THE ELONGATION RATE OF PROTEINS OF DIFFERENT MOLECULAR WEIGHT CLASSES IN YEAST

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1. Introduction

It is generaly assumed that the peptide-chain growth-rate does not change with the size of the polypeptide being synthesised. Indeed, Gausing has shown that this is the case in *Escherichia coli* [1]. However, recent publications have suggested that in eukaryotic cells the protein elongation rate may very according to the nature of the polypeptide being synthesised [2] or according to its molecular weight [3]. We wished to see whether direct measurements of protein elongation rate in *Saccharomyces cerevisiae* could substantiate the hypothesis that the elongation rate varies as a function of polypeptide length.

The method we used involves labelling cellular protein for different short periods of time and then separating the soluble polypeptides by polyacrylamide gel electrophoresis. The labelling kinetics of proteins of different molecular weight classes can subsequently be analysed by the technique of Bremer and Yuan [4]. Using this method we find that after polypeptides have attained a molecular weight of 40 000 they are further elongated at a rate of 10.5 amino acids per sec at 30°C. It seems that polypeptides smaller than mol. wt. 40 000 are synthesised at slower rates. Yeast polypeptides of average molecular weight are synthesised at an overall rate of 6.9 amino acids per sec at 30°C.

2. Materials and methods

2.1. *Growth and labelling of cells*

Saccharomyces cerevisiae strain FL 530 (a wild-type diploïd) was grown in liquid minimal medium of Yeast Nitrogen Base (Difco) supplemented with 2% glucose. Cultures were incubated on a reciprocal shaker at 30°C and grew with a generation time of 108 min.

To label proteins in their steady-state distribution 50 μCi of [^{3}H]leucine was added to a concentration of 0.8 μg/ml in 38 ml of an exponentially-growing culture containing 10^7 cells/ml. This radioactive amino acid was completely exhausted from the medium at least one hr before the kinetic experiment was begun. Four hrs later, when the culture reached a cell density of 4×10^7 cells/ml, 100 μCi of [^{14}C]-leucine was added to a concentration of 1.2 μg/ml, together with unlabelled isoleucine and valine (each to a concentration of 0.6 μg/ml). The unlabelled amino acids were added to ensure that their intracellular concentrations were not drastically lowered lowered during the kinetic experiment as a result of feedback inhibition of their synthesis by the exogenously-supplied leucine. At intervals during the following twelve min 2 ml samples were removed from the culture and mixed with 2 ml of ice-cold 20% trichloracetic acid (TCA) to prevent further protein synthesis. From each acidtreated sample 0.2 ml was taken for measurement of the overall rate of [^{14}C]leucine incorporation.

2.2. *Preparation of total soluble protein from cells*

From the remaining 3.8 ml of each acid-treated suspension the cells were collected by centrifugation and washed with SSC buffer (15 mM NaCl and 15 mM trisodium citrate, pH 7.0). The washed pellets were resuspended in 1.2 ml SSC buffer; glass beads were added and the cells broken by 5 min agitation with a Chemap Vibromixer (Chemap A. E., Manne-

dorf, Switzerland). Each sample was pipeted away from the glass beads and mixed with 4 ml ice-cold 10% TCA. After 30 min at 0°C the samples were centrifuged at 18 000 *g* for 12 min. The pellets were washed with 3 ml ether to remove traces of TCA and recentrifuged. The supernatants were discarded and, when all remaining ether had evaporated, the dry preparations were stored at −20°C.

The precipitates were taken up in 0.08 ml sample buffer (90 mM Tris–borate, 2.5 mM Na_2EDTA, 2% (w/v) sodium dodecyl sulphate (SDS), 5% (v/v) β-mercaptoethanol, 10% (w/v) sucrose and 0.05% (w/v) Bromophenol Blue, pH 8.3). These preparations contain not only proteins in solution but also unbroken cells and debris in suspension. Just before electrophoresis the samples were placed in a boiling-water bath for 2 min to dissociate the proteins.

2.3. *Polyacrylamide gel electrophoresis*

The polypeptides in total soluble protein preparations were separated according to molecular weight by electrophoresis through slabs of 5% polyacrylamide gel containing 0.1% (w/v) SDS.

