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Analysis of Enzyme Induction in Bacteria

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The theoretical relations between the induced initiation and accumulation of *lac* mRNA and its translation are derived, taking the kinetics of repressor–operator dissociation and enzyme maturation into account. These relations are used to evaluate observed data on *lac* induction and to estimate a number of parameters that characterize the transcription and translation of the β-galactosidase gene in the bacterium *Escherichia coli* B/r growing at three different rates (0.7–2.1 doublings/h).

Two parameters of interest for bacterial physiology, the peptide chain-elongation rate (Kepes, 1963; Coffman *et al.*, 1971) and the functional life of mRNA (Kepes & Beguin, 1966; Kennell & Bicknell, 1973), have been determined from the induction kinetics of the enzyme β-galactosidase. In addition, these kinetics contain information about the speed of repressor–operator dissociation and about the initiation of mRNA chains after de-repression. Most of this information is contained in the early section (0–3 min) of the induction kinetics or in the kinetics observed after pulse induction ('capacity' experiments). Both experimental and theoretical analyses of these kinetics have been reported (Boezi & Cowie, 1961; Pardee & Prestidge, 1961; Kepes, 1963; Kaempfer & Magasanik, 1967; Leive & Kollin, 1967; Kepes, 1969; Coffman *et al.*, 1971; Jacquet & Kepes, 1971; Mosteller & Mandula, 1973; Schleif *et al.*, 1973; Singh, 1973; Achord & Kennell, 1974; Dalbow & Young, 1975). Here we analyse the theory of enzyme induction in greater detail and use this theory for a quantitative evaluation of recent experimental data from our laboratory obtained with the bacterium *Escherichia coli* B/r.

The following analysis has three parts. In the first part we derive the relations between the initiation of mRNA chains and the accumulation of finished enzyme after induction. In the second part we analyse the changes in mRNA and enzyme synthesis after pulse induction. The third part contains the analysis of observed induction kinetics.

1. Kinetics of Enzyme Induction

(*a*) *General procedure and assumptions*

To show the effects of the different molecular events involved in enzyme induction, we will first show what happens if (in a hypothetical case) full de-repression should occur instantaneously and mRNA were stable, then the effect of mRNA decay, and finally the effect of a finite time for the dissociation of the repressor–inducer complex will be explored (dotted, dashed and solid curves in Fig. 1). Further, the induction will be followed (theoretically) at three levels: initiation of mRNA, accumulation of mRNA termini and accumulation of β-galactosidase monomers (Figs. 1*a*, 1*b* and 1*c* respectively).

The following three simplifying assumptions are made. (1) The culture growth will be set at zero. This is permissible for the short time-interval considered; besides, experimental data can always be plotted on an expanded time-scale which corrects for the growth of the culture (McCarthy & Britten, 1962) such that they become directly comparable with the theoretical kinetics derived for zero growth rate. (2) The basal enzyme activity will be set at zero, i.e. only induced enzyme is considered. (3) The following events are assumed to occur randomly in time: dissociation of the repressor–inducer complex from the operator, initiation of transcription, initiation of translation at the 5′-end of mRNA, and functional inactivation of mRNA. The agreement of the observed with the theoretical kinetics justifies these assumptions [see Kepes (1969) and the discussion in Dalbow & Young (1975)].

(*b*) *Instantaneous de-repression and maturation*

In this section we will ignore the short times required for the repressor–inducer complex to dissociate from the operator and for maturation of the finished polypeptide into active, tetrameric enzyme (see sections *c* and *d* below). We will also ignore the time required for the intracellular inducer concentration to reach its final value, since even at very low suboptimal concentrations of inducer this time is negligible (Branscomb & Stuart, 1968; Hirsh & Schleif, 1973).

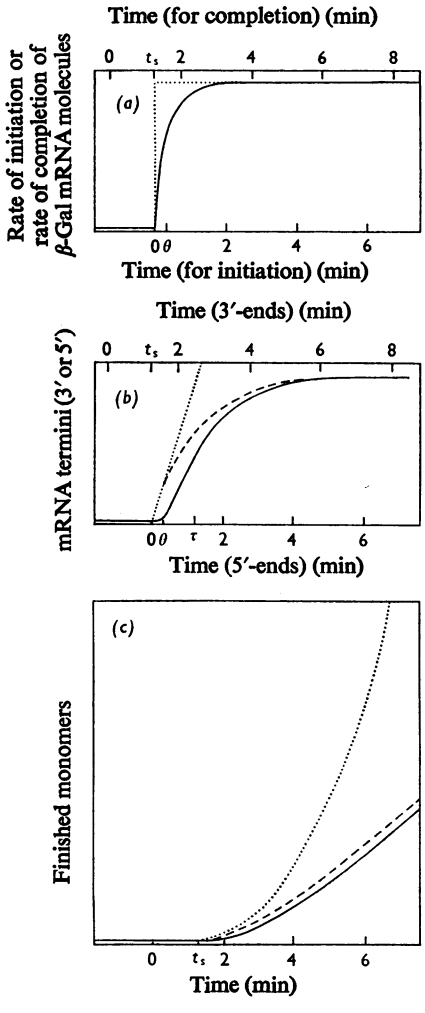
(*i*) *Initiation and completion of mRNA.* We define the rate of *lac* mRNA initiation, i (in mRNA molecules/min per average *lac* gene), the rate of β-galactosidase mRNA completion, c (that is, completion of the section of *lac* mRNA molecules corresponding to β-galactosidase, in mRNA molecules per average *lac* gene) and the synthesis time, t_s (in min) of the β-galactosidase section of *lac* mRNA. Instantaneous de-repression means that i and c increase in a step function to the final post-induction values i_∞ or c_∞ respectively, as shown in Fig. 1(*a*) (····; zero time defined as the moment of induction):

$$i = 0 \quad \text{for } t < 0 \qquad (1a)$$

$$i = i_\infty \quad \text{for } t > 0 \qquad (1b)$$

$$c = 0 \quad \text{for } t < t_s \qquad (2a)$$

$$c = c_\infty \quad \text{for } t > t_s \qquad (2b)$$



Normally, every mRNA molecule initiated is completed t_s min later such that $i_\infty = c_\infty$.

There have been reports of synchronous initiation (i.e. initiation in repeated bursts) after induction of a given operon (Morse *et al.*, 1969; Kepes, 1969; Schwartz *et al.*, 1970; Singh, 1973; Achord & Kennell, 1974). These bursts might be explained by scatter in the data [e.g. Kepes (1969) observed that the amount of induced enzyme increased in repeated steps, but only in two out of ten experiments], except for experiments reported by Singh (1973; his Fig. 6), which are not easily explained by random scatter alone. Burst initiation is implausible when one considers that such an effect requires that initiation by RNA polymerase be non-random and indeed tightly scheduled. Thus a promoter could not support RNA polymerase initiation until the previous polymerase had moved to a precise point some hundreds or thousands of nucleotides distant. Precise measurements of both induced mRNA (Cooper & Magasanik, 1974) and induced enzyme activity (Schleif *et al.*, 1973; Dalbow & Young, 1975) convincingly demonstrate that no periodicities or discontinuities are evident in *lac* operon induction. Accordingly we assume the intuitively more plausible concept, that initiation by RNA polymerase at a promoter is a random event.

