STRINGENT-LIKE RESPONSE IN SOYBEAN SEEDLINGS

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(*Received for publication Dec. 12, 1979*)

Abstracts: The meristematic hook portion of soybean seedlings from 0 to 0.5 cm below cotyledons were excised and incubated in phosphate buffer (1 mM, pH 6.0) or various culture media, such as B5, MS, ER, and White, and in addition of either yeast extract or coconut milk or casein hydrolysate for 12 hours at 28°C. Then polyribosomal level (as protein synthetic activity) in the tissues and the RNA polymerase activity in the isolated nuclei were examined.

Comparison was made on the efficiency of various medium to maintain the polyribosomal level and the RNA polymerase I activity in the excised hook tissues. Our results indicated that the B5 medium plus yeast extract was the most effective for the maintenance of both polyribosomal level and RNA polymerase I activity. At the end of 12 hours incubation period, RNA polymerase I activity was increased 40% (51% of its original activity) and polyribosomal level was increased 50% (79% of its original level).

For examination of the relationship between protein synthesis and rRNA synthesis in excised root tips, ^{3}H-leucine or ^{3}H-uridine was used. There was a positive relationship between the protein synthetic activity and the total RNA synthetic activity. The total RNA synthesis decreased rapidly and reached very low level within 4 hours of incubation in phosphate buffer. The majority of this decrease was due to the cessation of rRNA synthesis based on use of the base analogue, 5-fluorouracil, for inhibition of rRNA synthesis. At end of six hours incubation RNA polymerase I activity decreased to only 16% of its original activity and protein synthesis was also decreased to 10% of its original level in the excised root tips. From these results of this experiment a stringent-like response was operative in meristematic regions of higher plant.

INTRODUCTION

In some prokaryotic organisms, such as wild type or certain strains of *Escherichia coli*, when it suffered a starvation of an amino acid (Holmes and Levinson, 1967) or glucose (Schaeffer *et al.*, 1965) and phosphate limitation (Mac-Kechnie and Hanson, 1969) lead to sporulation. But under the excess nutrients prevented the process. This response was first noted as a stringent dependence of RNA accumulation on amino acid availability (Sands and Roberts 1952; Pardee and Prestidge 1956) and later termed the "stringent response" (Stent and Brenner 1961). The main characteristic of this response is a sharp decrement of the rate of synthesis of stable RNA species. But mutant can continue to accumulate RNA during amino acid starvation. This phenomenon is called relaxed. Cashel and Gallant (1969) found that amino starvation in stringent strains caused the accumulation of two unusual guanine nucleotide, ppGpp (magic spot I, MS I) and pppGpp (magic spot II, MS II), which did not appear in relaxed strains.

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These nucleotides were also produced by *Bacillus subtilis* near the end of exponential growth. Other two highly phosphorylated nucleotides were also synthesized in *B. subtilis* after amino acid starvation in vivo and by isolated ribosomes from sporulating cells in vitro. (Rhaese, Hans-J, and R. Groscurth. 1976). Those highly phosphorylated nucleotides were shown to be adenosine-5′-diphosphate-3′-diphosphate (ppApp) and adenosine-5′-triphosphate-3′-diphosphate (pppApp). Several lines of evidences implicated that these nucleotides were inducers of the stringent response (Cashel 1975).

Although this stringent response in prokaryotes is at least partially due to RC gene product and/or its synthesis of highly phosphorylated nucleotides. There is little known about the controlling mechanism involved in the transcription of ribosomal RNA as well as that of messenger RNA for ribosomal proteins. In lower eukaryotes, several organisms such as yeast (Warner and Gorenstein, 1978), chlamydomonas (McMahon and Langstroth, 1972), slime mold (Klein, 1974) were shown to have a true stringent response. In higher eukaryotes, a stringent response was demonstrated in some mammalian cells such as untransformed mouse fibroblasts (Hershko *et al.*, 1971), Chinese hamster ovary (CHO) (Stanners and Thompson, 1974), HeLa cells (Smulson, 1970), but not in higher plants. In this paper we showed the stringent-like response existed in the soybean seedlings.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* L. cv. Taita-Kaohsiung No. 3) were sterized with NaOCl and then were germinated in rolls of moist paper. (Ingle and Key, 1965). The seedlings were grown at 28°C in dark growth chamber for 60 hours. The meristematic hook portion of hypocotyl from 0 to 0.5 cm below cotyledons were excised, incubated in various culture medium in shaking water bath at 28°C for 12 hours. In some experiments the root sections (0.5 cm from the tips) were also used in this study.

