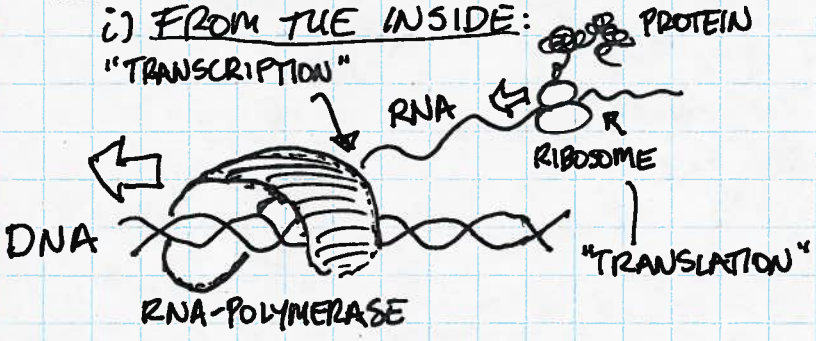
QUANTITATIVE METHODS IN BIOLOGY – BACTERIAL PHYSIOLOGY

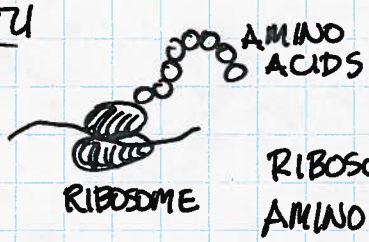
QUANTITATIVE METHODS HAVE A LONG HISTORY IN BIOLOGY – PARTICULARLY IN THE STUDY OF BACTERIAL COMPOSITION & GROWTH (CALLED "BACTERIAL PHYSIOLOGY"). MUCH OF THIS WORK PRECEDES OR RUNS IN PARALLEL WITH THE DEVELOPMENT OF OUR MODERN UNDERSTANDING OF BIOLOGY:

MATHEMATICS IS A SCAFFOLD FOR COMMON SENSE – IT ALLOWS US TO INFER THE UNKNOWN FROM SHADOWS CAST ON THE WALL.

TALKING ABOUT BACTERIAL GROWTH

i) FROM THE INSIDE:



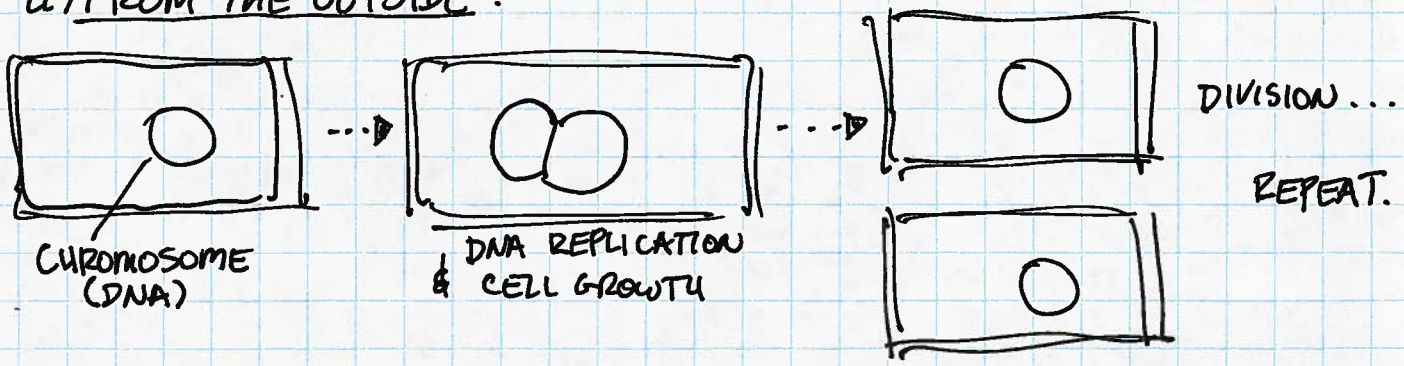


RIBOSOMES POLYMERIZE AMINOACIDS INTO PROTEINS

[RIBOSOMES THEMSELVES ARE MADE OF RNA (rRNA) & PROTEIN (rPROTEIN)]

