Regulation of Ribosomal Protein Synthesis in Escherichia coli B/r

PATRICK P. DENNIS* AND RYLAND F. YOUNG

Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706,* and Institute for Molecular Biology, University of Texas at Dallas, Richardson, Texas 75080

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The differential synthesis rate of ribosomal protein (r-protein), \( \alpha_r \) (synthesis rate of r-protein divided by synthesis rate of total protein), was measured during the cell division cycle. It was observed that \( \alpha_r \) remained essentially constant and was not measurably affected by duplication of the r-protein gene cluster (i.e., str-spC region) during the process of chromosome replication. It was further observed that the rate of total protein synthesis and r-protein synthesis increased continuously and uniformly during the entire cell cycle. This gene dosage independence of the synthesis rate of r-protein was similar to that observed earlier for the synthesis of ribosomal ribonucleic acid (rRNA). These observations indicate that the synthesis rates of the protein and RNA components of the ribosome are coordinately balanced during the entire cell division cycle and are not significantly perturbed by duplication of the r-protein or rRNA genes. Furthermore, this balanced synthesis insures that neither free rRNA nor free r-protein accumulate in appreciable amounts during balanced growth.

In the bacterium Escherichia coli, a large fraction of the cellular metabolic activity is devoted to the production of ribonucleic acid (RNA) and protein components of the ribosome which are rapidly incorporated into mature 70S particles (11, 12). The precise mechanisms regulating the expression of ribosomal protein (r-protein) and ribosomal RNA (rRNA) genes is not yet understood. In this study the differential rate of r-protein synthesis, \( \alpha_r \) (r-protein synthesis rate divided by total protein synthesis rate), has been measured during the division cycle of E. coli B/r to assess the effect of gene duplication on the regulation of r-protein gene expression.

Schleif (15) originally observed a constancy in \( \alpha_r \) during bacterial cell division cycle, implying that replication of the r-protein gene cluster has little or no influence on the synthesis rate of r-protein. More recently, however, Zaritski and Meyenburg (16) have presented data which suggest that the replication of the r-protein gene cluster results in a measurable perturbation in \( \alpha_r \). This implies that the mechanism regulating the synthesis of r-protein is sensitive to r-protein gene dosage. Using somewhat different techniques which are presumably more sensitive for detecting cell cycle-related events (6, 8) as well as small changes in \( \alpha_r \), we obtain results that are consistent with the original observations of Schleif (15). Furthermore, the results indicate that the rate of r-protein production increases continuously and uniformly during the entire cell division cycle. The net synthesis rate of 16 and 23S rRNA shows a similar independence of the cell cycle (3, 4). This means that r-protein production is at all times balanced to 16 and 23S rRNA production such that neither free rRNA nor free r-proteins accumulate in appreciable amounts.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strain was E. coli B/r (ATCC 12407). Cultures were grown at 37 C in a rotary shaker bath in minimal medium C (6) supplemented with 0.2% glycerol as sole carbon source. Cultures were begun by at least a 5000-fold dilution of a fresh stationary phase culture; growth was monitored as absorbance at 460 nm (A460), and cell concentrations were determined with a Coulter counter model B. The steady-state doubling times of bacteria growing in glycerol medium were 55 to 60 min, and an A460 of 1.0 corresponds to a density of approximately \( 6 \times 10^8 \) cells/ml.

Synthesis of DNA, protein, r-protein and \( \beta \)-galactosidase during the cell cycle. The rates of thymidine and leucine incorporation during the bacterial cell division cycle were determined by pulse labeling exponential-phase glycerol cultures (100 ml; \( 1.5 \times 10^8 \) cells/ml) for 2 min and then determining the amount of radioactivity incorporated into cells of different ages. This was accomplished by binding the cells to a
GS membrane filter (Millipore Corp., Bedford, Mass.) at the end of the labeling period and determining the radioactivity in newborn cells eluted continuously from the membrane. These procedures have been described \((6, 8)\). Briefly, cells were bound to the membrane by filtration and washed with 100 ml of medium. The filter assembly was inverted and elution with growth medium was begun. Samples of the effluent were collected continuously during regular time intervals and bacterial concentrations were determined.

