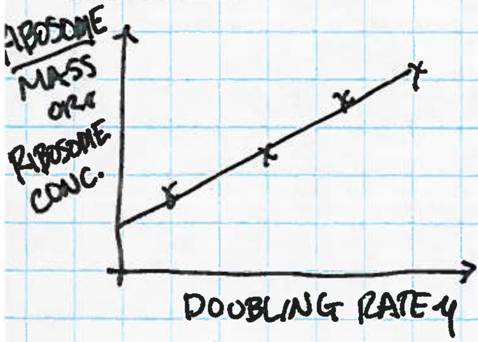
EMPIRICAL GROWTH LAWS

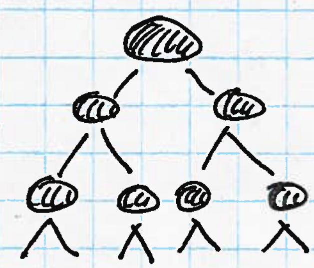


THE LINEAR RELATION BETWEEN DOUBLING RATE & RIBOSOME CONTENT IS OBSERVED IN ALL EXPONENTIALLY ORGANISMS

IS THIS AN EXAMPLE OF A 'LAW' IN BIOLOGY? IS THERE A SIMPLE EXPLANATION?

EXPONENTIAL GROWTH

THE FIRST STEP IS TO DESCRIBE EXPONENTIAL GROWTH MATHEMATICALLY:



SCALE TIME TO DOUBLING TIME

TIME $t=0$

$t=1$

$t=2$

LOOK AT HOW NUMBERS CHANGE:

	$t=0$	$t=1$	$t=2$	$t=3$	$t=4$	...
NUMBER BEFORE DIVISION $N(t)$	1	2	4	8	16	...
CHANGE IN POPULATION $N(t+\Delta t)-N(t)$	1	2	4	8	16	...
TIME STEP Δt	1	1	1	1	1	...

LOOKS LIKE: $\dfrac{N(t+\Delta t)-N(t)}{\Delta t} = N(t)$ $(*)$

IF $N(t)$ IS LARGE AND Δt IS SMALL ENOUGH THAT THE RELATIVE CHANGE IN $N(t)$ IS SMALL, THEN

$$\lim_{\Delta t} \frac{N(t+\Delta t)-N(t)}{\Delta t} = \frac{dN}{dt}$$

← "DERIVATIVE": THE INSTANTANEOUS RATE OF CHANGE IN N

THEN $(*)$ IS WRITTEN,

"EXPONENTIAL GROWTH RATE"

$$\frac{dN}{dt} = N \xrightarrow[\text{GENERALLY}]{\text{OR MORE}} \frac{dN}{dt} = \lambda N$$

WITH SOLUTION $N(t) = N_0 e^{\lambda t}$

$e \simeq 2.7$

COMPARE THIS EXPRESSION WITH $N(t) = N_0 2^{\mu t}$

TAKE LOGRITHM (BASE e) OF BOTH EXPRESSION FOR $N(t)/N_0$

$$\log_e(2^{\mu t}) = \log_e(e^{\lambda t})$$

$$\mu t \log_e(2) = \underbrace{\log_e(e)}_{1} \lambda t$$

OR $\lambda = \log_e(2) \times \mu$

$$\boxed{\lambda \simeq 0.693 \times \mu}$$

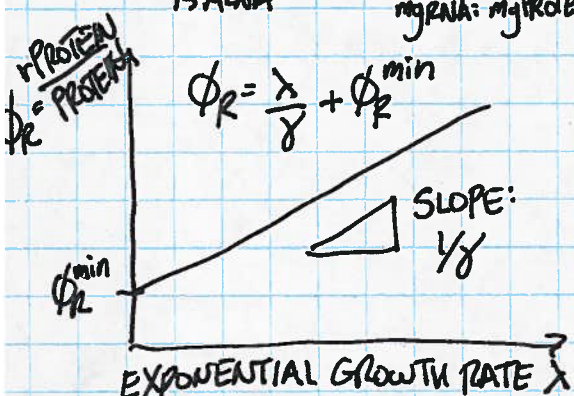
$dN/dt = \lambda N$ IS THE SIMPLEST DIFFERENTIAL EQUATION IMAGINABLE – BUT IT IS PROFOUND.

SUPPOSE, THROUGH REPEATED DILUTIONS, YOU HAVE A FLASK WITH BACTERIA THAT HAVE BEEN DOUBLING ONCE AN HOUR FOR 10 GENERATIONS. HOW QUICKLY IS THE DNA DOUBLING? THE RNA? THE PROTEIN?

