ALTOGETHER:

EXPERIMENTS

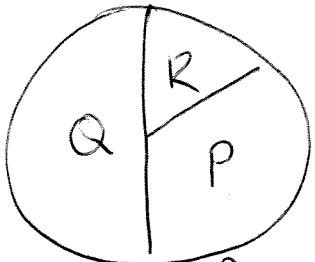
$\phi_R = \frac{\lambda}{\kappa_T} + \phi_R^{min}$

(CHANGE NUTRIENT)

$\phi_R = -\frac{\lambda}{\kappa_N} + \phi_R^{max}$

(CHANGE TRANSLATION)

HYPOTHESIS



PROTEOME PARTITION
$\phi_R + \phi_P = \phi_{max}$

INTERPRETATION:

κ_T: TRANSLATION RATE

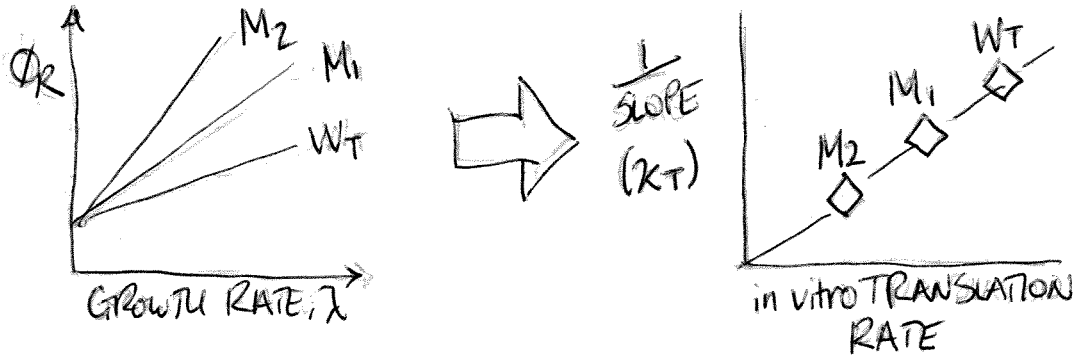
κ_N: NUTRIENT 'QUALITY'

ϕ_{max}: GROWTH-ALLOCATED PROTEOME FRACTION.

WE'LL LOOK AT THE PHENOMENOLOGICAL PARAMETERS κ_T, κ_N & ϕ_{max} IN MORE DETAIL

PROTEIN SYNTHESIS & κ_T

FROM THE TRANSLATIONAL MUTANTS, IT APPEARS THAT κ_T CORRELATES WITH THE in vitro TRANSLATION RATE

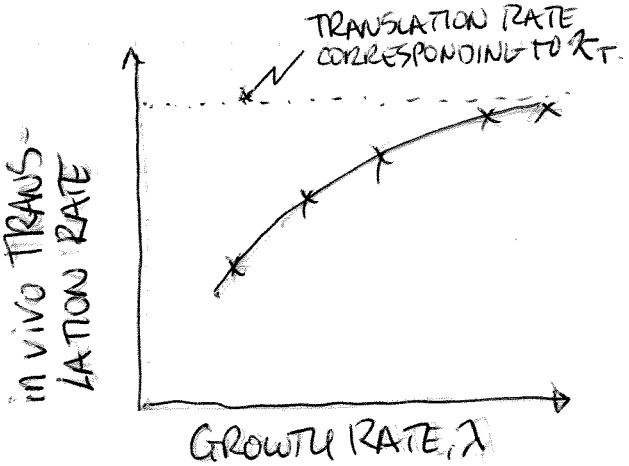


BUT THERE IS A PROBLEM: NEIDHARDT, MAGASANIK & MAALØE'S RATIONALIZATION OF THE LINEARITY (AND INTERPRETATION OF THE SLOPE)

$$\frac{dM_P}{dt} = \lambda M_P = k\,(N_{R_b} - N_{R_b}^0)$$

ASSUMES TRANSLATION RATE IS GROWTH-RATE INDEPENDENT.

