Regulation zweier Gene:
Das lac-Operon
Und das Endo16-Gen der Seeigels

Martin Gühmann

Wie Zellen denken: Struktur und Design von Regulationsnetzen

WS 2005/2006

Detailed Map of a Cis-Regulatory Input Function

Y. Setty, A.E. Mayo, M.G. Surette ad U. Alon

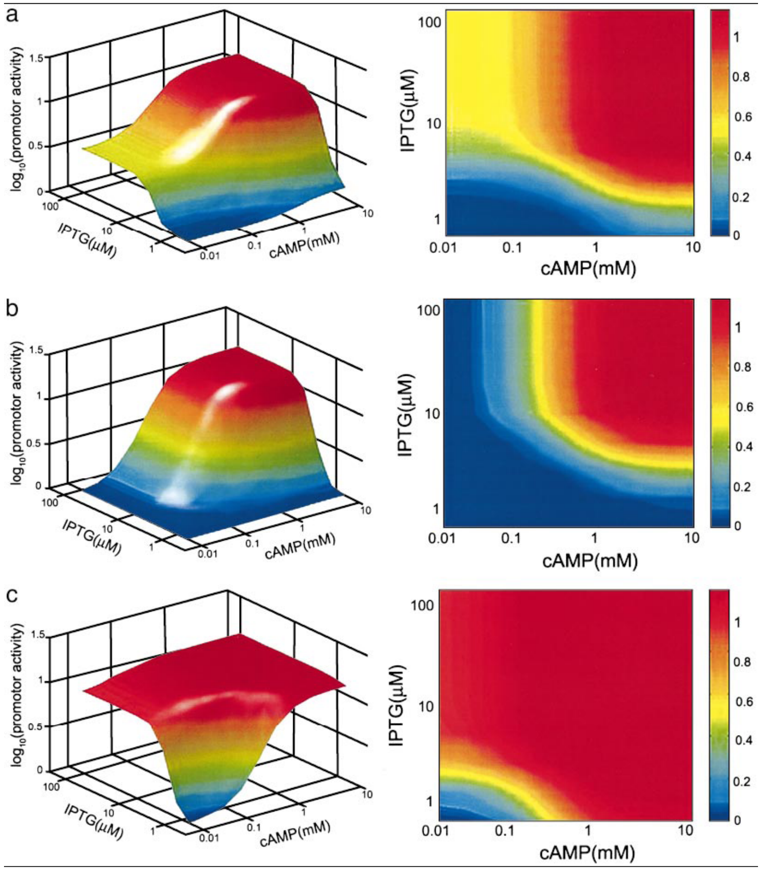
PNAS: 100(13) 7702 - 7707 (2003)

Influence of Catabolite Repression and Inducer Exclusion on the Bistable Behavior of the *lac* Operon

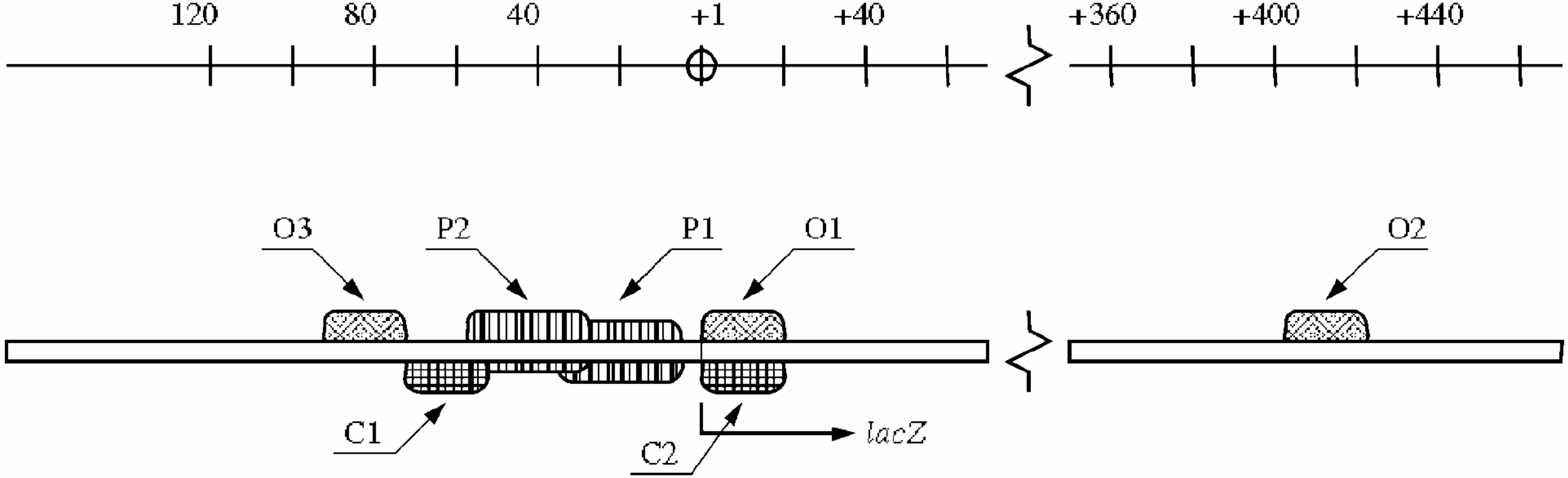
Moisés Santillán and Michael C. Mackey

Biophysical Journal: 86 1282 - 1292 (2004)

