Transcriptional effects of polyamines on ribosomal proteins and on polyamine-synthesizing enzymes in *Escherichia coli*

(ornithine decarboxylase/arginine decarboxylase/antizyme/ribosomal proteins S20 and L34)

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ABSTRACT We find that the transcription of various ribosomal proteins can be differentially affected by polyamines and by changes in growth rates. Using strain MG1655 of *Escherichia coli* K-12 (F⁻, λ⁻), we have determined the effects of polyamines and changes in growth rate on the transcription of several ribosomal genes and the polyamine-synthesizing enzymes ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17) and arginine decarboxylase (L-arginine carboxy-lyase; EC 4.1.1.19). Ribosomal proteins S20 and L34 can be differentiated from the other ribosomal proteins studied; the transcription of S20 and L34 is especially sensitive to polyamines and less sensitive to changes in growth rates. In contrast, the transcription of S10, S15, S19, L2, L4, L20, L22, and L23 is insensitive to polyamines although it is particularly sensitive to changes in growth rates. Like S20 and L34, the transcription of ornithine decarboxylase and arginine decarboxylase is especially sensitive to polyamines. Polyamines specifically enhance the transcription of ribosomal proteins S20 and L34, and decrease that of ornithine decarboxylase and arginine decarboxylase. It is evident that polyamines can exert both positive and negative regulation of gene expression in *E. coli* that can be differentiated from the effects caused by changes in growth rates.

Little information is available on transcriptional regulation in *Escherichia coli* by polyamines (1). Studies performed with polyamine-requiring *E. coli* strains (2–4) are difficult to interpret because any transcriptional changes that occur under such conditions could nonspecifically reflect the increased growth rates occasioned by the presence of polyamines.

We have found (5) that certain proteins that also act as noncompetitive inhibitors of ornithine decarboxylase (ODC; L-ornithine carboxy-lyase, EC 4.1.1.17) increased in amount when *E. coli* were grown in the presence of polyamines. Among these was a protein with an approximate molecular weight of 55,000 and an isoelectric point of 3.5 (5). We subsequently noted that two basic proteins in *E. coli* extracts had antizyme-like properties (6). They inhibited ODC and increased in amount when *E. coli* were grown in the presence of polyamines. These basic proteins were identified as the S20 and L34 ribosomal proteins (7). In our experiments these were the only basic ribosomal proteins that were highly inhibitory to ODC. In view of the autoregulation model for the synthesis of ribosomal proteins (8, 9) and the well-known *in vitro* effects of polyamines in protein synthesis (1), it became necessary to determine whether the increase in the levels of these two ribosomal proteins reflected a general effect of polyamines on ribosomal gene transcription.

We now compare the effects of polyamines and changes in growth rates on the transcription of several ribosomal genes

and of ODC and arginine decarboxylase (ADC; L-arginine carboxy-lyase, EC 4.1.1.19). This study was performed in *E. coli* K-12 strain MG1655 (F⁻, λ⁻) (10) whose growth rate is not affected by the presence of 1 mM putrescine plus 1 mM spermidine. Since the inhibition of ODC by ribosomal proteins may be related to the isoelectric points of the ribosomal protein, we have investigated the following set of ribosomal proteins with a wide range of isoelectric points (11), as indicated in parentheses: S10 (7.9), S15 (>12), S19 (>12), S20 (>12), L2 (>12), L4 (7.6), L20 (>12), L22 (11.5), L23 (9.6), and L34 (>12). The basic ribosomal protein L20 was specifically included because it was identified as a particularly effective inhibitor of ODC *in vitro* (3).

We found that, whereas the transcription of the bulk of the ribosomal genes is very sensitive to changes in growth rates, it is not affected by polyamines. However, the transcription of S20, L34, ODC, and ADC is more sensitive to the presence of polyamines than to changes in growth rates. Ribosomal proteins S20 and L34 appear to be uniquely related to polyamine biosynthesis. There appears to be no correlation between the effect of polyamines and changes in growth rates on the transcription of the genes under study and the isoelectric points of the ribosomal proteins.