Fig. 1. *Aspects of the induction of β-galactosidase; inducer is added at zero time*

(*a*) Rate of initiation (lower abscissa) or rate of completion (upper abscissa) of *z*-gene mRNA. If dissociation of the repressor–inducer complex is instantaneous (····), the initiation rate increases in a step at zero time. If the repressor–inducer operator complex has a finite average life (θ), the rate of initiation approaches the fully induced maximum asymptotically (——). Curves are drawn by using eqns. (1), (2) and (11) (see the text). (*b*) Number of mRNA termini: for 5'-termini, see the lower abscissa; for 3'-termini (end of *z*-gene), see the upper abscissa. For stable mRNA and instantaneous de-repression of the *lac* operator, the number of 5'-termini increases linearly with time from zero time (····). For unstable mRNA with an average life τ, the number of 5'-ends of the *z*-gene mRNA approaches a constant value (----). For a finite time required for de-repression ($\theta > 0$), the number of 5'-termini increases in a sigmoidal fashion (——). Curves are drawn from eqns. (3)–(6) and (12). (*c*) Number of finished *z*-gene monomers. For instantaneous induction and stable mRNA (····), the kinetics of accumulation of enzyme monomers is parabolic. For unstable mRNA with either instantaneous de-repression (----) or non-instantaneous de-repression ($\theta > 0$; ——), the kinetics are initially parabolic and finally ($t \to \infty$) linear. Curves are drawn by using eqns. (8), (9) and (13). All ordinate scales are arbitrary; the values refer to rates, numbers and amounts per unit culture volume or per cell (since the growth of the culture is assumed to be negligible).

Table 1. *Parameters for β-galactosidase synthesis in E. coli B/r, growing exponentially in glucose–amino acids medium, glucose, or succinate minimal medium, under conditions of continuous, maximum induction (high concentrations of cyclic AMP and inducer)*

Cells per mass unit (turbidity of culture at 460nm, 1cm light-path) and genomes (genome equivalent of DNA = 7.6×10^6 nucleotides) per cell are given to allow conversion of the other parameters in this Table, which are given in units per genome, into units per cell or units per turbidity of culture.

Parameter	Units	Symbol	Glucose–amino acids	Glucose	Succinate	Reference or equation
Growth rate	doublings/h	μ	2.14	1.36	0.67	
Cells per mass unit	1×10^8 Coulter counts/E_{460} unit	C	2.6	4.6	10.0	Dennis & Bremer (1974)
Genomes (= genome equivalents)	per cell	$\bar{G}$	2.95	2.02	1.61	Cooper & Helmstetter (1968)
Number of *lac* genes*	per cell	$N_{gen\,l}$	2.64	1.88	1.54	See footnote*
	per genome		0.89	0.93	0.96	$N_{gen\,l}$ (per cell)/$\bar{G}$
Differential rate of β-galactosidase synthesis	Fraction of β-galactosidase protein/total protein	α_{lac}	0.0037	0.0048	0.0092	Dalbow & Bremer (1975)
β-Galactosidase synthesis time	min	t_s	1.23	1.25	1.51	Dalbow & Young (1975)
Functional life of β-galactosidase mRNA	min	τ	1.27	1.25	0.99	$\tau = 2.5 - t_s$; Dalbow & Young (1975) (eqn. 10a)
Rate of β-galactosidase synthesis (max. induction)	1×10^4 amino acids/min	dE/dt	3.76	3.10	2.07	$dE/dt = \alpha_{lac}p$; p = protein synthesis rate = 10.16×10^6, 6.46×10^6 or 2.25×10^6 amino acids/min per genome (Dennis & Bremer, 1974)
	monomers/min per genome		32	26	18	dE(monomer)/dt = [dE(amino acids)/dt]/1170 amino acids assuming 1170 amino acid residues per monomer
Peptide chain elongation rate	amino acids/min per ribosome	c_p	951	936	775	c_p = 1170 amino acids/t_s
Translation rate	ribosomes/min per mRNA	k	30	26	17	$k = 3p/r_m\cdot\tau$; 3(nuc/amino acids) = coding ratio; r_m = rate of mRNA synthesis = 7.94×10^5, 6.04×10^5 or 4.05×10^5 nucleotides/min per genome (Dennis & Bremer, 1974)
Number of *lac* mRNA	molecules/genome	N_{mRNA}	1.1	1.0	1.1	$N_{mRNA} = dE/dt$(monomers)/k (eqn. 7)
Initiation rate of *lac* mRNA	molecules/min per *lac* gene	i	0.9	0.9	1.1	$i = N_{mRNA}/(\tau\cdot N_{gen\,l})$

* Number of *lac* genes per cell is calculated from the map position of the *lac* gene (10min on standard map), origin of DNA replication (74min; Bird *et al.*, 1972), the Cooper–Helmstetter (1968) theory of DNA replication and the age distribution of cells in a non-synchronized exponential culture [$n(a) = (\ln 2)\cdot 2^{1-a}$; Sueoka & Yoshikawa, 1965]. The *lac* gene is 26 map units (min) distant from the replication origin, corresponding to 26/45 = 0.58 (fraction) of one-half a chromosome. One-half of a chromosome is replicated in 41min ($\mu>1$) or 59min ($\mu=0.67$, i.e. in succinate medium), assuming bidirectional replication. Accordingly, the *lac* gene is replicated 24min ($\mu>1$) or 34min ($\mu=0.67$) after initiation of replication. Assuming the initiation age (a_i) to be 0.75, 0.53 and 0.0 (Cooper & Helmstetter, 1968), the cell age of *lac* replication (a_{lac}) is found to be at 0.60, 0.90 and 0.38 at the three growth rates. The fraction of cells in a population which have not yet replicated the *lac* genes (= integral over age distribution from 0 to a_{lac}) is then found to be 0.68, 0.12 and 0.46. Before replication, the number of *lac* genes per cell is 2, 1 or 1 for the three growth rates, after replication the numbers are 4, 2, 2. Thus in glucose–amino acid medium, for example, the average number of *lac* genes per cell is $[0.68\times2+(1-0.68)\times4] = 2.64$.