To measure the rate of deoxyribonucleic acid (DNA) synthesis, cultures were pulse labeled with \([\text{methyl-}^{3}H]\)thymidine (specific activity 1.9 Ci/mmol; 500 pmol/ml) to measure the rate of total protein and r-protein synthesis, cultures were pulse labeled with \([4.5\times{}^{3}H]\)leucine (specific activity 59.5 Ci/mmol; 170 pmol/ml). In both instances, cells were labeled during exponential phase growth and prior to the synchronization. Total incorporation of thymidine or leucine into cells of different ages was determined by collecting a measured portion from each sample of the effluent on a nitrocellulose filter, washing with minimal medium and counting as described previously \((3)\).

To determine total incorporation of \([^{3}H]\)leucine into r-protein, two additional portions (5 ml) from each effluent sample were mixed with a sample (5 ml) from either a \([^{4}C]\)uridine-labeled or \([^{4}C]\)leucine-labeled exponential-phase reference culture (see below). Three 100-µl samples were removed from each of the mixed cultures, precipitated with 5% trichloroacetic acid and collected on nitrocellulose filters. The \(^{3}H/^{14}C\) isotope ratios in these precipitates were determined after oxidation and scintillation counting. The remaining portions of the mixed cultures were cooled to 0°C and processed as described previously \((5)\). The \(^{3}H/^{14}C\) isotope ratio in 70S ribosomes isolated after zone sedimentation in \(10^{-2}\) M Mg\(^{2+}\) sucrose gradients was determined, and the quotient of the isotope ratios, \(S_{r}/S_{\text{tot}}\), was calculated:

\[
S_{r}/S_{\text{tot}} = \frac{^{3}H/^{14}C \text{ in 70S ribosomes}}{^{3}H/^{14}C \text{ in total cells}}
\]

For a description of the relationships between the \(S_{r}/S_{\text{tot}}\) values and the differential rate of r-protein synthesis, see Fig. 2.

Reference cultures, labeled with \([^{4}C]\)uridine or \([^{14}C]\)leucine, were prepared as follows. At an \(A_{660}\) of 0.1, 60 ml of bacteria growing in glycerol minimal medium was labeled with either \(2\times{}^{14}C\)uridine (specific activity 61 mCi/mmol; 670 pmol/ml) or \([^{4}C]\)leucine (specific activity 312 mCi/mmol; 150 pmol/ml). After one cell doubling, either uridine or leucine (400 nmol/ml) were added to the respective cultures, and incubation was continued for an additional cell doubling. Samples from the reference cultures were mixed with measured portions from the effluent (experimental) samples and used to normalize the recoveries of 70S ribosomes in the \(10^{-2}\) M Mg\(^{2+}\) sucrose gradients.

\(\beta\)-galactosidase inducibility during the cell cycle was measured as described previously \((7)\).

**RESULTS**

**Synthesis of DNA during the cell division cycle.** The rate of DNA synthesis during the cell division cycle was determined by pulse labeling an exponential-phase culture growing in glycerol minimal medium with \([^{3}H]\)thymidine and employing the membrane elution technique to measure the incorporation of radioactivity into cells of different ages \((6)\) (Fig. 1, DNA curve). The abrupt increases in the rate of incorporation at cell age 0.6 to 0.8 (5 to 20 min, 65 to 85 min, and 130 to 150 min elution time) are indicative of initiation of rounds of chromosome replication. In *E. coli* B/r the distribution in the time of initiation of chromosome replication during the cell cycle has a standard deviation of about 7% of the doubling time \((13)\). This small asynchrony in initiation with respect to cell age is apparent in the thymidine incorporation curve. The slight depression in the rate of thymidine incorporation at about cell age 0.5 (25 to 30 min and 85 to 90 min elution time) corresponds to the gap in DNA synthesis which occurs after completion of a round of chromosome replication in slowly growing cultures of *E. coli*. Thus, in exponential-phase bacteria growing in glycerol minimal medium, chromosome replication requires approximately 45 min and is followed by a 10- to 15-min period which is devoid of DNA synthesis. Initiation occurs at the origins of each of two chromosomes at cell age 0.7 and is followed by cell division at age 1.0; termination occurs in each of the daughter cells at age 0.5. This thymidine incorporation pattern is similar to previous measurements (for a complete discussion and analysis, see references 2, 6, and 9).

