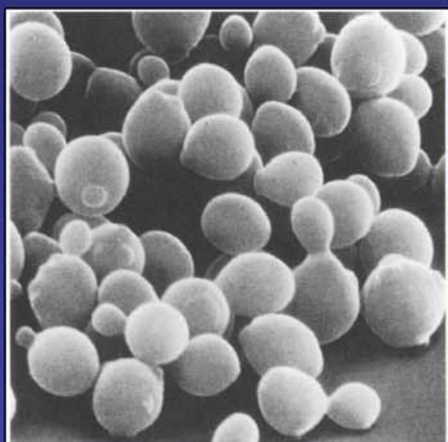
IGM

UMR CNRS U-PSUD

Stress-induced oscillatory nucleocytoplasmic behavior of the transcription factor Msn2 in yeast



Michel Jacquet

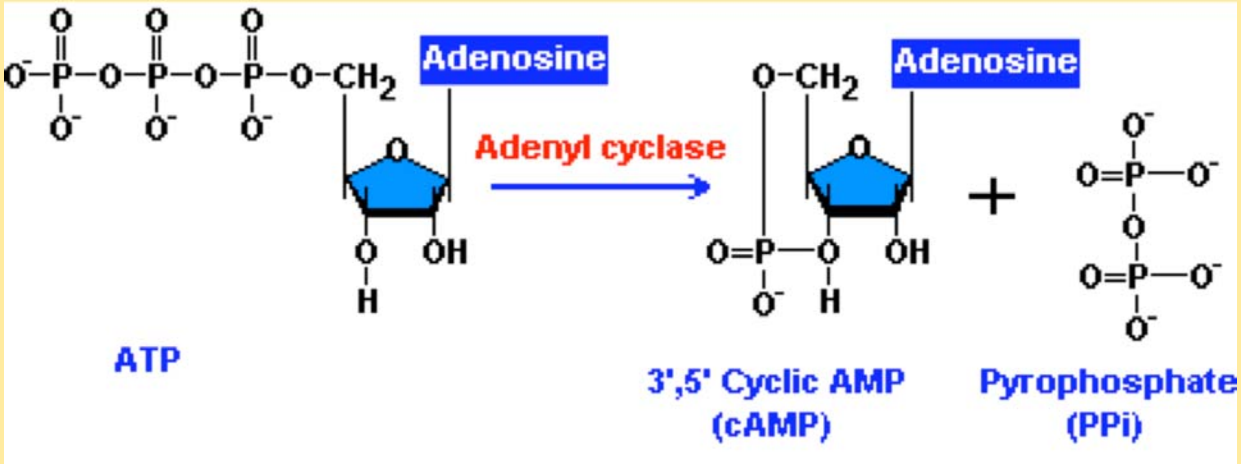
Georges Renault

Cecilia Garmendia-Torres

Albert Goldbeter

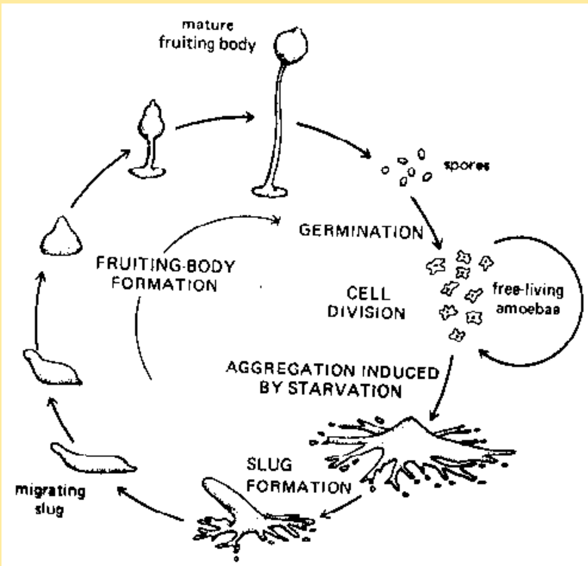
cAMP a signaling molecule (Sutherland 1957)

Formation of a Cyclic Adenine Ribonucleotide by Tissue Particles (Rall, T. W., and Sutherland, E. W. (1958) *J. Biol. Chem.* 232, 1065)
Fractionation and Characterization of a Cyclic Adenine Ribonucleotide Formed by Tissue Particles (Sutherland, E. W., and Rall, T. W. (1958) *J. Biol. Chem.* 232, 1077



The step sensitive to catabolite repression and its reversal by 3′–5′ cyclic AMP during induced synthesis of β-galactosidase in E. coli; **Biochemical and Biophysical Research Communications,** Volume 36, Issue 1, 7 **July 1969**, Pages 84-92. **Michel Jacquet** and Adam Kepes

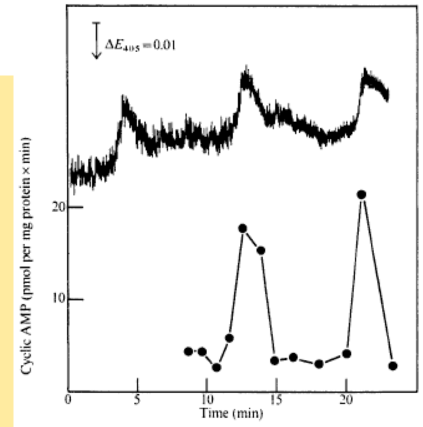
cAMP oscillations in *Dictyostelium discoideum*

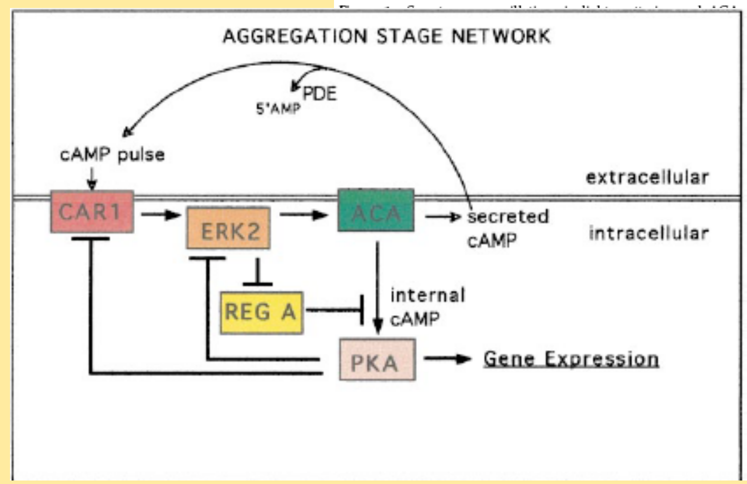


Molecular Biology of the Cell
Vol. 9, 3521–3532, December 1998

A Molecular Network That Produces Spontaneous Oscillations in Excitable Cells of *Dictyostelium*

Michael T. Laub and William F. Loomis*





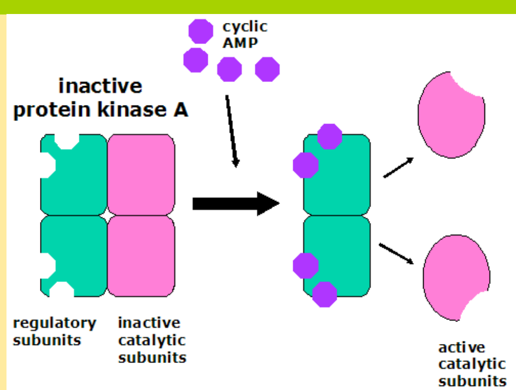
cAMP in yeast

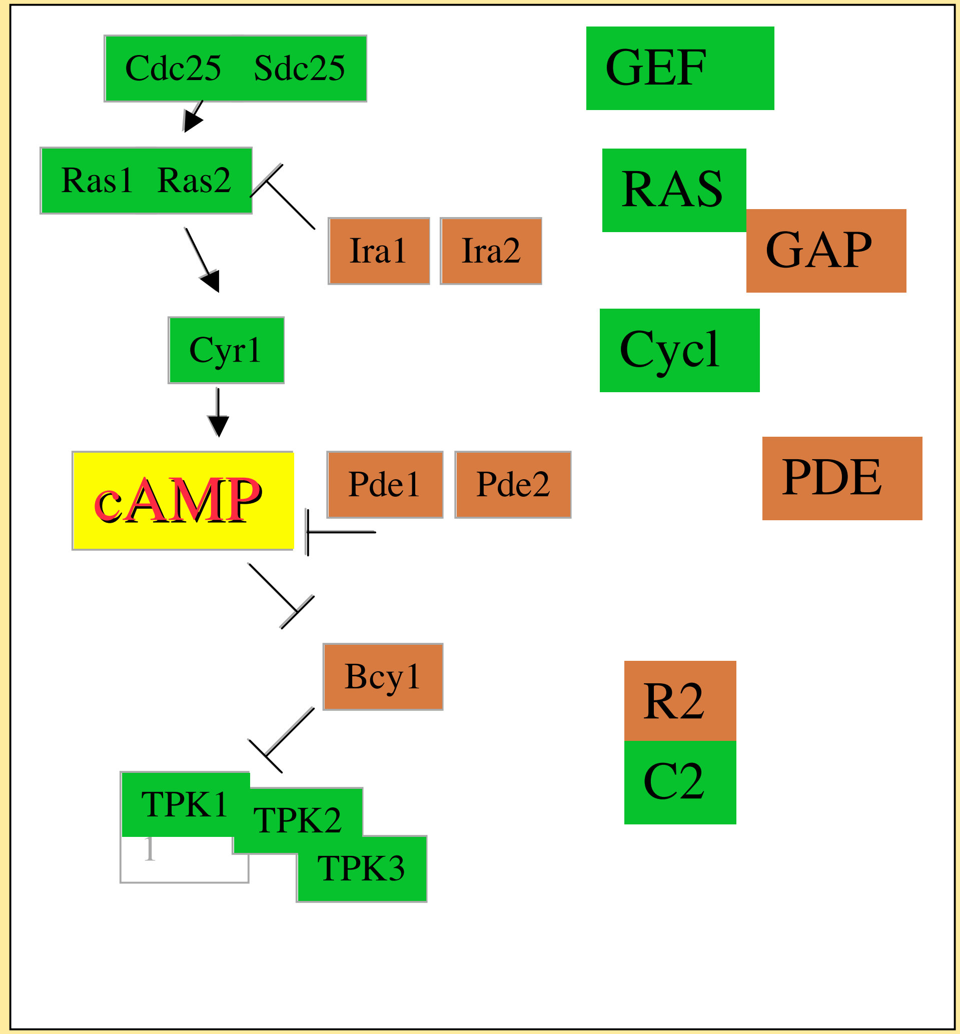
Classical genetic (BC) 75..

cdc25, cdc35 (Hartwell, Hilger),
cyr1 (Matsumoto et al.; 1982)
bcy1 (Matsumoto et al.; 1982)

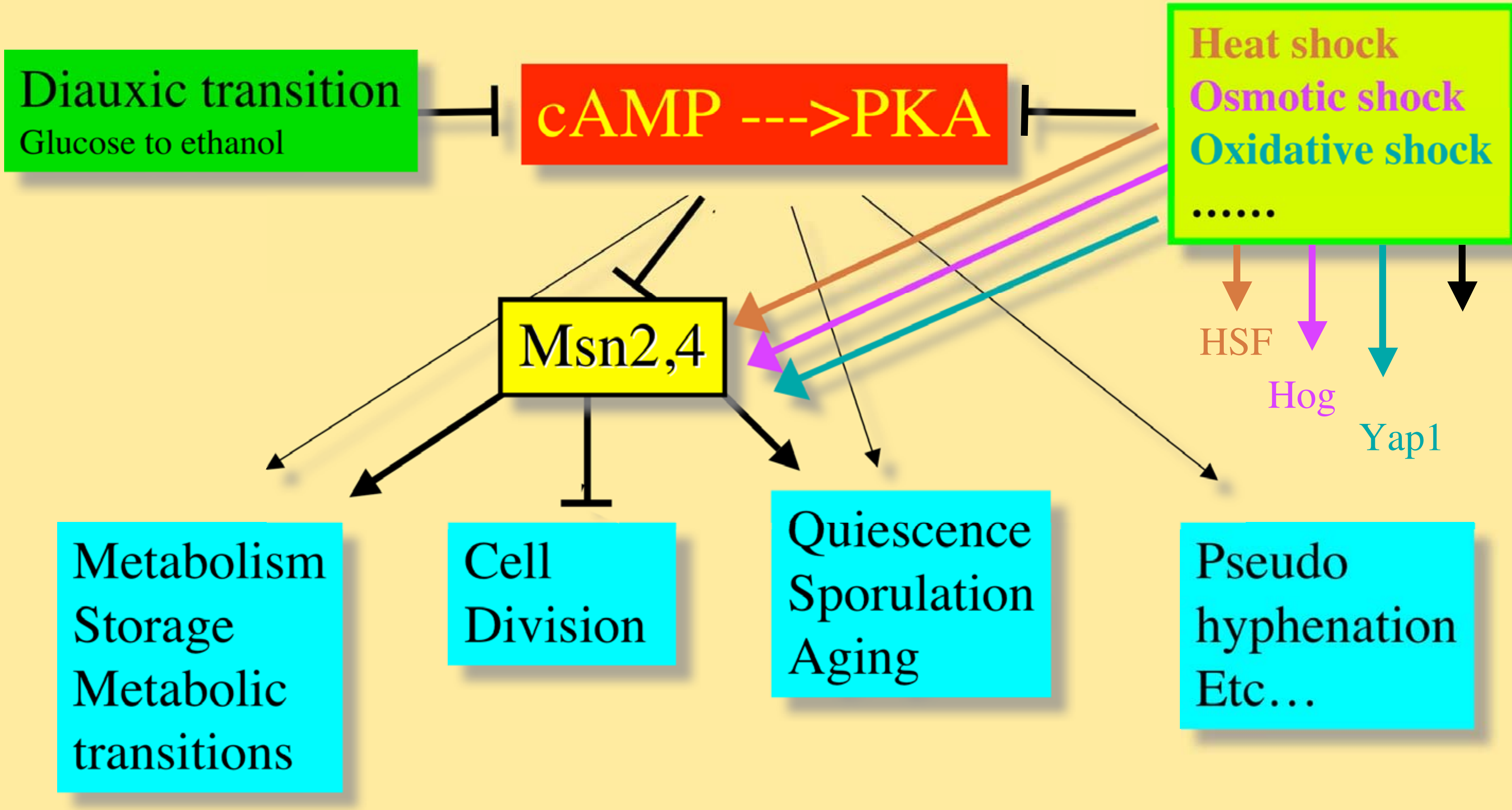
Mol. cloning and sequencing (..80-90)

CYR1/CDC35 (Masson 1984, Wigler 1985)
RAS1, RAS2 (…Wigler 1984)
CDC25, SDC25 (…Jacquet 1986)
BCY1 (…Wigler 1987)
TPK1,TPK2,TPK3 (…Wigler 1987)
PDE1, PDE2 (…Wigler 1987)
IRA1, IRA2 (Matsumoto 1990)





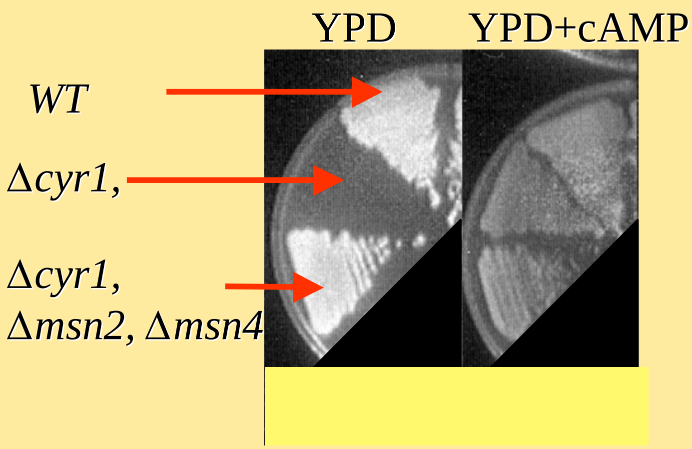
What is the role of the cAMP-PKA system in yeast ?



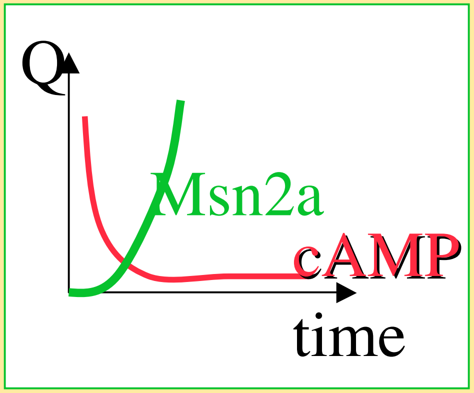
Msn2 and Msn4 are targets of cAMP

- **Msn2 and Msn4**

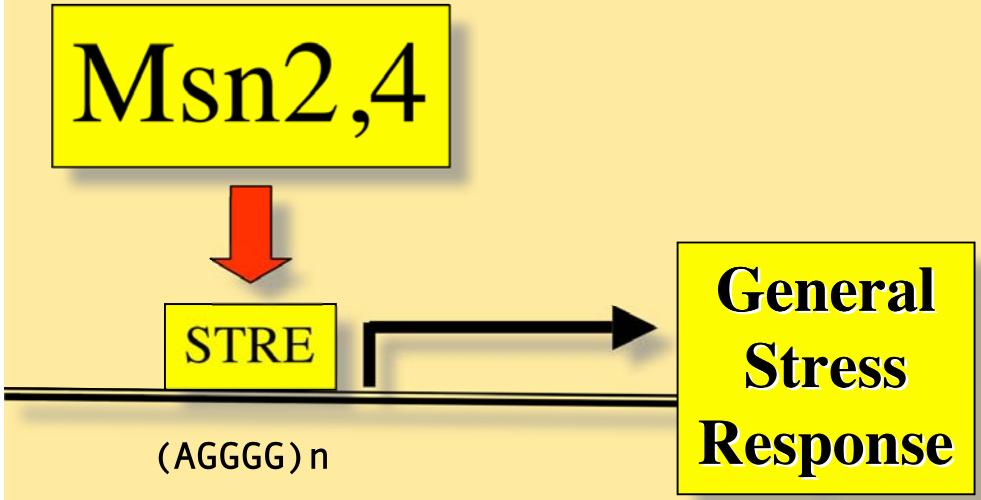
- Suppressor of growth defect of *Δcyr1*
- Mediates diauxic transition when cAMP is low
- Activated upon heat shock (low cAMP)
- Activated upon oxidative shock (low cAMP)
- …Osmotic shock
- …weak acid
- …

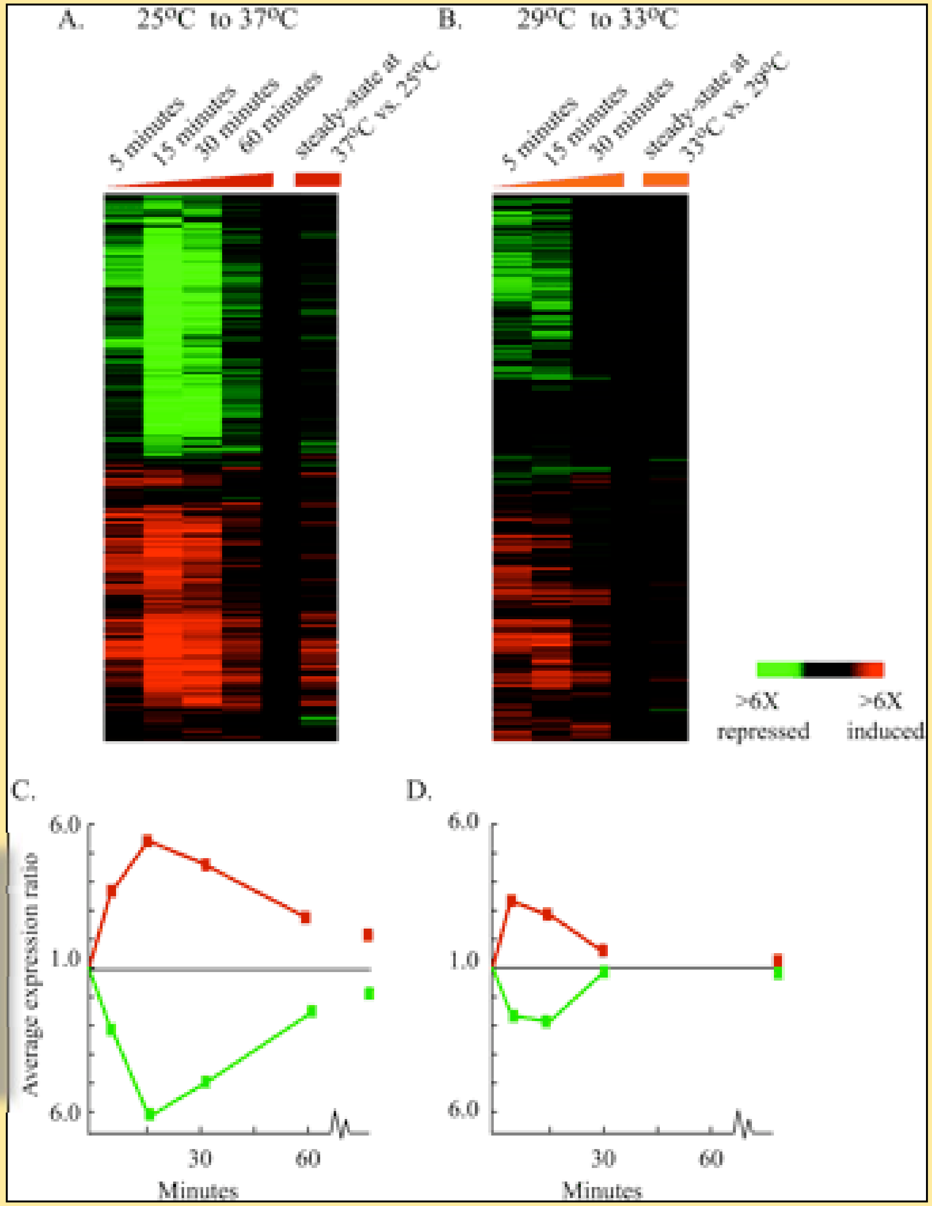


cAMP
⊥
Msn2,4
⊥
Growth



Msn2 and Msn4 activate the “STRE” régulon

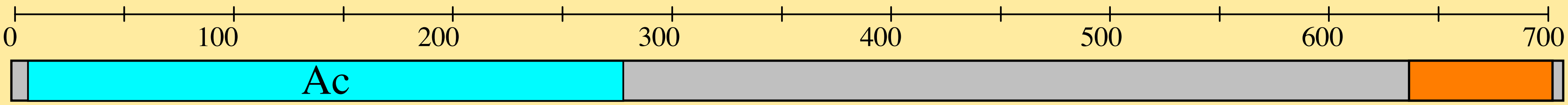




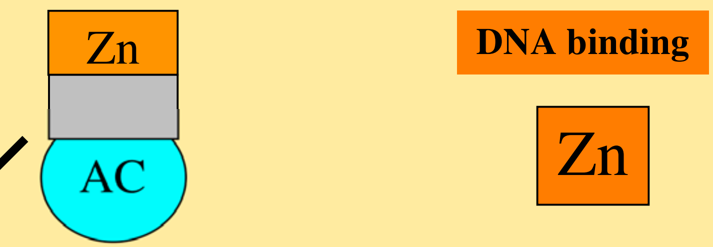
Molecular Biology of the Cell
Vol. 11, 4241–4257, December 2000
Audrey Gasch et al.

