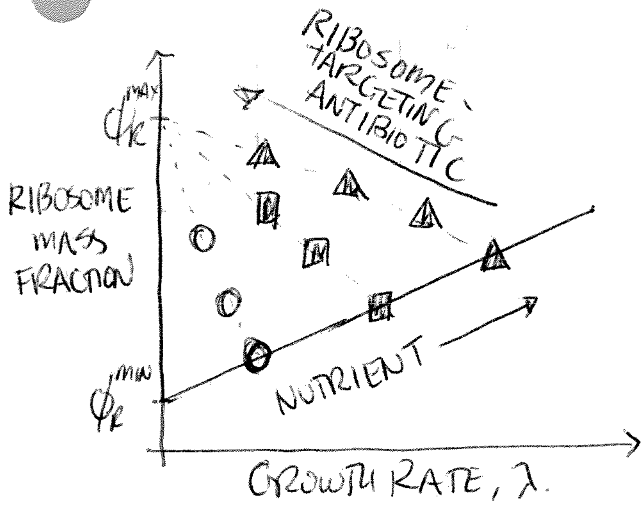
INTERFACING MECHANISTIC MODELS WITH HOST PHYSIOLOGY.

PREVIOUSLY, WE DISCUSSED HOW RIBOSOME ABUNDANCE IS AFFECTED BY THE GROWTH STATE OF THE BACTERIUM:



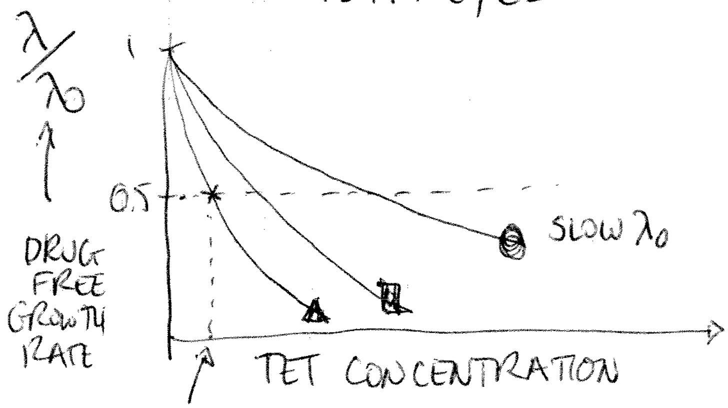
MANY ANTIBIOTICS TARGET RIBOSOMES.
<u>QUESTION</u>: IF THE TARGET EXHIBITS STRONG GROWTH DEPENDENCE, THEN HOW DOES THIS AFFECT SUSCEPTIBILITY?

eg. DO FASTER GROWING CELLS (ie. VIRULENT INFECTIONS) REQUIRE HIGHER OR LOWER DOSES COMPARED WITH SLOW GROWING CELLS?

EMPIRICALLY, THE SUSCEPTIBILITY <u>DOES</u> EXHIBIT GROWTH DEPENDENCE. A USEFUL MEASURE IS THE HALF-INHIBITION CONCENTRATION IC_{50}:

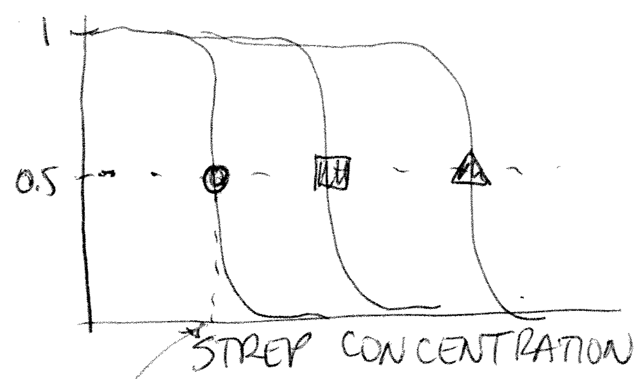
BOTH TARGET 30S, CLOSE TO A-SITE.

TETRACYCLINE



IC_{50} FOR ▲ (FAST GROWER)
(FAST λ_0)

STREPTOMYCIN



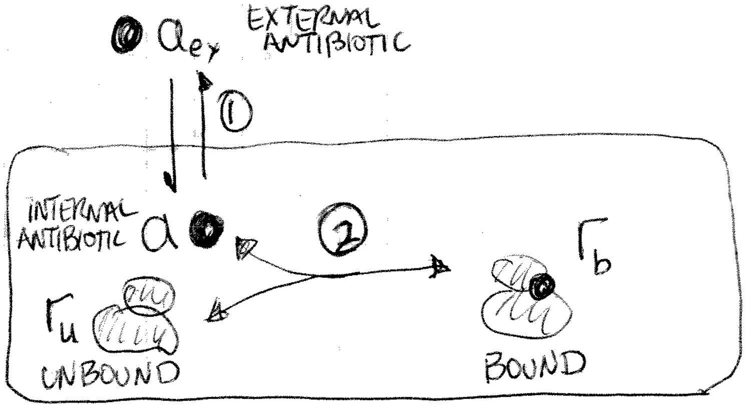
IC_{50} FOR ● (SLOW GROWER)
(SLOW λ_0)

