JOURNAL OF BACTERIOLOGY, Sept. 1988, p. 4136–4140
0021-9193/88/094136-05$02.00/0

Vol. 170, No. 9

RNA Polymerase Subunit Homology among Cyanobacteria, Other Eubacteria, and Archaebacteria

GEORGE J. SCHNEIDER† AND ROBERT HASELKORN*

Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, Illinois 60637

Received 18 April 1988/Accepted 6 June 1988

RNA polymerase purified from vegetative cells of the cyanobacterium *Anabaena* sp. strain PCC 7120 contains a dissociable σ factor and a core of five subunits: the β′, β, and two α subunits characteristic of all eubacteria and an additional 66,000-molecular-weight polypeptide called γ. Fifteen of fifteen strains of unicellular and filamentous cyanobacteria tested contained a serologically related γ protein. Antiserum to γ reacted with *Escherichia coli* β′ and the A subunit of RNA polymerase of the archaebacterium *Sulfolobus acidocaldarius*. Thus the evolution of the RNA polymerase β′ subunit has followed different paths in three groups of procaryotes: cyanobacteria, other eubacteria, and archaebacteria.

The basic structure of RNA polymerase is generally believed to be the same in all bacteria (2). The prototypical enzyme of *Escherichia coli* contains a core of four subunits ($\beta'\beta\alpha_2$) and a dissociable subunit, σ, which is required for initiation of transcription at specific sequences termed promoters (3). The RNA polymerase of cyanobacteria was thought to have the same structure (6). Recently, however, we purified the enzyme from vegetative cells of *Anabaena* species and found that its core contains an additional subunit, γ, of molecular weight 66,000, present in stoichiometric amounts (17). The β′, β, and α subunits of *Anabaena* RNA polymerase were named on the basis of size and stoichiometry by analogy with the *E. coli* enzyme. In addition, *Anabaena* RNA polymerase was analyzed by the method of Grachev et al. (5) with an *o*-formylphenyl ester of ATP to identify the nucleotide-binding domain. The major subunit labeled was β (A. Schäffner and G. J. Schneider, unpublished observations).

MATERIALS AND METHODS

Immunologic studies of *Anabaena* sp. strain PCC 7120 RNA polymerase. To determine the relationships among the γ subunit and the other subunits of the *Anabaena* enzyme, as well as the subunits of RNA polymerases of other procaryotes, four antisera to the *Anabaena* core $\beta'\beta\gamma\alpha_2$, β′+β, γ, and σ were raised in rabbits. Core enzyme was prepared as described previously (17). Holoenzyme subunits were separated on a 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel (20), and β′+β, γ, and σ subunits were separately electroeluted from gel slices. Immunological procedures were carried out as described by Kranz and Gennis (9). Blood drawn from rabbit ear arteries was allowed to clot for 30 to 60 min at 4°C and was then spun for 20 min at 16,000 × *g*. Antibodies were partially purified by precipitating lipoproteins from the serum with 0.25% sodium dextran sulfate and 80 mM $CaCl_2$. Immunoglobulins were precipitated with 50% saturating $(NH_4)_2SO_4$ and suspended in phosphate-buffered saline (PBS; 10 mM Na_2HPO_4–140 mM NaCl[pH 7.0]) at about 50 mg of protein per ml.

Western blots (immunoblots) were prepared by electroblotting proteins separated on SDS-polyacrylamide gels to nitrocellulose overnight in 20 mM Tris–150 mM glycine–20% methanol buffer at 70 V. The blots were rinsed in PBS for 5 min and then used immediately or air dried for storage. After being washed in 1% bovine serum albumin in PBS for 1 to 2 h, serum or immunoglobulins were added to fresh bovine serum albumin solution and incubated with the blot for 3 h. Blots were washed once with PBS, twice with 0.1% Triton X-100 in PBS, and finally with 1% bovine serum albumin in PBS (5 min each wash). Bound antibodies were labeled with 1% bovine serum albumin containing ^{125}I-protein A for 1 h and then washed as before except that the last wash was with PBS only. All washes were at room temperature with gentle shaking. Blots were air dried, and bound antibody-protein A was visualized by autoradiography. Blots were stained with amido black by being washed in a 0.1% solution of the dye in 20% methanol–7.5% acetic acid for 3 to 5 min. Destaining was accomplished by several short washes in the same methanol-acetic acid solution.

