LECTURES 1 AND 2 : COORDINATION OF GROWTH AND THE CELL CYCLE

Two landmark papers in bacterial physiology from 1958

1. Schaechter, Maaløe, Kjeldgaard
   Dependency on medium and temperature of cell size and chemical composition during balanced growth of Salmonella typhimurium
   J. gen. Microbiol., 19(3), 592-606 (1958)

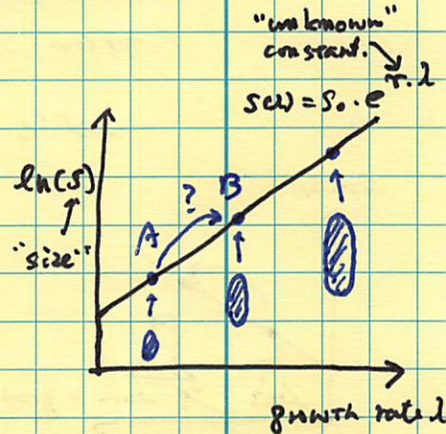
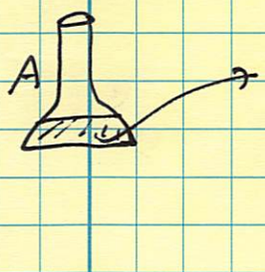


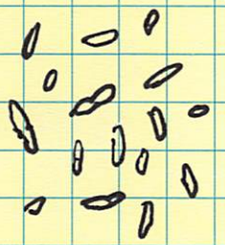
Figure 1.

2. Kjeldgaard, Maaløe, Schaechter
   The transition between different physiological states during balanced growth of Salmonella typhimurium
   J. gen. Microbiol. 18(3), 607-616 (1958)

→ This work is about how the cells respond to changes in growth conditions, say, from "A" to "B" in Figure 1.

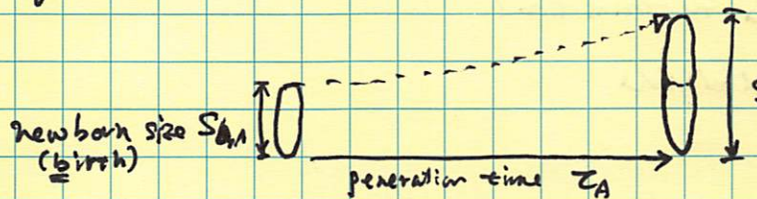
BUT, what defines the physiological state "A"?
We will come back to this later, but for now imagine a population of cells in a culture tube A





and focus on growth of a typical cell.

For simplicity, let's assume exponential elongation (good assumption)



