5. SEARCH FOR SYNCHRONY - HELMSTETTER & COOPER 1968

THE EMPIRICAL RELATIONS UNCOVERED BY MAALØE'S GROUP AND BY NEIDHARDT & MAGASANIK DEMONSTRATED A COORDINATION BETWEEN GROWTH RATE & CELL COMPOSITION SUGGESTING SOME FORM OF REGULATION.

ONE OF THE OBSTACLES THAT HAD TO BE OVERCOME WAS THE INHERENT <u>POPULATION-AVERAGING</u> IN BULK MEASUREMENTS. WHAT WAS NEEDED WAS A TECHNIQUE TO OBSERVE SINGLE CELLS, OR PERHAPS EVEN BETTER, A SYNCHRONOUS POPULATION. (IN FACT, THE SHIFT EXPERIMENTS BY MAALØE WERE INITIALLY PLANNED AS A WAY TO SYNCHRONIZE CELLS, WHICH DIDN'T WORK.)

CELL AGE DISTRIBUTION

~~IF THE DOUBLING TIME IS τ, THEN THE CELL AGE 'a' IS THE RELATIVE TIME BETWEEN BIRTH ($t=0$) AND DIVISION ($t=\tau$),~~

~~$a = t/\tau \;;\; a \in [0,1)$~~

THE CELL AGE 'a' IS THE TIME BETWEEN BIRTH ($a=0$) AND DIVISION ($a=\tau$). WE CALL $\phi(a)$ THE 'AGE DISTRIBUTION' i.e.

$\phi(a)da$: PROBABILITY A BACTERIUM IN THE POPULATION HAS AGE ~~AGE~~ BETWEEN $(a, a+da)$

IT IS NO MORE DIFFICULT TO DERIVE $\phi(a)$ FOR AN ARBITRARY DISTRIBUTION OF DOUBLING TIMES $f(\tau)$, i.e.

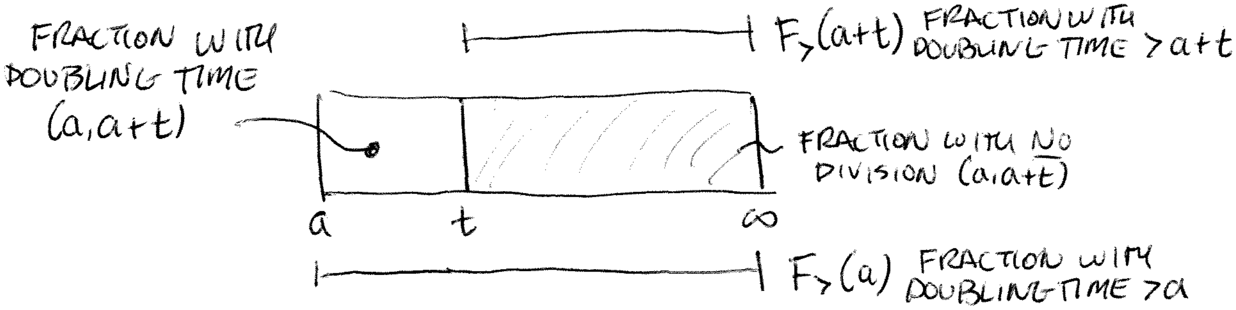
$f(\tau)d\tau$: PROBABILITY THAT A BACTERIUM DIVIDES AT AGE $a \in (\tau, \tau+d\tau)$.

THROUGHOUT, WE WILL ASSUME THE CULTURE IS IN BALANCED GROWTH $N(t) = N_0 e^{\lambda t}$ (AND THAT $\phi(a,t) \to \phi(a)$)

THE CUMULATIVE DENSITY $F_>(\tau)$, IS A MEASURE OF THE FRACTION OF BACTERIA WITH DOUBLING TIMES GREATER THAN τ:

$$F_>(\tau) = \int_\tau^\infty f(\tau')\,d\tau'$$ = FRACTION OF BACTERIA WITH DOUBLING TIME GREATER THAN τ.

SUPPOSE A BACTERIUM HAS REACHED AGE 'a', THE PROBABILITY IT WILL REACH $a+t$ WITHOUT DIVIDING IS:



GIVEN BACTERIUM REACHES AGE 'a'; PROBABILITY NO DIVISION OCCURS IN $(a, a+t)$ $= \dfrac{F_>(a+t)}{F_>(a)}$

FOR CULTURE WITH N BACTERIA, THE NUMBER OF BACTERIA AT AGE $(a, a+da)$ IS $N\phi(a)\,da$, SO THE NUMBER UNDIVIDED AT $(a+t)$ IS:

NUMBER OF BACTERIA STILL UNDIVIDED AT AGE $(a+t)$ $= N\phi(a)\,\dfrac{F_>(a+t)}{F_>(a)}\,da$

OVER THE INTERVAL $(a, a+t)$, THE POPULATION HAS INCREASED TO $Ne^{\lambda t}$; THE FRACTION OF UNDIVIDED CELLS IS THEN,

FRACTION OF CELLS UNDIVIDED AT $(a+t)$ $= \dfrac{N\phi(a)\dfrac{F_>(a+t)}{F_>(a)}\,da}{Ne^{\lambda t}} = \phi(a)\,\dfrac{F_>(a+t)}{F_>(a)}\,e^{-\lambda t}\,da$

ie. THE PROBABILITY THAT A CELL HAS AGE $(a+t, a+t+da)$, OR $\phi(a+t)\,da$.