WORK BY BREMER & YOUNG, AND LATER BY PEDERSEN USING A CLEVER GEL-BASED METHOD SHOWED THAT THE in vivo TRANSLATION RATE EXHIBITS STRONG GROWTH RATE DEPENDENCE!



HOW COULD THIS BE POSSIBLE?

THIS CAUSED A BITTER DIVIDE IN THE COMMUNITY - IS THERE ANY WAY A LINEAR RNA/PROTEIN RATIO CAN BE RECONCILLED WITH A GROWTH-DEPENDENT TRANSLATION RATE?

IT TOOK ALMOST FORTY YEARS TO PUT THE CONTROVERSY TO REST: STEFAN KLUMPP BEGAN BY ASKING: IS THERE AN ADMISSIBLE GROWTH DEPENDENCE IN THE TRANSLATION RATE THAT WILL STILL RETURN A LINEAR RNA/PROTEIN RATIO?

$$\frac{\lambda}{\kappa(\lambda)} = \phi_R - \phi_R^{min} \quad \text{OR} \quad \phi_R = \frac{\lambda}{\kappa(\lambda)} + \phi_R^{min}$$

THE ONLY CHOICE IS: $\kappa(\lambda) = \kappa^{max}\frac{\lambda}{\lambda + K_\lambda}$ ← MICHAELIS-MENTEN FORM. (RECOVER MAXIMUM'S CONSTANT WHEN $K_\lambda = 0$)

THEN $\phi_R = \frac{\lambda}{\kappa^{max}} + \left[\phi_R^{min} + \frac{K_\lambda}{\kappa_{max}}\right] \equiv \frac{\lambda}{\kappa^{max}} + \hat{\phi}_R^{min}$

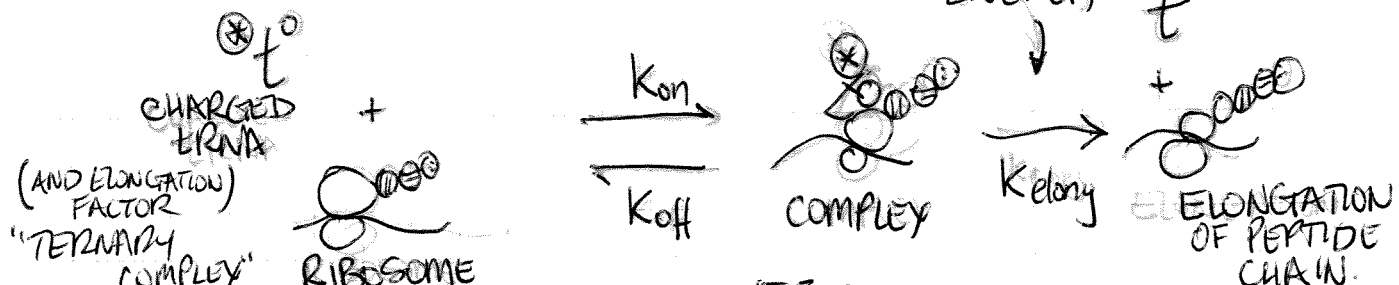
MODIFIED OFFSET.

$\frac{1}{\text{SLOPE}} \propto$ MAXIMUM TSL. RATE.

WHAT COULD LEAD TO A MICHAELIS-MENTEN FORM FOR THE TRANSLATION RATE? NOTICE THAT ANY VARIABLE $\alpha = a\lambda + b$ WOULD WORK AS WELL (IF $\frac{b}{a} \approx 0$, & λ IS $>> \phi_\lambda K_\lambda/a$)

$$\kappa(\alpha) = \kappa^{max}\frac{(a\lambda+b)}{(a\lambda+b)+K_\lambda} \simeq \kappa^{max}\frac{\lambda}{\lambda + K_\lambda/a}$$

PEPTIDE ELONGATION IN MORE DETAIL



ASIDE:

OVERALL REACTION SCHEME IS IDENTICAL TO ENZYME KINETICS

$$E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} ES \xrightarrow{k_{elong}} E + P$$

