Control and regulation of PGK gene expression in *Trypanosoma brucei*

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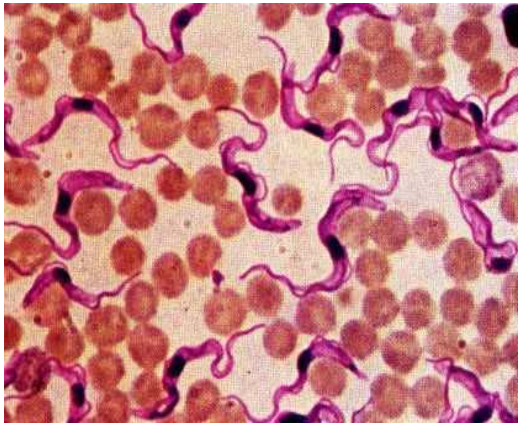
AIM

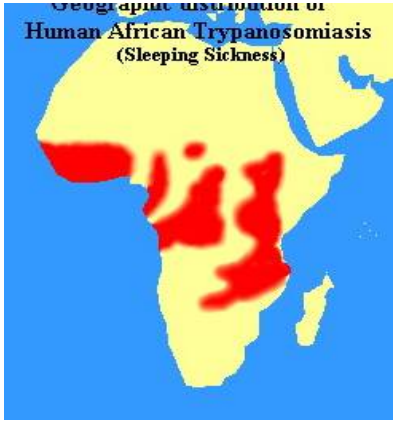
To unravel at which level(s) gene expression is *controlled* and *regulated* in *Trypanosoma brucei*, using the PGK locus as a case study

Control – Steps have control if a change in that step will give a change in the pathway output

Regulation – The steps that are actually used by a system (e.g. cell) to change the pathway output are regulating

Trypanosoma brucei





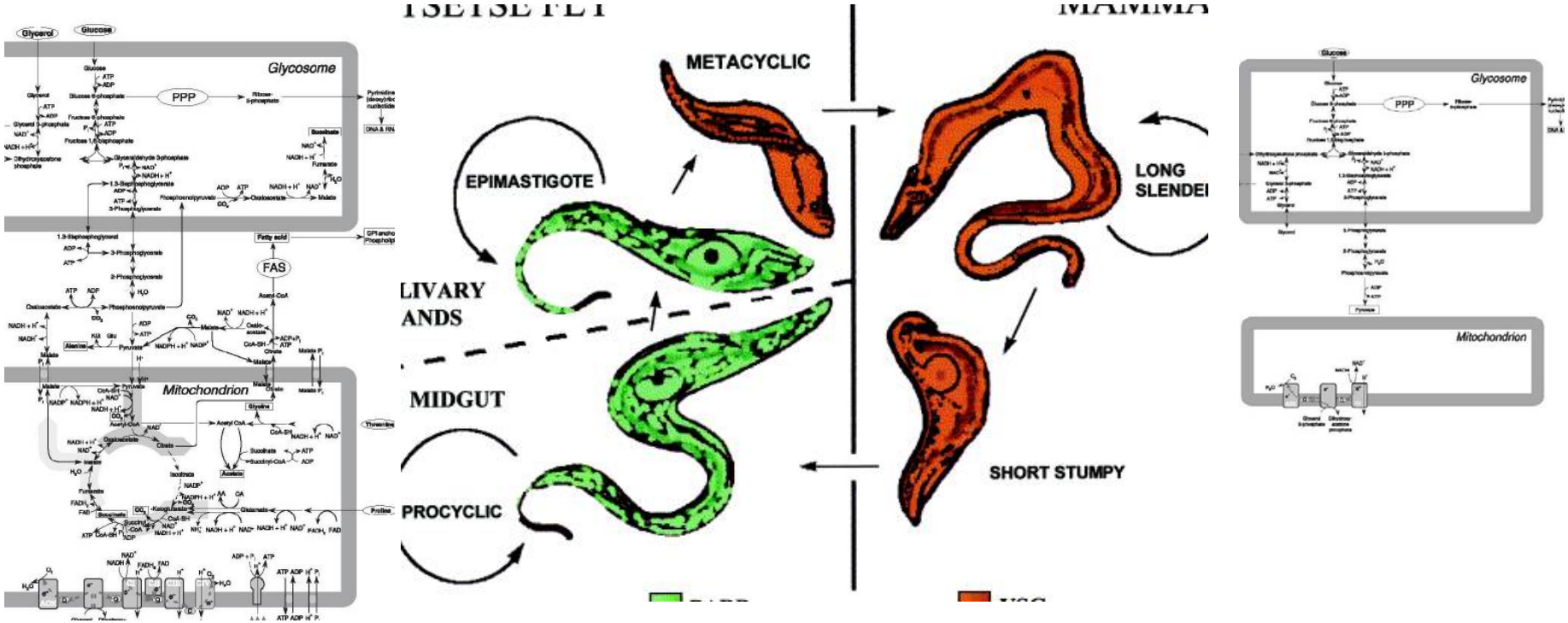
Trypanosoma brucei is the causative agent of African sleeping sickness.

