MODERN BACTERIAL PHYSIOLOGY

~~IN THE CLASSICAL EXPERIMENTS~~ WE HAVE LOOKED

TWO MAJOR ADVANCES IN THE LAST DECADE - CHARACTERIZATION OF RESOURCE ALLOCATION CONSTRAINTS (PARTICULARLY AT THE LEVEL OF PROTEIN SYNTHESIS) & REAL-TIME SINGLE CELL OBSERVATION. FOCUS WILL BE ON CONSTRAINTS (THAT IS THE WORK I KNOW BEST), BUT WILL BRIEFLY DISCUSS THE SINGLE-CELL VARIANT OF HELMSTETTER'S BABY MACHINE.

MODULATION OF GROWTH RATE

IN THE CLASSICAL EXPERIMENTS WE HAVE LOOKED AT, GROWTH RATE WAS MODULATED BY NUTRIENT QUALITY. GIVEN THE ~~NATURE~~ ECOLOGY OF E. COLI, THIS MAY BE A NATURAL CHOICE.

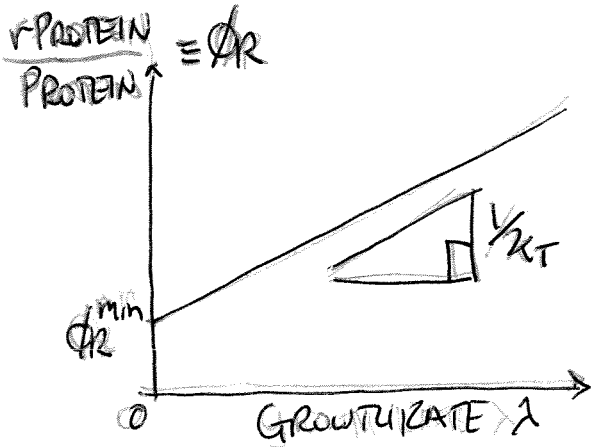
FOR OVER TWENTY YEARS, THE GROUP OF HANS BREMER MEASURED MANY PHYSIOLOGICAL PARAMETERS AT DIFFERENT GROWTH RATES MODULATED BY NUTRIENT QUALITY (ie. COMBINED DATA OF MAALØE, NIEDHARDT & MAGASANIK, COOPER & HELMSTETTER AT THE SAME TEMPERATURE, WITH THE SAME STRAIN OVER 5 WELL-DEFINED GROWTH CONDITIONS).

THE RESULT IS A NEAR-COMPLETE CHARACTERIZATION OF THE PHYSIOLOGY IN THE SPIRIT OF THE COPENHAGEN SCHOOL. BUT THERE ARE MANY OTHER WAYS TO MODULATE GROWTH, eg.

PHYSICAL PERTURBATION: TEMP., OSMOLARITY, PRESSURE...
CHEMICAL PERTURBATION: ANTIBIOTICS & GROWTH INHIBITORS...
BIOLOGICAL PERTURBATION: PROTEIN OVEREXPRESSION, TOXIC PROTEINS

IT IS POSSIBLE TO REPEAT BREMER'S ANALYSIS FOR A GIVEN GROWTH PERTURBATION. WE MAY, HOWEVER, GAIN SOME INSIGHT BY OBSERVING A SUBSET OF KEY REPORTERS eg. THE RIBOSOME.

EMPIRICAL 'GROWTH LAWS' - RIBOSOME ABUNDANCE



RETURNING TO THE ANALYSIS OF NEIDHARDT & MAGASANIK FOR THE RNA/PROTEIN, EMPIRICALLY WE OBSERVE SIMILAR GROWTH DEPENDENCE IN THE rPROTEIN/PROTEIN ϕ_R,

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

WE CAN RATIONALIZE THIS LINEARITY IN THE SAME WAY AS THE RNA/PROTEIN RATIO. IN BALANCED GROWTH

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

(k: TRANSLATION RATE; M_P: PROTEIN MASS; N_{Rb}°: INACTIVE RIBOSOMES.)