The composition of modified B, B5, MS, ER (Gamborg *et al.*, 1968; Gamborg and Wetter, 1975; Gamborg *et al.*, 1976), and White (Barley *et al.*, 1972a, 1972b; White, 1963) culture media were shown in Table I. Yeast extract or coconut milk or casein hydrolysate was put in culture medium as the additional nutrient source. The concentrations of 2, 4-D and cytokinin were 4.4×10^{-6} M and 0.9×10^{-7} M, respectively. The potassium phosphate buffer (1 mM, pH 6.0) was used as the control culture medium in each experiment. (Lin *et al.*, 1976).

Preparation of Nuclei and Assay of RNA Polymerase I Activity in Isolated Nuclei

The nuclei were isolated by a method of Chen *et al.* (1975). For the assay of RNA polymerase activities, the nuclear pellet was suspended in 1 M sucrose containing 25 mM MES-NaOH buffer (pH 6.0), 20 mM KCl, 30% glycerol, and 10 mM 2-mercaptoethanol as described in previous paper (Chen *et al.*, 1975). The radioactivities were counted in a liquid scintillation spectrometer (Beckman LS-100).

Isolation and Fractionation of Ribosomes

Ribosomes were isolated according to Lin and Key (1971) with slightly modification. Two grams of tissues were homogenized in a polytron (Brinkmann Instrument) in a buffer A containing 0.15 M tris-HCl at pH 8.5, 0.25 M sucrose, 40 mM KCl, 20 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. The homogenate was filtered through two layers of cheesecloth and then two layers of miracloth. The filtrate was centrifuged at 17,300×g for 25 minutes. The supernatant was layered over 5 ml of 65% (W/V) sucrose in buffer B containing 40 mM tris-HCl at pH 8.5, 20 mM KCl, 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol, and centrifuged at 220,000xg for 90 minutes in a type 65 rotor in a MSE model 65 ultracentrifuge. The ribosome pellets were suspended in buffer B and layered over linear sucrose density gradient from 15%

Table 1. The compositions of modified B5, MS, white and ER culture media

Macronutrients	B5		MS		White		ER	
	mg/ℓ	mM	mg/ℓ	mM	mg/ℓ	mM	mg/ℓ	mM
NH_4NO_3	—	—	1650	20.6	—	—	1200	15.0
KNO_3	2500	25.0	1900	18.8	80	0.3	1900	18.8
$CaCl_2 \cdot 2H_2O$	150	1.0	440	3.0	—	—	440	3.0
$MgSO_4 \cdot 7H_2O$	250	1.0	370	1.5	720	3.1	370	1.5
KH_2PO_4	—	—	170	1.3	—	—	340	2.5
$(NH_4)_2SO_4$	134	1.0	—	—	—	—	—	—
$NaH_2PO_4 \cdot H_2O$	150	1.1	—	—	16.5	0.12	—	—
KCl	—	—	—	—	65	0.9	—	—
$Ca(NO_3)_2 \cdot 4H_2O$	—	—	—	—	300	1.3	—	—
Na_2SO_4	—	—	—	—	200	1.4	—	—
Micronutrients	mg/ℓ	μM	mg/ℓ	μM	mg/ℓ	μM	mg/ℓ	μM
KI	0.75	4.5	0.83	5.0	0.75	4.5	0.75	4.5
H_3BO_3	3.00	50.0	6.20	100.0	1.5	25.0	3.00	50.0
$MnSO_4 \cdot 4H_2O$	13.38	60.0	22.30	100.0	7.0	31.4	13.38	60.0
$ZnSO_4 \cdot 7H_2O$	2.00	7.0	8.60	30.0	3.0	10.5	2.00	7.0
$Na_2MoO_4 \cdot 2H_2O$	0.25	1.0	0.25	1.0	—	—	0.25	1.0
$CuSO_4 \cdot 5H_2O$	0.025	0.1	0.025	0.1	—	—	0.025	0.1
$CoCl \cdot 6H_2O$	0.025	0.1	0.025	0.1	—	—	0.025	0.1
Fe•EDTA	40.00	100.0	40.00	100.0	—	—	40.00	100.0
$Fe_2(SO_4)_3$	—	—	—	—	2.5	6.0	—	—
Sucrose	2×10^4		2×10^4		2×10^4		2×10^4	
pH	6.0		6.0		6.0		6.0	