RNA polymerase subunit structures of other cyanobacteria. *Anabaena* sp. strain PCC 7120 was originally obtained from the Botany Department of Iowa State University. *Anabaena* sp. strain PCC 7118 was obtained from the Pasteur Culture Collection. *Anabaena azollae* 1A was obtained from Jack Newton of the U.S. Department of Agriculture Laboratory in Peoria, Ill. *Nostoc* sp. strain MAC PCC 7911 was obtained from Jack Meeks of the University of California, Davis. *Anabaena torulosa*, *Anabaena* sp. strain L31, and *Plectonema boryanum* were received from Shree Apte of the Molecular Biology and Agriculture Division of the Bhabha Atomic Research Center, Bombay, India. *Anabaena variabilis* ATCC 29413 was provided by Peter Wolk of Michigan State University. *Anabaena cylindrica* SAUG 1403-2, *Nostoc muscorum* PCC 7119, *Phormidium foveolum* SAUG 1442-1, *Calothrix membranacea* SAUG 1410-1, and *Oscillatoria tenuis* SAUG B-1459-4 were supplied by Herbert Böhme. *Synechococcus* sp. strain PCC 7942 (formerly *Anacystis nidulans* R2) was supplied by Susan Golden. *Synechococcus* sp. strain PCC 7002 (formerly *Agmenellum quadruplicatum*) was obtained from Don Bryant of Pennsylvania State University. *Anabaena azollae* symbiont was prepared from the fern *Azolla* sp. as described previously (13).

The strains described above were maintained in 100-ml cultures of Chu medium (15). Large-scale cultures were grown in Kratz and Myers medium (10) supplemented by 2.5

* Corresponding author.
† Present address: Department of Biology, University of California at San Diego, La Jolla, CA 92093.

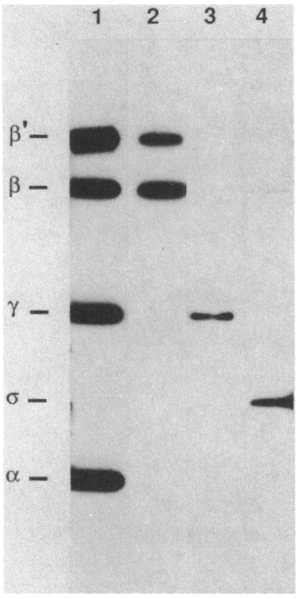

FIG. 1. Western blot with antisera to *Anabaena* RNA polymerase components. Lanes: 1, anticore; 2, anti-β'+β; 3, anti-γ; 4, anti-σ. Purified *Anabaena* RNA polymerase was separated by electrophoresis on a 12.5% SDS-polyacrylamide gel and electroblotted to nitrocellulose.

mM $(NH_4)_2SO_4$, stirred, and bubbled with 1% CO_2 in air under fluorescent light. Extracts from *Anabaena* sp. strain PCC 7120, *Anabaena* sp. strain PCC 7118, *A. variabilis* ATCC 29413, *A. azollae* 1A, *Anabaena* sp. strain L31, *A. torulosa*, *N. muscorum*, *Nostoc* sp. strain MAC PCC 7911, *C. membranacea*, and *O. tenuis* were prepared simply by boiling concentrated cells with SDS loading buffer (50 mM Tris [pH 6.8], 1% SDS, 0.14 M β-mercaptoethanol, 2 mM EDTA, 10% glycerol). Crude extracts from *A. cylindrica*, *A. azollae* symbiont, *Plectonema boryanum*, *Phormidium foveolum*, *Synechococcus* sp. strain PCC 7002, and *Synechococcus* sp. strain PCC 7942, which did not fully lyse on boiling, were prepared by French pressure cell lysis before being boiled in SDS loading buffer. After being boiled for 5 min, the cell extracts were loaded directly onto a 12.5% SDS-polyacrylamide gel. Approximately 100 μg of total cell protein was loaded per lane.

RESULTS AND DISCUSSION

Western blots of the four antisera demonstrated that γ is serologically unrelated to the other RNA polymerase subunits (Fig. 1). Antiserum to the γ protein did not significantly bind to any of the other RNA polymerase subunits (lane 3). A longer exposure did detect some weak recognition of the *Anabaena* β subunit, but since antiserum to the β' and β subunits did not react with γ, this recognition was almost certainly due to β proteolysis fragments that comigrated with the γ subunit during the electrophoresis used to separate the subunits in the preparation of antigen for immunization. Lane 4 shows a similar strong reaction of anti-σ serum to the σ protein, as well as a weak reaction to the β subunit and a still weaker reaction to γ detected on a longer exposure. Both weak reactions were ascribed to β and γ proteolysis products in the material used for immunization, since antisera to the larger subunits did not react with σ.