Both cattle and humans can be infected by bites of the tsetse fly

60 million people are at risk, ~50.000-70.000 infections/year

Fatal if left untreated, resistance to current medication is spreading

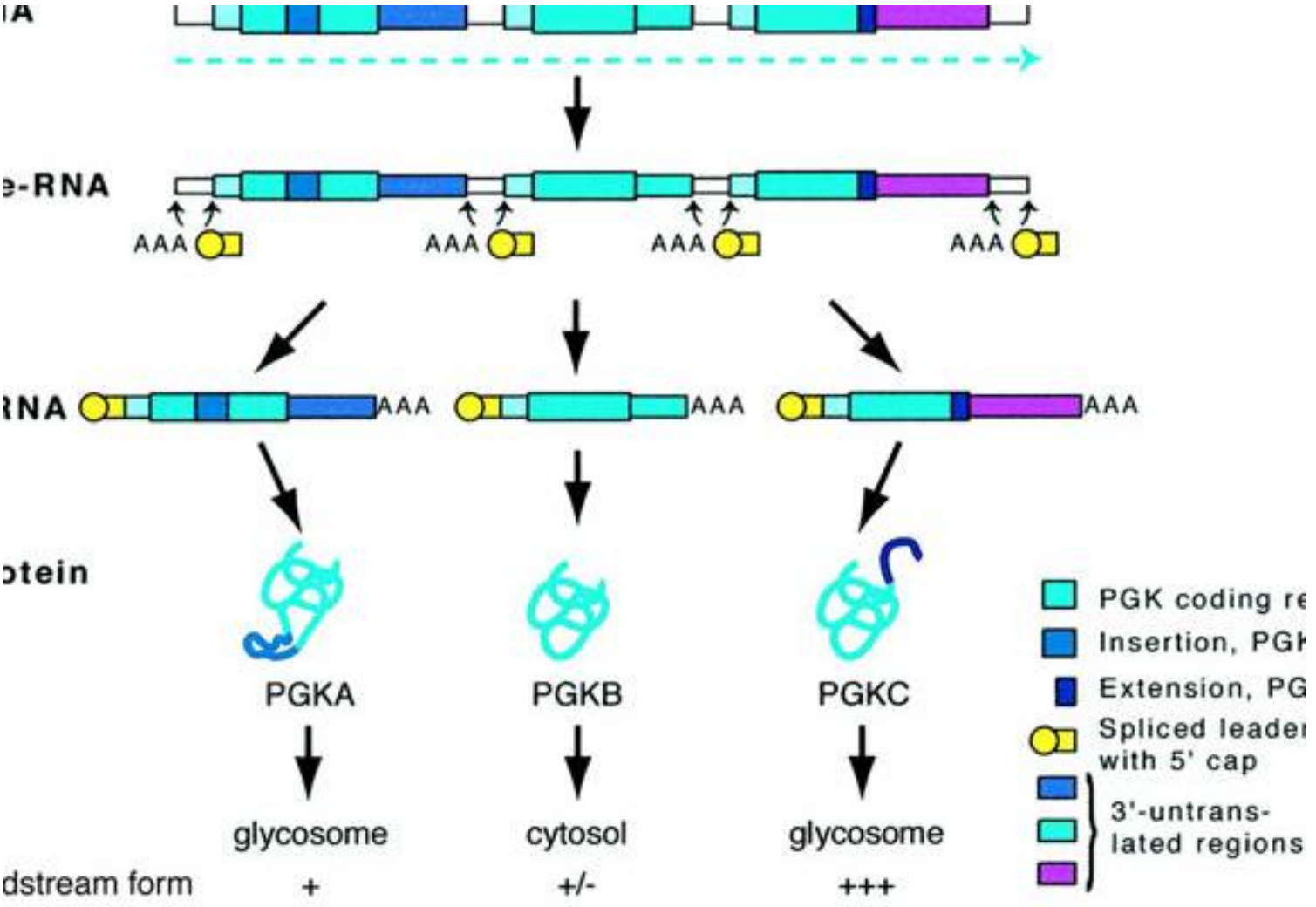
Life cycle of *T. brucei*



Gull, Int J Parasitol **31**, 2001

Van Hellemond et al., Adv. Microb. Physiol. **50**, 2005

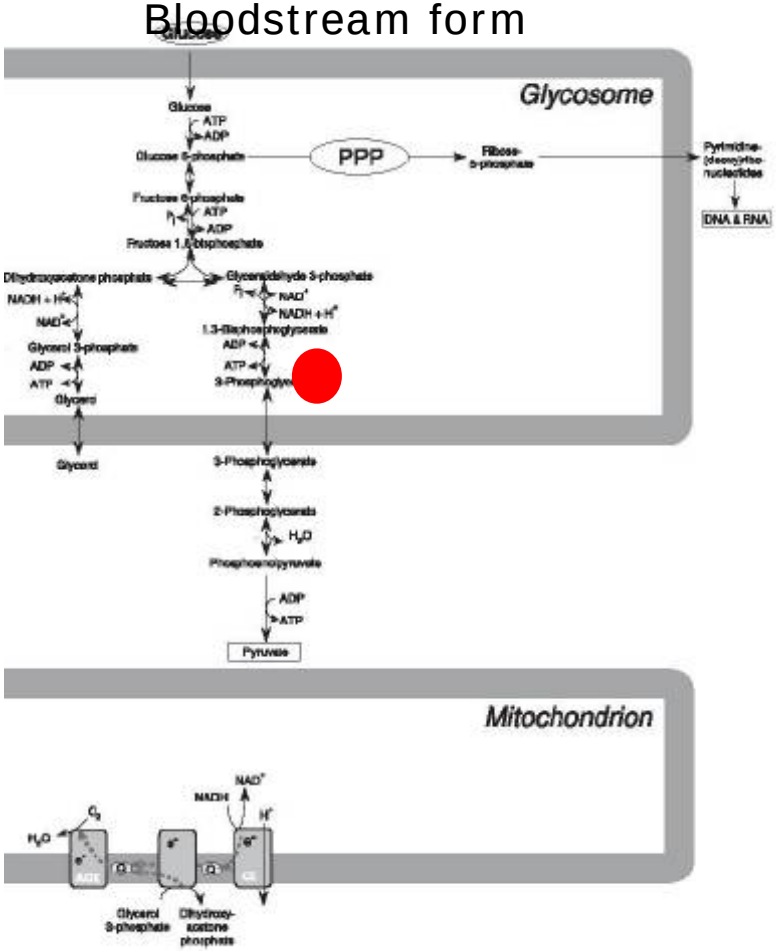
The PGK locus



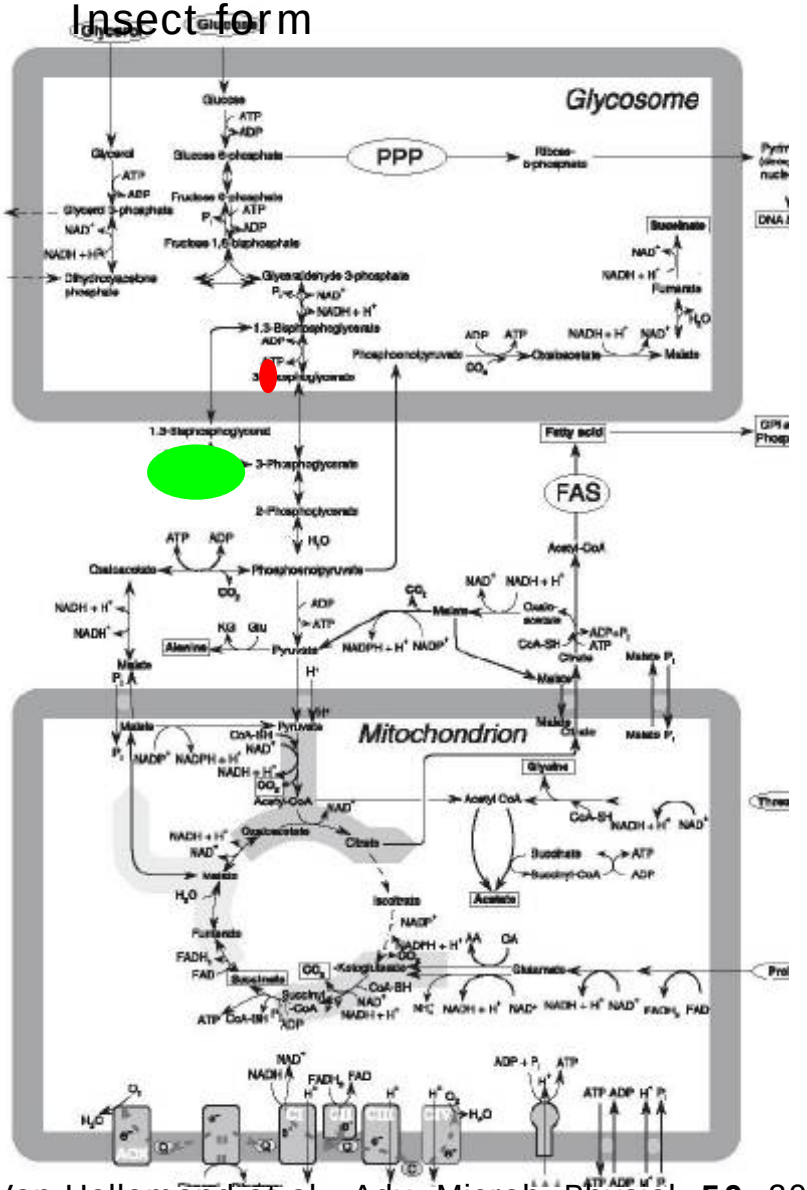
From Clayton, EMBO J. (2002)