The slabs (18 cm × 14 cm × 0.3 cm) were made as described by De Wachter and Fiers [5]. The gels, prepared in the absence of SDS, contained acrylamide and bis-acrylamide (19:1 w/w) in TEB buffer (90 mM Tris–borate and 2.5 mM Na_2EDTA, pH 8.3). *N,N,N′,N′*-tetramethylethylenediamine (0.033 ml/g acrylamide) and freshly-dissolved ammonium persulfate (3.3% w/v; 0.1 ml/g acrylamide) were used as catalysts. Polymerization was allowed to procede overnight at 20°C. Gels were then prerun at 250 V for 1 hr at 4°C using an electrophoresis buffer of TEB containing 0.1% (w/v) SDS. After the prerun this buffer was replaced by fresh TEB containing 0.1% SDS. Eight samples of 20 μl were applied to each gel and electrophoresed at 250 V (about 20 mA/gel) for 5 hr at 4°C.

Staining of gels was carried out overnight at room temperature by gentle shaking in a solution of 0.05% (w/v) Coomassie Brillant Blue in 50% methanol–7.5% acetic acid. The gels were destained by repeated washings in 25% methanol–7.5% acetic acid. Finally, the gels were dried down onto filter paper in a press at 80°C in a vacuum oven.

2.4. *Determination of radioactivity*

Acid-precipitated cells were collected on Whatman GF/C filters and washed with 5% TCA then cold water. The filters were dried overnight and then dissolved in 0.2 ml H_2O and 10 ml toluene-PPO scintillation fluid containing 10% (v/v) Biosolv BBS-3 (Beckman). Pieces of dried gel were dissolved by overnight incubation at 60°C in 1 ml 30% H_2O_2 containing 5% (v/v) NH_4O_H. To each 1 ml aqueous sample was then added 10 ml toluene-PPO scintillation fluid containing 33% (v/v) Triton X-100. Samples were counted in an Intertechnique SL30 Spectrometer. Correction was made for about 5% of the ^{14}C appearing in the ^{3}H channel and 0.35% of the ^{3}H counts appearing in the ^{14}C channel.

2.5. *Chemicals*

[G^3H] L-leucine specific activity 208 mCi/mmole and [$U^{14}C$] L-leucine specific activity 296 mCi/mmole were obtained from CEA-France. Acrylamide (purum) was purchased from Fluka A.G. *N,N′*-methylene-bis-acrylamide (Fluka practicum) was recrystallized from acetone before use. All other reagents were of analytical grade.

3. Results

Bremer and Yuan developed a method for determining the elongation rate of RNA molecules [4]. This method has been successfully applied to measure the peptide-chain growth-rate in *Escherichia coli* [1]. The fraction F (m,t) is defined by $F(m,t) = r(m,t)/r_{tot}(t)$ where $r(m,t)$ is the amount of radioactivity incorporated into growing and completed chains of molecular weight m at time t, and $r_{tot}(t)$ is the total radioactivity incorporated into all chains at time t. For a chain of any given molecular weight the variation of $F(m,t)$ with time is a diphasic curve. The breaking point in this curve occurs at the time (T_m) it takes to synthesise a chain of the given molecular weight.

To measure the elongation rate of polypeptides in *Saccharomyces cerevisiae* we have used the same method; the kinetics of appearance of [^{14}C]leucine in peptides of different molecular weights were followed. The fraction F (m,t) is then the ratio of ^{14}C counts in any molecular weight class to the ^{14}C counts in total protein. This measure is very sensitive to

variations in sample preparation and gel cutting. Therefore we have normalised all short-term labelling (^{14}C counts) to the corresponding steady-state label (^{3}H counts).

The overall rate of incorporation of [^{14}C]leucine into acid-precipitable material was measured during the course of a short-term labelling experiment (fig. 1). During most of this 12 min period the rate of incorporation was constant. There was an initial period during which the incorporation rate increased, probably due to equilibration of label with the pre-existing pool. Such an effect should not influence our estimations of the elongation rate since in calculating F (m,t) the low rate of incorporation into any molecular weight class is partly compensated by the low rate of incorporation into total protein.