Our earlier experiments showed that the *Anabaena* RNA polymerase core $\beta'\beta\gamma\alpha_2$ was unable to initiate transcription at a specific promoter unless σ was restored (17). Further evidence that the *Anabaena* holoenzyme contains all five kinds of subunits was provided by immunoprecipitation. Radioactively labeled extracts of total *Anabaena* soluble proteins were incubated with each of the four antisera used for Fig. 1. Following the addition of *Staphylococcus aureus* protein A and centrifugation, the immune precipitates were solubilized and examined by gel electrophoresis. In each case, the resulting gel showed all five subunits in roughly the same ratio (data not shown).

TABLE 1. Subunit molecular weights of cyanobacterial RNA polymerases

Species or strain	Mol wt (10^3) of RNA polymerase subunit:				
	β'	β	γ	σ	α
Anabaena sp. strain PCC 7120	171	124	66	52.5	41
Anabaena sp. strain PCC 7118	171	124	66	52.5	41
A. variabilis ATCC 29413	171	125	67.5	53	41
A. cylindrica	170	126	66	52	40
A. azollae 1A	171	126	67.5	53	41.5
A. azollae symbiont	171	124	68.5	52	41
Anabaena sp. strain L31	175	130	68	53	41.5
A. torulosa	177	130	67.5	53	41.5
Nostoc muscorum PCC 7119	171	124	66	52.5	41
Nostoc sp. strain MAC PCC 7911	173	126	68	52	40
Plectonema boryanum	170	127	65	50	42
Phormidium foveolum	170	127	65	50.5	42
Calothrix membranacea	165	124	68	54	40
Oscillatoria tenuis	171	125	67.5	53.5	40
Synechococcus sp. strain PCC 7002	173	126	66	53.5	40
Synechococcus sp. strain PCC 7942	171	126	68	51.5	39.5

RNA polymerase subunit structure of other cyanobacteria. Antisera to the *Anabaena* RNA polymerase proteins were used to probe Western blots of crude extracts of other cyanobacteria. Extracts of each cyanobacterium were probed with each of the four antisera separately to identify the RNA polymerase subunits. This process also allowed the identification of β' and β proteolysis products in *A. variabilis*, *A. azollae* 1A, *N. muscorum*, *Nostoc* sp. strain MAC PCC 7911, *C. membranacea*, and *O. tenuis*. Finally, extracts of all the cyanobacteria tested were probed simultaneously with anticore and anti-σ sera (Fig. 2). Proteins corresponding to each of the *Anabaena* RNA polymerase subunits were present in every extract. The RNA polymerase subunit molecular weights of the tested cyanobacteria varied only slightly (Table 1). In addition, γ protein was coprecipitated with the other RNA polymerase subunits from crude extracts of *Synechococcus* sp. strain PCC 7942 by anti-β'+β serum, indicating that γ is part of the RNA polymerase core even in the more distantly related unicellular cyanobacteria.

Subunit structures for two cyanobacterial RNA polymerases have been reported previously. *Anacystis nidulans* (*Synechococcus* sp. strain PCC 7942) was reported to possess a core enzyme similar to that of other bacteria plus a σ factor which purified in a 1:1 ratio with the core and failed to separate chromatographically from the core (6). In our work this protein was identified as homologous to the *Anabaena* γ subunit. The RNA polymerase structure of *Fremyella diplosiphon* (*C. membranacea*) as reported by Miller and Bogorad (12) lacked a σ subunit. The 91,000-molecular-weight protein present in the *F. diplosiphon* preparation previously de-

