Transcription of the lac Operon of Escherichia coli*

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SUMMARY

The effects of induction and of transient and catabolite repression on lac-specific RNA in cells of Escherichia coli were investigated. The rate of lac-specific RNA synthesis increases linearly for 3 min after induction and then becomes constant. The accumulation of RNA complementary to the promoter proximal lac Z gene begins before accumulation of RNA complementary to the promoter distal lac Y-A genes. When transient repression is imposed upon the cells by the addition of glucose, the lac Y-A-specific RNA begins to decay before the lac Y-A-specific RNA. Catabolite repression reduces the rate of formation of lac-specific RNA relative to the rate of formation of bulk RNA. Both lac Z-specific RNA and lac Y-A-specific RNA are equally affected. These results confirm the concept that the sole effect of transient repression and of catabolite repression is the inhibition of the initiation of RNA synthesis at the lac promoter site.

The lac operon of Escherichia coli has long served as a model for the study of the control of gene expression (1). Using a variety of inhibitors blocking cellular processes at known points, it has been possible to obtain a reasonably complete description of the response of the operon to a whole gamut of physiological conditions. In addition to determining the steady state level of expression of the operon, these approaches have also provided much information regarding the kinetics of that expression. With the isolation of specialized transducing phages carrying the genes of the lac operon, it has been possible to look at the transcriptional products of the operon directly. In the present report the carefully explored techniques described in the preceding paper (2) were used to study the kinetics of lac-specific RNA synthesis, accumulation and degradation in response to induction, transient repression by glucose, and catabolite repression (1).

A similar study of induction and transient repression was carried out by Adesnik and Levinthal (3). As will be seen, our results are in complete agreement with their findings. Our results disagree with those reported by Contesse et al. (4). Our findings concerning catabolite repression also disagree with those reported by the French group (4), but are in excellent agreement with the view that the promoter is the unique target of catabolite repression in the lac operon (5, 6).

EXPERIMENTAL PROCEDURE

Bacterial Strains—In most experiments the lac-inducible Escherichia coli strain MaF was used (2). In the experiment dealing with catabolite repression, E. coli strain 242, kindly supplied by Dr. Charles Yanofsky of Stanford University, was used. In this organism the enzymes of tryptophan biosynthesis are insensitive to repression by tryptophan (trp R−). E. coli strains Δ171 and EZ-2A were used as the source of unlabeled RNA preparations devoid of lac Z-specific messenger RNA and of lac Y-A-specific messenger RNA, respectively (2). The bacteria were grown at 30° as described (2).

Phage Preparations—The isolation and purification of Aplaco and of h53dlac has been described (2). Phage pT190 (p80 ptrp A-EII), a gift of Dr. Yanofsky, was prepared by infecting a 4-liter culture of M5107, grown on LB broth to an optical density of 0.45 at 500 nm, with 25 ml of phage at a titer of 3.3 X 1011 phage per ml. The incubation at 37° was terminated 4.5 hours after infection by addition of 25 ml of chloroform and 1.5 liters of ice. The precipitation of phage was collected by centrifugation for 1 hour at 13,000 x g.

The incubation at 37° was terminated 4.5 hours after infection by addition of 25 ml of chloroform and 1.5 liters of ice. At the time of termination, foaming due to cell lysis could no longer be easily controlled with Dow Corning Antifoam A. Following removal of cellular debris by centrifugation for 15 hours at 30,000 x g, the supernatant was brought to a concentration of 2.1% NaCl, then 0.05% ethylene glycol 0000 and stored, with gentle stirring, overnight at 4°.

The precipitated phage were collected by centrifugation at 10,000 x g for 15 hours and resuspended by stirring overnight in a medium of 0.5% NaCl, 0.02% Tris, pH 7.5, 0.01% gelatin, and 0.01 M MgCl2. Finally, the mixture was clarified by centrifugation for 15 min at 27,000 x g.

Purification of DNA, RNA, and Hybridization—The methods described in the preceding paper were employed (2).