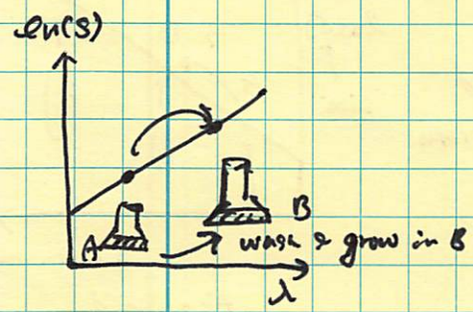
$$S_A(t) = S_{b,A} \cdot 2^{t/\tau_A}$$

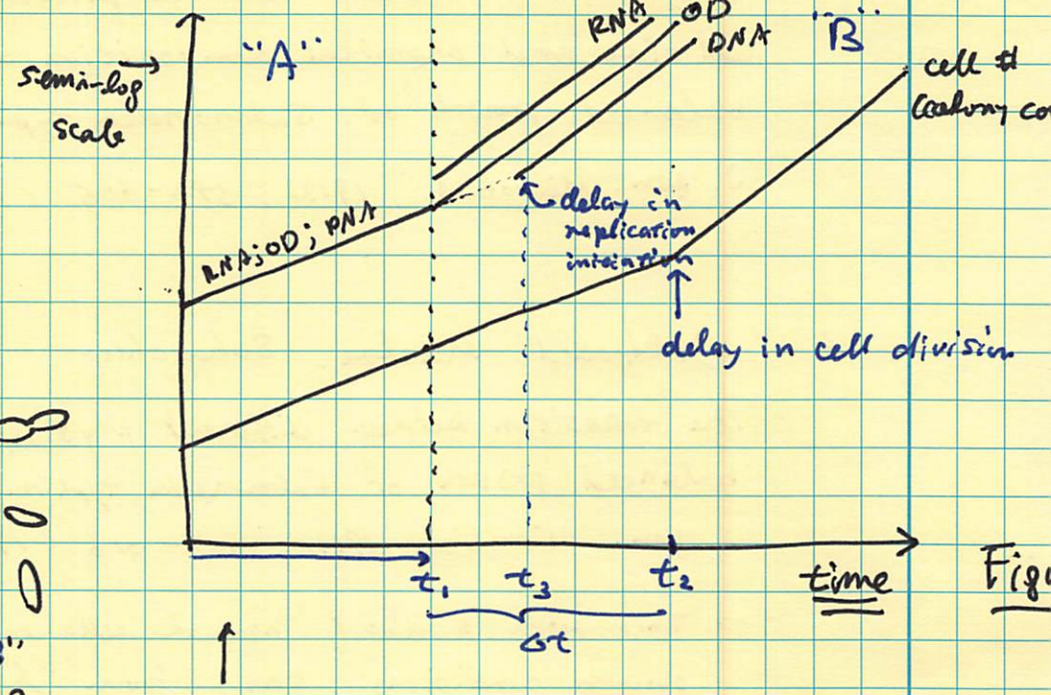
instantaneous elongation rate → $\lambda_A \equiv \frac{1}{S_A} \cdot \frac{dS_A}{dt} = \frac{\ln 2}{\tau_A}$ = growth rate

For comparison, imagine cells growing at condition "B":
They would look like ...

An obvious question is, how does a cell make the transition from A to B? Can we predict anything?

Let's look at the data from Kjeldgaard et al 1958.





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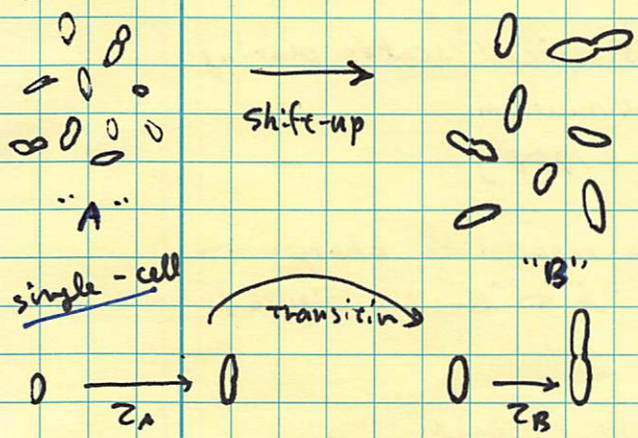


This is one of the greatest experiments in all time, revealing so much about coordination between growth, size, and the cell cycle.

Many questions:

Q1. How does the cell morphology change from ● to ⬭ ? ("fatter" & longer)

Q2. How many generations does it take to complete the transition?

Q3. When the cell will divide after shift-up?

Q4. When will a new round of DNA replication start after shift-up? (DNA/cell)

Q5. Why does the transcription rate jump?

Q6. How does the growth rate change? etc etc etc...

We will tackle one question here: why is cell division delayed?

For this, let's first accept that the growth rate itself increases immediately after shift-up to the new steady-state value, i.e, from $\lambda_A \rightarrow \lambda_B$. (A6)

06 → A6

The average "size" of the cell can be defined as

$$\text{avg } S(t) \equiv \frac{OD(t)}{N(t)}$$

$OD(t)$: total amount of cells (or biomolecules) in the culture

$N(t)$: total number of cells in the culture.

