Polypeptide-Chain-Elongation Rate in *Escherichia coli* B/r as a Function of Growth Rate

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By evaluating the kinetics of radioactive labelling of nascent and finished polypeptides, the peptide-chain elongation rate for *Escherichia coli* B/r at three different growth rates (μ) was determined to be 17 amino acids/s for the fast-growing cells ($\mu = 1.3$ and 2.0 doublings/h) and 12 amino acids/s for slow-growing cells ($\mu = 0.67$ doublings/h). The results agree with the growth-rate-dependence of the rate of peptide-chain elongation found for the translation of newly induced β -galactosidase messenger in this strain and under these conditions of growth [Dalbow & Young (1975) *Biochem. J.* **150**, 13–20]. Together with the previously observed ribosome efficiency at these growth rates [Dennis & Bremer (1974) *J. Mol. Biol.* **84**, 407–422] the results indicate that the fraction of ribosomes engaged in protein synthesis is about 0.8 at all three growth rates.

Maaløe (1969) proposed that bacteria, under conditions of balanced growth, maximize the function of ribosomes; this implies that ribosome function is independent of growth rate. This idea not only agreed with available information but also seemed in harmony with principles of synthetic economy.

The cell could achieve maximum economy of ribosome synthesis by monitoring function and adjusting synthesis accordingly. For example, the rate of peptide-chain elongation (i.e., the growth rate of a nascent protein) could be the monitored function: if the ribosome function diminished, owing to a limitation in charged tRNA, a chemical signal (like guanosine tetraphosphate; Cashel & Kalbacher, 1970) might be generated which leads to a repression of ribosome synthesis.

For the evaluation of any model relating ribosome synthesis and function, it is necessary to measure ribosome function at different cellular growth rates. The peptide-chain-elongation rate has been measured in various bacterial strains, at different temperatures, and in various growth media; therefore the results vary (12–23 amino acids/s per chain) and are difficult to compare (Table 1). Here we measure the peptide chain elongation rate by using a very direct method for *Escherichia coli* B/r under the conditions of growth that have been used in this laboratory to measure ribosomal RNA and protein synthesis in this strain (Bremer *et al.*, 1973; Dennis & Bremer, 1974). The results show that the peptide-chain-elongation rate is 17 amino acids/s and independent of the bacterial

growth rate in fast-growing bacteria, and significantly decreased (to 12 amino acids/s for bacteria growing at 0.7 doublings/h) in slowly growing bacteria.

Materials and Methods

Bacteria and growth conditions

E. coli B/r (A.T.C.C. no. 12407) was grown at 37°C in supplemented medium C (Helmstetter, 1967) and monitored by measuring the E_{460} by using a Gilford spectrophotometer with a 1 cm-light-path cuvette. The nutritional supplements and resultant growth rates were 0.2% succinate (growth rate, $\mu = 0.67$ doubling/h), 0.2% glucose ($\mu = 1.3$ doublings/h), and glucose plus 19 amino acids ($\mu = 2.0$ doublings/h). The succinate medium was adjusted to pH 6.8 with Tris base. The amino acids (minus leucine) were present in an initial concentration of 20 μ g/ml. Cultures were started by a dilution of at least 500:1 of a fresh, stationary-phase culture. All radioactively labelled compounds were obtained from Schwartz/Mann, Orangeburg, NY, U.S.A.

¹⁴C labelling

At an E_{460} of 0.2, a 15 ml portion of the exponential-phase culture was labelled with 100 μ l of a [¹⁴C]leucine solution (final radioactivity 0.3 μ Ci/ml; final leucine concentration 5 μ M). The label was exhausted within one doubling time in all three media.

³H pulse-labelling and sampling

At an E_{460} of 0.6, the 15 ml pre-labelled culture was placed in an automatic sampling device (Dalbow & Young, 1976) with aeration at 37°C.

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[^3H]Leucine was rapidly mixed into the culture at zero time to a final concentration of $5\mu\text{M}$ ($5\mu\text{Ci/ml}$). Samples (approx. 0.5ml) were taken automatically at 3 s intervals for 1 min into test tubes suspended in a solid CO_2 /methanol bath (so that the samples were frozen completely within 1 s). The samples were stored at -70°C .