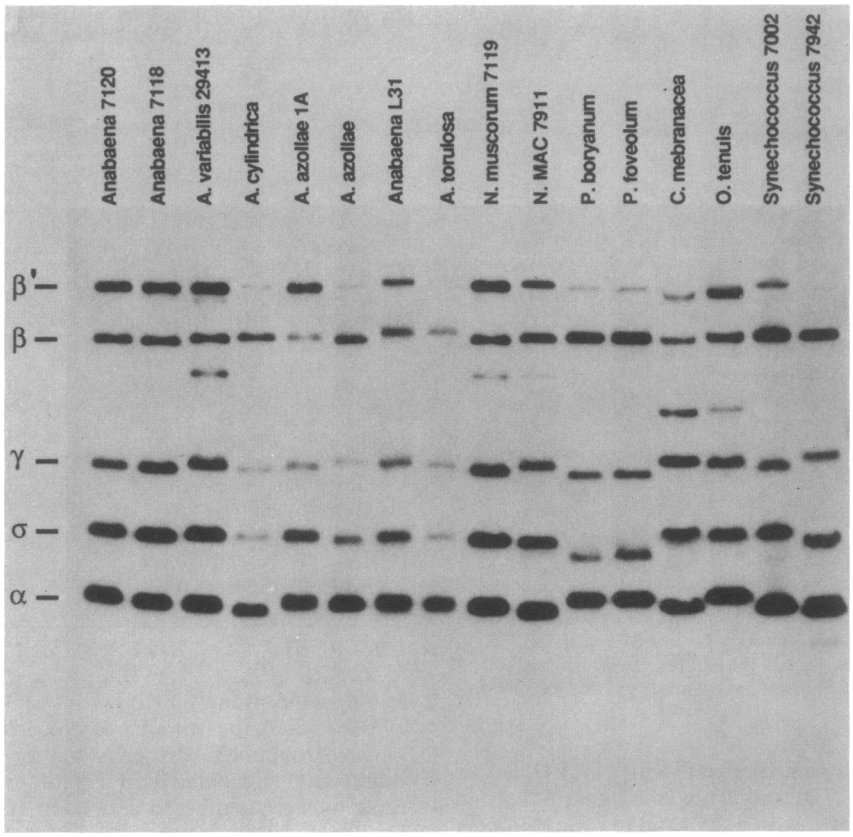

FIG. 2. Western blot of cyanobacterial extracts probed with antisera to the *Anabaena* sp. strain PCC 7120 core and σ proteins. About 100 μg of protein from each strain indicated at the top was loaded in each lane.

scribed is probably a β proteolysis product sometimes present in our extracts. The immunological data presented here show that both polymerases possess σ subunits similar in size to the 52,500-molecular-weight *Anabaena* sp. strain PCC 7120 σ subunit. Thus, all the cyanobacteria examined possess the same structure for RNA polymerase subunits, including the γ protein. Moreover, these cyanobacteria represent three of the five major subgroups (16). Section I, unicellular cyanobacteria that divide in one plane only, includes *Synechococcus* spp. Section III, filamentous nonheterocystous cyanobacteria, includes *Phormidium*, *Plectonema*, and *Oscillatoria* spp. Section IV, the filamentous heterocystous cyanobacteria, includes *Anabaena*, *Nostoc*, and *Calothrix* spp.

β′ and β homologies among cyanobacteria, eubacteria, and archaebacteria. *E. coli* RNA polymerase subunits also exhibited cross-reactivity with the *Anabaena* RNA polymerase antisera. *E. coli* core and σ subunits reacted with the corresponding antisera in Western blot experiments. When blots of *E. coli* extracts were probed with the anti-γ serum, a significant reaction occurred with the β′ subunit (Fig. 3). In contrast, the anti-β′+β serum, while reacting strongly with both *Anabaena* subunits, reacted mostly with *E. coli* β, indicating strong homology between the *Anabaena* and *E. coli* β subunits but limited homology between β′ subunits. It seems that the antigenic determinant most homologous to *E. coli* β′ resides, in *Anabaena* spp., on the γ subunit. Since *Anabaena* spp. has a β′ subunit as well as a γ subunit, which is antigenically related to *E. coli* β′, it appears that the domains of *E. coli* β′ are split in *Anabaena* spp. between the β′ and γ subunits.

Similar separations have been described for the σ and β subunits of other bacterial RNA polymerases. In some eubacteria, the functions of *E. coli* RNA polymerase σ seem to be divided between two polypeptides. In addition to a σ subunit, the enzyme from *Lactobacillus curvatus* has a large subunit called y (4), and the enzyme from *Bacillus subtilis* has a small subunit called δ (22). In both cases the function of these subunits is to prevent the nonspecific association of the enzyme with nonproductive sites on DNA, a function which is attributed to σ in *E. coli*.

