Translational, Autogenous Regulation of Gene 32 Expression during Bacteriophage T4 Infection

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Functional half-life measurements of the bacteriophage T4 gene 32 messenger RNA indicate that this mRNA is extremely stable. Regulation of gene 32 expression at the transcriptional level cannot account for the rapidity with which P32 synthesis can be repressed. Furthermore, derepression of P32 synthesis occurs in the presence of rifampicin, a drug which inhibits transcriptional initiation. In addition, T4-infected cultures in which P32 synthesis is repressed possess almost as much gene 32 mRNA as derepressed cultures. We conclude that expression of the T4 gene 32 protein is regulated at the level of translation.

During infection of Escherichia coli by the bacteriophage T4 a large number of virus-encoded proteins are synthesized (1, 2). Among these proteins is a DNA-binding protein (the gene 32 protein, P32) which was first characterized by Alberts and his colleagues (3, 4). Gene 32 expression is autogenously regulated; infections by gene 32 mutants yield abnormally large quantities of the altered polypeptide (5, 6). Regulation of gene 32 expression is normally regulated by the quantities of intracellular single-stranded DNA (7-10), we asked whether regulation of gene 32 expression occurs at the transcriptional or translational level. Our results show that autoregulation of gene 32 expression is translational; to our knowledge, gene 32 expression is the first example in E. coli of translational control in which the regulation could have been transcriptional.

MATERIALS AND METHODS

Bacteriophage Strains—The sources of bacteriophage strains are indicated in the preceding paper (5). In the text and figure legends we have designated the genotype for specific mutations as X - for amber and deletion mutants or X + for temperature-sensitive mutants. The wild type genotype is designated X +.

Conditions of Phage Infection—All infections were performed with Escherichia coli strain AS19 (11) at a cell density of 3 x 10⁷/ml. The cells were grown in MS + tryptophan (12). The culture temperature was 30o unless otherwise indicated. Phages were used at a multiplicity of infection of 8 to 10. In experiments in which rifampicin was used, the final concentration was 270 μg/ml. Mixed ³²P-amino-acids were used to pulse-label the cultures at different times postinfection (2, 13);

autoradiograms of SDS-acrylamide gels of the radioactive samples were prepared as described previously (13).

Quantification of P32 Synthesis—Autoradiograms were prepared from samples containing equivalent numbers of infected cells. Microdensitometer scans of appropriately exposed x-ray films were integrated under the P32 peaks as described previously (2, 13). To the extent that amino acid pools do not change during infection, the rates of P32 synthesis given for different times postinfection reflect the absolute rates. Data for the synthesis of gene 32 amber fragments were corrected for the molecular weight difference (5). Rates of P32 synthesis shown in Figs. 3B, 4, and 8 are in arbitrary density units and cannot be compared with one another. In some cases, the data have been plotted as ratios of P32 synthesis between infections by phages of different genotypes; no corrections were made for the very slight differences in total rates of protein synthesis observed between cultures infected with different phages.

The absolute rates of macromolecule synthesis in T4-infected E. coli are temperature-dependent. We have made extensive measurements of the rates of DNA and protein synthesis (and phage development) for all temperatures used in these experiments. For the experiments shown in Figs. 3, 4, and 8, all data have been corrected to 30o rather than alter the pulse intervals to achieve constant incorporation of labeled precursors during each pulse. This manipulation removes from the data rate changes brought on solely by temperature shift and leaves rate alterations brought on by the temperature shift-dependent physiological stress of interest (i.e. the onset of DNA synthesis in Fig. 3 and the cessation of DNA synthesis in Fig. 4).

Cell-free Protein Synthesis Assays for Gene 32 mRNA—Total RNA was extracted from appropriately infected cells as described previously (14). For the experiments shown in Figs. 6 and 7, RNAs were extracted from only 10 ml of cells for each time point; tRNA (50 μg) was added as a carrier RNA for the ethanol precipitation. These RNAs were dissolved in water and lyophilized directly into the tubes to be used subsequently for cell-free protein synthesis. These incubations did not receive any additional RNA.

Cell-free protein synthesis was performed as described previously (14, 15); rifampicin was included in the incubation to preclude any DNA-directed transcription and translation. Furthermore, extra E. coli RNA polymerase was not added to the extracts. After 30 min of cell-free protein synthesis at 37o, the samples were analyzed as described previously (15) and as above.