Electrophoresis

Each sample was thawed at 0°C in the presence of 0.75M-trichloroacetic acid, 10mM-sodium azide, and 1.5 E_{460} units of carrier cells from an iced, azide-killed exponential culture grown in the appropriate medium. The samples were pelleted at 5000g for 20 min, washed twice with cold 1mM-trichloroacetic acid, and re-suspended in 0.05M-Tris buffer, pH 6.8. Each sample was then mixed with one-quarter volume of a lysing solution (250mM-Tris/Cl, pH 6.8/5% sodium dodecyl sulphate/5% mercaptoethanol/20% (v/v) glycerol containing 0.001% Bromphenol Blue), heated at 100°C for 3 min, and applied to polyacrylamide slab gels formed with 25 sample wells in the stacking gel. The separating gel contained 0.1% sodium dodecyl sulphate, 12.5% (w/v) acrylamide, and 0.6% bisacrylamide. Each gel was electrophoresed for 3–4 h at a constant current of 25mA. Proteins of known molecular weight were co-electrophoresed with the samples. The slabs were then fixed for 30 min in 15% (w/v) trichloroacetic acid and stained in 0.12%

was dissolved in a scintillation 'cocktail' mixture containing 8g of butyl PBD [5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] and 60g of naphthalene made up to 1 litre with dioxane. The remaining gases were bubbled into a trap filled with a CO_2 -complexing scintillation cocktail containing 8g of butyl PBD, 53 ml of ethanolamine and 420 ml of ethylene glycol/monoethyl ether, made up to 1.0 litre with toluene. Both kinds of scintillation cocktails were counted for trapped radioactivity in a Nuclear-Chicago mark II scintillation counter with a full window and maximum amplification. The recoveries for both ^3H and ^{14}C were always above 90%.

For total incorporation assays, a small amount (usually 5–10 μl) of the sodium dodecyl sulphate lysate of each sample was precipitated in cold 0.5M-trichloroacetic acid, collected on nitrocellulose filters and dried. Each filter was then oxidized in the same way as the dried gel chips.

Evaluation of the data

The method used is based on the protocol of Gausing (1972), modified for the rapid sampling necessary for measurement of the peptide-chain-elongation rate (c_p), at 37°C ; the theoretical basis for the calculations stems from Bremer & Yuan (1968) and is extended in the Appendix for these purposes. Essentially a fraction $F^*(m, t)$ is determined at various times after the addition of the ^3H label, where:

$$F^*(m, t) = \frac{(^3\text{H c.p.m. in molecular-weight class } m \text{ at time } t) / (^{14}\text{C c.p.m. in class } m)}{(^3\text{H c.p.m. in total protein at time } t) / (^{14}\text{C c.p.m. in total protein)}}$$

Coomassie Brilliant Blue dissolved in a solution made up of 11.25 ml of acetic acid gel was de-stained in acetic acid/methanol/water (3:10:40, by vol.) and dried on Whatman no. 1 filter paper under heat and vacuum.

Radioactivity assay

A given band was selected for oxidation, on the basis of staining intensity and apparent molecular weight. (The apparent molecular weight was calculated from the mobilities of the protein standards of known molecular weight that were co-electrophoresed in adjacent sample wells.) The band was cut from the dried slab with care exercised to ensure that the edges of the cut stayed within the stained borders of the band. (This precaution is essential because the ^{14}C isotope is localized entirely within the stained bands, but the ^3H isotope is distributed both in the stained bands and in the intervening unstained areas, where there are only nascent polypeptides.) Each dried gel chip was then burnt in a Harvey biological-material oxidizer. $^3\text{H}_2\text{O}$ was frozen by passing the oxidation products through a glass trap immersed in a methanol/solid CO_2 bath; the radioactive condensate

For a given molecular weight, $F^*(m, t)$ should initially increase linearly with time, from a value $F^*(m, 0)$ (depending on the ratio of nascent chains to finished chains of the given molecular weight) until a time t_m , equal to the average synthesis time of a polypeptide of molecular weight m , when:

$$F^*(m, t = t_m) = F^*(m, t = 0) + 0.5$$

(see the Appendix). At t_m there is a break in the slope of the kinetics and after t_m the function gradually approaches 1.0. The peptide-chain-elongation rate (c_p , in amino acids/s per active ribosome) is then found by:

$$c_p = \frac{m}{t_m \cdot a}$$

where m is the selected molecular weight, t_m is the time at the break in the kinetics (in), and a is the molecular weight of the average amino acid residue in *E. coli* protein (113; Spahr, 1962).