We estimate that, on maximum induction, the *lac* mRNA initiation rate in *E. coli* (i) is only about 1molecule/min per gene (Table 1). If the initiation rate were much higher, for example several mRNA molecules/s, then it is obvious that on removal of the repressor from the operator, the rate of mRNA initiation would increase stepwise, the step occurring within the first second. However, when the final initiation rate is on the average one chain every 60s, such that it takes 30–60s until most promoters have

had at least one initiation, it might seem that it takes that long for the initiation rate to reach its final value [e.g. see Coffman *et al.* (1971): 'Only if every cell in the population initiates mRNA synthesis fully and quickly upon induction will the average lag time give accurate information about mRNA lifespan.' Also Singh (1973): 'it should be possible to demonstrate a periodicity in the transcription of *lac* mRNA at very low concentrations of inducer'.] This is not the case; in a large population of cells the initiation rate will always increase to its final value as fast as the repressor is removed, no matter how low the initiation rate. The initiation rate is determined by the probability of RNA chain initiation at the promoter; this probability has only one (maximum) value after removal of the repressor. Hence, in contrast with published arguments there is absolutely no contribution from the initiation frequency to the delay after the addition of inducer before induced enzyme appears [for example, Schleif *et al.* (1973) evaluated this contribution to be less than 0.2min], in agreement with the experimental results of Branscomb & Stuart (1968).

(*ii*) *Accumulation of functionally active RNA.* The number of mRNA molecules initiated and finished at a given time after induction is given by the number (N_5, N_3) of 5′- or 3′-termini of mRNA. Here, the termini refer to the boundaries of the *z*-gene, not to the physical ends of *lac* mRNA. Thus, $dN_5/dt = i$ and $dN_3/dt = c$. In the hypothetical case of stable mRNA the number of mRNA molecules would increase linearly with time (Fig. 1*b*, ····):

$$N_5 = \int_0^t i\,dt = it \qquad \text{for } t \geqslant 0 \tag{3}$$

$$N_3 = \int_{t_s}^t c\,dt = c(t-t_s) \qquad \text{for } t \geqslant t_s \tag{4}$$

In the actual case of a finite average functional life (τ, in min) of mRNA the rate of inactivation of mRNA must be subtracted from the rate of initiation. The inactivation is proportional to N and inversely proportional to τ:

$$N_5 = \int_0^t \left(i - \frac{N_5}{\tau}\right) dt = i\tau(1-e^{-t/\tau}) \quad \text{for } t>0 \tag{5}$$

$$N_3 = \int_{t_s}^t \left(c - \frac{N_3}{\tau}\right) dt = c\tau(1-e^{-(t-t_s)/\tau}) \text{ for } t>t_s \tag{6}$$

These equations are illustrated in Fig. 1(*b*) (----). The number of 5′- and 3′-ends are found to reach a constant value, equal to the product $i\tau$ ($=c\tau$), for long times after induction ($t \to \infty$). For *E. coli* B/r, this value is estimated to be equal to about 1 mRNA molecule per *lac* gene or 2–3 *lac* mRNA molecules per cell (Table 1).

The value of N_5 includes mRNA molecules initiated but not finished; N_3 includes mRNA molecules finished (to the end of the *z* gene) but whose initial section is inactive or degraded. Thus, although the numbers N_5 and N_3 become equal later after induction, the molecules whose 5′-ends are counted (N_5) are not physically identical with the molecules whose 3′-ends are counted (N_3). If the chemical lifetime of mRNA is longer than the functional life, then the cell may contain additional *lac* mRNA molecules (or mRNA fragments), included neither in N_5 nor in N_3, whose ribosome attachment site is inactive but not yet degraded [see Jacquet & Kepes (1971) for a detailed discussion].

(*iii*) *Accumulation of β-galactosidase monomers.* Ribosomes begin translation of mRNA with a constant probability per unit of time as soon as the initiation sequence is synthesized (this situation is analogous to the initiation of mRNA by RNA polymerase discussed above). At each 3′-end (polypeptide termination sequence) of β-galactosidase mRNA, ribosomes with finished monomer chains arrive at a constant rate, k, in chains/min per mRNA molecule. (Again, the magnitude of k does not affect the delay between induction and appearance of enzyme.) Hence the rate of β-galactosidase polypeptide formation, dE/dt, is equal to the product kN_3. The amount of enzyme at any time t after induction is obtained by integrating the function describing the rate from zero to t:

$$E = \int_0^t kN_3\,dt \tag{7}$$

For stable mRNA, we substitute eqn. (5) for N_3 and obtain:

$$E = kc\int_{t_s}^t (t-t_s)\,dt = \frac{kc}{2}(t-t_s)^2 \quad \text{for } t>t_s. \tag{8}$$

This is the parabolic kinetics shown in Fig. 1(*c*) (····).

Taking mRNA decay into account, by substituting eqn. (6) for N_3, one obtains:

$$E = kc\tau[(t-t_s) - \tau(1-e^{-(t-t_s)/\tau})] \qquad \text{for } t>t_s \tag{9}$$

This is the more complex kinetics shown in Fig. 1(*c*) (----). These theoretical kinetics (eqn. 9) express to a very good approximation the actually observed kinetics of β-galactosidase induction, and will therefore be discussed in more detail.

Before t_s, the amount of induced enzyme is zero ($E = 0$ for $t < t_s$), i.e. there cannot be finished enzyme before any mRNA is finished. After t_s the kinetics of enzyme accumulation as given by eqn. (9) are determined by the parameters t_s, τ and product kc. Further, these kinetics show an initial parabolic phase [for $(t-t_s) \to 0$], the origin of the parabola

being at t_s, a fact that has been used to determine t_s (Schleif *et al.*, 1973; Dalbow & Young, 1975), and a final linear phase (for $t \to \infty$) determined by the parameters τ and kc.

At times short in comparison with the functional life of mRNA ($t - t_s \ll \tau$), mRNA decay has only a negligible effect, such that one can expect the initial kinetics (eqn. 9) to be parabolic, identical with the kinetics given by eqn. (8). That this is the case is seen by rewriting eqn. (9) (developing the exponential term as polynomial):

$$E = kc\left[\frac{(t-t_s)^2}{2} - \frac{(t-t_s)^3}{3!\tau} + \frac{(t-t_s)^4}{4!\tau^2} - \cdots\right] \quad (9a)$$

i.e. for $(t-t_s) \to 0$ or for $\tau \to \infty$ (stable mRNA) all terms except the first one become negligible, and eqn. (9) becomes eqn. (8).

Late after induction ($t \to \infty$) enzyme accumulates at a constant rate according to the (linear) equation:

$$E = kc\tau[t-(t_s+\tau)] \qquad \text{for } t \to \infty \qquad (9b)$$

This final rate of β-galactosidase synthesis is seen to be equal to the product $kc\tau$. For maximum induction in *E. coli* B/r this rate is 7–24 (depending on the growth medium) tetrameric enzyme molecules/min per cell (Table 1).

