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Relationship Between Changes in the Translational Apparatus and Actinomycin Production in *Streptomyces antibioticus*

GEORGE H. JONES

Division of Biological Sciences, Department of Cellular and Molecular Biology, The University of Michigan, Ann Arbor, Michigan 48109

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As previously reported (G. H. Jones, 1975), transfer ribonucleic acids (tRNA's) and ribosomes from actinomycin-producing cultures of *Streptomyces antibioticus* show a decreased ability to function in aminoacylation and translation as compared with the corresponding components from younger cells. Further, specific changes in the isoacceptor patterns are revealed when tRNA's from actinomycin-producing cells are compared with those of younger cells by reverse-phase column chromatography. A specific glycyl-tRNA species is eliminated from the reverse-phase profile of tRNA's from actinomycin-producing *S. antibioticus* cells as compared with younger cells. Changes in isoacceptor patterns were also observed for the amino acids methionine, valine, phenylalanine, and leucine. Actinomycin synthesis was inhibited by growing *S. antibioticus* cells in the presence of α-methyl-DL-tryptophan. Inhibition of actinomycin synthesis reversed the changes in tRNA observed in normally grown control cultures, although it had no demonstrable effect on the growth of the cells. Thus, tRNA from 48-h-old, α-methyl-tryptophan-grown cells had amino acid acceptor activity that was equal to or greater than that of tRNA from 12-h-old, normally grown cells. Similarly, the reverse-phase chromatographic pattern for glycyl-tRNA's from 48-h-old, α-methyl-tryptophan-grown cells was identical to that of the glycyl-tRNA's from 12-h-old, normally grown cells. In contrast, the ability of ribosomes from 48-h-old, α-methyl-tryptophan-grown cells to function in polypeptide synthesis in vitro was essentially identical to that of 48-h-old, normally grown cells. Ribosomes from 12-h-old, normally grown cells were severalfold more active in in vitro polypeptide synthesis.

Studies from several laboratories have revealed a number of changes in the translational apparatus that occur as cultures of *Streptomyces antibioticus* prepare to synthesize actinomycin. Thus, prior to actinomycin synthesis, *S. antibioticus* cells show decreased incorporation of precursor amino acids into cellular protein (5, 7), decrease of the amino acid acceptor activity of certain transfer ribonucleic acids (tRNA's), and diminished capacity of translational components from actinomycin-producing cells to function in polypeptide synthesis in vitro as compared with components from younger, nonproducing cells (3). In the latter case, it was shown that specific changes occur in the ribosomes from actinomycin-producing cells which decrease their ability to function in protein synthesis in vitro (3). However, it was not known whether these changes in translational capacity were related to actinomycin production or simply to the age of the cells. The present report presents evidence that the change in the activity of the ribosomes is age dependent and cannot be reversed by inhibiting actinomycin formation. Furthermore, the occurrence of changes in the pattern of isoaccepting tRNA's for certain amino acids is described, and these changes can be prevented by inhibiting actinomycin synthesis.

MATERIALS AND METHODS

S. antibioticus cultures were grown as previously described (2, 3, 6). Some cultures were grown for 48 h in the presence of 50 μg of α-methyl-DL-tryptophan (MT) per ml. tRNA's, aminoacyl-tRNA synthetases, and components for in vitro polypeptide synthesis were isolated as previously described (3). Conditions for aminoacylation and polyuridylic acid-directed polyphenylalanine synthesis were also described in the previous report (3). [^{3}H]methionine was used as a precursor of protein and actinomycin in incorporation studies performed as described (4). The assay for actinomycin synthesis was performed essentially

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as described by Katz and Weissbach (7). This method involves ethyl acetate extraction of labeled actinomycin from the cell growth medium after exposure of *S. antibioticus* cultures to a radioactive precursor amino acid. Portions of the ethyl acetate extracts were examined by liquid scintillation counting.

Reverse-phase chromatography of tRNA's was performed as previously described (1, 9). Gradient conditions for the separation of specific isoacceptors are given in appropriate figure legends.

[^{3}H]methionine (6 Ci/mmol), [^{3}H]phenylalanine (16.6 Ci/mmol), [^{3}H]leucine (46 Ci/mmol), and [^{35}S]methionine (200 Ci/mmol) were from Amersham/Searle; [^{3}H]glycine (33 Ci/mmol) and [^{14}C]glycine (104 mCi/mmol) were from Schwarz/Mann; and [^{14}C]leucine (320 mCi/mmol) was from New England Nuclear Corp. MT was from Sigma Chemical Co.