METHODS

Bacterial Strains, Plasmids, and Culture Conditions. *E. coli* K-12 strain MG1655 (F⁻, λ⁻) (5, 12) was used in most of this work. Strain LL308 (F' *pro⁺-lacI^q Z^{ΔM15} y⁺/Δ(pro-lac) gyrA recA supE thi*) (13) and its derivatives LL519, LL469, LL497, and LL405 carrying plasmids pLL36, pLL153, pLL161, and pLL133 with the genes of ribosomal proteins S10, L2, and L23; L22 and S19; and L4 (14), respectively, were generously provided by L. Lindahl (University of Rochester, Rochester, NY). Plasmid pODC-1 (a pBR322 plasmid carrying the ODC gene) (*speC*) (15) and pKA5 (carrying the agmatine ureohydrolases, ADC, and S-adenosylmethionine synthase genes) (*speAB* and *metK*) (15) were kindly supplied by S. M. Boyle (Virginia Tech). Plasmid pGP2 (pS20) (16) was supplied by G. A. Mackie (University of Western Ontario, Canada) and plasmid pRB1A (17, 18), containing the gene for ribosomal protein L34 (*rpmH*), was a gift of A. Wright (Tufts University, Boston, MA). Plasmid A127S (19), containing the *rpsO* gene of ribosomal protein S15, was provided by R. Takata (Saga Medical School, Saga, Japan), and M. Springer (Institute de Biologie, Paris) provided plasmid pUA6 (20) carrying the *rplT* gene of ribosomal protein L20.

Strain MG1655 was grown overnight in minimal medium 56 (21) supplemented with 0.4% glucose and thiamine (1 μg/ml). The cultures were subsequently grown for 2.5 generations to an A_{600} of 0.5 in minimal medium 56 alone or containing 1 mM putrescine and 1 mM spermidine.

Abbreviations: ODC, ornithine decarboxylase; ADC, arginine decarboxylase.

Probes. *For DNA·RNA filter hybridization.* M13mp18 containing the 1.3-kilobase (kb) *Sma* I–*Hin*dIII fragment from pODC-1 (15) and the 325-base-pair (bp) *Eco*RV–*Eco*RI fragment from pRB1A (17) were used as ODC and L34 probes, respectively. M13mp19 containing the 4.0-kb *Bal* I–*Eco*RI fragment of pKA5 (15) and the 550-bp *Hin*cII–*Hin*dIII fragment of pGp2 (16) were used as ADC and S20 probes, respectively. Control probes for ODC, S20, and L34 consisted of the nonhybridizing strand inserted in M13mp19, M13mp18, and M13mp19, respectively; and the control probe for ADC was the 2.5-kb *Bal* I–*Pst* I fragment inserted into M13mp19.

For Northern and dot-blot hybridization. The purified pGEM-4 plasmids containing the 1.3-kb *Sma* I–*Hin*dIII fragment of pODC-1 (pHPC-0), the 4.0-kb *Bal* I–*Eco*RI fragment of pKA5 (pHPC-1), the 550-bp *Hin*cII–*Hin*dIII fragment of pGp2 (pHPC-2), and the 325-bp *Eco*RV–*Eco*RI fragment of pRB1A (pHPC-3) were used as templates for ^{32}P-labeled complementary RNA transcription of ODC, ADC, S20, and L34, respectively, following the procedure indicated by Promega Biotec. Plasmids carrying the other ribosomal genes were nick-translated with [^{32}P]dCTP (22).

RNA Isolation and Analysis. Total mRNA was quantitated as follows. Cell cultures were grown to an A_{600} of 0.5 and poured over an equal volume of crushed frozen medium 56 containing 50 mM sodium azide, and the cells were harvested at 0°C by centrifugation at 8000 × *g* for 5 min. The cell pellet was suspended at 0°C in a small volume of 50 mM Tris·HCl (pH 8.0) and lysed by adding it to an equal volume of boiling SDS buffer (14). The RNA was phenol-extracted, dissolved in 4 M guanidinium thiocyanate, and freed of DNA by pelleting it through a cushion of CsCl (23). The RNA pellet was dissolved in 1× TE buffer (22). The mRNA was quantitated either by Northern blots as described by Maniatis *et al.* (22) or by dot-blots as described by Cheley and Anderson (24) and hybridized with the radioactive probes by following the procedure of Melton *et al.* (25), and the radioactivity was quantitated.

The half-lives of the total mRNA were determined by growing the cells to an A_{600} of 0.5 at which time [5,6-^{3}H]uridine at 200 μCi/ml (1 Ci = 37 GBq) was added. Rifampicin (200 μg/ml) and nonradioactive uridine (200 μg/ml) was added 2 min later and 5-ml samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, and 10 min after the addition of rifampicin and immediately lysed by the hot SDS method at 100°C (14). The RNA was purified by phenol extraction, ethanol-precipitated, and hybridized (14) to nitrocellulose filters to which an excess of the appropriate DNA probes had been immobilized. This approach permitted the determination of the half-life of the mRNA by the methods of Zengel *et al.* (14) and of Singer and Nomura (26).