Labeling Procedures—To obtain cultures whose messenger RNA was uniformly labeled, the method described in the preceding paper was used, in which the cells were exposed to an amount of [3H]uridine sufficient for 75 to 90 min of incorporation, 30 min before the addition of IPTG. For pulse labeling experiments, inducer was added, and at indicated times, portions of the culture (usually 5 ml) were transferred to a centrifuge tube kept at 30°, containing [3H]uridine. After an incubation period of 15 s, RNA synthesis was halted by plunging the sample into a mixture of ice and 1 M KCN (2). A period of 15 s was chosen, because at this time the incorporation of [3H]uridine into trichloroacetic acid-precipitable material became constant (Fig. 1). It is worthy of note, however, that whether or not incorporation is linear is not significant with regard to this experiment as long as the duration of the pulse is maintained rigorously constant from sample to sample. In the experiment on catabolite repression, cells growing in different media containing IPTG were labeled for 3 min with [3H]uridine.

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1 The abbreviation used is: IPTG, isopropyl thiogalactoside.
uniformly labeled. Rather, the radioactivity will be localized only at a vessel containing uridine at high specific radioactivity; and This procedure measures only that RhA synthesized during the initiating the experiment by the addition of inducer; (b) transcribing samples of the experimental culture at the indicated times to a vessel containing uridine at high specific radioactivity; and (c) collecting the sample after a short (15 s) period of incubation. This procedure measures only that RNA synthesized during the 15-s incubation period. The RNA chains produced will not be uniformly labeled. Rather, the radioactivity will be localized only at the growing end. This procedure, because of the shortness of the labeling period measures the rate of lac specific RNA synthesis. The second method is designated pulse labeling. It involves: (a) initiating the experiment by the addition of inducer; (b) transferring samples of the experimental culture at the indicated times to a vessel containing uridine at high specific radioactivity; and (c) collecting the sample after a short (15 s) period of incubation. This procedure measures only that RNA synthesized during the 15-s incubation period. The RNA chains produced will not be uniformly labeled. Rather, the radioactivity will be localized only at the growing end. This procedure, because of the shortness of the labeling period measures the rate of lac specific RNA synthesis. The second method is designated equilibrium labeling. It involves incubating the cells in the presence of a large excess of radioactive uridine for a length of time (30 min) sufficient to allow the nucleotide pools serving as RNA precursors to be labeled to constant specific activity. At the end of this period, the experiment is initiated by the addition of inducer and samples are collected at the indicated times thereafter. This procedure will determine the total amount of lac specific RNA present in the cells at a time of collection. It is important to note that the RNA chains possess a uniform distribution of label throughout their entire length.

Fig. 2 depicts the results of a pulse labeling experiment. IPTG was added to a culture of strain MoF lac growing exponentially on glycerol and samples of the culture were labeled for 15 s at various times after addition of the inducer. As shown in this figure the rate of lac specific RNA synthesis increases linearly with time up to 3 min. After this time synthesis proceeds at a constant rate. When the experiment described in Fig. 2 was repeated employing equilibrium labeling techniques, the data in Fig. 3 were obtained. As shown here, accumulation begins immediately; it proceeds first at an increasing rate and then at decreasing rate so that the total amount of lac specific RNA in the cell becomes constant after 7 to 8 min. The fact that accumulation is seen to proceed first at an increasing rate is in good agreement with the observation that the rate of lac specific RNA synthesis is increasing at a constant rate for the first 3 min.