THEN,

$$\frac{\lambda}{k} = \frac{N_{Rb}}{M_P} - \frac{N_{Rb}^{\circ}}{M_P}$$

WE CAN CONVERT N_{Rb} TO THE RIBOSOMAL PROTEIN (rPROTEIN) MASS USING THE PER RIBOSOME PROTEIN MASS M_{Rb}:

$M_{rPROTEIN} = M_{Rb} \cdot N_{Rb}$

$$\frac{\lambda}{(k/M_{Rb})} = \frac{M_{rPROTEIN}}{M_P} - \frac{M_{rPROTEIN}^{\circ}}{M_P} = \phi_R - \phi_R^{min}$$

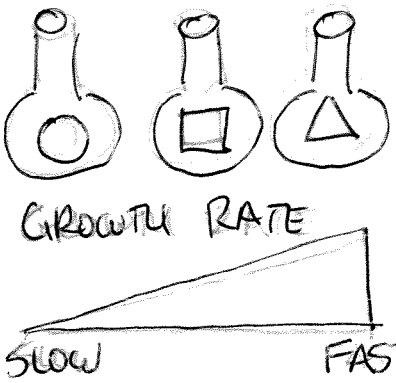
OR

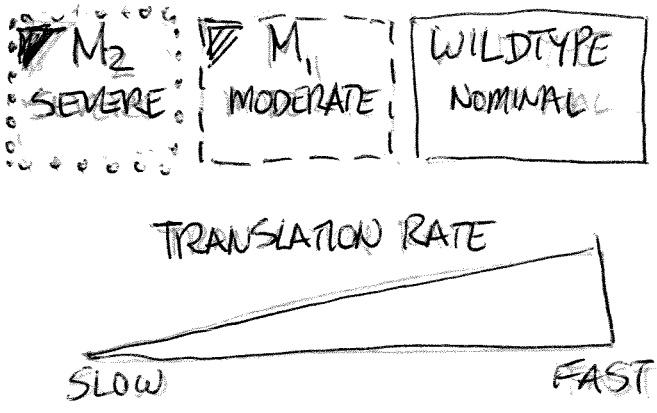
$$\boxed{\phi_R = \frac{\lambda}{\kappa_T} + \phi_R^{min}}$$

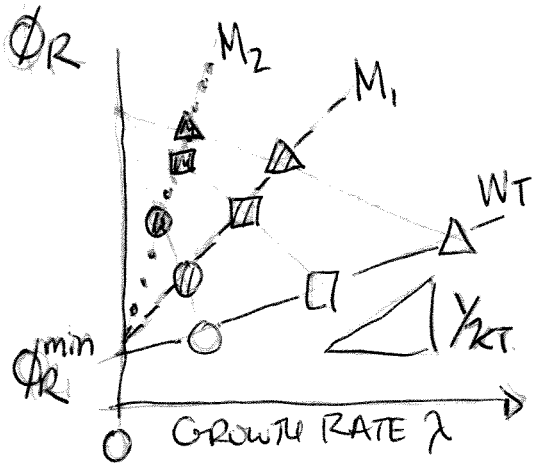
IF: i) $\kappa_T = k/M_{Rb}$
(ie. SLOPE INVERSELY-CORRELATED WITH TRANSLATION RATE)
ii) $\phi_R^{min} = M_{Rb}^{\circ}/M_P$
(ie. INTERCEPT CORRELATED WITH INACTIVE R_b FRACTION)

EASIER TO TEST THAN ii);
CAN USE MUTANTS THAT SYNTHESIZE PROTEIN MORE SLOWLY.