ENZYME SUBSTRATE COMPLEX PRODUCT

E <=> RIBOSOME S <=> CHARGED tRNA P <=> UNCHARGED tRNA (CHAIN GROWTH)

USING MASS-ACTION KINETICS, THE RATES OF FORMATION OF EACH SPECIES ARE:

$$\frac{d[E]}{dt} = -k_{on}[E][S] + k_{off}[ES] + k_{elong}[ES]$$

$$\frac{d[S]}{dt} = -k_{on}[E][S] + k_{off}[ES]$$

$$\frac{d[ES]}{dt} = k_{on}[E][S] - k_{off}[ES] - k_{elong}[ES]$$

$$\frac{d[P]}{dt} = k_{elong}[ES]$$

AND THE TOTAL ABUNDANCE OF ENZYME IS CONSERVED: $[E] = [E]^\circ - [ES]$

WE ARE INTERESTED IN THIS RATE; THIS IS THE RATE OF PROTEIN CHAIN ELONGATION

IF THE SUBSTRATE $[S] >> [E]$, THEN COMPLEX CONCENTRATION $[ES]$ DOES NOT CHANGE ON THE TIMESCALE OF PRODUCT FORMATION

$$\frac{d[ES]}{dt} \simeq 0 = k_{on}[E][S] - k_{off}[ES] - k_{elong}[ES]$$

BECAUSE $[E] = [E]^\circ - [ES]$,

$$(k_{off} - k_{elong})[ES] \simeq k_{on}[E]^\circ[S] - k_{on}[ES][S]$$

$$[ES]\left(\{k_{off} + k_{elong}\} + k_{on}[S]\right) \simeq k_{on}[E]^\circ[S]$$

$$\text{or} \quad [ES] = \frac{[E]^\circ[S]}{[S] + k_m} \quad \text{with } k_m = \frac{k_{off} + k_{elong}}{k_{on}}$$

FINALLY, $\frac{d[P]}{dt} = k_{elong}[E]^\circ \frac{[S]}{[S]+K_m}$

END OF ASIDE

OR, IN THE CASE OF PROTEIN TRANSLATION,

$$\text{RATE OF PROTEIN SYNTHESIS} = k_{elong}[Rb]\frac{[t^*]}{[t^*]+K_m}$$

$$\text{RATE OF PROTEIN SYNTHESIS PER RIBOSOME MASS} = \frac{k_{elong}}{t}\frac{[t^*]}{[t^*]+K_m}$$

THE DENSITY OF THE CELL IS GROWTH-RATE INDEPENDENT SO CONCENTRATION IS PROPORTIONAL TO MASS FRACTION,

$$\text{RATE OF PROTEIN SYNTHESIS PER RIBOSOME MASS} = k_{elong}\frac{\phi_T}{\phi_T + \hat{K}_m}$$

ϕ_T ← PROTEIN MASS FRACTION OF "TERNARY COMPLEX"

THE MASS ACCUMULATION EQUATION BECOMES,

$$\frac{dM_P}{dt} = \lambda M_P = k_T \frac{\phi_T}{\phi_T + \hat{K}_m} N_{Rb}$$

OR,

$$\frac{\lambda}{k_T} = k_T \frac{\phi_T}{\phi_T + \hat{K}_m}\phi_R$$

IF $\hat{K}_m/\phi_T \ll 1$, THEN $\frac{1}{1+\hat{K}_m/\phi_T} \simeq 1 - \frac{\hat{K}_m}{\phi_T} + \mathcal{O}\left(\frac{\hat{K}_m^2}{\phi_T^2}\right)$

AND

$$\frac{\lambda}{k_T} = \left(1 - \frac{\hat{K}_m}{\phi_T}\right)\phi_R = \phi_R - \hat{K}_m\frac{\phi_R}{\phi_T}$$

QUESTION: IS ϕ_R/ϕ_T A CONSTANT? YES! THE PROTEINS IN THE TERNARY COMPLEX ARE CO-REGULATED WITH RIBOSOMAL PROTEINS i.e. $\phi_T = \epsilon\phi_R$, SO THAT

$$\frac{\lambda}{k_T} = \phi_R - \phi_R^{min} \leftarrow \frac{\hat{K}_m}{\epsilon}$$

INTERPRETATION: 'INACTIVE' RIBOSOMES AWAITING CHARGED tRNA

PHYSICAL LIMITS IMPOSED BY DIFFUSION ENSURE $\hat{K}_m \neq 0$.