From Figure 2, $OD_A(t) = OD_0 \times 2^{t/\tau_A}$

$$N_A(t) = N_0 \times 2^{t/\tau_A}$$

$$\therefore S_A(t) = \frac{OD_0}{N_0} = \text{const.}$$

After shift-up, new steady-state average size is

$$OD_B(t) = OD_A(t_1) \times 2^{(t-t_1)/\tau_B}$$

$$N_B(t) = N_A(t_2) \times 2^{(t-t_2)/\tau_B}$$

$$S_B(t) = \frac{OD_B(t)}{N_B(t)} = \frac{OD_0 \times 2^{t_1/\tau_A} \times 2^{(t-t_1)/\tau_B}}{N_0 \times 2^{t_2/\tau_A} \times 2^{(t-t_2)/\tau_B}}$$

$$= \frac{OD_0}{N_0} \times 2^{(t_1-t_2)/\tau_A - (t_1-t_2)/\tau_B}$$

$$= \frac{OD_0}{N_0} \times e^{(t_2-t_1)\cdot(\lambda_B-\lambda_A)}$$

$$= \frac{OD_0}{N_0} \times e^{\Delta t \cdot (\lambda_B-\lambda_A)}$$

($\frac{OD_0}{N_0} = S_A(t)$)

$$\therefore \frac{S_B(t)}{S_A(t)} = e^{\Delta t \cdot (\lambda_B-\lambda_A)}$$

(However, the growth law states that $S(t) = S_0 \cdot e^{r \cdot \lambda}$

$$\therefore \frac{S_B(t_B)}{S_A(t_A)} = e^{r \cdot (\lambda_B-\lambda_A)}$$

→ A3

Therefore, we obtain an interesting result, $\underline{\Delta t = r}$, to satisfy the growth law.

This is one of the powerful aspect of consistency test. Without knowing anything about the cell cycle, the growth-law alone imposes a strong constraint on the timing of adaptation to a new growth condition / steady state.

Note that this delay in division $\Delta t = \tau$ is independent of what "B" is. It can be any steady state other than "A". This is a prediction, to be tested by experiments.

We will revisit another important consistency test in the next lecture.

Q2: How many generations does it take to complete the transition?

A2: E. coli by the number.

DNA (bp) per chromosome $= 4.6 \times 10^6$

proteins / cell $\sim 10^5 - 10^6$

genes ≈ 4000

DNA (bp) / gene $\approx \frac{4.6 \times 10^6 \text{ bp}}{4000} \approx$ 1000 bp / gene after taking into account the gene packing ra (~ 0.8

∴) # proteins / gene × cell $\sim 10^2 - 10^3$

$10^2 - 10^3$ per protein per gene for "A"

In steady state, the physiological state is defined by the proteome. Some genes are ON, and some genes are OFF As the growth condition changes, a new set of genes are turned ON, and some genes for the old steady state are not in need anymore. However, proteins in bacteria in general are NOT actively degraded. The only way to remove the proteins from the cell is by dilution by growth.

~10 divisions to dilute out

transition to "B"

expression for 10^2-10^3 per gene for "B" takes ~1 generation.

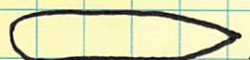
To dilute out $10^2 \sim 10^3$ proteins, it takes $\gamma \sim 10$ generations ($2^7 = 128 \sim 10^2$, $2^{10} = 1024 \sim 10^3$).

Indeed, the rule of thumb from the Copenhagen School of Bacterial Physiology states "10 generations" to reach a S.S

Q5: Why does the transcription rate "jump"?

A5: Assuming the data is correct (non-trivial), one interpretation has been that there is an excess of RNA polymerases that are inactive. These RNAPs can be utilized more in a nutrient rich medium w/ faster growth of the cell. But this has to be tested experimentally.

Q1: How does the morphology change during shift-up?

A1: This was addressed by Conrad Woldringh. They observed cells with tapered tip in one of the cell poles such as  . Can you guess what this morphology represent?

Q4: Why is there a delay in increase of DNA synthesis rate? (Figure 2)

A4: We cannot answer unless we understand how the cell cycle is coupled to growth. The delay indicates that initiation might be delayed. However, this delay is <u>logically independent</u> of delay in division, which is the consequence of consistency requirement with the growth law. We will come back to this. For now, what is missing is <u>DNA</u>.

The Baby Machine and the Helmstetter - Cooper Model

In the 1960s, Charles Helmstetter developed the celebrated baby machine, the technology that enabled acquisition of synchronized cell populations. Combined with radiolabeling of DNA during replication, the baby machine produced data critical for the development of the cell cycle model.