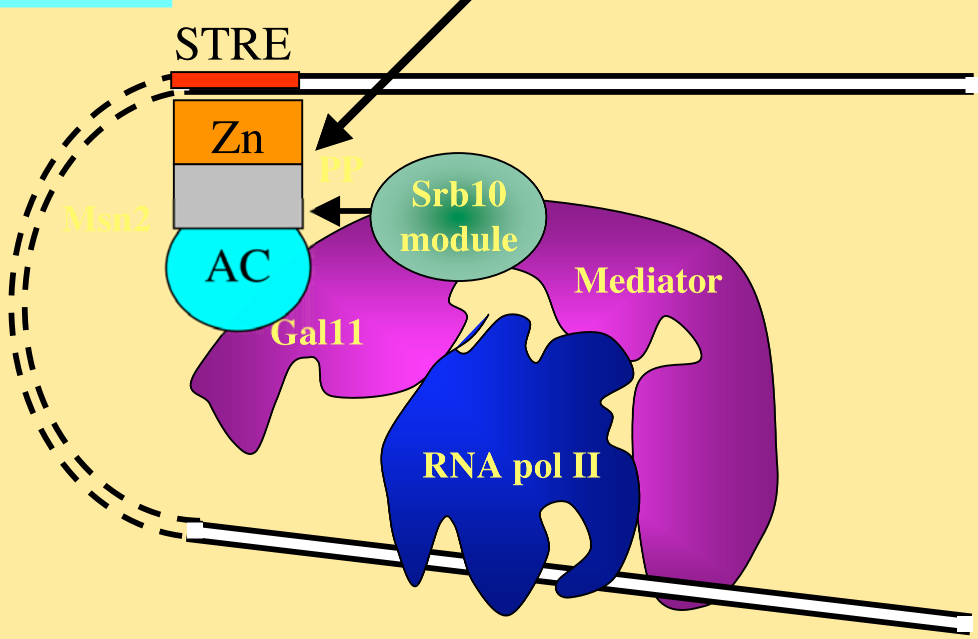
Functional anatomy of Msn2

Msn2



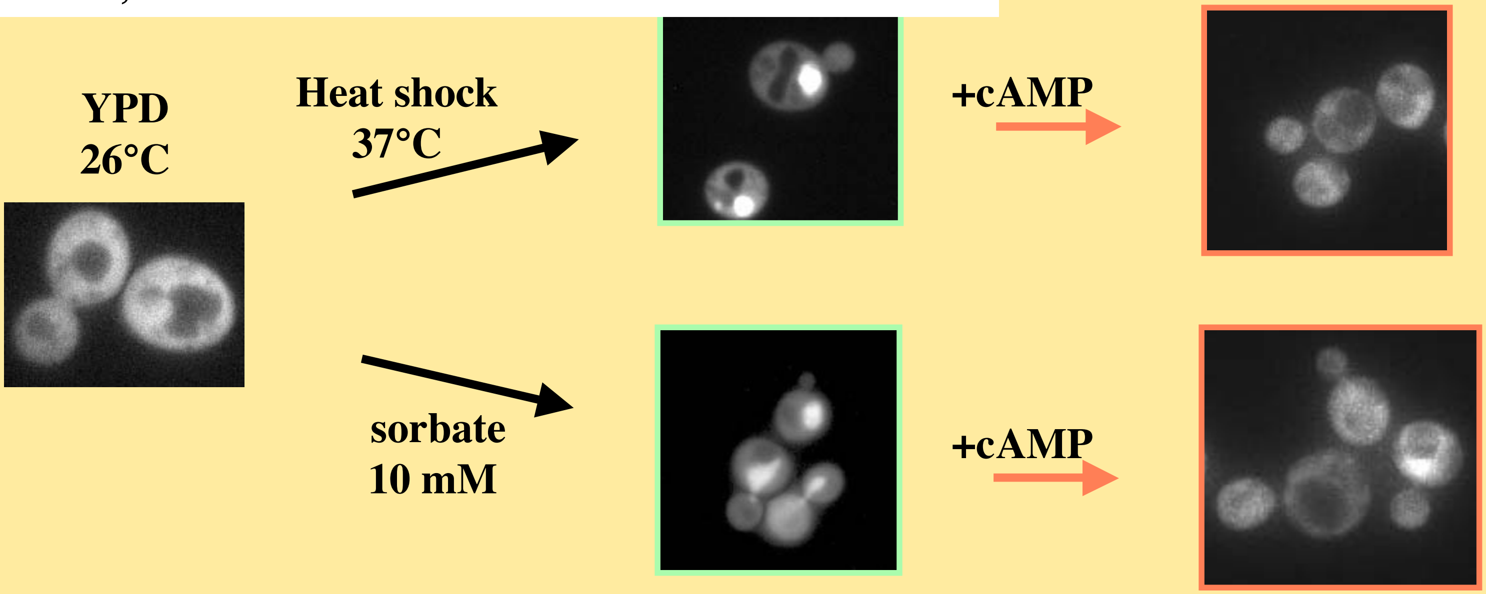
Activation domain
Stress response:
- Stress dependent localization
- Stress dependent activation

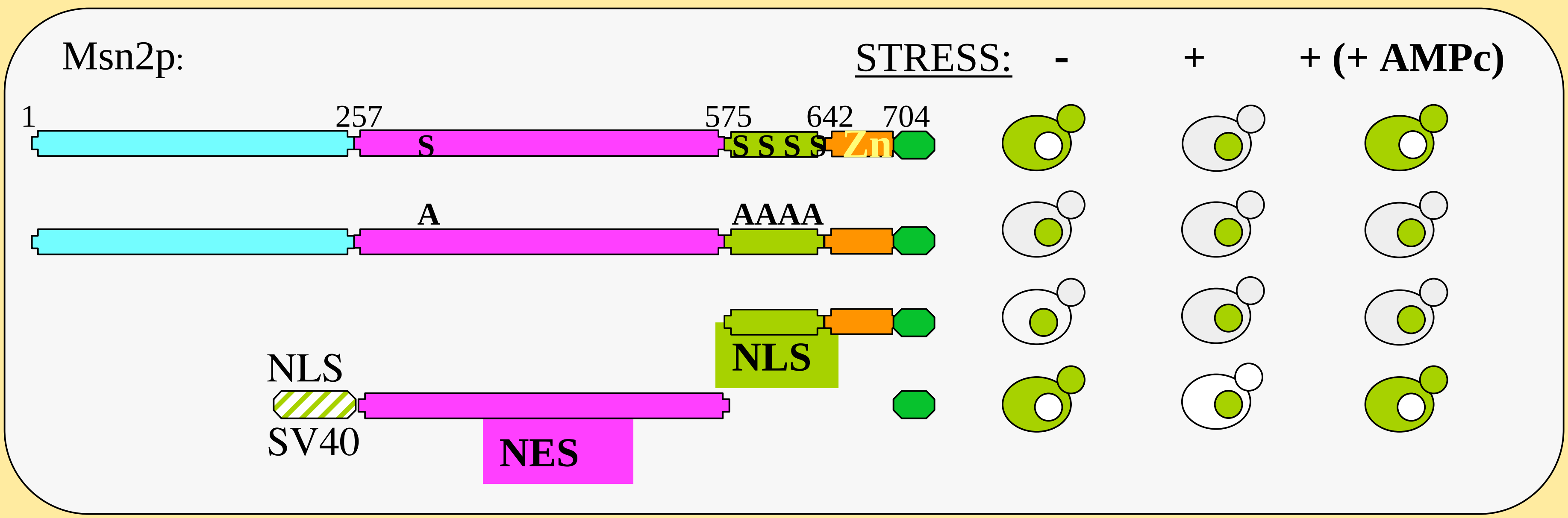




Nucleocytoplasmic localization of Msn2

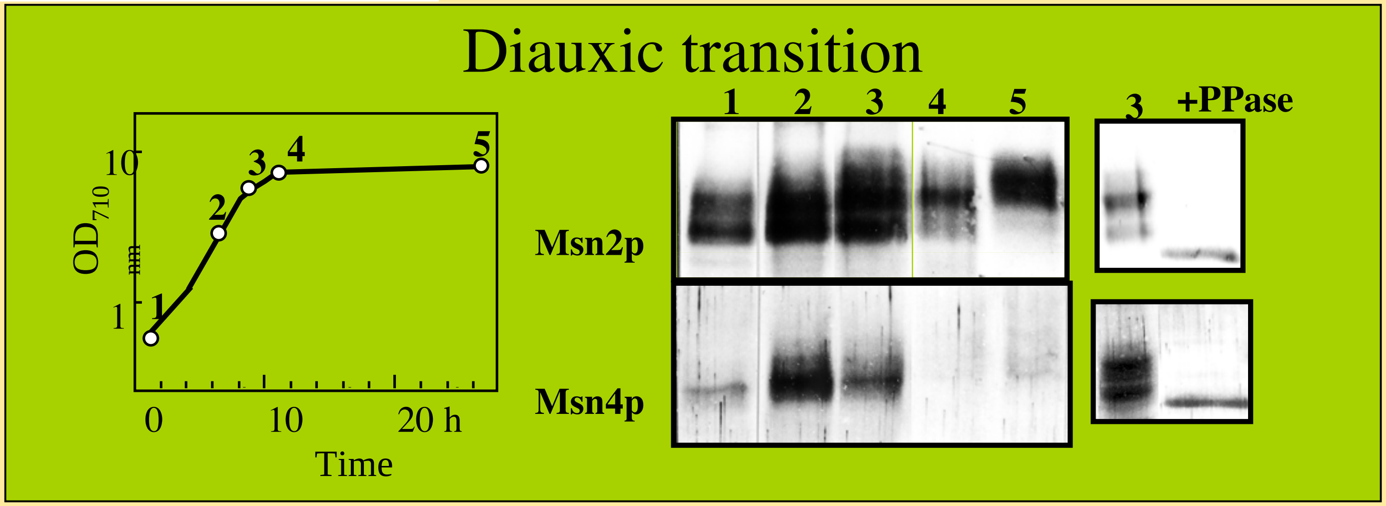
Gorner *et al*, Genes Dev. 1998 12: 586-97.1998

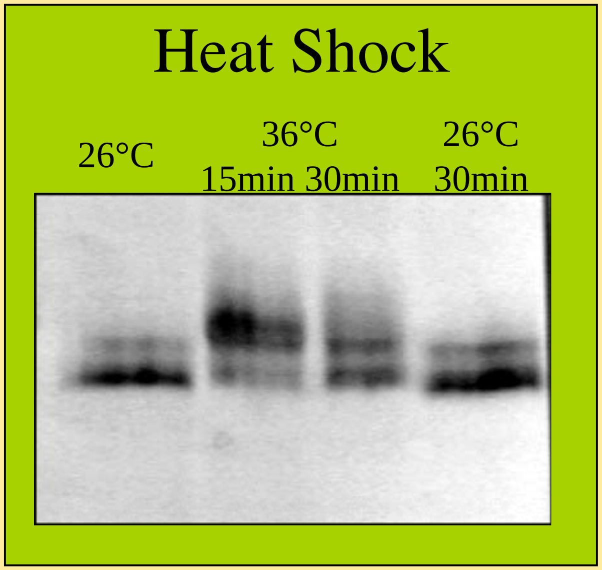


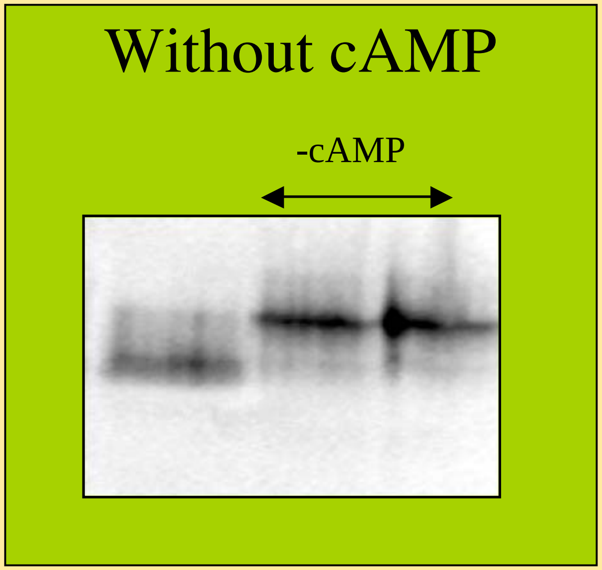


Msn2 is hyperphosphorylated when activated

Garreau et al Microbiology. 2000 146 2113-20.







The search for the hyperphosphorylating kinase (S. Lallet and H. Garreau)

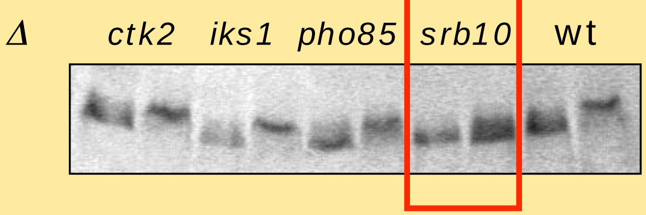
Lallet et al Mol Microbiol. 2006 Oct;62(2):438-52.

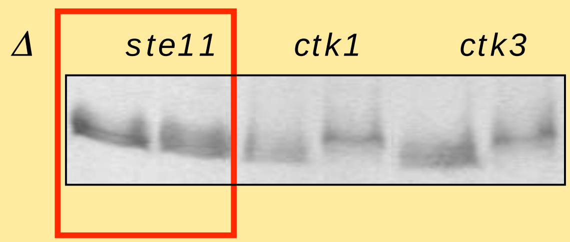
Using 120 mutants for protein kinase

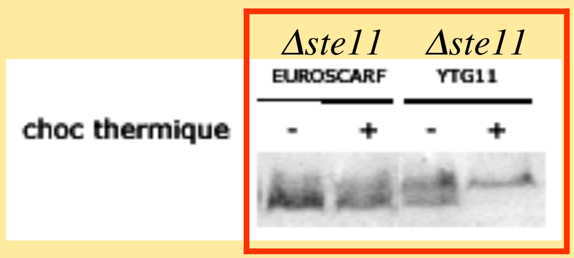
The only two mutant strains

srb10/ssn3

ste11

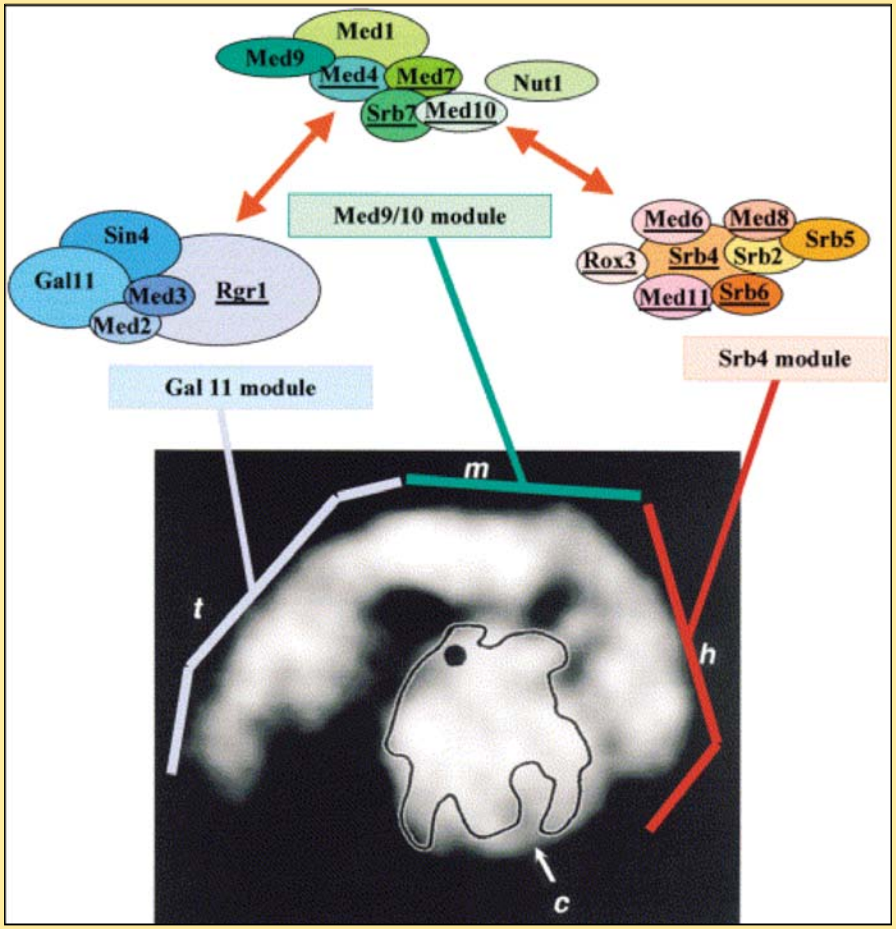


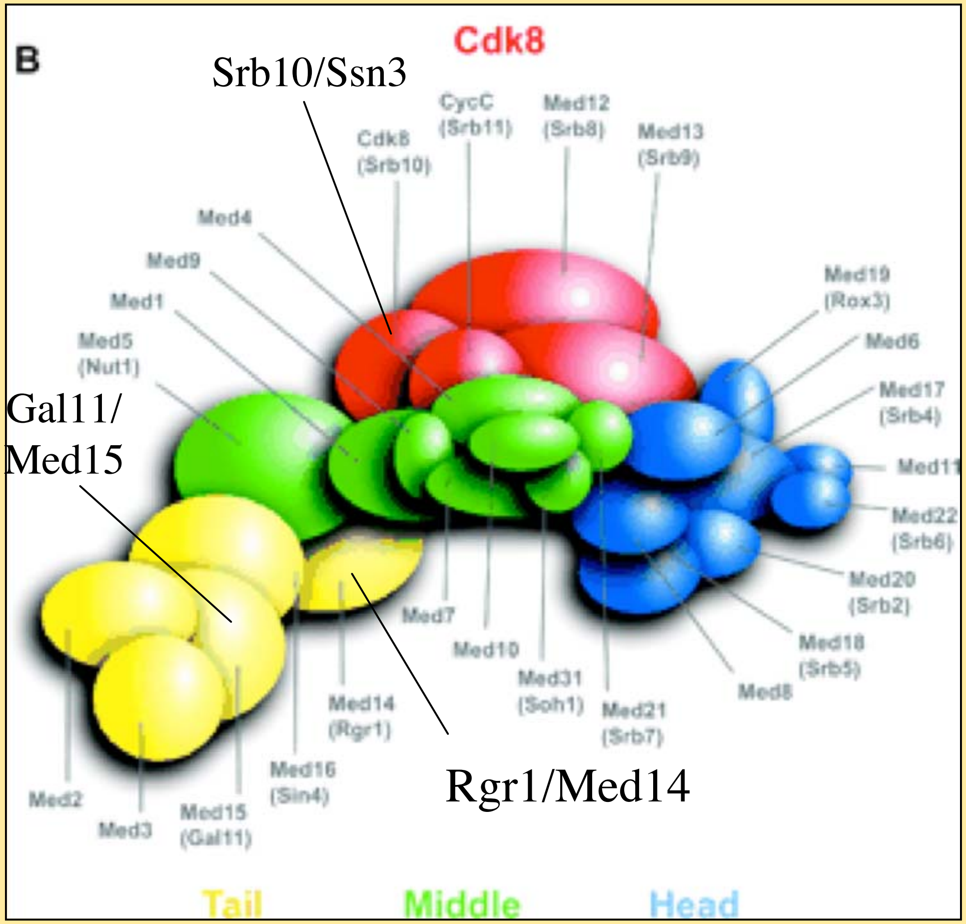




Ste11=X=? Gal11

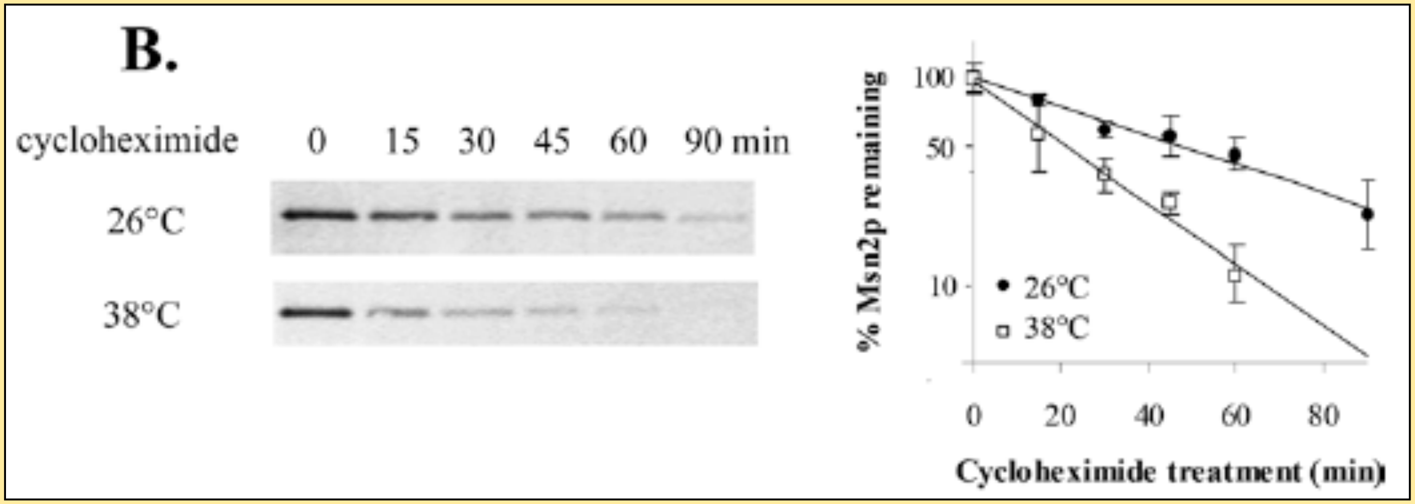
The mediator (R. Kornberg)

