[CANCER RESEARCH 32, 2348–2352, November 1972]

# Transcriptional Transformation of Walker Tumor Chromatin by Nonhistone Proteins[1]

**Nina C. Kostraba and T. Y. Wang**

*Biology Department, State University of New York, Buffalo, New York 14214*

## SUMMARY

Nonhistone proteins fractionated from normal rat liver and Walker carcinosarcoma can stimulate chromatin-templated RNA synthesis *in vitro*. This has been demonstrated by using RNA polymerase prepared from rat liver, Walker tumor, or *Micrococcus luteus*. The RNA isolated from these fractionated nonhistone proteins does not activate transcription from chromatin. As judged by DNA-RNA hybridization studies, the altered transcription of chromatin effected by the nonhistone proteins reflects the characteristic transcription from chromatin homologous to the nonhistone proteins. Thus, when rat liver nonhistone proteins were used to activate transcription from Walker tumor chromatin, the activated transcript was found to contain RNA species similar to that synthesized *in vitro* from rat liver chromatin. Conversely, Walker tumor nonhistone proteins can activate the synthesis of RNA from rat liver chromatin to that partly characteristic of Walker tumor chromatin transcript. It is concluded that the nonhistone proteins are tissue specific in the alteration of transcription of chromatin.

## INTRODUCTION

The transformation of cells from the normal to the neoplastic state is accompanied by both repression and derepression of normal genome transcription (14). The control mechanisms regulating these alterations in RNA synthesis may operate on several levels. One such mechanism is the involvement of the chromatin components functioning as regulatory molecules. Apart from the histones, which have been suggested as gene repressors (1, 6, 9, 23), recent studies have indicated that the acidic chromosomal nonhistone proteins, which have been shown to be tissue variable and specific (5, 8, 12, 13, 17, 20, 25, 27), are capable of augmenting transcription *in vitro* from chromatins and DNA in both normal and tumor tissues (10, 15, 25–27). Furthermore, such gene activation by nonhistone proteins is reflective of the tissue from which the acidic chromosomal proteins are derived (8, 27). These results suggest specificity of the nonhistone proteins in the determination of gene expression. If this reasoning is correct, nonhistone proteins isolated from normal tissues should then alter neoplastic transcription to that more characteristic of normal transcription. Conversely, a transformation from normal to neoplastic expression on the transcriptional level should be effected by tumor nonhistone proteins. This paper describes such a study with Walker tumor as the neoplastic tissue and liver as the normal tissue. Results obtained from this investigation show that the Walker tumor nonhistone proteins are able to alter transcription *in vitro* from rat liver chromatin such that its transcribed RNA product contains RNA species found in tumor transcript. Similarly, the liver nonhistone proteins can effect the transcription of tumor chromatin to that partially characteristic of the normal liver chromatin. These data indicate that the nonhistone proteins are one of the regulatory factors participating in the control of gene activity.

## MATERIALS AND METHODS

**Animals.** Sprague-Dawley male rats, obtained from Holtzman Company, Madison, Wis., were used in this study. The Walker 256 carcinosarcoma was transplanted and harvested as reported previously (14).

**Preparation of Chromatin.** Nuclei were isolated from Walker tumor and rat liver by the method of Chauveau *et al.* (4) as modified by Busch and Steele (3). Chromatin was prepared from the isolated nuclei following the procedure described by Seligy and Miyagi (22). The procedure consists of extracting nuclei with 0.14 M NaCl in 0.05 M Tris-HCl (pH 8.0), homogenizing in 10 volumes of 0.05 M Tris-HCl (pH 8.0), and centrifuging through 1.7 M sucrose solution. The pelleted chromatin was washed free of sucrose with the Tris buffer and suspended in distilled water overnight. Suspension of the chromatin was adjusted to a DNA concentration of 200 μg/ml, as determined by the method of Burton (2).

**Preparation of Nonhistone Protein Fraction.** The nonhistone fractions were prepared as described elsewhere (10). Briefly summarized, the nuclei, isolated as described above, were extracted 3 times with 0.14 M NaCl in 0.02 M Tris-HCl, pH 7.6, discarding the extract, followed by extraction with 1.0 M NaCl in 0.02 M Tris-HCl, pH 8.0. After centrifugation of the 1.0 M NaCl suspension at 30,000 rpm in a Spinco 30 rotor for 90 min, the supernatant was collected and adjusted to 0.4 M NaCl by slow addition of 0.02 M Tris-HCl, pH 8.0. The resulting precipitate was removed by centrifugation and the supernatant was treated with Bio-Rex 70 (Bio-Rad Laboratories, Richmond, Calif.), according to the method of Langan (16), and lyophilized. Just prior to use, the lyophilized powder was dissolved in a minimal volume of 0.05 M NaCl in TME[2] and extensively dialyzed against same solvent. The dialyzed protein was adsorbed on a

[1] Supported by USPHS Grant GM-11698-09.
Received April 3, 1972; accepted July 11, 1972.

[2] The abbreviations used are: TME, 0.001 EDTA-0.001 M β-mercaptoethanol-0.05 M Tris-HCl (pH 8.0); SSC, 0.15 M NaCl-0.015 M sodium citrate, pH 7.0.

DEAE-cellulose column and washed successively with 0.05 M and 0.1 M NaCl in TME; the nonhistone protein fraction was then eluted by 0.3 M NaCl in TME.

**Preparation of RNA Polymerase.** Mammalian RNA polymerase was isolated from 0.9% NaCl solution-extracted nuclei of Walker tumor and of rat liver following the procedure of Roeder and Rutter (21). The Form II nucleoplasmic RNA polymerase was used in the template study. *M. luteus* RNA polymerase Fraction V, prepared according to the procedure of Nakamoto *et al.* (19), was used for synthesizing RNA used in DNA-RNA hybridization experiments.