Ref. C. Helmstetter, A ten-year search for synchronous cells: obstacles, solutions, and practical applications. Frontiers in Microbiology 6, Article 238 (2015)

Basic idea: Filter all culture through a membrane filter. (Helmstetter used pore diameter 0.22 μm)

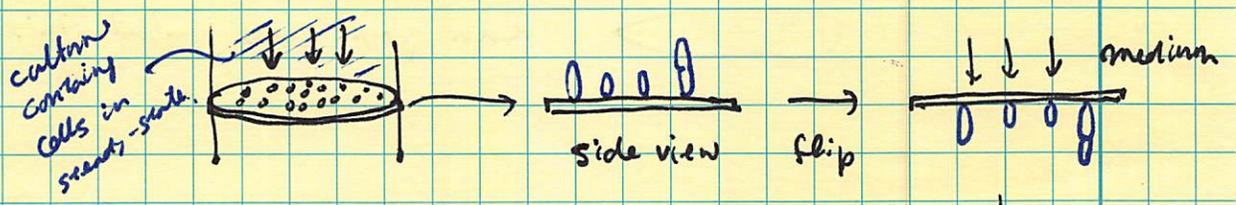
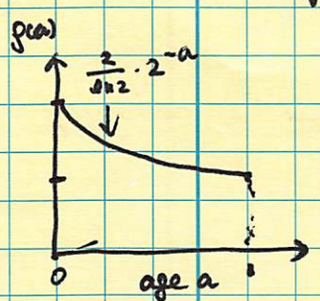


FIGURE 3

flow medium through the filter. the cells grow & divi
collect the cells tha

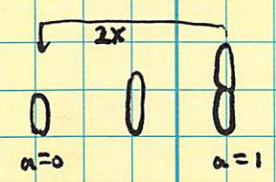
What is the distribution (eg size) of the cells to be collected?

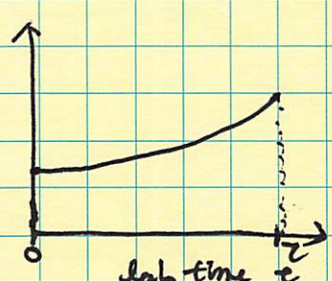


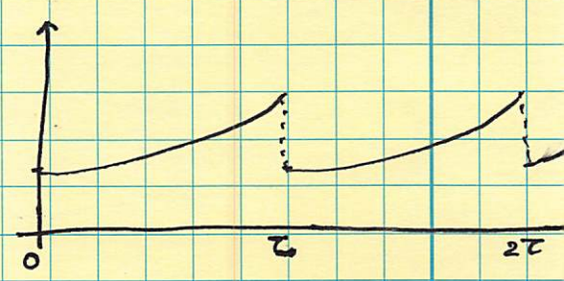
→ This is the age distribution of the cells on the filter.

If we collect the <u>eluted cells</u> from $t=0$ to $t=\tau$, the age distribution would be a mirror image because we effectively run backward in cell age.

$a \equiv t/\tau$ ← gen. time







Pulse labeling

Imagine a cell growing in poor media.

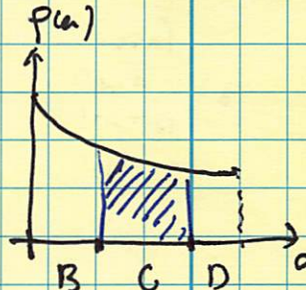
FIGURE 4

replication diagram focusing on the forks

cell cycle / nuclear cycle: bacteria → B period (G_1); (eukaryotes) → C (S); D (G_2)

* Only during C period, DNA is radio-labelled.

* For no overlapping replication cycle in steady state population →



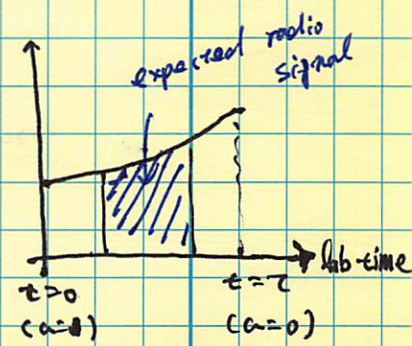


FIGURE 5

HOWEVER, the measured radio signal distribution in nutrient rich media from the baby machine experiments was like this, e.g.,