PGK activity

Bloodstream form



Insect form



PGKB

PGKC

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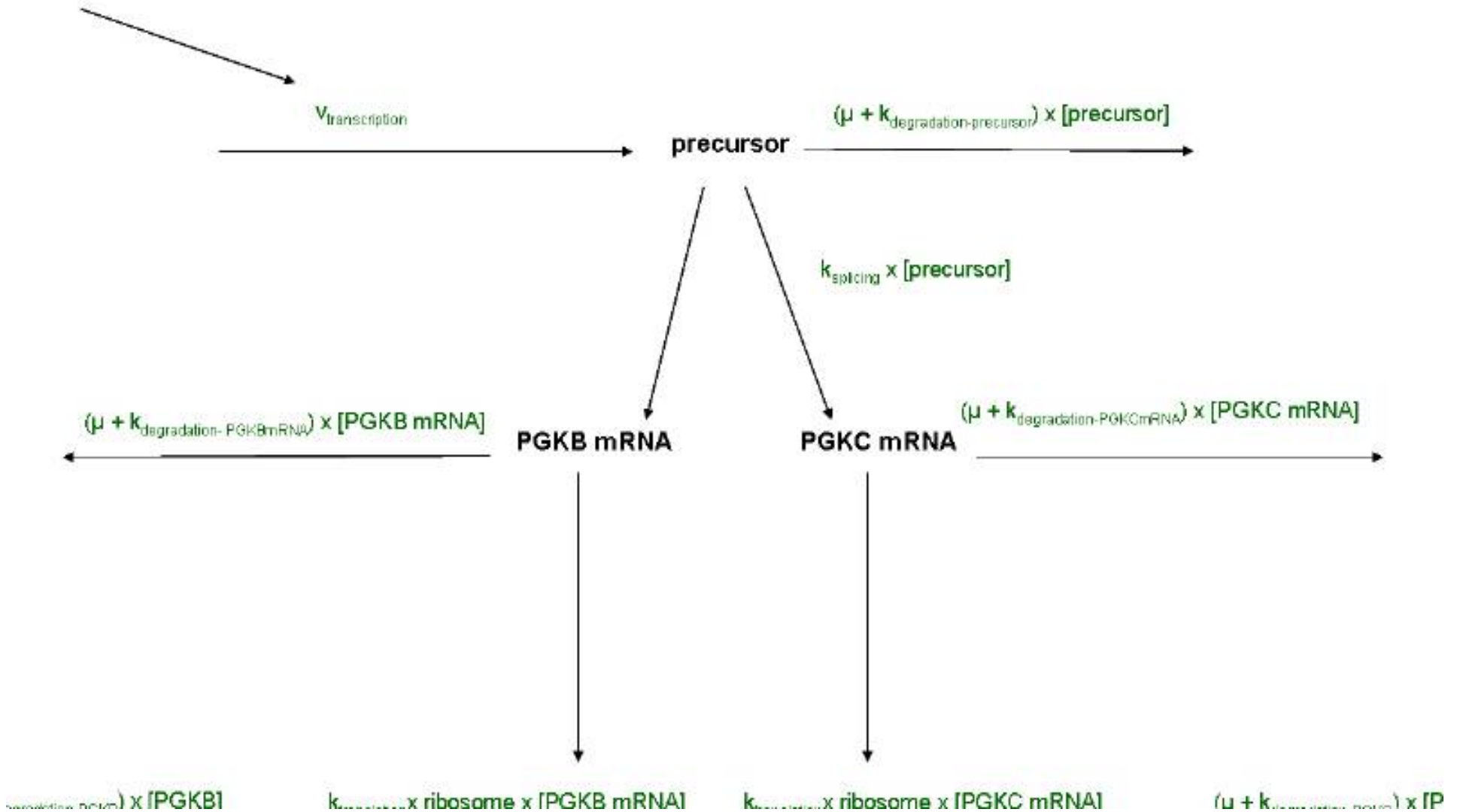
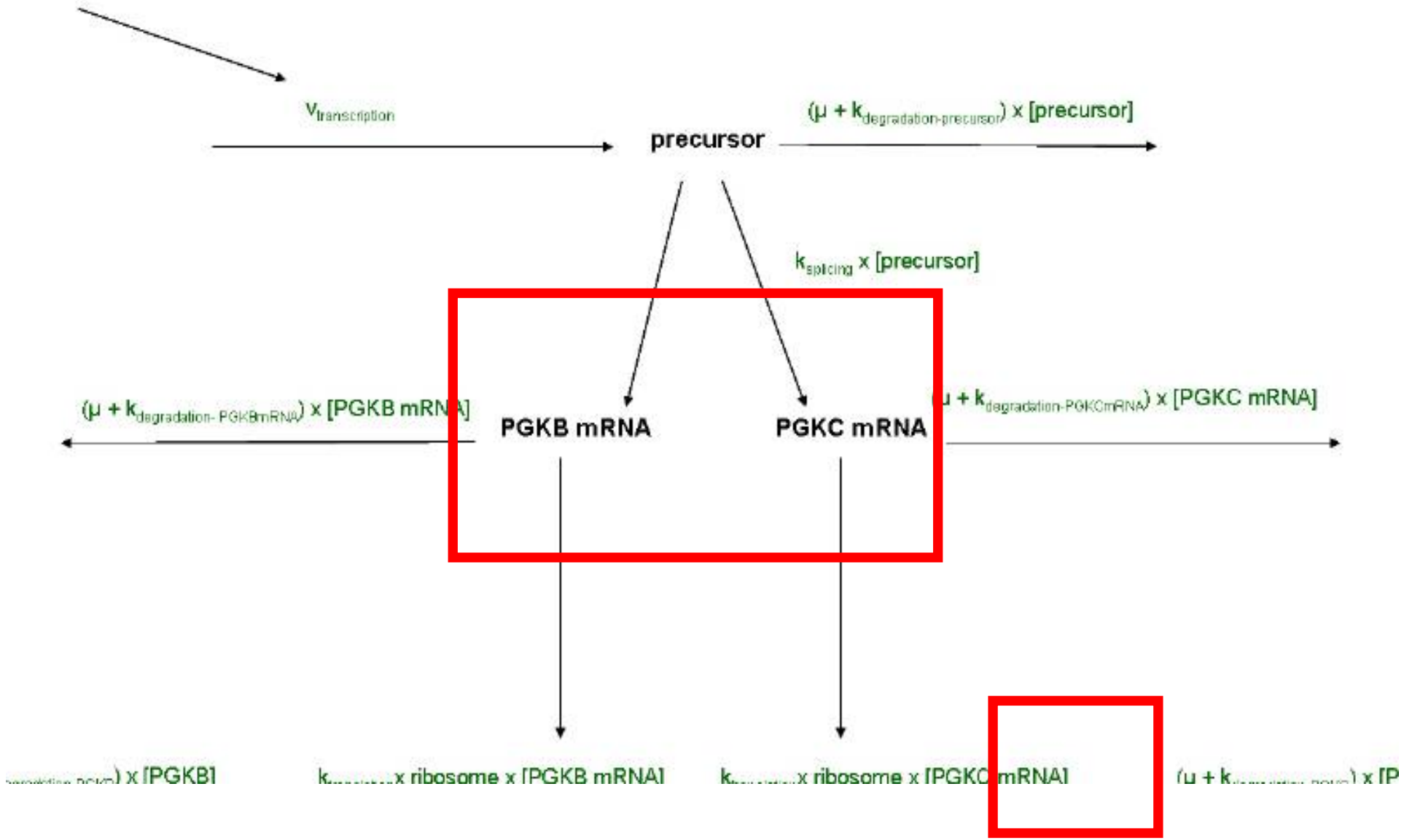
PGKB and PGKC expression