Regulationsfunktionstypen

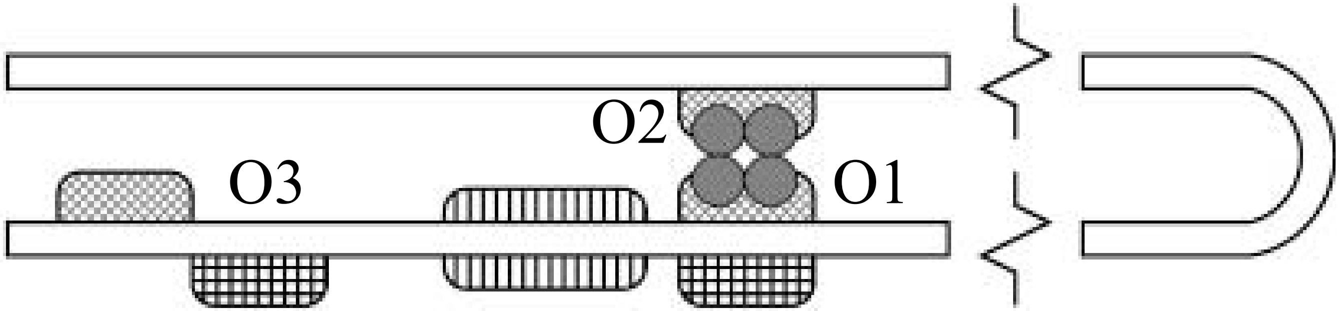


Regulatorische Elemente des *lac*-Operons

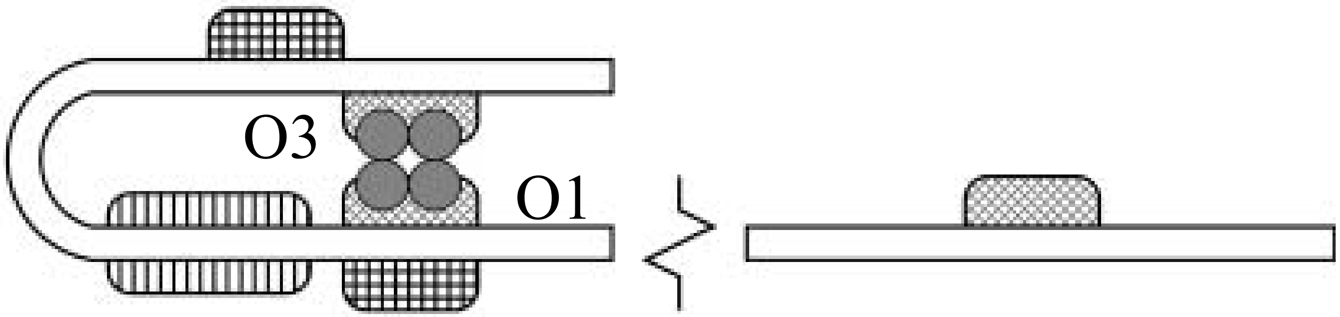


Repression des *lac*-Operons *Lac*I

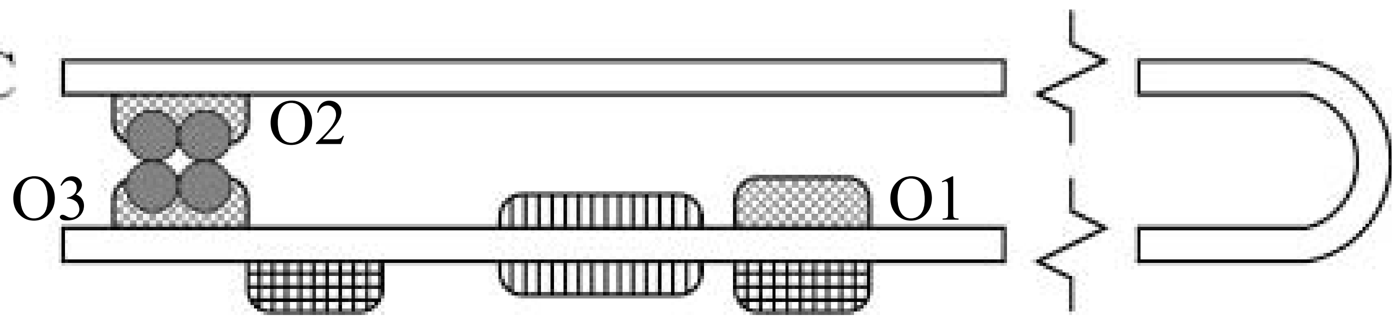
A



B

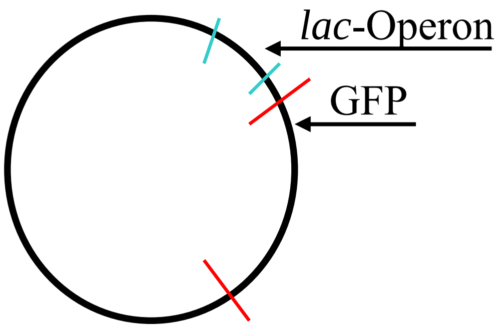


C



GFP-Plasmid-System versus Kolorimetrischer Assay

- Benutzt GFP
- Zusätzliche Plasmide
- Kontinuierliche Messung
- Keine Zelllyse



- Kol. A. misst *Lac*Z-Konzentration
- Keine zusätzlichen Plasmide notwendig
- Zelllyse zur Messung nötig.
- Beides Fluoreszenz-Messmethoden

Einfaches Modell der *lac*-Regulator-Region

$$f = \alpha[SP] + \beta[SPC] + \gamma([S]+[SC]+[SR])$$

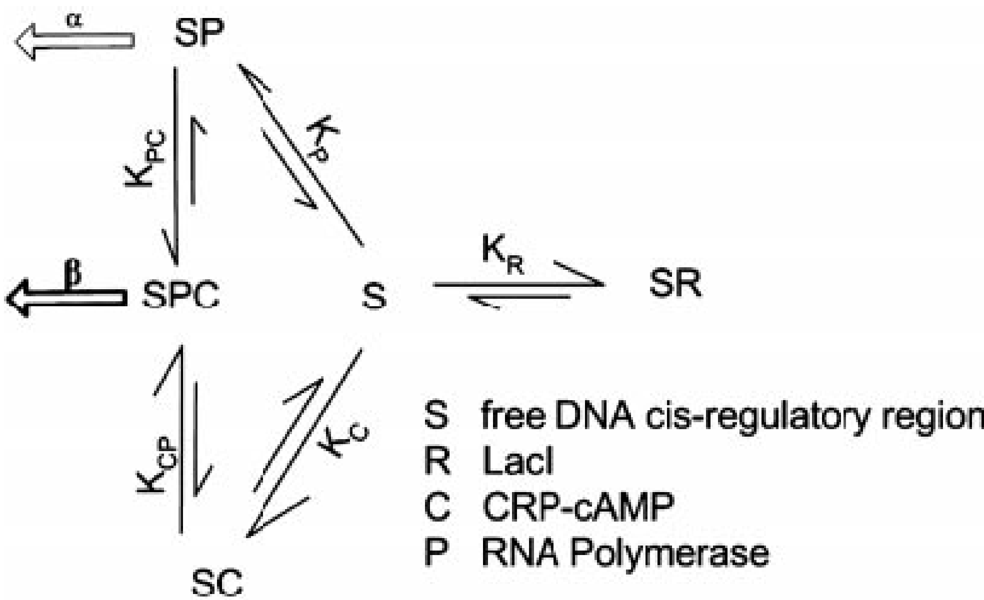
$$f = \frac{\alpha a + 2\beta b d \mathrm{A} + \gamma(c\Lambda + d\mathrm{A} + 1)}{1 + a + (2b+1)d\mathrm{A} + c\Lambda}$$

$$\mathrm{A} = \frac{[C]}{[C_T]} = \frac{X^n}{(1+X^n)}, \quad X = \frac{[cAMP]}{K_{cAMP}}$$

$$\Lambda = \frac{[R]}{[R_T]} = \frac{Y^m}{(1+Y^m)}, \quad Y = \frac{[IPGT]}{K_{IPGT}}$$

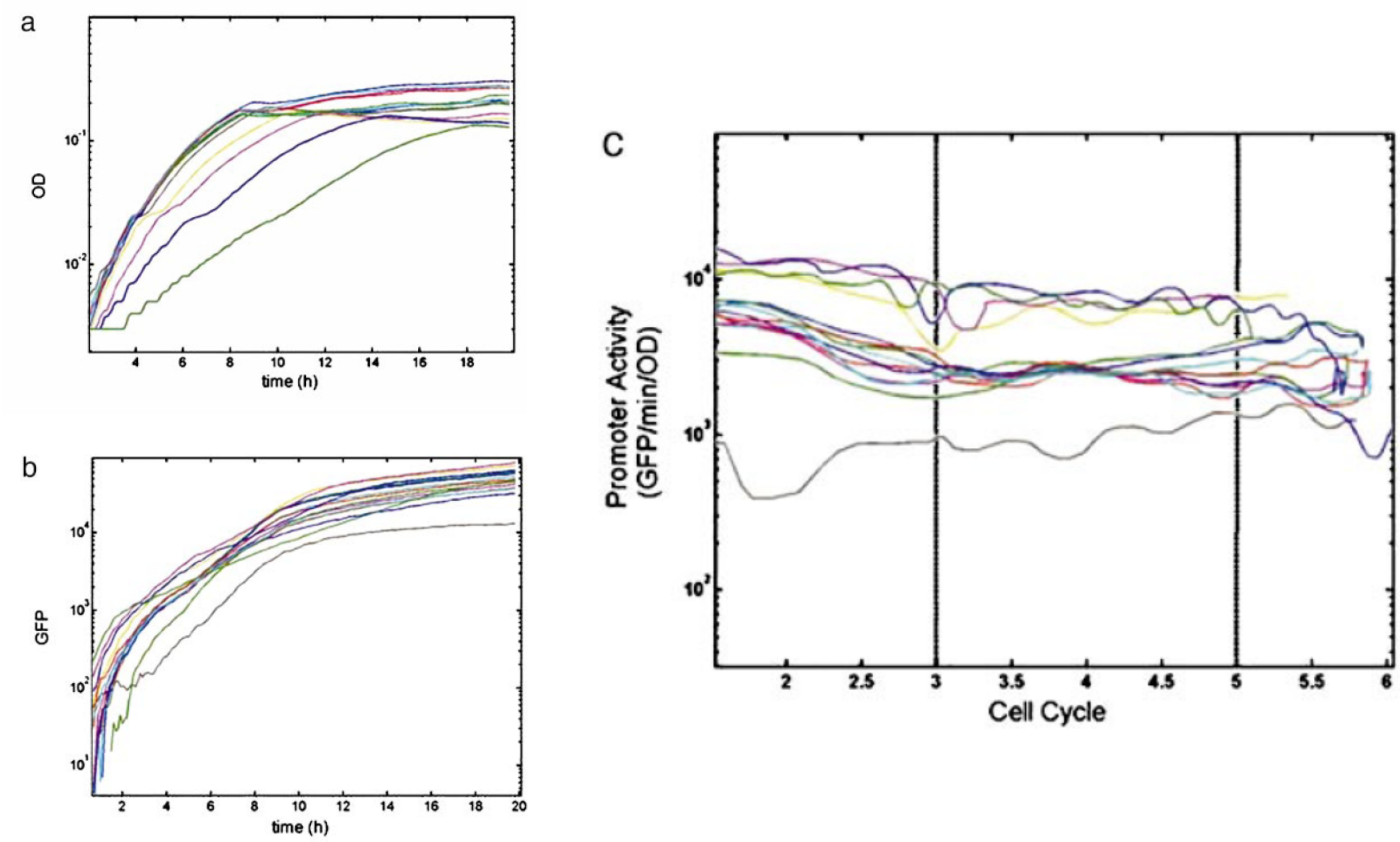
T: *total*, n, m: *Hillkoeffizienten*

$$a = \frac{[P]}{K_P}, \quad b = \frac{[P]}{K_{CP}}, \quad c = \frac{[R_T]}{K_R}, \quad d = \frac{[C_T]}{K_C}$$