'CENTRAL DOGMA': INFORMATION FLOWS DNA → RNA → PROTEIN.

ii) FROM THE OUTSIDE:



IT IS USEFUL TO QUANTIFY HOW RAPIDLY THIS OCCURS – DOUBLING TIME (τ) OR DOUBLING RATE ($\gamma = 1/\tau$)

IF γ = 3 doublings/hour, THEN BACTERIA DOUBLE EVERY 20 minutes (τ = 20 minutes).

IN MATHEMATICS,

NUMBER OF CELLS $N = N_0 2^{t/\tau} = N_0 2^{\gamma t}$

(N_0: INITIAL NUMBER AT TIME $t=0$.)

IF TIME t,
$t=\tau$, $N = N_0 \times 2$
$t=2\tau$, $N = N_0 \times 4$
$t=3\tau$, $N = N_0 \times 8$
⋮
$t=n\tau$, $N = N_0 \times 2^n$

RECALL HOW WE DEFINE THE LOGRITHM –

$$\log_2(2^{\gamma t}) = \gamma t \underbrace{\log_2(2)}_{=1.} = \gamma t.$$

HOW DID THIS PICTURE EMERGE? AMAZING EXPERIMENTS FROM 1940-1970

- AVERY-MACLEOD-MCCARTY (1943) CLEAR EVIDENCE DNA CARRIES HEREDITARY INFO.
- HERSHEY-CHASE (1952) CONFIRM DNA IS GENETIC MATERIAL.
- WATSON & CRICK (1953) HYPOTHESIS: PROPOSE DNA DOUBLE-HELIX.
- CRICK (1957) HYPOTHESIS: LAYS OUT 'CENTRAL DOGMA'

- PARDEE-JACOB-MONOD (1959) DEMONSTRATE ENZYME INDUCTION
- PALADE-SIEKEVITZ (1960) SHOW RIBOSOMES POLYMERIZE AMINO ACIDS
- CRICK-BRENNER-BARNETT & WATTS-TOBIN (1961) DEMONSTRATE THAT 3 RNA 'BASES' ENCODE 1 AMINO ACID.

- COOPER-HELMSTETTER (1968) DETERMINE RULES FOR DNA REPLICATION IN THE BACTERIUM E.COLI.

THESE EXPERIMENTS WERE DIRECT, OR LOGICALLY COMPELLING, DEMONSTRATIONS, BUT SOME DISCOVERIES WERE ANTICIPATED BY USING QUANTITATIVE METHODS TO INFER MECHANISM.

WE'LL EXAMINE THREE CASE STUDIES IN DETAIL:

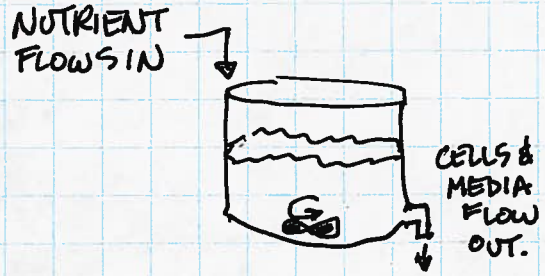
1. MONOD (1949) GROWTH OF BACTERIAL CULTURES.
2. SCHAECHTER, MAALØE & KJELDGAARD (1958) DEPENDENCY ON MEDIUM & TEMPERATURE OF CELL SIZE & CHEMICAL COMPOSITION DURING BALANCED GROWTH OF SALMONELLA.
3. NEIDHARDT & MAGASANIK (1960) STUDIES ON THE ROLE OF RIBONUCLEIC ACID (RNA) ON GROWTH OF BACTERIA.