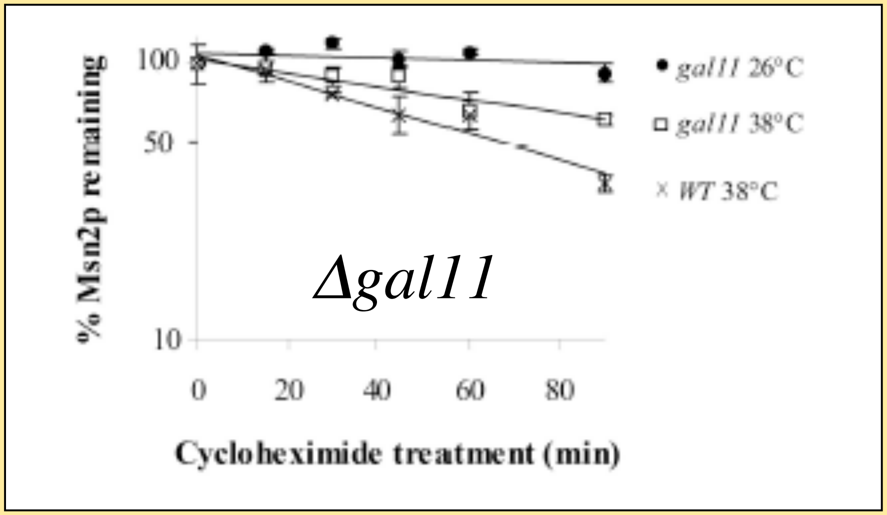


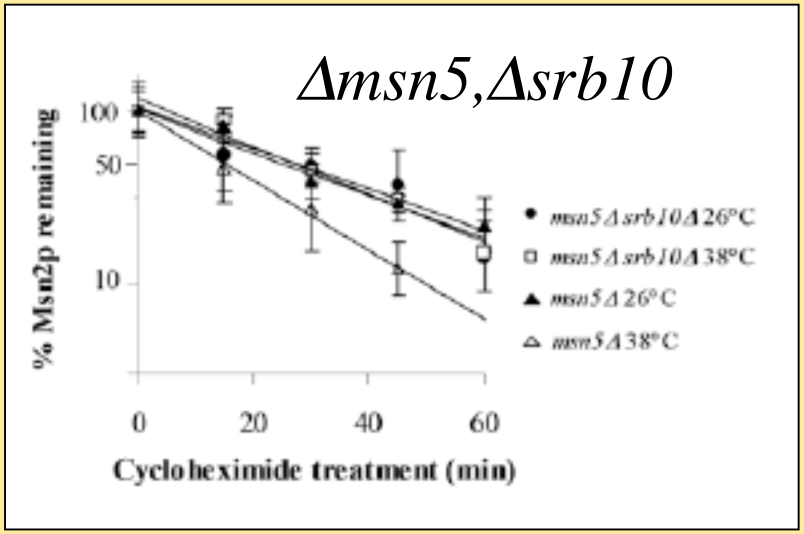


Degradation of Msn2 occurs upon activation in the nucleus and depends upon Srb10 and Gal11/med15

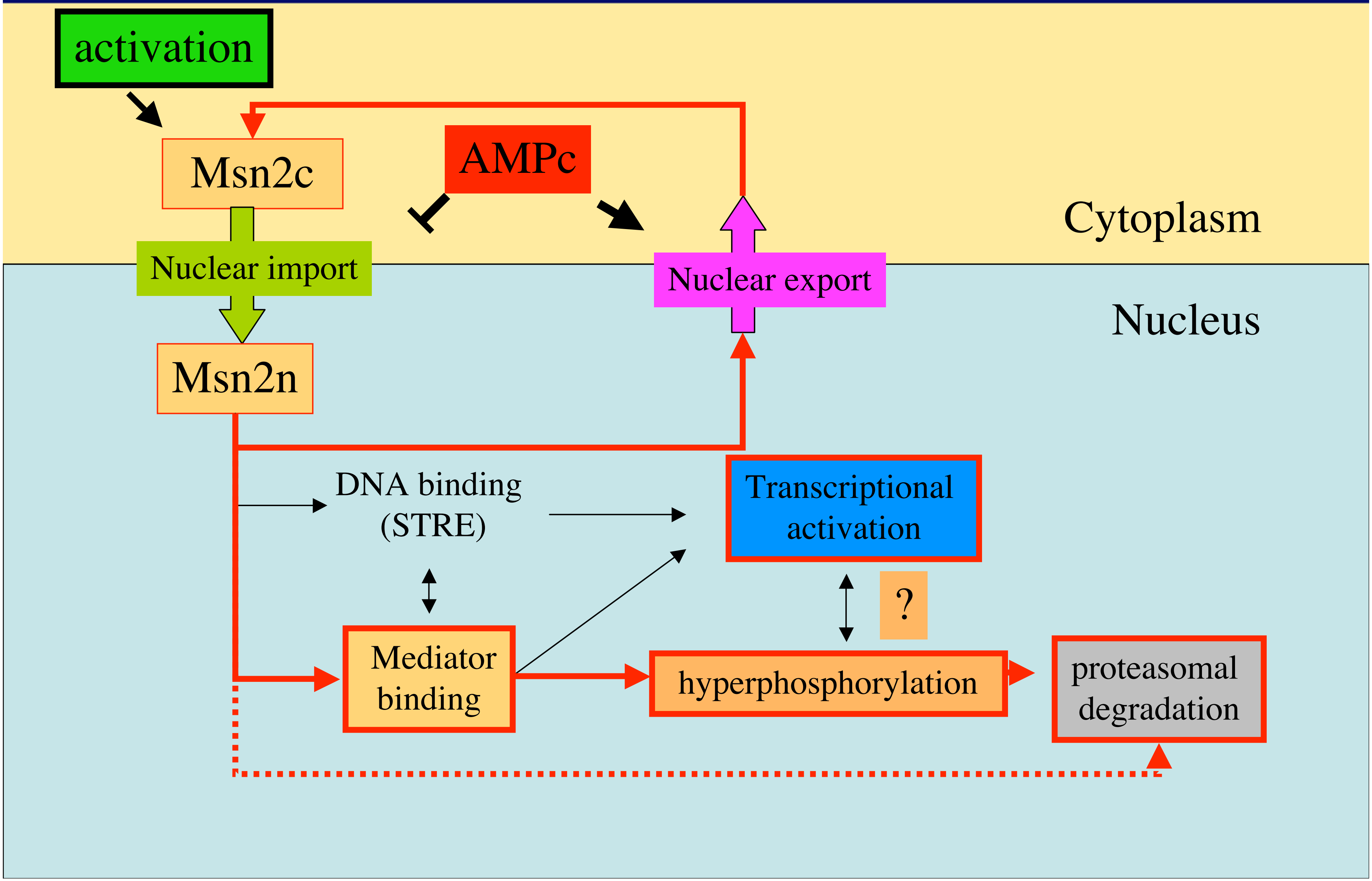
Lallet Mol Genet Genomics. 2004 Oct;272(3):353-62.



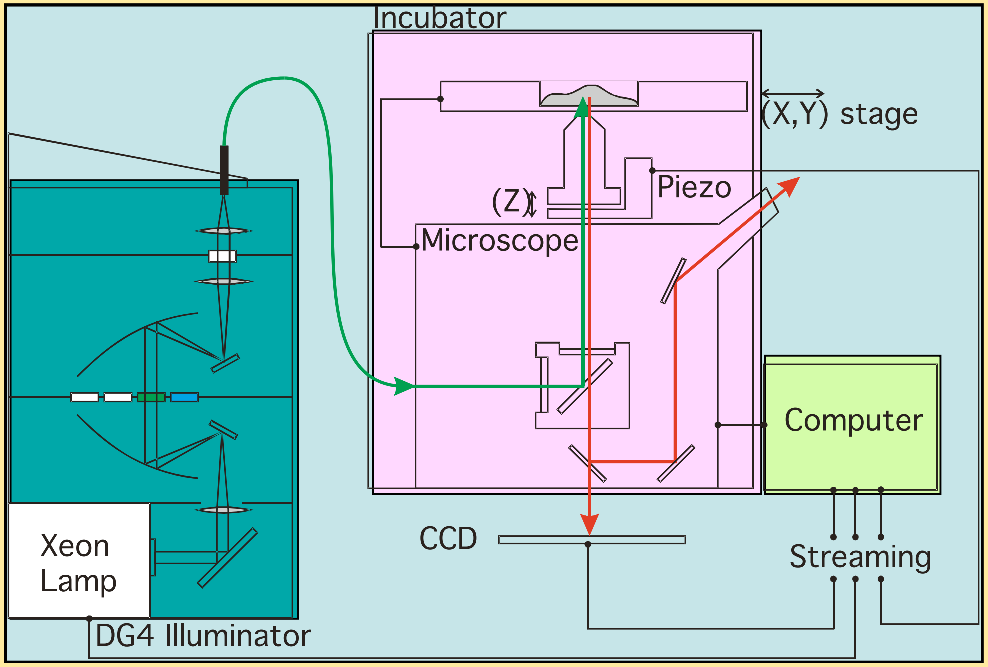


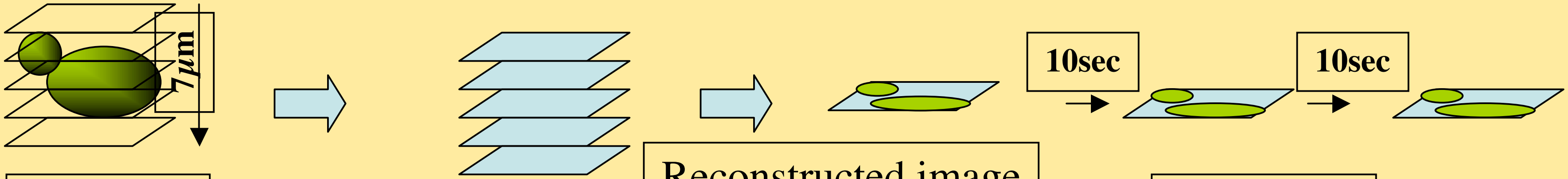


The life of Msn2



3D Ultra-fast video microscope (Jan De Mey)





21 slices
50ms each

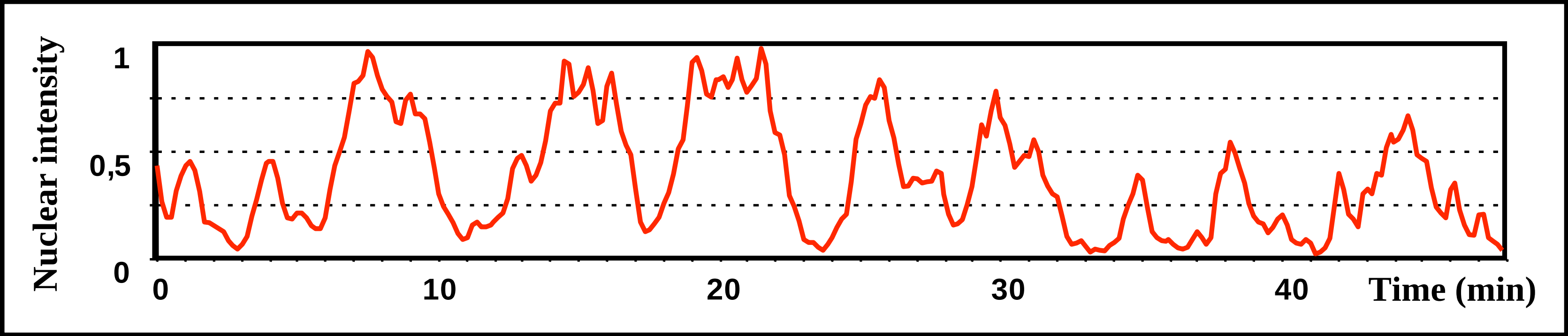
Deconvolution

Reconstructed image
Max pixel

Time lapse

Kinetics of nucleocytoplasmic localization

Msn2-GFP in WT strain
48 min



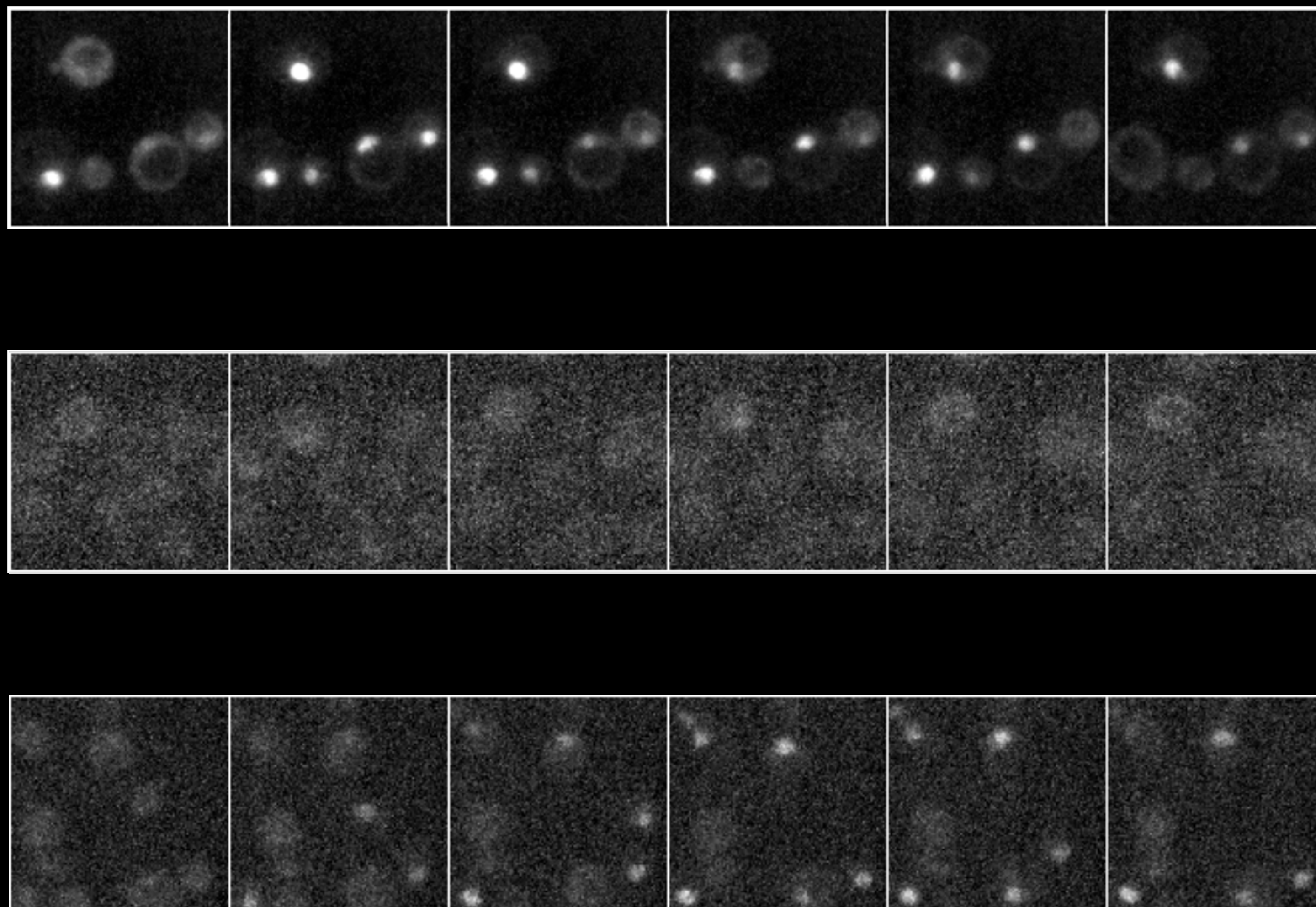
Is the light a stress for the cell ?

Time (min) 0 4 8 12 16 20

Light. every 10 sec

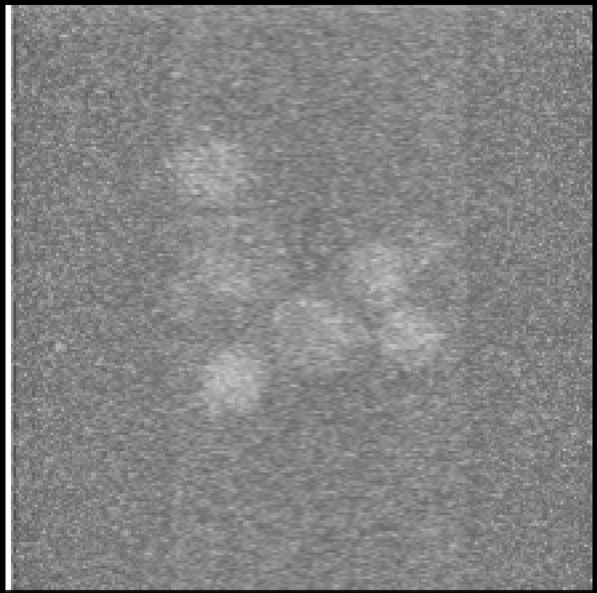
Light every 2 min
4 time lower

Light every 2 min
4 time lower
+ NaCl 0.5M

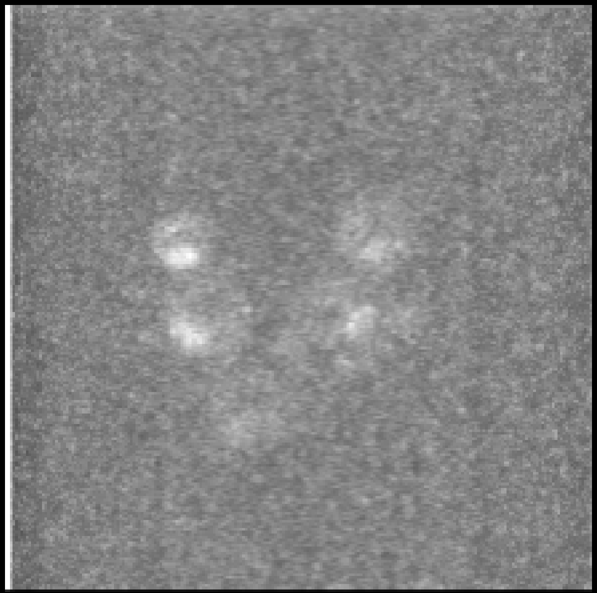


Is Protein synthesis required ?

Periodic nucleocytoplasmic shuttling of transcriptional activators generally involves feedback loop with delay produced by transcription-translation of an inhibitory product

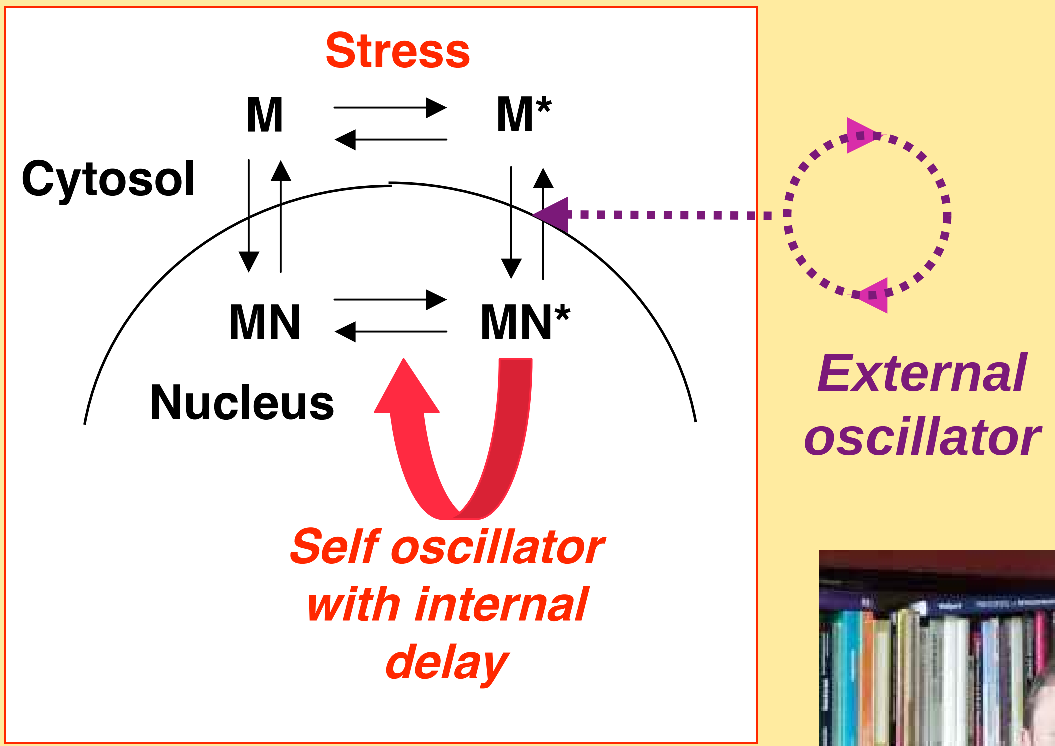


Control

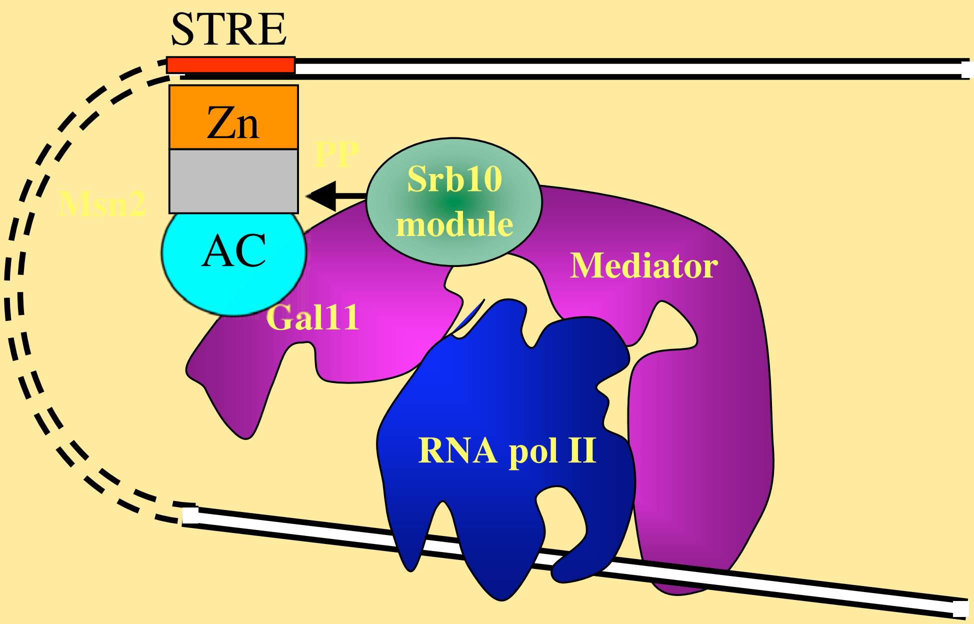


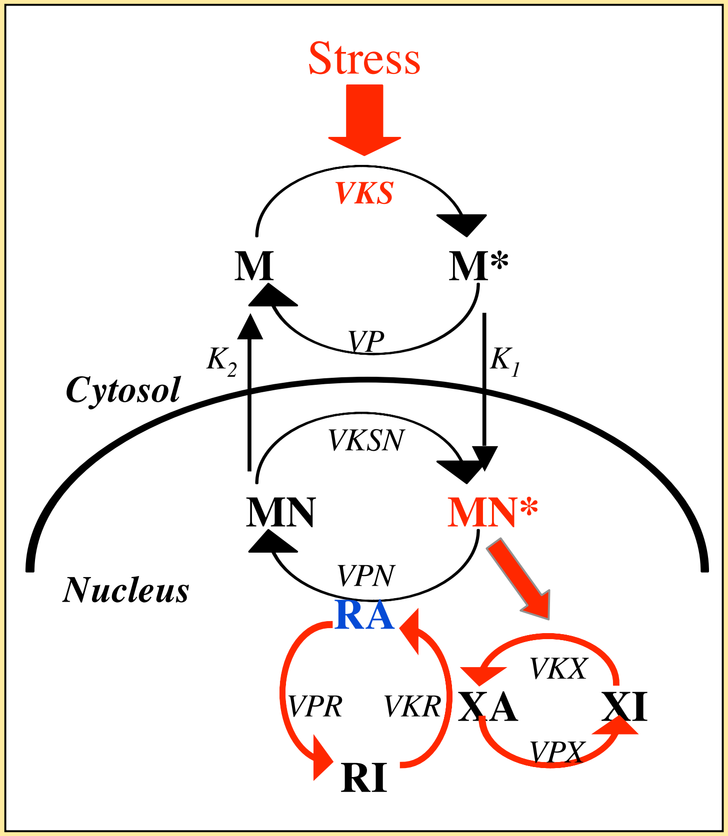
Cycloheximide
400μg/ml

How to generate an oscillatory behavior ?