Vitamins	mg/ℓ
Inositol	100.0
Nicotinic acid	1.0
Pyridoxine•HCl	1.0
Thiamine•HCl	10.0
Growth regulator	**mg/ℓ**
Kinetin	0.1
2,4-D	1.0
Supplements	**g/ℓ**
Yeast extract	2.0
Casein hydrolyzate	2.0
Coconut milk	10% (v/v)
Bacteria growth inhibitor	**μg/ℓ**
Chloramphenicol	50.0

to 60% (W/V) for centrifugation in SW 30 rotor at 27,000 rpm for 3 hours. The sucrose gradients were fractionated by a ISCO density gradient fractionator (model 185) and scanned at 254 nm with an ISCO model UA-4 absorbance monitor.

Time-Course Study on Synthesis of RNA and Protein in the Excised Tissues

Sixty sections of root tip (0-0.5 cm) from etiolated seedlings were incubated in 5 ml of 0.01 M phosphate buffer (pH 6.0) for various duration, and then RNA or protein synthetic activiy was determined. For determination of total ribosomal RNA synthesis, the root tips were incubated with phosphate buffer or phosphate buffer plus 2.5×10^{-3} M 5-fluorouracil for various duration and then ^{3}H-uridine (3 μCi per mililiter) was added for 90 minutes. The samples were washed with cold uridine solution for 3 times and then homogenized with 2.5 ml of phosphate buffer (0.05 M, pH 7.0). The homogenates were filtered with miracloth and the filtrates were centrifuged at 15,000 × g for 20 minutes. 0.2 ml of supernatant was precipitated with 2 ml of 10% trichloroacetic acid and collected on a GF/A glass fiber disk. For determination of protein synthesis, the excised root tips were incubated in phosphate buffer (0.01 M, pH 6.0) for various duration, and ^{3}H-Leucine (2 μCi/ml) was added in the last 1 hour incubation period. They were then homogenized with 2.5 ml of 0.05 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 × g for 20 minutes. 0.2 ml of supernatant was precipitated with 2 ml of 10% trichloroacetic acid, heated at 90°C for 10 minutes and then precipitates were collected on a GF/A glass fiber disk.

RESULTS

1. The Comparison of RNA Polymerase Activities in the Excised Hook Tissues After Incubating in the Various Culture Media in Combination with Supplement Nutrient Solutions

For analysis the effect of different supplement nutrient sources on RNA polymerase activities in the excised hook tissues, the B5 or MS or ER or white medium was used in combination with either yeast extract or casein hydrolysate or coconut milk. The results were shown in the Table 2.

Table 2. The effects of different supplement nutrient sources on RNA polymerase activities in the isolated hook tissues which were cultivated in the B5 or MS or white or Er medium

Treatment	RNA polymerase activities (cpm)					
	I	II	I	II	I	II
	Yeast extract		Coconut milk		Casein hydrolysate	
B5	1,630±148	2,359±125	1,453± 64	1,915±243	1,315±312	1,545± 98
MS	1,478±501	1,925±178	1,378±165	1,421± 87	1,462±122	1,534±155
White	1,121±113	1,638±244	1,278±198	1,546±172	1,099± 78	1,388± 21
ER	1,095±130	1,421±432	1,598± 87	1,514± 96	1,299±154	1,529±161

The hook tissues were incubated in various culture media containing 50 μg/ml chloramphenicol and two grams of yeast extract or 2 grams of casein hydrolysate per liter or 10% (v/v) of coconut milk. After 12 hours of incubation, the nuclei were isolated from the hook tissues and the RNA polymerase activities were measured as indicated in the text. The initial RNA polymerase I and II activities were 3,233±487 cpm and 2,890±789 cpm respectively. The isolated nuclei (about 4×10^6 nuclei) were assayed for RNA polymerase activities with ^{3}H-UTP as described in the Materials and Methods with the addition of ammonium sulfate. RNA polymerase I activity was based on the presence of 4 μg/ml α-amanitin at 50 mM $(NH_4)_2SO_4$. RNA polymerase II activity was determined by the difference of activity in the presence and absence of 4 μg/ml α-amanitin at 200 mM $(NH_4)_2SO_4$. The reaction mixtures were incubated at 28°C for 20 minutes.

