Antisera were raised against native RNA polymerases A or B, as well as against each individual subunit of RNA polymerase A from the yeast *Saccharomyces cerevisiae*. The affinity spectrum of antibodies was evaluated by reacting electrophoretically separated enzyme subunits, transferred to a membrane, with ¹²⁵$I$-labeled immunoglobulins. Alternatively, the subunit* immunoglobulin complex was revealed by ¹²⁵$I$-labeled Protein A. Antibodies directed against native RNA polymerase A recognized the majority of the polypeptides forming the enzyme. When challenged with RNA polymerases B or C, this antibody preparation demonstrated the presence of polypeptides common to the three enzymes. A small cross-reaction was also found at the level of the large subunits of Enzyme B as well as some additional polypeptides of Enzyme C. Similar experiments with antibodies directed against native RNA polymerase B confirmed the presence of common subunits and also showed that the large polypeptides of the three enzymes share a few immunological determinants. Common subunits are AC₄₀, ABC₂₃, ABCₓ₃, AC₉, and ABC₁₄₅.

Immunologically related sites were conserved in the large subunits of RNA polymerase A from remote yeast species. Similarly, yeast and wheat germ RNA polymerase B share immunological determinants on the large subunit as well as on a small peptide. On the other hand, there was no significant cross-reaction between yeast and mammalian Enzyme B or *Escherichia coli* RNA polymerase.

Antibodies raised against the different polypeptide components of RNA polymerase A reacted specifically with the corresponding subunits. Inhibition studies with these subunit-specific antibodies showed that the common subunits are not always similarly exposed to antibody attack within the three enzymes. The data are discussed in terms of the structural similarity, organization and evolution of eukaryotic RNA polymerases.

Three forms of RNA polymerases, A, B, and C, are responsible for the transcription of yeast nucleic DNA (Roeder, 1976; Popa et al., 1971; and Adman et al., 1972). Among eukaryotic RNA polymerases, the yeast enzymes, for practical reasons, have been the most extensively studied at the molecular level (Sentenac et al., 1976; and Hager et al., 1976). These studies have revealed the extreme molecular complexity of these multisubunit enzymes which are the major component

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in a system concerned with the regulation of gene expression (Huet et al., 1975; Dezéée et al., 1976; Valenzuela et al., 1976b). The majority of the subunits of the RNA polymerases have unique mass. Some polypeptides are phosphorylated in *vivo* (Buhler et al., 1976a; Bell et al., 1977). Structural studies have also demonstrated the presence of common subunits which belong to the pool of the small peptides. Enzymes A and B possess three subunits which are indistinguishable on the basis of molecular weight, isoelectric point, and fingerprint pattern (Buhler et al., 1976b; Sentenac et al., 1976). Enzyme C also probably shares polypeptides with enzymes A and B (Valenzuela et al., 1976a).

The existence of common subunits is a possible explanation for the previous findings that yeast RNA polymerases A and B (Hildebrandt et al., 1973; Buhler et al., 1976b) as well as the corresponding mammalian enzymes (Ingles, 1973) exhibit extensive cross-reactivity. However, it was also possible that the functionally homologous subunits were structurally related. It was suggested that the polypeptides involved in the interaction with a common subunit in the three enzymes may have retained the same subunit binding site (Buhler et al., 1976b). The fact that enzymes B and C from mammalian cells (Seifart et al., 1972; Roeder, 1976) and enzymes B and A from yeast (Huet et al., 1975) are both sensitive to α-amanitin also suggested a certain conservation of the α-amanitin binding site. Partial evidence that RNA polymerases A and B are primarily constructed of distinct gene products was again immunological. Antibodies raised against the largest subunit from RNA polymerase A do not cross-react with or inhibit RNA polymerase B (Buhler et al., 1976b). This immunological approach needed to be extended to all the individual subunits in order to present a complete picture of the structural relationship of the three forms of RNA polymerase. For this purpose, we used a protein blotting technique to allow antibodies to react with the subunits separated by SDS-polyacrylamide gel electrophoresis. The results establish that the different subunits of yeast RNA polymerase A are unique proteins and identify the polypeptides in the three enzymes which are immunologically related. These immunological studies have been extended to RNA polymerases from other organisms.