From each sample taken during the short-term labelling experiment total soluble protein was prepared. The polypeptides present in these preparations were separated into different molecular weight classes by SDS-polyacrylamide gel electrophoresis. When such gels are stained a number of conspicuous bands can be seen, each probably containing several

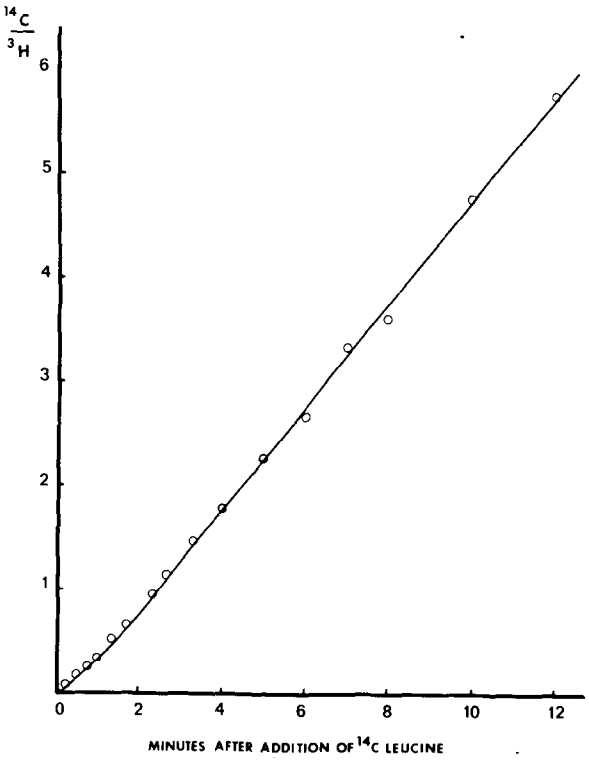

Fig. 1. The overall rate of incorporation of [^{14}C]leucine during a short-term labelling experiment. A culture was incubated with [^{3}H] leucine (1.3 μCi/ml; 0.8 μg/ml) for 4 hr to label proteins in their steady-state distribution. At time zero of the short-term experiment [^{14}C]leucine (2.6 μCi/ml; 1.2 μg/ml) was added to the culture. Samples were taken at the times indicated and mixed with an equal volume of cold 10% TCA. Aliquots were filtered, dried and counted. The ^{14}C counts were divided by the ^{3}H counts to normalize the short-term labelling to the steady-state label.

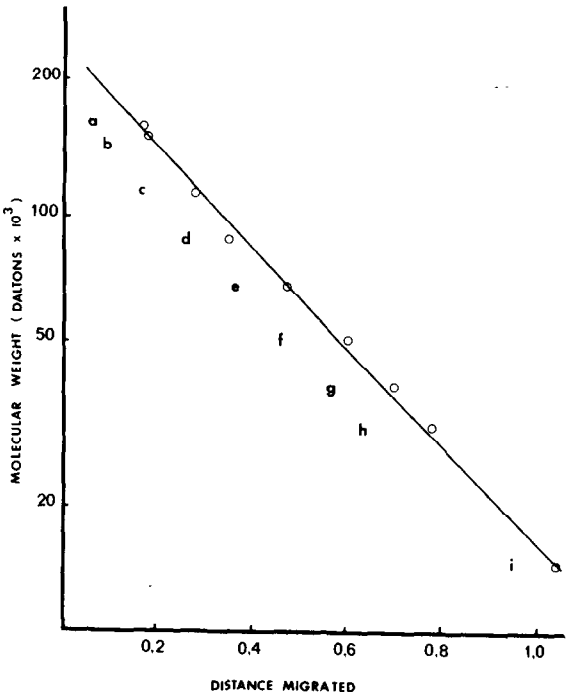

Fig. 2. Calibration of an SDS-polyacrylamide gel according to the migration of proteins of known molecular weights. The standard proteins were dissolved at a concentration of 1 mg/ml in sample buffer (See Materials and methods, section 2.2). After heating in a boiling-water bath for 2 min, 2 μl of each protein solution was applied to the gel. Electrophoresis and gel staining were carried out as described in Materials and methods (section 2.3). The migration of each protein band was measured relative to that of the Bromophenol Blue marker. The following molecular weights were taken for the standard polypeptides: (a) 165 000 for the β' subunit of *E. coli* RNA polymerase; (b) 155 000 for the β subunit of RNA polymerase; (c) 114 000 for valyl–tRNA synthetase from *E. coli*; (d) 90 000 for the subunit of RNA polymerase; (e) 68 000 for bovine serum albumen; (f) 50 000 for bovine glutamate dehydrogenase; (g) 39 000 for the α subunit of RNA polymerase; (h) 31 000 for bovine deoxyribonuclease I, and (i) 14 300 for lysozyme.