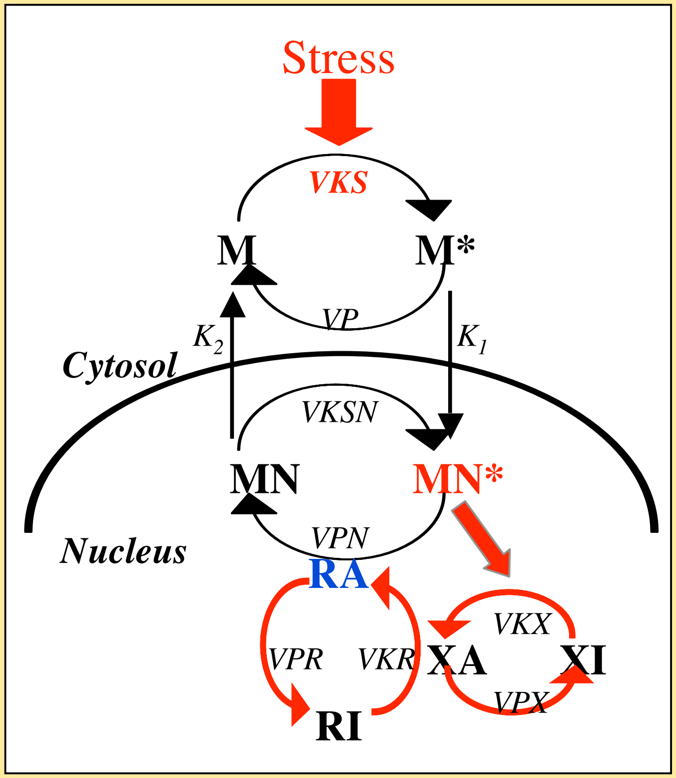
Some phosphorylation-dephosphorylation process at the transcriptionnal preinitiation complex

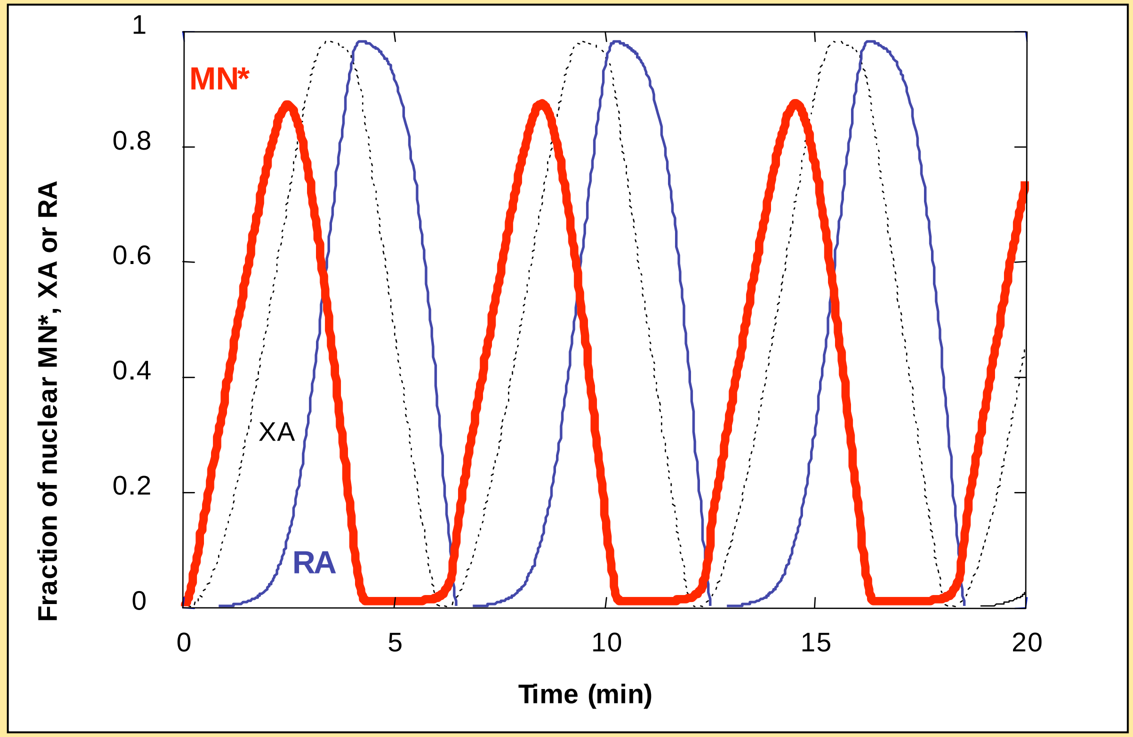




Jacquet M, Renault G, Lallet S, De Mey J, Goldbeter A.
J Cell Biol. 2003 161(3):497-505.

Simulation





$$\frac{dM}{dt}=-V_1+V_2+k_2.MN$$

$$\frac{dM^*}{dt}=V_1-V_2-k_1.M^*$$

$$\frac{dMN^*}{dt}=k_1.M^*+V_3-V_4$$

$$\frac{dMN}{dt}=V_4-V_3-k_2.MN$$

$$\frac{dXA}{dt}=V_5-V_6$$

$$\frac{dRA}{dt}=V_7-V_8$$

$$V_1=V_{KS}\left(\frac{M}{K_1+M}\right)$$

$$V_2=V_P\left(\frac{M^*}{K_2+M^*}\right)$$

$$V_3=V_{KSN}\left(\frac{MN}{K_3+MN}\right)$$

$$V_4=V_{PN}\left(\frac{RA}{K_{a1}+RA}\right)\left(\frac{MN^*}{K_4+MN^*}\right)$$

$$V_5=V_{KX}\left(\frac{MN^*}{K_{a2}+MN^*}\right)\left(\frac{1-XA}{K_5+1-XA}\right)$$

$$V_6=V_{PX}\left(\frac{XA}{K_6+XA}\right)$$

$$V_7=V_{KR}.XA\left(\frac{1-RA}{K_7+1-RA}\right)$$

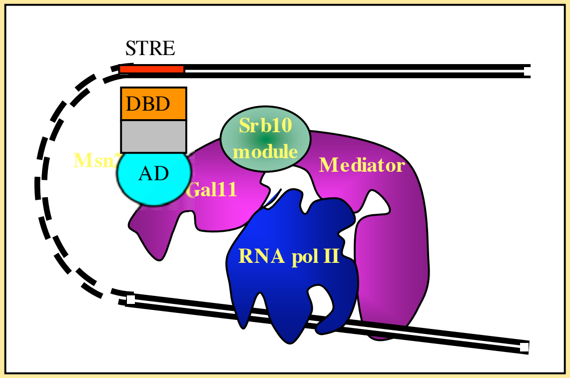
$$V_8=V_{PR}\left(\frac{RA}{K_8+RA}\right)$$

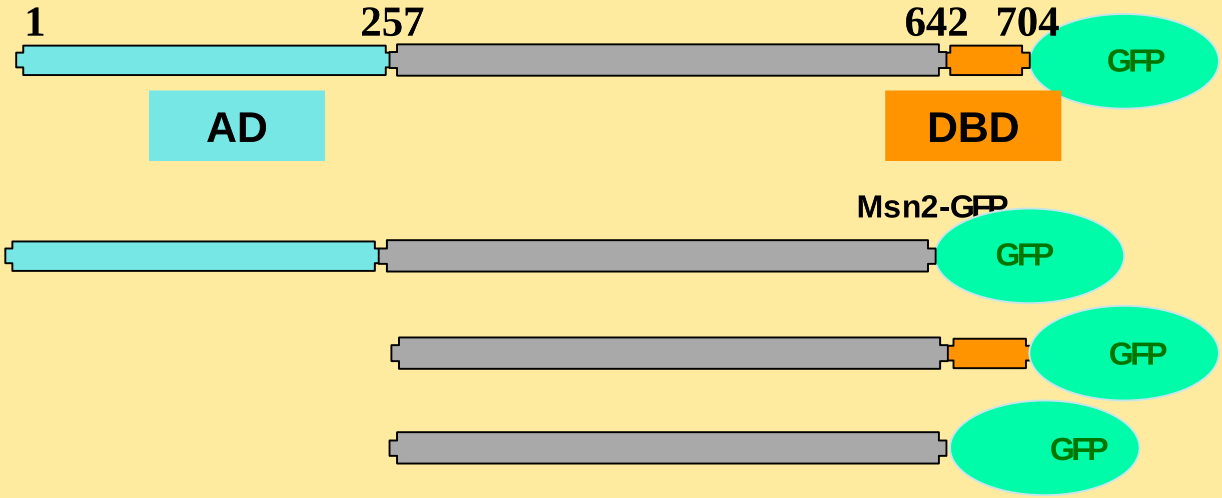
6 variables,

10 equations

Jacquet M, Renault G, Lallet S, De Mey J, Goldbeter A.
J Cell Biol. 2003 161(3):497-505.

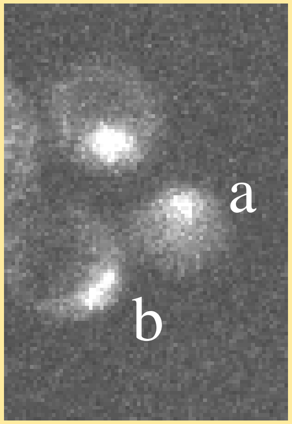
Domains of MSN2 dispensables for oscillations

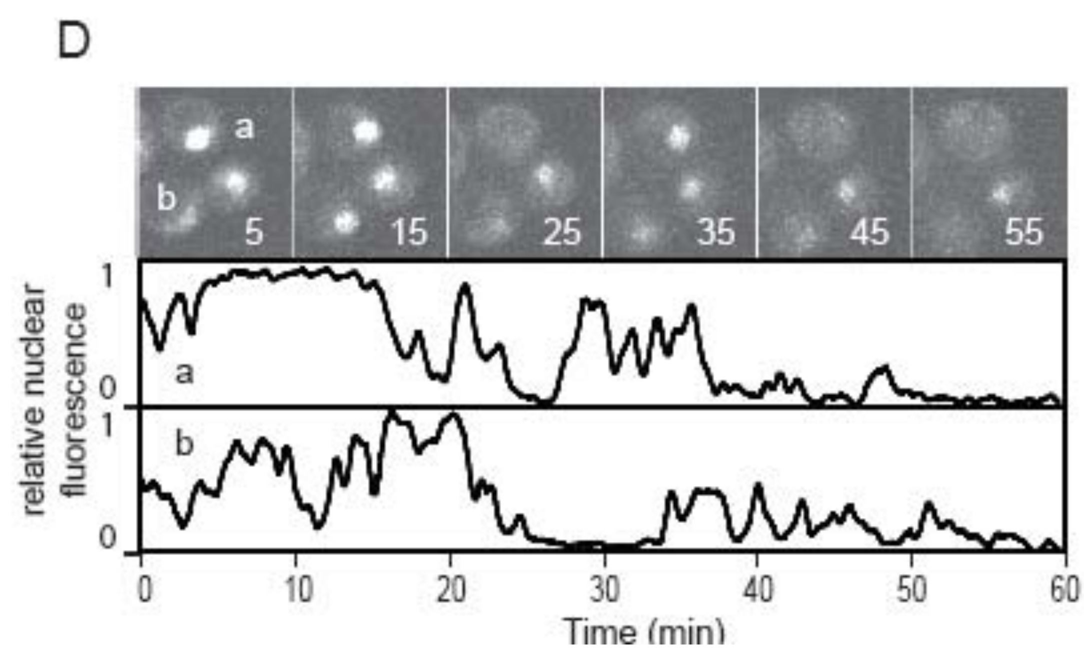




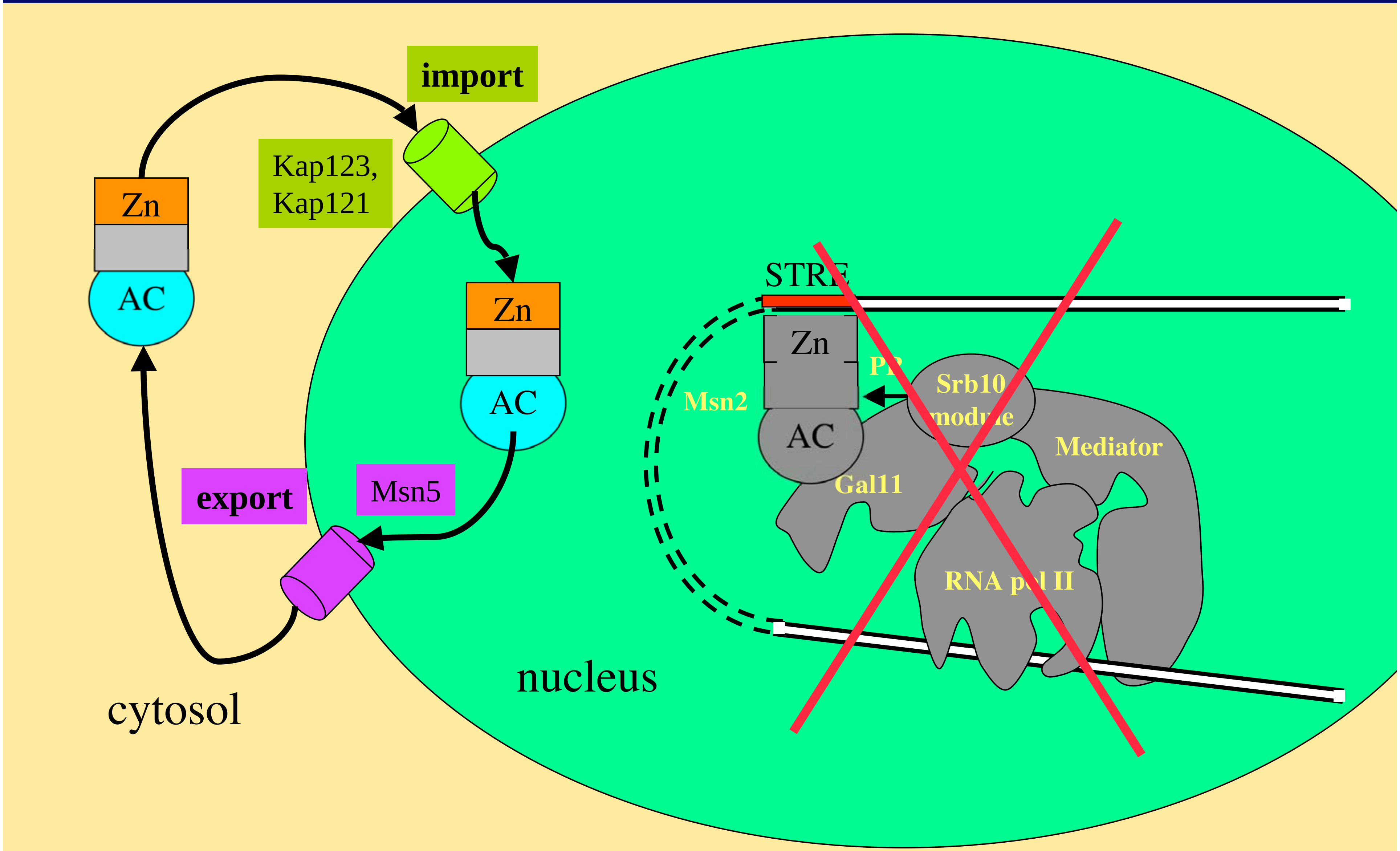
Cecilia Garmendia-Torres

ΔAD,ΔDBD 1h

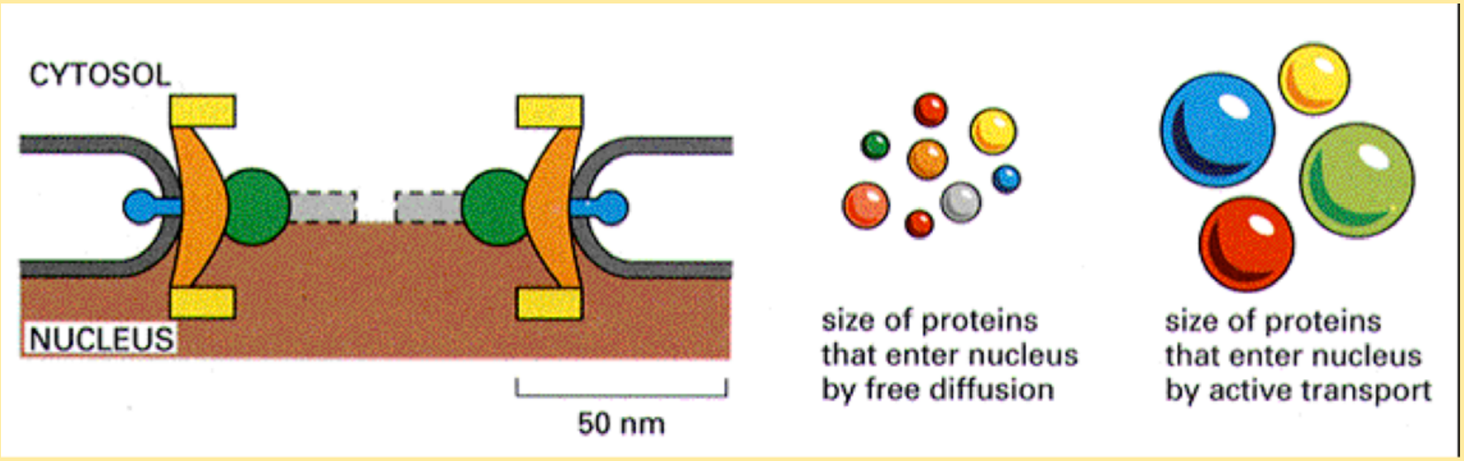


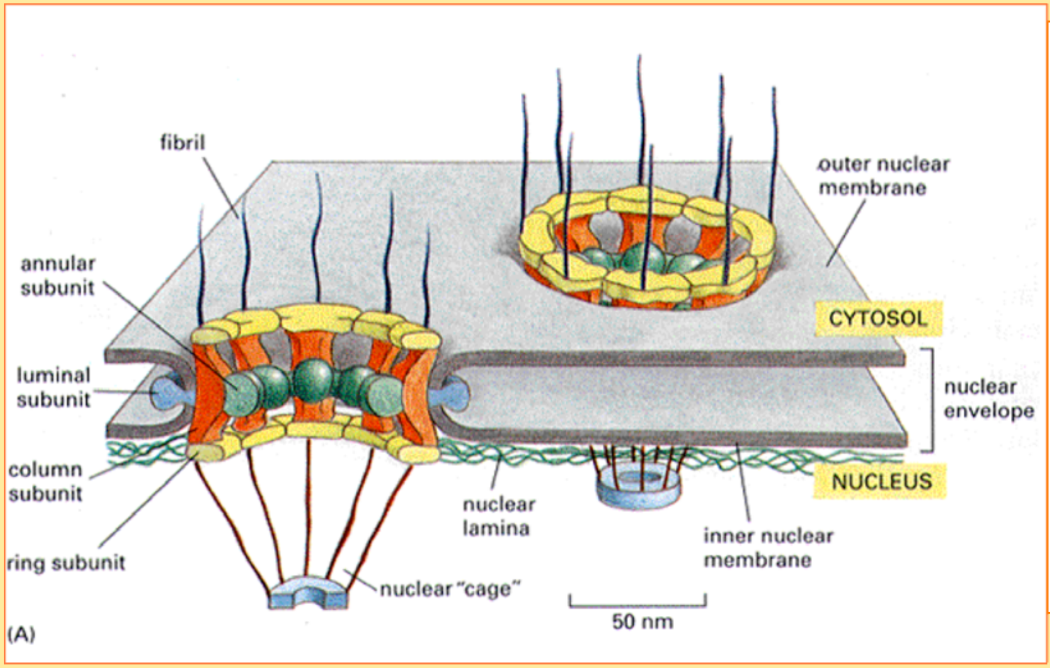


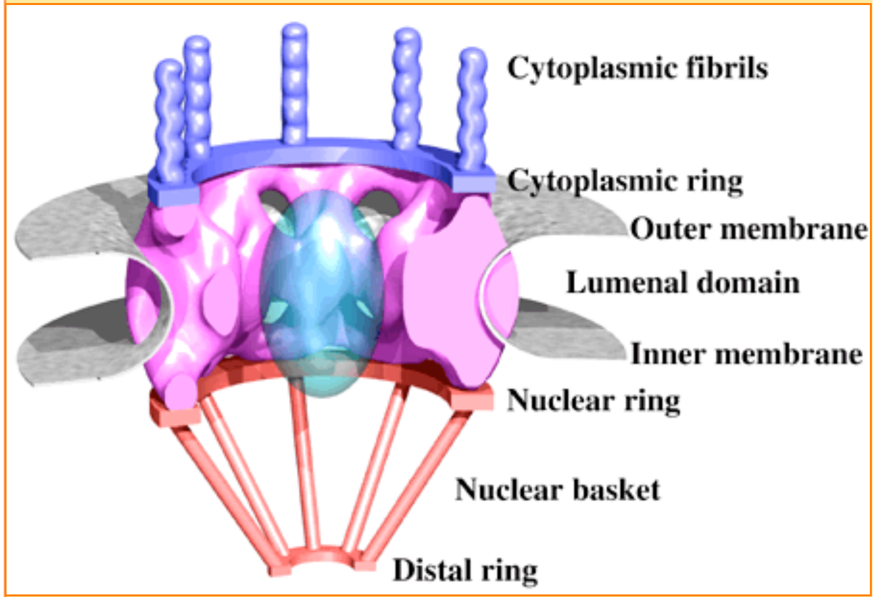
Interaction with the preinitiation complex of transcription is not essential for the oscilatory behavior