**EXPERIMENTAL PROCEDURES**

*RNA Polymerases and Subunits*—Yeast RNA polymerases A and B were purified from *Saccharomyces cerevisiae* as previously described (Huet et al., 1975; Dezéée et al., 1976). RNA polymerase C was purified according to Hager et al. (1977). *Escherichia coli* RNA polymerase was a gift of A. Ruet (this laboratory). Wheat germ and calf thymus RNA polymerase B were donated by C. Kedinger (Strasbourg University).

Subunits of yeast RNA polymerase A were isolated on a preparative scale by SDS-polyacrylamide gel electrophoresis on a 1.0-mm...
thick, 11.5% acrylamide gel, using 2 mg of RNA polymerase per run. The different polypeptides were visualized after electrophoresis by immersing the gel into a KCI solution (0.1 M) in the cold for 30 min. Polycrylamide strips containing the subunits were cut out and crushed with a glass homogenizer. A portion of each mixture was subjected to an analytical SDS-polyacrylamide gel electrophoresis to check the purity of the subunits before injection. A total of 10 mg of RNA polymerase was needed to obtain the antibodies.

**Antiserum—**Antibodies against native RNA polymerases A and B were raised in rabbits by injecting 250 µg of protein, emulsified with complete Freund’s adjuvant (Difco), intratracheally at several sites in the back of the rabbit. Booster injections were performed in the same way, every 21 days, after injection of 250 µg of protein, emulsified with incomplete Freund’s adjuvant (Difco), after 21 days and subsequently every 10 days. The animals were bled 12 days after the sixth and seventh injections.

Antibodies directed against the isolated subunits of yeast RNA polymerase A were obtained similarly, by injecting the immunogen under the form of a suspension of crushed polyacrylamide with 1 volume of Freund’s complete adjuvant. Booster injections were done with incomplete adjuvant after 21, 36, 51, and 66 days. The protein mass injected, which depended, of course, on the molecular weight of the subunits, corresponded to 2 mg of RNA polymerase. Rabbits were bled 15 days after the fifth injection.

Preparation of immunoglobulins (IgG) with 125\(^I\) were carried out according to Broome and Gilbert (1979). Protein A from Staphylococcus aureus, a gift from J. M. Dubert (University of Paris VII), was labeled with 125\(^I\) by the same procedure to a specific activity of about 4 X 10\(^6\) cpm/µg.

**Protein Blotting Procedures—**RNA polymerases (150 µg) were first subjected to electrophoresis in a 11.5% polyacrylamide slab gel (10 X 9 X 0.06 mm) with sodium dodecyl sulfate, according to Laemmli (1970). The subunits were then transferred to a membrane by direct blotting or electrophoresis. Direct blotting was carried out essentially as described by Southern (1975) for blotting DNA fragments. The proteins were covalently linked to a cellulose acetate membrane (SM 11106, Sartorius) activated with cyanogen bromide just before the transfer. The blotting buffer (PBS) was 0.8% NaCl, 10 mM sodium phosphate, pH 7.5. The flow rate through the acrylamide gel was 0.2 ml/h cm\(^2\). After 20 h of blotting, the membrane was removed, and washed for 4 h in PBS containing glycine (15 g/liter) at 4\(^\circ\)C, then rinsed with and stored in PBS + 0.5% rabbit serum and 0.1% serum albumin until used for immunological detection. The electrophoretic transfer of RNA polymerase subunits was carried out as described by Towbin et al. (1979). Electrophoresis of RNA polymerase A under non-denaturing conditions was performed as previously described (Huet et al., 1979). The enzyme was transferred to activated cellulose acetate membrane by direct blotting as for the subunits.