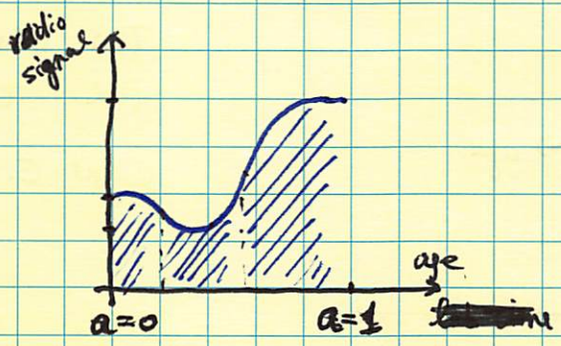


FIGURE 6

OBSERVATION

1. Replication is continuous for all cell age
2. The number of replication forks changes between birth & division (assuming the forks move at the same speed during the cell cycle).

The Helmstetter-Cooper model explains the data by assuming two internal timers for the cell, which run independently.

Timer τ : triggers replication initiations at every τ minutes

Timer C+D : triggers division after C+D minutes since repl initia

For fast growth condition, these timers can be visualized as follows:

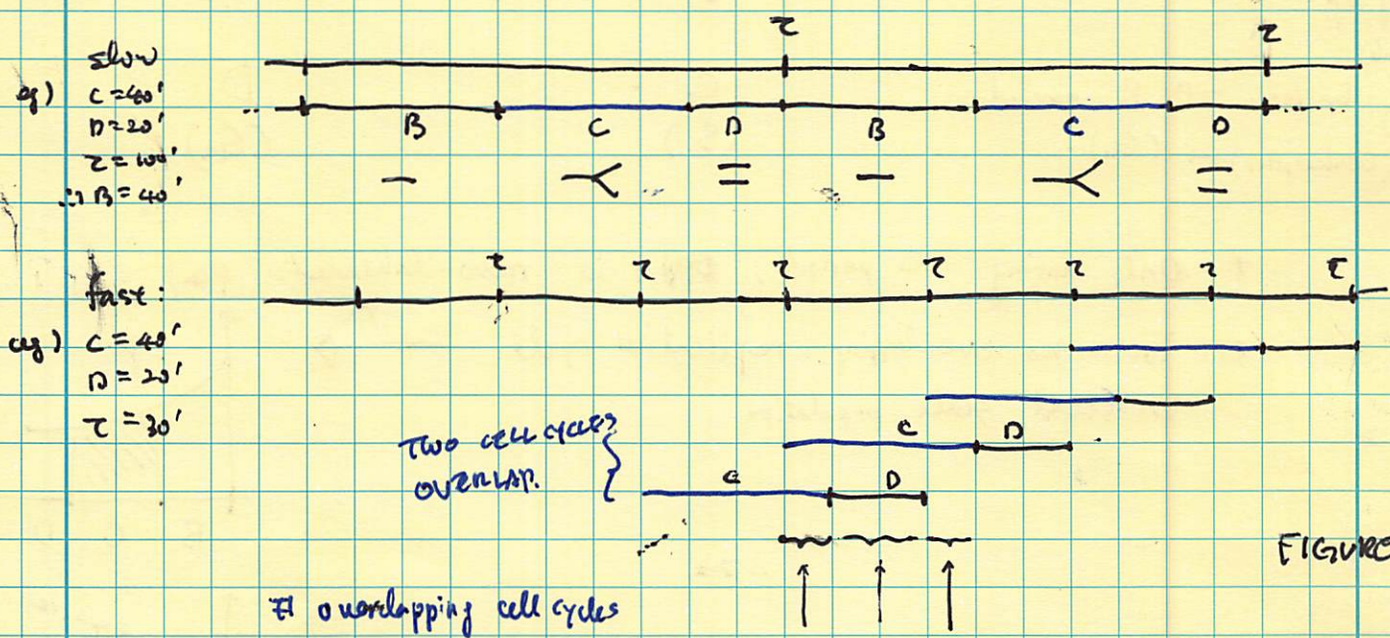


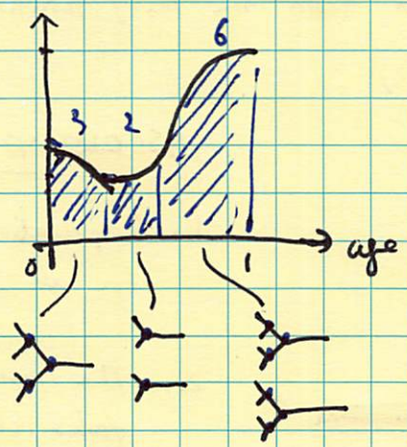
FIGURE 7

overlapping cell cycles

$$n_{oc} = \left\lceil \frac{C+D}{\tau} \right\rceil$$

eg) C+D = 60'
τ = 30'
$n_{oc} = 2$

For FIGURE 6, the replication rate changes 3 : 2 : 6 between birth and division. These are the # ratio of replicating forks.



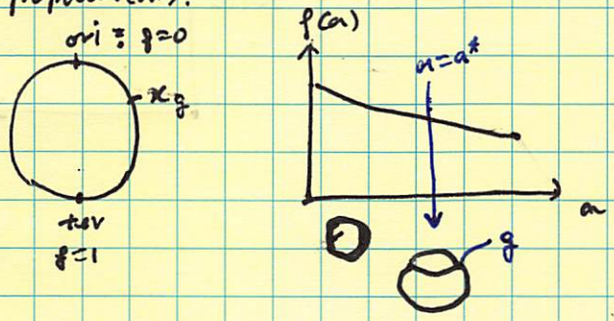
EXERCISE.

TRY DRAWING A REPLICATION DI
FOR $\tau < C+D$, BUT $\tau <$

CAN E. COLI BE A DIPLOID?

The Helmstetter-Cooper model is like Kepler's laws. It is kinematics and does not explain the origin of the phenomenological rules. However, it does allow experimentally testable quantitative predictions.