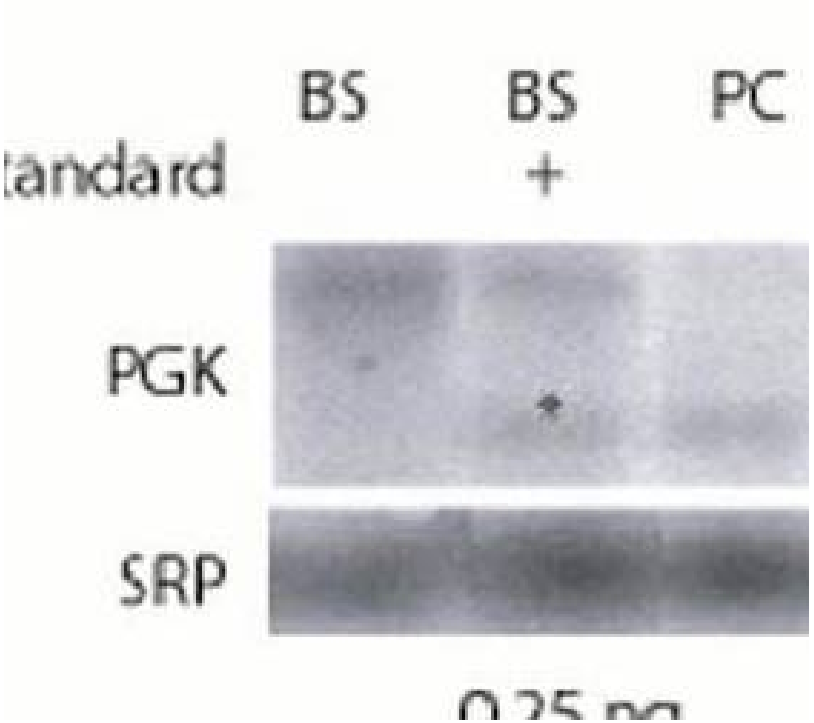


PGKB and PGKC expression



mRNA and protein levels

mRNA



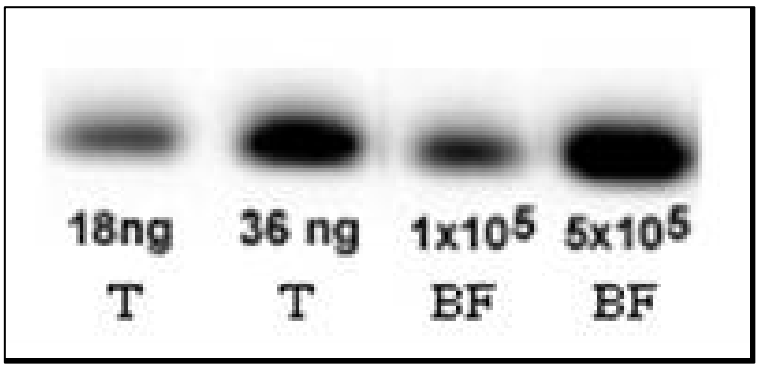
PGKC

PGKB

BF: 12.1 ± 7.1 molecules/cell

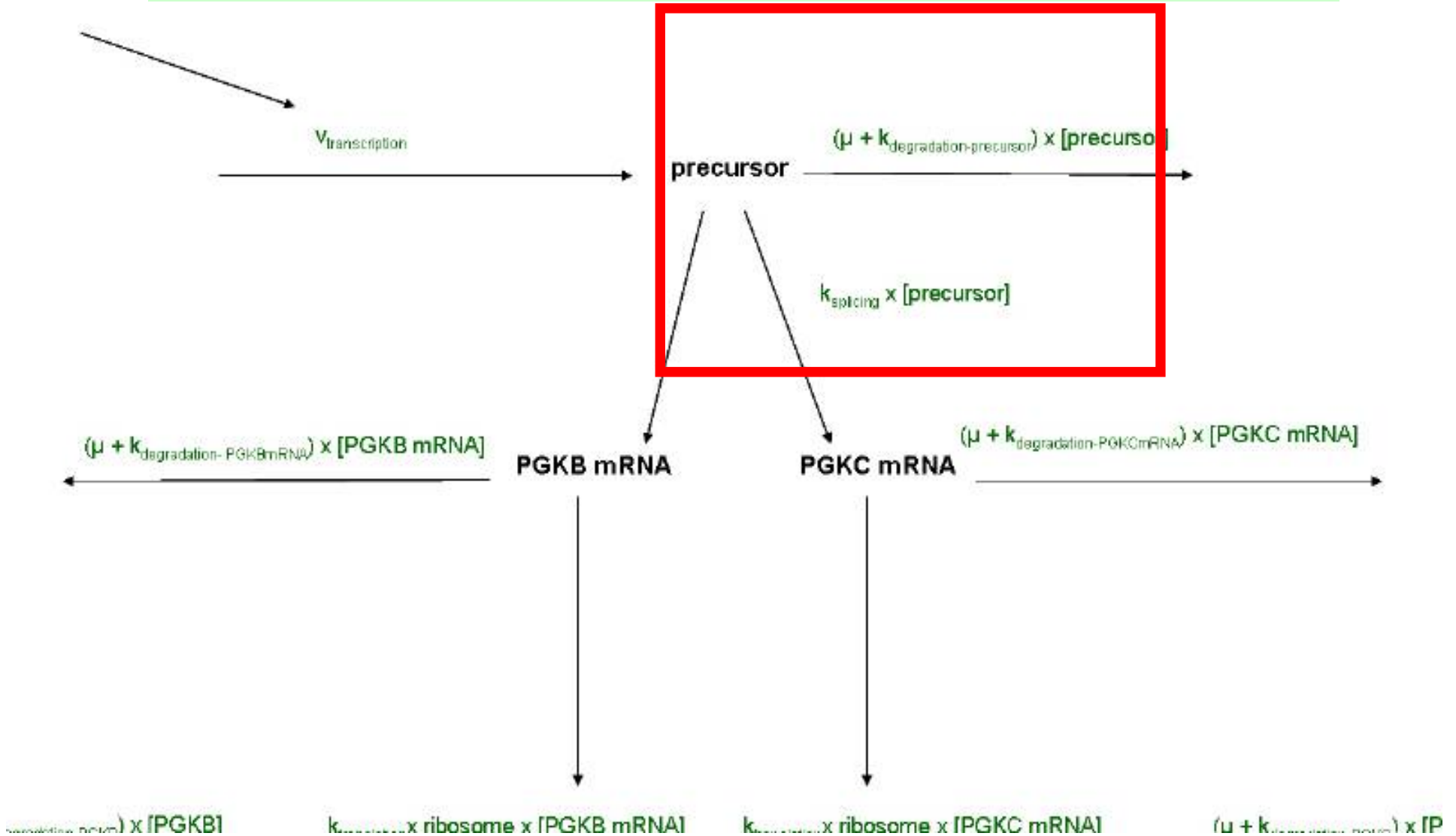