The linear section of the kinetics can be extrapolated back to the time axis (actually to the basal enzyme activity) at a value [t_x, known as 'total lag of induced enzyme synthesis' (Kepes, 1969)], which is seen from eqn. (9b) (setting $E = 0$) to be equal to the sum $t_s + \tau$. Thus if t_s has been determined (see section 3*a* below), one can find the functional life of β-galactosidase mRNA from t_x and t_s, by using the relationship:

$$\tau = t_x - t_s \qquad (10a)$$

Mosteller & Mandula (1973) apparently assumed t_x to be equal to t_s rather than to the sum $(\tau + t_s)$; using the intersection of the linear kinetics with the basal enzyme activity, they find translational 'lag times' that are greater than the transcription times measured by other workers for the inducible *trp* genes. The difference found is 1–1.5 min, ascribed by Mosteller & Mandula (1973) to retardation of the ribosomes under the conditions used, such that the ribosomes cannot keep pace with the RNA polymerase. However, the analysis here shows that the difference that they ascribe to ribosome retardation is just equal to τ and that they are determining t_x, not t_s.

In summary, the derivation presented here predicts that after induction, there is a finite lag (the transcription or translation time, t_s) before the appearance of the first completed polypeptide. Finished polypeptides then increase initially as a parabolic function, which gradually becomes a linear function of time as the number of 3′-termini of functional messenger approaches a steady state.

Similar final expressions for the accumulation of completed polypeptides after induction have been derived previously. A. Koch, in an Appendix to Coffman *et al.* (1971), derived an equation (on p. 16 of that paper) which, on substitution for the parameters he defined, can be shown to be identical with our eqn. (9). Also, Singh (1973, 1974) has derived expressions for enzyme induction. Although the units used are obscure (e.g. time is expressed in units of the interval between translation terminations), some of his conclusions agree with those presented here. However, there is considerable emphasis given to 'synchronous' transcription initiation (Singh, 1973), which is manifestly unrealistic (see above). In the above and other previous theoretical treatments of enzyme-induction kinetics there is no analysis of the role of 3′-termini of mRNA, which we consider central to the understanding of the induction kinetics.

(*c*) *Delayed repressor–operator dissociation*

Here we will consider only the events after the binding of inducer to the repressor, since the delay owing to the build-up of the intracellular inducer concentration is negligible regardless of the extracellular inducer concentration (Branscomb & Stuart, 1968; Hirsch & Schleif, 1973).

Measurements *in vitro* with purified *lac* repressor indicate that, on binding of inducer to the repressor, the repressor dissociates within about 5 s from the operator (Jobe & Bourgeois, 1972). Those experiments were done at an ionic strength of 0.05 M; at the ionic strength *in vivo* (about 0.2 M) the dissociation is presumed to be faster, perhaps 2–4 s. This means that the assumption of a stepwise derepression (eqn. 1) is essentially correct.

In spite of the relatively small effect to be expected from a delayed repressor dissociation we have explored this case because a delay of even a few seconds somewhat changes the shape of the graph of the early induction kinetics and thereby affects the accurate determination of the synthesis time of β-galactosidase (Dalbow & Young, 1975); besides, this analysis makes it possible in the future to study *in vivo* the slower dissociation of mutationally altered repressor–operator systems (Smith & Sadler, 1971).

Assuming that the dissociation of the repressor from the operator follows first-order reaction kinetics with a time constant θ (= average life of the repressor–operator complex), the initiation of β-galactosidase mRNA will increase after induction in an exponential function (eqn. 11 below; Fig. 1*a*, ——), which is the mirror image of the dissociation reaction (repressor–operator complex as a function of time).

The accumulation of β-galactosidase monomers (Fig. 1*c*, ——) is then altered in three ways.

(1) Initially (i.e. during the first few seconds after t_s) the amount of enzyme increases with the third power of time rather than the second power. (2) Later (about 1 min after t_s) enzyme activity increases somewhat faster than expected from eqn. (8). This, for a while, compensates for the effect of mRNA decay such that the kinetics appear parabolic longer than otherwise expected. (3) At late times the whole kinetics is shifted along the time axis, the shift being equal to θ, such that the extrapolation value (t_x, eqn. 10a) becomes the sum of the three time constants considered:

$$t_x = t_s + \tau + \theta \qquad (10b)$$

These relationships are mathematically expressed as follows. Completion of mRNA (Fig. 1*a*, ——):

$$c = c_\infty(1 - e^{-(t-t_s)/\theta}) \qquad \text{for } t > t_s \qquad (11)$$

Accumulation of complete, functional β-galactosidase mRNA molecules (3′-termini; Fig. 1*b*, ——):

$$N_3 = \int_{t_s}^{t} \left(c - \frac{N_3}{\tau}\right) dt = c_\infty \tau \left\{\left[\frac{\tau}{(\tau-\theta)} e^{-(t-t_s)/\tau}\right] + \left[\frac{\theta}{(\tau-\theta)} e^{-(t-t_s)/\theta}\right]\right\} \quad \text{for } t > t_s \qquad (12)$$

Accumulation of finished enzyme (Fig. 1*c*, ——):

$$E = \int_{t_s}^{t} kN_3\,dt = kc_\infty\tau\left\{(t-t_s) - \frac{\tau^2}{(\tau-\theta)}\left[1 - e^{-(t-t_s)/\tau}\right] + \frac{\theta^2}{(\tau-\theta)}\left[1 - e^{-(t-t_s)/\theta}\right]\right\} \qquad (13)$$

Eqn. (13) has been used to calculate various expectations of the induction kinetics (different τ and θ values) to be compared with the observed induction kinetics (Dalbow & Young, 1975).

(*d*) *Enzyme maturation*

Some investigators have observed that β-galactosidase activity continues to increase for 30–40s when, at some time after induction, protein synthesis is inhibited by chloramphenicol (Kepes, 1963; Kaempfer & Magasanik, 1967); they concluded that a maturation process which does not require protein synthesis and which does not occur under assay conditions is required for the enzyme to become active. Other investigators did not observe this effect (Alpers & Tomkins, 1965; Dalbow & Young, 1975); possible reasons for this discrepancy have been discussed (Dalbow & Young, 1975).

In addition to producing the chloramphenicol effect, maturation is also expected to affect the induction kinetics. A mathematical treatment (see below) indicates that, in spite of an entirely different level of action, the effect of a delayed maturation on the induction kinetics is exactly the same as the effect of a delayed repressor–operator dissociation: if μ defines the average duration of the maturation process, it is only necessary to substitute μ for θ in eqn. (13) to obtain the expectation for induction kinetics including a finite maturation time (and zero θ). If μ is 30–40s (see above) it should have drastic effects on the induction kinetics: the amount of enzyme should increase as a cubic function of time for 30–40s after t_s. This has not been observed (Hirsh & Schleif, 1973; Dalbow & Young, 1975) and it has not been firmly established whether or not, or under what conditions, the synthesis of active β-galactosidase requires a significant time for maturation (see the discussion section in Dalbow & Young, 1975).

To derive the kinetics of accumulation of mature enzyme, one needs to distinguish between the time (t') of completion of inactive monomeric enzyme (E^*) and the time (t) of completion of mature tetrameric enzyme (E). A differential amount of monomer (dE^*), completed at t', is assumed to be converted into mature enzyme (dE) according to the maturation function:

$$dE = dE^*[1 - e^{-(t-t')/\mu}] \qquad (14)$$

($1/\mu$ = rate constant). Substituting $dE^* = kN_3\,dt'$ (eqn. 7), and eqn. (6) for N_3, then integrating from $t' = t_s$ to $t' = t$ gives the kinetics for the accumulation of mature enzyme (for $t > t_s$), which is formally identical with eqn. (13) (μ substituted for θ).