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1 The abbreviations used are: P32, protein encoded by wild type gene 32; “P32,” protein (or protein fragment) encoded by gene 32 mutants; SDS, sodium dodecyl sulfate; am, amber mutation; ts, temperature sensitive mutation.

2 H. Morrissett, manuscript in preparation.
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FIG. 1. Functional half-life of gene 32 mRNA. Bacteria (AS19) were infected with $am^HlE18-amE2059$ deletion $r638$ (32' 44 r1IB') phage at a multiplicity of eight. Five minutes after infection, rifampicin was added to the culture, and at the times indicated 1-ml aliquots were pulse-labeled with $^{32}$P-amino-acids (1 $\mu$Ci/ml). The data are expressed as a per cent of the values obtained for the radioactive pulse at 11 min postinfection. The symbols are: O, $"P32$; ○, total $^{14}$C-amino-acid incorporation; □, PrIIA; Δ, P46; ▲, P52. We have included a reference line (- -) to indicate the slope for a 3-min half-life.

Measurements of DNA Synthesis—DNA synthesis was measured in 1-ml aliquots of infected cells during 1-min pulses with $[^3H]$thymidine at 1 $\mu$Ci/ml (2). The radioactive thymidine was from Amersham ($[^methyl]$H)thymidine, TRK 120, about 15 Ci/mmol).

RESULTS

I. Gene 32 mRNA Half-life—Since rifampicin is a known inhibitor of all transcriptional initiation which occur during T4 infection (2, 16, 17), that antibiotic may be used to determine functional messenger half-lives (18, 19). The functional half-life of the gene 32 mRNA was determined during infection by an amber mutant in gene 32 (Fig. 1). Whereas the average functional decay of T4 mRNA, as measured by total protein synthesis, occurs with a half-life of 6% min (in agreement with the published data of others (19)), most specific T4 messengers are less stable than the average. On the other hand, mRNA encoding the gene 32 amber fragment is completely stable. Due to possible effects of rifampicin addition (from 5 to 30 min postinfection) did not affect the data. When measured under repressed conditions, that is, in a 45-32+ infection (5), the apparent half-life of gene 32 messenger RNA is shorter than its half-life when measured under derepressed conditions (that is, for example, in a 45-32- infection (5)); however, the gene 32 mRNA under any conditions is more stable than any other T4 mRNA. The data from more than 10 determinations yield a half-life of greater than 30 min for gene 32 mRNA under derepressed conditions, and a half-life of more than 15 min for the same mRNA under repressed conditions. The P-fold difference in gene 32 mRNA stability cannot account for the vastly different rates of $P32$ synthesis observed (5); for the relative half-lives calculated for repressed and derepressed gene 32 mRNA it would take 1 h to generate a 4-fold difference in the relative rates of $P32$ synthesis.

The stability of the gene 32 mRNA (under any condition) suggests that efficient transcriptional regulation cannot occur; rapid transcriptional repression of a gene which produces an extremely stable mRNA cannot quickly alter the rate of expression of that gene. Therefore, experiments (below) have been performed which explicitly test the hypothesis that the regulation of gene 32 expression is translational.

II. Denaturation of Thermolabile Repressor and Derepression in Presence of Rifampicin—$P32$ is its own repressor. Using a temperature-sensitive gene 32 mutation and an appropriate
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Regime of temperature shift, it is possible to derepress "P32" synthesis by inactivating thermolabile repressor. Cultures were infected at low temperature with a phage carrying a ts lesion in gene 32. Following rifampicin addition, the infected culture was transferred to high temperature. Control cultures were infected with either 32® or 32® phages; the strains were also 45 44 rIIIB to preclude DNA synthesis (20) and interference with densitometric analysis of P32 (5, 13). The rates of gene 32 expression (Fig. 2) show that denaturation of the gene 32 protein leads to increased synthesis of that protein in the absence of transcriptional initiations.

The rate of gene 32 expression in tsP7 infected cultures is about 7 times higher at 42° than at 20°; the rate of gene 32 expression in tsP7 at 42° is equivalent to the fully derepressed rate observed for the gene 32 amber mutant. The synthesis of the gene 32 amber fragment used as a control is not sensitive to temperature shift.

"P32" overproduction is not severe in tsP7 infected cells at 20°; thus this culture has the capacity for substantial derepression which can be uncovered by temperature shift. Approximately 20 min at 42° are required for the tsP7 infected culture to fully derepress gene 32 expression; this particular ts loses the capacity to replicate DNA after a much shorter interval at 42° (21, 22).