PF: 18 ± 1.4 molecules/cell

protein

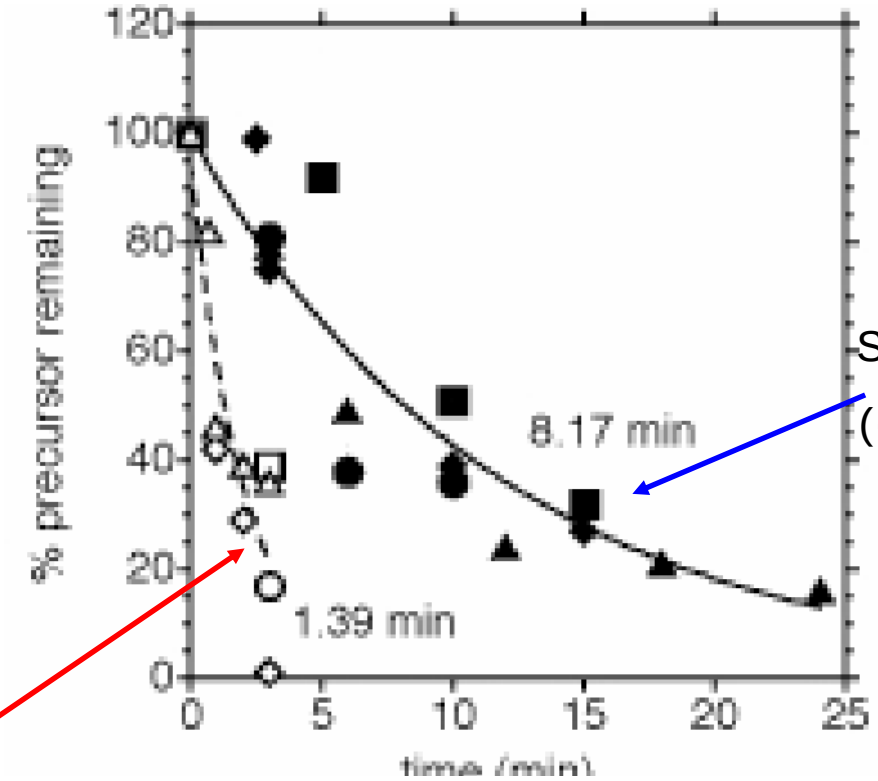


BF: $1x10^6 \pm 0.5$ molecules/cell

PGKB and PGKC expression



Precursor processing



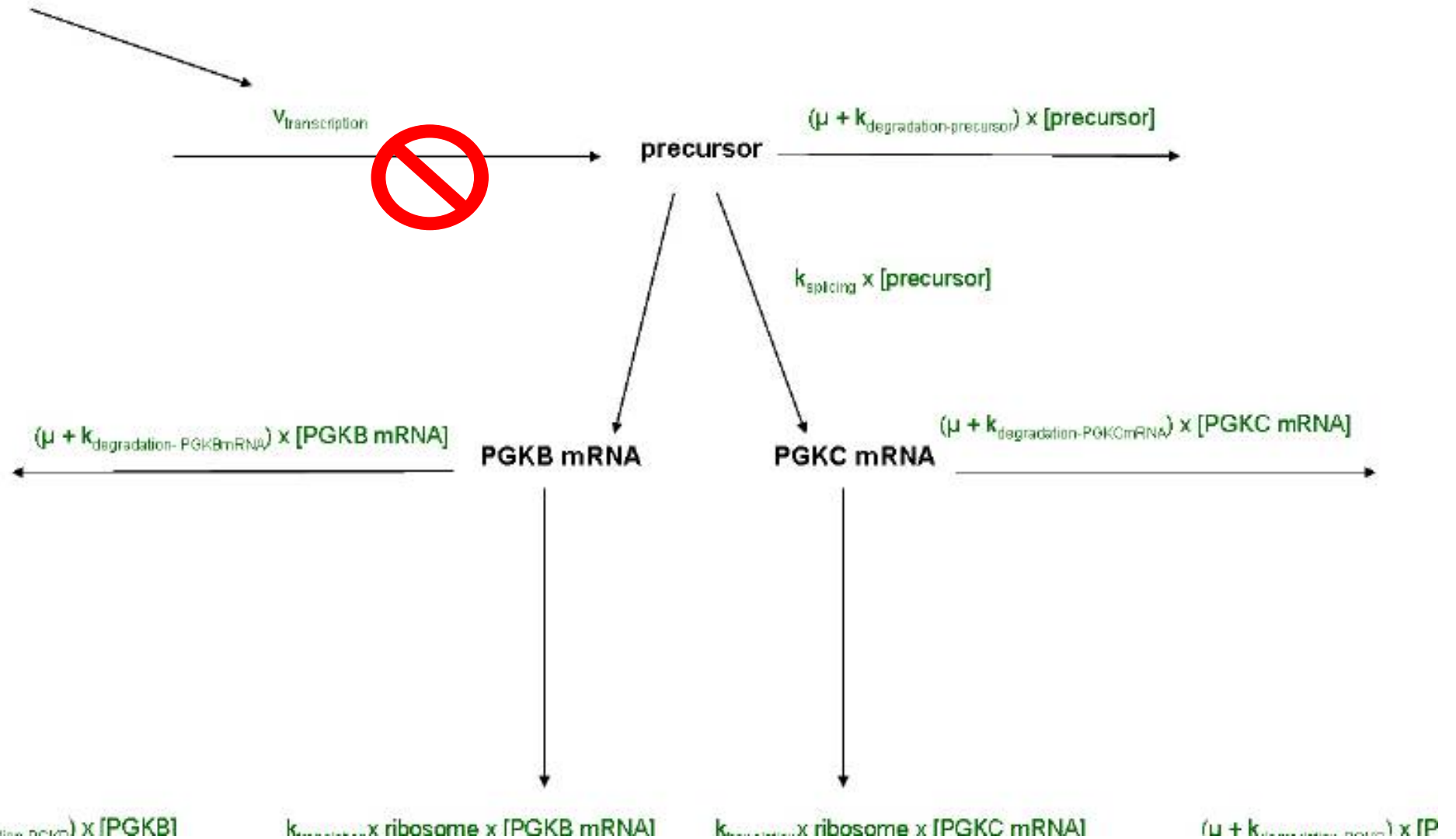
Sinefungin/Actinomycin D (degradation)