BOTH ANTIBIOTICS TARGET THE RIBOSOME, BUT FOR

TETRACYCLINE
- IC_{50} DECREASES WITH INCREASING λ_0
- INHIBITION CURVES ARE HYPERBOLIC

STREPTOMYCIN
- IC_{50} INCREASES WITH INCREASING λ_0
- INHIBITION CURVES ARE SIGMOIDAL.

SIMPLE MODEL:



1. <u>TRANSPORT</u>

$$J(a, a_{ex}) = P_{in} a_{ex} - P_{out} a$$

2. <u>BINDING</u>

$$k_{on} r_u \cdot a - k_{off} r_b$$

DIFFERENTIAL EQUATIONS FOR CONCENTRATIONS:

$$\frac{da}{dt} = \not{\gamma} - \lambda a \quad - k_{on} r_u a + k_{off} r_b \quad + J(a, a_{ex}) \quad \leftarrow \text{TRANSPORT}$$

$$\frac{dr_u}{dt} = -\lambda r_u \quad - k_{on} r_u a + k_{off} r_b \quad + s(\lambda)$$

$$\frac{dr_b}{dt} = \underbrace{-\lambda r_b}_{\text{DILUTION}} \quad \underbrace{+ k_{on} r_u a - k_{off} r_b}_{\text{BINDING}}$$

$s(\lambda)$: RIBOSOME SYNTHESIS RATE.

WHAT WE WANT IS THE INHIBITION CURVES $\lambda(a_{ex})$. WE HAVE THREE EQUATIONS, BUT <u>FOUR</u> UNKNOWNS a, r_u, r_b & $\underline{\lambda}$

<u>QUESTION</u>: 1. WHAT IS THE SYNTHESIS RATE $s(\lambda)$?
2. HOW DO WE "CLOSE" THE SYSTEM; i.e. WHAT IS THE MISSING EQUATION TO OBTAIN $\lambda(a_{ex})$?

* GREULICH, SCOTT, EVANS & ALLEN (2015) <u>MOLECULAR SYSTEMS BIOLOGY</u> <u>11</u>: 796

THE ANSWER TO BOTH COMES FROM THE "GROWTH LAWS" RELATING RIBOSOME ABUNDANCE TO GROWTH RATE!

1. WHAT IS THE SYNTHESIS RATE $s(\lambda)$?

LOOK AT TOTAL TARGET CONCENTRATION $r_u + r_b = r_{TOT}$

$$\frac{dr_u}{dt} = -\lambda r_u - k_{on} r_u a + k_{off} r_b + s(\lambda).$$

$$+ \quad \frac{dr_b}{dt} = -\lambda r_b + k_{on} r_u a - k_{off} r_b$$

$$\frac{dr_{TOT}}{dt} = -\lambda(r_{TOT}) + s(\lambda)$$ ← IN STEADY-STATE, SYNTHESIS MUST BALANCE DILUTION VIA GROWTH

AT STEADY-STATE $\boxed{s(\lambda) = \lambda r_{TOT}}$ BUT WE KNOW $r_{TOT} = r^{max} - \frac{\lambda}{\kappa_n}$

"GROWTH LAW" UNDER TRANSLATION-INHIBITION. κ_n SETS DRUG-FREE GROWTH RATE λ_0.

$$\boxed{s(\lambda) = \lambda\left[r^{max} - \frac{\lambda}{\kappa_n}\right]}$$

2. HOW TO CLOSE THE SYSTEM?

IN STEADY-STATE, GROWTH RATE λ SYNONYMOUS WITH PROTEIN-SYNTHESIS RATE $\boxed{\lambda = \kappa_t \cdot r_u}$

OUR STEADY-STATE SYSTEM IS:

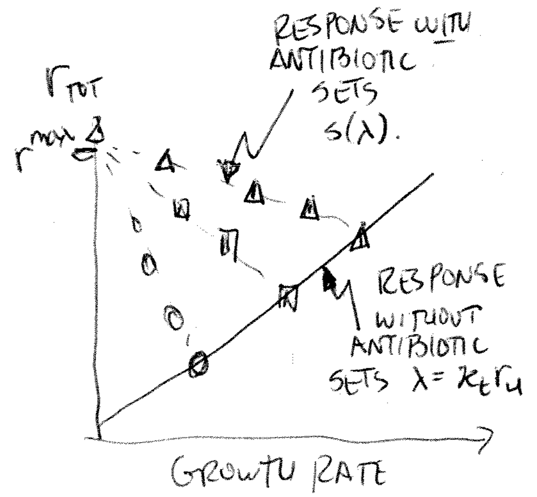
$$0 = -\lambda a - k_{on} r_u a + k_{off} r_b + J(a, a_{ex})$$

$$0 = -\lambda r_u - k_{on} r_u a + k_{off} r_b + s(\lambda; \lambda_0)$$

$$0 = -\lambda r_b + k_{on} r_u a - k_{off} r_u$$

$$\lambda = \kappa_t r_u$$

$$s(\lambda) = \lambda\left[r^{max} - \frac{\lambda}{\kappa_n}\right]$$



IN THIS WAY, OUR MECHANISTIC MODEL IS CONSISTENT WITH GROWTH LAW CONSTRAINTS!

THE RESULT IS A CUBIC EQUATION FOR (λ/λ_0) AS A FUNCTION OF ANTIBIOTIC a_{ex}:

$$0 = \left(\frac{\lambda}{\lambda_0}\right)^3 - \left(\frac{\lambda}{\lambda_0}\right)^2 + \left(\frac{\lambda}{\lambda_0}\right)\left[\frac{1}{4}\left(\frac{\lambda_0^*}{\lambda_0}\right)^2 + \frac{a_{ex}}{2IC_{50}^*}\left(\frac{\lambda_0^*}{\lambda_0}\right)\right] - \frac{1}{4}\left(\frac{\lambda_0^*}{\lambda_0}\right)^2$$

λ_0^* AND IC_{50}^* ARE NON-DIMENSIONALIZING PARAMETERS.

$$\underset{\text{CONCENTRATION}}{IC_{50}^*} = \frac{r^{max}\lambda_0^*}{2P_{in}} \qquad \underset{\text{RATE}}{\lambda_0^*} = 2\sqrt{P_{out}\tau_t K_D}$$

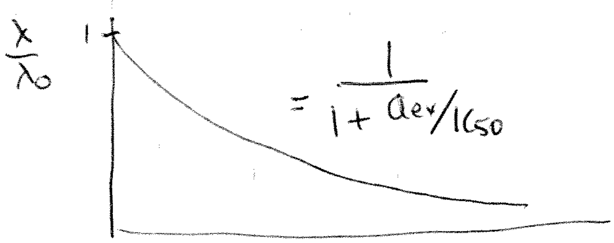
$K_D = k_{off}/k_{on}$

P_{in}, P_{out} & K_D ARE CHEMICAL PROPERTIES OF ANTIBIOTIC
r^{max} & τ_t ARE PHYSIOLOGICAL PROPERTIES OF PATHOGEN
λ_0 IS ~~EA~~ VIRULENCE MEASURE OF INFECTION

LOOK AT SOME LIMITS:

$\frac{\lambda_0^*}{\lambda_0} \gg 1$ "REVERSIBLE"

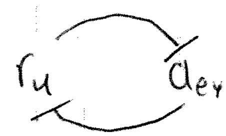
$r_u + a_{ex} \rightleftharpoons r_b$

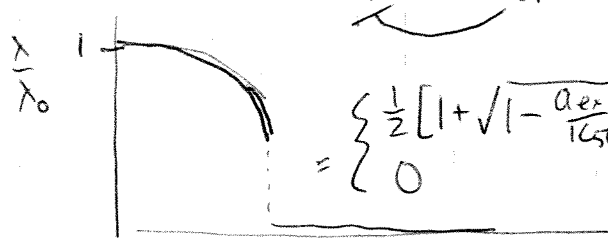


$$\frac{\lambda}{\lambda_0} = \frac{1}{1 + a_{ex}/IC_{50}}$$

$$IC_{50} \propto \frac{1}{\lambda_0}$$

$\frac{\lambda_0^*}{\lambda_0} \ll 1$ "IRREVERSIBLE."





$$\frac{\lambda}{\lambda_0} = \begin{cases} \frac{1}{2}\left[1 + \sqrt{1 - \frac{a_{ex}}{IC_{50}}}\right] \\ 0 \end{cases}$$

$$IC_{50} \propto \lambda_0$$

TAKE HOME: SIMPLE MECHANISTIC MODEL OF BINDING/TRANSPORT CAN EXPLAIN GROWTH-DEPENDENT SUSCEPTIBILITY ONCE IT IS MADE CONSISTENT WITH GROWTH LAW CONSTRAINTS.