There were no major differences in maintaining either RNA polymerase I or II activity found when coconut milk was supplemented with B5 or MS or ER or white medium. However, the addition of yeast extract in four different media both B5 and MS media showed the significant efficiency of maintaining RNA polymerase activities in the excised hook tissues, whereas the white or ER medium did not help in maintaining the activities. Table 2 showed the RNA polymerase I activity was low in the B5 medium supplemented with casein hydrolysate compared with other supplements. The B5 medium is widely used in a soybean tissue culture and the addition of yeast extract in B5 medium was the most effective supplement for maintenance of RNA polymerase activities in the excised tissues.

For preventing the bacterial growth during the cultivation of the excised tissues, the chloramphenicol at the concentration of 50 μg per milliliter was added to each culture medium. It is necessary to investigate whether or not this antibiotics can effect the RNA polymerase activities of the excised tissues during the cultivation. Table 3 indicated that the RNA polymerase I activity was not significantly inhibited by chloramphenicol. But the RNA polymerase II was very slightly inhibited by chloramphenicol at different level, especially in the phosphate buffer and ER medium, the RNA polymerase II was inhibited about 20% and 25% respectively (Table 3). Because RNA polymerase I activity was not inhibited by the addition of chloramphenicol in each culture medium, so that chloramphenicol at the concentration of 50 μg/ml was added to each culture medium for preventing the growth of bacteria.

Table 3. Effect of chloramphenicol on RNA polymerase activities in the excised hook tissues

RNA polymerase activity cpm / Treatment	Minutes chloramphenicol		Plus chloramphenicol (50 μg/ml)	
	I	II	I	II
Phosphate buffer	1,114± 44	2,597±772	1,192± 96	1,865± 9
B5	1,840±249	2,571±236	1,639±148	2,359±125
MS	1,249±244	2,267± 85	1,478±501	1,925±178
White	1,037± 98	1,949±109	1,121±113	1,638±344
ER	1,101±116	1,919± 17	1,095±130	1,421±432

The original RNA polymerase activities in isolated nuclei were 3,233±487cpm for RNA polymerase I and 2,890±798 cpm for RNA polymerase II. Two grams of yeast extract was added to each culture per liter expect phosphate buffer. The activities of RNA polymerase I and II were calculated by the method as described in the legend of Table 2.

2. The Relationship Between Protein Synthetic Activity (Polysome Level) and RNA Polymerase Activities in the Hook Tissues

The relationship between protein synthetic activity and RNA polymerase activities were shown in Table 4 and Fig. 1. The percentage of polysome in the hook tissues at initial was 59.41%. But when the hook tissues was excised and incubated in the phosphate buffer (1 mM, pH 6.0) for 12 hours, the polysome level in this excised hook tissues was dropped to about 31.41% (about 50% decrease), and the RNA polymerase I activity was decreased to 37% of its original activity. From these results there was the positive relationship between polysome level and RNA polymerase I activity in the hook tissues. The B5 medium plus yeast extract was found to be the most effective medium for maintenance of RNA polymerase I activity in the excised hook tissues. At the end of 12 hours incubation period, the RNA polymerase I activity up to 51% of its original activity and 79% of its original polysomal level were maintained.