The half-lives of mRNAs synthesized from each promoter of S20 and L34 were determined from 100-ml portions of the culture (A_{600} = 0.5) removed 0, 1, 2, 3, 4, 6, 8, and 10 min after the addition of rifampicin (200 μg/ml). After cell lysis in boiling SDS buffer, the RNA was isolated, and a Northern blot was prepared and incubated with the ^{32}P-labeled S20 or L34 complementary RNAs. The intensities of the hybridized RNA bands were measured by densitometry and the half-life of each RNA was calculated from linear regression analysis of the log[mRNA] vs. time.

Protein Quantitation. ODC and ADC were purified to homogeneity by slight modifications of standard methods (27, 28) and antibodies were prepared from sera of correspondingly immunized New Zealand White rabbits. The IgGs were partially purified by 50% (wt/vol) ammonium sulfate precipitation and chromatography on Whatman DE-52 columns. Antibodies against the ribosomal proteins S20 and L34 had been raised in sheep and were generously provided by G. Stoffler (University of Innsbruck, Austria).

Protein was measured in the 15,000 × *g* supernatant fraction of cell sonicates as described (12). SDS/polyacrylamide slab gel electrophoresis was performed on these fractions essentially as described by Laemmli (29) on polyacrylamide minigels (7 × 8 cm, Hoeffer Mighty Small). The proteins from the polyacrylamide gels were transferred to nitrocellulose membranes (30), incubated with the appropriate antibody, and then labeled either (*i*) with peroxidase-conjugated goat anti-rabbit IgG antibodies (The Jackson Laboratory) diluted 1:2000 in blocking solution [4% (wt/vol) bovine serum albumin/0.1% Tween 20/0.8% NaCl/0.02% KCl/0.115% Na_2HPO_4/0.02% KH_2PO_4] or (*ii*) with ^{125}I-labeled protein A at 0.1 μCi/ml (30 μCi/mg, ICN) in the same blocking solution. Peroxidase-conjugated rabbit anti-sheep IgGs (The Jackson Laboratory) were used as a second antibody in the S20 and L34 immunoblots.

Densitometry. The x-ray films of the ^{125}I-labeled protein A immunoblots and the mRNA of each individual species present in the Northern blots and the negatives of the photographic prints of the Western immunoblots were quantitated by densitometric tracing with a Zeineh soft laser scanning densitometer (model SL-TRF) connected to an Apple IIe computer and analyzed using a program created by Robert Dreyer of this department.

RESULTS

Effects of Polyamines on the Level of ODC, ADC, and Ribosomal Proteins S20 and L34. Table 1 shows that growth of strain MG1655 in the presence of 1 mM putrescine and 1 mM spermidine decreased the levels of ODC and ADC, as evidenced by the decrease in the molar ratio of these proteins and in the amount of these proteins. The enzyme protein levels are decreased by greater than 50%. Because the amount of ADC protein in *E. coli* is higher than ODC, the decrease in ADC when measured as pmol is greater than that of ODC, despite the equivalent decrease in the ratio of the two enzymes in the presence of polyamines. The changes noted in the levels of ADC were found to be equivalently distributed between the intracellular and the periplasmic forms (33) of the enzyme. In the presence of polyamines, there was a parallel increase in the levels of ribosomal proteins S20 and L34; these were increased by greater than 60%. As the amounts of ribosomal proteins are considerably higher than those of the two decarboxylases, whereas their molecular weights are smaller, these differences translate into a considerable molar increase of the ribosomal proteins relative to the molar decrease in the levels of the decarboxylases.