**Visualization of Antibody Binding—**After transfer of the proteins, the membrane was soaked in 3% bovine serum albumin in PBS for 1 h at 40\(^\circ\)C, rinsed three times with a Triton/SDS buffer (Dimitriadi, 1974), then incubated with a bovine serum albumin (0.2% TritonX-100, and 0.2% SDS) at room temperature. The membrane was then incubated with the 125\(^I\)-labeled IgG in the same buffer (10 cpm/ml 0.15 ml/cm\(^2\) of filter) into a sealed plastic bag with gentle rocking at 22\(^\circ\)C for 17 h. The membrane was washed four times with 50 ml of the above Triton/SDS buffer for a total of 45 min, blotted on filter paper, thoroughly air dried, and subjected to autoradiography on x-ray film (3M; type XM) with intensifying screen (3M; type T-12) for 4 h or overnight at room temperature. For detection with Protein A, the blot was incubated with unlabeled IgG diluted to 30 µg/ml with the Triton/SDS buffer and washed as indicated above, then incubated with Protein A (5 X 10\(^5\) M) 0.15 ml/cm\(^2\) of filter and SDS buffer for 1 h at room temperature. The membrane was washed with 50 ml of Triton/SDS buffer four times, dried, and subjected to autoradiography as above.

**Effect of Specific Antibodies on Enzyme Activity—**Purified IgG directed against isolated subunits of RNA polymerase A were preincubated at varying concentrations with 1.5 µg of RNA polymerase A, B, or C, in 50 µl of a solution containing 50 mM Tris-HCl (pH 7.4), 2 mM NaCl, and 0.1 mM EDTA, for 1 h at 37\(^\circ\)C. The final concentration of IgG was kept constant by complementing with control y-globulins from a normal rabbit. Then, the residual enzyme activity was estimated by a further incubation of 20 min at 30\(^\circ\)C, after addition of 50 µl of the following transcription mixtures. For RNA polymerase A, it contained 150 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol, 10 mM MgCl\(_2\), 10 µg of denatured calf thymus DNA, 2 mM each of ATP, GTP, and CTP, and 0.2 mM [\(\alpha\)32P]UTP (12 cpm/pmol). In this case, incubation was for 10 min at 30\(^\circ\)C. For RNA polymerase C, it contained 50 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol, 2 mM MnCl\(_2\), 10 mM MgCl\(_2\), 100 mM ammonium sulfate, 10 µg of denatured calf thymus DNA, 2 mM each of ATP, CTP, and GTP, and 1 mM [\(\alpha\)32P]UTP (25 cpm/pmol). After incubation, acid-precipitable radioactivity was collected on a membrane filter (HAWP 025, Millipore) and measured by liquid scintillation.

**RESULTS**

**Affinity Spectrum of Antibodies—**Antibodies raised against yeast RNA polymerase A cross-react with and inhibit RNA polymerase B (Hildebrandt et al., 1973). To interpret this observation, the affinity spectrum of the antibody preparation had to be determined at the subunit level. A method was developed to react the 125\(^I\)-labeled antibodies with the individual subunits of RNA polymerase. The proteins were first separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to an activated cellulose acetate membrane essentially as described by Southern (1975) for transfer of DNA fragments. The proteins covalently attached to the membrane were subsequently incubated with the 125\(^I\)-labeled immunoglobulins. Because yeast RNA polymerases are made of polypeptides of largely different mass, from 190,000 to 12,000 daltons, one could expect a preferential transfer of the small proteins. The efficiency of the transfer method was therefore quantified using a mixture of four labeled proteins from yeast separated as a function of molecular weight by SDS-polyacrylamide gel electrophoresis. Basic and acidic polypeptides were analyzed independently. The transfer of proteins to the activated membrane was estimated by scanning the autoradiogram of the membrane and of the gel before and after blotting. In the case of acidic proteins, the 14\(^C\)-protein pattern after transfer was similar to the original one and the extent of transfer was in the range of 50%. A more erratic transfer occurred from the pool of basic proteins. Although most of the proteins were detectable, the extent of transfer varied from protein to protein. However, this did not depend simply on the molecular size of the polypeptides. In fact, the method can be applied to very large proteins like RNA polymerase A (M, 550,000) (Huet et al., 1975) under electrophoresis under nondenaturing conditions (Fig. 1). The method is sensitive enough to tolerate, in this case, a low transfer efficiency.