III. Derepression of P32 Synthesis after Rifampicin Addition—Derepression of wild type gene 32 protein occurs if single-stranded DNA accumulates in cultures infected with phage of the appropriate genotype (5). The T4 double mutant 33 55® (12, 23, 24) yields extremely high levels of gene 32 expression; that high level expression is dependent on DNA replication (5). Accordingly, two triple mutants of T4 were compared: 33 55® 43®, in which the gene 43 mutation is an amber, and 33 55® 43® in which the gene 43 mutation (tsP36) renders the gene 43 protein thermolabile (gene 43 encodes the T4 DNA polymerase (25)). At high temperature the two phages are equivalent; neither synthesizes DNA, and as a result, gene 32 expression is low. However, if infections are initiated at high temperature and then shifted to low temperature, this thermolabile DNA polymerase renatures (26) and allows DNA replication (Fig. 3A). Thus, one may ask if gene 32 derepression in response to newly generated single-stranded DNA can occur in the absence of transcriptional initiations. Following rifampicin addition, the infected cultures were transferred from 39° to 30°. Gene 32 expression increases rapidly in the culture competent for DNA synthesis (Fig. 3B). The recovery of DNA synthesis in the 33 55 43® infection is not complete; nevertheless, the rate of P32 synthesis increases about 4-fold in the 10 min after the shift, and the ratio of P32 synthesis in the two cultures (33 55 43® to 33 55 43®) goes from about 1 to 10.

IV. Maintenance of P32 Derepression after Rifampicin Addition—In the preceding paper an experiment was described in which repression of gene 32 expression occurred following temperature shift of a 33 55 43® infection from 30° to 42° (see Fig. 6 in Ref. 5); DNA synthesis stopped in this infection after about 2 min at 42° (see also Fig. 8, below). The control infection (33 55®) continued to synthesize P32 at a high rate following the shift to 42°. If the rapid repression of gene 32 expression following the shift to 42° in the 33 55 43® infection was the result of immediate inhibition of gene 32 transcription, then the sustained P32 derepression in the 33 55® control must have resulted from continued transcriptional initiations. Thus, if the regulation of P32 expression were transcriptional, the
Fig. 4. Continuous derepression of P32 expression after rifampicin addition. Bacteria (AS19) were infected at 30° with amN134-amBL292 (33-55-43') or amN134-amBL292-60S66 (33-55-46') phage at a multiplicity of 10. Rifampicin was added to half of each culture at 18.5 minutes postinfection (RIF); at 20 min the cultures (±rifampicin) were transferred to 42°. Aliquots (1 ml) of the four cultures were pulse-labeled with 14C-amino acids (1 μCi/ml) for 1 min at various times. The data have been corrected for the rates of isotope incorporation at the two temperatures (see "Materials and Methods"). The symbols are: O, 33-55-, minus rifampicin; A, 33-55-, plus rifampicin; O, 33-55-43', minus rifampicin; Δ, 33-55-43', plus rifampicin.

Intracellular gene 32 mRNA levels also have been measured as a function of time and temperature in parallel infections by either 33-55- or 33-55-43' phage; the conditions used were similar to those shown in Fig. 4. Infections were initiated at 30°, 20 min after infection the cultures were shifted to 42°. At various times after infection, two separate measurements were made: first, the level of P32 synthesis in vivo, and, second, the level of P32 synthesis in vitro in response to RNA extracted from the infected cells. For both the in vivo and in vitro results, the data are plotted as the ratio obtained for 33-55-43' to 33-55- infections (Fig. 6). Whereas the 33-55-43' infected cells undergo a 10-fold reduction in the relative rate of P32 synthesis following temperature shift to 42°, only a small reduction in the intracellular level of gene 32 messenger RNA is observed. Although the data for translation in vitro scatter considerably, the same result has been obtained in three separate experiments. The level of intracellular gene 32 message, as assayed by cell-free translation, does not fall to a level corresponding to the diminution of P32 synthesis in vivo.

Similarly, the levels of gene 32 mRNA have been measured in cultures infected by either 33-55- or 33-55-46' phages. Each culture makes DNA at wild type rates (5, 28); however, at about 10 min postinfection the 33-55-46' infected cells begin to synthesize less P32 than the 33-55- infected cells; at 25 min postinfection the relative amount of P32 expression in the two infected cultures differs by approximately 5-fold (Fig. 7). The amount of gene 32 mRNA in 33-55-46' infected cells does not fall below half that of the 33-55- infected cells (Fig. 7). Once again, the intracellular gene 32 mRNA remains relatively high in cells which are repressed for gene 32 expression.