RESULTS

Patterns of isoaccepting tRNA's from young and old cells. Isoaccepting tRNA's for several amino acids were examined by reverse-phase chromatography. In these experiments, tRNA's from 12-h cells were acylated with a ^{14}C- or ^{3}H-labeled amino acid and tRNA's from 48-h cells were acylated with a ^{3}H- or ^{14}C-labeled (or ^{35}S-labeled) amino acid. The aminoacyl-tRNA preparations were combined and chromatographed simultaneously essentially as described previously (1, 9). Significant differences in the isoaccepting patterns of tRNA's from 12- and 48-h-old *S. antibioticus* cells were found for the amino acids leucine, methionine, glycine, phenylalanine, and valine. The chromatographic patterns for leucine and methionine isoacceptors are shown in Fig. 1. Figure 1 shows that although the major isoaccepting leucyl-tRNA species were present in similar amounts in tRNA populations from 12- and 48-h cells, a number of minor peaks were reproducibly observed in both tRNA preparations. Significant quantitative differences were observed when these minor peaks were examined. As regards the pattern of methionyl-tRNA's, Fig. 1 shows that the tRNA preparation from 48-h-old cells contains relatively more of tRNA^{met} species 2 and less of species 1, as compared with tRNA from 12-h cells, and almost no species 4.

A particularly striking difference was observed when the glycine isoacceptors from 12- and 48-h cells were compared (Fig. 2a). Three major glycyl-tRNA's were observed, but the relative amounts of the glycyl-tRNA's designated 1 and 2 in Fig. 2a was reversed in 12-h cells as compared with 48-h cells. Twelve-hour *S. antibioticus* cells contain relatively more of peak 1, whereas 48-h cells have a diminished amount of peak 1 relative to peak 2 (Fig. 2a). This pattern was reproducibly observed with three tRNA preparations, and the relative proportions of the tRNA peaks was not affected by reversing the labeled amino acids used to acylate the tRNA's from 12- and 48-h cells.

Effects of growth on MT on the translational apparatus of *S. antibioticus*. MT has

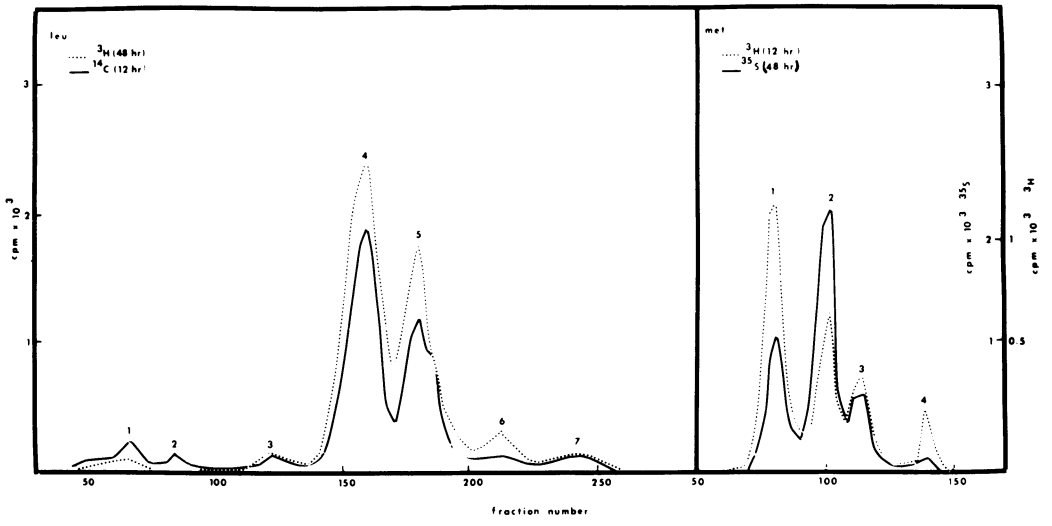

FIG. 1. *Reverse-phase chromatography of leucyl- and methionyl-tRNA's from 12- and 48-h S. antibioticus cells. tRNA's from 12- and 48-h cells were acylated as described previously (3) and chromatographed on an RPC-5 column (1 by 100 cm) (9) maintained at 27°C (1). tRNA's were eluted with a linear gradient of 0.5 to 0.8 M NaCl in 1 liter for leucine and 0.45 to 0.75 M NaCl in 1 liter for methionine. The contents of every third fraction were collected by precipitation with trichloroacetic acid. In each experiment about 100,000 cpm of ^{3}H-labeled aminoacyl-tRNA and 50,000 cpm of ^{14}C- or ^{35}S-labeled aminoacyl-tRNA were applied to the column.*