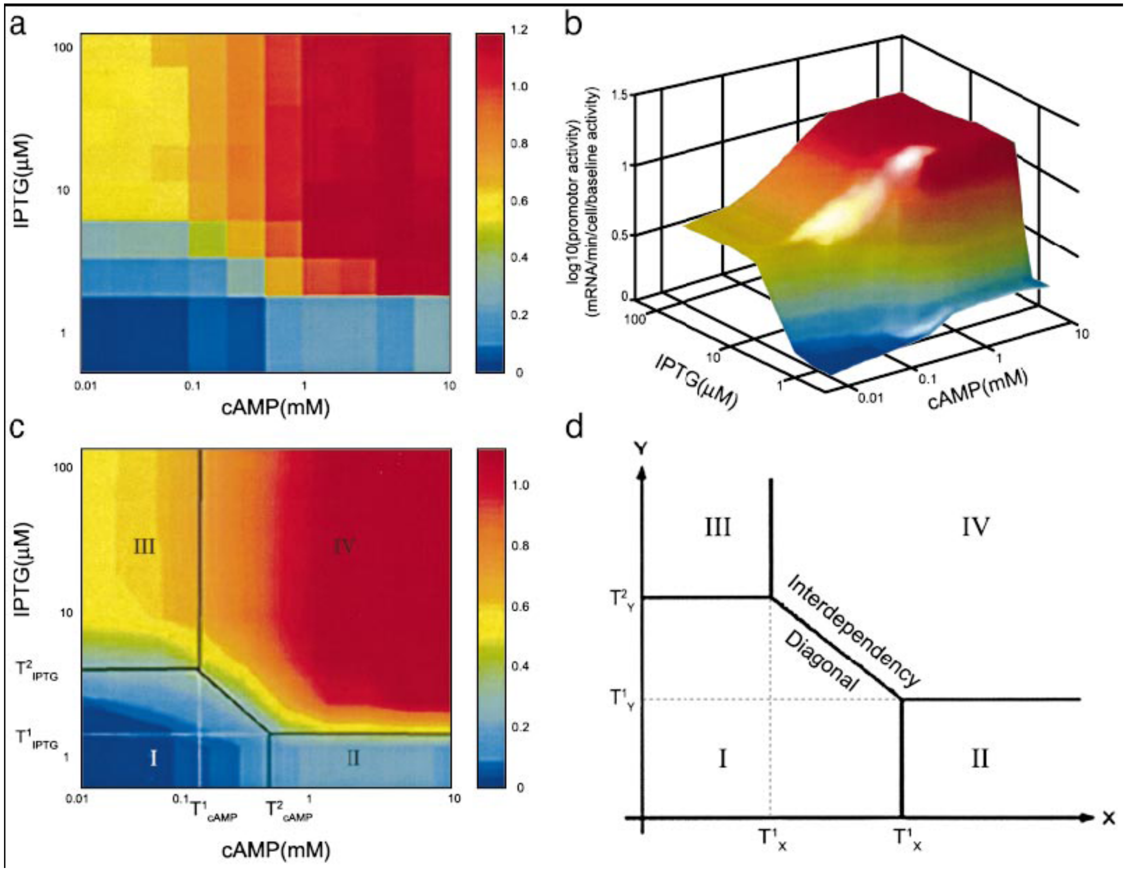


IPGT: Isopropyl-β-D-Thiogalaktosid
CRP: cAMP-Rezeptor-Protein

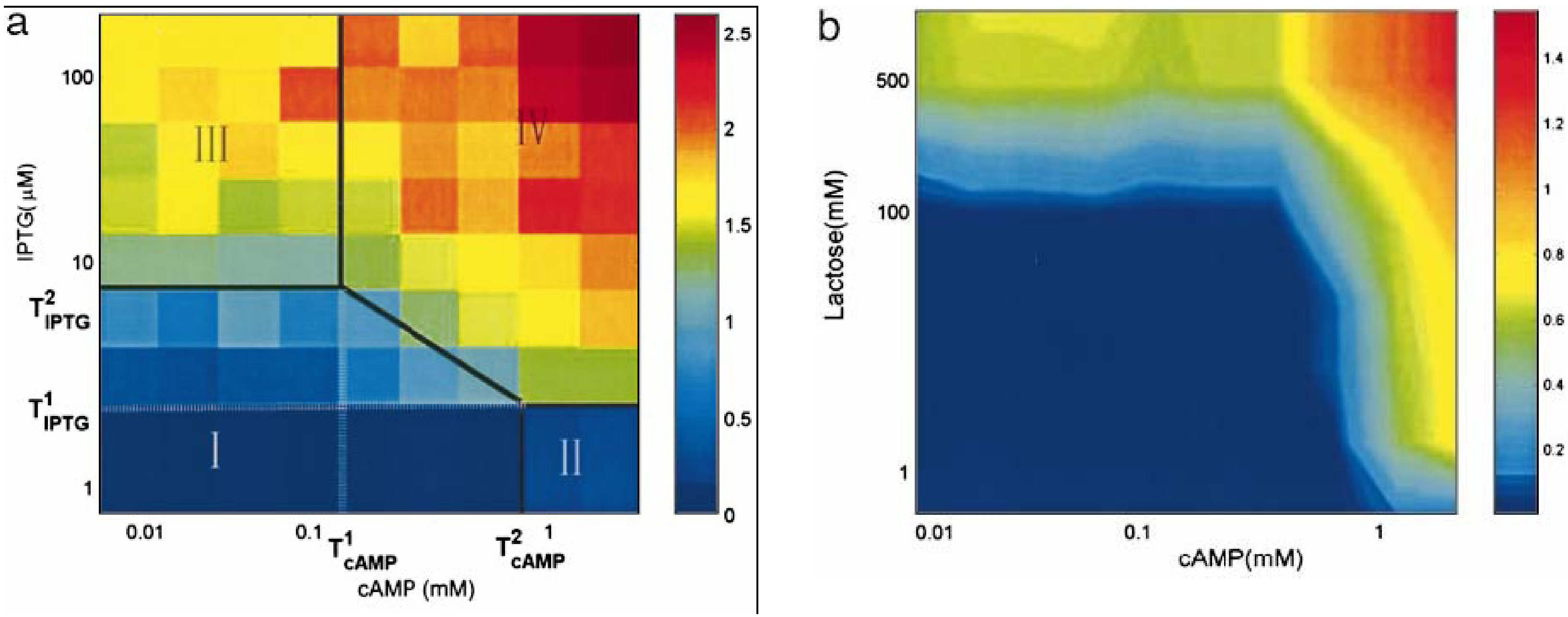
Promotoraktivität während des Zellzyklusses



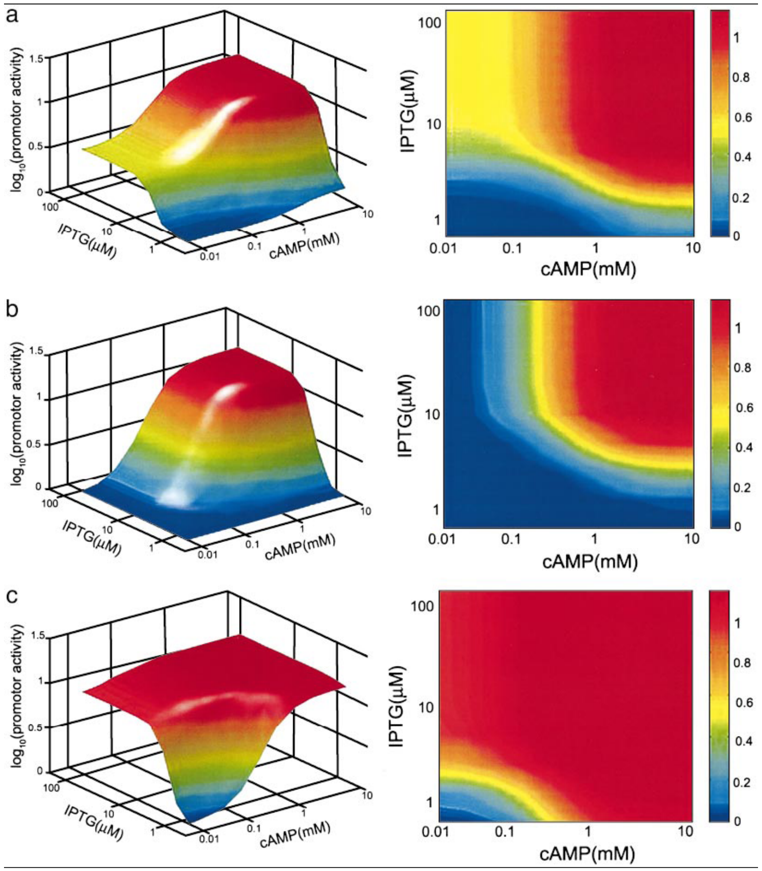
lac-cis-regulatorische Eingangsfunktion (GFP-gemessena)



lac-Eingangsfunktion (klorometrisch) und Laktosepromotoraktivität (GFP)