Results

Bacterial protein was first uniformly pre-labelled with [^{14}C]leucine. At zero time, [^3H]leucine was

added to the exponentially growing culture and samples were taken every 3 s. After electrophoretic separation the $^3\text{H}/^{14}\text{C}$ ratios for proteins of various

selected molecular weights were determined and each of these ratios was divided by the isotope ratio observed in total protein at the particular time. The

Fig. 1. F^* kinetics for selected protein bands for bacteria growing with three different doubling times

Bacteria were pre-labelled with $[^{14}\text{C}]$ leucine and later pulse-labelled with $[^3\text{H}]$ leucine beginning at zero time. Samples were taken at 3 s intervals and prepared for electrophoresis as described in the Materials and Methods section. For a band of a given molecular weight, the $^3\text{H}/^{14}\text{C}$ ratio was determined in each sample and divided by the $^3\text{H}/^{14}\text{C}$ ratio in the total protein, to give $F^*(m, t)$. All isotope ratios were determined by complete combustion of each sample followed by separate collection and counting of the radioactivity. The breakpoints of the F^* kinetics were determined by inspection and are designated by the vertical arrow. F^* kinetics for (a) cells grown on succinate medium ($\mu = 0.67$ doublings/h), (b) cells grown on glucose medium ($\mu = 1.4$ doublings/h), (c) cells grown on glucose/amino acids medium ($\mu = 2.0$ doublings/h). (a)–(c) each show data for four bands of polypeptides of the given molecular weight.

quotient of these isotope ratios is designated F^* and is plotted as a function of the labelling time for each molecular-weight class (m , each class corresponding to a selected band on the gel). A theoretical treatment shows that these kinetics have a breakpoint at the time equal to the synthesis time of peptides of the molecular weight m (see the Appendix).

Fig. 1 shows such kinetics (F^*) for four bands for each of the three different growth rates. The observed breakpoint times are designated by the arrow in each set of kinetics. Fig. 2 shows the relationship between the breakpoint time (t_m) for each set of kinetics and the size (m) corresponding to the given band, both in daltons and the average number of amino acids residues. The slope of the plot of m versus t_m is the peptide-chain-elongation rate, c_p . For succinate-grown cells, c_p is found to be 1330 daltons/s or 12.1 amino acids/s; for glucose and glucose/amino acids-grown cells, c_p is 1880 daltons/s, or 17.1 amino acids/s.

Discussion

Precision of results

The result, namely that nascent polypeptides are elongated at 17 amino acids/s in fast-growing cells but more slowly in slow-growing cells ($c_p = 12$ amino acids/s at $\mu = 0.67$ doubling/h), agrees with values for c_p calculated previously from the synthesis time of induced β -galactosidase (16 amino acids/s for $\mu = 2.1$ and 1.4 doublings/h, 13 amino acids/s for $\mu = 0.67$ doublings/h) in this bacterial strain grown under the same condition. The 17 amino acids/s value corresponds to 51 mRNA nucleotides/s, a value that is very close to the 55 nucleotides/s found for mRNA chain-growth rate (Bremer & Yuan, 1968) suggesting that transcription and translation are closely matched.

Determining the inherent error of these measurements is complicated by the subjective nature of the breakpoint determination. [It is not meaningful to determine the slopes in Fig. 2 and thus the breakpoints by a least-squares analysis. First, after t_m , the curves are hyperbolas for which we have no particular expectation without knowing t_m . Secondly, in general, there are very few measuring points before t_m ; therefore the best-fit straight line is largely influenced by the number of points included in the least-squares analysis. Since it would be necessary to exclude points after t_m , one would have to make a subjective pre-judgement of t_m . Thirdly, a much more powerful criterion to draw the best-fit straight lines before t_m is the condition that the theoretical increase in F^* is 0.5 between zero time and t_m (see the Appendix), although there are apparent exceptions from this expectation (e.g. Fig. 1a, 43 500 daltons; see the Appendix for possible reasons). Assuming that t_m is proportional to the molecular weight of the poly-

Fig. 2. Breakpoint times determined from the individual kinetics in Fig. 1 plotted against the corresponding size, in daltons and in average amino acids residues