WHAT ABOUT RIBOSOMES? THEY'RE MADE OF RNA & PROTEIN AND THEY MAKE PROTEIN. THERE IS INTRINSIC POSITIVE FEEDBACK. HOW QUICKLY DO THE RIBOSOMES DOUBLE?

WITH THE MATHEMATICAL FORMALIZATION OF EXPONENTIAL GROWTH, LET'S RETURN TO NEIDHARDT & MAGASANIK. IT WILL SIMPLIFY THE MATHEMATICS IF WE CONVERT TOTAL RNA TO THE MASS OF RIBOSOMAL PROTEINS $M_{rPROTEIN}$:

$$M_{RNA} \xrightarrow[\text{OF RNA IS rRNA}]{85\%} M_{rRNA} \xrightarrow[\text{IS 2:1 } m_g\text{RNA} : m_g\text{PROTEIN}]{\text{RIBOSOME}} M_{rProtein}$$



LOOK IN MORE DETAIL AT THE RATE OF PROTEIN SYNTHESIS:

$$\frac{dN}{dt} = \lambda N \xrightarrow[\text{FOR TOTAL PROTEIN}]{\text{SAME GOES}} \frac{dM}{dt} = \lambda M$$

HOW IS PROTEIN MADE? BY TRANSLATING RIBOSOMES!

$$\frac{dM}{dt} = \boxed{\lambda M = k\,(N_{Rb} - N^{o}_{Rb})}$$

k: RATE OF PROTEIN SYNTHESIS PER RIBOSOME

N_{Rb}: NUMBER OF RIBOSOMES

N^{o}_{Rb}: INACTIVE RIBOSOMES

SOLVE FOR λ:

$$\frac{\lambda}{k} = \frac{N_{Rb}}{M} - \frac{N^{o}_{Rb}}{M}$$

WE CAN CONVERT FROM N_{Rb} TO MASS OF RIBOSOMES $M_{rPROTEIN}$ WITH THE MASS OF PROTEIN PER RIBOSOMES: $m_R \times N_{Rb} = M_{rPROTEIN}$

~~WE~~ THEN:

$$\frac{\lambda}{(k/m_R)} = \frac{M_{rPROTEIN}}{M} - \frac{M^{o}_{rPROTEIN}}{M}$$

OR

$$\boxed{\frac{\lambda}{\gamma} = \phi_R - \phi_R^{min}}$$

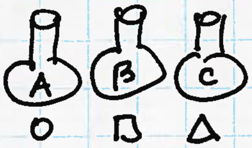
IF $\gamma = k/m_R$

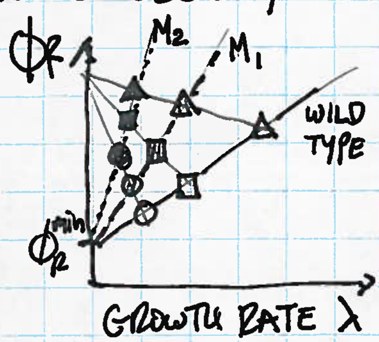
1^ST GROWTH LAW.

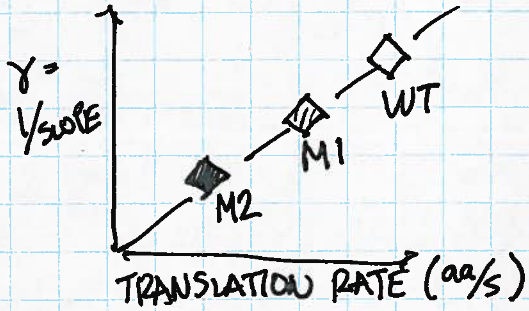
SLOPE TELLS YOU AVERAGE SPEED OF A RIBOSOME! $\gamma \approx 22$ aa./sec.

CORROBORATE BY LOOKING AT MUTANTS THAT SYNTHESIZE PROTEIN MORE SLOWLY:

MEDIA





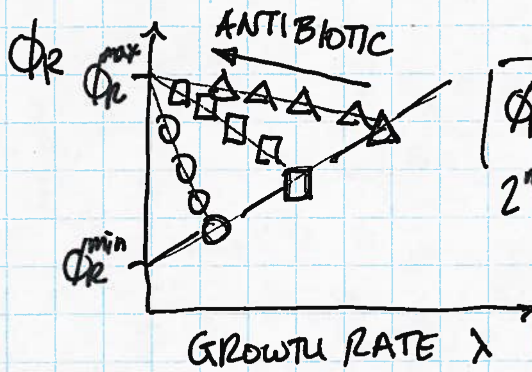


HERE WE HAVE A VERY NICE EXAMPLE OF HOW QUANTITATIVE MODELING & EXPERIMENT WORK TOGETHER.

