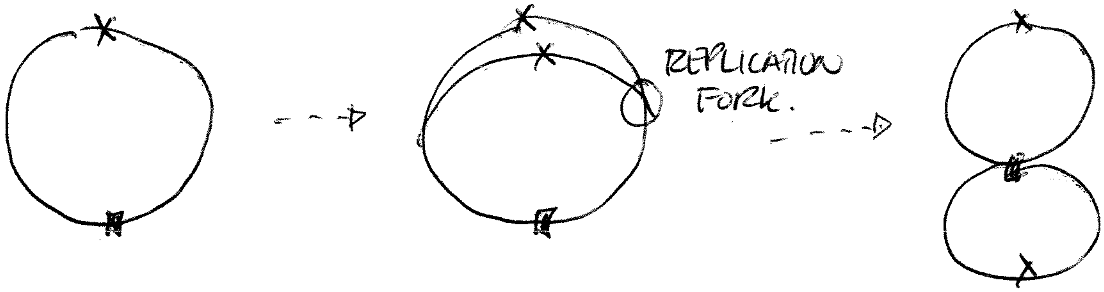
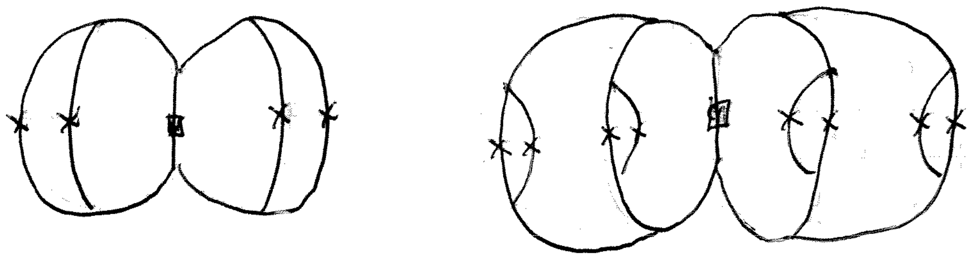
COOPER - HELMSTETTER RULES

WHAT WAS THOUGHT, AT THE TIME, WAS THAT THERE WAS A SINGLE REPLICATION POINT PER CHROMOSOME IN E. COLI



MAALØE SUGGESTED THE RATE OF DNA SYNTHESIS AT THE REPLICATION FORK IS CONSTANT IRRESPECTIVE OF GROWTH RATE. A PREDICTED CONSEQUENCE OF MAALØE'S VIEW IS THAT IF THE DOUBLING TIME IS VERY LONG, THERE SHOULD BE GAP IN DNA SYNTHESIS OVER A FRACTION OF THE CELL CYCLE; IN 1967, HELMSTETTER USED THE BABY MACHINE TO OBSERVE SUCH A GAP.

WHAT ABOUT FAST GROWING CELLS? SUPPOSE IT TAKES τ_{cyc} MINUTES TO GO FROM INITIATION OF DNA REPLICATION TO CELL DIVISION. IF THE DOUBLING TIME $\tau < \tau_{cyc}$, THE CELL WILL INITIATE <u>MULTIPLE ROUNDS OF REPLICATION</u>:



$\tau < \tau_{cyc}$ $\qquad\qquad$ $\tau \ll \tau_{cyc}$

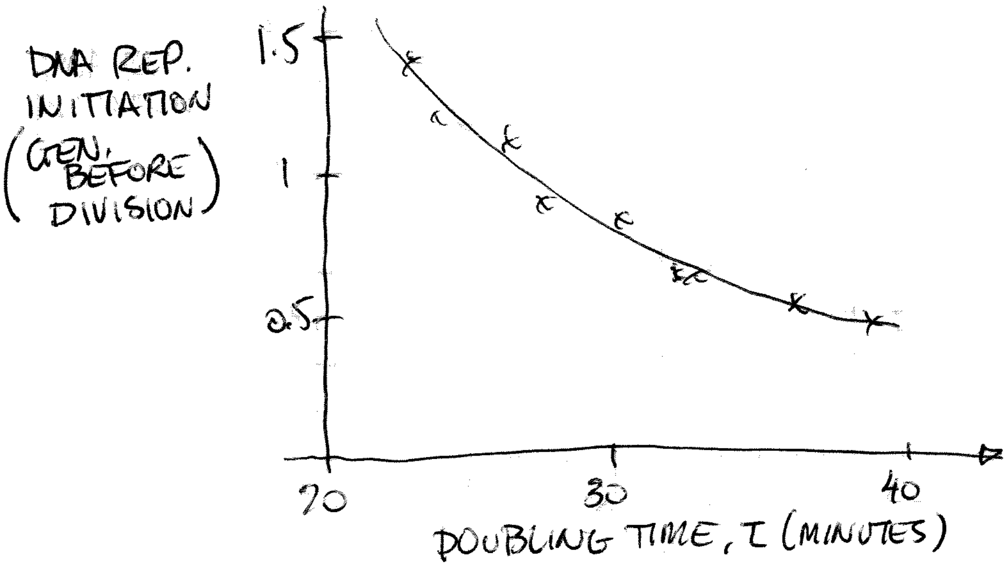
HOW FAR BACK DOES DNA REPLICATION INITIATION NEED TO OCCUR IN ORDER TO COINCIDE WITH THE PRESENT DIVISION CYCLE?