TAKEHOME: IF THE MICHAELIS CONSTANT (CHARACTERISTIC MASS FRACTION FOR EFFICIENT BINDING) IS LOW, $\hat{K}_m/\phi_T \ll 1$, THEN THE LINEAR RNA/PROTEIN RATIO VS GROWTH RATE CAN BE ~~JUST RATIONAL~~ RECONCILED WITH GROWTH-RATE DEPENDENT CHAIN ELONGATION BECAUSE $\phi_T \propto \phi_R$ [i.e. TERNARY COMPLEX PROTEINS CO EXPRESSED WITH rPROTEINS].

$$\frac{\lambda}{\kappa_T} = \frac{\phi_T}{\phi_T + \hat{K}_m}\phi_R \simeq \left(1 - \frac{\hat{K}_m}{\phi_T}\right)\phi_R = \phi_R - \phi_R^{min}$$

- λ/κ_T: MAX. ELONGATION RATE IF tRNA* IS SATURATING (AS IN in vitro EXP.)
- $\hat{K}_m/\phi_T \ll 1$
- $\phi_T = \epsilon\phi_R$: COREGULATION
- $\hat{K}_m/\epsilon$: PAUSED AWAITING tRNA*.

NUTRIENT QUALITY & κ_N

PROTEINS ARE MADE FROM AMINO ACIDS - BUT WHERE DO THE AMINO ACIDS COME FROM? LETS LOOK AT THE AMINO ACID FLUX:

$$\frac{dq}{dt} = J_{in} - J_{out}$$ A CONSERVATION LAW: RATE IN − RATE OUT.

FOR SIMPLICITY, IMAGINE THERE IS A SINGLE GROWTH LIMITING A.A. (IN UNITS OF $\mu g AA/mg$ PROTEIN)

WE KNOW THAT AMINO ACIDS ARE CONSUMED BY PROTEIN SYNTHESIS. FROM OUR RATIONALIZATION OF THE NUTRIENT GROWTH LAW,

$$J_{out} = \kappa_T(\phi_R - \phi_R^{min}) + \lambda a$$ ← DILUTION DUE TO GROWTH

WHAT ABOUT AMINO ACID SUPPLY? IN GENERAL, AMINO ACIDS ARE EITHER TRANSPORTED IN FROM THE ENVIRONMENT, OR BIOSYNTHESIZED WITHIN THE CELL VIA CENTRAL METABOLISM. SUPPOSE FOR NOW THAT THE GROWTH LIMITING AMINO ACID IS IN THE GROWTH MEDIUM SO THAT J_{in} IS THE TRANSPORT RATE.

$$J_{in} = k_{CAT}\,\phi_{TRANSPORTER} \cdot \frac{[AA]}{[AA] + K_m}$$

- $\phi_{TRANSPORTER}$: PROTEIN MASS FRACTION OF TRANSPORTER
- ← INSPIRED BY MONOD/MICHAELIS-MENTEN KINETICS.

IF THE TRANSPORTER PROTEIN IS PART OF THE METABOLIC P-SECTOR

THEN $\phi_{TRANSPORTER} = \epsilon \phi_P$

AND

$$J_{in} = \left[\frac{\epsilon k_{CAT} [AA]}{[AA] + K_m} \right] \phi_P$$

IN BALANCED GROWTH, THIS WILL BE A CONSTANT; CALL IT γ_N

FOR dq/dt:

$$\frac{dq}{dt} = \gamma_N \phi_P - \gamma_T (\phi_R - \phi_R^{min}) - \lambda q$$