1. MONOD (1949): SURVEYS GROWTH OF MANY BACTERIA & IDENTIFIES IMPORTANT FEATURES WORTHY OF OBSERVATION

TWO PRIMARY METHODS FOR GROWING BACTERIA:

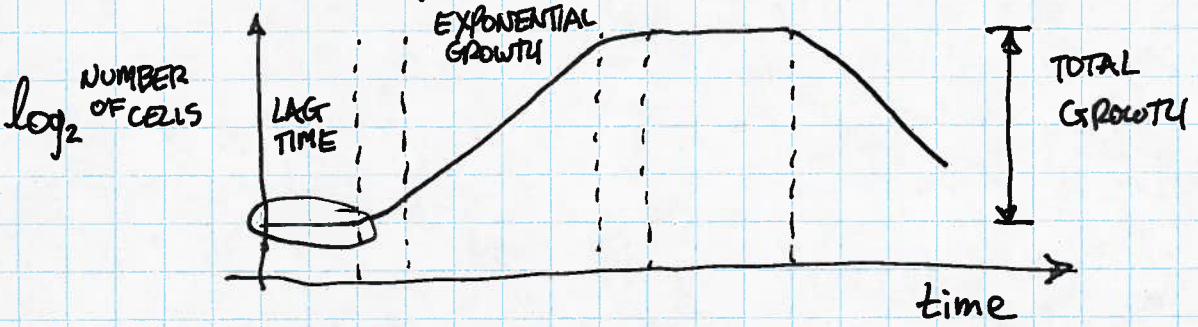
A) BATCH CULTURE: PUT BACTERIA & GROWTH MEDIA IN A FLASK OR TEST TUBE. SHAKE VIGOUROUSLY (TO AERATE) AT CONSTANT TEMPERATURE (OFTEN 37°C). IMPORTANT THAT ALL NUTRIENTS ARE IN EXCESS. IN THIS MODE OF GROWTH, BACTERIA DICTATE THEIR OWN GROWTH RATE.

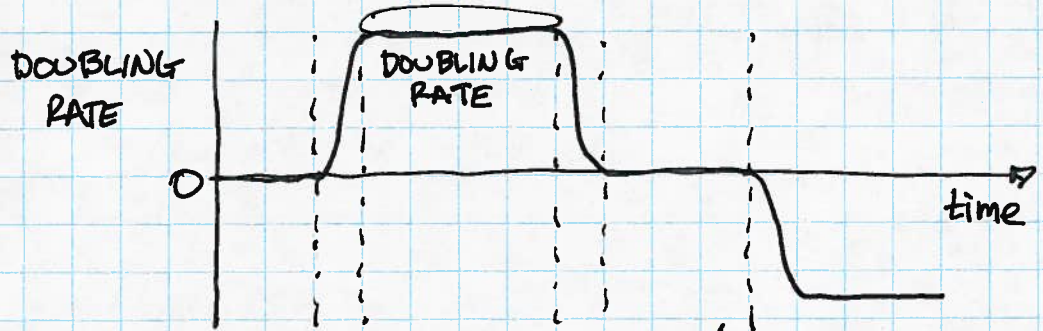
B) CONTINUOUS CULTURE (CHEMOSTAT OR TURBIDOSTAT):



BY ADJUSTING THE CONCENTRATION OF A GROWTH-LIMITING NUTRIENT IN THE INFLOW, BACTERIA ARE KEPT HUNGRY & GROWTH RATE IS REDUCED. IN THIS MODE OF GROWTH, THE GROWTH RATE IS ADJUSTED BY FLOW RATE.