The RNA polymerases of archaebacteria are relatively complex, but recent studies have shown that the multiplicity of subunits can be related to the simpler eubacterial structure. Consider first the subunits related to *E. coli* β. The extreme thermophile *Sulfolobus acidocaldarius* contains a single B subunit. This subunit is serologically related to two smaller proteins, B′ and B″, found in the enzymes from halobacteria and methanogens. However, the distribution of B-related antigenic sites differs among the B′ and B″ subunits of halobacteria and methanogens. One could imagine the simple B gene of *S. acidocaldarius* being split at two sites to give rise to the B′ and B″ genes of the halobacteria and the methanogens (24).

In the archaebacteria, homology to β′ is also split between two subunits, A and C. Serological studies showed that subunit A (M_r, = 104,000) is related to *E. coli* β′ (24). The C component (M_r, 44,000) had been thought previously to be related to σ (24), but recent cloning and sequencing experiments have shown that C is homologous to the C-terminal region of *E. coli* β′ while A is homologous to the N-terminal region. The order of the genes encoding these subunits is

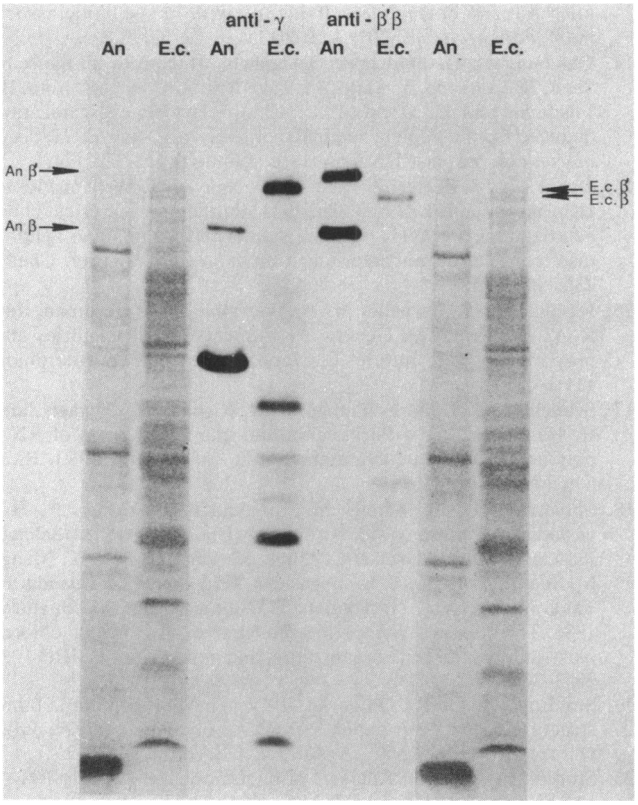

FIG. 3. Western blots of *Anabaena* sp. strain PCC 7120 (An) and *E. coli* (E.c.) extracts probed with anti-γ and anti-β'+β sera. The four center lanes are autoradiograms of the amido black-stained nitrocellulose blots shown in the side lanes. *E. coli* HB101 cells were grown overnight in LB medium and lysed by being boiled in SDS loading buffer. The band in anti-γ lane E.c. can be unambiguously identified as the β' subunit by aligning it with the β' and β subunits visible on the amido-black-stained blot. Several of the lower-molecular-weight bands in anti-γ lane E.c. match up with bands for major proteins in the *E. coli* extract and are probably due to nonspecific binding.

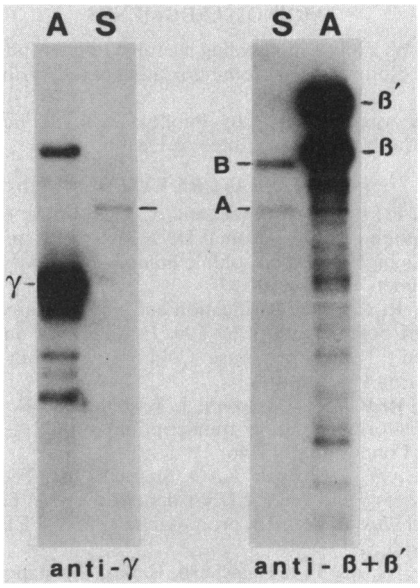

FIG. 4. Detection of serological cross-reactions of *Anabaena* RNA polymerase antisera with subunits of *S. acidocaldarius* (lanes S) RNA polymerase. Western blots were prepared with the antisera characterized in Fig. 1. Lanes A, *Anabaena* holoenzyme. The *S. acidocaldarius* B subunit molecular weight was 122,000; the A subunit molecular weight was 104,000. All of the *S. acidocaldarius* subunits were recognized by their homologous antisera (data not shown).