1. Average number (copy) of a specific genomic locus in steady state populations.



At age $a<a^*$, position x_g is duplicated.

average copy # of x_g

$$\langle x_g \rangle = \int_0^1 f(a)\, n_x(a)\, da$$

$$n_x(a) = n_{oc} \qquad a < a^*$$

$$n_x(a) = 2^1 * n_{oc} \qquad a \geq a^*$$

$$\langle x_g \rangle = 2^{\frac{(1-g)C+D}{2}}$$

for all growth conditions.

x_g) ori $\to g=0$. $\langle ori \rangle = 2^{\frac{C+D}{2}}$

ter $\to g=1$ $\langle ter \rangle = 2^{\frac{D}{2}}$

$$\frac{\langle ori \rangle}{\langle ter \rangle} = 2^{C/2}$$

more generally, $\boxed{\frac{\langle x \rangle}{\langle ter \rangle} = 2^{(1-g)\frac{C}{2}}}$

→ can be used to measure C period using qPCR.

2. Genome content: what is the average amount of DNA per cell?

$$\langle G \rangle = \int_{x=ori}^{x=ter} \langle x_g \rangle \cdot dx$$

$$= \frac{2}{\ln 2} \cdot \frac{\tau}{C} \cdot 2^{\frac{C+D}{2}} \cdot \left(1 - 2^{-\frac{C}{2}}\right) \quad (1)$$

(1): half of the full chromosome from ori to ter.

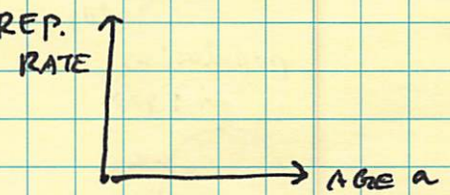
*** This does NOT follow the growth law like simple exponential relationship between $\langle G \rangle$ & λ.