Table 4. The relationship between polysome level (protein synthetic activity) and RNA polymerase I activity in the hook tissues

Treatment	RNA polymerase I activity (cpm)	Percentage of initial RNA polymerase I	Percent of polysome	Percentage of initial polysome level
Control	3,233±487	100	59.41	100
Phosphate buffer	1,292± 92	37	31.41	53
Phosphate buffer plus sucrose	1,289± 24	40	37.50	63
B5 minus yeast extract	1,338± 54	41	—	—
B5 plus yeast extract	1,639±148	51	47.05	79

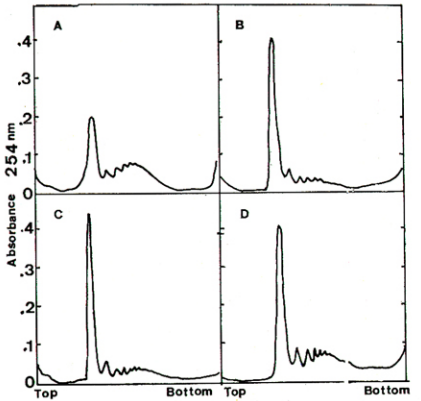

Fig. 1. The OD_{254} profiles of ribosomes on 15 to 60% sucrose density gradient. The ribosomes were isolated from the hook tissues (A), the hook tissues has been incubated in phosphate buffer (1 mM, pH 6.0) (B), in phosphate buffer plus 2% sucrose (C), in B5 medium plus yeast extract (D), for 12 hours and then layered on 15 to 60% (w/v) linear sucrose density gradient. After centrifugation at 27,000 rpm for 3 hours in a MSE model 65 SW 30 rotor, gradients were fractionated and scanned at 254 nm with an ISCO model UA-4 absorbance monitor.

3. The Protein and RNA Synthetic Activity in Excised Root Tips

The excised root tips were preincubated in 0.05 M phosphate buffer (pH 6.9) for various duration and then labelled with ^{3}H-uridine and ^{3}H-leucine to detect its protein and RNA synthetic activity. The results showed that the total RNA synthetic activity was decreased rapidly after incubating the excised roots in the phosphate buffer for one hour. At end of six hours incubation, only 15.9% of its original RNA polymerase activity and 10% of its protein

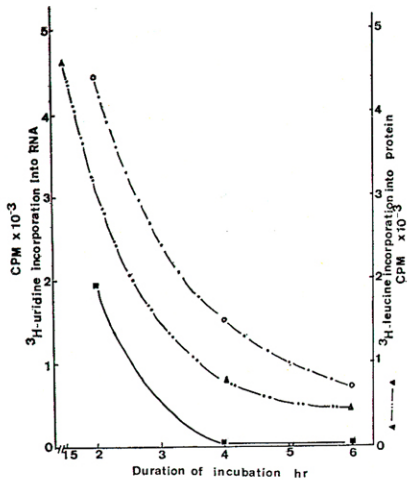

Fig. 2. The RNA and protein synthetic activities in the excised root tips after incubation in phosphate buffer for variour duration.
o—•—o Total RNA synthetic activity
▲••—••▲ Total protein synthetic activity
■——■ Ribosomal RNA synthetic activity (cpm inhibited by 5-FU)

synthetic activity were remained in the root tips. 5-FU sensitive incorporation of ^{3}H-uridine into RNA represented the ribosomal RNA synthetic activity. The ribosomal RNA synthetic activity was decreased rapidly after short time of incubation in the phosphate buffer and this ribosomal RNA synthesis dropped almost to null after about 4 hours of incubation. At the end of 4 hours incubation, other than rRNA synthesis was a dominant part of RNA synthesis in the excised root tips. Fig. 2 also showed that the total protein synthetic activity was paralleled with the RNA synthetic activity in excised root tips.

DISCUSSION

The soybean seedlings under anaerobiosis and then aerobiosis treatment, Chen (1979) demonstrated that there was the positive correlation between the level of RNA polymerase I activity expressed and the functional state of the protein synthetic apparatus as assayed by the level of polysome level in the region of soybean hypocotyl. The results presented in this paper and those previous studies demonstrate a stringent-like response in the soybean seedlings.