IN BALANCED GROWTH: $dq/dt = 0$

IF SUPPLY & CONSUMPTION RATES MUCH FASTER THAN GROWTH (WHICH THEY MUST BE), THEN $\lambda q \approx 0$ COMPARED WITH THE OTHER TERMS.

$$\gamma_N \phi_P - \gamma_T (\phi_R - \phi_R^{min}) - \lambda q = 0$$

$\gamma_N \phi_P$: FROM PROTEOME CONSTRAINT $\phi_R^{max} - \phi_R = \phi_P$

$\gamma_T (\phi_R - \phi_R^{min})$: FROM NUTRIENT GROWTH LAW $\lambda = \gamma_T (\phi_R - \phi_R^{min})$

$$\gamma_N (\phi_R^{max} - \phi_R) = \lambda \quad \text{OR} \quad \boxed{\phi_R = -\frac{\lambda}{\gamma_N} + \phi_R^{max}}$$

THE INTERPRETATION IS THAT γ_N IS A LUMPED PARAMETER CHARACTERIZING THE ABILITY OF THE ORGANISM TO CONVERT NUTRIENTS TO AMINO ACIDS

IN SUMMARY:

EXPERIMENT

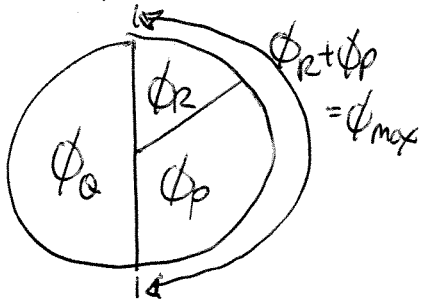
$\phi_R = \frac{\lambda}{\gamma_T} + \phi_R^{min}$

CHANGE NUTRIENT

$\phi_R = \frac{-\lambda}{\gamma_N} + \phi_R^{max}$

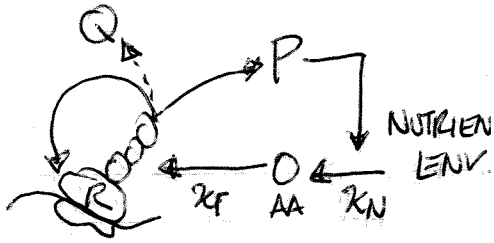
CHANGE TRANSLATION

HYPOTHESIS



COARSE-GRAINED PROTEOME PARTITION

MODEL/INTERPRETATION



ϕ_R DRIVES PROTEIN SYNTHESIS
ϕ_P ASSIMILATES NUTRIENTS TO FEED PROTEIN SYNTHESIS

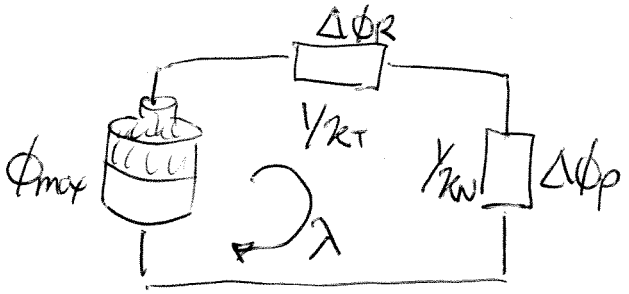
PROTEOME PARTITION HAS SUBSEQUENTLY BEEN VALIDATED BY PROTEOMIC STUDIES UNDER VARIOUS GROWTH CONDITIONS.

QUMICS: ELECTRICAL CIRCUIT ANALOGIES

THE TWO GROWTH LAWS CAN BE RE-WRITTEN AS:

$$\lambda = \gamma_T \Delta\phi_R \quad (\Delta\phi_R = \phi_R - \phi_R^{min})$$
$$\lambda = \gamma_N \Delta\phi_P \quad (\Delta\phi_P = \phi_{max} - \Delta\phi_R)$$