** However, if we know C, this allows us to estimate D or C+D.

3. # replication forks & replication rate

EXERCISE. CALCULATE THE AVERAGE NUMBER OF REPLICATION FORKS IN A STEADY-STATE POPULATION (# fork at age

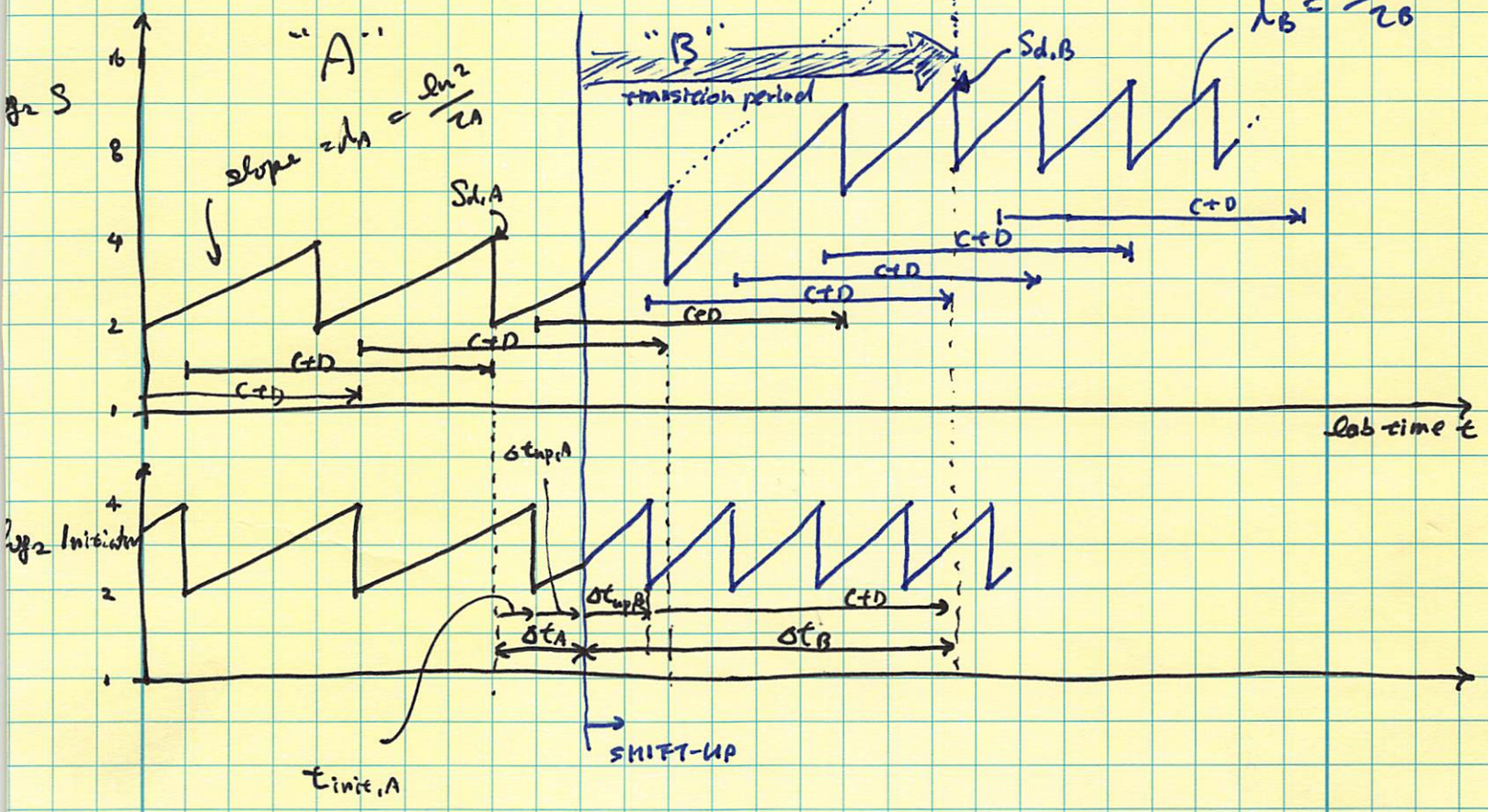
EXERISE. USIING THE RESULT FROM THE ABOVE EXERCISE GRAPHICALLY REPRESENT THE REPLICATION RATE AS



APPLICATION OF THE HELMSTETTER-COOPER MODEL TO NUTRIENT SHIFT-UP EXPERIMENT.
IMPORTANCE OF CONSISTENCY CHECK, PART II.