We examined the transcription of the separate elements of the lac operon by determining separately the amount of lac Z-specific and lac Y + A-specific RNA present at different times after induction. To this end samples of the RNA described in Fig. 3 were hybridized with wild type lac DNA which had been previously hybridized with nonradioactive RNA derived from strains carrying either deletions of the lac Y + A regions (Δ-171) or the lac Z region (EZ-2A). As shown in the previous study (1) this technique allows the measurement of the RNA transcribed from the Z or Y + A cistrons, respectively. The results of this experiment are shown in Fig. 4. Upon induction, Z-specific messenger RNA begins to accumulate at an increasing rate. A steady state level is reached about 6 min after the onset of induction. In contrast there is a delay of 2½ min before any Y + A-specific

Fig. 1. Incorporation of [3H]uridine into trichloroacetic acid-precipitable material. A culture of MoF was grown on glycerol minimal medium to a cell density of 112 Klett units. At this time 1.0 ml of the culture was added to a centrifuge tube containing [3H]uridine (100 μCi and 0.9 μg). The cells and radioactive uridine were incubated together at 30° for the indicated periods of time and the incorporation of label was terminated by addition of 1 ml of cold 5% trichloroacetic acid. At the conclusion of the experiment two additional 1-ml portions of trichloroacetic acid were added to each tube and the tubes were stored overnight at 4°. On the next morning the precipitates were collected on Millipore filters and washed with cold 5% trichloroacetic acid. After drying the filters, the amount of radioactivity on each was determined.

RESULTS

Induction of lac Operon—In our experiments the kinetic data of specific RNA synthesis and degradation were determined by isolating specific RNA radioactively labeled in two different ways. The first method is designated pulse labeling. It involves: (a) initiating the experiment by the addition of inducer; (b) transferring samples of the experimental culture at the indicated times to a vessel containing uridine at high specific radioactivity; and (c) collecting the sample after a short (15 s) period of incubation. This procedure measures only that RNA synthesized during the 15-s incubation period. The RNA chains produced will not be uniformly labeled. Rather, the radioactivity will be localized only at the growing end. This procedure, because of the shortness of the labeling period measures the rate of lac specific RNA synthesis. The second method is designated equilibrium labeling. It involves incubating the cells in the presence of a large excess of radioactive uridine for a length of time (30 min) sufficient to allow the nucleotide pools serving as RNA precursors to be labeled to constant specific activity. At the end of this period, the experiment is initiated by the addition of inducer and samples are collected at the indicated times thereafter. This procedure will determine the total amount of lac specific RNA present in the cells at the time of collection. It is important to note that the RNA chains possess a uniform distribution of label throughout their entire length.

Fig. 2. Incorporation of [3H]uridine into lac-specific RNA. A culture of MoF was grown on glycerol minimal medium to a cell density of 96 Klett units. At this time IPTG was added to the culture to a final concentration of 5 mM. This addition and mixing required 16 s. Every 15 s after the addition of inducer, a 5-ml sample of the culture was transferred to a prewarmed centrifuge tube containing [3H]uridine (100 μCi and 0.9 μg). Incorporation of radioactive uridine was permitted for 15 s and then terminated by addition of 0.5 ml of cold 1 M KCN. RNA was prepared from each of these samples using the techniques described earlier and hybridized to a large excess of wild type lac (Δ T-68) DNA bound to nitrocellulose filters. In this and all subsequent experiments the DNA is in large excess unless indicated otherwise. The points were plotted at a time equal to the time elapsed between addition of inducer and the midpoint of the labeling period. The hybridization procedures used have been described under "Experimental Procedure" of the accompanying paper (2).
RNA begins to accumulate. This RNA reaches a steady state level 8 to 9 min after the onset of induction. Another interesting result of this experiment is the finding that when steady state has been reached the ratio of Z to Y + A specific RNA is 2.45. This ratio is nearly identical with the ratio of the length of the Z-specific and Y + A-specific DNA segments which were measured by competition experiments (Fig. 12 of the preceding paper). This result indicates that the two RNA segments are present in the cell in equimolar amounts. These results agree with those of Adesnik and Levintal (3), that the transcription of the Z region is initiated immediately upon induction and that the transcription of the Y + A region begins 2-5 min later. They support the view that transcription proceeds through the Z region to the Y + A region of the operon.