Actinomycin D alone (splicing +degradation)

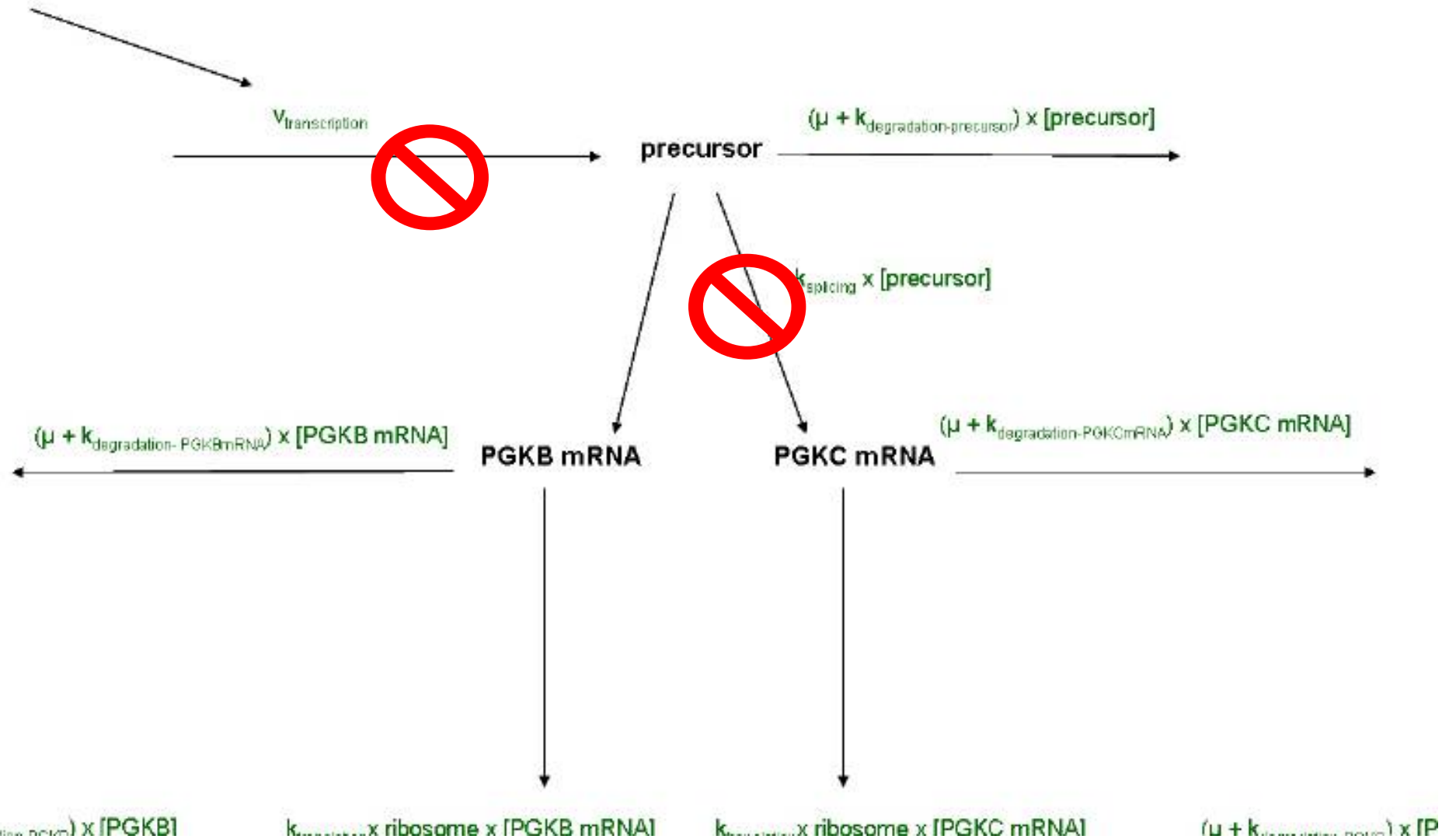
Sinefungin inhibits splicing

Actinomycin D inhibits transcription

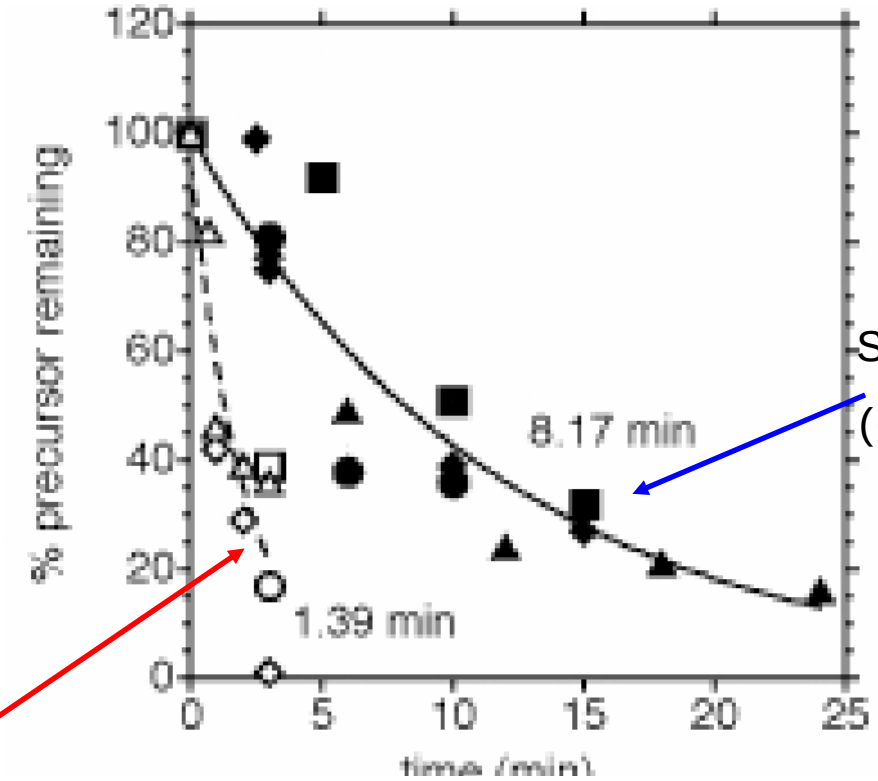
Actinomycin D



Actinomycin D + Sinefungin



Precursor processing



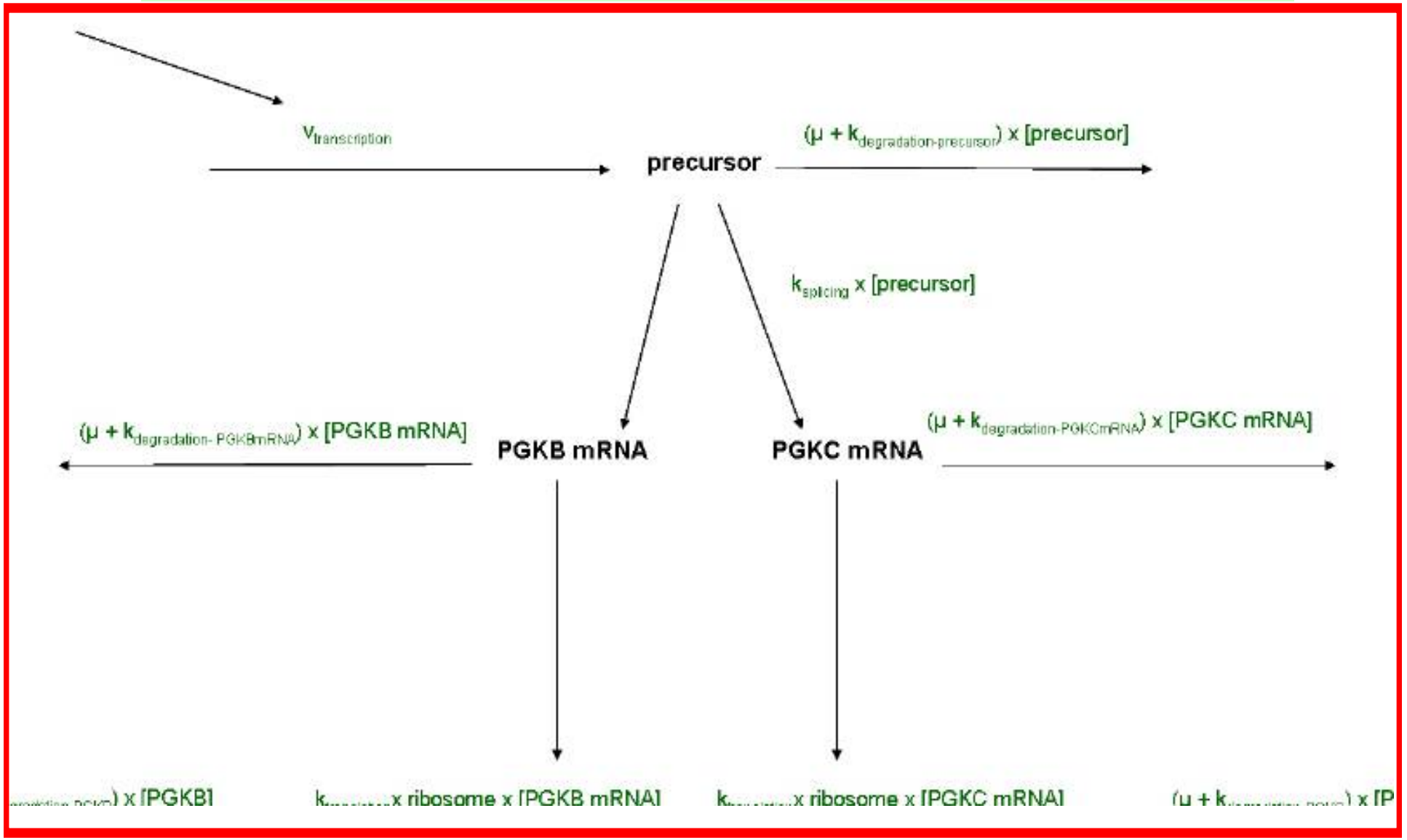
Sinefungin/Actinomycin D (degradation)

Actinomycin D alone (splicing +degradation)

Sinefungin inhibits splicing

Actinomycin D inhibits transcription

PGKB and PGKC expression



Concentration Control Coefficients

$$\zeta = \frac{\Delta X / X}{\Delta p_i / p_i}$$