IF:	THEN REPLICATION INITIATION IS:
$0 < \tau_{cyc}/\tau \leq 1$	SELF-INITIATED (SLOW GROWTH
$1 < \tau_{cyc}/\tau \leq 2$	INITIATED IN THE MOTHER
$2 < \tau_{cyc}/\tau \leq 3$	INITIATED IN GRAND-MOTHER
⋮	⋮

OR, MORE COMPACTLY,

$$\boxed{\text{GENERATIONS } \underline{\text{BEFORE}} \text{ DIVISION THAT DNA REPLICATION INITIATED} = \cancel{1 - T_{cyc}/\tau}\ \ T_{cyc}/\tau - 1}$$

GROWING CELLS IN MEDIA SUPPORTING DOUBLING TIMES $\tau = 22-40$ minutes, THEIR DISCONTINUOUS DATA COLLAPSED ONTO A SINGLE PLOT, WITH $\underline{T_{cyc} \approx 60 \text{ minutes}}$.



THE ORIGINAL DATA MEASURED THE AGE (IN GENERATIONS) AT WHICH DNA SYNTHESIS INCREASED – HOW DO WE GET THAT PLOT?

$$\text{DNA REP. INITIATION (GEN. AFTER DIVISION)} = -\left(T_{cyc}/\tau - 1\right) = 1 - \frac{T_{cyc}}{\tau}$$

BUT ANY NEGATIVE <u>INTEGER</u> PART OF $-T_{cyc}/\tau$ JUST TELLS YOU THE RELATIVE AGE (MOTHER, GRANDMOTHER, ETC..)

IF WE THROW OUT THE GENERATIONAL MARKERS, THEN

$$a_i = 1 - \text{Frac}\left[T_{cyc}/\tau\right]$$

"FRACTIONAL PART."

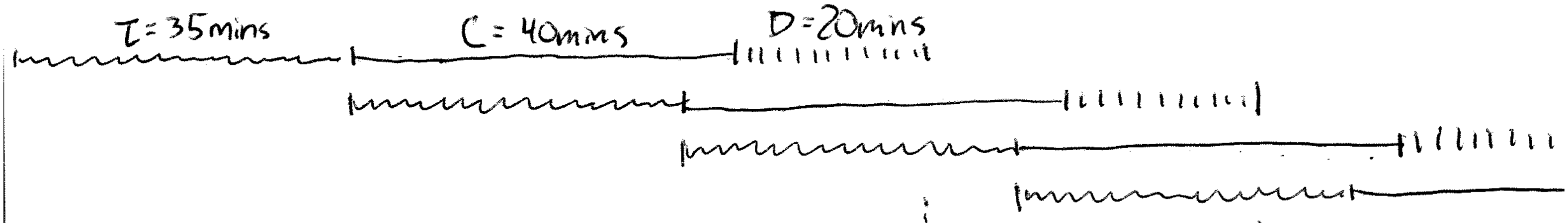
DISCONTINUITIES OCCUR WHEN $T_{cyc} = n \cdot \tau$ (i.e. $\tau = 20, 30, 60$ minutes.)