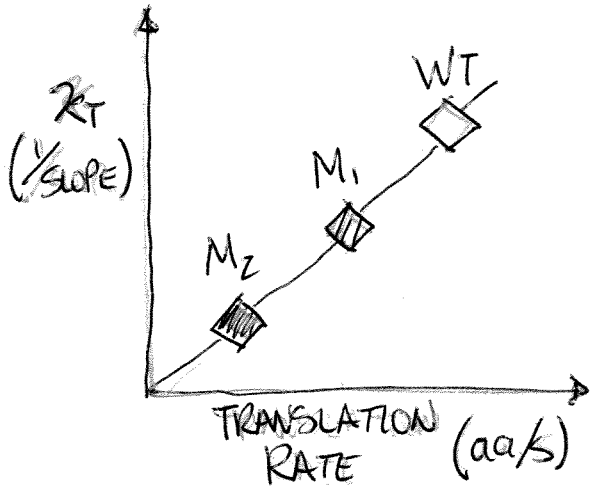
GIVEN: DIFFERENT GROWTH MEDIA





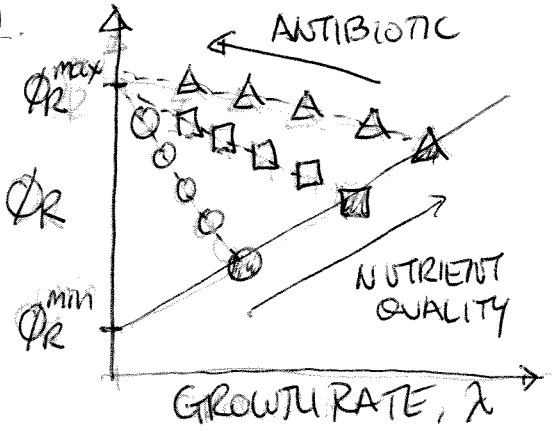


LINEARITY PERSISTS & THE SLOPE CORRELATES VERY WELL WITH THE in vitro TRANSLATION RATE



FROM THE MUTANT DATA, IT APPEARS THERE IS A SECOND FAMILY OF LINES WITH NEGATIVE SLOPE CONNECTING MUTANTS IN THE SAME GROWTH MEDIUM.

WE CAN INVESTIGATE BY USING A RIBOSOME-TARGETING ANTIBIOTIC TO INHIBIT TRANSLATION eg CHLORAMPHENICOL.



SECOND EMPIRICAL RELATION:

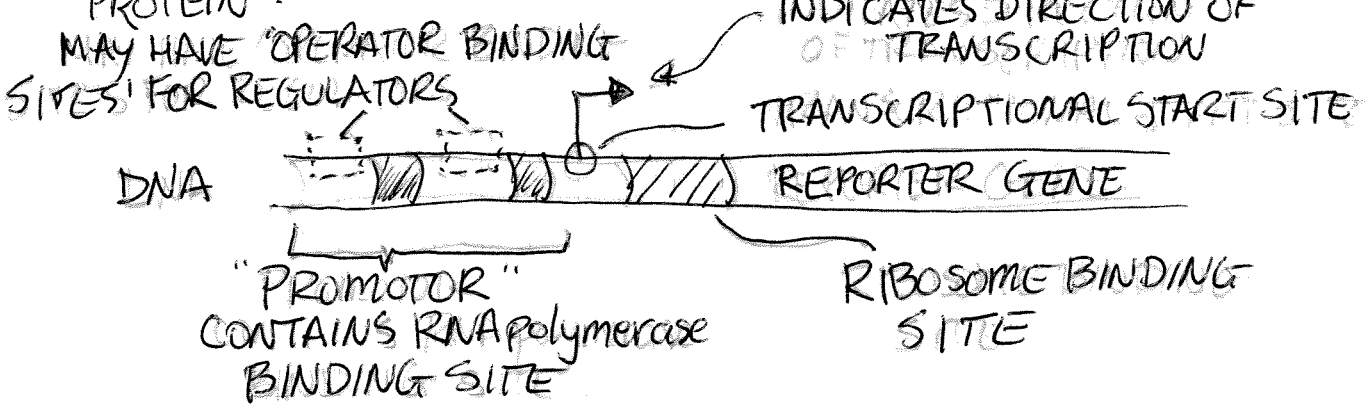
$$\phi_R = -\frac{\lambda}{\kappa_N} + \phi_R^{max}$$

THE PARAMETER κ_N APPEARS TO CORRELATE WITH NUTRIENT QUALITY...

THESE TWO EMPIRICAL 'GROWTH LAWS' ARE AT THE LEVEL OF RIBOSOMAL PROTEIN ABUNDANCE.