2. Messenger RNA and Capacity

(*a*) *Relation between termini and amount of mRNA*

Since mRNA includes in various proportions, depending on the time after induction, unfinished and partially degraded molecules, the kinetics of the accumulation of the amount of β-galactosidase mRNA must be different from the kinetics of accumulation of mRNA termini (given by eqn. 7). The amount of mRNA can be calculated if one assumes that degradation of decaying mRNA proceeds with equal speed (and in the same 5′ → 3′ direction) as the elongation of nascent mRNA chains and further, that initiation of chemical degradation at the 5′-termini is a random event.

The rate of accumulation of mRNA for β-galactosidase, r_m ($r_m = dR_m/dt$; R_m = amount of mRNA) is proportional to the difference 'number of growing molecules (N_g) minus number of decaying molecules (N_d)' and to the mRNA chain growth rate (c_m):

$$r_m = (N_g - N_d)c_m \qquad (15)$$

Molecules which grow at the 3′-end and are simultaneously degraded at their 5′-end do not contribute to the accumulation of mRNA, i.e. their equal contributions to N_g and N_d cancel in the difference.

After induction, the number of growing *lac* mRNA molecules increases at first linearly with time until t_s, then it becomes constant, reflecting that the *lac* genes are now filled with RNA polymerase molecules (Fig. 2*b*):

$$N_g = it \qquad \text{for } t \leqslant t_s \qquad (16a)$$

$$N_g = \text{constant} = it_s \qquad \text{for } t \geqslant t_s \qquad (16b)$$

The validity of eqns. (16a) and (16b) has been experimentally verified by pulse-labelling *lac* mRNA at various times after induction (Cooper & Magasanik, 1974; the amount of pulse label is proportional to the number of growing mRNA chains).

The net change in the number of decaying molecules, dN_d/dt, is given by:

$$\frac{dN_d}{dt} = \frac{1}{\tau}(N_5 - N_3) \qquad (17)$$

where $1/\tau$ is the rate of decay (1 decay per lifetime; here chemical, not functional life) and the quantity in parentheses reflects the appearing mRNA molecules minus disappearing, decaying mRNA molecules. (A molecule disappears from consideration when the 3′-end of the β-galactosidase section of a *lac* mRNA molecule decays.)

Substituting eqns. (5) and (6) for N_5 and N_3 into eqn. (17), integrating and combining with eqns. (15) and (16) gives the rate of β-galactosidase mRNA synthesis (see Figs. 2*c* and 2*d*):

$$r_m = c_m i\tau(1 - e^{-t/\tau}) \qquad \text{for } t \leqslant t_s \qquad (18a)$$

$$r_m = c_m i\tau[e^{-(t-t_s)/\tau} - e^{-t/\tau}] \qquad \text{for } t \geqslant t_s \qquad (18b)$$

and, by integration, the amount of β-galactosidase mRNA as a function of induction time (see Fig. 2*e*):

$$R_m = c_m i\tau[t - \tau(1 - e^{-t/\tau})] \qquad \text{for } t \leqslant t_s \qquad (19a)$$

$$R_m = c_m i\tau[t_s - \tau(e^{-(t-t_s)/\tau} - e^{-t/\tau})] \qquad \text{for } t \geqslant t_s \qquad (19b)$$

The units for the amount are mRNA nucleotides per cell if c_m is given in nucleotides/min, i in molecules/min per cell and all times in min.

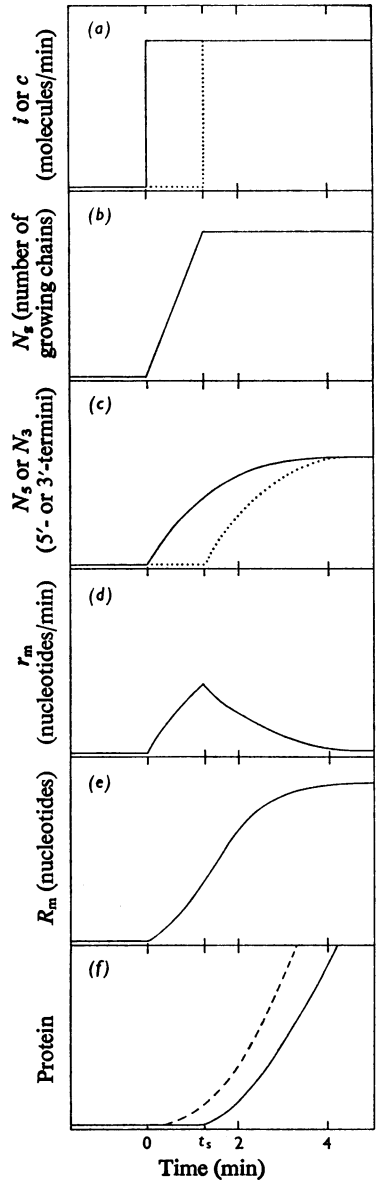


Fig. 2. *lac mRNA during β-galactosidase induction*

Inducer is added at zero time. τ is the average lifetime of mRNA, t_s is the synthesis time of β-galactosidase. It is assumed that induction is instantaneous (i.e. $\theta = 0$), and culture growth is negligible. (*a*) Initiation rate (——) or completion rate (····) of *z*-gene mRNA (eqns. 1 and 2). (*b*) Number of nascent *z*-gene mRNA molecules (i.e. number of RNA polymerase molecules transcribing the *z*-gene; eqns. 16a and 16b). (*c*) Number of 5′- (——) or 3′- (····) termini of *z*-gene mRNA (eqns. 5 and 6). (*d*) Net rate of synthesis (r_m) of *z*-gene mRNA; r_m approaches zero as the amount of *z*-gene mRNA becomes constant (mRNA breakdown equal to mRNA synthesis; eqns. 18a and 18b). (*e*) Amount of *z*-gene mRNA (R_m); i.e. total RNA hybridizable to *z*-gene DNA (eqns. 19a and 19b). (*f*) β-Galactosidase protein accumulation; the finished polypeptides (——) increase parabolically from time = t_s, approaching a linear rate of accumulation at $t \gg t_s$ (eqn. 9). The amount of total β-galactosidase protein (nascent plus finished protein; - - - -) initially increases with the third power of time, later the kinetics becomes parallel to the kinetics describing the accumulation of finished protein (——, calculated by integration of eqn. 19).