Nuclear pore

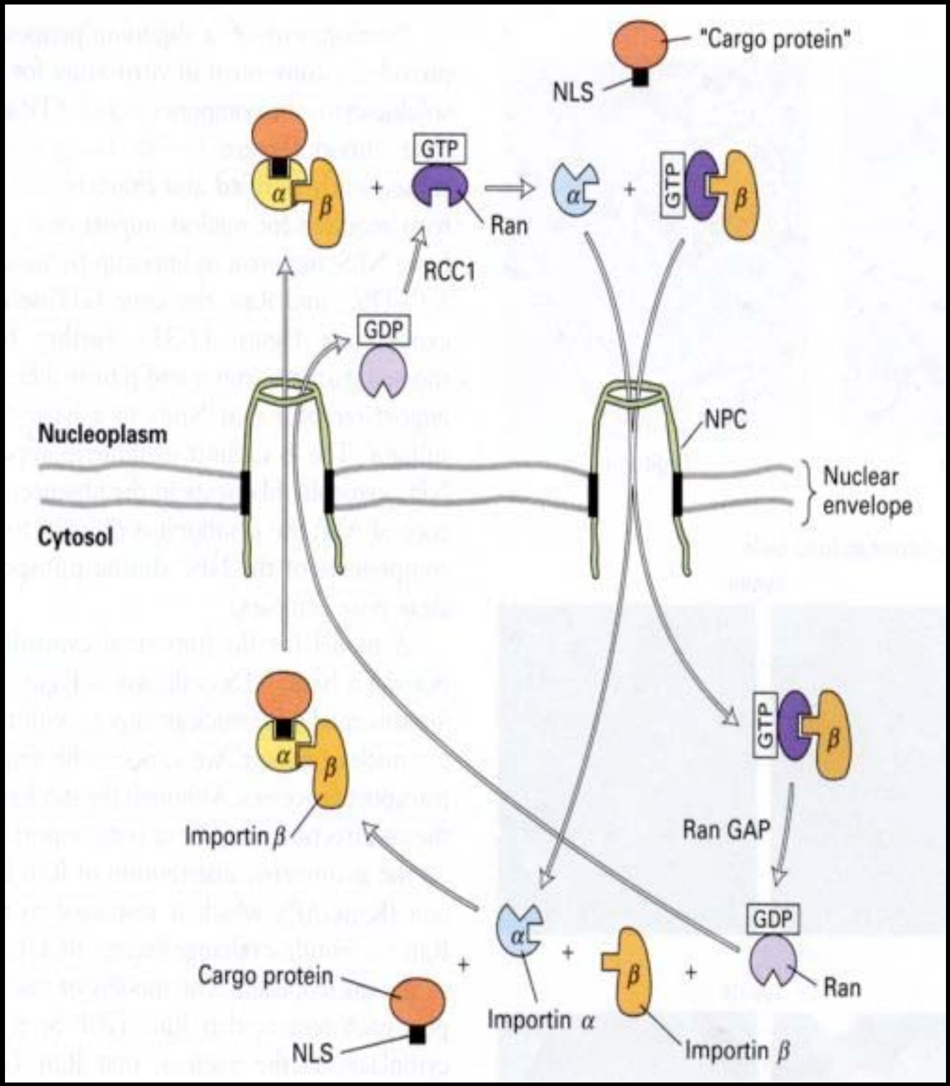






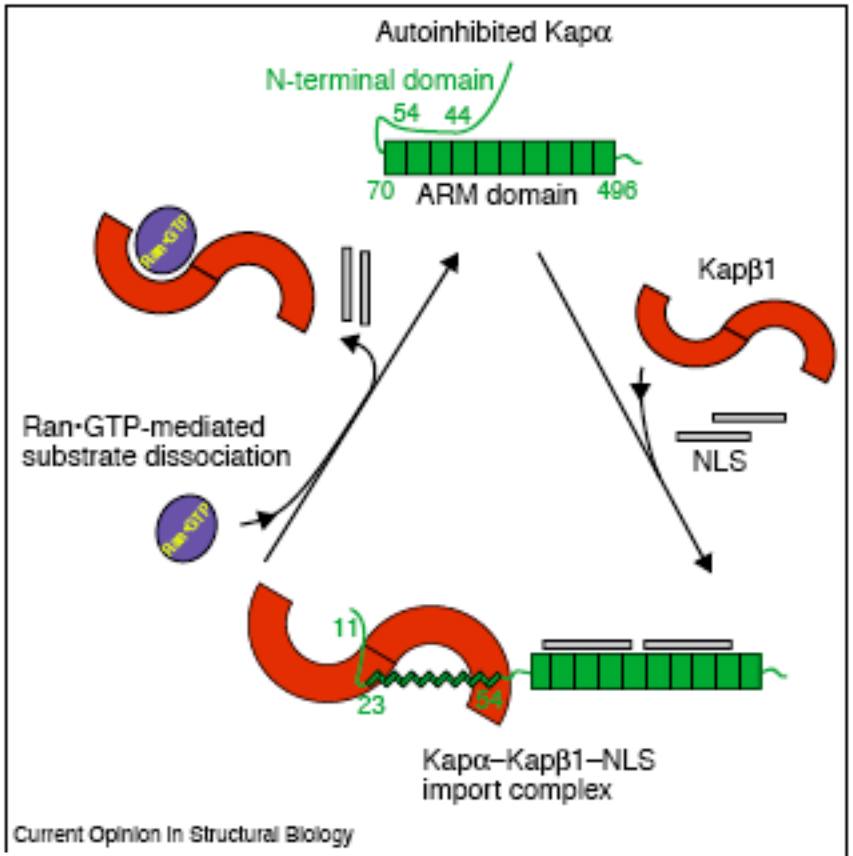
Nuclear Import Mechanisms

- Proteins to be exported into nucleus have Nuclear Localization Signals (NLS's) that enable nuclear import.
- NLS's bind to importin α subunit of an importin α-β complex.
- Transport through the NPC is mediated by interaction of degenerative sequences in the NPC proteins with the importin β subunit.
- Key to function and regulation are RAN GTP [high in nucleus by RCC1 (Ran nucleotide exchange factor)] & RAN GDP [high in cytoplasm by RAN GAP (RAN GTP activating protein)].
- The asymmetric distribution of RCC1 in the nucleus and RAN GAP in the cytoplasm drives the nuclear import process.



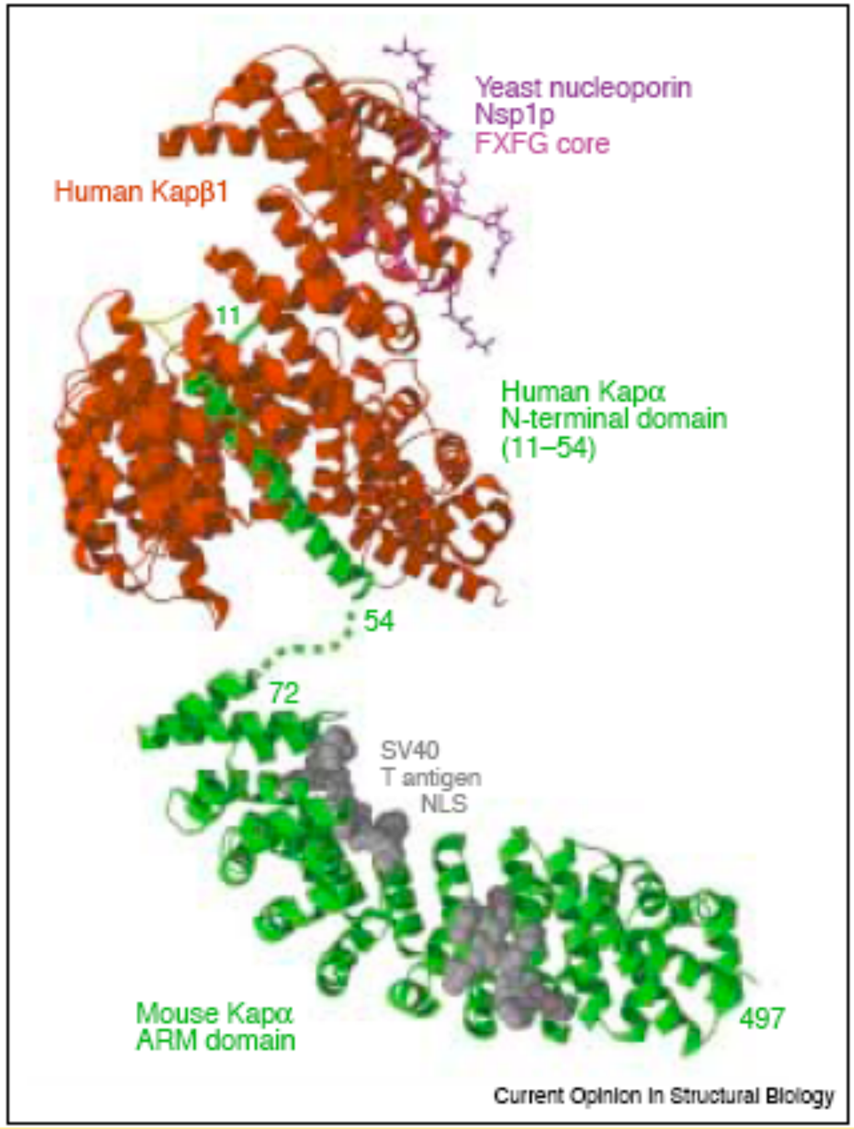
Karyopherins and nuclear import

Yuh Min Chook* and Günter Blobel†



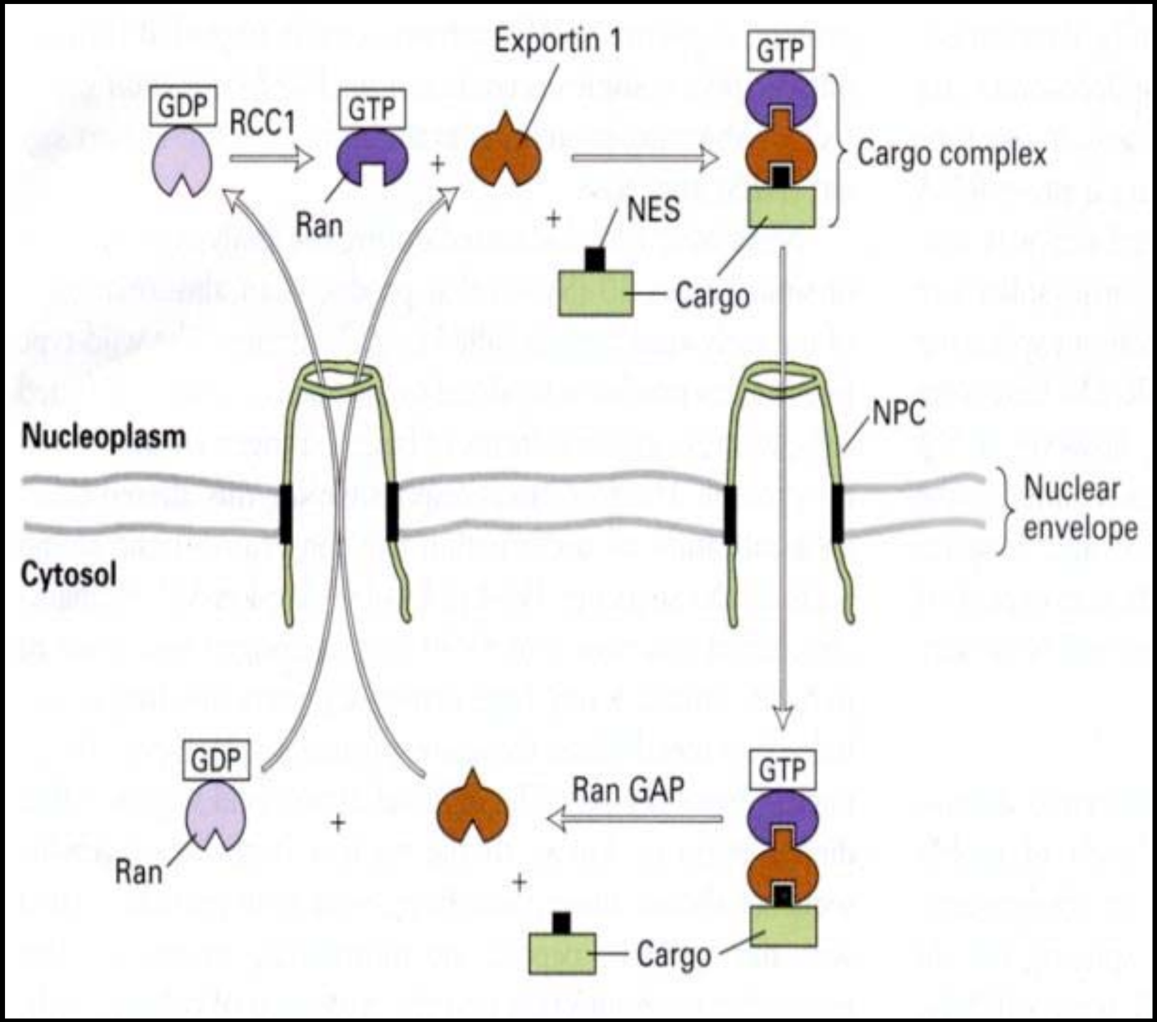
Current Opinion in Structural Biology 2001, 11:703–715

0959-440X/01/$ – see front matter

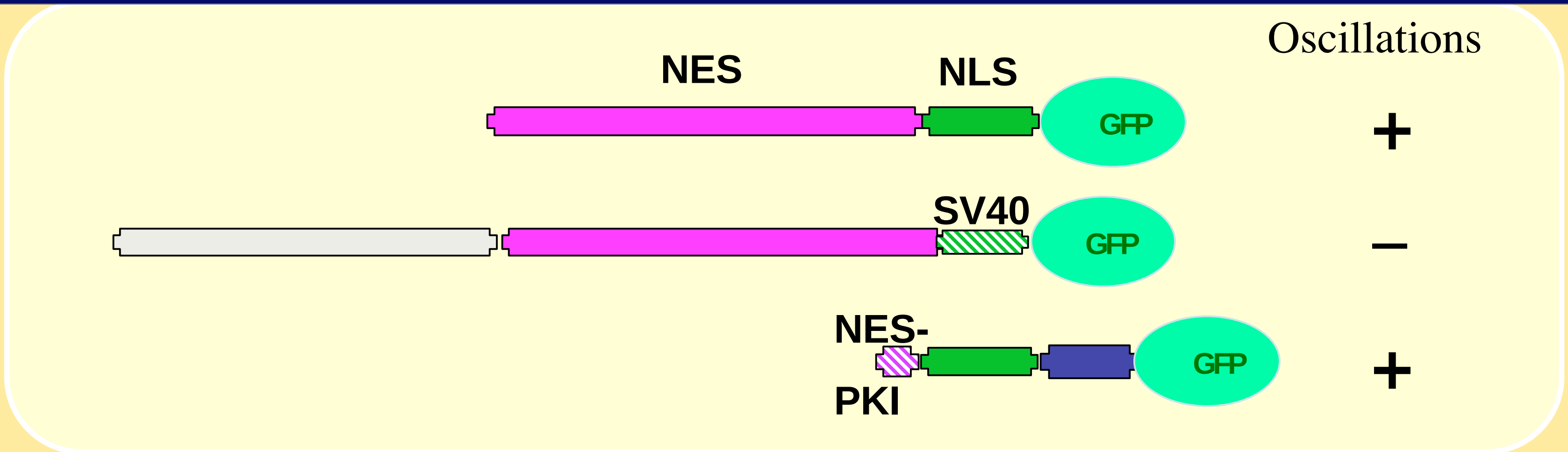


NUCLEAR EXPORT

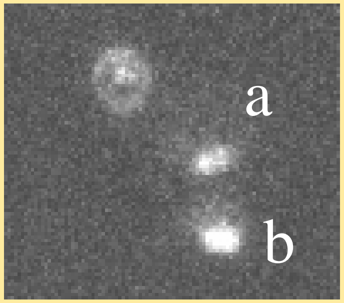
- The exporting proteins have special sequences called Nuclear Export Signals (NES's) that mediate export through binding to a class of proteins that function in export called *exportins.*
- Exportins are typically monomeric and function in a reverse manner to importin under the control of RAN.
- Thus the cargo complex requires RAN-GTP which is found only in the nucleus.
- Disassociation of the 'cargo" from the exportin requires RAN-GDP which occurs only in the cytoplasm.