Repressed gene 32 mRNA is somewhat less stable than derepressed gene 32 mRNA (see I, above), thus the slow loss of mRNA on following repression (Figs. 6 and 7) and the concomitant accumulation of perhaps 3-fold more gene 32 mRNA in fully derepressed versus fully repressed conditions (Fig. 5, D and E) are not surprising. However, repressed cultures contain severalfold more intracellular gene 32 mRNA than predicted by transcriptional regulation models.

Repressed mRNA can be derepressed (that is, translated) in the absence of transcription. A comparison has been made between cells infected with 33-55- and 33-55-43' phage during a double temperature shift regime. Infections were started at 30°, rifampicin was added, and the cultures were shifted to 42°. In the 33-55-43' infected cells, DNA synthesis stops abruptly and P32 expression drops within 4 min to 20% that of the 33-55- control infection (Fig. 8). The cultures were then shifted back to 30°, at the time of the return to 30° the level of intracellular gene 32 mRNA in the 33-55-43' infected cells should have been (Fig. 6) about 70% that of the control. In striking support of translational regulation, after resumption of DNA synthesis in the 33-55-43' infection, gene 32 expression climbs to >90% of the P32 expression of the control infection (Fig. 8).

The autoradiogram (Fig. 5) contains more than a demonstration that gene 32 mRNA can be assayed in vitro. In addition, a comparison is offered between gene 32 expression in vivo and the relative quantities of intracellular gene 32 mRNA. Cultures were infected with either 33-55- or 46' phage (27); at 30 min postinfection the 46' infected culture yields P32 at 2.5% the rate observed in the 33-55- infected culture (Fig. 5, F and G) (see also Table I, Table IV, and Fig. 8 of the preceding paper (8)). By contrast, the RNA extracted from the 46' infected cells directs the synthesis of P32 at nearly 40% the level of RNA extracted from 33-55- infected cells (Fig. 5, D and E).

Continued overproduction of gene 32 protein in a 33-55' infection would be sensitive to rifampicin and would decay with kinetics similar to those seen in a 33-55-43' infection. Therefore, P32 expression was monitored in cells infected with either 33-55- or 33-55-43' phage. The cells were infected at 30°; after 20 min at 30° the cultures were shifted to 42°. Gene 32 protein synthesis drops rapidly in the 33-55-43' infected cells, whereas P32 synthesis remains high in the 33-55- infected cells (Fig. 4). The important additional result is that rifampicin addition prior to the temperature shift has no effect on P32 expression (Fig. 4). Therefore, the maintenance of high level P32 synthesis in a 33-55' infection does not result from continued, rapid transcriptional imitations.

V. Intracellular quantities of gene 32 mRNA—Since the gene 32 mRNA is stable (see I, above), repression of gene 32 expression should not be accompanied by an equivalent loss of translatable gene 32 mRNA. Intracellular gene 32 messenger RNA concentrations have been measured using cell-free translation of that messenger as an assay (14). The gene 32 mRNA (present in crude RNA extracted from appropriate T4-infected cells) is an efficient messenger for the gene 32 protein (Fig. 5). Gene 32 messenger isolated from cells infected with a gene 32 amber mutation directs the synthesis of the correct amber fragment; efficient suppression of that amber site is achieved in vitro with Su+t tRNA (Fig. 5) or SuVIII+ tRNA. The assays described below have been carried out under limiting mRNA input; these conditions allow quantification of the relative abundance of gene 32 mRNA in a given crude RNA preparation (14).

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FIG. 5. Translation of the gene 32 mRNA in vitro. Cell-free protein synthesis (autoradiograms A to E) was performed in 0.05-ml reaction mixtures as described previously (16). Sample A shows the pattern of protein synthesis for no added template. Reactions B and C were primed by 1.3 A$_{260}$ units of total RNA extracted 40 min postinfection from bacteria infected by amElO-amN82-deletion r638-amHL618 (45-44 rIB-32') phage; reaction C contained, in addition, 0.005 A$_{260}$ unit of purified (1142 pmol of serine aminoacylated/A$_{114}$ unit) Escherichia coli tRNA$^{\triangledown}$ of (gift from Dr. D. Hatfield). Reactions D and E were primed by the total RNA extracted 31 min postinfection from a total of 1.0 × 10$^7$ bacteria infected by amB271 (46-') and amN134-amBL292 (33-55') phage, respectively. Reactions B to E contained limiting quantities of added mRNA. Aliquots (1 ml) of the 46'- (G) and 33-55' (F) infected cultures (above), were labeled with $^3$H-amino-acids (1 μCi/ml) from 29 to 31 min postinfection. The autoradiogram is from a 10% SDS-acrylamide gel.