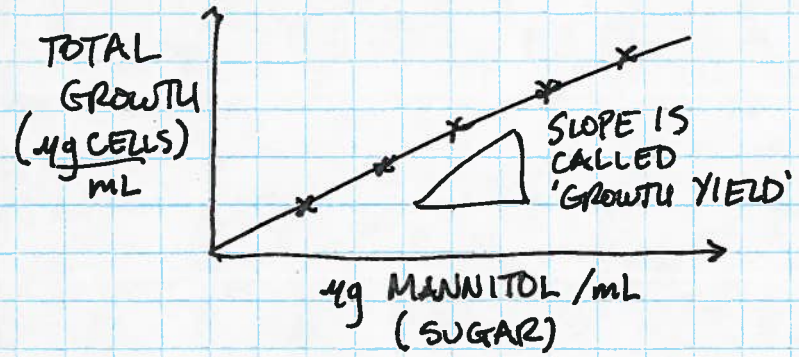
IN BATCH CULTURE, MONOD IDENTIFIED THE FOLLOWING:





DOUBLING RATE IS INCREDIBLY ROBUST - ALMOST NO VARIATION (<5%) DAY-TO-DAY, DECADE-TO-DECADE, LAB-TO-LAB...

DOUBLING RATE IS ADJUSTED BY THE QUALITY OF THE GROWTH MEDIUM; TOTAL GROWTH IS ADJUSTED BY THE QUANTITY



GROWTH YIELD TELLS YOU HOW EFFICIENT THE ORGANISM IS AT TURNING:

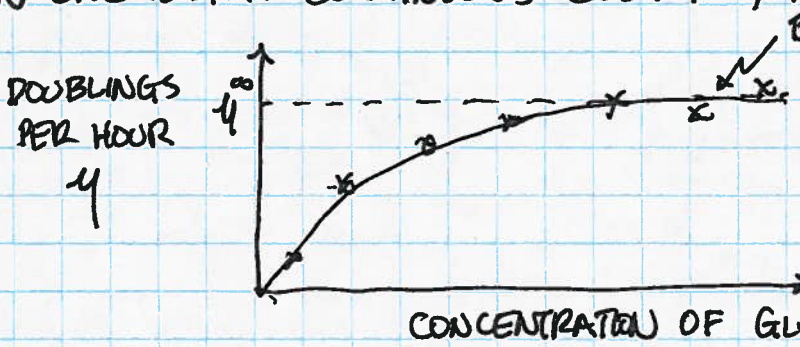
NUTRIENT → BIOMASS

i.e.

$$\text{GROWTH YIELD} = \frac{\mu g \text{ BACTERIA}}{\mu g \text{ NUTRIENT}}$$

ANALOGY: GROWTH RATE IS 'TOP SPEED' & GROWTH YIELD IS 'MILES-PER-GALLON'.

IN CHEMOSTAT CONTINUOUS CULTURE, MONOD OBSERVED:



$$\mu = \mu^{\infty} \frac{[\text{NUTRIENT}]}{[\text{NUTRIENT}] + K_D}$$

K_D ↑ CONSTANT.

TAKE-HOME: DESPITE IMMENSE UNDERLYING COMPLEXITY, CHARACTERISTICS OF BACTERIAL GROWTH OBEY SIMPLE "LAWS"

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2. SCHAECHTER, MAALØE & KJELDGAARD (1958) USING 20 DIFFERENT GROWTH MEDIA IN BATCH CULTURE, THEY SHOW THAT MACROMOLECULAR COMPOSITION IS DEPENDENT ON GROWTH RATE ALONE!