BAC, where B is the single β-related subunit and A+C corresponds to β' (W. Zillig, personal communication). Thus the β'-related subunit appears to be split in two in *S. acidocaldarius*. We determined the serological relationships among the cyanobacterial and archaebacterial subunits, using the enzyme from *S. acidocaldarius* and antisera kindly provided by W. Zillig. Anti-*Anabaena* γ reacted with the A subunit of *S. acidocaldarius*. Anti-*Anabaena* β'+β reacted with the A and B subunits of *S. acidocaldarius*. Neither serum reacted with the C subunit (Fig. 4).

These results are consistent with the *Anabaena* sp.-*E. coli* homologies and suggest that γ is homologous to the N-terminal portion of *E. coli* β'. Evidence that this may be the case comes from chloroplast RNA polymerase genes. A phylogenetic tree based on 16S rRNA sequence comparison suggests that the chloroplasts of higher plants descended from a cyanobacterial symbiont (23). Shinozaki et al. (18) and Ohyama et al. (14) have shown that the tobacco and liverwort chloroplast genomes contain a gene homologous to *E. coli rpoB* (the β gene) and show homology to *E. coli rpoC* (the β' gene) in several downstream reading frames. In the spinach chloroplast genome, Hudson et al. (7) have found three adjacent cotranscribed genes, named *rpoB*, $rpoC_1$, and $rpoC_2$, encoding proteins of 121, 78, and 154 kilodaltons, respectively. Chloroplast *rpoB* shows homology to *E. coli rpoB*. Chloroplast $rpoC_1$ and $rpoC_2$ gene products show homology to the N- and C-terminal portions, respectively, of *E. coli* β'. Proteins of sizes near those predicted for the products of these genes are present in chloroplast RNA polymerase preparations from spinach (1, 11), maize (8, 19), and pea (21).

Given the close relationship of cyanobacteria and chloroplasts and the similarity between the predicted sizes of chloroplast gene products and the observed *Anabaena* subunit molecular sizes, the chloroplast gene order seems most likely to also represent the cyanobacterial gene order, i.e., genes coding for β, γ, and β', in that order. The archaebacteria have a similar arrangement, but one in which the larger subunit, A, is homologous to the N-terminal portion of *E. coli* β' and to *Anabaena* γ, while homology to the C-terminal region of *E. coli* β' is present in the smaller subunit, C. This situation could have arisen by the splitting of a primordial β' gene, represented today by the *E. coli rpoC* gene, at two different sites, generating the genes for A and C in archaebacteria and for the smaller and larger β'-related subunits in cyanobacteria (γ and β') and chloroplasts (the $rpoC_1$ and $rpoC_2$ gene products). This sequence of events is similar to the proposed splitting of the archaebacterial B into two sets of genes, B' and B'', which are different in the halobacteria and the methanogens.

We conclude that the evolution of the β' subunit of RNA polymerase in cyanobacteria, other eubacteria, and archaebacteria has followed separate but parallel paths. The antisera described here should be useful tools in further studies of bacterial and chloroplast evolution.

ACKNOWLEDGMENTS

We thank W. Zillig for providing useful materials and unpublished data and W. Bottomley for communicating results prior to publication.

This work was supported by Public Health Service grant GM 21823 from the National Institutes of Health.