When the excised hook tissues were incubated in the B5 medium plus yeast extract, the RNA polymerase I activity in the hook tissues could be maintained to a high level. (Table 2

and 3). Because the B5 medium is a widely used medium for soybean tissue culture. Comparison the composition of B5 with MS or White or ER medium, it contains a high concentration of K^+ ion (25 mM). The concentration of K^+, NH_4^+, and NO_3^- ions in the B5 medium were at the optimal concentration for the growth of soybean tissue culture. Bayley *et al.* (1972a, 1972b) and Gamborg *et al.* (1968, 1976) had shown that the effects of ionic concentration of K^+, NH_4^+, and NO_3^- on the growth of soybean suspension culture were more important than other inorganic salts. The ionic concentration of K^+, NH_4^+, and NO_3^- in MS or White or ER medium were either too high or too low, so that these media were not suitable for cultivation of soybean tissues. For cultivation of younger meristematic tissues, Gamborg *et al.* (1968, 1976), Murashige (1974), and Street (1973) had reported that the younger soybean meristematic tissues were used for in vitro cultivation, the more organic supplement, i. e. glutamine was necessary in the culture medium. Although the B5 medium containing adequate various inorganic salts, vitamins, sucrose, and growth regulators, when omitting these organic nutrient supplement the RNA polymerase activities (particularly RNA polymerase I activity) in the excised hook tissues were found decrement. Yeast extracts contained higher contents of L-glutamine, L-aspartic acid, and L-glycine than those in the casein hydrolysate or coconut milk and these amino acids were absorbed and used by the soybean tissues. So that the B5 medium plus yeast extracts was the most effective culture medium for maintenance of RNA polymerase I activity in the excised soybean hook tissues.

There is a positive relationship between polysome level (protein synthetic activity) and RNA polymerase I activity in the soybean hook tissues. Table 4 and Fig. 1 showed that the B5 medium plus yeast extract can maintain 51% of its original RNA polymerase I activity. This phenomenon is similar to the "stringent response" of certain strain of *E. coli* when it suffers an amino acid starvation. The main characteristic of this response is a sharp decrement of the rate of synthesis of stable ribosomal RNA. The relaxed locus in *E. coli* is the control element whose product, the stringent factor or the enzyme which catalyzes the formation of ppGpp (Block and Haseltine, 1974) may serve to couple the synthesis of stable RNA species to protein synthesis. From the results of ^{3}H-uridine incorporation into RNA with or without 5-fluorouracil treatment in the excised root tips (shown in the Fig. 2) indicated that ribosomal RNA synthesis was decreased rapidly in the initial short time incubation of the excised root tips in the phosphate buffer. Other than rRNA was only decreased gradually. At the end of six hours of incubation, the total incorporation of ^{3}H-uridine into RNA was almost in the fraction of RNA other than rRNA. This observation is very similar to the stringent response of *E. coli*. The prokaryotic organisms under normal growth condition, the transcription and translation are under positive control. In other words, when the expression of RNA polymerase activity was in active condition (i. e. rRNA synthesis), the polysome level (i. e. protein synthetic activity) was also high. Under the starvation of an amino acid, the aminoacylation of transfer RNA was limited. In this situation a lot of uncharged transfer RNA were binded into entry site (or A site) of ribosomes under non-enzymatic codon specific binding, and this condition could retard the protein synthesis. Under this condition could stimulate the stringent factor or ribosomes to produce ppGpp (Block and Haseltine, 1974; Cashel, 1975). This unusual nucleotide could inhibit the synthesis of ribosomal and transfer RNAs. When the addition of an amino acid into the culture medium, the accumulation of ppGpp in bacteria disappeared rapidly and its RNA synthetic activity was recovered (Cashel, 1975). In many eukaryotic organisms an amino acid starvation could also induce the stringent response (Smulson, 1970; Hershko, *et al.*, 1971; Stanners and Thompson, 1974; Klein, 1974; Gross and Pogo, 1974, 1976; Chambon, 1975; Hallberg and Bruns, 1976; Shulman *et al.*, 1977; Warner and Gorenstein, 1978). In general, the eukaryotic cells contain three different kinds of RNA polymerases, namely RNA polymerase I, II, and III, localized in the nucleus. In doubtlessness, the control