The affinity spectrum of the antibodies directed to native RNA polymerase A was determined after blotting the subunits on a membrane (Fig. 1). The antibody preparation recognized the majority of the subunits. The antibodies bound most efficiently subunits A1m and A43. A clear binding response was obtained on A26, A12, A13, A25, A27, A32, A15, and A17, although the signal intensity varied from one experiment to another, especially on A12, using the same antibody preparation. One polypeptide, A43, was poorly and only occasionally detected. Three different antibody preparations gave grossly a similar binding pattern mainly with variations in binding intensity on different subunits. The enzyme could be boiled in 1% SDS (w/v) or concentrated by precipitation with 10% trichloroacetic acid (w/v) and still retain the same pattern of antibody recognition (Fig. 1). The low and variable binding response of subunit A2, reflected a poor transfer to the membrane as evidenced by staining the gel for proteins after blotting.

While this protein blotting method was developed, two alternative procedures were described (Renart et al., 1979; Towbin et al., 1979). We were interested in using the electrophoretic technique of Towbin et al. (1979) in comparison with our direct blotting procedure to determine whether the method of transfer influenced the apparent affinity spectrum.
Immunological Relationship of Yeast RNA Polymerases

**Fig. 1.** Detection of native RNA polymerase and its subunits with 125I-labeled antibodies. Left, RNA polymerase A was subjected to electrophoresis on a 5% polyacrylamide slab gel under nondenaturing conditions and blotted on an activated cellulose acetate membrane. The membrane was incubated with 125I-labeled IgG directed against native enzyme A, as described under "Experimental Procedures." The autoradiogram shows the antibody binding response obtained with 1.2 μg (1) or 0.12 μg (2) of enzyme. Right, subunits of RNA polymerase A (10 μg of enzyme) were separated on a SDS-polyacrylamide gel, transferred to a membrane, and allowed to react with 125I-labeled IgG directed against native RNA polymerase A, as described under "Experimental Procedures." The autoradiogram shows the antibody binding response obtained with 1.2 pg subunits of enzyme A.

**Fig. 2.** Immunological relationship, at the subunit level, of RNA polymerases from various organisms. Left panel, different RNA polymerases (10 μg) were subjected to electrophoresis on a SDS-polyacrylamide slab gel. Their subunits were transferred to an activated cellulose acetate membrane as described under "Experimental Procedures." The blots were revealed with 125I-labeled IgG directed against S. cerevisiae RNA polymerase A, as described under "Experimental Procedures." RNA polymerases were enzyme A from: 1. *C. tropicalis*, 2. *S. pombe*, 3. *S. cerevisiae*, or 4. *E. fibuligera*; or enzyme B from 5. *calf thymus* or 6. *E. coli* holoenzyme. Right panel, subunits of RNA polymerase B from: 7. *S. cerevisiae*, 8. *calf thymus*, and 9. wheat germ, were separated by SDS-gel electrophoresis, transferred electrophoretically to a nitrocellulose sheet, and allowed to react with 125I-labeled IgG directed against *S. cerevisiae* RNA polymerase B. The autoradiogram shows the subunits which have bound the labeled antibodies.

tropicalis and *E. fibuligera* enzymes and to a lesser extent with the second largest one. A faint signal was also detected, in another experiment, on two smaller polypeptides (the common subunits A32 and A64). On the other hand, there was no detectable cross-reaction with RNA polymerases from *S. pombe* (enzyme A), *E. coli* holoenzyme, or *calf thymus* (enzyme B). The immunological relationship of RNA polymerases B from *S. cerevisiae*, *calf thymus*, and wheat germ was similarly investigated at the subunit level. Using 125I-labeled antibodies directed against native enzyme B from yeast, a clear cross-reaction signal was found on the largest subunit of wheat germ enzyme B, as well as on the smallest of the polypeptides (Fig. 2). Only a faint binding response was detected on the large subunits of the mammalian enzyme. There was no significant signal with *E. coli* RNA polymerase (not shown).