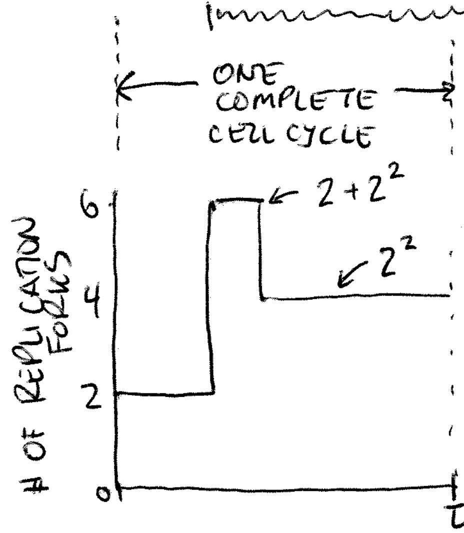
COOPER & HELMSTETTER DIVIDED T_{cyc} INTO TWO PARTS

C – TIME TO REPLICATE (ORI → TER)

D – TIME TO SEPTATE/DIVIDE (TER → CELL)

SEE THE RULES IN ACTION: DNA SYNTHESIS RATE WILL BE DIRECTLY PROPORTIONAL TO THE NUMBER OF REPLICATION FORKS. HELMSTETTER DEVELOPED A USEFUL VISUALIZATION STRATEGY TO COUNT THE REPLICATION FORKS ACROSS THE CELL CYCLE FOR A GIVEN DOUBLING TIME τ:



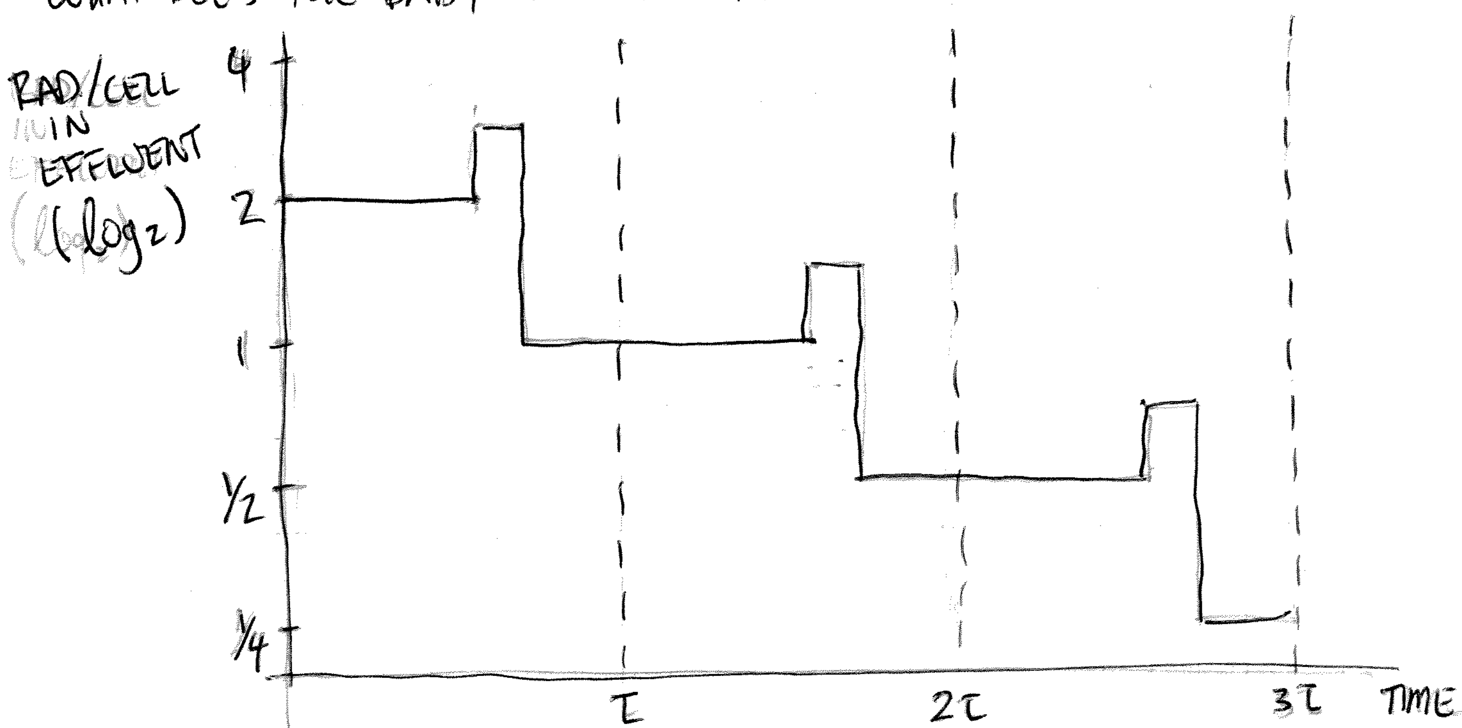


WHAT IS THE CELL AGE WHEN DNA REPLICATION RATE INCREASES?

$$a_i = \frac{10}{35} = \frac{2}{7} \approx 0.2857$$

$$= 1 - \mathrm{Frac}\left[\frac{60}{35}\right]$$

WHAT DOES THE BABY MACHINE DATA LOOK LIKE?