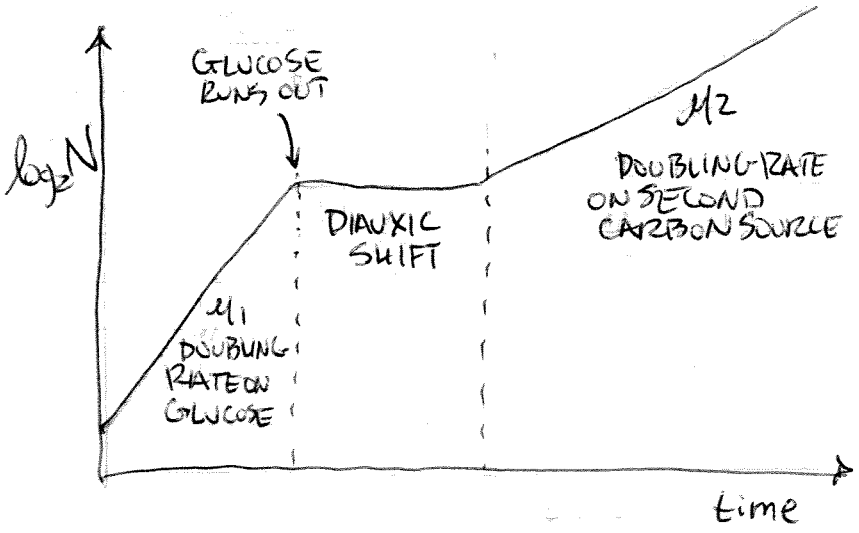
THEN, TOGETHER WITH THE PARTITIONING CONSTRAINT $\phi_{max} = \Delta\phi_R + \Delta\phi_P$, THE GROWTH LAWS ARE IDENTICAL TO KIRCHOFF'S LAWS APPLIED TO TWO RESISTORS IN SERIES -



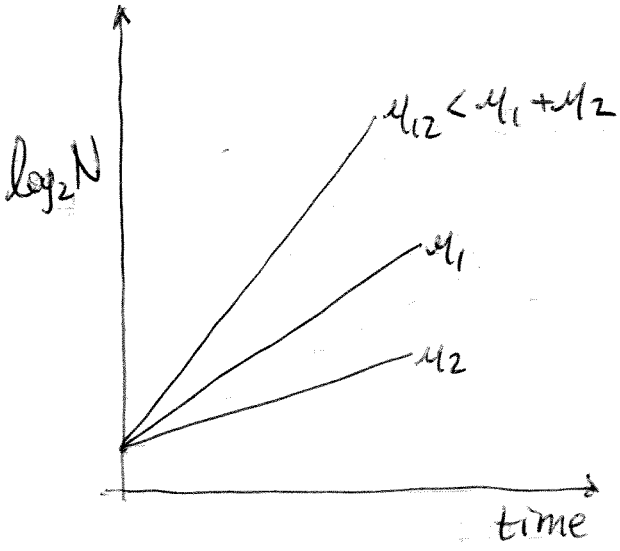
THE PHENOMENOLOGICAL PARAMETERS γ_T & γ_N PLAY THE ROLE OF THE CONDUCTANCE, THE MASS FRACTIONS $\Delta\phi_R$ & $\Delta\phi_P$ ARE ANALOGOUS TO THE POTENTIAL DROP ACROSS THE RESISTORS, AND THE GROWTH RATE λ PLAYS THE ROLE OF THE CURRENT. THE SIMPLICITY OF THIS PICTURE (& THE TOOLS AVAILABLE FOR NETWORK ANALYSIS) ALLOW EXTENSIONS OF THE BASE MODEL.