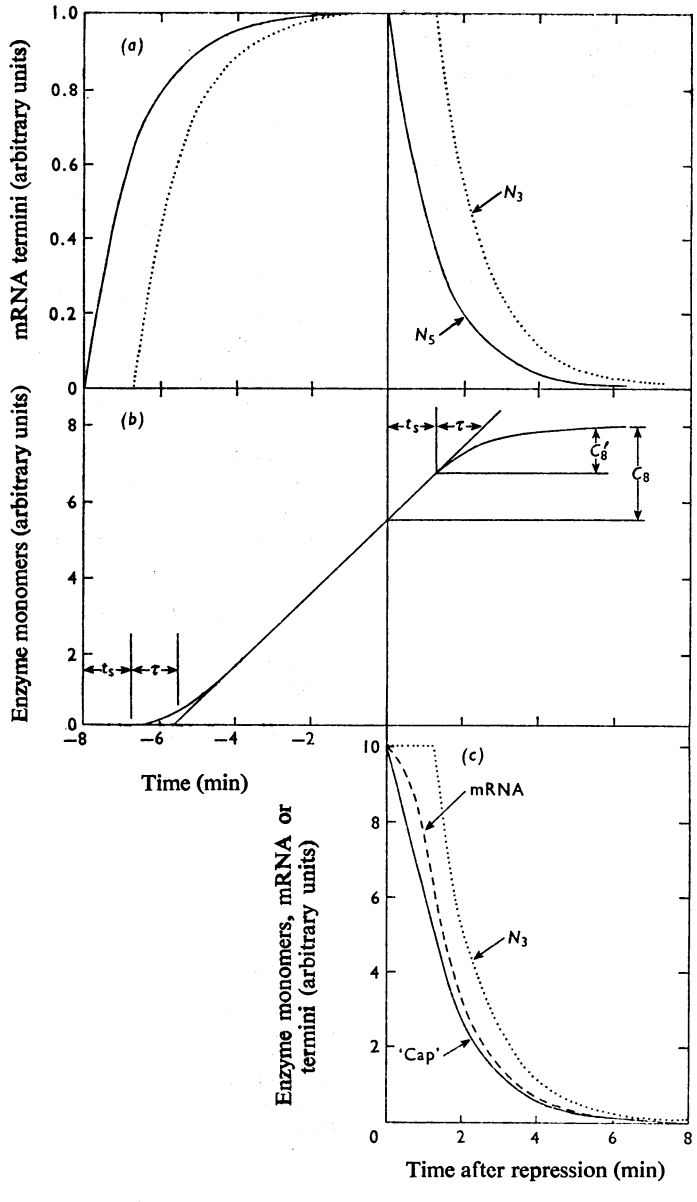


Fig. 3. *Synthesis of β-galactosidase after a pulse induction of 8 min duration*

Inducer is added at −8 min and removed at zero time. (*a*) Number of *z*-gene 5′-termini (——) and 3′-termini (····), obtained by using eqns. (5) and (6). (*b*) Number of completed β-galactosidase monomers; finished monomers first appear at a time t_s after induction (t_s = synthesis time for the polypeptide). After removal of the inducer, β-galactosidase continues to accumulate at a constant rate for a period equal to t_s; the rate of completed monomer production then decreases with an average lifetime equal to τ. C_8 is the enzyme-forming capacity at the time of de-induction defined as the difference between final amount of enzyme minus the amount present at zero time; C_8' is the residual capacity at the time when the rate of enzyme begins to decrease exponentially (i.e. with the average lifetime τ). Curves are drawn by using eqn. (7), substituting N_3 from Fig. 3(*a*). (*c*) Superposition of the curves showing the decrease in the number of 3′-termini for *z*-gene mRNA (N_3) (····), the amount of *z*-gene mRNA (– – –), and the enzyme-forming capacity ('Cap') (——). Replot of the data from Fig. 3(*b*).

For long induction times ($t \to \infty$) the amount of mRNA approaches a plateau:

$$R_m(\infty) = c_m i\tau t_s \qquad (19c)$$

or, setting $i\tau = N_5(\infty)$ and $c_m \cdot t_s = m$ (length of z-gene section of *lac* mRNA in nucleotides):

$$R_m(\infty) = mN_5(\infty) \qquad (19d)$$

In contrast with the number of mRNA molecules (eqn. 7, Fig. 2*c*), the amount of mRNA (eqn. 19) increases as a sigmoidal function of time (Fig. 2*e*), with an initial parabolic section. This initial parabolic increase of the amount reflects a linear increase of the rate of β-galactosidase mRNA synthesis, or an increasing number of RNA polymerase molecules filling the *lac* gene until it becomes saturated with polymerase at $t = t_s$. These theoretical kinetics (eqn. 19) are in good agreement with observations of Cooper & Magasanik (1974).

The accumulation rate of finished β-galactosidase monomers was shown above to be proportional to the number of 3′-termini of β-galactosidase mRNA. In contrast, the accumulation rate of total (finished plus nascent) β-galactosidase protein is proportional to the amount of β-galactosidase mRNA (Fig. 2*f*). The distinction between these two relations is important for the evaluation of so-called capacity measurements (see below).

(*b*) *Capacity*

Fig. 3(*a*) shows the calculated changes in the number of β-galactosidase mRNA termini during a period of 8 min after induction (eqn. 6), which is followed by another 8 min period of repression (i.e. removal of inducer = de-induction). The number of 5′-ends (N_5) is seen to reach the final plateau value during the induction period; N_5 then decreases exponentially to zero during the repression period. The kinetics describing the number of 3′-ends (N_3) as a function of time is identical but shifted by t_s (= 1.25 min, Table 1).

The kinetics of β-galactosidase monomer accumulation (Fig. 3*b*) has been obtained by integration of the function for the number of 3′-ends (eqn. 7). The amount of monomer is zero until the time t_s, then increases parabolically and finally linearly, as expected from eqn. (9). After de-induction, enzyme initially continues to increase linearly for the time t_s, then the rate of accumulation decreases (exponentially) to zero; at this time the amount of enzyme has reached a final plateau value. The increase in the amount of enzyme after de-induction has been designated 'capacity' and is assumed to be a measure for the amount of β-galactosidase mRNA present at the time of de-induction (see, e.g., Kepes & Beguin, 1966; Morris & Hansen, 1973).

With increasing time after de-induction, the remaining capacity decreases, which is seen better in the replot in Fig. 3(*c*) ('Cap' curve) on a linear scale, or in Fig. 5 (C_8 curve; the '8' signifies de-induction after 8 min of induction), in semi-logarithmic manner. The C_8 curve in Fig. 5 shows an initial 'shoulder', reflecting the linear decrease (which is non-linear on this logarithmic scale), followed by a straight section reflecting the exponential decrease in the remaining capacity corresponding to decay of β-galactosidase mRNA.

In Fig. 3(*c*) the decrease of the capacity after de-induction is compared with the decrease in the number of 3′-ends of β-galactosidase mRNA and in the amount of mRNA. Fig. 3(*c*) illustrates that the capacity is not a measure for the amount of mRNA. Rather the pertinent relationship is between capacity and number (N_3) of mRNA molecules: the N_3 curve corresponds to the negative slope of the capacity curve.