Transcript Repression of lac Operon—Transient repression is the rapid cessation of β-galactosidase synthesis upon addition of glucose to a culture growing on a different carbon source (5). To establish directly that this loss of ability to synthesize β-galactosidase is due to loss of the appropriate messenger, the kinetics of lac-specific RNA synthesis and degradation was followed before and after addition of glucose to a glycerol-grown culture. As shown in Fig. 5, addition of glucose at a time when the accumulation of lac-specific RNA reached 90% of its steady state level results in a precipitous decrease in the lac-specific RNA concentration beginning 2-4 min after addition of glucose. The data in Fig. 6 show that the limiting step in this degradative process may be accounted for by a first order rate equation. When the RNA preparations from the experiments depicted in Fig. 5 were hybridized against DNA preparations previously hybridized against nonradioactive RNA derived from cultures of Δ-171...
(Y + A-specific RNA) and EZ-2A (Z-specific RNA), the data shown in Fig. 7 were obtained. Following glucose addition 13% and 23% min elapsed before there was measurable degradation of Z- and Y + A-specific RNA, respectively. These are the expected results if the addition of glucose prevented further initiation of the lac operon. The continuing synthesis of already initiated lac-specific RNA chains would yield the observed increase in hybridizable RNA. This would be followed by the disappearance of lac-specific messenger RNA since no new chains would be initiated. Synthesis of Y + A-specific RNA would be expected to continue longer than the synthesis of Z-specific RNA and this was in fact observed.

Catabolite Repression of lac Operon—There is considerable indirect evidence that catabolite repression is exerted at the level of transcription (5). However, it has not been possible to measure the expected decrease in messenger RNA due to the difficulties encountered in labeling cultures under different conditions of growth. These difficulties arise from our lack of understanding of the effects of growth rate on the rate of synthesis of messenger RNA and of stable RNA. Without this information it is difficult to interpret labeling data, because there is no simple way to relate the specific activities of RNA preparations produced under different conditions of growth. In the present work these difficulties were circumvented in several ways. In order to assess the effect of catabolite repression on lac-specific RNA synthesis we compared the rate of lac-specific RNA synthesis with the synthesis of RNA specific for an operon known to be refractive to regulation under the conditions of our experiments. We chose for this purpose RNA-specific for the trp operon in a trp R- mutant. It has been shown previously (6) that the trp operon is not subject to catabolite repression and that the trp R- mutation renders the operon insensitive to repression by tryptophan. Therefore, E. coli strain 242, a trp R- strain possessing a wild type lac operon was grown for many generations on either glycerol, glucose, or glucose-6-phosphate in the presence of 5 mm IPTG. At a cell density of 100 to 120 Klett units the cells were labeled for 3 min with [3H]uridine. RXA was prepared from each of these cultures using standard procedures (2). Following purification, the specific activity of the RNA preparations was determined and found to be higher in the preparations from the glycerol and glucose-6-phosphate-grown cultures than in that from the glycerol grown culture (Table I). This presumably reflects the higher rate of growth in glucose and glucose-6-phosphate than in glycerol. A portion of each RNA preparation was hybridized simultaneously against filters containing either an excess of trp-specific DNA, prepared from plasmid DNA or of lac-specific DNA from lambda 5. Following standard hybridization procedures, the amount of radioactivity on each pair of filters was determined. The results of this experiment are presented in Table I. They show that the rate of trp messenger RNA synthesis increases with each successively better carbon source while the rate of lac-specific RNA synthesis decreases. The ratio of lac- to trp-specific RNA synthesis decreases 2- and 7-fold when RNA derived from cells grown on glycerol is compared to RNA derived from cells grown on glucose and glucose-6-phosphate, respectively. It can be seen that growth on carbon sources known to exert catabolite repression, i.e. glucose and glucose-6-phosphate, reduces the rate of lac-specific RNA synthesis relative to that of trp specific RNA synthesis. Taking the rate of trp RNA synthesis as a measure of uncontrolled messenger RNA synthesis, these findings indicate that catabolite repression specifically reduces the rate of synthesis of lac-specific RNA.