**Immunological Relationship between Yeast RNA Polymerases A, B, and C**—One of the purposes of this study was to examine the immunological relationship between the three forms of yeast nuclear RNA polymerases. Their subunits were separated on an SDS-polyacrylamide slab gel and challenged with antibodies raised against RNA polymerases A or B. The bound immunoglobulins were visualized with 125I-labeled Protein A (Fig. 3). Antibodies against RNA polymerase A bound subunits B27, B32, and B41.5. The intensity of the binding signal on these small subunits was the same for enzymes A and B. In addition, a clear binding response was distinguished on the two large subunits, especially on B30 (or its proteolyzed form, B29). However, in this case, antibody binding was much less strong than on the large subunits of enzyme A, suggesting that the cross-reactivity was limited to one or a few immunological determinants. Reciprocally, antibodies against RNA polymerase B which recognized subunits B23, B19, B15, B4.5, B32, B27, B23, B10, B14.5, and B12.6 also bound subunits A27, A32, and A14.5 from enzyme A with the same efficiency as for B27, B23, and B4.5. In addition, a small cross-reaction was distinguished on A96 and A135.
In another experiment, RNA polymerase C subunits were probed with antibodies against enzyme A or B (Fig. 3). Anti-RNA polymerase A antibodies revealed subunits C$_{12}$, C$_{27}$, and C$_{33}$ with the same efficiency as A$_{19}$, A$_{27}$, and A$_{23}$, hence demonstrating the immunological relationship of these subunits. A$_{19}$ and A$_{15}$ were poorly detected in this experiment but another antibody preparation cross-reacted with these two putative common subunits in enzyme C (results not shown). Interestingly, another polypeptide of our enzyme C preparation, C$_{51}$, bound antibodies. This polypeptide was designated as a component of RNA polymerase C by Hager et al. (1977). There was also a weak binding signal on other polypeptides, C$_{120}$, C$_{49}$, and C$_{54}$ but these results need further investigation.

Anti-RNA polymerase B revealed subunits C$_{27}$ and C$_{33}$. A weak cross-reaction signal also revealed C$_{51}$, suggesting an immunological relationship with one of the B subunits. These experiments were repeated using the direct transfer technique or the electrophoresis, with similar results.

Studies using Antibodies Directed against Individual Subunits—To probe in more detail the structure of yeast RNA polymerases, antisera were raised against each individual subunit of RNA polymerase A, separated by gel electrophoresis. The reactivity of the immunoglobulins towards the polypeptide components of enzymes A and B was evaluated by the electrophoretic transfer technique or the electrophoresis, with similar results.

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other antibodies were inactive, including those directed against the common subunits A27 and A14.5. RNA polymerase C was inhibited by anti-A60, anti-A23, and anti-A19 immunoglobulins, practically to the same extent as RNA polymerase A. RNA polymerase C was even more sensitive to anti-A19 IgG than enzyme A. Again, the other antibodies were inactive.

DISCUSSION

Two important general conclusions can be drawn from these immunological studies. The first one concerns the uniqueness of the polypeptide components of yeast RNA polymerase. Our preliminary fingerprint data suggested that most of the subunits of RNA polymerase A were distinct proteins and did not derive from limited proteolysis of the larger molecular weight polypeptides (Buhler et al., 1976b). This is now strongly confirmed by the specific reactivity of antibodies against isolated subunits. It is clear that A190, A135, A60, A40, A14.5, A17, A30, and A19 are unique proteins which carry unique immunological determinants. The data do not yet allow an extension of this conclusion with certainty to the smallest subunits A14.5 and A12.2 but it is likely that these polypeptides are also distinct gene products. If one extrapolates these results to RNA polymerases B and C, the transcription machinery in yeast appears to be one of the most complex enzymatic systems of the cell with some 25 distinct proteins.