LITERATURE CITED

1. **Briat, J.-F., C. Bisanz-Seyer, and A.-M. Lescure.** 1987. *In vitro* transcription of the ribosomal DNA operon of spinach chloroplast by a highly purified soluble homologous RNA polymerase. Curr. Genet. **11**:259–263.
2. **Burgess, R. R.** 1976. Purification and physical properties of *E. coli* RNA polymerase, p. 69–100. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Burgess, R. R., A. A. Travers, J. J. Dunn, and E. K. F. Bautz.** 1969. Factor stimulating transcription by RNA polymerase. Nature (London) **221**:43–46.
4. **Gierl, A., W. Zillig, and K. O. Stetter.** 1982. The role of the components σ and y of the DNA-dependent RNA polymerase of *Lactobacillus curvatus* in promoter selection. FEBS Lett. **125**: 41–47.
5. **Grachev, M. A., T. I. Kolocheva, E. A. Lukhatanov, and A. A. Mustaev.** 1987. Studies on the functional topography of *Escherichia coli* RNA polymerase. Eur. J. Biochem. **163**:113–121.
6. **Herzfeld, F., and W. Zillig.** 1971. Subunit composition of DNA-dependent RNA polymerase of *Anacystis nidulans*. Eur. J. Biochem. **24**:242–248.
7. **Hudson, G. S., T. A. Holton, P. R. Whitfeld, and W. Bottomley.** 1988. Spinach chloroplast *rpoBC* genes encode three subunits of the chloroplast RNA polymerase. J. Mol. Biol. **200**:639–654.
8. **Kidd, G. H., and L. Bogorad.** 1980. A facile procedure for purifying maize chloroplast RNA polymerase from whole cell homogenates. Biochim. Biophys. Acta **609**:14–30.
9. **Kranz, R. G., and R. B. Gennis.** 1982. A quantitative radioimmunological screening method for specific gene products. Anal. Biochem. **127**:247–257.
10. **Kratz, W. A., and J. Myers.** 1955. Nutrition and growth of several blue-green algae. Am. J. Bot. **42**:282–287.
11. **Lerbs, S., E. Bräutigam, and B. Parthier.** 1985. Polypeptides of DNA-dependent RNA polymerase of spinach chloroplasts: characterization by antibody-linked polymerase assay and determination of sites of synthesis. EMBO J. **4**:1661–1666.
12. **Miller, S. S., and L. Bogorad.** 1978. Purification and characterization of RNA polymerase from *Fremyella diplosiphon*. Plant Physiol. **62**:995–999.
13. **Neirzwicki-Bauer, S. A., and R. Haselkorn.** 1986. Differences in mRNA levels in *Anabaena* living freely or in symbiotic association with *Azolla*. EMBO J. **5**:29–35.
14. **Ohyama, K., H. Fukuzawa, T. Kohchi, H. Shirai, T. Sano, S. Sano, K. Umesono, Y. Shiki, M. Takeuchi, Z. Chang, S. Aota, H. Inokuchi, and H. Ozeki.** 1986. Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature (London) **322**:572–574.
15. **Orr, J., L. M. Keefer, P. Keim, T. D. Nguyen, T. Wellems, R. L. Heinrikson, and R. Haselkorn.** 1981. Purification, physical characterization, and NH_2-terminal sequence of glutamine synthetase from the cyanobacterium *Anabaena* 7120. J. Biol. Chem. **256**:13091–13098.
16. **Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier.** 1979. Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. **111**:1–61.
17. **Schneider, G. J., N. E. Tumer, C. R. Richaud, G. Borbely, and R. Haselkorn.** 1987. Purification and characterization of RNA polymerase from the cyanobacterium *Anabaena* 7120. J. Biol. Chem. **256**:4633–4639.
18. **Shinozaki, K., M. Ohme, M. Tanaka, T. Wakasugi, N. Hayashida, T. Matsubayashi, N. Zaita, J. Chunwongse, J. Obokata, K. Yamaguchi-Shinozaki, C. Ohto, K. Torazawa, B. Y. Meng, M. Sugita, H. Deno, T. Kamogashira, K. Yamada, J. Kusuda, F. Takaiwa, A. Kato, N. Tohdoh, H. Shimada, and M. Sugiura.** 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J. **5**: 2043–2049.
19. **Smith, H. J., and L. Bogorad.** 1974. The polypeptide subunit structure of the DNA-dependent RNA polymerase of *Zea mays* chloroplasts. Proc. Natl. Acad. Sci. USA **71**:4839–4842.
20. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
21. **Tewari, K. K., and A. Goel.** 1983. Solubilization and partial purification of RNA polymerase from pea chloroplasts. Biochemistry **22**:2142–2148.
22. **Whitely, H., E. Achberger, and M. Hilton.** 1982. The effect of the delta subunit in the interaction of *Bacillus subtilis* RNA polymerase with SP82 early gene promoters, p. 267–282. *In* R. Rodriguez and M. Chamberlin (ed.), Promoters: structure and function. Praeger Publishers, New York.
23. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.
24. **Zillig, W., R. Schnabel, K. Stetter, M. Thomm, F. Gropp, and W. D. Reiter.** 1985. The evolution of the transcription apparatus, p. 45–72. *In* K. H. Schleifer and E. Stackebrandt (ed.), Evolution of prokaryotes. Academic Press, Inc., Orlando, Fla.