different polypeptides with almost identical molecular weights. The molecular weights of these bands were determined by comparing their mobilities during electrophoresis with the mobilities of polypeptides of known molecular weights (fig. 2). Knowing the molecular weight of polypeptides in any gel fraction, it is possible to calculate the average molecular weight of yeast polypeptides from the distribution of protein in a gel. This distribution can be measured if one assumes that leucine constitutes the same proportion of the amino acids in all polypeptides. In this case the amount of protein in any fraction is proportional to the amount of steady-state ^{3}H label in that fraction. The average molecular weight (M_A) of soluble polypeptides can then be estimated from the function ($M_A = \Sigma$ (^{3}H cpm$_b$ × M_b)/^{3}H cpm$_{tot}$ where ^{3}H cpm$_b$ is the number of ^{3}H counts in each band, in which the polypeptide molecular weight is M_b, and ^{3}H cpm$_{tot}$ is the total number of ^{3}H counts in the gel. From the gel shown in fig. 3, we calculate the average molecular weight of yeast polypeptides to be about 57 000. It can also be shown from fig. 3 that the total ^{14}C/^{3}H ratio in the gel is of the same order of magnitude as the ^{14}C/^{3}H ratio in whole cells after identical labelling. Hence there is not a selective loss of nascent ^{14}C-labelled polypeptides in our experiments.

To determine the protein elongation rate selected bands were cut from dried gels of the short-term labelling experiment. The F (m,t) for each band was then calculated by dividing the ^{14}C/^{3}H ratio in the band by the ^{14}C/^{3}H ratio in the total protein of that preparation. In fig. 3 less than 2% of the total radioactivity stays at the origin during electrophoresis (probably in unbroken cells). Hence the ^{14}C/^{3}H ratio in total protein could be measured in the crude preparations and did not require determination of the amount of radioactivity in all the gel. The variations of F (m,t) with time for some bands are shown in fig. 4. In each of these graphs the breaking point of the curve equals the time required to synthesise a polypeptide of the molecular weight in that band. The relationship between these chain completion times (T_m) and molecular weight is given in fig. 5. In the molecular weight range of 40 000 to 200 000 the points fall on a straight line whose slope corresponds to an elongation rate of 10.5 amino acids per second at 30°C. All polypeptides of molecular weight greater than 40 000 are further elongated at this rate. However, the straight line function of chain completion time versus molecular weight does not extrapolate to the origin, but would cut the time axis at about 30 sec (fig. 5). This implies that the elongation rate of polypeptides smaller than mol. wt. 40 000 is slower than 10.5 amino acids per sec. The slower rate extends by 30 sec the time required to complete long polypeptides.

A yeast polypeptide of average molecular weight (57 000) contains 533 amino acids, assuming that the average molecular weight of amino acids in yeast