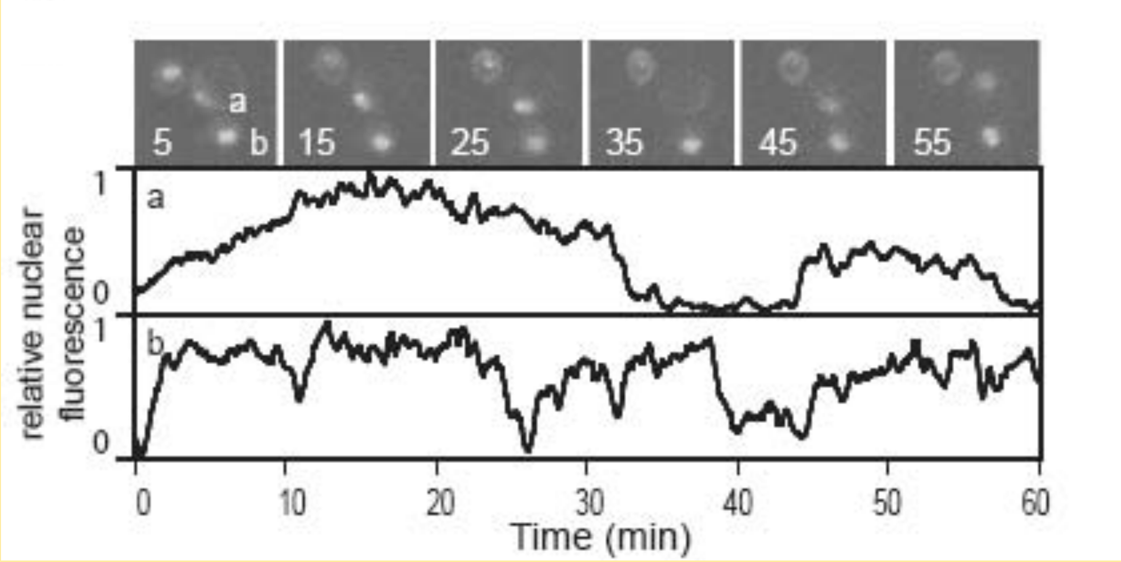
Swapping NLS and NES



NLS-Msn2, NES-PKI

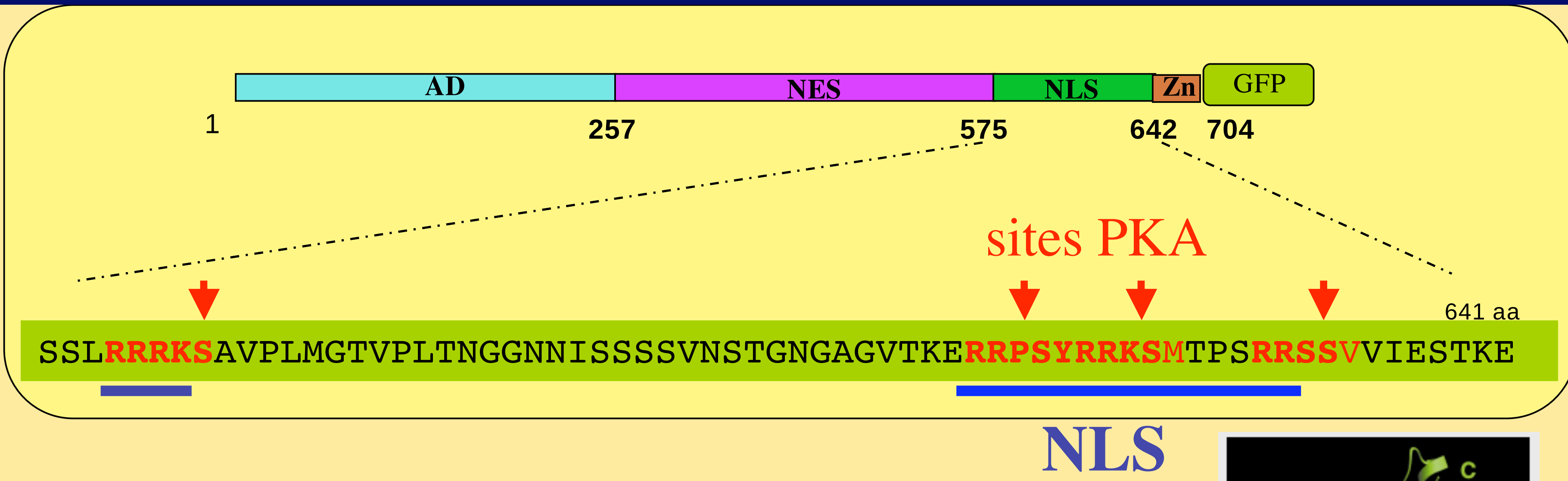


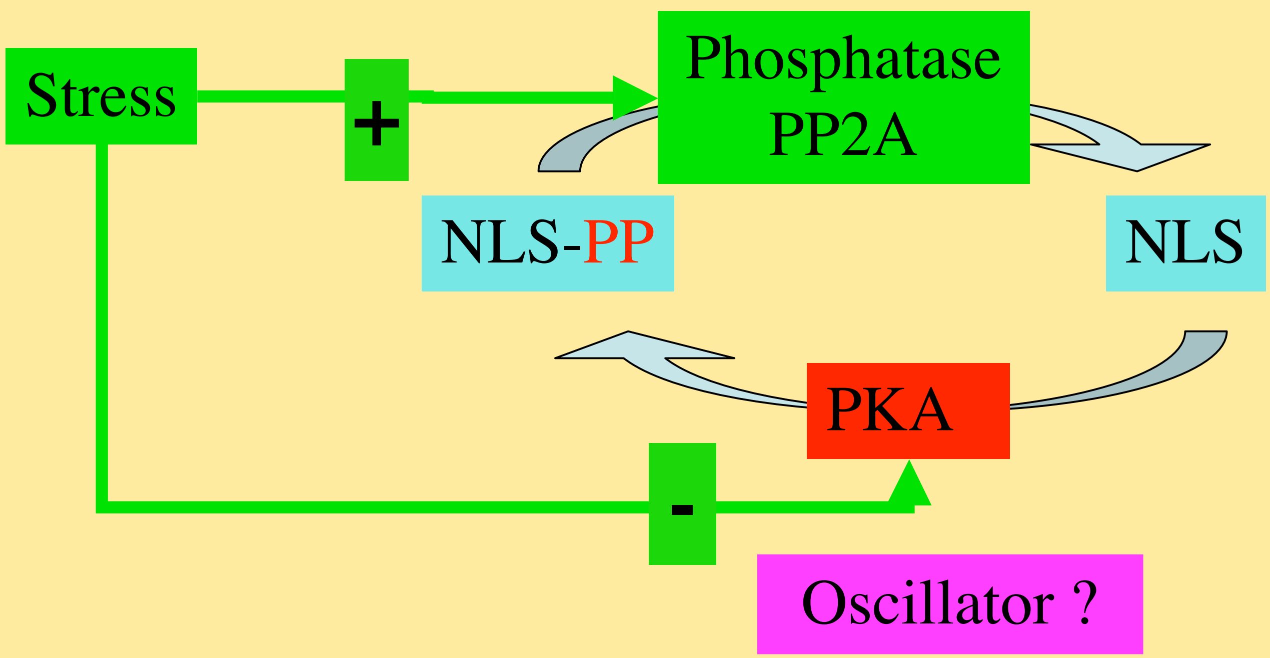
1h



The NLS region is sufficient for the oscillatory behavior

The NLS domain of Msn2





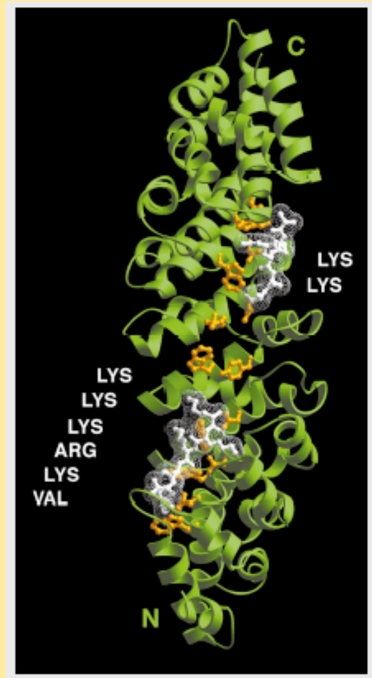
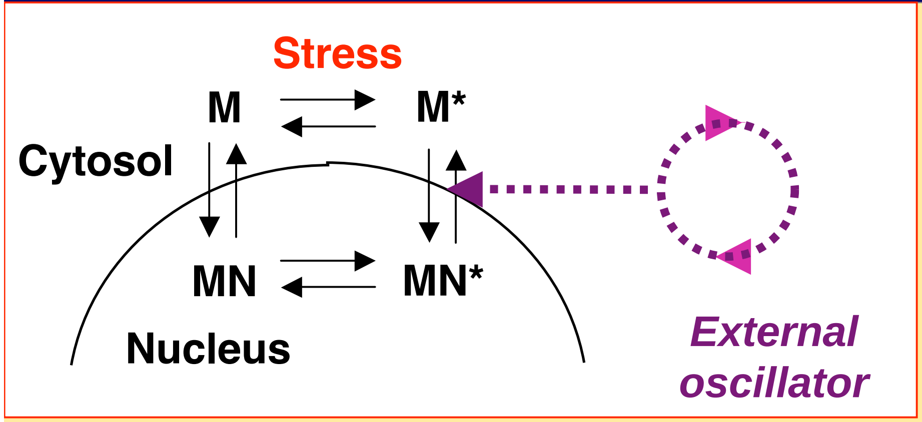


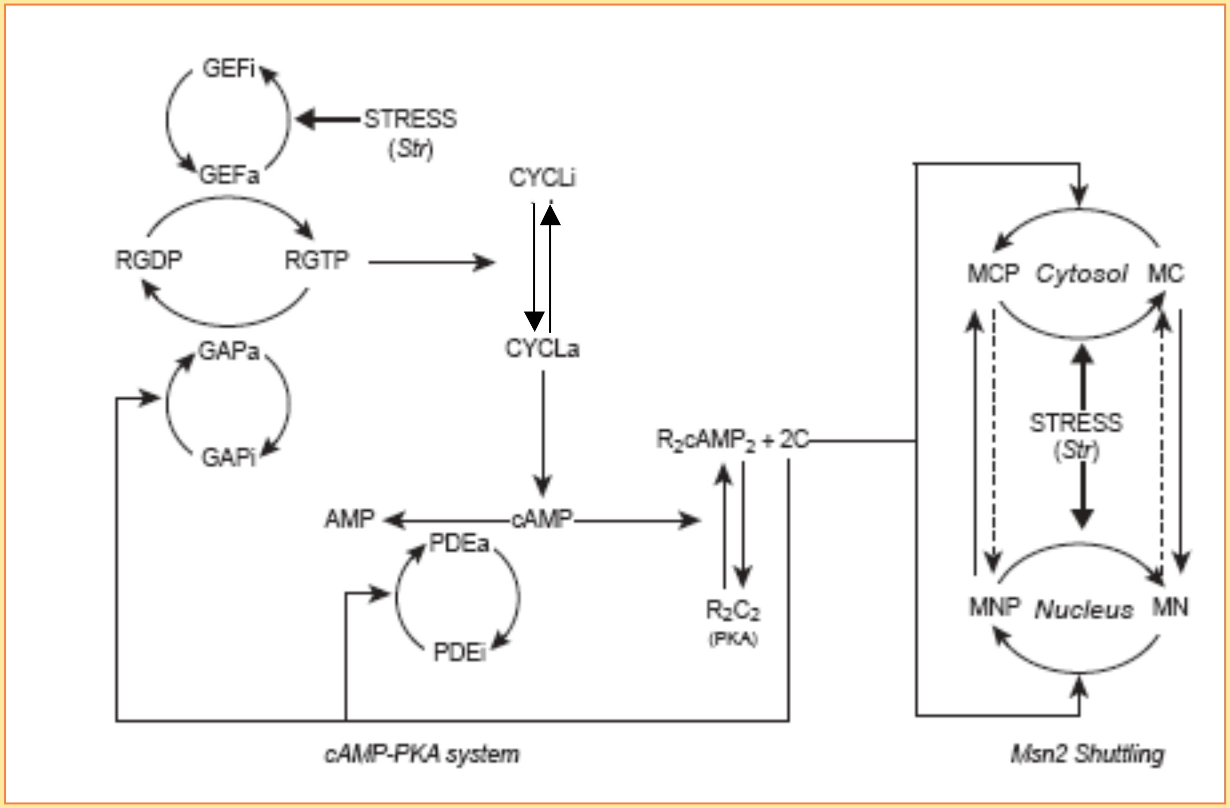
FIGURE 5

Yeast (*Saccharomyces cerevisiae*) karyopherin-α. Image kindly provided by Elena Conti, Heidelberg, Germany.

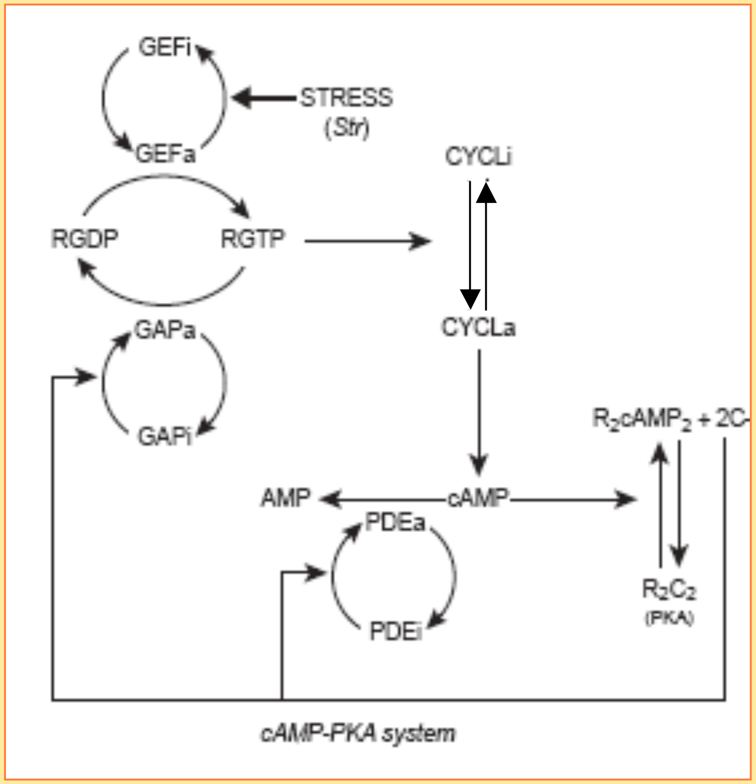
The new model



Curr Biol. 2007 Jun 19;17(12):1044-9.
Nucleocytoplasmic Oscillations of the Yeast Transcription Factor Msn2: Evidence for Periodic PKA Activation.
Garmendia-Torres C, Goldbeter A, Jacquet M.



Negative feedback upon cAMP accumulation

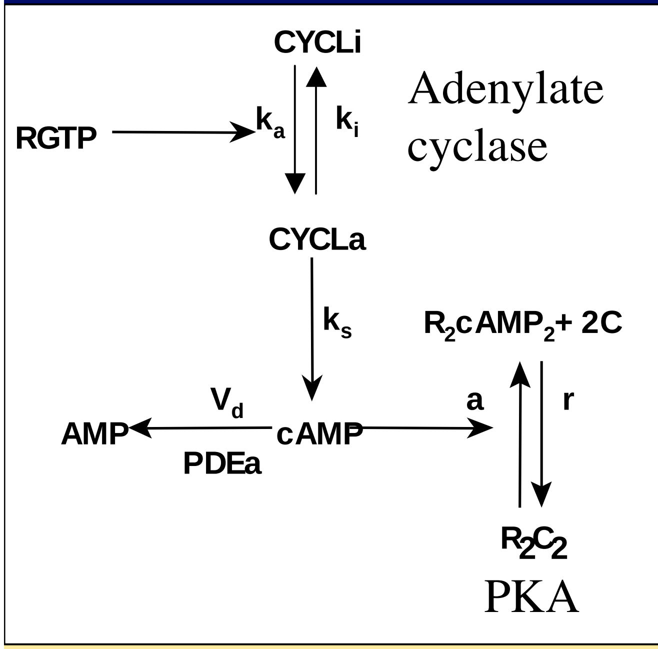


Strain	TPK1	TPK2	TPK3	BCY1	cAMP level (pmole/mg prot
S7-1A	+	-	-	+	2,5
S13-58A	+	-	-	-	0,5
S18-3B	W1	-	-	+	1500
RS13-58A1	W1	-	-	-	570
S7-1B	-	+	-	+	3,7
S13-7C	-	+	-	-	0,3
S13-7C	-	W1	-	+	3400
S15-5BRS13-7C-1	-	W1	-	-	3400

	Genotype				cAMP level (pmole/mg prot)		
Strain	RAS1	RAS2	PDE1	PDE2	Exp1	Exp2	Exp3
SP1	+	G	+	+	1,7	1,6	1,8
TK161-R2V	+	V	+	+	2,7	ND	3,5
DJ23-3C	+	G	-	-	3,6	ND	ND
DJ31-4D	+	V	-	-	2300	450	3000
DJ31-6A	+	V	-	-	ND	3500	ND

Rigorous feedback control of cAMP levels in Saccharomyces cerevisiae.
Nikawa J, Cameron S, Toda T, Ferguson KM, Wigler M.
Genes Dev. 1987 Nov;1(9):931-7

cAMP variations and PKA activation



Equations

$$\frac{dCYCLa}{dt} = k_a(RGTP)(RASt)(1-CYCLa) - k_i CYCLa$$

$$\frac{dcAMP}{dt} = k_s(CYCLa)(CYCLt) - k_d(PDEt)\frac{cAMP}{K_{md} + cAMP} - 2V_{PKAt}PKAt$$

$$\frac{dR_2C_2}{dt} = -a(R_2C_2)(cAMP)^2 + rC^2(R_2cAMP_2)(PKAt)^2$$

$R_2cAMP_2 = 1-R_2C_2$, $C=2(1-R_2C_2)$

Parameters

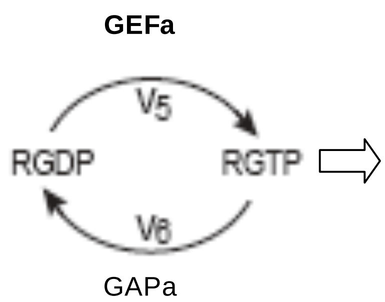
Kinetic steps	Parameters	Values
Activation of adenylate cyclase	CYCLt (Cyr1)	0.7 μM
	k_a	0.01$\mu M^{-1} min^{-1}$
	k_i	1 min^{-1}
Production of cAMP	k_s	4 min^{-1}
Activation of phosphodiesterase	PDEt	0.5 μM
	k_{c7}	3.333 min^{-1}
	V_{Max8}	1.5 μM min^{-1}
	K_7	0.01
	K_8	0.01
Degradation of cAMP	k_d	100 min^{-1}
	K_{md}	20 μM
Activation of PKA	PKAt (Bcy1,Tpk)	0.3 μM
	a	1 μM^{-2} min^{-1}
	r	1 min^{-1}

Simplifications

Only one type Ras protein

Only one type Phosphodiesterase protein

The ras module



Equations

$$\frac{dRGTP}{dt} = V_{M5}\, GEFa \frac{(1 - RGTP)}{K_5 + (1 - RGTP)} - V_{M6}\, GAPa \frac{RGTP}{K_6 + RGTP}$$

$V_5 = k_{gef}$ GEFt/RASt, $V_6 = k_{gap}$ GAPt/RASt

Parameters

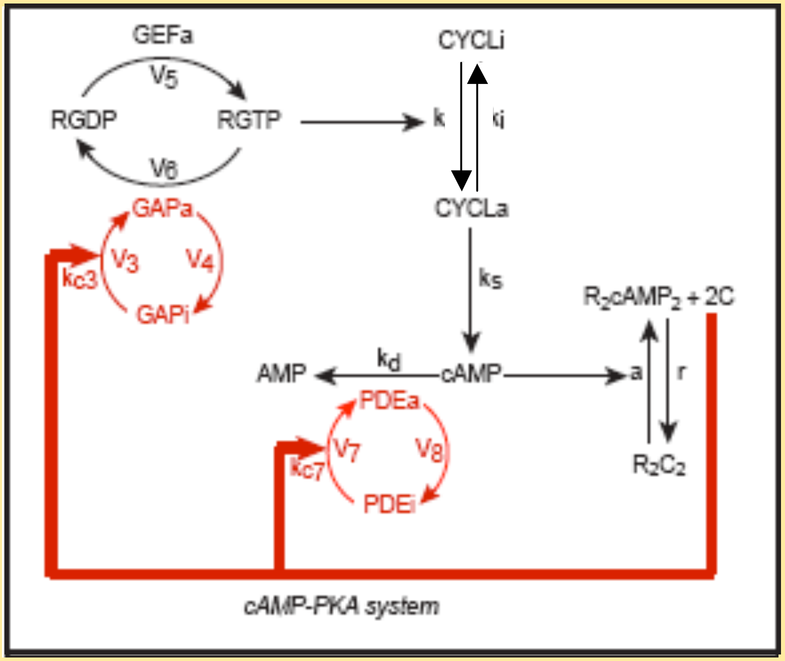
Ghaemmaghami, *et al.*, *Nature* **425**, 737-741 (2003)	N per cell	Cellular concentration (30fl)	Cortical concentration (X 250)
GEF = Cdc25	320	16 nM	4μM
GAP = Ira1 + Ira2	nd		*1.5 μM*
RAS = Ras1 + Ras2	2000 + 20000	1.1μM	250μM

$Kd(R_{GDP})$= 0.16μM; $Kcat_{CDC25}$= 240 min^{-1}
$Kd(R_{GTP})$= 0,25μM; $Kcat_{GAP}$ = 600 min^{-1}

Ultrasensitivity:
[RAS] >[GEF] et [GAP] 250 > 4 ; 1.5
[RAS]>>$Kd(R_{GDP})$ et Kd (R_{GTP}) 250 >> 0.16; 0.25

Goldbeter et Koshland PNAS 78 1981 6840

Modeling the negative feedback loop



$$\frac{dGAPa}{dt} = V_{M3}C\frac{(1-GAPa)}{K_3+(1-GAPa)} - V_{M4}\frac{GAPa}{K_4+GAPa}$$

$$\frac{dPDEa}{dt} = V_{M7}C\frac{(1-PDEa)}{K_7+(1-PDEa)} - V_{M8}\frac{PDEa}{K_8+PDEa}$$

$V_3 = k_{c3}$ PKAt/GAPt, $V_4 = V_{max4}$/GAPt

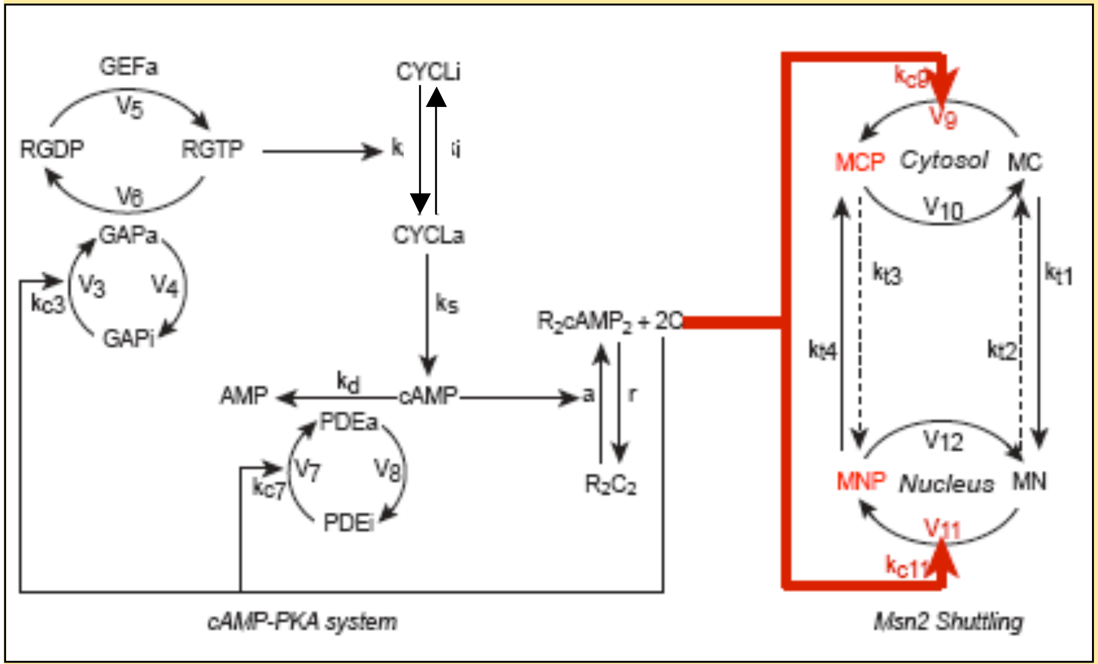
$V_7 = k_{c7}$ PKAt /PDEt, $V_8 = V_{max8}$/PDEt

Assumption

Two different targets PDE and GAP

Similar effect of PKA on each

Coupling Msn2 to the cAMP-PKA pathway



$$\frac{dMC}{dt} = -k_{t1}MC + k_{t2}MN - V_{11}C\frac{MC}{K_{11}+MC} + V_{12}\frac{MCP}{K_{12}+MCP}$$

$$\frac{dMN}{dt} = k_{t1}MC - k_{t2}MN - V_{9}C\frac{MN}{K_{9}+MN} + V_{10}\frac{MNP}{K_{10}+MNP}$$

$$\frac{dMNP}{dt} = V_{9}C\frac{MN}{K_{9}+MN} - V_{10}\frac{MNP}{K_{10}+MNP} + k_{t3}MCP - k_{t4}MNP$$

$$\frac{dMCP}{dt} = -k_{t3}MCP + k_{t4}MNP + V_{11}C\frac{MC}{K_{11}+MC} - V_{12}\frac{MCP}{K_{12}+MCP}$$