The second conclusion concerns the structural relationship of RNA polymerases. It was known that the multiple forms of RNA polymerases are immunologically related (Hildebrandt et al., 1973). However, it was not clear whether this overall cross-reactivity was due to the presence of common subunits or to the conservation of large common structural domains, or both. We have previously shown that antibodies against the largest subunit of RNA polymerase A do not noticeably cross-react with nor inhibit RNA polymerase B (Buhler et al., 1976b). This study has now been extended to all the subunits of RNA polymerase A and the cross-reaction has been studied at the subunit level with a highly sensitive technique. Two different types of antibodies were used as a probe. Antibodies against native RNA polymerase are expected to recognize the immunological determinants accessible on the enzymes under their native configuration. Antibodies against SDS-denatured subunits will be directed against all the immunoreactive sites of the denatured polypeptide. The conclusion which emerges clearly from the data is that the immunological relationship between the three forms of yeast RNA polymerase is mostly accounted for by the existence of common subunits. However, the large polypeptides of the three enzymes share a few immunological determinants.

Let us examine first the case of the common subunits. On the basis of identical molecular weight, the putative common subunits are AC40, ABC7, ABC25, AC9, and ABC14.5 (Buhler et al., 1976b; Valenzuela et al., 1976a). Now, on the basis of antibody binding, the immunological relationship of the following subunits is firmly established: AC40, ABC25, ABC25, AC9, and ABC14.5. Inhibition data with antibodies directed against RNA polymerase A subunits confirm the structural relationship of AC40, ABC25, and AC9. It also suggests that these polypeptides are similarly exposed to antibodies within the three enzymes. Hager et al. (1976) and Valenzuela et al., (1976a) have reported the co-migration, by two-dimensional gel electrophoresis, of subunits C40 and A40, as well as of C10 and A19, while C27 was found to be less acidic than AB27. The charge difference between AB27 and C27 probably stems from a different degree of phosphorylation or another covalent modification of the polypeptide. The same authors also could not detect C27 on their two-dimensional subunit map. Therefore, the only indication that C27 is identical to A27 and B27 was their strikingly similar migration during SDS-polyacrylamide gel electrophoresis. The good binding response of C27 to the antibodies directed against enzymes A or B is now a convincing proof of identity. A covalent modification of C27 may explain the abnormal electrophoretic migration in urea. There remains the case of anti-A27 and anti-(A14.5 + A10) antibodies which only inhibit RNA polymerase A, although there is convincing evidence that A27 and A14.5 are common to the three forms of enzyme (Buhler et al., 1976b; and this work). The lack of inhibition probably reflects a different setting of these subunits within enzymes A, B, and C, thereby being less accessible to antibody attack.

There were cases of antibodies cross-reacting with polypeptides other than the previously recognized common subunits. Hence, anti-polymerase A antibodies recognized immunolog-
Immunological Relationship of Yeast RNA Polymerases

It is suggested that large subunits of enzyme A from remote yeast species suggests with the common polypeptides. Nevertheless, widely divergent response was at least 1 order of magnitude lower than on the ancestral set of proteins to meet the different functional and regulatory requirements of the enzymes which are located in different cell compartments and probably interact with different chromatin components. The lack of inhibition of RNA polymerase B or C by anti-A190 IgG or anti-A19 IgG, which confirms our preliminary observation (Buhler et al., 1976b), is probably due to the low concentration of the antibodies recognizing the common sites. Further studies are in progress using antibodies against isolated subunits of enzymes A and B to explore more detail the immunological relationships of the large subunits of the three forms of RNA polymerase.