Effects of Polyamines on the Levels and Half-Lives of mRNAs for ODC, ADC, S20, and L34. The RNA probe synthesized by

Table 1. Effect of growth of *E. coli* in the presence of polyamines on the levels of the ODC, ADC, S20, and L34 proteins

| Protein | Protein, pmol/mg of total protein | | | Ratio |
	+ PA	− PA	(+ PA) − (− PA)	+ PA/− PA
ODC	1.8	3.8	−2.0	0.48
ADC	17	38	−21	0.45
S20	126	79	+47	1.6
L34	185	93	+92	2.0

Strain MG1655 was grown in the presence (+ PA) or in the absence (− PA) of polyamines. The amounts of the indicated proteins were determined in the 15,000 × *g* supernates of cell sonicates by Western immunoblots and are expressed as pmol/mg of total cell protein. The values given are within 10% of the averages of three experiments. Molecular weights of the various proteins are as follows: ODC, M_r 80,000 (15); ADC, M_r 71,000 and M_r 75,000 (28); S20, M_r 9553 (31); and L34, M_r 5408 (32).

Table 2.   Effect of polyamines on mRNA half-lives

mRNA	Half-life, sec	
	+ PA	− PA
S20 total	80	83
From promoter P1	73	75
From promoter P2	85	90
L34 total	82	85
From promoter P1	94	99
From promoter P2	69	71
ODC	70	66
ADC	185	170

Strain MG1655 was grown in minimal medium in the presence (+ PA) or absence (− PA) of polyamines (1 mM putrescine plus 1 mM spermidine) and the half-lives of the various mRNAs were determined.

the transcription of plasmid pHPC-1 with T7 RNA polymerase permits quantitation of the ADC mRNA alone (Tables 2 and 3) since *speA* is transcribed counterclockwise (48) and *metK* is transcribed clockwise (34). This conforms to our finding that the RNA probe synthesized by the transcription of pHPC-1 with T7 RNA polymerase hybridizes only with a 2.4-kb species corresponding to ADC mRNA, whereas the RNA probe synthesized by the transcription of the *Pst* I-digested plasmid pHPC-1 with Sp6 RNA polymerase hybridized only with a 1.2-kb RNA species corresponding to the *S*-adenosylmethionine synthase mRNA (results not presented). The half-lives of the S20 and L34 mRNAs from their promoters were determined on Northern blots of the total RNA hybridized to the radioactive probes corresponding to each promoter region. The half-lives of the ODC, ADC, S20, and L34 mRNAs were determined by hybridizing the [5,6-^{3}H]uridine-labeled RNA to nitrocellulose blots of single-stranded M13 DNA carrying the appropriate hybridizing probe. Examples of the half-lives of S20 and ADC mRNAs are presented in Fig. 1. Growth in the presence of polyamines had a minimal effect on the half-lives of these various mRNAs (Table 2). The levels of the ODC and ADC mRNA in strain MG1655 grown in the presence of polyamines are decreased, compared to control, whereas the corresponding levels of the S20 and L34 mRNAs are increased (Table 3). These results indicate that polyamines affect the levels of these mRNAs at the transcriptional level by altering the transcriptional rate of these genes.

Differentiation of the Polyamine Effects from Growth Rate Effects on Gene Transcription. The doubling time of strain MG1655 was unaltered by growth in the presence of polyamines (Fig. 2). The doubling time, in the presence and absence of polyamines, was equivalently increased by the addition of valine, which inhibits protein synthesis by feedback inhibition of the first enzyme in the isoleucine pathway (35); the doubling time returned to normal after the addition of isoleucine. RNA taken at time points a, b, and c or a', b', and c' (Fig. 2) provided common samples in which poly-

Table 3.   Relative change in mRNA levels of *E. coli* in response to polyamines and to changes in growth rates

mRNA	Experimental mRNA/control mRNA ratio		
	Polyamines	Decrease in growth rate	Increase in growth rate
S20	2.21	0.81	1.15
L34	2.0	0.89	1.08
Average	2.1	0.85	1.11
L20	1.02	0.57	1.67
L22,S19	1.02	0.49	1.65
L2,L3	0.97	0.51	2.15
L4	1.01	0.53	1.56
S15	1.02	0.52	1.69
S10	1.01	0.47	1.73
Average	1.0	0.51	1.74
ODC	0.53	0.77	1.02
ADC	0.27	0.72	1.27
Average	0.4	0.75	1.19