EXAMPLE - CARBON CO-UTILIZATION

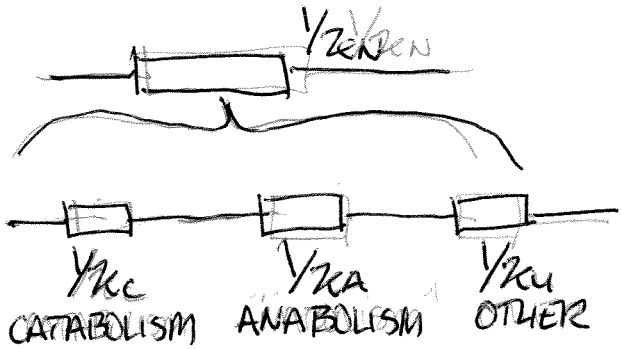
IN SOME CASES, MONOD NOTICED THAT E. COLI APPARENTLY PREFERENTIALLY USES ONE CARBON SOURCE OVER ANOTHER:



SOMETIMES, HOWEVER, BOTH CARBON SOURCES WERE USED SIMULTANEOUSLY.



LOOK AT THE METABOLIC RESISTOR IN MORE DETAIL: You et al (2013) SUBDIVIDED FURTHER INTO A CATABOLIC SECTOR (RESPONSIBLE FOR "BREAKING DOWN" NUTRIENTS), AN ANABOLIC SECTOR (RESPONSIBLE FOR "BUILDING UP") AND A REMAING UNASSIGNED SECTOR:

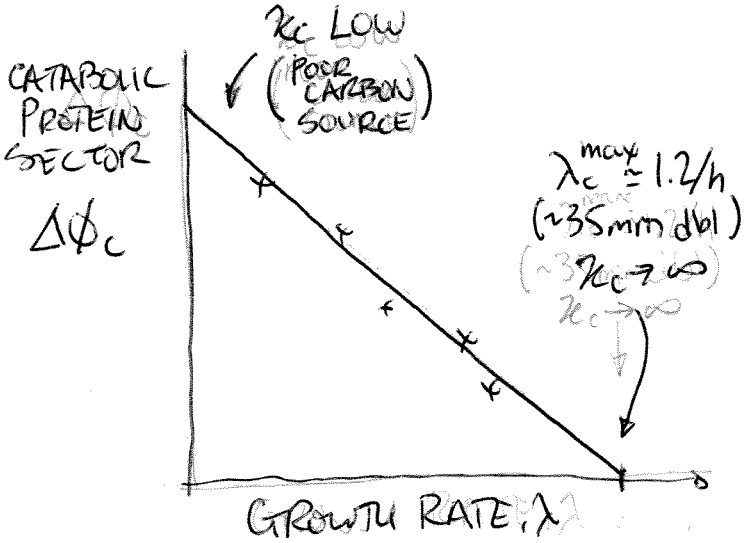


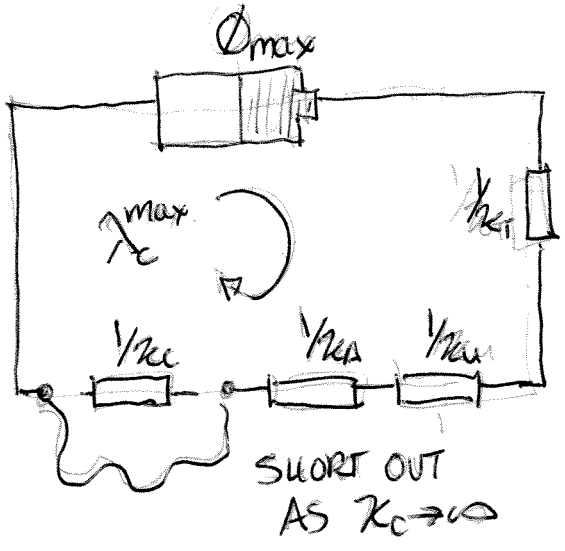
NOTICE THAT CHANGING κ_C OR κ_A WILL RESULT IN CHANGES IN THE PROTEIN FRACTION ASSIGNED TO $\Delta\phi_C$, $\Delta\phi_A$, $\Delta\phi_U$ (& $\Delta\phi_R$); WE CALL THIS 'INDIRECT' REGULATION OF GENE EXPRESSION.

THIS FRAMEWORK WAS USED TO RESOLVE A LONGSTANDING MYSTERY IN BACTERIAL PHYSIOLOGY CALLED 'CARBON CATABOLITE REPRESSION' REFERRING TO THE APPARENT HIERARCHICAL UTILIZATION OF DIFFERENT CARBON SOURCES.