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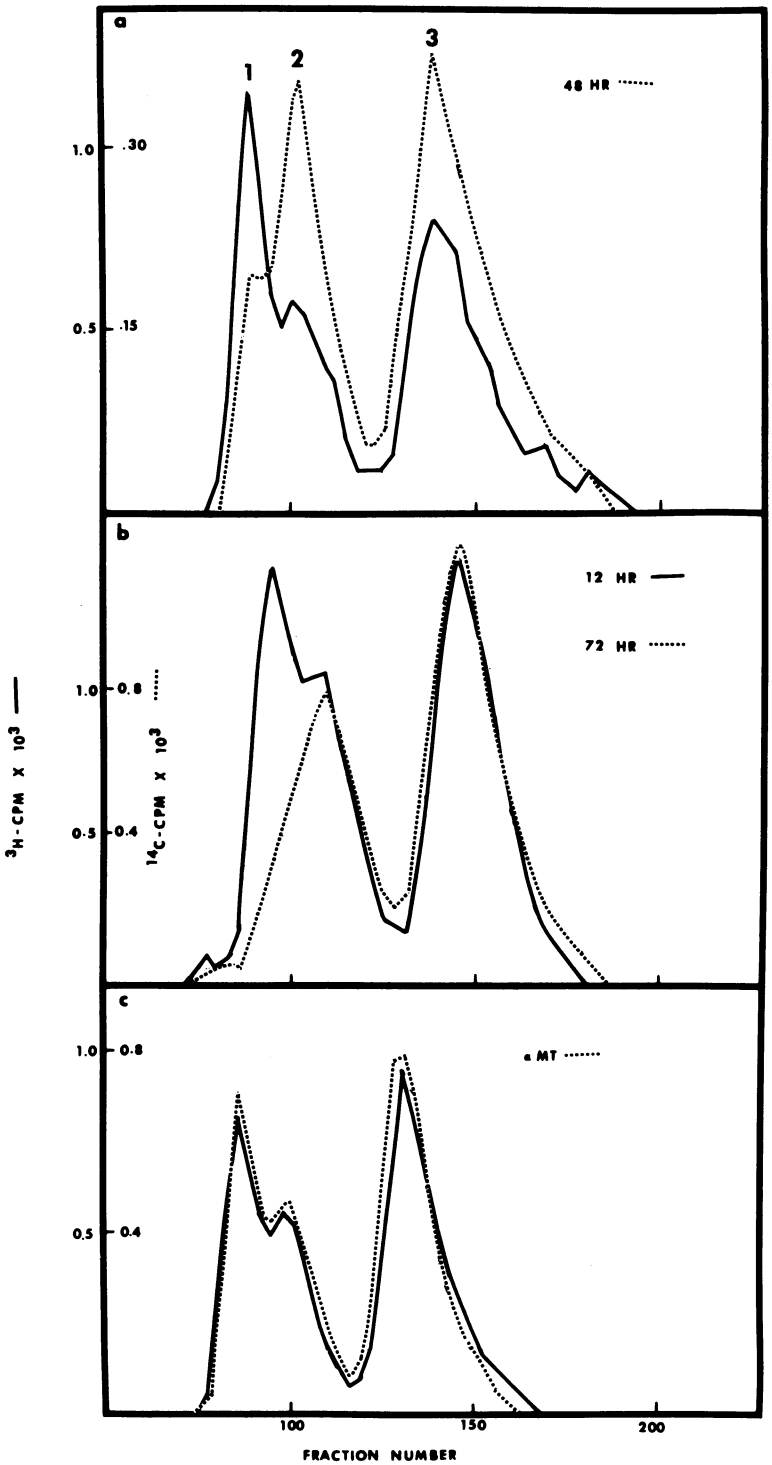