Lac-Regulationsmodell versus UND- und ODER-Modell



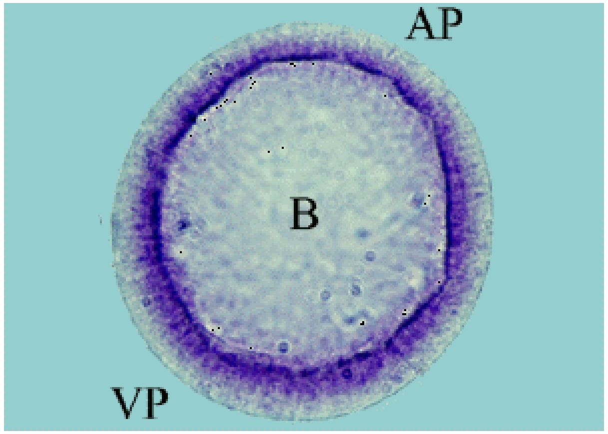
Genomic Cis-Regulatory Logic: Experimental and Computational Analysis of a Sea Urchin Gene

Chiou-Hwa Yuh, Hamid Bolouri, Eric H. Davidson

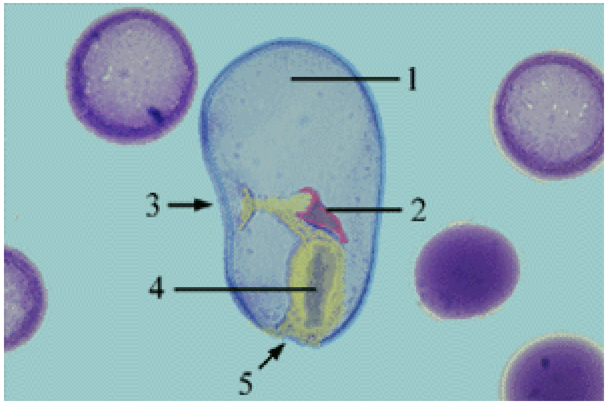
Science: 279 1896 - 1902 (1998)

Endo16-Expression

Endo16: Protein, sekretiert im Mitteldarm des späten Embryos und der Larve



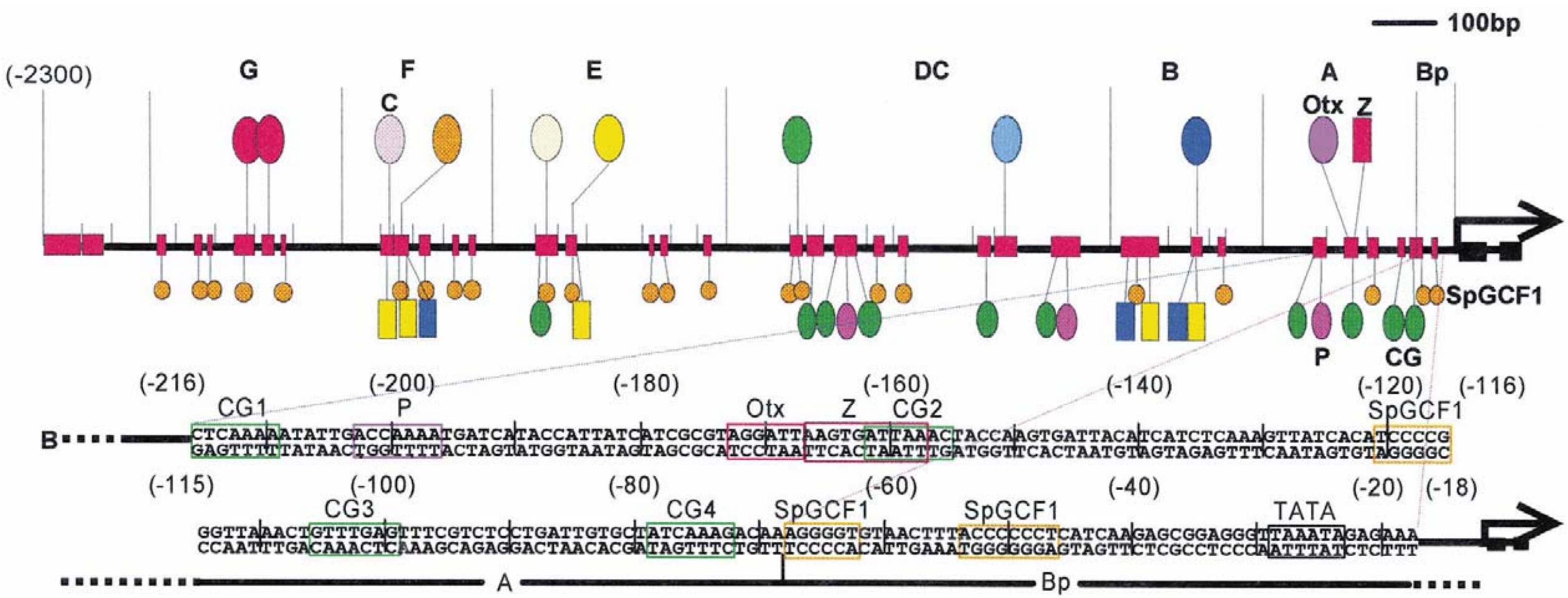
Späte Seeigel Blastula
AP: Animaler Pol
B: Blastocoel
VP: Vegetativer Pol



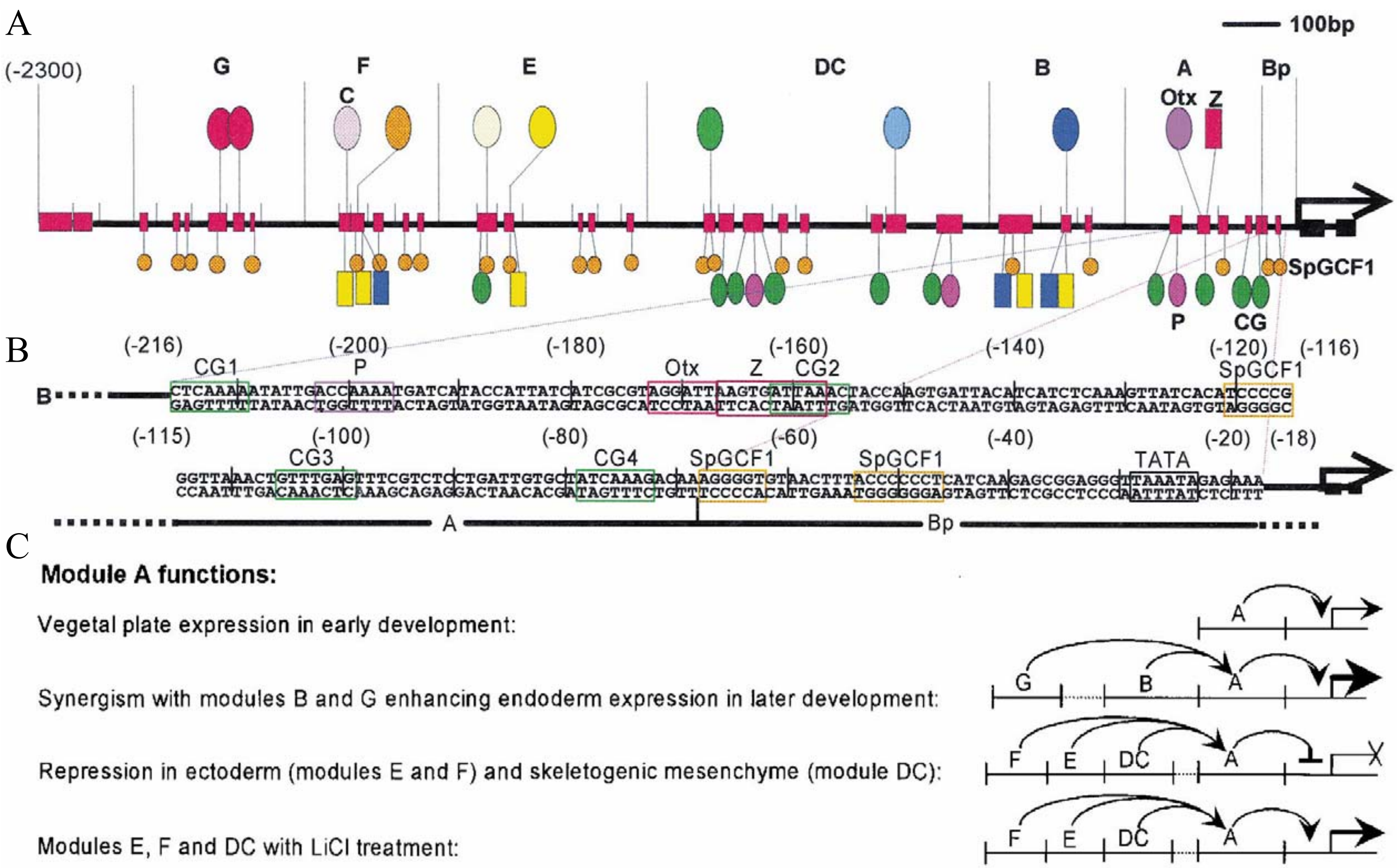
Seeigellarve
Blau: Exoderm
Rot: Mesoderm
Gelb: Endoderm

http://www.zum.de/Faecher/Materialien/hupfeld/index.htm?/Faecher/Materialien/hupfeld/Inhalt-Entwicklung.html