The conservation of immunologically related sites in the large subunits of enzyme A from remote yeast species suggests that a high degree of functional constraint was exerted on these subunits, especially on A190 which appears the most immunologically conserved. Similarly, yeast and wheat germ RNA polymerase B share immunological determinants on their largest subunit as well as on a very small peptide. This is in keeping with the observation that antibodies directed against Drosophila RNA polymerase B inhibit yeast and calf thymus enzyme B (Greenleaf et al., 1976). On the other hand, we found no significant cross-reaction between yeast enzymes B and E. coli or mammalian RNA polymerase B. It is interesting in this respect to note that yeast and wheat germ RNA polymerase B are not competent to transcribe accurately a mammalian gene when added to a crude mammalian reconstituted system (Weil et al., 1979).

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REFERENCES

Adman, R., Schultz, L. D., and Hall, B. D. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1702-1706
Bell, G. I., Valenzuela, P., and Rutter, W. J. (1977) J. Biol. Chem. 252, 3092-3091
Broome, S., and Gilbert, W. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2746-2749
Buhler, J. M., Iborra, F., Sentenac, A., and Fromageot, P. (1976a) FEBS Lett. 71, 37-41
Buhler, J. M., Iborra, F., Sentenac, A., and Fromageot, P. (1976b) J. Biol. Chem. 251, 1712-1717
Dezèlle, S., Wyers, F., Sentenac, A., and Fromageot, P. (1976) Eur. J. Biochem. 65, 543-552
Dimitriadias, G. J. (1979) Anal. Biochem. 98, 445-451
Greenleaf, A. L., Kramer, A., and Bautz, E. K. F. (1976) in RNA Polymerase (Losick, R., and Chamberlin, M., eds) pp. 783-801, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
Hager, G. L., Holland, M. J., and Rutter, W. J. (1977) Biochemistry 16, 1-8
Hager, G. L., Holland, M. J., Valenzuela, P., Weinberg, F., and Rutter, W. J. (1976) in RNA Polymerase (Losick, R., and Chamberlin, M., eds) pp. 745-762, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
Hildebrandt, A., Sebastian, J., and Halvorson, H. O. (1973) Nature 246, 73-74
Huet, J., Buhler, J. M., Sentenac, A., and Fromageot, P. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3034-3038
Ingles, C. J. (1973) Biochem. Biophys. Res. Commun. 55, 364-371
Lompré, A. M., Bouveret, P., Leger, J., and Schwartz, K. (1979) J. Immunol. Methods 28, 143-148
Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685
Fonta, H., Ponta, U., and Wintersberger, E. (1971) FEBS Lett. 18, 204-208
Renart, J., Reiser, J., and Stark, G. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3116-3120
Roeder, R. G. (1976) in RNA Polymerase (Losick, R., and Chamberlin, M., eds) pp. 285-329, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
Seifert, K. H., Benecke, B. J., and Juhas, P. P. (1972) Arch. Biochem. Biophys. 151, 519-532
Sentenac, A., Buhler, J. M., Ruet, A., Huet, J., Iborra, F., and Fromageot, P. (1978) in Gene Expression (Brian, F., Clark, C., eds) pp. 187-201, Pergamon Press, Oxford
Sentenac, A., Dezèlle, S., Iborra, F., Buhler, J.-M., Huet, J., Wyers, F., Ruet, A., and Fromageot, P. (1976) in RNA Polymerase (Losick, R., and Chamberlin, M., eds) pp. 763-778, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
Southern, E. M. (1975) J. Mol. Biol. 98, 185-191
Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354
Valenzuela, P., Bell, G. I., Weinberg, F., and Rutter, W. J. (1976a) Biochem. Biophys. Res. Commun. 71, 1319-1325
Valenzuela, P., Hager, G. L., Weinberg, F., and Rutter, W. J. (1976b) Proc. Natl. Acad. Sci. U. S. A. 73, 1024-1028
Weil, P. A., Luse, D. S., Segall, J., and Roeder, R. G. (1979) Cell 18, 469-484