The molecular weight for the average amino acid residue was taken as 113. The slope of the line for cells grown on glucose/amino acids ($\mu = 2.0$ doubling/h; ■) and on glucose ($\mu = 1.4$ doubling/h; △) is 1900 daltons/s or 17.1 amino acids/s. The corresponding slope for the succinate-grown cells ($\mu = 0.67$ doubling/h; ●) is 1330 daltons/s or 12.1 amino acids/s.

peptides, that all polypeptides have the same mol fraction of labelled amino acids (leucine) evenly distributed along their chain length, no anomalies in electrophoretic behaviour and negligible turnover, it would be possible in principle to write a computer program which generates the best fit of the observed data with theoretically expected kinetics. But such effort would be formidable, and is not justified, since the assumptions above are only partially met.] The molecular-weight estimate for each band is probably accurate to within 500, since all the bands (between 11 000 and 60 000) are spread over a 7 cm space on the slab gel, and the breakpoint is usually determinable unambiguously to within one sample, so that the calculated values for the peptide-chain-elongation rate are probably accurate to within 15%. If all of the succinate culture breakpoints were actually one sample earlier, then the difference between the peptide-chain-elongation rates for fast- and slow-growing bacteria would be only marginally significant. Inspection of the succinate kinetics (Fig. 1c), however, reveals no such consistent ambiguity, and it seems fair

to conclude that the difference for the peptide-chain-elongation rate in fast- and slow-growing bacteria is real.

Fig. 3. Diagram of the labelling of a polyribosome

Label is added at time zero (a). At time $t_m/2$ (b) the nascent polypeptides of the molecular weight m is half-labelled. At time t_m (c) the m -sized nascent polypeptide is saturated with label. Thus m/t_m is equal to the polypeptide-chain-elongation rate. All messengers coding for m -sized polypeptides or larger contribute to the labelling of m -sized nascent polypeptides. The dashed lines delimit the region of the polyribosome that contains m -sized nascent polypeptide chains.

Characteristics of the method

The method used in the present study has distinct advantages over several others that have been used to measure c_p . The theoretical basis is that nascent polypeptides of molecular weight m become saturated with label at a time after the addition of a radioactive amino acid equal to the translation time for a polypeptide of molecular weight m . The determination of the breakpoint of F^* kinetics for a band m allows calculation of the average polypeptide-chain-elongation rate for all nascent polypeptides up to the molecular weight m (see Fig. 3). In a sense the method is a direct measurement of the elongation rate of polypeptide chains and by its directness it escapes most interference caused by artifacts. The use of the ^{14}C pre-label precludes any recovery problem, since the ^3H labelling kinetics for each band is normalized first to the recovery of ^{14}C pre-labelled protein in that band and then to the $^3\text{H}/^{14}\text{C}$ ratio of the entire culture at that time. The use of slab-gel electrophoresis allows the co-electrophoresis of all the samples for a given experiment, plus known protein standards to permit precise molecular-weight determination and excision of the identical band from each sample. Finally, complete combustion of the bands ensures that no differential radioactive-isotope quenching and/or spill-over problems interfere with the measurement of the radioactive-isotope ratios, since the two isotopes are counted only when they are sequestered into separate vials. The difficulties in this approach arise from limitations imposed by the theory and from the deviation from the ideal situation that the real bacterial cell represents. Theory dictates (see the Appendix) that the larger the molecular-weight class studied, the less distinct the sharpness of the breakpoint; add to this the fact that there is less and less nascent protein as one studies classes of increasing molecular weight (and hence even less distinct breakpoints), one finds a severe limitation on the upper limit of available molecular weights. Here no polypeptide class of greater than 42000 mol.wt. was used. The lower limit is set by the sampling time; here the 3 s sampling interval necessitates discarding polypeptide classes of less than 6 s synthesis times (probably 8000–12000 daltons or less).

Previous estimates of the rate of peptide-chain elongation

Measurement of the rate of total protein synthesis and the number of active ribosomes. In principle one can find the average peptide-chain-elongation rate by determining the total number of amino acids polymerized per s per cell and then dividing this rate by the number of active ribosomes per cell, which requires an additional estimate of the fraction of bacterial ribosomes that are active in protein synthesis at any instant (see Table 1). Unfortunately it is difficult to measure the fraction of active ribosomes

Table 1. Comparison of estimates for the rate of peptide-chain elongation in bacteria