REFS. S. COOPER, Cell Division and DNA Replication following a Shift to a Richer Medium, J. Mol. Biol. 43, 1-11 (1969)

S. TAHERI-ARAGHI, Self-consistent examination of Donachie's Constant Initiation Size at the Single-Cell Level, FRONTIERS in Microbiology 6, article 1342 (2015)



Book keeping:

$$\begin{cases} \delta t_A = t_{init,A} + \delta t_{up,A} \\ \delta t_B = \delta t_{up,B} + (C+D) \\ \text{(*)} \quad \alpha \quad \lambda_A \times \delta t_{up,A} + \lambda_B \times \delta t_{up,B} = \lambda_B \times \tau_B = \ln 2 \end{cases}$$

Now the relationship between $Sd_{,B}$ and $Sd_{,A}$:

$$Sd_{,B} = \frac{\frac{Sd_{,A}}{2} \times 2^{\frac{\delta t_A}{\tau_A}} \times 2^{\frac{\delta t_B}{\tau_B}}}{2^{\#\text{divisions in } \delta t_B}}$$

$$\text{use (*)} \quad \Rightarrow \quad Sd_{,A} \times e^{C(C+D)(\lambda_B - \lambda_A)}$$

We derived the Growth Law $S(t) = S_0 \times e^{r \cdot t}$ if $r = C+D$

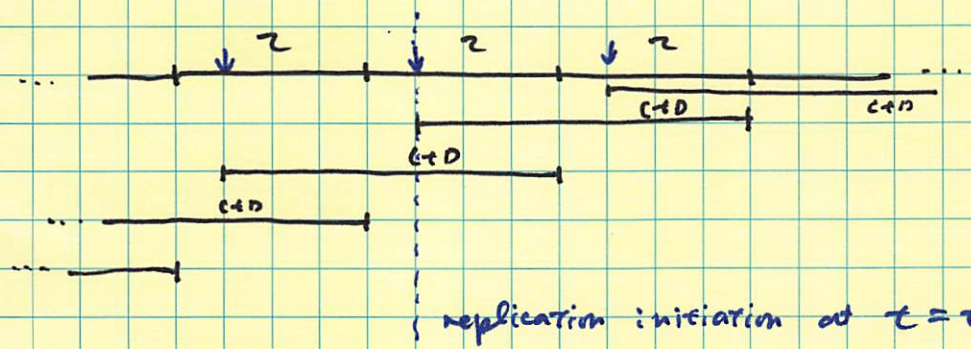
This explains the Kjeldgaard et al. 1958

i) The delay in cell division up to CTD from shift-up

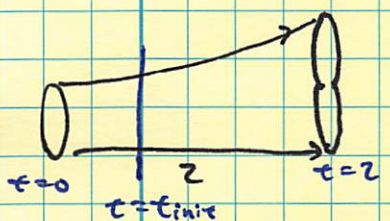
ii) Delay in initiation by $\Delta\tau_{up,B}$.
(or lag)

Donachie's constant initiation mass
IMPORTANCE OF CONSISTENCY CHECK, PART II.

Consider overlapping cell cycles



Q: What is the cell size at $t = t_{init}$, and how does it change at different growth condition?



$$S(t) = S_b \times 2^{t/\tau}$$

$$S_{init} = S(t = t_{init}) = S_b \times 2^{t_{init}/\tau} = S_b \times 2^{\frac{n_{oc}\times\tau - (C+D)}{\tau}}$$

, where n_{oc} = # overlapping cycle $= \left\lceil \frac{C+D}{\tau} \right\rceil$

Now, S_b changes under different growth condition (growth rate λ) following the growth law $S_b(\lambda) = S_{b,0} \times e^{\tau\cdot\lambda}$; $\lambda = \frac{\ln 2}{\tau}$

Thus, $S_{init}(\lambda) = S_{b,0} \times e^{\tau\cdot\lambda} \times 2^{n_{oc} - \frac{C+D}{\tau}}$

$$= S_{b,0} \times 2^{n_{oc}} \times 2^{\frac{\tau - (C+D)}{\tau}}$$

Now, if $\tau = C+D$, then $S_{init}(\lambda) = S_{b,0} \times 2^{n_{oc}}$, i.e., independent of the growth rate

Furthermore, $2^{n_{oc}}$ = # origins during multifork replication.

That is, if $\tau = C+D$, then the size per replication origin is

$$\boxed{\frac{S_{init}}{\#ori} = S_{b,0}}$$

, independent of λ.
This is an experimentally testable prediction.