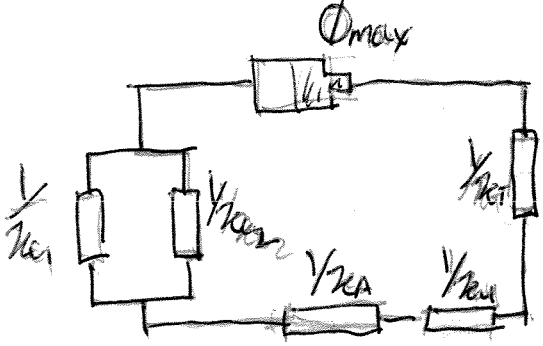
FOR THE COUTILIZATION PROBLEM, WE CAN QUANTIFY THE 'CONDUCTANCE' OF EACH SUBSECTOR BY CHANGING THE GROWTH ENVIRONMENT.

FOR EXAMPLE, IN MINIMAL MEDIA (BUFFER + NITROGEN), CHANGING THE <u>CARBON SOURCE</u> CHANGES κ_C





IMAGINE TWO COUTILIZED CARBON SOURCES - THE SIMPLEST HYPOTHESIS IS TO IMAGINE THEY REQUIRE AN ORTHOGONAL SET OF CATABOLIC GENES. IN THE CIRCUIT MODEL, THE CATABOLIC RESISTOR R_C IS REPLACED BY TWO RESISTORS IN PARALLEL:

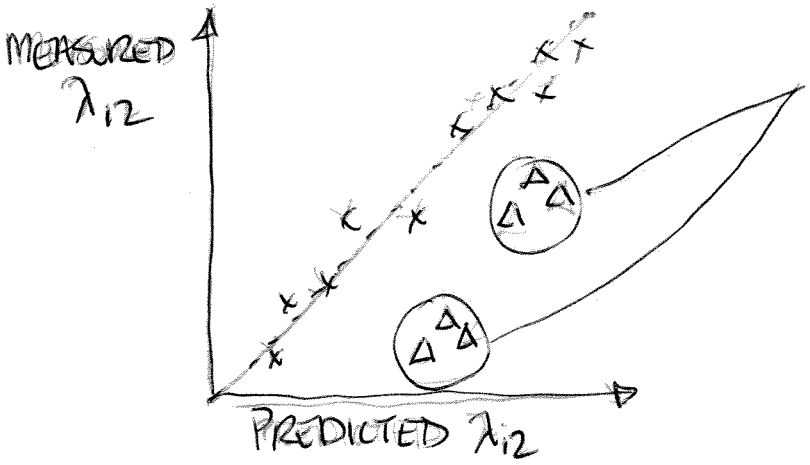


WHERE $1/R_{C_1}$ & $1/R_{C_2}$ ARE INFERRED FROM THE GROWTH RATE IN THE CORRESPONDING SINGLET CARBON SOURCE:

$$\lambda_i = \frac{\Phi_{max}}{1/R_T + 1/R_A + 1/R_M + 1/R_{C_i}} \qquad \left(\text{GIVEN } \lambda_C^{max} = \frac{\Phi_{max}}{1/R_T + 1/R_A + 1/R_M}\right)$$

THEN, THE PREDICTED GROWTH RATE FOR COUTILIZATION (ASSUMING ONLY INDIRECT PROTEOME PARTITIONING CONSTRAINTS) IS:

$$\frac{\lambda_{12}}{\lambda_C} = \frac{\hat{\lambda}_1 + \hat{\lambda}_2 - 2\hat{\lambda}_1\hat{\lambda}_2}{1 - \hat{\lambda}_1\hat{\lambda}_2} \qquad \left(\hat{\lambda}_i = \frac{\lambda_i}{\lambda_C}\right)$$



MECHANISMS OF DIRECT REGULATION THAT PREVENT/INHIBIT COUTILIZATION (PRIMARILY WITH GLUCOSE AND GLYCEROL)