Method	Strain	μ	τ^*	c_p	Reference
(A) Measurement of Rs/P and β_r at each growth rate	<i>E. coli</i> 15	2.5	37	21	Forchhammer & Lindahl (1971)
	TAU BAR	2.1	37	20	
		1.5	37	21	
		0.9	37	16	
	<i>E. coli</i> B	0.6	37	12	Harvey (1973)
		0.39	30	18	
(B) Appearance of N-terminal label in completed polypeptides		0.82	30	14	
	<i>E. coli</i> 300 μ	0.75	37	14.3	Lacroute & Stent (1968)
	<i>Salmonella typhimurium</i> LT2	1.9	37	16–20	Engbaek <i>et al.</i> (1973)
		1.0	37		
		0.5	37		
	<i>E. coli</i> B	0.75	30	11	Harvey (1970)
				(ribosomal protein)	
		0.75	30	22	
(C) Induction of β -galactosidase				(non-ribosomal protein)	
	<i>E. coli</i> ML3	?	37	13	Kepes (1963)
	3000	0.8	37	13–15	Kepes & Beguin (1966)
	ML3	0.9	37	9–14	Coffman <i>et al.</i> (1971)
	B	?	37	5–11	West & Stein (1973)
	B/r	2.1	37	16	Dalbow & Young (1975)
		1.4	37	16	
		0.7	37	13	
	DK6	2.2	37	17	Morris & Hansen (1973)
	K12	0.7	37	17	Schleif <i>et al.</i> (1973)
(D) Labelling kinetics of nascent polypeptides	<i>E. coli</i> K12	0.7	37	10–13	Schleif <i>et al.</i> (1973)
	BAU	0.8	30	11.4	Gausung (1972)
	B/r	2.1	37	17	(Present work)
		1.4	37	17	
		0.7	37	12	
	DK6	2.2	30	9–11	Jorstad & Morris (1974)

* τ = doubling time.

directly, since polyribosomes (the active form of ribosomes) are very fragile; for instance, Forchhammer & Lindahl (1971) found a 20% increase in the observed polyribosome fraction by lowering the handling temperature from 4–7°C to 0°C. Their finding, that c_p is 20 amino acids/s at high growth rates but decreased below $\mu = 1.0$ doubling/h, is qualitatively similar to the present results, and would be identical if their lysis method caused fractional polyribosome breakdown.

If the direct method of observing the sedimentation properties of the ribosome fraction yields estimates for the active fraction that are subject to considerable interference from artifacts, indirect methods to the same end are even more questionable. Harvey (1973) determined the number of 'mol' of nascent protein per total ribosome (leading to an estimation of the active fraction of ribosomes) by pulse-labelling cells with radioactive leucine and then isolating the ribosome fraction, performing puromycin-induced chain

termination and release from the ribosome fraction *in vitro*, pelleting the ribosomes, and determining the specific radioactivity of the supernatant, which presumably contained only the released nascent protein. This protocol involves so many different radioactivity and colorimetry measurements that its accuracy is difficult to evaluate; the results obtained are in several respects quite different from the results observed with more direct methods, especially that c_p decreased continuously with increasing growth rate.

Determination of the lag between the addition of label and the appearance of N-terminal radioactivity in an isolated protein of known molecular weight. Another approach to determining the peptide-chain-elongation rate is to label the N-terminal amino acid of some isolatable protein, and follow both the kinetics of labelling of total protein precipitated and of the N-terminus of the total protein isolated. For this method accurate back-extrapolation of either curve to very short times after the addition of the

label is necessary, and there is the difficulty of obtaining purified completed polypeptides separated from nascent protein contamination. Lacroute & Stent (1968) reported a value of 80s for the lag between the addition of radioactive threonine residues and the appearance of finished β -galactosidase molecules labelled in the *N*-terminal threonine residue; this corresponds to a value of 15 amino acids/s for c_p (at 37°C with $\mu = 0.75$ doubling/h). Engbaek *et al.* (1973) applied this method to the measurement of c_p over a range of growth rates; even though β -galactosidase is probably one of the most easily isolated and one of the largest polypeptides in the cell, they concluded that the 20–25% difference in measured translation times for β -galactosidase was not significant. Harvey (1970), studying the appearance of *N*-terminal alanine residues in soluble protein and ribosomal protein, estimated values of 22 amino acids/s for non-ribosomal protein and 11 amino acids/s for ribosomal protein; evidently his calculation did not allow for the fact that *N*-terminal labelled finished ribosomal protein does not appear as a structural element of ribosomes until after the completed polypeptide undergoes a maturation process and then assembly into ribosomes.