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Fig. 8 illustrates the relation of the rate of synthesis of trp-specific RNA to the over all rate of RNA synthesis. It has been shown in Table I that the over all rate of RNA synthesis depends upon the carbon source present in the growth medium. The data in Fig. 8 show that a constant fraction of the radioactivity incorporated into bulk RNA can be hybridized to trp-specific DNA. Thus the rate of synthesis of trp-specific RNA is directly proportional to the rate of synthesis of bulk RNA. In contrast, the data illustrated in Fig. 9 show that as the degree of catabolite repression increases from glycerol through glucose to glucose-6-phosphate, less and less of the bulk RNA is lac-specific. As expected, the ratios of the slopes in Fig. 9 are the same as the ratios obtained by comparing lac- and trp-specific RNA to one another (Table I).

These conclusions were further verified by employing the technique of Stubbs and Hall (7). This approach involves using nonradioactive RNA preparations derived from cultures grown on the three carbon sources as competitors of a standard radioactive preparation for hybridization to a limited amount of lac-specific DNA. The success with which each of the nonradioactive preparations is able to compete with the standard, will be a function of the proportion of the total RNA in each preparation that is lac-specific. This technique has the advantage that labeling considerations are totally eliminated. Employing this method the data in Fig. 10 were obtained. It is clear that here, as with the other approaches, bulk RNA derived from a glycerol-grown culture contains a larger fraction of lac-specific RNA than is found in the RNA derived from glucose- or glucose-6-phosphate-grown cells. It is also significant that the ratio of the slopes obtained using RNA derived from glycerol- and glucose-grown cultures is similar to that observed in Table I and Fig. 9. The same argument, however, cannot be made in the case of RNA derived from a glucose-6-phosphate culture, because in this instance the competition is so slight that it is difficult to obtain sufficiently precise data.

It has been reported (4) that under conditions of catabolite repression, the ratio of the amounts of Z- to Y + A-specific RNA is altered from that observed under nonrepressive conditions. We have reinvestigated this question using the covering tech-

### Table I

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>RNA specific activity</th>
<th>Counts per min hybridized</th>
<th>lac:trp ratio</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>plac 5 DNA</td>
<td>lac-specific</td>
</tr>
<tr>
<td>Glycerol</td>
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<td>1678</td>
<td>1136</td>
</tr>
<tr>
<td>Glucose</td>
<td>58.3</td>
<td>1432</td>
<td>1086</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>05.8</td>
<td>688</td>
<td>2548</td>
</tr>
</tbody>
</table>

A culture of strain 242 was grown to a cell density of 100 Klett units on minimal media containing IPTG (2.5 mm) and glucose, glycerol, or glucose-6-P as the sole carbon source. At this time [3H]uridine (200 µCi and 1.8 µg per ml) was added to the culture and after a 3½-min incubation period the culture was poured over ice and KCN. RNA was prepared using standard procedures. The specific activities of the three preparations were determined by measuring the amount of trichloroacetic acid precipitable and the absorbance at 260 nm that were present in an aliquot of the preparations. Five microliters of each preparation were hybridized with a large excess of either lac (plac 5) DNA or trp (pT 190) DNA using our standard procedures.
the RNA complementary to the IPTG, to a culture of E. coli. Our results. (a) Upon addition of the inducer of the lac system, RNA increases linearly for 3 min and then becomes constant. (b) During catabolite repression, the rate of synthesis of lac-specific RNA is lowered compared to the rate of total RNA synthesis or the rate of synthesis of the catabolite insensitive trp RNA; the rates of Z-specific and A-specific RNAs are lowered to the same degree.

The first two findings are in complete agreement with those reported by Adesnik and Levinthal (3). All three findings are in complete disagreement with those reported by Contesse et al. (4). They find that the rate of lac-specific RNA synthesis increases stepwise upon induction, that transient repression arrests RNA synthesis intermittently, and that catabolite repression reduces the rate of synthesis of lac A-specific RNA more than that of lac Z-specific RNA. We cannot account for the difference between our findings and those of Contesse et al. (4). However, the method we and Adesnik and Levinthal use for the estimation of lac-specific RNA, (2, 3) appears to account more completely for this RNA than the method used by Contesse et al. (4). Thus we find in the experiment illustrated in Fig. 2, an approximately 30-fold increase in the rate of lac RNA synthesis upon induction, whereas Contesse et al. record only a 4-fold increase.