**Synthesis of total protein and ribosomal protein during the cell division cycle.** In a similar experiment, the rate of protein synthesis was determined by labeling the exponential-phase culture with \([^{3}H]\)leucine and measuring the incorporation of radioactivity into cells of different ages (Fig. 1, total protein curve). The results indicate that the rate of protein synthesis, unlike DNA synthesis, increases continuously and uniformly during the cell cycle.

Incorporation of \([^{3}H]\)leucine into r-protein in cells of different ages was determined by removing portions from the effluent samples collected over a period of 72 min (representing the entire age distribution of the original \([^{3}H]\)leucine-labeled exponential-phase culture) and analyzing the fraction of radioactivity in ribosomes. The \(^{3}H\)-labeled samples were mixed with samples from either a \([^{14}C]\)uridine- or \([^{14}C]\)leucine-labeled exponential-phase culture (the \(^{14}C\) ra-
Fig. 1. Synthesis rate of DNA, total protein, r-protein and β-galactosidase during the division cycle of E. coli B/r. The rate of DNA synthesis was determined by pulse labeling an exponential-phase culture for 2 min with [3H]thymidine. The culture was bound to a membrane filter and washed with 100 ml of medium, and elution of newborn cells was begun. The elution rate was 3.5 ml per min, and effluent samples were collected continuously for 4-min periods. Samples (10 ml) from each effluent sample were collected on nitrocellulose filters (0.45-µm pore size), washed with minimal medium, dried, and counted in 6 ml of toluene base scintillation fluid. Cell concentrations were determined in the effluent samples with a model B Coulter counter and the [3H]thymidine per cell (histogram, top) in the effluent samples was calculated. The rate of protein synthesis was determined by pulse labeling an exponential-phase culture with [3H]leucine and incorporation per effluent cell was determined as described above with the following modifications. Between 6 to 36 min and 108 to 132 min elution time, 6-min samples of the effluent were collected continuously. The radioactivity in a 5-ml portion and cell concentration were determined in each sample. Beginning at 36 min, alternate 5-min and 1-min samples of the effluent were collected. Two portions (5 ml) from the 5-min samples were mixed with portions from the 14C-reference cultures, and incorporation of [3H]leucine into r-protein was determined as described in the text. Portions (2.0 ml) were removed from the 1-min sample for determination of total incorporation. The rate of r-protein synthesis (relative units) was determined as the product of the total leucine incorporation per cell curve and relative
dioactivity was used exclusively as a reference for normalization of the recovery of 3H-labeled 70S ribosomes. The 3H to 14C isotope ratios in total cells (S\textsubscript{tot}) and in 70S ribosomes (S\textsubscript{r}), isolated after zone sedimentation of crude cell extracts in 10 \textsuperscript{-2} M Mg\textsuperscript{2+} sucrose gradients, were determined. The quotients of the isotope ratios were calculated as follows:

\[
S_{r} = \frac{[3H] \text{leucine/}[14C] \text{uridine}}{[3H] \text{leucine/}[14C] \text{uridine in 70S ribosomes}}
\]

and

\[
\frac{S_{r}}{S_{\text{tot}}} = \frac{[3H] \text{leucine/}[14C] \text{leucine in 70S ribosomes}}{[3H] \text{leucine/}[14C] \text{leucine in total cells}}
\]