mechanism of transcriptional step in the eukaryotic cells were more complicated than that of the prokaryotic systems. In 1977, McMillian *et al.*, found a low molecular weight RNA 4–5 S) which was isolated from a pathogenic fungus, *Histoplasm capsulatum*, and was shown a guanine-rich RNA could inhibit the RNA polymerase I activity. The inhibition mechanism was similar to ppGpp which inhibit the rRNA synthesis in *E. coli*. The stringent response in yeast has been extensively investigated. But there was never found ppGpp accumulation in the starved yeast cells. The RNA polymerase activity in the isolated nuclei from soybean hypocotyl was not inhibited by exogenously added ppGpp. (Chen, 1979). However from the data shown in this paper and previous study (Chen, 1979) revealed that in soybean meristematic region actually existed a stringent-like response when the amino acids sources were under starvation condition. At this situation, it caused the differential expression of RNA polymerase activities in the nucleus. Under this starvation condition, whether the stringent substances were produced by ribosomes or modification of protein synthetic apparatus occured, the further studies were needed. Nevertheless there is a existence a stringent-like response in a higher plant.

REFERENCES

1. ALFÖLDI, L., G. S. STENT, and R. C. CLOWES. 1962. The chromosomal site for the RNA control (RC) locus ih *Escherichia coli*. J. Mol. Biol. **5**: 348.
2. BAYLEY, J. M., J. KING, and O. L. GAMBORG. 1972a. The effect of the source of inorganic nitrogen on growth and enzymes of nitrogen assimilation in soybean and wheat cells in suspension cultures. Planta (Berl.) **105**: 15–24.
3. BAYLEY, J. M., J. KING, and O. L. GAMBORG. 1972b. The ability of amino compounds and conditioned medium to alleviate the reduced nitrogen requirement of soybean cells grown in suspension cultures. Planta (Berl.) **105**: 25–32.
4. BLOCK, R., and W. A. HASELTINE. 1974. In vitro synthesis of ppGpp and pppGpp. In: M. Nomura, A. Tissieres, and P. Lergyle ed., Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. pp. 747-761.
5. CASHEL, M., and J. GALLANT. 1969. Two compounds implicated in the function of the RC gene of *Escherichia coli*. Nature **221**: 838-841.
6. CASHEL, M. 1975. Regulation of bacterial ppGpp and pppGpp. Ann. Rev. Microbiol. **29**: 301-318.
7. CHAMBON, P. 1975. Eukaryotic nuclear RNA polymerases. Ann. Rev. Biochem. **44**: 613-638.
8. CHEN, Y. M., C. Y. LIN, H. HUANG, T. J. GUILFOYLE, and J. L. KEY. 1975. Isolation and properties of nuclei from control and auxin-treated soybean hypocotyl. Plant Physiol. **56**: 78–82.
9. CHEN, Y. M. 1979. Studies on the control mechanism of RNA polymerase I activity in higher plants. Taiwania **24**: 27–34.
10. GAMBORG, O. L., R. A. MILLER, and K. OJIMA. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50**: 151–158.
11. GAMBORG, O. L., and I. R. WETTER, 1975. Plant tissue culture methods, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon Saskatchewan.
12. GAMBORG, O. L., T. MURASHIGE, T. A. THORDE, and I. K. VASIL. 1976. Plant tissue culture media. In Vitro. **12**: 473-478.
13. GROSS, K. J., and A. O. POGO. 1974. Control mechanism of ribonucleic acid synthesis in eukaryotes. The effect of amino acid and glucose starvation and cycloheximide on yeast deoxyribonucleic acid-dependent ribonucleic acid polymerases. J. Biol. Chem. **249**: 568-576.
14. GROSS, K. L., and A. O. POGO. 1976. Control of ribonucleic acid synthesis in eukaryotes. 2. The effect of protein synthesis on the activities of nuclear and total DNA-dependent RNA polymerases in yeast. Biochemistry **15**: 2070–2081.
15. HALLBERG, R. L., and P. J. BRUNS. 1976. Ribosome biosynthesis in *Tetrahymena pyriformis*: Regulation in response to nutritional changes. J. Cell Biol. **71**: 333-894.
16. HERSHKO, A., P. MAMONT, R. SHIELDS, and G. M. TOMKINS. 1971. Pleiotypic response. Nature (London) New Biol. **232**: 206-211.