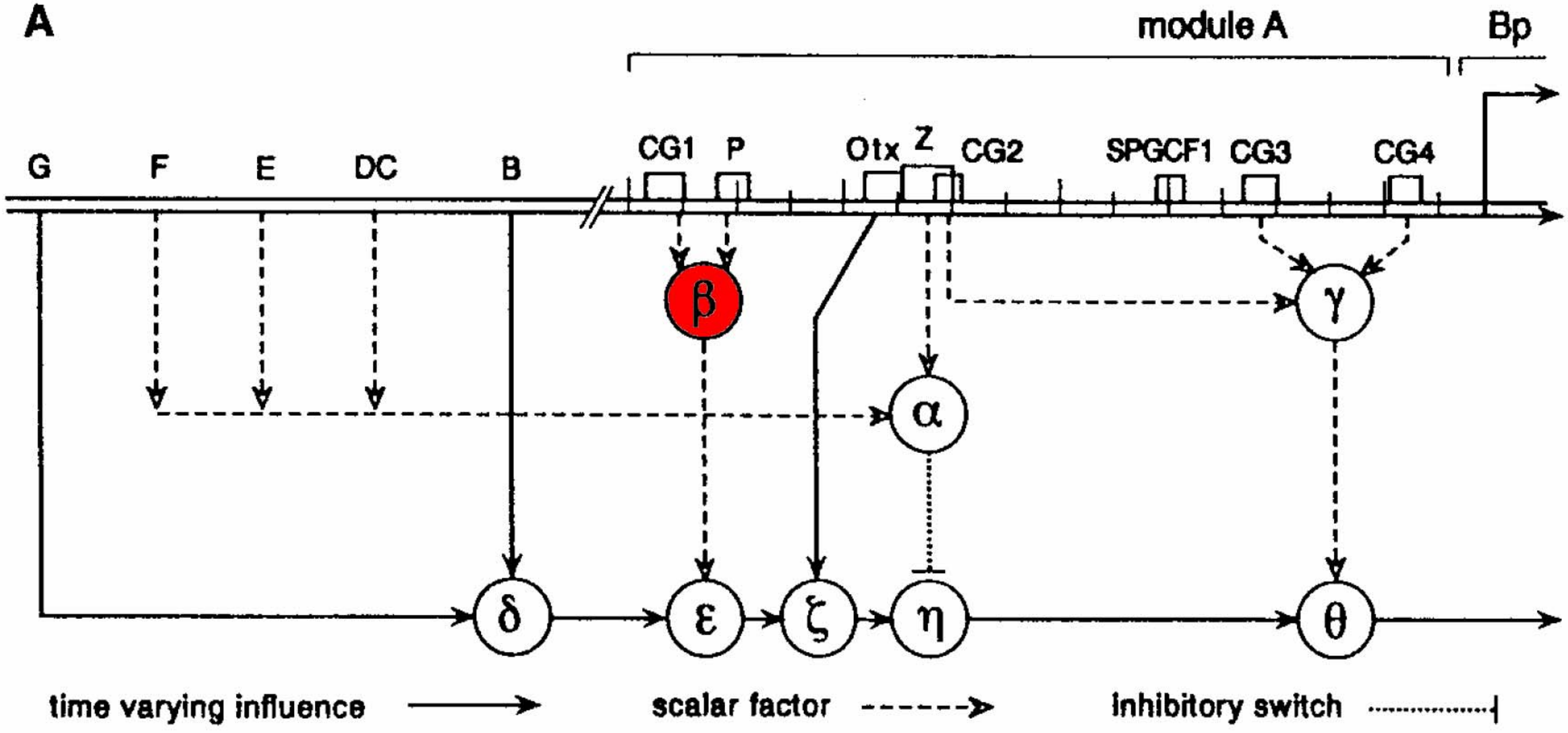
Endo16 cis-Regulationssystem



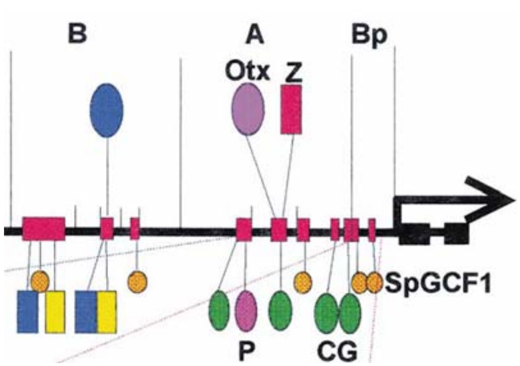
Endo16 cis-Regulationssystem

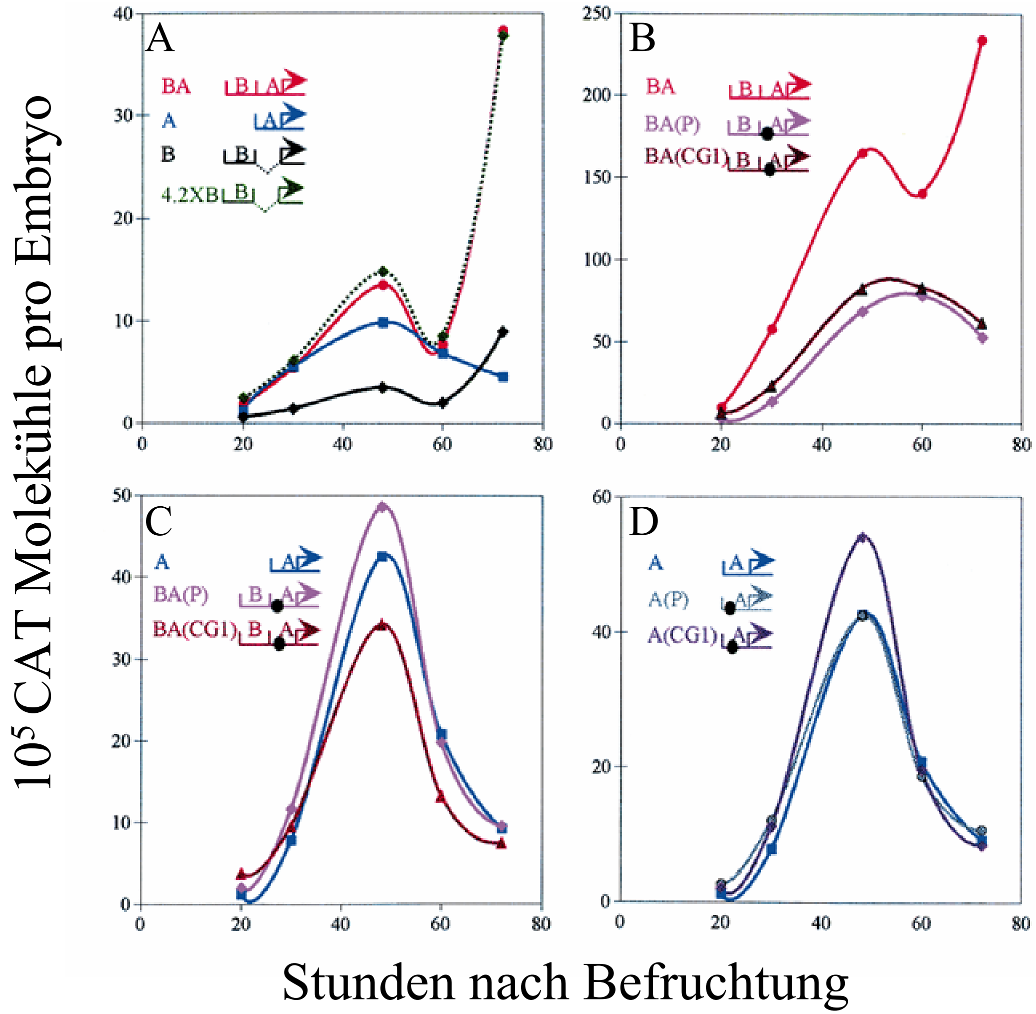


Das Modell: Eine Übersicht



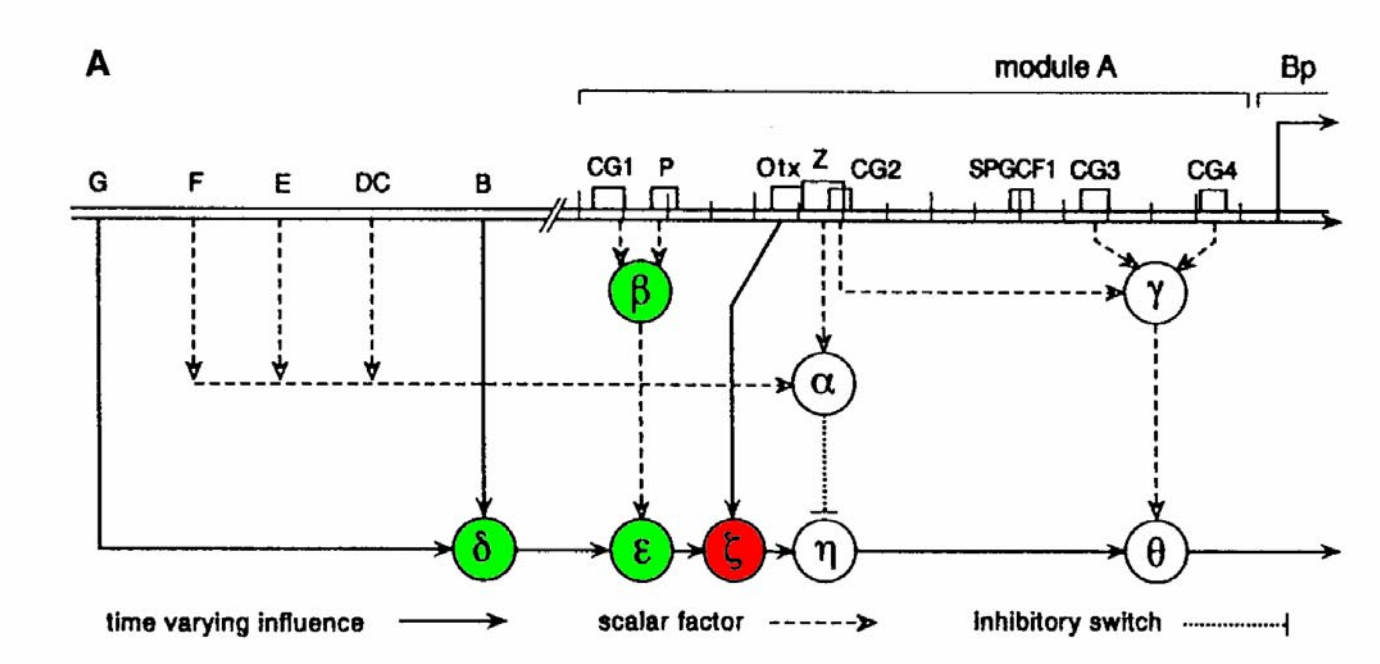
Interaktion zwischen Modul A und B





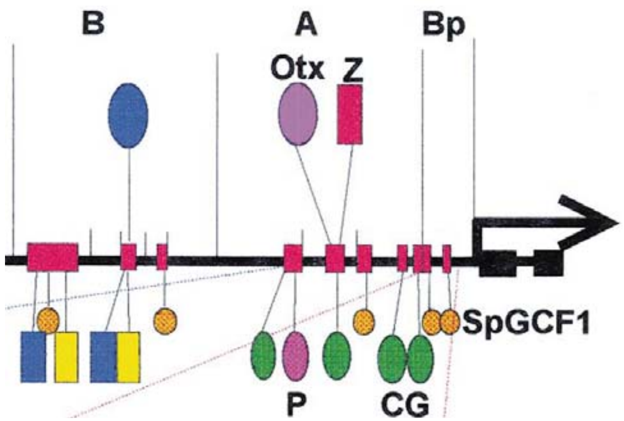
CAT: Chloramphenikol-Acetyl-Transferase

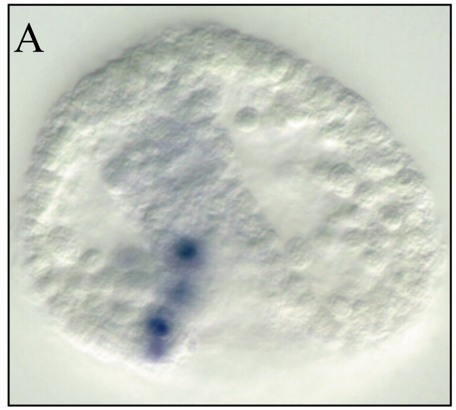