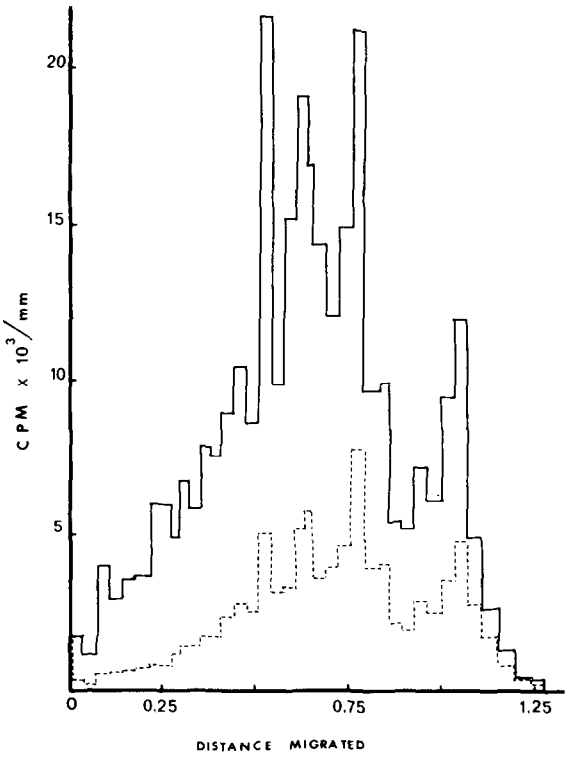

Fig. 3. The distribution of long-labelled and short-labelled polypeptides in an SDS-polyacrylamide gel. A culture was incubated for 4 hr with [^{3}H]leucine (1.3 μCi/ml; 0.8 μg/ml) and then [^{14}C]leucine (2.6 μCi/ml; 1.2 μg/ml) was added for 60 sec. Incorporation was stopped by mixing the culture with an equal volume of cold 10% TCA. The soluble polypeptides were prepared from cells and electrophoresed (Materials and methods, sections 2.2 and 2.3). The distribution of radioactivity in the gel was determined as described in the Materials and methods (section 2.4).

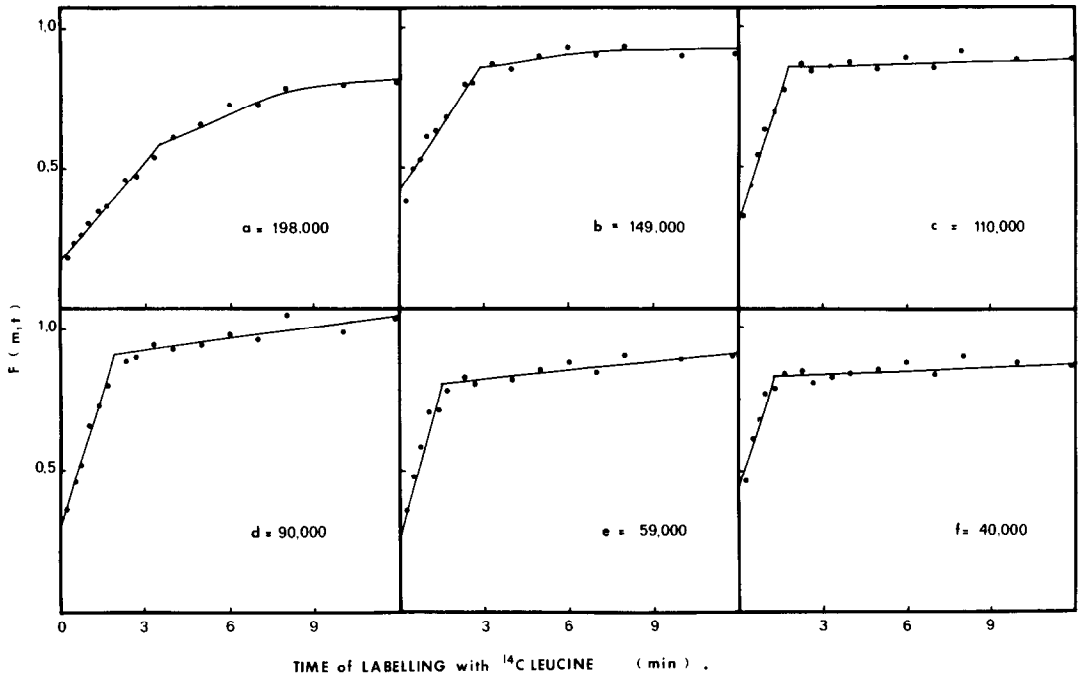

Fig. 4. The variation of F (m,t) with time for polypeptides of different molecular weight classes. Cells were labelled in steady-state with [^{3}H]leucine and then for different short periods of time with [^{14}C]leucine, as described in Materials and methods (section 2.1). The soluble proteins were prepared from each sample of cells and separated according to molecular weight by electrophoresis (sections 2.2 and 2.3). The radioactivity was measured in selected pieces of dried gel (section 2.4). For each selected band in each preparation the F (m,t) was calculated by dividing the ^{14}C/^{3}H ratio in that band by the total ^{14}C/^{3}H ratio in that preparation. In each frame the variation of F (m,t) with time is a diphasic curve with a breaking point at the time (T_m) required to complete a polypeptide of the molecular weight given.

protein is 107 [6]. From fig. 5 it can be seen that such a polypeptide chain is completed in 78 sec at 30°C, corresponding to an overall elongation rate of 6.9 amino acids per sec.