17. HOLMES, P. K., and H. S. LEVINSON. 1967. Metabolic requirements for microcycle sporogenesis of *Bacillus megaterium*. J. Bacter. **94**: 434-440.
18. INGLE, J., and J. L. KEY. 1965. A comparative evaluation of the synthesis of DNA-like RNA in excised and intact plant tissues. Plant Physiol. **40**: 1212-1219.
19. KLEIN C. 1974. Presence of magic spot in *Dictyostelium discoideum*. FEBS Lett. **38**: 149-152.
20. LIN, C. Y., and J. L. KEY. 1971. Dissociation of N_2 gas-induced monomeric ribosomes and functioning of the derived subunits in protein synthesis in pea. Plant Physiol. **48**: 547-552.
21. LIN, C. Y., Y. M. CHEN, T. J. GUILFOYLE, and J. L. KEY. 1976. Selective modulation of RNA polymerase I activity during growth transitions in soybean seedlings. Plant Physiol. **58**: 614-617.
22. MACKECHNIE, J., and R. S. HANSON. 1968. Microcycle sporogenesis of *Bacillus cereus* in a chemically defined medium. J. Bact. **95**: 355-359.
23. MCMAHON, D., and P. LANGSTROTH. 1972. The effects of canavanine and arginine starvation on macromolecular synthesis of *Chlamydononas reinhard*. J. Gen. Microbiol. **73**: 239-259.
24. MCMILLIAN, R., B. V. KUMAR, G. MEDOFF, D. SCHLESSINGER, and G. S. KOBAYASHI. 1977. Inhibition of *Histoplasma capsulatum* ribonucleic acid polymerases by homologous and heterologous ribonucleic acid. J. Bacteriol. **130**: 1387-1389.
25. MURASHIGE, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol. **25**: 135-166.
26. PARDEE, A., and L. PRESTIDGE. 1956. The dependence of nucleic acid synthesis on the presence of amino acids in *Escherichia coli*. J. Bact. **71**: 677-683.
27. RHAESE, HANS. J. and R. GROSCURTH. 1976. Control of development. Role of regulatory nucleotides synthesized by membranes of *Bacillus subtilis* in initiation of sporulation. Proc. Natl. Acad. Sci. U. S. A. **73**(2): 331-335.
28. ROEDER, R. G., and W. J. RUTTER. 1969. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature **224**: 234-237.
29. SANDS, M. K., and R. B. ROBERTS. 1952. The effects of a tryptophan histidine deffeciency in a mutant of *Escherichia coli*. J. Bact. **63**: 505-511.
30. SCHAEFFER, P., J. MILLET, and J. P. AUBERT. 1965. Catabolic repression of Bacterial sporulation Proc. Natl. Acad. Sci. U. S. A. **54**: 704-711.
31. SHULMAN, R. W., C. E. SRIPATI, and J. R. WARNER. 1977. Noncordinated transcription in the absence of protein synthesis in yeast. J. Biol. Chem. **252**: 1344-1349.
32. SILVERMAN, R. H. and A. G. ATHERLY. 1979. The search for guanosine tetraphosphate (ppGpp) and other unsual nucleotides in eukaryotes. Microbiol. Rev. **43**(1): 27-41.
33. SMULSON, M. 1970. Amino acid deprivation of human cells: effects on RNA synthesis, RNA polymerase, and ribonucleoside phosphorylation. Biochim. Biophys. Acta **199**: 537-540.
34. STANNERS, C. P., and L. H. THOMPSON. 1974. Studies on a mammalian cell mutant with a temperature-sensitive leucyl-tRNA synthetase. In: B. Clarkson, and R. Baserga ed., Control of Proliferation in Mammalian Cells. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. pp. 191-203.
35. STENT, G. S., and S. BRENNER. 1961. A genetic locus for the regulation of ribonucleic acid synthesis. Proc. Natl. Acad. Sci. U. S. A. **47**: 2005-2014.
36. STREET, H. E. 1973. Plant Tissue and Cell Culture. Botanical monographs Vol. II. Blackwell Scientific Publication, Lodon.
37. WARNER, J. R., and C. GORENSTEIN. 1978. Yeast has a true stringent response. Nature **275**: 338-339.
38. WHITE, P. R. 1963. The cultivation of animal and plant cells. 2nd. ed. Ronald Press, N. Y.