$V_9=k_{c9}$PKAt/MSNt

$V_{11}=k_{c11}$PKAt/MSNt

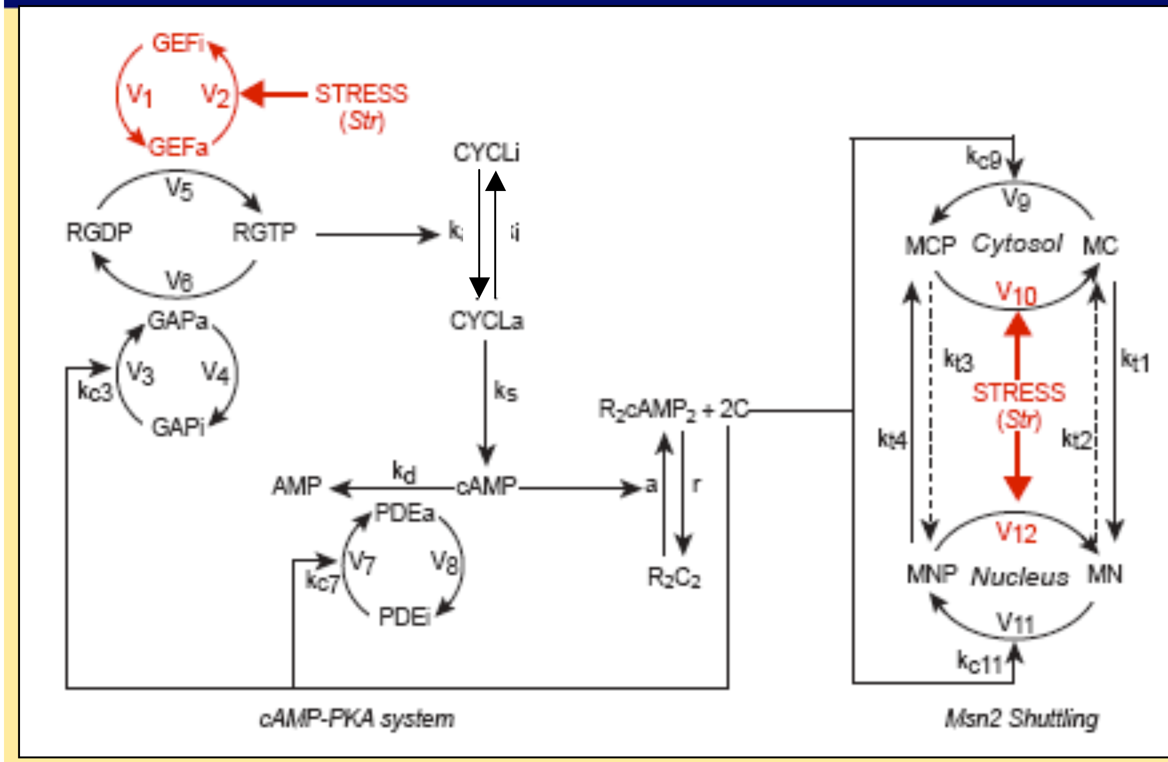
Simplified assumptions

Only one phosphorylation dephosphorylation event

Same concentration of PKA and Phosphatase in cytosol and nucleus

Direct effect of stress upon the phosphatase in and out the nucleus

Applying stress



$$\frac{dGEFa}{dt} = V_{M1}\frac{(1-GEFa)}{K_1+(1-GEFa)} - V_{M2}\frac{GEFa}{K_2+GEFa}$$

V_{M1}= **Str** V_{max1}/GEFt, V_{M2}=V_{max2}/GEFt

V_{10}=*Str* V_{max10}/MSNt

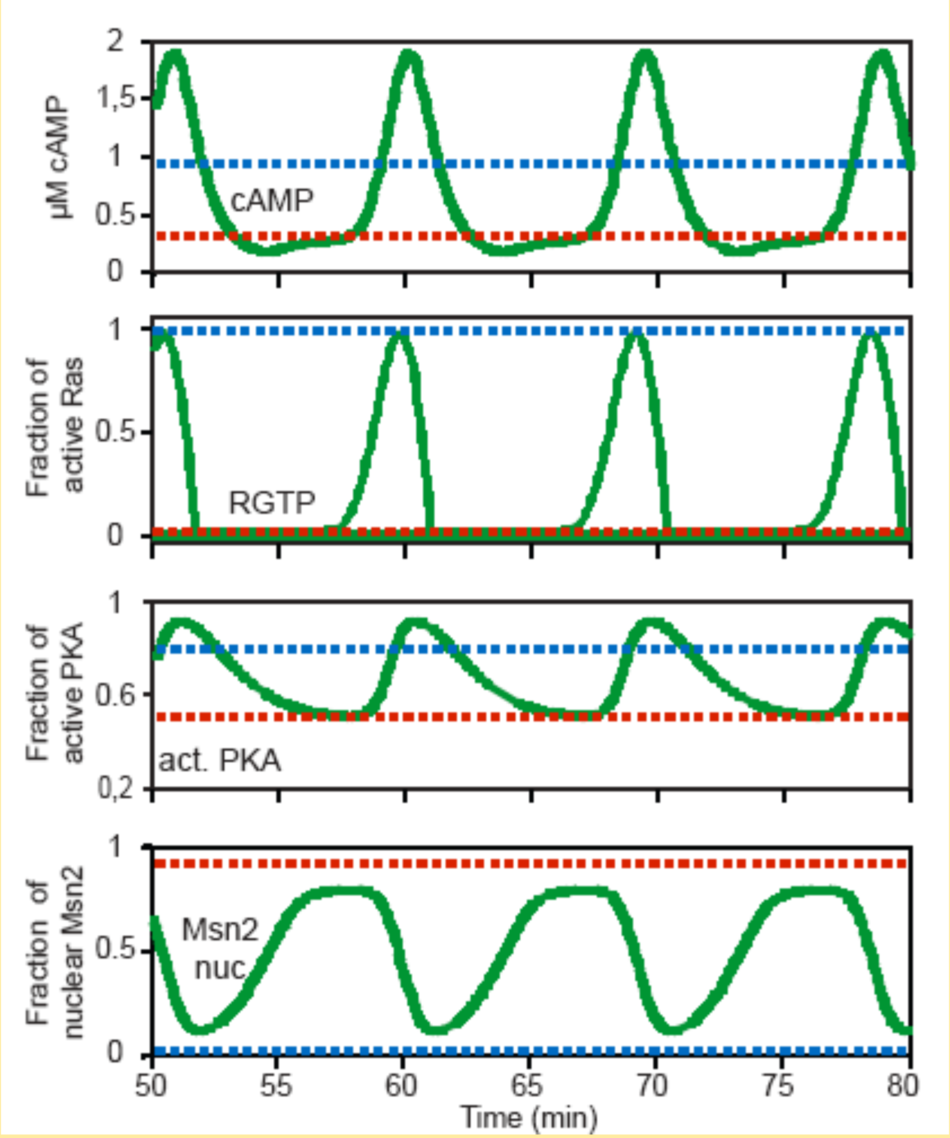
V_{12}=*Str* V_{max12}/MSNt

Simplified assumptions

Stress reduces GEF activity (Wang et al. Microbiology. 2004 150: 3383-91.)

Stress is acting on the dephosphorylation step of Msn2 (PKA independent)

Linear and oscillatory behaviors as a function of stress



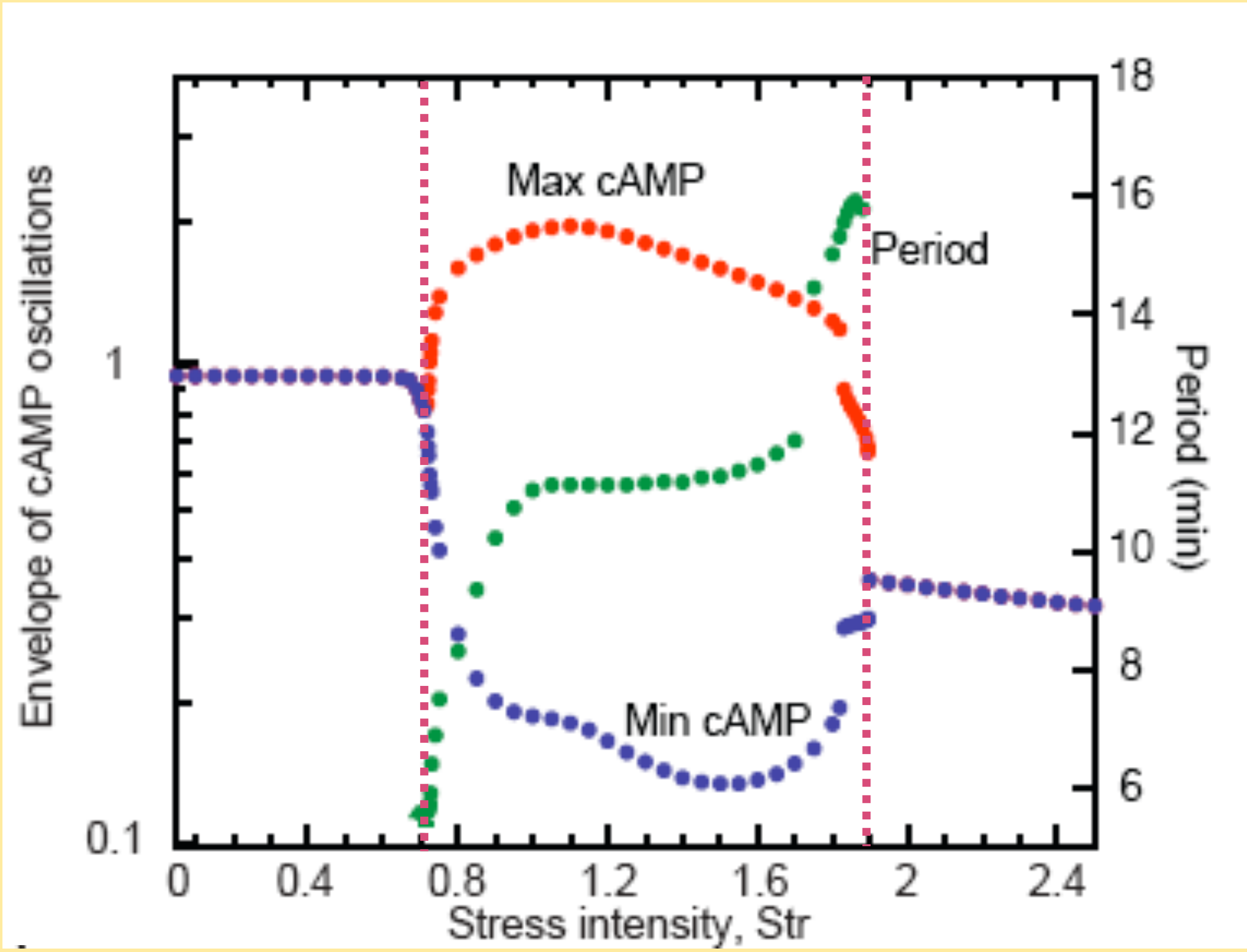
STR=0.5

STR=1

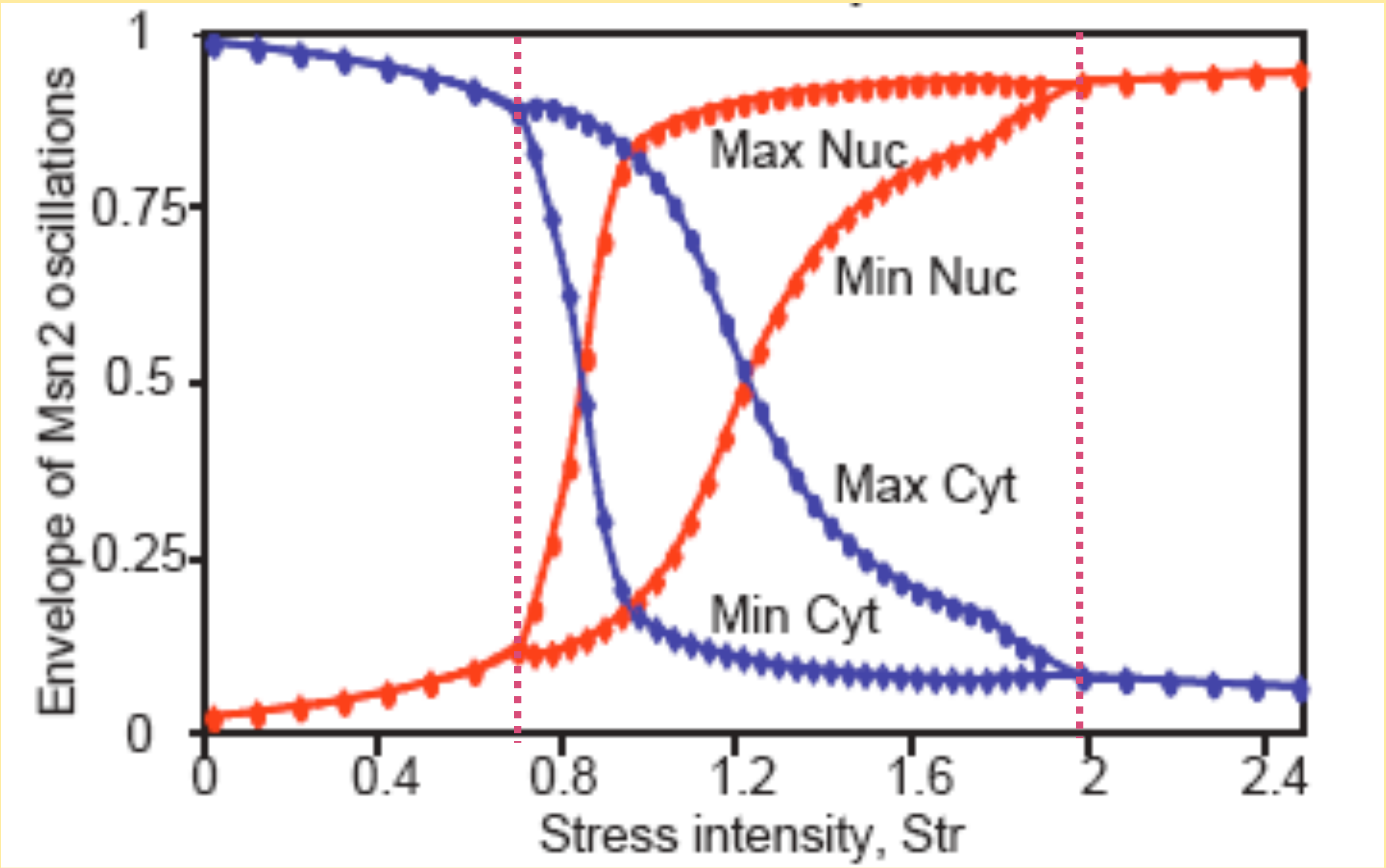
STR=2.5

Berkeley madonna

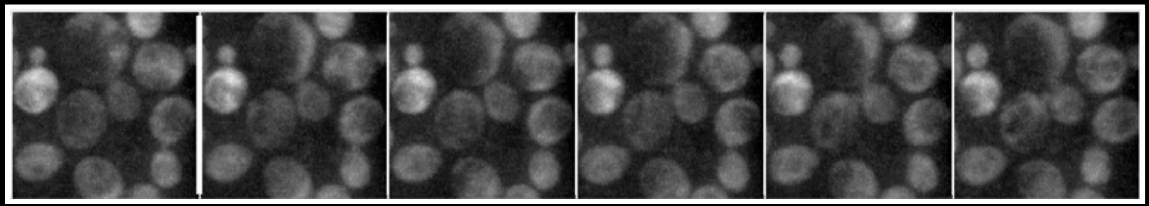
Envelope of cAMP oscillations



Envelope of nucleocytoplasmic oscillations of Msn2

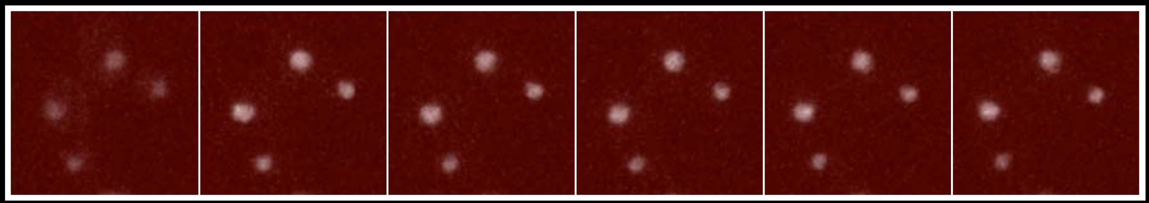


Role of the cAMP-PKA system



0 4 8 12 16 20 Min.

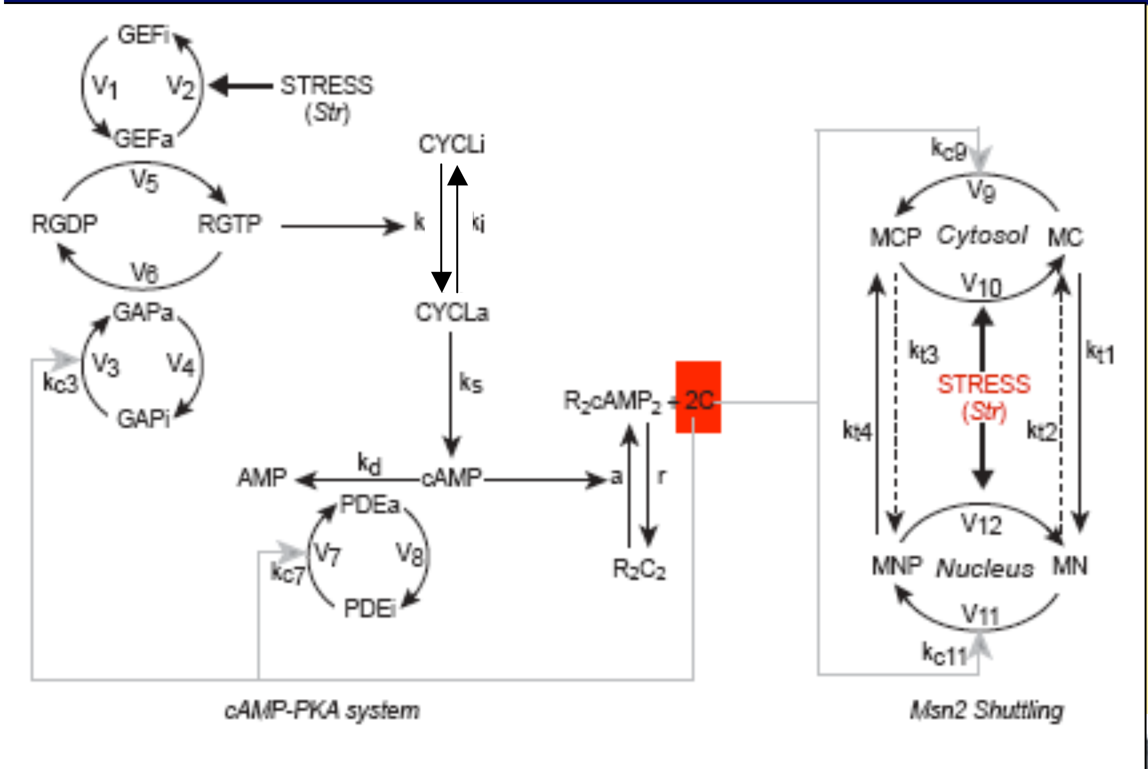
pde2 High cAMP



0 4 8 12 16 20 Min.

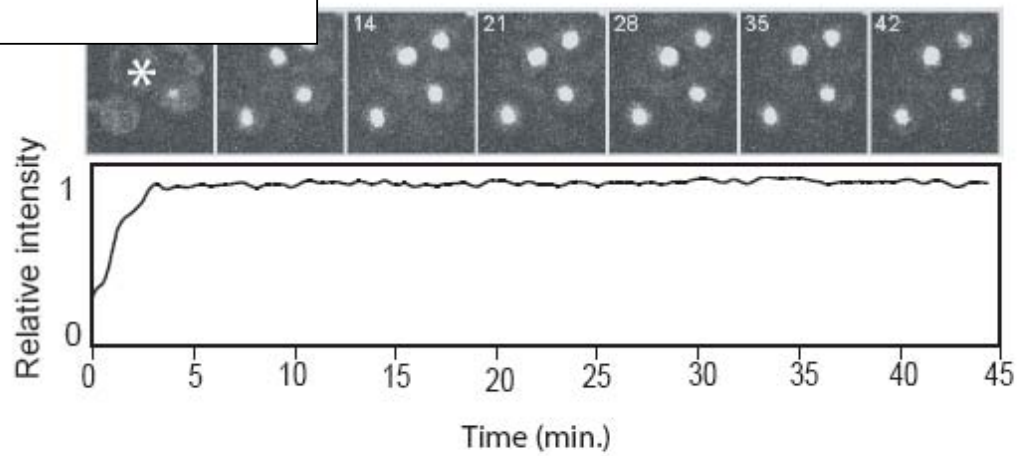
yak1Δ, tpk1Δ, tpk2Δ, tpk3Δ No PKA

Suppressing the negative feedback loop prevents oscillation but not the stress response



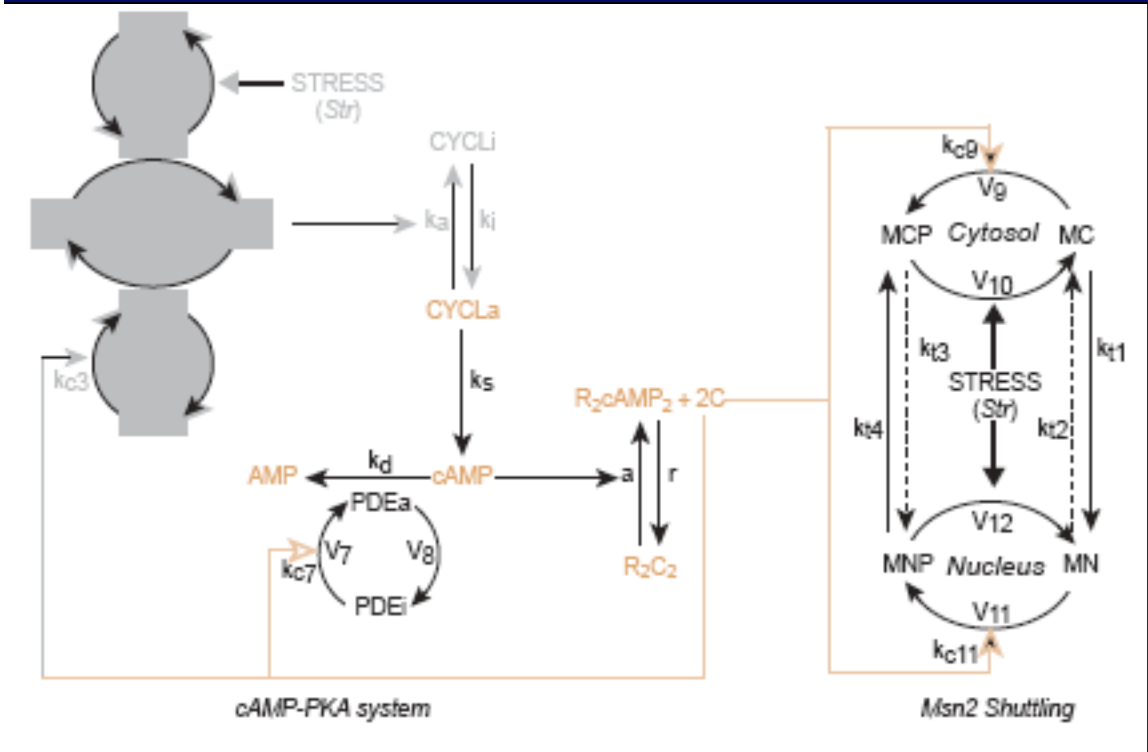
tpk2^w

45 min

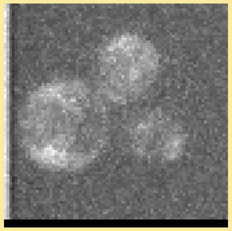


0 osc/ 67 Cell
7 experiments

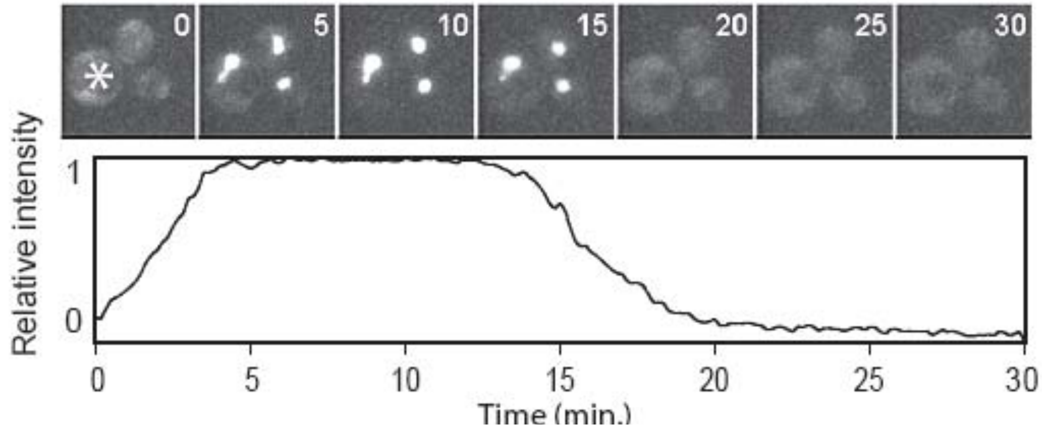
Alteration of the feedback prevents oscillation but the shuttling is conserved



F1D:
Δras1Δras2CYR1m
45min



0 osc /71 cell
20 exp.