Expressionssteuerung durch CG_1- und P-Stelle



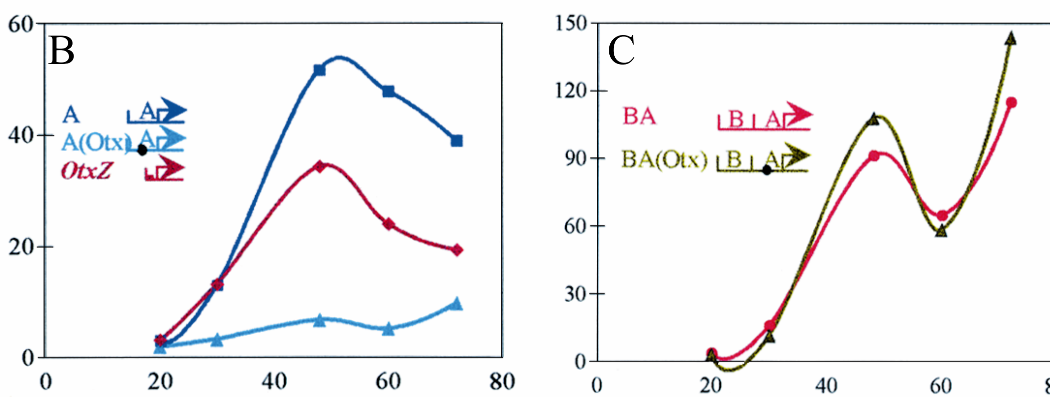
- if (P == 1 and CG1 == 1)
- $\beta := 2$ // Halbe Erklärung des Effektes
- else $\beta := 0$
- $\delta(t) := B(t) + G(t)$
- $\varepsilon(t) := \beta * \delta(t)$

Expressionsaktivität der Z- und der Otx-Stelle





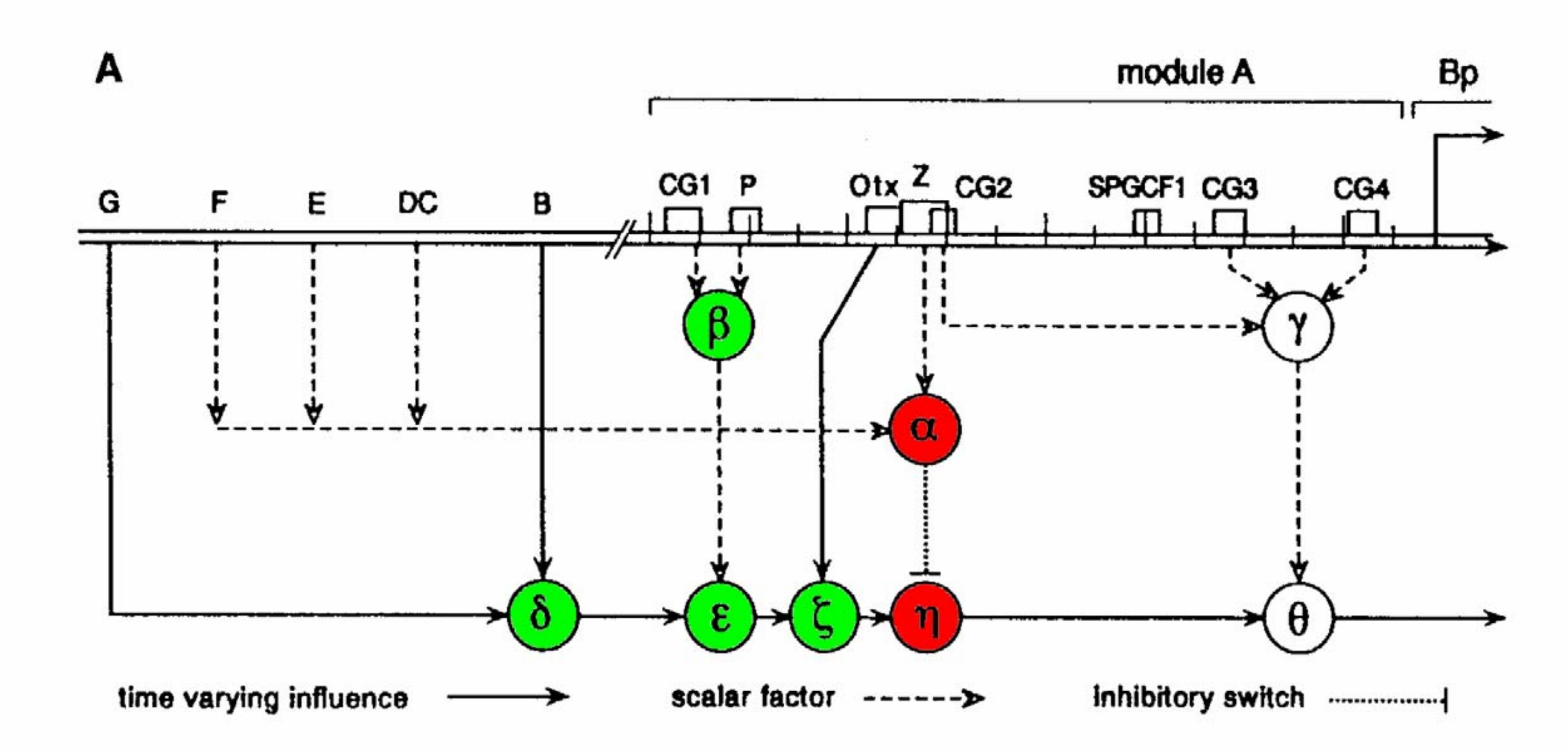
Constructs	Endoderm
OtxZ	56.5%
(Otx)Z	3.6%
Otx(Z)	72.0%
A	78.9%
A(Otx)	5.1%
ENDO16	97.0%