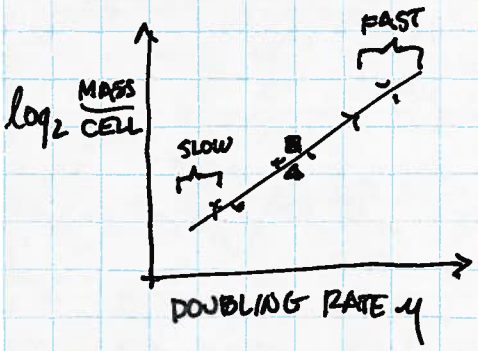
DOUBLING RATE IN BOTH IS ABOUT 1 dbl/hour

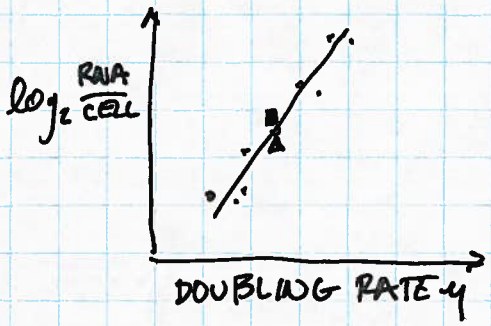
DESPITE HUGE DIFFERENCES IN HOW THE NUTRIENTS ARE PROCESSED, LARGESCALE COMPOSITION:

- DNA/CELL
- RNA/CELL
- PROTEIN/CELL
- MASS/CELL

} ALL THE SAME! THE BACTERIA GROWING IN FLASKS A & B ARE INDISTINGUISHABLE!

MORE THAN THAT...





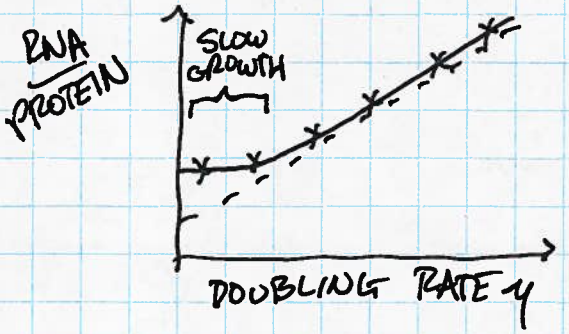
i) $\frac{\text{MASS}}{\text{CELL}} \propto 2^{\mu}$: FASTER GROWING CELLS ARE BIGGER! 20 min/dbl TWICE AS LARGE AS 30 min/dbl.

ii) UNITS LOOK FUNNY: $2^{\mu/\mu_0}$ SEEMS LIKE $\mu_0 = 1$ dbl/hr IS NATURAL.
YES: HELMSTETTER & COOPER (1968):
40 mins REPLICATE DNA + 20 mins SEPARATE DAUGHTERS = 60 mins dbl.

iii) $\frac{\text{RNA}}{\text{CELL}} \propto 2^{1.54}$ OR $\mu 2^{\mu}$
→ INCREASES MORE RAPIDLY THAN MASS....

TAKE-HOME: MACROMOLECULAR COMPOSITION & GROWTH RATE PLAY THE ROLE OF 'STATE VARIABLES' LIKE PRESSURE, VOLUME, TEMPERATURE IN PHYSICS - PERHAPS UNIVERSAL LAWS IN BIOLOGY ARE POSSIBLE IF THE CORRECT OBSERVABLES ARE CHOSEN.

3. NEIDHARDT & MAGASANIK (1960) OBSERVE THAT AWAY FROM SLOW GROWTH, THE RATIO RNA/PROTEIN CORRELATES VERY STRONGLY WITH DOUBLING RATE



i) WHEN MEDIA IS SHIFTED-POOR TO GOOD - THE ACCUMULATION OF RNA PRECEDES PROTEIN MASS INCREASE
→ RNA DRIVES PROTEIN MASS ACCUMULATION & GROWTH

ii) 85% OF THE TOTAL RNA IS RIBOSOMAL RNA (rRNA), IRRESPECTIVE OF GROWTH RATE
→ TOTAL RNA IS A READ-OUT OF RIBOSOME CONTENT

TAKE-HOME:
THEY CONCLUDE THAT RNA (RIBOSOME) PLAYS A PASSIVE OR TEMPLATE ROLE IN PROTEIN SYNTHESIS
ie: RATE OF PROTEIN SYNTHESIS IS DETERMINED BY NUMBER OF RIBOSOMES.