e parameters that were varied were: $v_{transcription}$, μ, k_{degrP}, $k_{splicing}$, k_{degrB} and k

$$\sum C_i^X = 0$$

Concentration control coefficients sum up to 0!

Control on [mRNA]

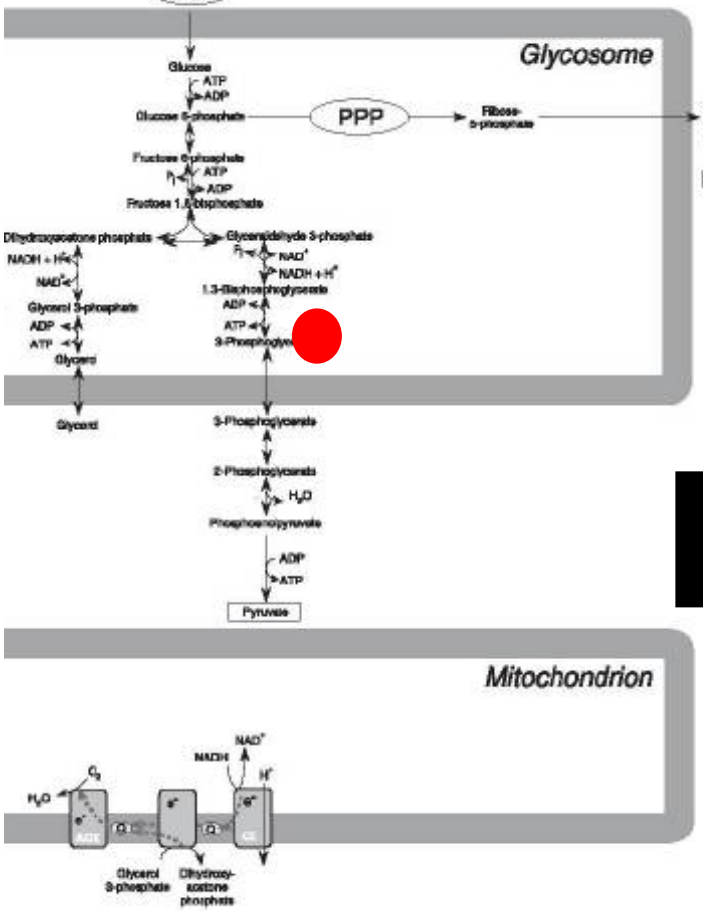
control on	control of: transcription	growth	precursor degradation	precursor splicing	degradation PGKB mRNA	degradation PGKC mRNA	sum
[precursor]	1.000	-0.004	-0.163	-0.834	0.000	0.000	0.000
[PGKBmRNA]	1.000	-0.023	-0.163	0.166	-0.981	0.000	0.000
[PGKCmRNA]	1.000	-0.116	-0.163	0.166	0.000	-0.888	0.000