Stunden nach Befruchtung

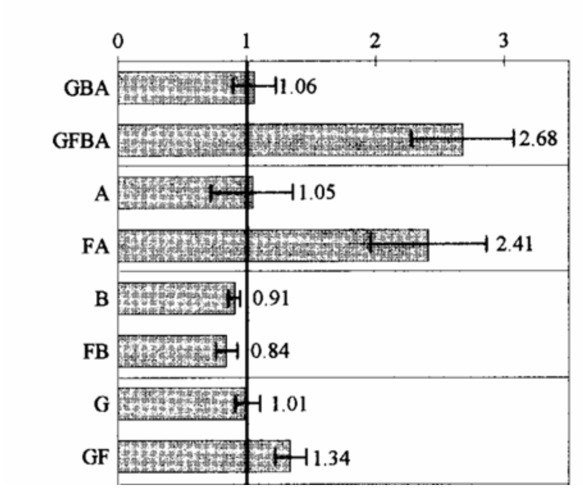
10^5 CAT Molekühle pro Embryo

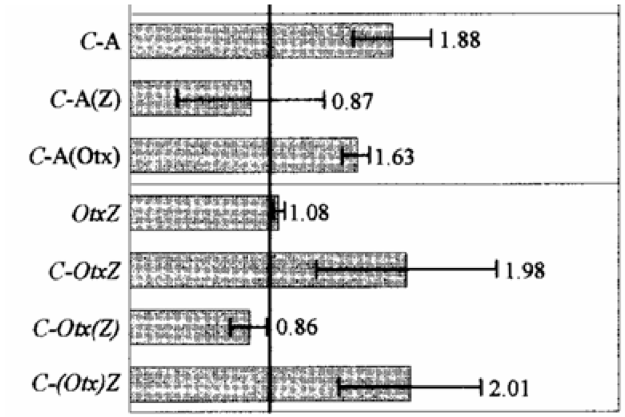
Einfluss der Otx-Stelle auf die Expression

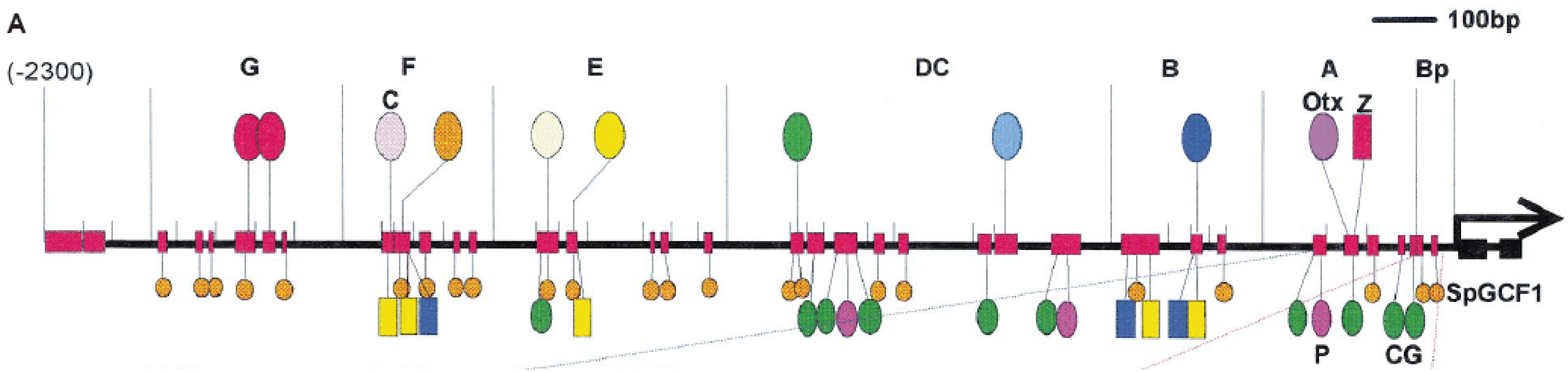


- if (ε(t) == 0)
- ζ(t) := Otx(t)
- elseζ(t) := ε(t)

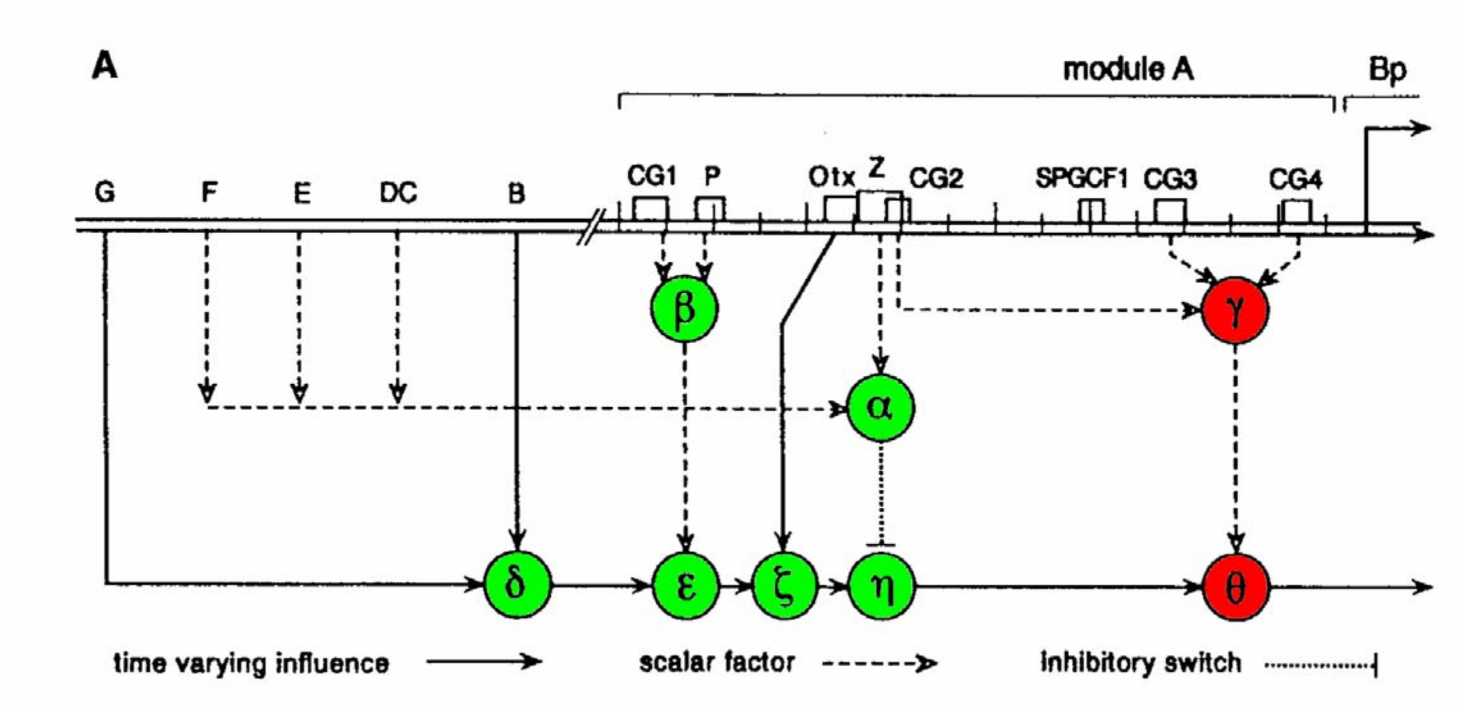
CAT-Aktivität unter LiCl Einfluss: Synergismus mit Modulen D, F oder C