RNA from the six samples corresponding to points a, b, c, a', b', and c' (see Fig. 2) was diluted serially with 10× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). These serial dilutions were dot-blotted on nitrocellulose sheets. The individual nitrocellulose sheets were separately hybridized with each of the 10 ^{32}P-labeled probes, i.e., S20; L34; L20; L22,S19; L2,L3; L4; S15; S10; ODC; and ADC. The radioactivity in the individual dots corresponding to the amount of hybridized radioactive probe was determined. Each growth curve was performed in duplicate. The averages of two such experiments are presented. (*i*) Polyamines refers to the ratio of mRNA levels after growth in the presence of added polyamines vs. mRNA levels after growth in the absence of added polyamines (i.e., ratio of mRNA levels at time points a' and a in Fig. 2). (*ii*) Decrease in growth rate refers to the ratio of mRNA levels after inhibition of growth by the addition of valine vs. mRNA levels during the uninhibited stage of growth (i.e., time points b and a or b' and a' in Fig. 2). (*iii*) Increase in growth rate refers to the ratio of mRNA levels after the enhancement of growth subsequent to the addition of isoleucine vs. mRNA levels during the valine-inhibited stage of growth (i.e., time points c and b or c' and b' in Fig. 2).

amine-induced changes in the levels of the various mRNAs were compared by hybridizing the RNA to radioactive DNA probes for the various genes under study. These samples also provided a means of determining changes in the levels of the mRNAs in response to experimentally induced changes in the growth rates.

Table 3 shows that the changes in the mRNA levels in response to polyamines and to increased or decreased growth rates can be placed in three distinct categories that are tightly grouped around a mean value. One category contains the S20 and L34 mRNAs that increase when *E. coli* are grown in the presence of polyamines; these were affected relatively slightly by changes in growth rates, regardless of whether the growth rates were increased or decreased. A second category includes the mRNAs of the other ribosomal proteins tested; the pres-
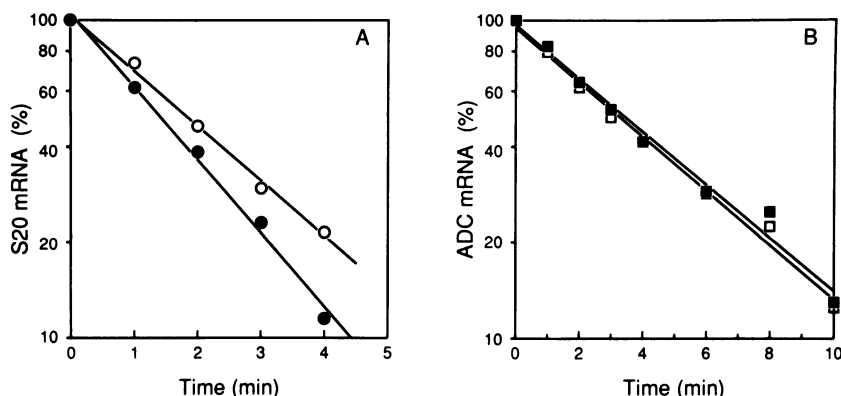

FIG. 1.   Half-lives of S20 and ADC mRNAs in strain MG1655. Cultures were treated with rifampicin (200 µg/ml) at A_{600} = 0.5, and portions were harvested as indicated. RNA was extracted and analyzed. The data points represent average values obtained from three preparations of RNA, probed twice, each time on independent blots. The data are normalized with the individual promoter-initiated S20 mRNA and ADC mRNA set as 100 at zero time. (*A*) Half-lives of S20 mRNA species. ●, mRNA initiated at promoter P1; ○, mRNA initiated at promoter P2. (*B*) Half-lives of ADC mRNA from MG1655 cells grown in the presence (■) or absence (□) of polyamines.

ence of polyamines did not affect the mRNA levels of L20; L4; L22,S19; L2,L23; S10; S15; or S19. However, the mRNAs of these ribosomal proteins were strongly affected by changes in growth rates (e.g., when the growth rates were decreased after the addition of valine or increased after the addition of isoleucine). The mRNA levels of ODC and ADC responded differently from the other two categories of mRNAs. Like S20 and L34 mRNAs, they were most sensitive to the presence of polyamines, except that they decreased rather than increased, and were affected relatively slightly by changes in growth rates.

DISCUSSION

Our results indicate that growth of *E. coli* in the presence of polyamines differentially affects the rates of gene transcription; it lowers the levels of ODC and ADC mRNAs and raises the level of S20 and L34 mRNAs without affecting the half-lives of these mRNAs. These changes are associated with lowered protein levels of ODC and ADC and increased levels of S20 and L34 proteins. Thus transcription of these genes is regulated by polyamine concentration. These same conditions do not affect the mRNA levels of eight other ribosomal proteins, including S10, S15, S19, L2, L4, L20, L22, and L23; their transcription is affected by changes in growth rates, in coordination with changes in the rates of protein synthesis.