THAT GIVES THE DIFFERENCE EQ.

$$\phi(a)\,\frac{F_>(a+t)}{F_>(a)}\,e^{-\lambda t} = \phi(a+t) \qquad \text{FOR ALL } t \geq 0.$$

NOT EASY TO SOLVE; TAKE TAYLOR SERIES, $t \to 0$,

$$F_>(a+t) = F_>(a) + t\,F_>'(a) + \mathcal{O}(t^2)$$
$$\phi(a+t) = \phi(a) + t\,\phi'(a) + \mathcal{O}(t^2)$$
$$e^{-\lambda t} = 1 - \lambda t + \mathcal{O}(t^2)$$

THE DIFFERENCE EQ BECOMES A SEPARABLE DIFFERENTIAL EQ, (DROPPING $\mathcal{O}(t^2)$ TERMS),

$$\frac{\phi'(a)}{\phi(a)} = \frac{F_>'(a)}{F_>(a)} - \lambda$$

OR,
$$\frac{d}{da}\left[\ln \phi(a)\right] = \frac{d}{da}\left[\ln\left\{F_>(a)e^{-\lambda a}\right\}\right]$$

INTEGRATING:
$$\boxed{\phi(a) = \phi(0)\,e^{-\lambda a}\,F_>(a) = \phi(0)\,e^{-\lambda a}\int_a^\infty f(\tau')\,d\tau'}$$

THE INTEGRATION CONSTANT $\phi(0)$ IS CHOSEN TO NORMALIZE THE DISTRIBUTION.

REMARK: IRRESPECTIVE OF $f(\tau)$, WE HAVE:

$$\phi(a) = \phi(0)\;e^{-\lambda a}\int_a^\infty f(\tau')\,d\tau'$$

TWO POSITIVE, MONOTONIC DECREASING FUNCTIONS OF a.

SO $\max \phi(a) = \phi(0)$ [ALWAYS MORE NEWLY-BORN BACTERIA].

USING AN IDEAL DOUBLING TIME DISTRIBUTION $f(\tau') = \delta(\tau - \tau')$ (i.e. ALL CELLS DIVIDE AT EXACTLY $a = \tau$)

$$\phi(a) = \begin{cases} \phi(0)\, e^{-\lambda a} & 0 \le a < \tau \\ 0 & \text{OTHERWISE} \end{cases}$$

OR, AFTER NORMALIZATION:

$$\lambda = \frac{\ln 2}{\tau}$$

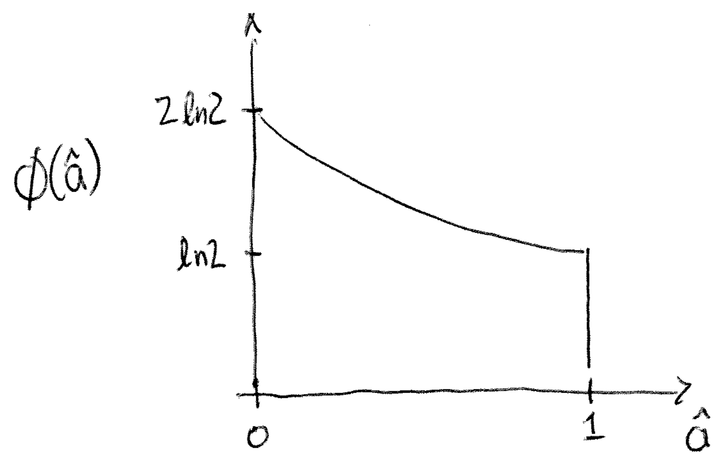
$$\phi(a) = \frac{\lambda}{1 - e^{-\lambda\tau}}\, e^{-\lambda a} \overset{\checkmark}{=} \frac{(2\ln 2)}{\tau}\, 2^{-a/\tau} \qquad 0 \le a < \tau.$$

USUALLY NORMALIZE CELL AGE TO DOUBLING TIME:

$$\hat{a} = a/\tau \quad , \quad 0 \le \hat{a} < 1.$$

AND,

$$\phi(\hat{a}) = (2\ln 2)\, 2^{-\hat{a}}$$



SYNCHRONY WOULD MEAN NARROWING THE AGE DISTRIBUTION

$$\phi(\hat{a}) \to \delta(\hat{a} - a_0)$$

HELMSTETTER DEVELOPED A WAY TO INSTEAD SAMPLE NARROW STRIPS OF $\phi(\hat{a})$.