WHAT ABOUT OTHER PROTEINS IN THE CELL?
WHAT ABOUT COMPLEX REGULLATORY NETWORKS?
DOES GROWTH RATE CHANGE AFFECT THESE?

PLACE TO BEGIN IS WITH AN UNREGULATED (CONSTITUTIVE) PROTEIN:



THE 'REPORTER' GENE SHOULD: • BE EASY TO MEASURE
• ACTIVITY UNAFFECTED BY PHYSIOLOGY.

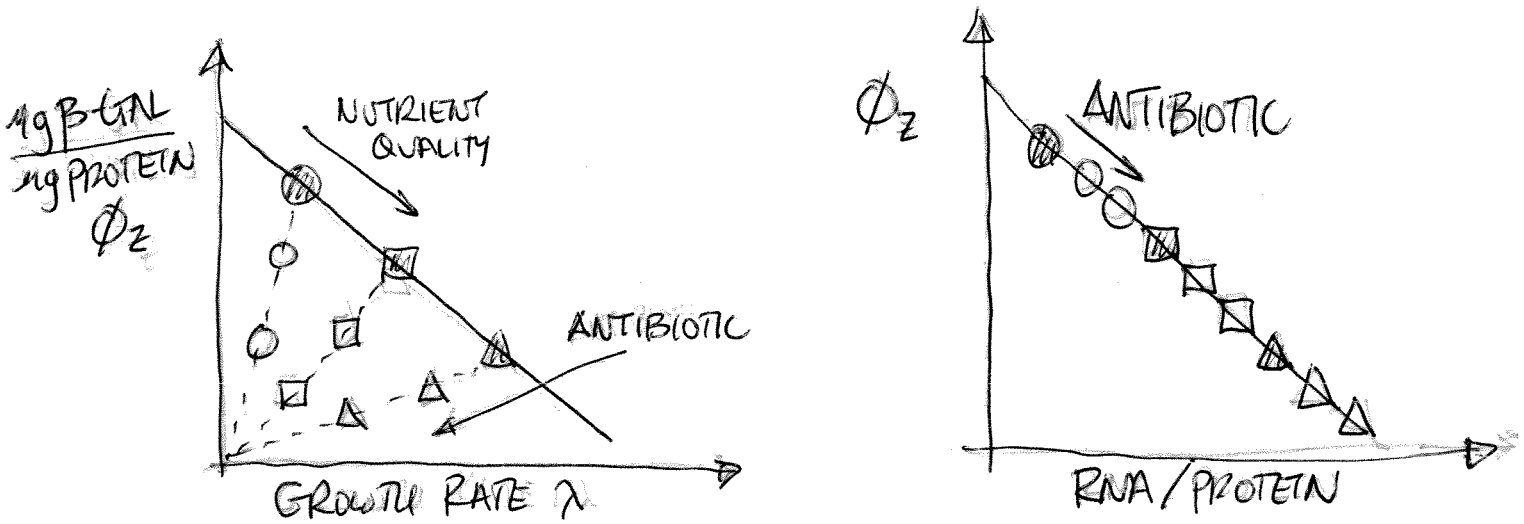
COMMON REPORTERS: ENZYMES
eg β-GALACTOSIDASE ONPG → O-NITROPHENOL
colorless yellow

FLUORESCENT PROTEINS eg gfp.
* VERY SENSITIVE TO O_2 & pH; NOT CLEAR FLUORESCENCE CAN BE COMPARED ACROSS GROWTH RATES.