4. Discussion

We have previously estimated that the average protein elongation rate in *Saccharomyces cerevisiae* is 7 to 9 amino acids per sec at 30°C [7]. This value was calculated from the overall rate of protein synthesis, assuming 83% of ribosomes were actively engaged in making protein. We have now used another method to determine the protein elongation rate in yeast, namely measurement of the times required to synthesise polypeptides of known molecular weight. By this method we find that a polypeptide of average molecular weight is completed in 78 sec at 30°C. Petersen and McLaughlin [3] have estimated a similar figure for yeast sphaeroplasts, where the average peptide chain is synthesised in 2 min at 23°C (equivalent to 74 sec at 30°C). From our data we calculate that a polypeptide of average molcular weight is elongated at an overall rate of 6.9 amino acids per second at 30°C, a value which is good agreement with the indirect estimate [7].

Our results indicate that the time required to synthesise a polypeptide chain in yeast is not proportional to the size of the polypeptide (fig. 5). After polypeptides have reached a mol. wt. of 40 000 they are further elongated at a rate of 10.5 amino acids per sec. at 30°C. The polypeptides smaller than a mol. wt. of 40 000 are two types–completed chains and incomplete precursors of high molecular weight polypeptides. For both these types of small polypeptide the

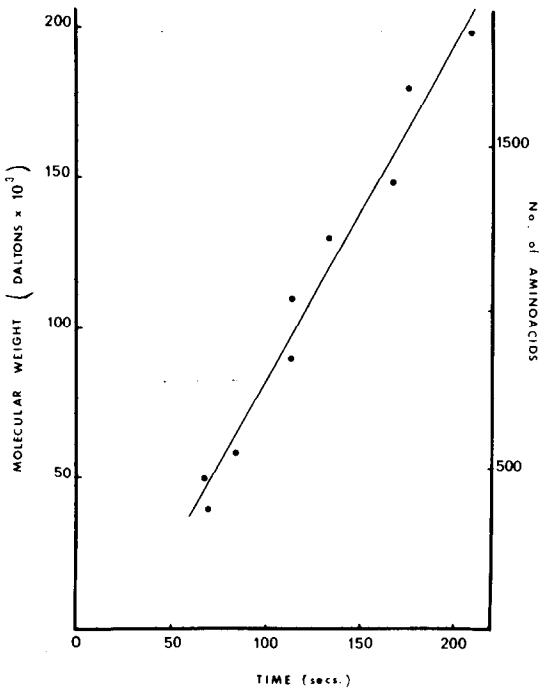

Fig. 5. The relationship between chain completion time and polypeptide molecular weight. From graphs such as those in fig. 4 the time (T_m) required to complete a polypeptide chain of molecular weight m has been determined for a number of molecular weight classes. The number of amino acids corresponding to each molecular weight has been calculated on the assumption that the average molecular weight of amino acids in yeast protein is 107 [6].

elongation rate seems to be slower than 10.5 amino acids per sec, extending by about 30 sec the time required to complete long polypeptides. The 30-sec extension does not appear to be an artefact since our previous estimate of 7 to 9 amino acids per sec for the average protein elongation rate [7] is considerably slower than the 10.5 amino acids per sec measured for long polypeptides. The molecular basis for a slower elongation rate of small proteins could be that nascent polypeptides interfere with positioning of the next aminoacyl-tRNA in the ribosomal A site. This interference could be reduced as the polypeptides reach sufficient length to assume more complex secondary and tertiary structures.

Petersen and McLaughlin have also reported a variation of protein elongation rate with the size of polypeptide being made in yeast [3]. However, they found that the rate of protein synthesis in yeast sphaeroplasts is noticeably faster on small polysomes than on larger polysomes. This variation in protein elongation rate is opposite to that deduced from our experiments. These contrasting observations probably result from the use of different experimental conditions. Each of our kinetic analyses were performed on a single culture of exponentially-growing whole yeast cells at 30°C, whereas the experiment of Petersen and McLaughlin was performed at 23°C with several cultures of yeast sphaeroplasts, each containing different amounts of labelled aminoacids [3]. It may be that changing the medium in their experiment has introducted culture-specific changes in the elongation rate of polypeptides. It is now important to have more information about the times required to complete small molecular weight polypeptides. Unfortunately, with the technique used here, this is very difficult since the little radioactivity incorporated during short labelling periods does not permit reliable estimates of small elongation times to be made.

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