LET'S GO FURTHER.... THIS IS ALMOST MIRACULOUS!

FROM THE MUTANT DATA, IT LOOKS LIKE THERE IS ANOTHER FAMILY OF LINES WITH NEGATIVE SLOPE.

TRY REDUCING PROTEIN SYNTHESIS RATE WITH ANTIBIOTICS...



$$\boxed{\phi_R = \phi_R^{max} - \frac{\lambda}{\nu}}$$

2^{nd} GROWTH LAW

TWO EMPIRICAL RELATIONS AT THE LEVEL OF RIBOSOME CONTENT:

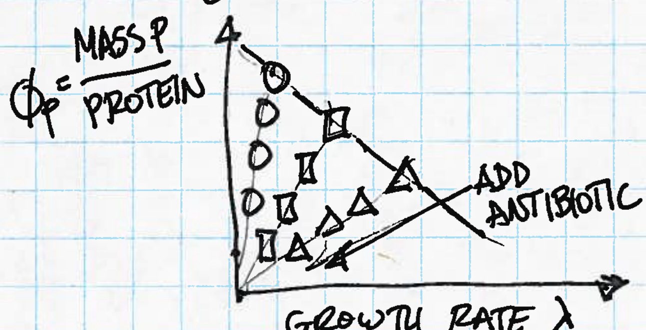
1. $\phi_R = \phi_R^{min} + \dfrac{\lambda}{\gamma}$

CHANGE NUTRIENT

2. $\phi_R = \phi_R^{max} - \dfrac{\lambda}{\nu}$

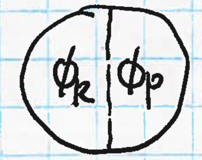
CHANGE TRANSLATION

REPEAT MONITORING A GENERIC UNREGULATED PROTEIN P



HOW WOULD YOU FIT THIS TOGETHER?

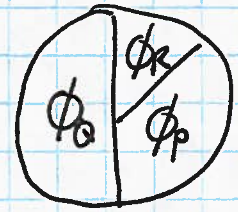
SIMPLEST MODEL: TWO KINDS OF PROTEIN



$\phi_R + \phi_P = 1$

BUT WHEN $\phi_P \to 0$, $\phi_R \to \phi_R^{max} \simeq 0.5$ (NOT 1)

OKAY - ADD A THIRD FRACTION ϕ_Q.



ϕ_Q: FIXED
ϕ_R: PROTEIN SYNTHESIS
ϕ_P: METABOLISM

WITH $\boxed{\phi_R + \phi_P = 1 - \phi_Q = \phi_R^{max.}}$

WE CAN USE THIS COARSE-PARTITIONING OF THE PROTEIN TYPES TO RATIONALIZE THE SECOND GROWTH LAW

$$\phi_R = \phi_R^{max} - \lambda/\nu.$$

PROTEINS ARE MADE FROM AMINO ACIDS - LET'S LOOK AT AA. FLUX:

$$\underbrace{\frac{da}{dt} = J_{in} - J_{OUT}}_{\text{CONSERVATION LAW: RATE IN - RATE OUT}}$$

WE KNOW AMINO ACIDS ARE CONSUMED BY PROTEIN SYNTHESIS. FROM RATIONALIZATION OF 1ST GROWTH LAW,

$$J_{OUT} = \gamma(\phi_R - \phi_R^{min})$$

WHAT ABOUT AA SUPPLY - J_{in}. SUPPOSE AA ARE IN THE GROWTH ENVIRONMENT SO J_{in} IS TRANSPORT RATE.