**DISCUSSION**

**P32 Probably Represses Translation Directly—**A rather simple notion may be utilized to explain the data presented here and in the preceding paper (5). The gene 32 protein has two quite different biochemical fates: P32 binds either to regions of single-stranded DNA, thereby promoting DNA replication, repair, or recombination, or, if no single-stranded DNA is available, P32 binds to its own messenger RNA and represses its own translation. A logical case has been made that overproduction of P32 results from the appearance of single-stranded DNA (5) and, conversely, that repression of P32 translation is brought about by the absence of single-stranded DNA; however, the notion that P32 directly represses its own synthesis is merely the least complicated and most appealing model consistent with the data. No mutants in essential T4 genes (other than gene 32 itself) alter P32 expression in a manner consistent with an activator or repressor function. Only mutants in gene 32 give increased expression of gene 32 independent of the state of the intracellular DNA (5). In addition, the gene 32 protein harbors a strong binding site for single-stranded DNA and RNA (3, 4, 7, 8). Thus, repression could utilize the nonspecific binding site and might be, thereby, “economical.” During infections by all temperature-sensitive gene 32 mutants at permissive temperatures (where DNA replication proceeds at wild type rates), the altered P32 is always somewhat overproduced. Such low temperature conditions are fully permissive for all parameters of phage growth. The amount of functional gene 32 protein in such infections must be equivalent to the wild type level (5). Therefore, the overproduction of thermolabile P32 species at permissive temperatures most likely results directly from the diminished repressor function of these temperature-sensitive proteins. Thus, we argue that P32 is its own repressor, and, as documented above, the molecular target for repression is the gene 32 mRNA.

**Observations Consistent with Translational Model—**RNAs synthesized after T4 infection have been examined on polyacrylamide gels; there is a prominent RNA, about 1200 nucleotides long, which has been tentatively identified as the gene 32 mRNA on the basis of cell-free translation experiments. Infected cells which make little P32 in vivo have
slightly reduced levels of putative gene 32 mRNA, but that RNA remains a major band; the reduction in intensity presumably is related to the shorter half-life of repressed mRNA (see above). This RNA is the most prominent large RNA at late times in a 33 S 55 infection and is completely stable after rifampicin addition. Lastly, this RNA is observed only if the labeling period is long; short pulses of [14C]uridine are never preferentially incorporated into this RNA. Further support of the translational model is provided by the observation that translation of gene 32 mRNA is efficiently and specifically repressed in vitro by added P32. These experiments will be reported subsequently.  

Molecular Aspects of P32 Repression of Gene 32 mRNA—The translational repression of gene 32 messenger RNA could be a function of specific sequence recognition by P32. Although gene 32 protein binds to single-stranded DNA and RNA without apparent sequence specificity (3, 4, 7–10), one might argue that the translational repression of gene 32 messenger RNA involves specific sequence recognition. Such a model would be merely derivative of the classic operon models for transcription (29); the repressor binding site (in the vicinity of the initiation site for translation) would be equivalent to an operator (30). In fact, repression of the translation of the R17 synthetase cistron by the R17 coat protein is similar to classic transcriptional repression (31, 32).

However, the gene 32 protein does not show sequence recognition in vitro, whether for DNA or RNA binding (3, 4, 7, 8); can specific repression emerge without sequence recognition? The answer is "perhaps" if one is willing to imagine that gene 32 mRNA contains unusual structural elements in the vicinity of the ribosome binding site. We offer below a speculative model that provides a gene 32 messenger RNA which is simultaneously (a) stable, (b) capable of extremely efficient (derepressed) translational initiations, (c) repressible by the gene 32 protein (Fig. 9). Other T4 mRNAs must be constructed in such a manner as to preclude long half-lives and P32-mediated repression.