FIG. 2. *Reverse-phase chromatography of glycyl-tRNA's from 12-, 48-, 72-, and 48-h MT-grown S. antibioticus cells. (a) Comparison of tRNA's from 12-h (solid line) and 48-h (dashed line) cells; (b) comparison of tRNA's from 12-h (solid line) and 72-h (dashed line) cells; (c) comparison of tRNA's from 12-h (solid line) and 48-h MT-grown (dashed line) cells. The tRNA's were eluted with a linear gradient of 0.45 to 0.75 M NaCl. Approximately 50,000 cpm each of ^{3}H- and ^{14}C-labeled glycyl-tRNA was applied to the column.*

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been previously shown to be an effective inhibitor of actinomycin synthesis in *S. antibioticus* (10). Figure 3a shows the effects of MT on actinomycin formation (measured as described in reference 7, using [^{3}H]methionine as a precursor) and on the growth of the cells, as compared with control cultures. It can be seen that the synthesis of actinomycin was inhibited by at least 90% by 50 μg of MT per ml as compared with control cultures, but there was essentially no difference in the yield of cells from MT and control cultures. Thus, based on the latter criterion, MT does not appear to adversely affect the growth of *S. antibioticus* cultures, although it effectively diminishes their ability to synthesize actinomycin.

Figure 3b shows the effects of MT on the ability of *S. antibioticus* cells to incorporate [^{3}H]methionine into protein, as compared with control cultures. Both cultures showed a decrease in ability to incorporate labeled amino acid into protein with increasing age, but, at all time points examined up to 72 h, the specific activity of the labeled protein synthesized during the labeling period was higher in MT-grown cells than in control cells. These data suggest that inhibition of actinomycin synthesis leads to a higher level of protein synthesis as compared with cells in which actinomycin synthesis can take place.

As previously reported (3), tRNA's from 48-h-old *S. antibioticus* cells are deficient in their abilities to accept certain amino acids as compared with tRNA's from 12-h-old cells. Table 1 shows that this decreased amino acid acceptor activity is even more pronounced in 72-h-old *S. antibioticus* cells than in 48-h cells but that the decrease can be prevented by growing the *S. antibioticus* culture for 48 h in the presence of MT. Indeed, for some amino acids tested, the amino acid acceptor activity of the tRNA's from 48-h-old cells grown in the presence of MT was

TABLE 1. *Acylation of S. antibioticus tRNA's with various amino acids*[a]

Amino acid	pmol of amino acid accepted/A_{260} unit of tRNA			
	12 h[b]	48 h	72 h	48-1 h MT
Glycine	10.9	7.4	4.0	10.2
Leucine	7.3	5.3	2.9	8.3
Phenylalanine	3.2	3.0	1.2	5.4
Methionine	38.3	34.0	20.7	43.7
Valine	43.2	29.6	17.4	52.2

[a] tRNA's were acylated with tritiated amino acids as previously described (3), using an amino-actl-tRNA synthetase preparation from *E. coli*. Cells designated 48-h MT were grown for 48 h in the presence of 50 μg of MT per ml. A_{260}, Absorbance at 260 nm.

[b] Age of cells from which tRNA's were isolated.