Control on [mRNA]

control on	control of: transcription	growth	precursor degradation	precursor splicing	degradation PGKB mRNA	degradation PGKC mRNA	sum
[precursor]	1.000	-0.004	-0.163	-0.834	0.000	0.000	0.000
[PGKBmRNA]	1.000	-0.023	-0.163	0.166	-0.981	0.000	0.000
[PGKCmRNA]	1.000	-0.116	-0.163	0.166	0.000	-0.888	0.000

Regulation of mRNA

Bloodstream form



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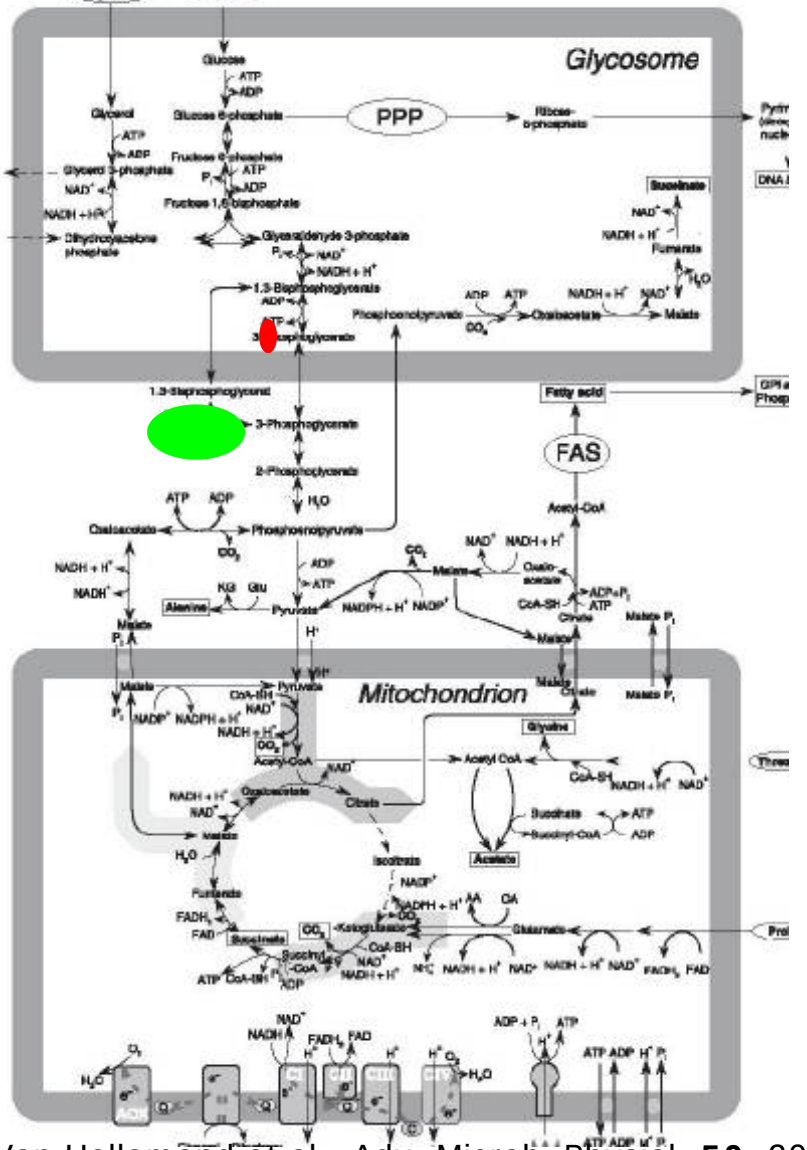

PGKB

PGKC

Van Hellemond et al., Adv. Microb. Physiol. **50**, 2005

Regulation analysis for differentiation from BF -> PF (I)

$$\frac{d[precursor]}{dt} = v_{transcription} - (k_{splicing} + k_{\deg rP}) \cdot [precursor] = 0 \qquad 1)$$

follows that:

$$mRNA_i] = \frac{v_{transcription} \cdot k_{splicing}}{(k_{splicing} + k_{\deg rP}) \cdot (\mu + k_{\deg r\_i})} \qquad 2)$$

logarithmic space:

$$\ln[mRNA_i] = \ln v_{transcription} + \ln\left(\frac{k_{splicing}}{k_{splicing} + k_{\deg rP}}\right) - \ln(\mu + k_{\deg r\_i}) \qquad 3)$$

we consider transitions from one state to another, *e.g.* from one life stage t

$$\Delta \ln[mRNA_i] = \Delta \ln v_{transcription} + \Delta \ln\left(\frac{k_{splicing}}{k_{splicing} + k_{\deg rP}}\right) - \Delta \ln(\mu + k_{\deg r\_i}) \qquad \text{(Eq. 14)}$$

Regulation analysis for differentiation from BF -> PF (II)

vision through $\Delta \ln[mRNA_i]$ yields:

$$\frac{\ln[mRNA_i]}{\ln[mRNA_i]} = \frac{\Delta \ln v_{transcription}}{\Delta \ln[mRNA_i]} + \frac{\Delta \ln\left(\frac{k_{splicing}}{k_{splicing} + k_{\deg rP}}\right)}{\Delta \ln[mRNA_i]} - \frac{\Delta \ln(\mu + k_{\deg r\_i}}{\Delta \ln[mRNA_i]}$$

iis can be written as

$= \rho \quad . \quad + \rho \quad . \quad + \rho . \quad .$

Regulation coefficients

	μ (min^{-1})	kdegr (min^{-1})		steady state levels (molecules $cell^{-1}$)	
		PGKB	*PGKC*	*PGKB*	*PGKC*
BF	0.0019	0.09[a]	0.02	1.2[c]	12
PF	0.0010	0.01[b]	0.14	18	1.0[d]

	$\rho_{degradation}$	$\rho_{transcription} + \rho_{precursorprocessing}$
PGKB	0.97	0.03
PGKC	0.84	0.16

$$1 = \rho_{transcription} + \rho_{precursorproces\sin g} + \rho_{\deg radation}$$

Conclusions/Discussion

We have experimentally determined PGKC mRNA and protein levels, ribosome density on mature mRNA and precursor processing kinetics

We made a transcription model for PGKB and PGKC expression based on the data from this study and literature data.

Although PGKC mRNA levels is *controlled* at several levels (positively by transcription, precursor splicing and negatively by precursor/mRNA degradation and growth), *regulation* seems to be exclusively at the mRNA degradation level.

The small precursor processing regulation coefficient for PGKC mRNA is very sensitive to errors in the measurement: a half life of the PGKC mRNA increased to 3', completely abolishes the regulation by precursor processing