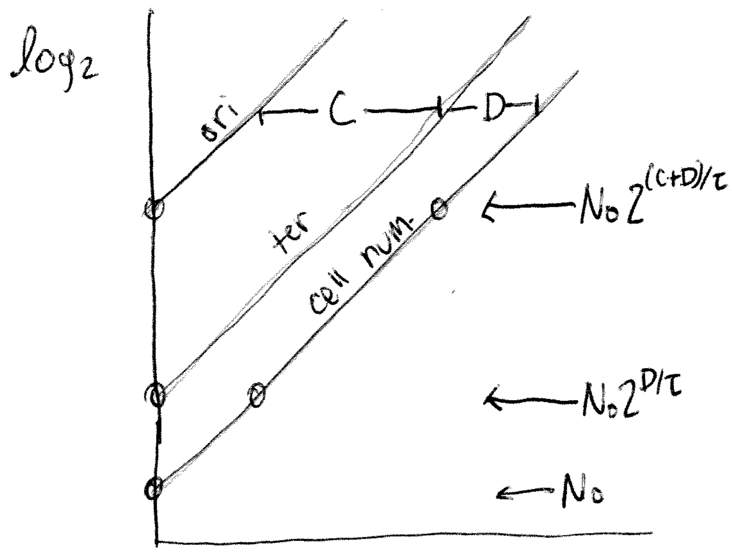


COULD AVERAGE OVER THE AGE DISTRIBUTION TO GET, FOR EXAMPLE, AVERAGE DNA/CELL. BREMER & CHURCHWARD

DEVELOPED A MUCH SIMPLER METHOD: IF 'C' IS DEFINED AS THE TIME IT TAKES TO TURN AN ORIGIN OF REPLICATION INTO A TERMINUS, AND 'D' IS THE TIME TO TURN A TERMINUS INTO A NEW CELL, AND EVERYTHING IS IN BALANCED GROWTH AT DOUBLING-TIME τ, THEN

$$\frac{\text{ori}}{\text{mL}} = O_0\, 2^{t/\tau} \qquad \frac{\text{ter}}{\text{mL}} = T_0\, 2^{t/\tau} \qquad \frac{\text{num}}{\text{mL}} = N_0\, 2^{t/\tau}$$

PLOTTING ON A log-SCALE:



i.e. $O_0 = N_0\, 2^{(C+D)/\tau}$

$T_0 = N_0\, 2^{D/\tau}$

AND TAKING QUOTIENTS

$$\boxed{\underset{(\bar{O})}{\frac{\text{ori}}{\text{cell}}} = 2^{(C+D)/\tau} \qquad \underset{(\bar{T})}{\frac{\text{ter}}{\text{cell}}} = 2^{D/\tau}}$$

THE NUMBER OF FORKS IS 2x THE DIFFERENCE $(\bar{O}-\bar{T})$:

$$\vec{F} = 2(\bar{O}-\bar{T})$$

THE RATE (PER CELL) OF DNA SYNTHESIS IS:

$$\frac{d\bar{G}}{dt} = \bar{F}\underbrace{\frac{1}{2C}}_{\text{1/2 GENOME PER C MINUTES} \times \text{\# OF FORKS}} = \frac{1}{C}\left[2^{(C+D)/\tau} - 2^{D/\tau}\right] = \lambda \bar{G}$$

IN BALANCED GROWTH. (λ: EXP. GROWTH RATE).

SO,

$$\bar{G} = \frac{1}{C\lambda}\left[2^{(C+D)/\tau} - 2^{D/\tau}\right] = \frac{1}{C\lambda}\left[e^{(C+D)\lambda} - e^{D\lambda}\right]$$

SMK II

ALTOGETHER, $\bar{M}\ \frac{\text{mass}}{\text{cell}} \propto 2^{\mu} = 2^{60/\tau}$ $\quad \bar{G}\ \frac{\text{DNA}}{\text{CELL}} = \frac{1}{C\lambda}\left[e^{(C+D)\lambda} - e^{D\lambda}\right] \doteq 2^{0.84}$

$\frac{\text{RNA}}{\text{PROTEIN}} \propto \mu$ $\qquad \bar{O} = 2^{(C+D)/\tau} \qquad \bar{T} = 2^{D/\tau} \qquad \vec{F} = 2(\bar{O}-\bar{T})$