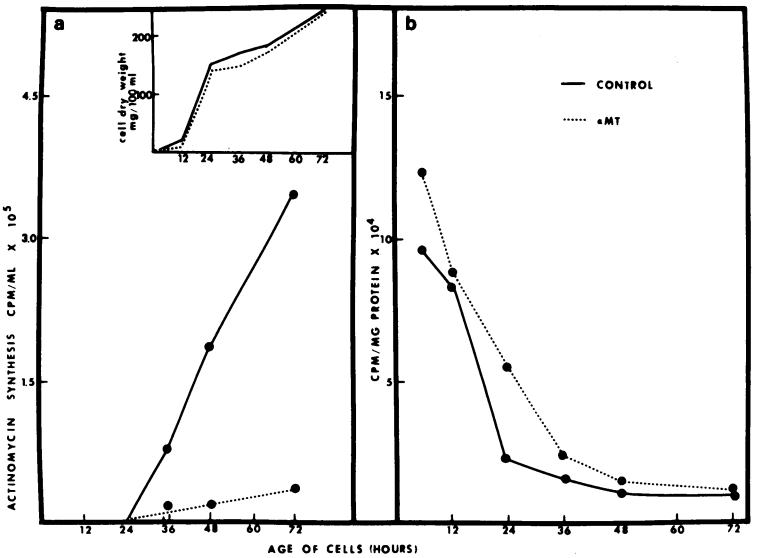

FIG. 3. *Effects of MT on actinomycin production and cell dry weight in S. antibioticus. Two hundred-milliliter cultures were grown and labeled with [^{3}H]methionine as previously described (4). An analysis of the incorporation of labeled amino acids into actinomycin was performed as described by Katz and Weissbach (7), and cell dry weight was measured as in reference 4. (b) Incorporation of [^{3}H]methionine into protein by control S. antibioticus cells and cells grown in the presence of 50 μg of MT per ml. Labeled protein was analyzed as described in reference 4.*

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higher than that of tRNA's from 12-h-old control cells (Table 1). Thus, inhibition of actinomycin synthesis also effectively reverses the changes observed in the amino acid acceptor activity of tRNA's from cells that normally produce actinomycin.

Additional information on the effects of inhibiting actinomycin synthesis on *S. antibioticus* tRNA's was obtained by examining the glycyl-tRNA patterns for tRNA's from 12-, 48-, and 72-h *S. antibioticus* cells and tRNA's from 48-h cells grown in the presence of MT. The results of such experiments are shown in Fig. 2a to c. As was mentioned above, the relative amount of the earliest eluting glycyl-tRNA species decreases in 48-h *S. antibioticus* cells as compared with 12-h cells. By 72 h of growth, this tRNA has essentially disappeared from the isoacceptor pattern (Fig. 2a and b). In these experiments, all acylations were performed with an enzyme from *Escherichia coli* so that the observed patterns could not be due to differences in the aminoacyl-tRNA synthetases in the cells studied. In contrast to the results obtained with control cultures, growing *S. antibioticus* cells in the presence of MT for 48 h leads to a complete preservation of the first glycyl-tRNA species as compared with 12-h control cultures (Fig. 2c). Identical results were obtained when glycyl-tRNA's from 12-h control cultures were compared with those from 72-h-old MT-grown cells (data not shown). Thus, inhibition of actinomycin synthesis also effectively reverses the changes observed in the glycyl-tRNA patterns in cells that normally produce actinomycin.

Finally, it was of interest to determine whether inhibiting actinomycin synthesis would reverse the decreased ability of ribosomes from actinomycin-producing cells to function in polypeptide synthesis in vitro. In these experiments, ribosomes from 12- and 48-h control cells were compared with those of 48-h-old cells grown in the presence of MT. A soluble enzyme fraction from 12-h cells was used in all incubations, and *E. coli* tRNA was used to supplement all reaction mixtures as previously described (3). Results of a typical experiment are shown in Fig. 4. As previously reported (3), ribosomes from 12-h *S. antibioticus* cells were considerably more active in supporting polyuridylic acid-dependent polyphenylalanine synthesis than ribosomes from 48-h cells. In contrast to the results presented above for RNA, inhibiting actinomycin synthesis did not increase the activity of ribosomes from 48-h cells. Ribosomes from 48-h-old, MT-grown cells were essentially equivalent to those of 48-h control cells in cell-free polyphenylalanine synthesis (Fig. 4).

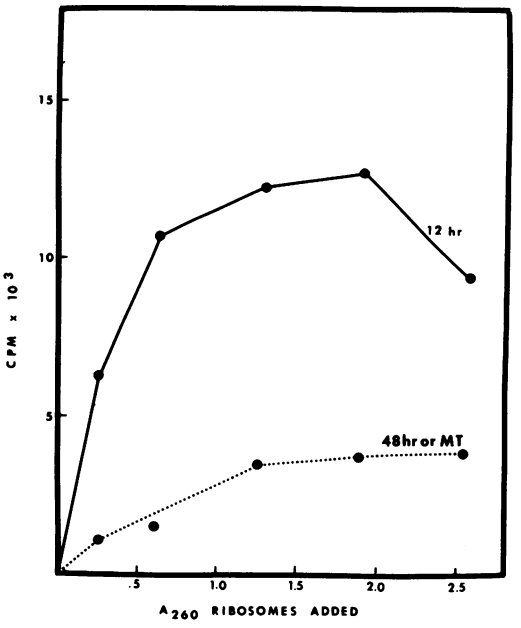

FIG. 4. *Synthesis of polyphenylalanine with ribosomes from 12-, 48-h, and 48-h MT-grown S. antibioticus cells. Incubation mixtures containing increasing amounts of ribosomes were constructed as described previously (3).*