The details of this model are as follows: the gene 32 mRNA initiation region contains an unusually long region devoid of base pairs (33); the deca nucleotide sequence UAAGGAGGUG (34) is positioned seven bases distal to the initiator AUG to ensure strong homology with the 3' end of 16 S RNA (35); the region is constructed so that either 1 ribosome or 5 P32 molecules can just cover the site; no base pairing is allowed within this region, unlike random RNA (33) and presumably unlike other T4 mRNA initiation regions. The structure is
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**Fig. 8.** Repression and derepression of P32 synthesis: repressed gene 32 mRNA can be translated after derepression. Bacteria (AS19) were infected at 30° with amN134-amBL292 (33-55°) or amN134-amBL292-tsP36 (33-55°-43°) phage at a multiplicity of 10. At 18.5 min postinfection rifampicin was added (RIF), and 1.5 min later the cultures were transferred to 42°. At 24 min postinfection the cultures were returned to 30°. Aliquots were pulse-labeled with [14C]amino-acids (1 μCi/ml) or with [3H]thymidine (1 μCi/ml) for 1 min at various times during the experiment. The data have been corrected for the rates of isotope incorporation at the two temperatures (see “Materials and Methods”). In the upper panel the rates of [3H]thymidine incorporation are plotted as the ratio [3H]thymidine33-SS/[3H]thymidine33-3t8. In the lower panel the rates of P32 synthesis in the two cultures are shown: O, cells infected by 33-55° phage; O, cells infected by 33-55°-43° phage. In the middle panel the data from the bottom panel are replotted as the ratio P3233-55-3t8/P3233-55...

quite analogous to the ribosomal binding site of the A cistron of the bacteriophage R17 (34), except that the secondary structure of gene 32 mRNA surrounding the initiator region does not interfere with ribosome access. By suitable adjustment of binding constants of P32 toward single-stranded DNA and RNA, rapid derepression by gapped DNA (through competition for P32 with the gene 32 mRNA initiation region) may be facilitated (5). Random nucleotide sequences the length of an initiation region (approximately 35 nucleotides) contain considerable secondary structure (33). Thus, in the absence of positive evolutionary pressure, other T4 messenger RNAs would not contain similarly constructed single-stranded sites in their initiation regions.

This model provides, through one unique structure and sequence homology with 16 S RNA, plausible explanations for several aspects of gene 32 regulation. Ribosomal attack of the derepressed mRNA must be extremely fast; we have calculated that the polypeptide elongation rate probably limits initiation on the derepressed gene 32 mRNA. The rapid and reversible repression of P32 synthesis (Fig. 8) suggests that the primary competitor for ribosomal attack is P32 itself; therefore, under derepressed or repressed conditions endonucleolytic attack is restricted by the ribosomes or repressor.

Furthermore, the model utilizes known molecular aspects of the gene 32 protein. The nonspecific binding site for single-stranded nucleic acids may be sufficient for repressor function; in fact, missense mutants in gene 32 are each deficient for DNA synthesis and repression. Cooperative binding of P32 to nucleic acids (3, 4), coupled with an initiation region lacking secondary structure, might enhance the selectivity of P32 binding to its own mRNA; the cooperativity also would provide more complete protection against endonuclease(s).

A last feature of the model relates to potential regulatory mutations. If the initiation region lacks internal base pairing, a single base change could not sufficiently increase the secondary structure to prevent P32 attack. Thus this model, unlike classic operon models, does not have the potential for mutations to constitutive gene 32 expression.

An entirely distinct model, in which the gene 32 protein attacks the ribosome and thereby alters translation, has not...
been considered seriously; however, we must note that earlier workers probably identified P32 as a translational "initiation factor" (36).

Translational Regulation of P32 Expression Solves Difficult Problem—The gene 32 protein must cover single-stranded DNA quickly in vivo or that DNA will be attacked by nucleases (5, 37); on the other hand, for a variety of reasons, gross overproduction of P32 relative to the number of single-stranded DNA binding sites, might be lethal (5). Thus, the P32 regulatory system provides rapid derepression and repression, since the risks of imprecise P32 regulation are large. Translational regulation is more rapid than transcriptional regulation (except in the case of a gene whose mRNA is infinitely unstable). Furthermore, the first events in derepression by gene 32 mRNA, these regions might include single-stranded non-base-paired regions) could hold a limited reservoir of P32, ready to instantaneously cover gapped DNA. The P32 reservoir could be replenished by subsequent translation. One must assume that P32 molecules which associate and dissociate rapidly from noninitiation regions of all cellular mRNA have no effect on the translation of those mRNAs; such a nonspecific reservoir is reminiscent of the postulated role of nonspecific DNA in the regulation of the lac operon (38).

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