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

INSPIRED BY MONOD - DOESN'T REALLY MATTER; ASSUME $J_{in} \propto \phi_{TRANS.}$

IF TRANSPORTER IS IN P-SECTOR,

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

THEN

$$J_{in} = \underbrace{\left[\epsilon k_{cat} \frac{AA}{AA + k_M}\right]}_{\substack{\text{CONSTANT} \\ \nu}} \phi_P$$

FOR da/dt:

$$\frac{da}{dt} = \nu\phi_P - \gamma(\phi_R - \phi_R^{min}) \xRightarrow[da/dt = 0]{\text{AT STEADY-STATE}} \nu\phi_P = \gamma(\phi_R - \phi_R^{min})$$

$$\nu \phi_P = \gamma(\phi_R - \phi_R^{min})$$

FROM PROTEIN PARTITION $\phi_P = \phi_R^{max} - \phi_R$

FROM 1ST GROWTH LAW $\lambda = \gamma(\phi_R - \phi_R^{min})$

$$\boxed{\nu(\phi_R^{max} - \phi_R) = \lambda}$$ 2nd GROWTH LAW

INTERPRETATION IS: ν IS A LUMPED PARAMETER CHARACTERIZING THE ABILITY OF THE ORGANISM TO CONVERT NUTRIENTS TO AA.

SUMMARY –

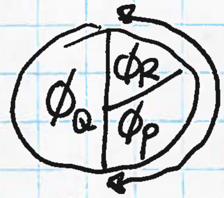
EXPERIMENT

$$\phi_R = \phi_R^{min} + \lambda/\gamma$$

$$\phi_R = \phi_R^{max} - \lambda/\nu$$

GROWTH LAWS

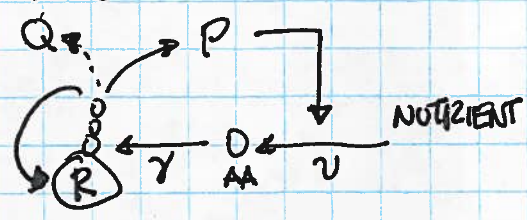
HYPOTHESIS



$\phi_R + \phi_P = \phi_R^{max}$

COARSE-GRAINED PROTEIN PARTITION

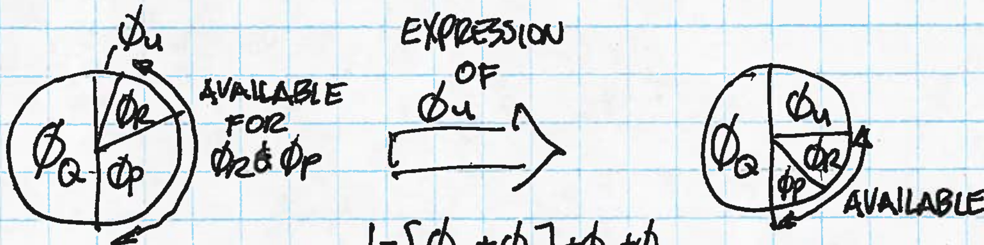
MODEL



ϕ_R DRIVES PROTEIN SYNTHESIS
ϕ_P ASSIMILATES NUTRIENTS TO FEED PROTEIN SYNTHESIS

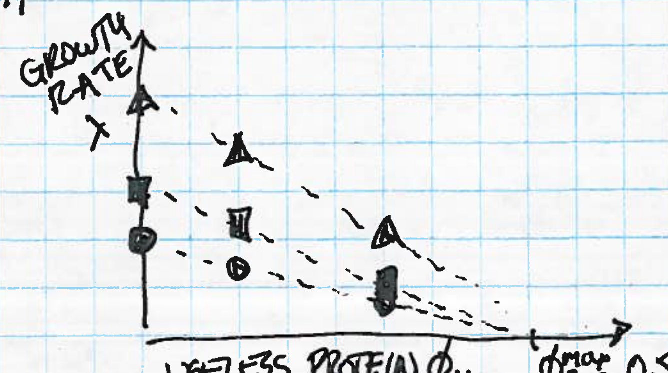
<u>LOOK AT SOME IMPLICATIONS</u>

1. <u>OVEREXPRESSION OF USELESS PROTEIN</u>: BACTERIA IS OFTEN USED TO PRODUCE HIGH-VALUE BIOPRODUCTS – VALUABLE TO US (LIKE INSULIN), BUT PROVIDE NO BENEFIT TO THE BACTERIUM. THE MASS FRACTION OF USELESS PROTEIN IS ϕ_u; IT BASICALLY INCREASES THE FIXED OVERHEAD ϕ_Q:

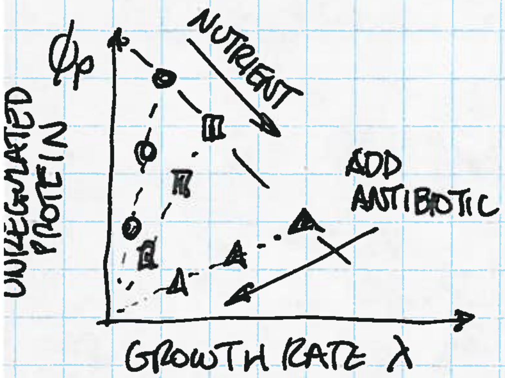


$$1 = [\phi_Q + \phi_u] + \phi_R + \phi_P$$

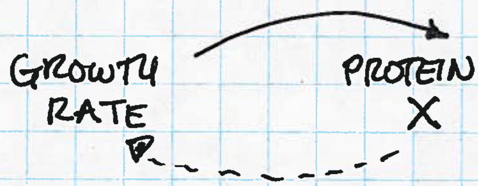
FROM THE 1ST GROWTH LAW,
$\lambda \propto \phi_R$
WE WOULD PREDICT A <u>LINEAR</u> DECREASE IN GROWTH RATE λ AS ϕ_u INCREASES – WITH $\lambda = 0$ WHEN $\phi_u = \phi_R^{max} \simeq 0.5$



2. GROWTH-MEDIATED FEEDBACK: CHANGES IN GROWTH RATE CHANGE PROTEIN LEVELS, EVEN UNREGULATED PROTEINS



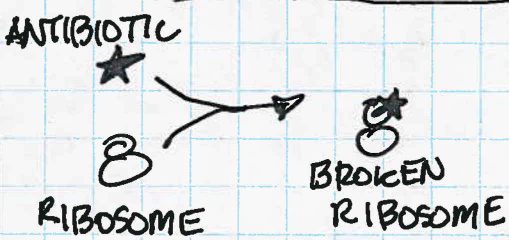
WHAT IF ONE OF THOSE PROTEINS CHANGES GROWTH RATE? eg. ANTIBIOTIC RESISTANCE.



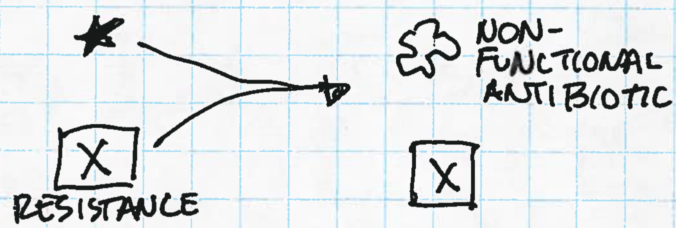
FEEDBACK LOOP THAT IS NOT CODED DIRECTLY IN THE GENOME!

CONSIDER RIBOSOME-TARGETING ANTIBIOTICS:

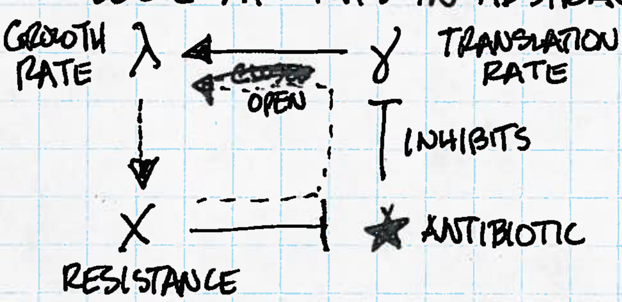
MECHANISM OF ACTION



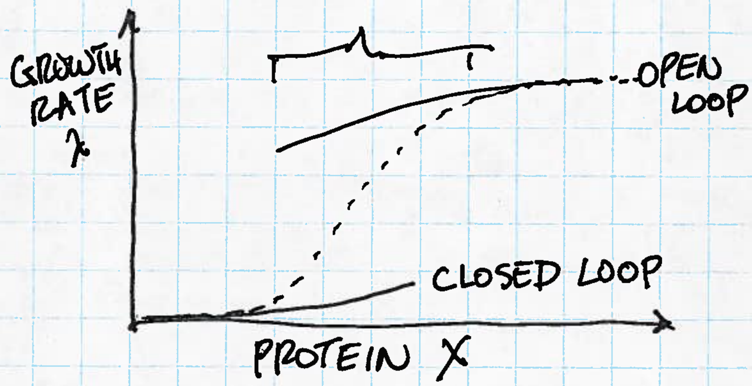
RESISTANCE



LOOK AT THIS IN ABSTRACT



BISTABLE!



CLOSED LOOP EXHIBITS POSITIVE FEEDBACK: eg. MICROPHONE POINTED AT A SPEAKER.
POTENTIALLY BISTABLE; CERTAINLY VERY SENSITIVE.

THIS TYPE OF FEEDBACK IS MEDIATED BY GROWTH