Our results suggest that upon induction RNA polymerase molecules become consecutively attached to the promoter site of the lac operon, filling the lac operon in approximately 3 min when the rate of lac RNA synthesis becomes constant. This finding is in good agreement with the observation that the accumulation of RNA complementary to the Z and Y-A regions is first observed 25 min after induction. Taking into account the length of the Z and Y-A regions (1), an estimate of 3 min for the gene. (c) During catabolite repression, the rate of synthesis of lac-specific RNA is lowered compared to the rate of total RNA synthesis or the rate of synthesis of the catabolite insensitive trp RNA, the rates of Z-specific and A-specific RNAs are lowered to the same degree.

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The experiments presented in this paper have had the following results. (a) Upon addition of the inducer of the lac system, IPTG, to a culture of E. coli the rate of synthesis of lac-specific RNA increases linearly for 3 min and then becomes constant. (b) Upon induction, the RNA complementary to the promoter proximal Z gene of the lac operon begins to accumulate before the DNA complementary to the promoter distal A gene; upon imposition of transient repression, the RNA complementary to the Z gene begins to decay before the DNA complementary to the A gene. (c) During catabolite repression, the rate of synthesis of lac-specific RNA is lowered compared to the rate of total RNA synthesis or the rate of synthesis of the catabolite insensitive trp RNA, the rates of Z-specific and A-specific RNAs are lowered to the same degree.

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time required for an RNA polymerase molecule to transverse the whole region seems reasonable. It is, however, possible that this agreement is fortuitous and that the period of 3 min of increasing rate of RNA synthesis reflects delays in the response of the individual cells in the population to induction.

Our observations are in excellent accord with the evidence presented earlier that the promoter is the unique target of transient repression and of catabolite repression (6). We find that imposition of transient repression does not immediately lead to an arrest of the accumulation of lac-specific RNA. The accumulated Z-specific RNA begins to disappear before the Y-A-specific RNA. The lac-specific RNA decays exponentially at the same rate as the capacity for β-galactosidase synthesis (8). These results are predicted by the hypothesis that the sole effect of transient repression is the arrest of the initiation of synthesis of lac-specific RNA (8).

Our findings indicate that catabolite repression reduces the rate of synthesis of lac-specific RNA relative to the rate of synthesis of unregulated trp-specific RNA to the same extent as it reduces the differential rate of synthesis of β-galactosidase synthesis (5). Furthermore, both the synthesis of Z-specific RNA and of Y-A-specific RNA is reduced to the same degree. These are again the results expected from the hypothesis that catabolite repression has as its target a single site of the operon and that it affects primarily transcription rather than translation (5, 6). It is of additional interest that in cells whose rate of bulk RNA synthesis is widely different due to the composition of the growth medium, the rate of synthesis of the unregulated trp-specific RNA is proportional to the rate of synthesis of bulk RNA. The reduction of the rate of lac-specific RNA synthesis relative to the rate of bulk RNA synthesis in cells whose lac system is fully induced is thus a proper measure of the effect of catabolite repression.

Our results suggest that the global rate of RNA synthesis is determined by the composition of the growth medium: it is twice as high in a medium containing glucose 6-phosphate as the carbon source than in the medium containing glycerol as the carbon source. This can be ascertained from the observation that a constant fraction of the RNA made in a 3-min period is trp-specific (Fig. 9) and that twice as much trp-specific RNA has been made by the cells growing on glucose 6-phosphate than by the cells growing on glycerol. The portion of the RNA synthesized allotted to each species of RNA is then determined by specific regulatory mechanisms such as induction and repression.

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