The differential rate of r-protein synthesis (α\textsubscript{r}) is related to S\textsubscript{r}/S\textsubscript{tot} (leucine-uridine) by the proportionality factor 0.6 [5]; Fig. 2. The average α\textsubscript{r} calculated from the 11 samples spanning the entire age distribution was 0.11 with a range of less than ± 0.01. The value of α\textsubscript{r} measured previously in exponential-phase cultures growing in glycerol minimal medium was 0.108 ± 0.01 (5). Thus, it seems that α\textsubscript{r} remains essentially constant during the cell division cycle.

The S\textsubscript{r}/S\textsubscript{tot} (leucine-leucine) quotients directly measure the value of α\textsubscript{r} in cells representing small intervals in the age distribution relative to the value of α\textsubscript{r} in an exponential-phase population. Again, these relative values (i.e., differential synthesis rates of r-protein) show no cell age dependency (Fig. 2) and remain essentially constant during the cell cycle.

\[
\alpha_{r} = \frac{\text{cde lane, O or absolute } \alpha_{r} (\text{leucine-uridine, D})}{\text{Values of } \alpha_{r} \text{ from Fig. 2.)}}
\]

That is:

\[
\alpha_{r} \times \frac{dP}{dt} = \frac{d(\text{r-protein})}{dt}
\]

where α\textsubscript{r} = r-protein synthesis rate/total protein synthesis rate; dP/dt = total protein synthesis rate; and d(r-protein)/dt = r-protein synthesis rate. The capacity for induction of β-galactosidase is from the data of Helmstetter (upper curve; [7]) and our own experiments (lower curve). The exponential-phase culture growing in glycerol minimal medium was exposed to isopropylthiogalactoside (10 \textsuperscript{-3} M) for 2 min, and the enzyme activity per effluent cell was determined. The elution rate was 7.5 ml/min, and samples were collected continuously at regular time intervals.
The rates of incorporation of $[^3H]$leucine into r-protein (relative units) in cells of different ages (Fig. 1, r-protein curves) are obtained as the product of the $\alpha_r$ values (either the leucine-leucine or leucine-uridine values) and the corresponding rate of incorporation of $[^3H]$leucine into total protein (from the total protein curve in Fig. 1). From this calculation it is apparent that the rate of r-protein synthesis, like the rate of total protein synthesis, appears to increase continuously and uniformly during the entire cell cycle (Fig. 1).

**Synthesis of $\beta$-galactosidase during the cell cycle.** The capacity for induction of $\beta$-galactosidase is known to be dependent upon the number of $\beta$-galactosidase genes (10); in a synchronous population the capacity increases twofold after replication of the $\beta$-galactosidase structural gene (7, 14). Experiments illustrating the inducibility of $\beta$-galactosidase as a function of cell age are illustrated in Fig. 1. (The upper $\beta$-galactosidase curve is the data of Helmstetter; [7].) Exponential-phase cultures growing in glycerol minimal medium were exposed to the gratuitous inducer, isopropylthiogalactoside, for 2 min, and the amount of enzyme in cells of different ages was determined. The amount of enzyme per effluent cell increases twofold early in the division cycle at cell age 0.1 to 0.3 (45 to 55 min and 105 to 115 min elution time) and then remains essentially constant for the remainder of the cell cycle. Again these curves illustrate the quality of selection of newborn cells with this technique (3), and the dispersion in the time at which a genetic locus (i.e., $\beta$-galactosidase) is duplicated during the cell division cycle (13).