Mitsui *et al.* (2) indicated that growth of *E. coli* in the presence of polyamines increases the protein level of the L20 and S1 ribosomal proteins; polyamines were also shown to increase the levels of an as yet unidentified P1 protein in *E. coli* (4). However, these studies were performed with polyamine-requiring *E. coli* strains, and the comparison was made between nongrowing polyamine-depleted cultures and polyamine-induced cultures in logarithmic growth. Our results indicate that, in these experiments (2–4), the increase in the levels of L20 and probably of the other proteins was a response to a polyamine-induced increase in the growth rate and was not a specific polyamine effect on gene transcription.

The synthesis of most ribosomal proteins is considered to be balanced and coordinately regulated (8, 9). In the autogenous regulation model, the free ribosomal proteins bind to sites on their mRNAs and thereby decrease the efficiency of initiation of their own translation. In the ribosome feedback regulation model, the free nonfunctional ribosomes regulate the synthesis rate of ribosomal RNA. However, it should be noted that the differential response of S20 and L34 to polyamines is only one of many characteristics that seem to differentiate L34 and especially S20 from the bulk of the other ribosomal proteins. S20 is located on the 30S ribosomal subunit at the interface with the 50S subunit (36, 37) and also purifies as the L26 ribosomal protein of the large subunit. L34 is a loosely attached protein that is easily lost from the ribosomes during isolation (32). Mackie and his collaborators (39) have also shown that, although the S20 ribosomal protein is monocistronic (38) and although it binds to 16S RNA, it does not bind to its own mRNA (39). They further showed (40) that, although the synthesis of the S20 protein is autoregulated, its mRNA does not degrade more rapidly during translational repression as do mRNAs of other ribosomal proteins. Since these properties of S20 do not conform to the requirements of the autoregulation model (8, 9), the authors suggest that perhaps the S20 protein does not interact directly with its mRNA but rather interacts through an intermediate in the initiation of its own synthesis (41).

The relationship of S20 and L34 to polyamine metabolism has been indicated by our earlier results (42) in which we have shown that *E. coli* transformed with the pODC plasmid increased the levels of the S20 and the L34 mRNAs and protein, whereas transformation with plasmids pS20 or pL34 decreased both the mRNA and the protein levels of ODC.

Other evidence also indicates an interrelationship between the polyamines and ribosomal proteins. For instance, *E. coli* mutants that have been deleted for their polyamine-synthesizing enzymes become polyamine-dependent when they are also selected for streptomycin resistance (43, 44); the latter is associated with mutations in ribosomal proteins (45, 46) especially the S12 and with the methylation of the 16S RNA (47). Although our studies have only covered a relatively small number of ribosomal proteins, our finding that polyamines can alter the transcription and the steady-state levels of two ribosomal proteins suggests a mechanism by which polyamines can more generally affect protein synthesis.
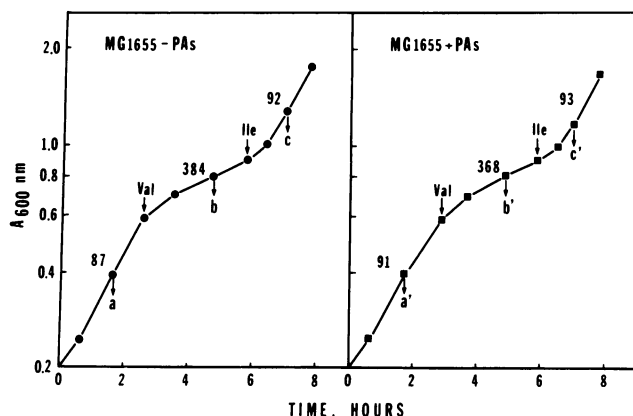
FIG. 2.   Growth curves of strain MG1655 grown at 34°C, in the absence of polyamines (− PA) or in the presence of 1 mM putrescine plus 1 mM spermidine (+ PA). Valine (Val; 500 μg/ml) or isoleucine (Ile; 500 μg/ml) was added to alter the growth rates. The numbers refer to the doubling times of the cultures at the point of sampling. Samples were removed at points a, b, c, a', b', and c', fast-cooled by pouring into an equal volume of crushed frozen medium 56 containing 50 mM sodium azide, and centrifuged at 0°C. The RNA was isolated by the hot SDS method and used for hybridization with the appropriate DNA probes immobilized on nitrocellulose paper (see Table 3).

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