Measurement of the synthesis time of an inducible enzyme of known molecular weight. Determining the appearance of induced β -galactosidase activity has frequently been used to calculate c_p (Table 1, method C). There is considerable theoretical complexity in the evaluation of enzyme-induction kinetics, to the extent that the synthesis time is frequently overestimated (see the Discussion section in Dalbow & Young, 1975). For example, Coffman *et al.* (1971) measured the synthesis time for induced β -galactosidase for a range of bacterial growth rates and found 90–130s, corresponding to a chain-elongation rate of 9–14 amino acids/s, with no obvious growth-rate dependency. However, analysis of the induction kinetics of β -galactosidase for *E. coli* B/r over a range of growth rates in this laboratory yielded values for c_p of 16, 16 and 13 amino acids/s, for $\mu = 2.1$, 1.4, and 0.7 doubling(s)/h (Dalbow & Young, 1975).

One indeterminate factor of the β -galactosidase induction is the time-lag required for the inducer to penetrate the cell membrane and cause de-repression of the operator. Morris & Hansen (1973) eliminated this problem by also measuring the appearance of the 60-amino acid *N*-terminal fragment of β -galactosidase (auto- α ; Ullman *et al.*, 1965; Morrison & Zipser, 1970) after induction and calculating the chain-growth rate by the difference in the lag times for auto- α and β -galactosidase. The 65s difference between the appearance of auto- α and of β -galactosidase corresponds to a chain-elongation rate of 17 amino acids/s, in good agreement with the other values obtained for the chain-elongation rate of the β -galactosidase polypeptide.

One criticism of the determination of c_p by the induction of β -galactosidase is that the value results from the time required to translate a specific polypeptide; the codon constitution of the *z*-gene mRNA, the unusually great length of the β -galactosidase polypeptide, or the fact that it is an inducible enzyme, might be factors detracting from the generality of the calculated c_p value. The close agreement between the careful c_p determinations using the β -galactosidase-induction kinetics and those using more direct methods indicate that none of these factors are relevant.

Labelling kinetics of nascent polypeptide (F).* Gausung (1972) first used the double-label F* method to measure c_p , finding $c_p = 11.4$ amino acids/s at 30°C, which is consistent with the values reported here for 37°C. A similar value was obtained by Schleif *et al.* (1973) for slow-growing bacteria (0.7 doubling/h); however, since the bacterial proteins were not separated into distinct molecular-weight bands but rather into broad sectors, the results are relatively imprecise (10–13 amino acids/s). Jorstad & Morris (1974), by using a single-label version of the method, found a value for c_p (9–11 amino acids/s) similar to Gausung's (1972) result, but their interpretations of the F* curves are questionable (see the Appendix) and thus the agreement may be fortuitous.

Fraction of active ribosomes

The quantity denoted 'ribosome efficiency' (e_r) is defined as the quotient, rate of protein synthesis/cell divided by 70S ribosome equivalents/cell. The ribosome efficiency is equal to the peptide-chain-elongation rate if all ribosomes are actively engaged in protein synthesis; in general:

$$e_r = \beta_r \cdot c_p$$

where β_r = fraction of ribosomes that are active and c_p = peptide-chain-elongation rate as defined above. Dennis & Bremer (1974) have measured e_r and found 13.5, 13.5 and 9.5 amino acids/s per 70S ribosome for $\mu = 2.1$, 1.4 and 0.67 doublings/h respectively. By using the values for c_p reported here (17, 17 and 12 amino acids/s), β_r , the fraction of active ribosomes, appears to be about 80% at all growth rates. It is not known why 20% of the cellular ribosomes are inactive at any instant; the inactive fraction may be in equilibrium with the active fraction or it may represent a permanently inactive class.