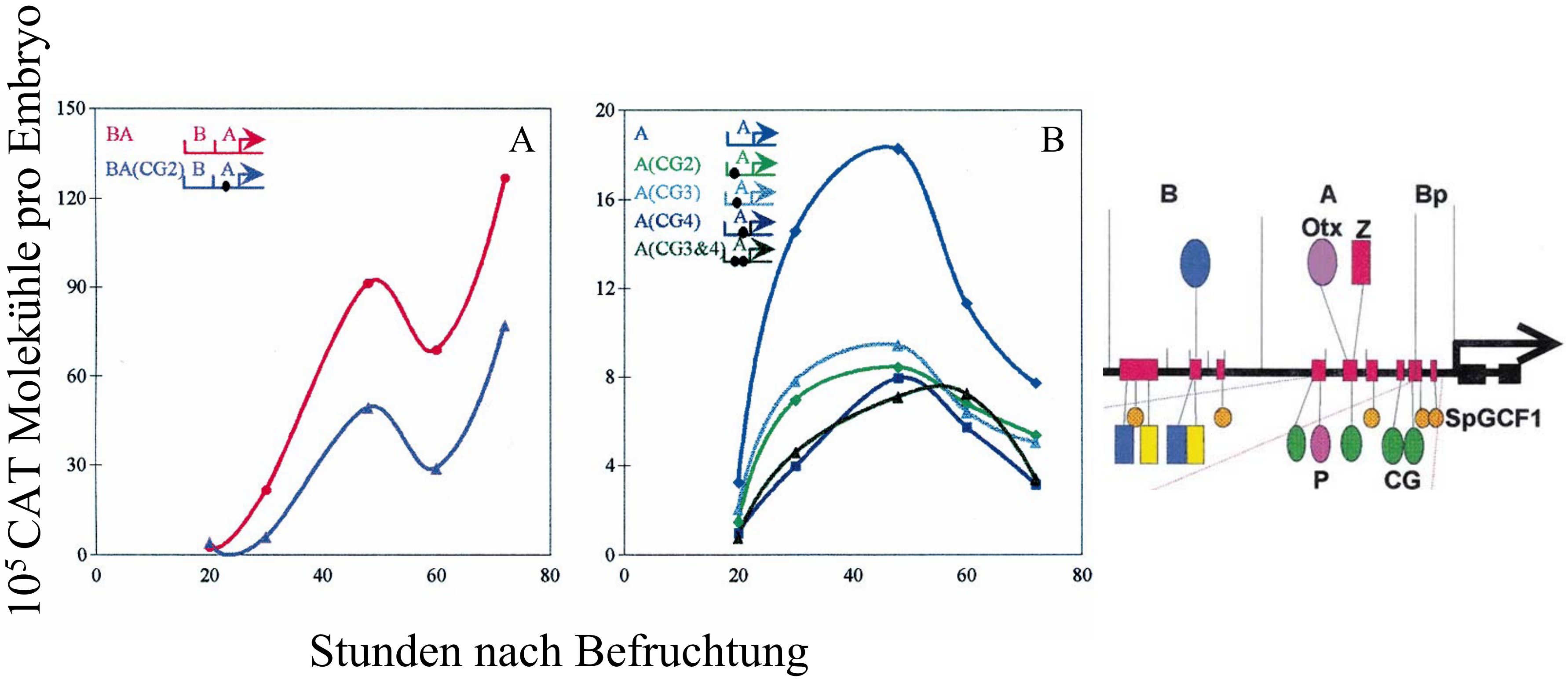


Interaktionsmodell zwischen der Z-Stelle und der Module F, E und DC

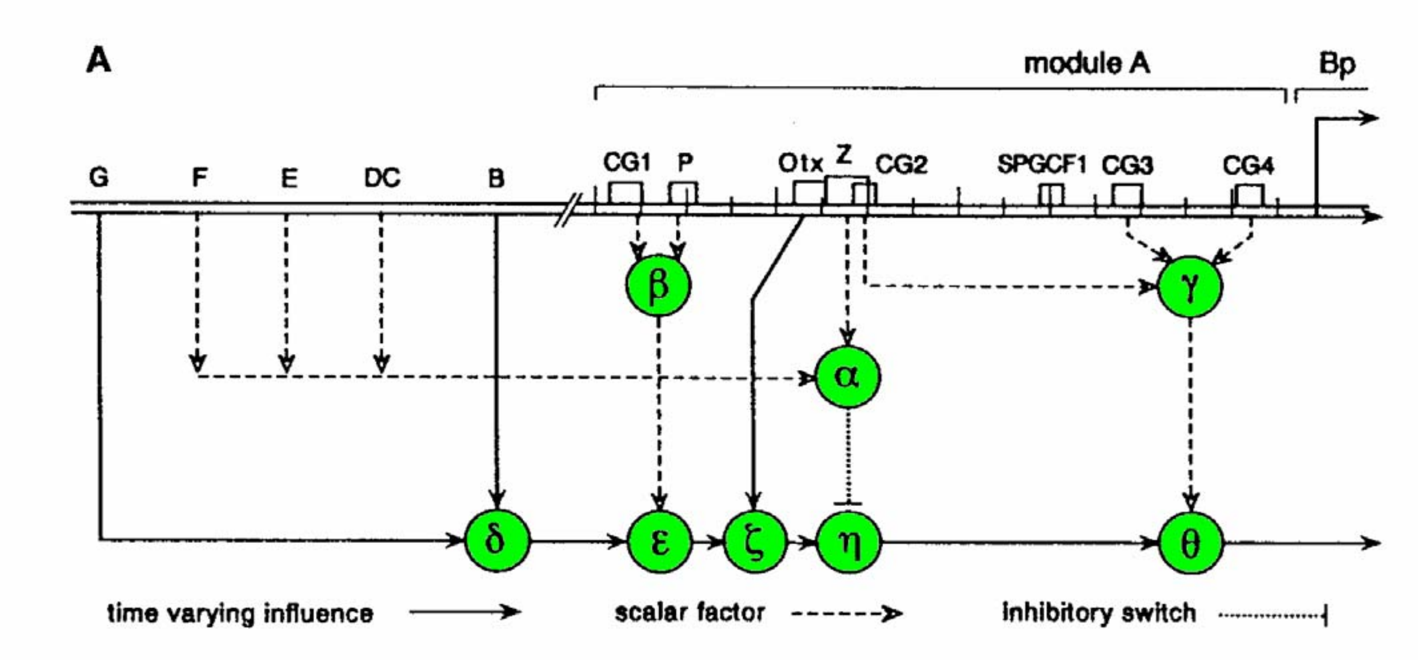


- if (F == 1 or E == 1 or CD == 1) and (Z == 1)
- α := 1
- else α := 0
- if (α == 1)
- η(t) := 0
- else η(t) := ζ(t)

Regulation durch $CG_{2,3,4}$-Stellen

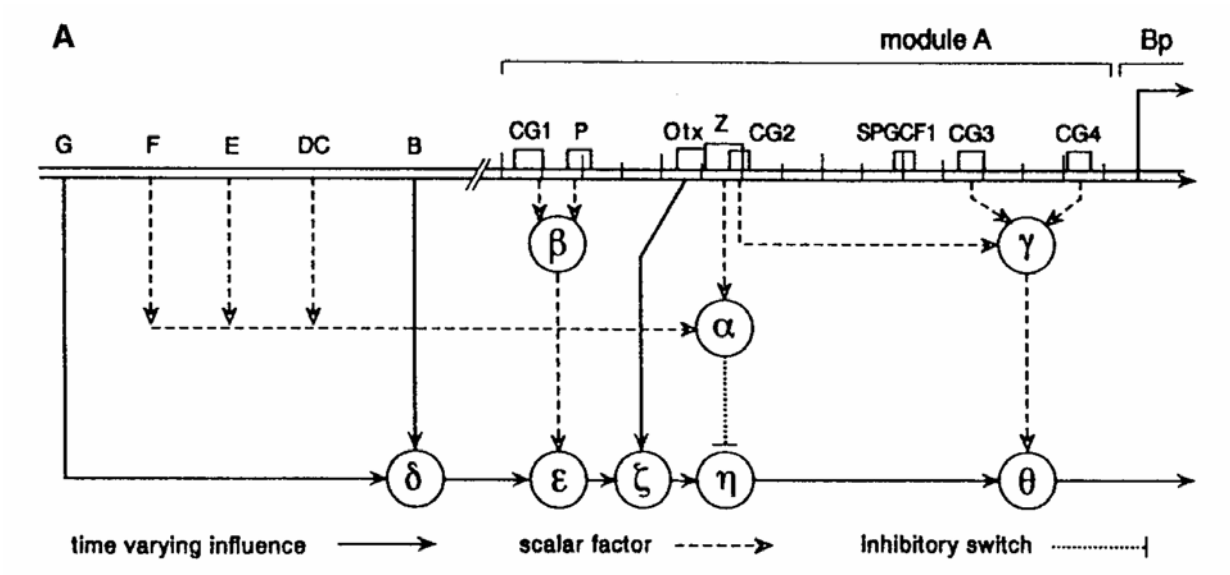


Einfluss der $CG_{2,3,4}$-Stellen



- if (CG2 == 1 and CG3 == 1 and CG4 == 1)
- $\gamma := 2$
- else $\gamma := 1$
- $\theta(t) := \gamma * \eta(t)$

Das Modell: Zusammengefasst



if (F == 1 or E == 1 or CD == 1) and (Z == 1)
$\alpha := 1$
else $\alpha := 0$
if (P == 1 and CG_1 == 1)
$\beta := 2$
else $\beta := 0$
if (CG_2 == 1 and CG_3 == 1 and CG_4 == 1)
$\gamma := 2$
else $\gamma := 1$
$\delta(t) := B(t) + G(t)$

$\varepsilon(t) := \beta * \delta(t)$

if ($\varepsilon(t) == 0$)
$\zeta(t) := Otx(t)$
else $\zeta(t) := \varepsilon(t)$
if ($\alpha == 1$)
$\eta(t) := 0$
else $\eta(t) := \zeta(t)$
$\theta(t) := \gamma * \eta(t)$