SUPPOSE WE HAVE AN ENZYME REPORTER (eg. β-GALACTOSIDASE) WITH ACTIVITY CONVERTED TO μg OF ENZYME PROTEIN, AND NORMALIZED TO TOTAL PROTEIN:

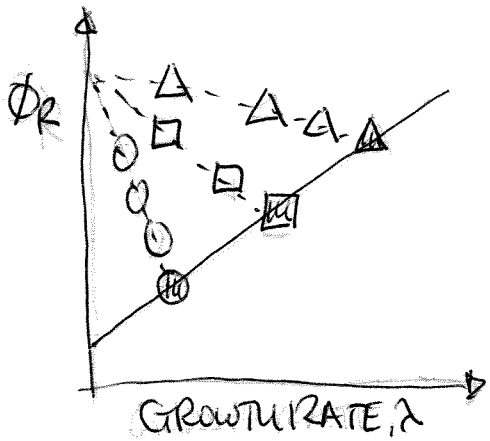
$$\frac{\mu g\ \beta\text{-GAL}}{\mu g\ \text{PROTEIN}} \equiv \phi_z$$

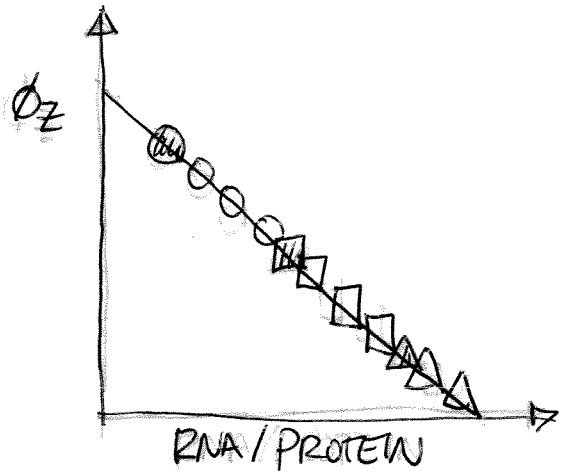
USING SAME GROWTH MEDIA & ANTIBIOTIC:

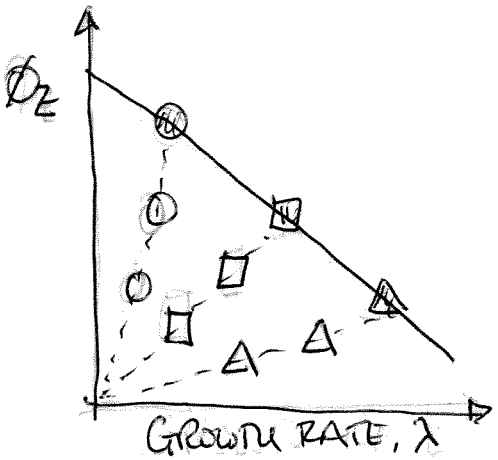


How would you explain this?
Can you find a minimal hypothesis linking the ribosomal growth laws to the constitutive protein growth laws?

PROTEOME PARTITIONING - CONSTRAINTS





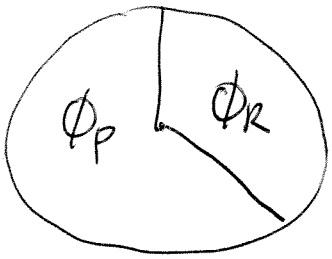


PERHAPS THE SIMPLEST MODEL IS A TWO-COMPONENT PROTEOME

ϕ_R: ALL RIBOSOME & RIBOSOME AFFILIATED PROTEINS

ϕ_P: ALL OTHERS (INCLUDING CONSTITUTIVELY-EXPRESSED β-GALACTOSIDASE)
i.e. $\phi_P = S \cdot \phi_Z$

THEN: TOTAL PROTEIN MASS IS

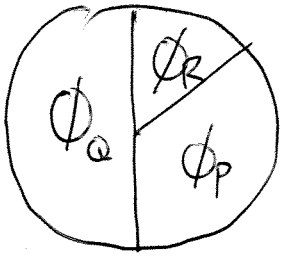


$\phi_R + \phi_P = 1$

PROBLEM: WHEN $\phi_P \to 0$

$\phi_R \to \phi_R^{max} \simeq 0.5$
(NOT 1)

OKAY: ADD A THIRD (GROWTH-RATE INDEPENDENT) SECTOR ϕ_Q



ϕ_Q: FIXED

ϕ_R: PROTEIN SYNTHESIS

ϕ_P: METABOLISM

$$\phi_R + \phi_P = 1 - \phi_Q = \phi_{R,max}^{MAX}$$