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APPENDIX

Theoretical Analysis of $F^*(m, t)$

Mathematical equation for the function $F^*(m, t)$

The kinetics of labelling of a particular molecular-weight class of polypeptides with a radioactive amino acid can be characterized by the fraction

$$F(m, t) = \frac{\text{label in polypeptides of mass } m \text{ at time } t}{\text{label incorporated into total protein at time } t}$$

(Bremer & Yuan, 1968). The numerator of this fraction has two components, the label in growing (nascent) polypeptides and the label in finished polypeptides. Scheme 1(a) shows the kinetics of labelling of the nascent polypeptides of mass m ; the label increases until t_m , when the nascent chains of the indicated molecular weight are saturated with label. Scheme 1(b) shows the increase of the radioactivity in finished chains of mass m ; the kinetics are initially parabolic (since more and more chains are completed with increasing amounts of radioactivity per chain) but become linear with time at t_m . The sum of these two kinetic parameters gives the kinetics of labelling for the molecular-weight class m (Scheme 1c), i.e. the numerator of the fraction F above. The label in total cell protein, which is the

denominator of fraction F , increases linearly with labelling time (Scheme 1d; for a short time-period, culture growth can be ignored). The kinetics of the (time-dependent) fraction F [i.e. the quotient of the

value for each time-point in Scheme 1(c) divided by the value for the same time-point in Scheme 1(d), is shown in Scheme 1(e). It is seen that these kinetics have a breakpoint at t_m , i.e. at the transition point between the initially linear and subsequently hyperbolic portions of $F(m, t)$. From the breakpoint time, equal to t_m , one can calculate the polypeptide-chain-elongation rate, c_p , as quotient m/t_m .

It is useful to normalize the ordinate of the kinetics. This is done here by dividing each value $F(m, t)$ by the final value $F(m, t \rightarrow \infty)$, equal to the fraction F obtained from the ^{14}C prelabel used in these experiments:

$F(m, t \rightarrow \infty) = (^{14}\text{C in mol. wt. class } m) / ^{14}\text{C in total protein}$.

The normalized fraction will be designated as F^* :

$$F^*(m, t) = \frac{F(m, t)}{F(m, t \rightarrow \infty)} = \frac{(^3\text{H in mol. wt. class } m \text{ at time } t) / (^3\text{H in total protein at } t)}{(^{14}\text{C in mol. wt. class } m) / (^{14}\text{C in total protein})}$$

Scheme 1. Derivation of F^* kinetics

(a) Specific radioactivity of nascent polypeptides of mass m increases linearly until t_m and then stays constant; (b) specific radioactivity of finished polypeptides of mass m increases parabolically until t_m and linearly with time thereafter; (c) specific radioactivity of total polypeptides of mass m , the sum of (a) and (b); (d) specific radioactivity of total cell protein increases linearly with time (culture growth is negligible during labelling period); (e) F^* kinetics, equal to the ratio at each point in time of the specific radioactivity of m -sized polypeptides (nascent+finished; c) and the specific radioactivity of total protein (d). F^* is linear with time until t_m , hyperbolically approaching 1.0 as $t \rightarrow \infty$. The difference between $F^*(t_m)$ and $F^*(0)$ is 0.5 (dimensionless) unit.

Rearranging shows F^* to be the quotient of two isotope ratios:

$$F^*(m, t) = \frac{{}^3\text{H}/{}^{14}\text{C in mol.wt. class } m \text{ at time } t}{{}^3\text{H}/{}^{14}\text{C in total protein at time } t}$$

Bremer & Yuan (1968) defined a fraction $F^*(m, t)$ in which F was normalized to $F(t \rightarrow 0)$ rather than to $F(t \rightarrow \infty)$. They derived a mathematical expression which describes the value of their F^* as a function of t_m and of another parameter, a_m . Before the breakpoint, for $t < t_m$:

$$F^*(m, t) = \frac{F(m, t)}{F(m, t \rightarrow 0)} = 1 + a_m t$$

after the breakpoint, for $t > t_m$:

$$F^*(m, t) = 2a_m t_m + t_m(1 - a_m t_m) \frac{1}{t}$$

The parameter a_m is a time-invariant function of the molecular weight (i.e. a property of the bacteria under the particular growth conditions) and of c_p (see eqn. 11 of Bremer & Yuan, 1968); its dimensions are $1/t$, such that all terms in the above equations are dimensionless. By using their relation, one obtains for $t \rightarrow \infty$:

$$F^*(m, \infty) = 2a_m t_m$$

Hence, dividing their equations by $2a_m t_m$ gives the equations for the normalization used here, based on the isotope ratios. For $t \leq t_m$:

$$F^*(m, t) = \frac{F(m, t)}{F(m, t \rightarrow \infty)} = \frac{1}{t_m} \left(\frac{1}{2a_m} + \frac{t}{2} \right) \quad (1a)$$

After t_m :

$$F^*(m, t) = 1 + \left(\frac{1}{2a_m} - \frac{t_m}{2} \right) \frac{1}{t} \quad (1b)$$

Determination of t_m from the shape of the kinetics F^*

If the breakpoints of the kinetics F^* in Fig. 1 of the main paper were always well defined, there would be no need for further evaluation of the kinetics. However, this is not the case for three reasons. (1) For theoretical reasons, the breakpoint disappears for high-molecular-weight proteins which otherwise could be expected to give the most accurate determination of c_p ; (2) random scatter in the data severely limits the accuracy in the breakpoint determination; (3) systematic deviations from the theoretical expectation can make the significance of a breakpoint questionable.