(*c*) *Capacity and mRNA life*

The shape of the capacity curve depends on the duration of the induction period before de-induction. Figs. 3(*a*), 3(*b*), 4(*a*) and 4(*b*) show calculated examples for 8 and 1 min induction respectively. Enzyme curves very similar to those in Figs. 3(*b*) and 4(*b*) have been observed (e.g. Kepes, 1963). The decrease of the remaining capacity plotted in semi-logarithmic fashion (Fig. 5) shows a small shoulder for the 8 min induction (C_8) and a much larger shoulder for the 1 min induction (C_1). These shoulders do not reflect an initial lag in the onset of mRNA decay, but result from the delay with which events at the 5′-ends of mRNA (stop of initiation) are relayed to the 3′-ends; this delay is the synthesis time t_s. Only the final exponential decrease in the capacity can be used to determine the lifetime τ of β-galactosidase mRNA.

With increasing t_s (actually increasing ratio t_s/τ), the period of non-exponential decrease in the capacity curve (Fig. 5) becomes longer. For example, for long induction times (when the accumulation of enzyme has become linear with time) and a ratio $(t_s/\tau) = 1$, which is typical for β-galactosidase synthesis at 37°C (Table 1), the non-exponential phase goes down to 0.5 (= 50% remaining capacity; Fig. 5, C_8 curve). The operator–distal transacetylase of the *lac* operon has a longer t_s period such that the non-exponential phase is expected to be longer and to go down to about 30% remaining capacity. Since the average slope in the non-exponential phase of the curve is less than in the exponential phase, a long non-exponential section may simulate a longer mRNA life. Blundell & Kennell (1973) have concluded that transacetylase mRNA has a longer functional life than β-galactosidase mRNA. It is

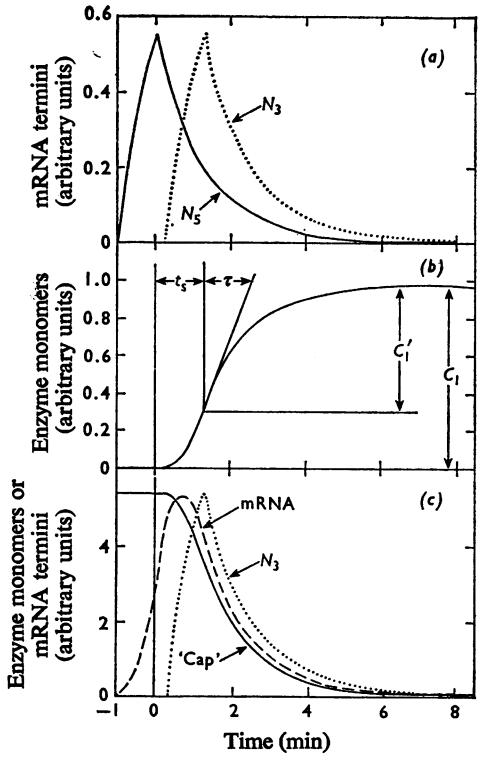


Fig. 4. *Synthesis of β-galactosidase after a pulse induction of 1 min duration*

Inducer is added at −1 min and removed at time zero. The legend to Fig. 3 applies, except in Fig. 4(*b*), where C_1 and C_1' refer to the enzyme-forming capacity instead of C_8 and C_8'. Note that in Fig. 4(*c*), the amount of *z*-gene message is still increasing shortly after de-induction, although the enzyme-forming capacity is decreasing.

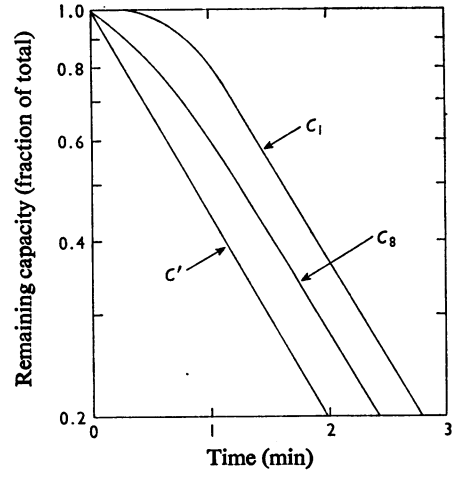


Fig. 5. *Enzyme-forming capacity after de-induction*

C_1 and C_8 refer to the enzyme-forming capacity as measured from the time of de-induction for a 1 min- and an 8 min-pulse induction respectively. C' is the 'residual capacity' (see legend to Fig. 3), measured first at a time t_s after de-induction. (The abscissa thus refers to time after de-induction for C_1 and C_8 but corresponds to a further delay equal to t_s for C'.)

not clear whether their data permit this conclusion. (These authors also inferred, from capacity data, that the ratio of the lifetimes of these two different sections of *lac* mRNA depends on the growth temperature of the bacteria. An alternate interpretation is that t_s and τ have different temperature-dependencies.) The same kind of question must be directed to the conclusion of Achord & Kennell (1974; see their Fig. 2*c*) that the functional half-life of mRNA for the three genes in the *gal* operon is 59, 36 and 91 s respectively. On the basis of 1100 nucleotide pairs for each of the three genes of the *gal* operon, one can calculate that, assuming an average functional lifetime of 75 s (Dalbow & Young, 1975), the exponential decrease of capacity should begin after the capacity had been decreased to 85, 70 and about 55% for the galactose epimerase, transferase and kinase respectively. The apparently longer decay time for the mRNA corresponding to the gene most distal from the operator, the kinase gene, is probably a result of this non-exponential decay predicted for about one-half of its total capacity. In the light of these theoretical considerations and the scatter of their data (Fig. 2 of Achord & Kennell, 1974), a fair conclusion to be drawn from that work is that within the experimental precision there is no detectable difference in the decay rate for the three induced enzyme capacities.

To avoid misinterpretations of the capacity curves it is best first to determine t_s and then to ignore the initial decrease in the capacity for a time equal to t_s and to plot only the decrease in the residual capacity C' (defined in Figs. 3*b* and 4*b*). This residual decrease is independent of the duration of the induction period, i.e. $C_1' = C_8'$ (Fig. 5), and shows no shoulder in the semi-logarithmic plot. It is, however, the difference of two larger numbers and as such is subject to large experimental errors which cannot be avoided in any evaluation of capacity measurements. In particular, the precise value of the final plateau for the pulse-induced enzyme is vital for the determination of the capacity decay. For example, ±5% accuracy in the estimation of the final amount of the enzyme synthesized after pulse-induction would lead to enormous difficulties in establishing the decay rate of the capacity: for

β-galactosidase one must rely on points representing between 50 and 10% of the final value to determine slopes; if at time t_1 there was 40% of the capacity left, and at time t_2, 10%, one would conclude that (t_1-t_2) is equal to twice the half-life of the mRNA. But if there had been a 5% over-estimate of the final value originally, then the decrease in capacity represented a change from 35 to 5%, a sevenfold decrease, such that (t_1-t_2) is almost equal to three times the half-life.

