
- Pathways
  - Alberts: Molecular Biology of the Cell (5th edition), Chapter 15 and 18