Information about t_m is contained in the shape of the whole kinetics F^* . However, no single plot can simplify both $F^*(t < t_m)$ and $F^*(t > t_m)$ to straight lines. Since the late part of the F^* kinetics should always approach 1.0, it is more informative to plot F^* directly against time, so that the initial $t < t_m$ kinetics are linear. Theoretically the slope of the

initial kinetics (equal to $\frac{1}{2}t_m$) provides an estimate for the synthesis time t_m . Accordingly, Fig. 1 (main paper) shows steeper slopes of the kinetics for low molecular weights than for high molecular weights, but systematic deviations and scatter in the data often prevent accurate initial slope determinations. An additional criterion for the accuracy of the slope is whether the value of F^* at t_m is 0.5 (dimensionless) unit greater than F^* at zero time. By this criterion, Fig. 1 (main paper) shows that it is not practical to obtain t_m values from initial slopes in those cases where such a method would be of real value, i.e. when the breakpoint itself is indistinct (e.g. Fig. 1a: 43 500 mol.wt.). However, in several cases one's confidence in some of the breakpoint determinations is increased by applying this criterion (Fig. 1a, 24 400; Fig. 1b, 19 000, 24 400; Fig. 1c, 27 000, 32 200 mol.wts.).

Experimental deviations from theory

In addition to more-or-less trivial reasons that may cause systematic deviations of the observed kinetics F^* from theory (eqn. 1; e.g. turnover of the protein in the band selected, or slight changes in the differential rate of synthesis of the protein band between the ^{14}C prelabel and the ^3H pulse), the following more complex reasons are considered.

Small number of leucine residues in the selected protein band. The theory was based on the assumption that the radioactive label incorporated into a growing polypeptide with the finished chain length m increases linearly with its length. This assumption does not hold when the polypeptide contains a very small number of leucine (labelled amino acid) residues. The effect can be qualitatively assessed by assuming the extreme, that the peptide contains only one leucine residue. If this were the last (C-terminal) residue, radioactivity in finished peptides (Scheme 1, main paper) should increase linearly with time from $t = 0$; if it were the first (N-terminal) residue, radioactivity in finished peptides should not appear before t_m and then increase linearly. In neither case would the increase be parabolic (as in Scheme 1b). In either case, the kinetics of F^* would still show a break at t_m , but the initial

slope would be either shallower or steeper than expected. This effect should mainly affect the labelling of low-molecular-weight proteins, since those have on the average a smaller number of leucine residues.

Bandwidth effects. The theory was derived for a continuous molecular-weight distribution of *E. coli* proteins. In this case the width of the band cut out from the gel cancels in the normalization. If the proteins are in a series of sharp bands, however, then it makes a difference whether a broad or a narrow band is cut out of the gel for radioactive counting, since only the centre of the band contains finished proteins (^{14}C label), whereas the sides of the bands contain mainly growing chains (^3H label); hence, the ratio nascent finished chains (Scheme 1a versus Scheme 1b; main paper) increases with increasing band-width. This effect tends to make the initial slope shallower than expected.

Previous applications of the theory

Results in the literature show varying correlation with the theory. Gausing (1972) presents results for bands ranging from 43 000 to 160 000 daltons (her Fig. 5); with increasing molecular weight, the breakpoints become less distinct, as the theory predicts, becoming essentially indeterminable at mol.wts. greater than 80 000. However, the results of Jorstad & Morris (1974; their Fig. 2a) are interpreted in terms of two intersecting straight lines, which may indicate that they have seriously overestimated the peptide-chain-elongation rate they calculate from kinetics not shown. Schleif *et al.* (1973), by using a single label adaptation of the Bremer & Yuan (1968) theory, also interpret the kinetics in terms of two intersecting straight lines.

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