3. Evaluation of Observed Induction Kinetics

(*a*) *Accumulation of mRNA termini*

The kinetics of enzyme accumulation after induction was calculated above by integration of the function describing the number of 3′-termini of the corresponding mRNA. By reversing this process and differentiating an observed enzyme-accumulation kinetics, one can obtain the kinetics of accumulation of mRNA termini from observed data. In this way an induction kinetics obtained with *E. coli* B/r growing in succinate medium (37°C, 90min doubling time; data from Dalbow & Young, 1975) was analysed (Fig. 6).

(1) Finished mRNA begins to accumulate 90s after induction. The synthesis time (t_s) of β-galactosidase mRNA is therefore about 90s [see Dalbow & Young (1975) for exact definition of β-galactosidase synthesis time].

(2) For about 1min, β-galactosidase mRNA accumulates linearly with time, indicating that the initiation rate of β-galactosidase mRNA increases in a nearly stepwise manner, and also indicating that the enzyme kinetics during this period is a second-order parabola.

(3) The differential curve does not exactly agree with the exponential function expected for random mRNA inactivation. This could be caused by a short delay (3–5s) in the repressor–operator dissociation [see section 1*c* above, and the Discussion section in Dalbow & Young (1975)].

(*b*) *Parameters determining β-galactosidase synthesis in E. coli B/r*

From an evaluation of the β-galactosidase induction kinetics observed (Dalbow & Bremer, 1975; Dalbow & Young, 1975) and from the growth parameters for *E. coli* B/r (Dennis & Bremer, 1974), we have calculated a number of parameters related to β-galactosidase synthesis in *E. coli* B/r growing at 0.67, 1.36 and 2.14 doublings/h (Table 1). Some of the values given are based on the assumption that the average functional life of *E. coli* mRNA is the same as that of *lac z*-gene mRNA (Dalbow & Young, 1975). These parameters may be recalculated with alternative values for the average functional life by using the equations provided above.

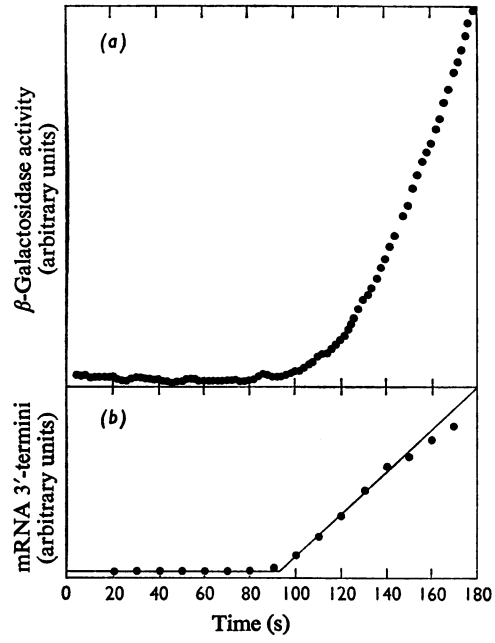


Fig. 6. *Analysis of an observed induction kinetics*

Inducer (10mM) is added at zero time to a culture growing at 0.67doubling/h in succinate medium (from Dalbow & Young, 1975; their Fig. 2*a*). (*a*) Observed kinetics of accumulation of β-galactosidase activity, with each point averaged over the two adjacent points. (*b*) Number of 3′-termini for the *z*-gene mRNA, obtained by graphic differentiation of the curve generated in Fig. 6(*a*). Extrapolation of the initial slope to the basal value gives a synthesis time $t_s = 93$s.

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References

Achord, D. & Kennell, D. (1974) *J. Mol. Biol.* **90**, 581–600
Alpers, D. & Tomkins, G. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **53**, 797–802
Bird, R. E., Louarn, J., Martuscelli, J. & Caro, L. (1972) *J. Mol. Biol.* **75**, 547–566
Blundell, M. & Kennell, D. (1973) *J. Mol. Biol.* **83**, 143–162

Boezi, J. A. & Cowie, D. B. (1961) *Biophys. J.* **1**, 639–647
Branscomb, E. W. & Stuart, R. N. (1968) *Biochem. Biophys. Res. Commun.* **32**, 731–738
Coffman, R., Norris, T. & Koch, A. (1971) *J. Mol. Biol.* **60**, 1–19
Cooper, S. & Helmstetter, C. (1968) *J. Mol. Biol.* **31**, 519–540
Cooper, T. G. & Magasanik, B. (1974) *J. Biol. Chem.* **249**, 6556–6561
Dalbow, D. G. & Bremer, H. (1975) *Biochem. J.* **150**, 1–8
Dalbow, D. G. & Young, R. (1975) *Biochem. J.* **150**, 13–20
Dennis, P. P. & Bremer, H. (1974) *J. Bacteriol.* **119**, 270–281
Hirsh, J. & Schleif, R. (1973) *J. Mol. Biol.* **80**, 433–444
Jacquet, M. & Kepes, A. (1971) *J. Mol. Biol.* **60**, 453–472
Jobe, A. & Bourgeois, S. (1972) *J. Mol. Biol.* **72**, 139–152
Kaempfer, R. O. R. & Magasanik, B. (1967) *J. Mol. Biol.* **27**, 475–494
Kennell, D. & Bicknell, I. (1973) *J. Mol. Biol.* **74**, 21–31
Kepes, A. (1963) *Biochim. Biophys. Acta* **76**, 293–309
Kepes, A. (1969) in *Progress in Biophysics* (Butler, J. A. V. & Noble, D., eds.), vol. 19, pp. 199–236, Pergamon Press, Oxford
Kepes, A. & Beguin, S. (1966) *Biochim. Biophys. Acta* **123**, 546–560
Leive, L. & Kollin, V. (1967) *J. Mol. Biol.* **24**, 247–259
McCarthy, B. J. & Britten, R. J. (1962) *Biophys. J.* **2**, 35–47
Morris, D. R. & Hansen, M. T. (1973) *J. Bacteriol.* **116**, 588–592
Morse, D. E., Mosteller, R. D. & Yanofsky, C. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 725–740
Mosteller, R. D. & Mandula, B. B. (1973) *J. Mol. Biol.* **80**, 801–823
Pardee, A. B. & Prestidge, L. S. (1961) *Biochim. Biophys. Acta* **49**, 77–88
Schleif, R., Hess, W., Finkelstein, S. & Ellis, D. (1973) *J. Bacteriol.* **115**, 9–14
Schwartz, T., Craig, E. & Kennell, D. (1970) *J. Mol. Biol.* **54**, 299–312
Singh, U. N. (1973) *J. Theor. Biol.* **40**, 553–571
Singh, U. N. (1974) in *Basic Life Sciences* (Biswas, B. B., Mandal, R. K., Stevens, E. & Cohn, W. E., eds.), vol. 3, pp. 93–103, Plenum Press, London
Smith, T. F. & Sadler, J. R. (1971) *J. Mol. Biol.* **58**, 273–305
Sueoka, N. & Yoshikawa, H. (1965) *Genetics* **52**, 747–757