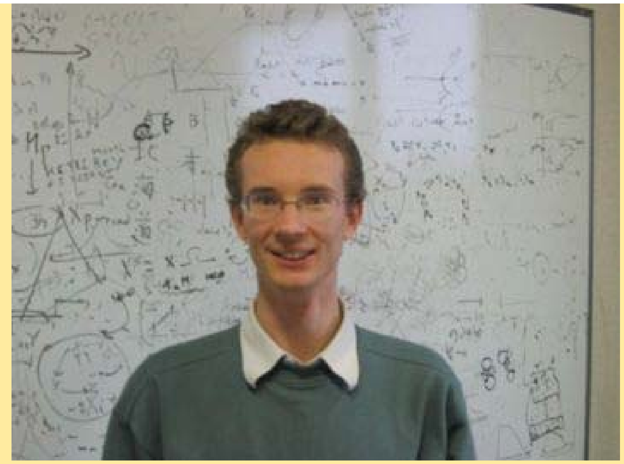
Domain of oscillation: Range of variation of the components

Component	Value in the model (μM)	Minimal value (μM)	Maximal value (μM)	Ratio
GEFt	4.0	0.41	7.14	17.5
RASt	250.0	2.10	1429	680.00
GAPt	1.5	0.85	20.19	23.75
CYCLt	0.7	0.02	4.93	246.50
PDEt	0.5	0.23	18.30	79.50
PKAt	0.3	0.19	2.38	12.50

Domain of oscillation: range of variation of the parameters

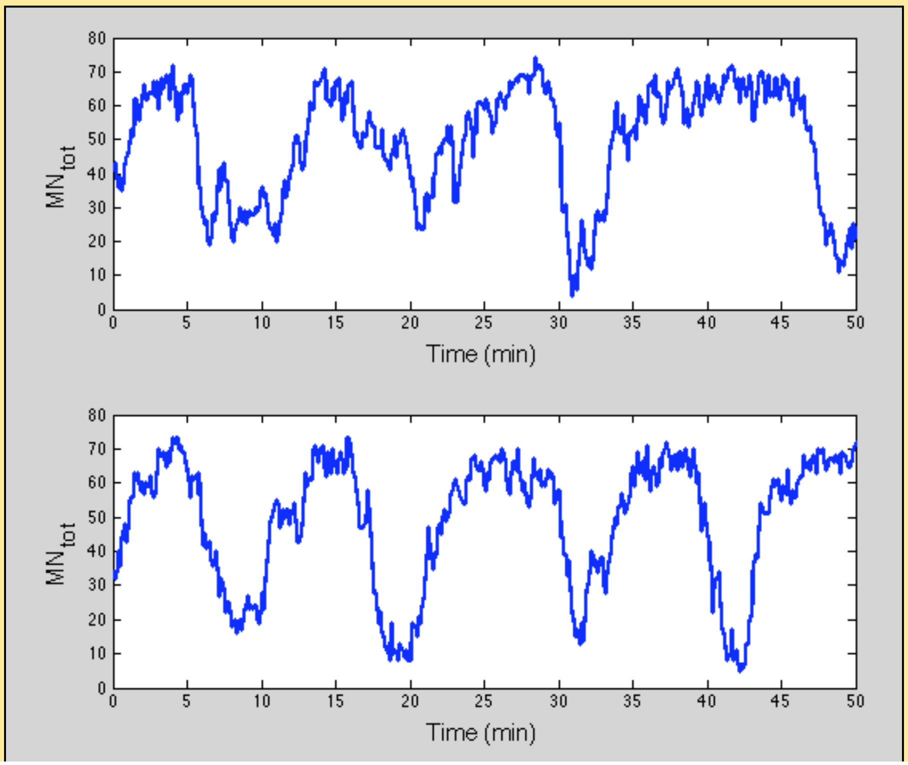
Parameter	Basal value considered in the model	Minimal value	Maximal value	range
r (μM^{-2} min^{-1})	1	0.092	>3000	>30000
a (μM^{-2} min^{-1})	1	< 0.001	10.7	>10700
K_8	0.01	< 0.006	> 100	>10000
K_5	0.001	< 0.001	8.5	>8500
k_{c7} (min^{-1})	3.333	2.763	> 3000	>1000
K_{md}	20	0.11	42.6	387
k_i (min^{-1})	1	0.4	123	307
k_s (min^{-1})	4	0.11	28.17	256
K_7	0.01	< 0.001	0.21	>210
K_1	0.05	0.006	0.945	157.5
K_2	0.05	0.003	0.417	139
K_3	0.01	< 0.001	0.125	>125
k_d (min^{-1})	100	47.4	>3000	>70
K_4	0.01	< 0.001	0.062	>62
k_a (μM^{-1} min^{-1})	0.01	< 0.001	0.025	>25
K_6	0.001	< 0.0001	0.0023	>23
k_{GEF} (min^{-1})	240	25	427	17
k_{GAP} (min^{-1})	600	337	>3000	>10
k_c (min^{-1})	3.5	2.75	4.05	1.4

Stochastic simulation at KITP
Didier Gonze



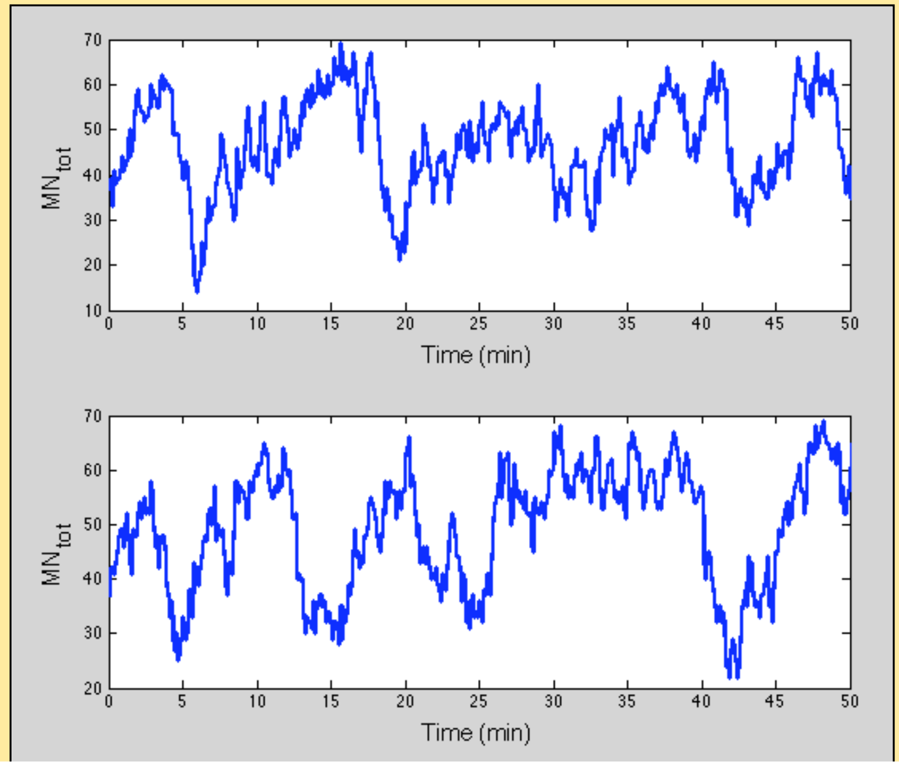
$\Omega = 75$

$K_9=K_{10}=K_{11}=K_{12} = 0.005$

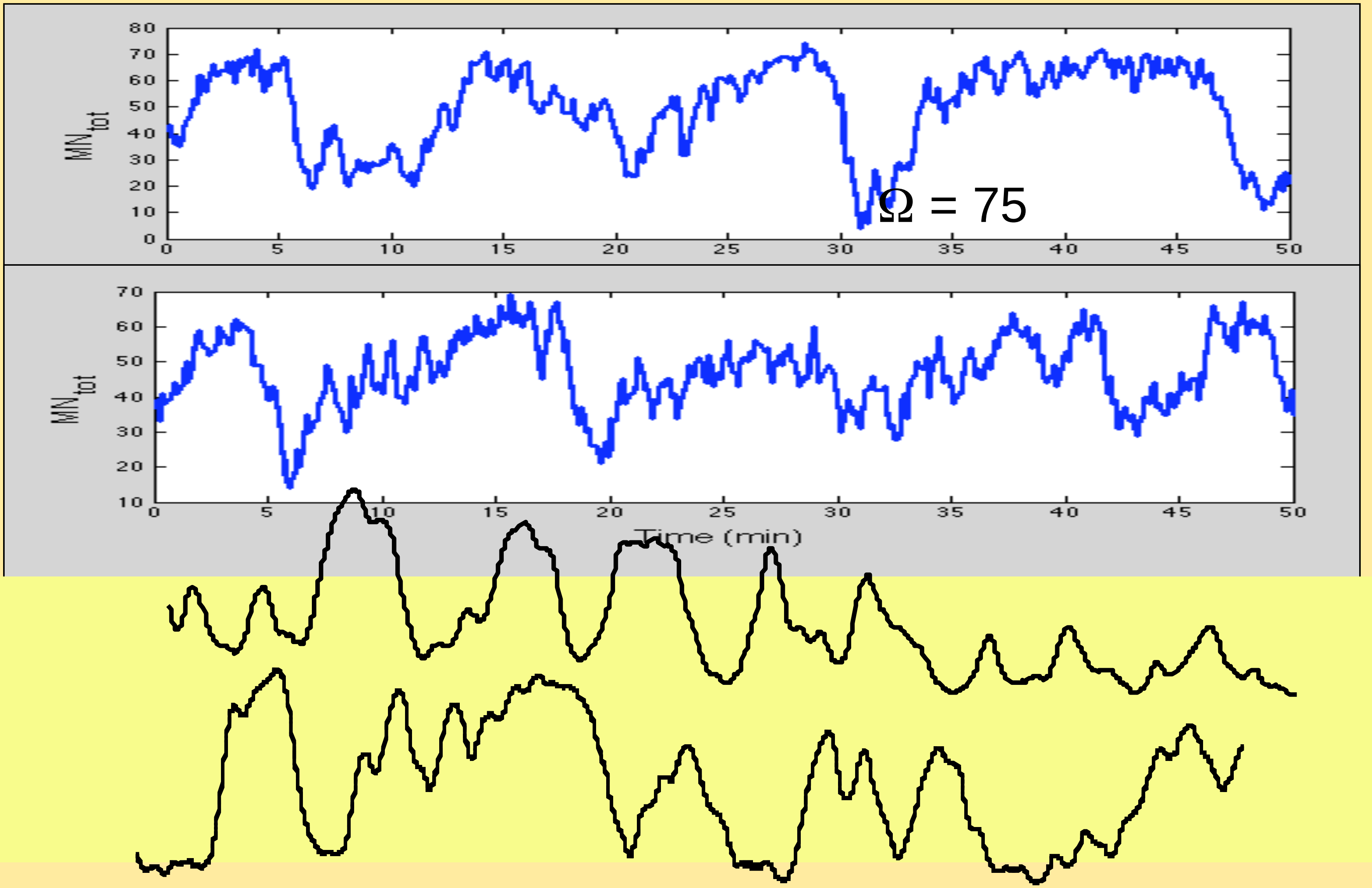


$\Omega = 75$

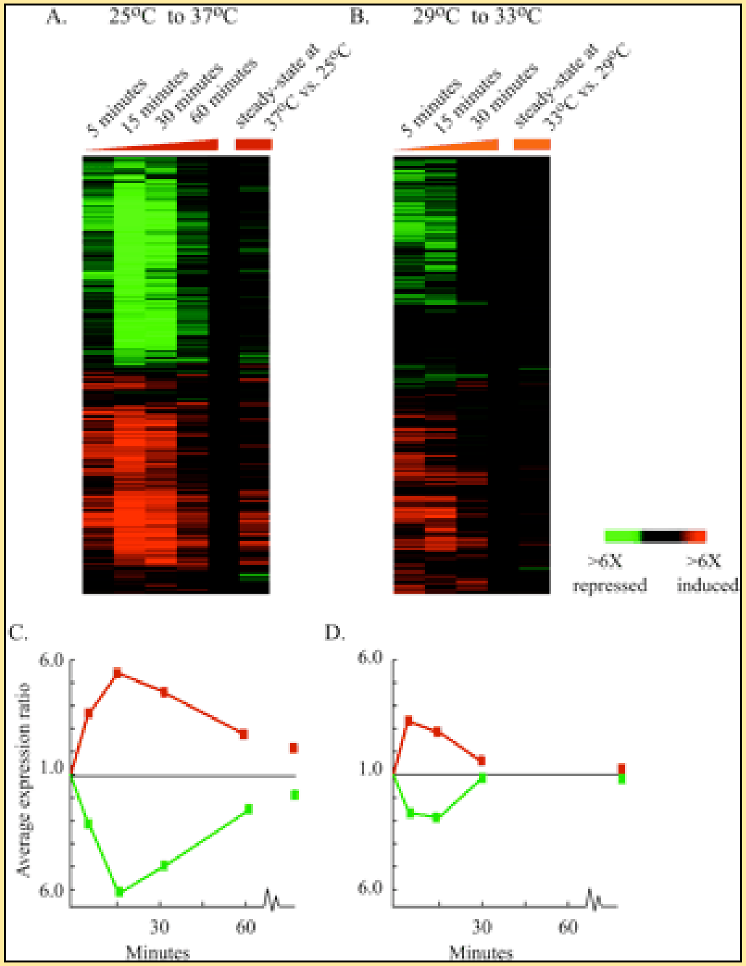
$K_9=K_{10}=K_{11}=K_{12} = 0.025$

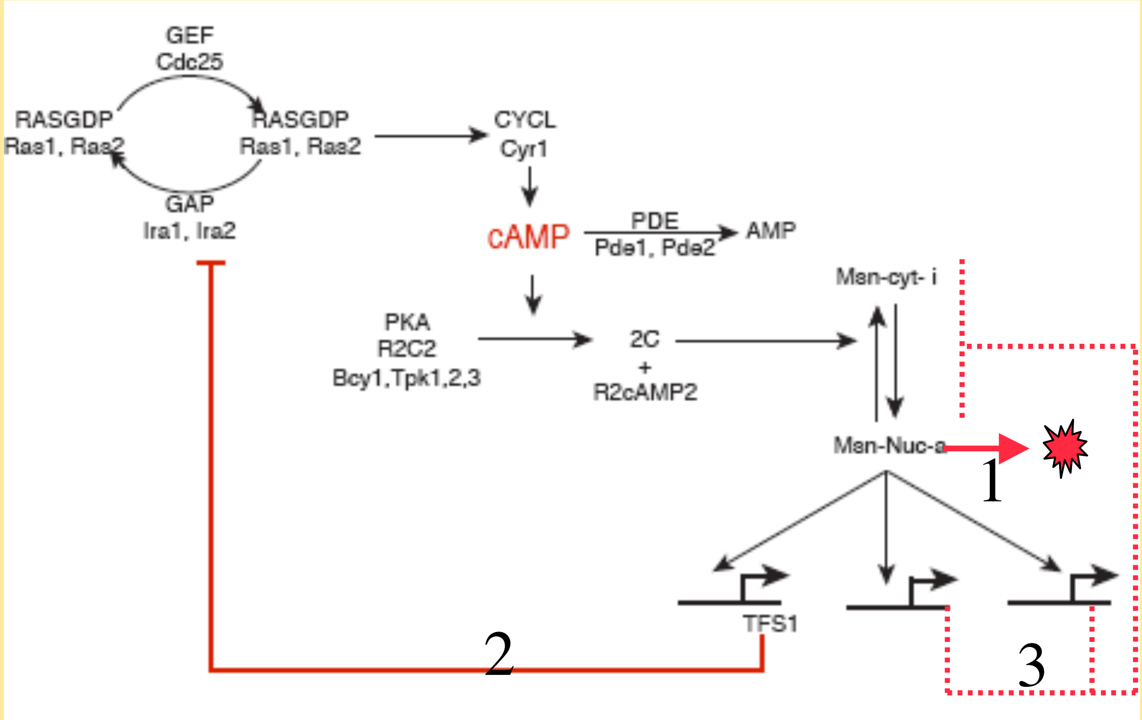


Stochastic simulation at KITP
Didier Gonze

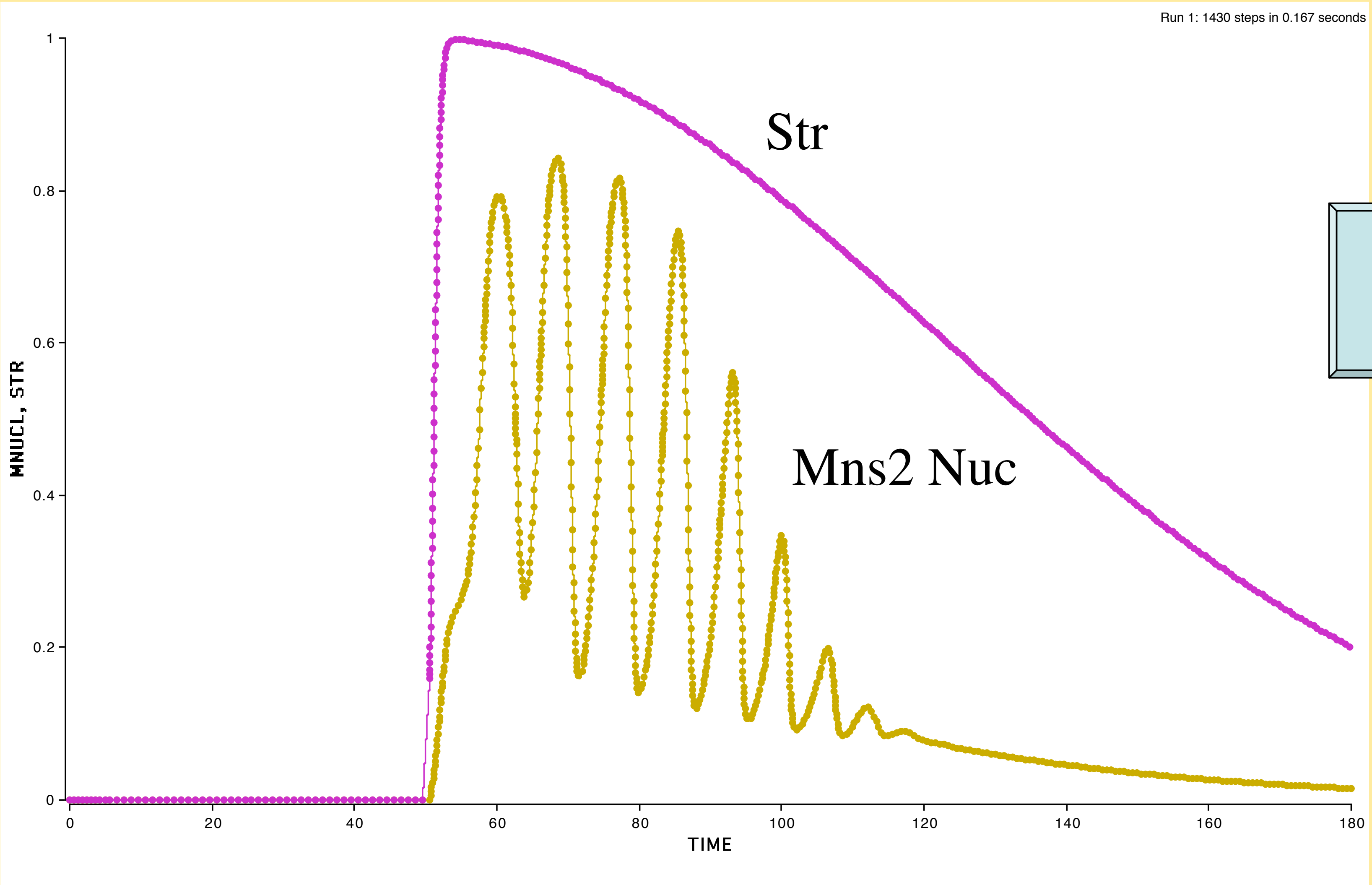


The stress response is transient





Simulations with transient stress response



What would be the interest for the cell to have oscillators ?

Cover the space of targets of Msn2 (N#<nSTRE)

Other cAMP-PKA controlled pathways

cAMP-PKA a clutch for different cellular programs
Disengage the cluch twice for reducing gear

Msn2 the neutral position before new program

Conclusions

Evidence for the existence of oscillatory nucleocytoplasmic shuttling of transcription factors independent of transcription

Another example of the complex behavior of the cAMP-PKA system

The emergence of additional oscillatory systems in living cells

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