The differential rate of $\beta$-galactosidase synthesis, $\alpha_{ac}$ (equal to $\beta$-galactosidase synthesis rate/total protein synthesis rate), was calculated as the quantity of enzyme per cell divided by $[^3H]$leucine per cell in samples of the effluent (i.e., the total protein synthesis rate) collected between 36 and 108 min elution time in Fig. 1 and is illustrated (Fig. 2). The differential value $\alpha_{ac}$ equals the $\beta$-galactosidase synthesis rate divided by total protein synthesis rate. The range in $\alpha_{ac}$ is $\pm20\%$ of the average value. An essentially identical $\alpha_{ac}$ curve is obtained using our own $\beta$-galactosidase data, illustrated in Fig. 1. The dashed lines represent the theoretical fluctuation in $\alpha_r$ and $\alpha_{ac}$ if the differential synthesis rates are gene dosage dependent and if the total protein synthesis rate increases continuously and uniformly during the cell cycle. The theoretical curves deviate $\pm33\%$ from the average values of 1.5. Duplication of the str-spc region occurs at cell age 0.95 and that of the lac region occurs at cell age 0.20.
synthesis rate of β-galactosidase begins to increase between cell age 0 and 0.1, reaches a maximum at cell age 0.3 and then gradually decreases during the remainder of the cell division cycle. This abrupt increase in α_{G2} occurs early in the cell cycle presumably results from duplication of the β-galactosidase gene during the process of chromosome replication.

**DISCUSSION**

In the bacterium *E. coli* B/r growing in glycerol minimal medium (DT ~ 55 min), chromosome replication is initiated at cell age 0.6 to 0.8 (40 min after cell division; [6]). Replication is initiated near the ilv locus at 75 min on the genetic map and proceeds in both directions at nearly equal rates (1). Termination occurs near the his locus. Duplication of the str-spc region of the chromosome which contains most all of the known r-protein genes occurs 10 min after initiation (cell age 0.95), whereas duplication of the β-galactosidase gene occurs 25 min after initiation (cell age 0.20).

The capacity for induction of β-galactosidase is dependent upon the number of β-galactosidase genes and increases twofold after Z-gene duplication early in the cell cycle (Fig. 1; [7, 14]). The differential rate of β-galactosidase synthesis (α_{G2}) fluctuates in a regular manner during the cell cycle and agrees well with the theoretical expectation (Fig. 2). In contrast, the differential rate of r-protein synthesis (α_{r}) measured during time intervals spanning the entire age distribution was observed to be essentially constant and apparently unaffected by duplication of the r-protein gene cluster (Fig. 2); the fluctuations in α_{r} appear to be random in nature and are considerably less than the regular fluctuation observed in α_{G2}). This means that if r-protein gene duplication influences the differential synthesis rate, the effect is probably small, is rapidly damped out, and is obscured by the dispersion in chromosome replication (13).

Schleif (15), using a somewhat less sensitive technique to measure α_{r} during the cell division cycle, also reached the conclusion that α_{r} is independent of the r-protein gene dosage. In contrast, Zaritski and Meyenburg (16) have suggested that α_{r} increases abruptly after duplication of the r-protein gene cluster. However, the maximum observed deviation of α_{r} was less than ±15% of the average, whereas the theoretical deviation should have been ±33%. In any case, the Zaritski and Meyenburg method involves chilling to 0°C and resuspension of the synchronized cells eluted from the membrane filter (Millipore) prior to labeling; our method allows the labeling to be done in the exponential, steady-state condition prior to any manipulation, and thus eliminates the influence of any perturbations introduced into the normal physiological processes by the synchronization procedures.

The continuous and uniform increases in the rate of protein synthesis (Fig. 1) and the constancy of α_{r} during the cell cycle implies that the rate of r-protein synthesis increases continuously and uniformly during the cell cycle. The rate of rRNA synthesis also increases continuously and uniformly during the cell cycle (3, 4); this means that the synthesis rate of both the RNA and protein components are closely balanced during the entire cell division cycle.

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**LITERATURE CITED**

RIBOSOMAL PROTEIN SYNTHESIS