DISCUSSION

The findings presented above indicate that the inhibition of actinomycin synthesis can reverse the changes in tRNA which normally occur in growing cultures of *S. antibioticus*, but that the changes which occur in the ribosomes are not related to actinomycin synthesis. These conclusions, of course, hinge on the demonstration that MT acts exclusively to inhibit actinomycin formation in *S. antibioticus* without directly affecting the translational apparatus. Figure 2 shows that the growth curve for *S. antibioticus* is virtually the same in the presence of MT as in its absence and that MT-grown cells synthesize protein at least as well as do their counterparts grown in the absence of the inhibitor. Parallel experiments have shown that cells grown in the presence of MT also incorporate precursors of RNA in a fashion that is similar to that observed for control cultures (data not shown). These findings suggest some specificity in the action of MT on *S. antibioticus* cells.

The possibility cannot be completely excluded, however, that MT has some direct effect

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on the translational and transcriptional apparatus in addition to its effects on actinomycin synthesis. It seems possible that MT might affect the process of tRNA synthesis, maturation, or degradation, although no direct evidence for this possibility has been obtained to date. Studies performed with *S. antibioticus* RNA polymerase and with cell-free systems for polypeptide synthesis have indicated no effect of MT on transcription and translation in vitro (unpublished data). Experiments are in progress to obtain additional information on the mode of action of MT on *S. antibioticus* cells.

The results presented above suggest the interesting possibility that specific tRNA's may somehow be involved in actinomycin synthesis in normally growing cultures of *S. antibioticus*. In this regard, it is of interest to note that, of the amino acids for which differences in the isoaccepting tRNA patterns were found, three (valine, methionine, and glycine) are precursors of the actinomycin molecule (11). Although tRNA's are not directly involved in the formation of those peptide antibiotics whose biosynthetic pathways have been studied (8), it is possible that specific tRNA's may be involved in the synthesis of the enzymes required for actinomycin formation. This possibility is presently being investigated.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Bridges, K. R., and G. H. Jones.** 1973. Transfer ribonucleic acids from mouse plasmacytoma tumors producing κ and λ immunoglobulin chains. Biochemistry 12:1208–1212.
2. **Gallo, M., and E. Katz.** 1972. Regulation of secondary metabolite biosynthesis: catabolite repression of phenoxazinone synthetase and actinomycin formation by glucose. J. Bacteriol. 109:659–667.
3. **Jones, G. H.** 1975. Macromolecular synthesis in *Streptomyces antibioticus*: in vitro systems for aminoacylation and translation from young and old cells. J. Bacteriol. 124:364–372.
4. **Jones, G. H.** 1976. RNA synthesis in *Streptomyces antibioticus*: in vitro effects of actinomycin and of transcriptional inhibitors from 48 hr cells. Biochemistry 15:3331–3341.
5. **Jones, G. H., and H. Weissbach.** 1970. RNA metabolism in *Streptomyces antibioticus*: effects of 5-fluorouracil on the appearance of phenoxazinone synthetase. Arch. Biochem. Biophys. 137:558–573.
6. **Katz, E., P. Pienta, and A. Sivak.** 1958. The role of nutrition in the synthesis of actinomycin. Appl. Microbiol. 6:236–241.
7. **Katz, E., and H. Weissbach.** 1963. Incorporation of ^{14}C-labeled amino acids into actinomycin and protein by *Streptomyces antibioticus*. J. Biol. Chem. 238:666–675.
8. **Lipmann, F.** 1971. Attempts to map a process evolution of peptide biosynthesis. Science 173:875–884.
9. **Pearson, R. L., J. F. Weiss, and A. D. Kelmers.** 1971. Improved separation of transfer ribonucleic acids on polychlorotrifluoroethylene-supported reversed-phase chromatography columns. Biochim. Biophys. Acta 228:770–774.
10. **Sivak, A., and E. Katz.** 1962. Biosynthesis of the actinomycin chromophore. Influence of α-, 4-, 5-, and 6-methyl-DL-tryptophan on actinomycin synthesis. Biochim. Biophys. Acta 62:80–90.
11. **Waksman, S. A.** 1968. Actinomycins—nature, formation and activities. Wiley (Interscience), New York.

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