**Template Activity of Chromatin.** The template activities of chromatins of Walker tumor and rat liver were assayed with homologous RNA polymerase according to the method of Roeder and Rutter (21). The assay mixture, in a total volume of 0.25 ml, contained 5 μg pyruvic kinase; 14 μmoles Tris-HCl, pH 8.0; 0.4 μmole $MnCl_2$; 2 μmoles KCl; 1.5 μmoles NaF; 1.0 μmole phosphoenolpyruvate; 0.4 μmole β-mercaptoethanol; 0.15 μmole each of GTP, CTP, ATP, and UTP-$^3$H (1 μCi); chromatin equivalent to 20 μg DNA; Walker tumor or rat liver RNA polymerase; and nonhistone proteins as indicated. After incubation for 10 min at 30°, the reaction was terminated by chilling in ice water and processed for radioactivity counting, as described elsewhere (14).

**Isolation of RNA Synthesized *in Vitro*.** For hybridization experiments, RNA was synthesized *in vitro* from chromatin in a reaction mixture scaled up 20-fold following the procedure of Tan and Miyagi (24). The reaction mixture, in a total volume of 10 ml, contained 500 μmoles Tris-HCl, pH 7.5; 1000 units RNA polymerase; 10 μmoles each of tritium-labeled GTP, CTP, ATP, and UTP (0.125 mCi each); 25 μmoles $MnCl_2$; 20 μmoles spermidine phosphate; and chromatin, equivalent to 1.5 mg DNA. The reaction mixture was incubated at 30° for 1 hr, and sodium dodecyl sulfate and NaCl were added to the mixture to a final concentration of 0.5% and 0.14 M, respectively. The isolation and purification of the *in vitro*-synthesized RNA has been reported elsewhere (14). This involved extraction of nucleic acid with water-saturated redistilled phenol at room temperature. The nucleic acid was precipitated from the aqueous phase with 2 volumes of ethanol, allowed to stand at −20° for 2 hr, and collected by centrifugation. The resulting pellet was dissolved in 0.01 M Tris-HCl, pH 7.5, containing 0.01 M $MgCl_2$, and treated with DNase (50 μg/ml) at room temperature; the mixture was then brought to 0.15 M NaCl and 0.5% sodium dodecyl sulfate concentrations. Following extraction with phenol, the RNA was precipitated from the aqueous phase as described above. The pelleted RNA was dissolved in 0.01 X SSC and dialyzed against 6 liters of 0.01 X SSC over a 24-hr period. The specific radioactivity of the *in vitro*-synthesized RNA was approximately $2 \times 10^4$ cpm/μg RNA.

**Hybridization of RNA Transcribed *in Vitro* with DNA.** DNA-RNA hybridization was performed according to the procedure of Gillespie and Spiegelman (7) with annealing conditions as described by Tan and Miyagi (24). One μg of alkali-denatured DNA was immobilized on a 25-mm nitrocellulose membrane filter (Schleicher and Scheull, type B-6). Two DNA filters and 2 blank filters were incubated in 1 ml reaction mixture containing a saturating amount of unlabeled RNA in 30% formamide and 2 X SSC at 37° for 24 hr. At the end of the incubation period, the DNA filters were transferred to vials containing increasing amounts of competing RNA-$^3$H. The double saturation was allowed to proceed for an additional 24 hr of incubation at 37° and prepared for radioactivity counting according to the method of Gillespie and Spiegelman (7). The background "noise" was 0.05 to 0.1% of the input RNA.

The molecular hybridization technique used throughout this study is able to distinguish complementary sequences mostly from the more reiterated DNA sequences (18). The technique, while not suitable for comparison of the total transcript of chromatin, can be used to distinguish relative differences between 2 populations of RNA.

## RESULTS

**The Transcription of Rat Liver Chromatin Altered by Walker Tumor Nonhistone Fraction.** Chart 1 shows the results of double saturation hybridization studies of rat liver DNA saturated with unlabeled rat liver transcript (RNA transcribed from rat liver chromatin). Three sets of the DNA-RNA filters were used to anneal with $^3$H-labeled rat liver RNA, tumor protein-activated RNA (RNA transcribed from rat liver

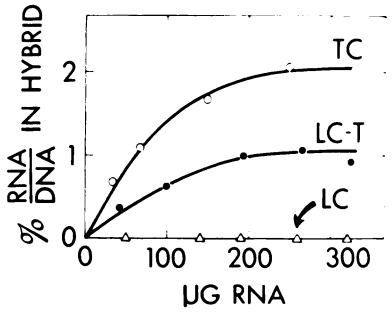


Chart 1. RNA-$^3$H synthesized from rat liver chromatin (*LC*), from rat liver chromatin activated by 2.5 mg of Walker tumor nonhistone protein fraction (*LC-T*), and from Walker tumor chromatin (*TC*) were annealed to 1 μg of rat liver DNA saturated with unlabeled liver chromatin RNA. In all cases, chromatin equivalent to 1.5 mg of DNA was used. A 1% hybrid formation represents about 200 cpm annealed to DNA.

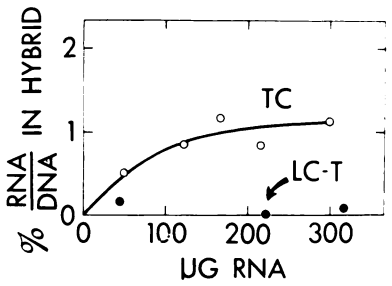


Chart 2. RNA-$^3$H synthesized from rat liver chromatin activated by 2.5 mg of Walker tumor nonhistone protein fraction (*LC-T*), and from Walker tumor chromatin (*TC*) were annealed to 1 μg of rat liver DNA saturated with unlabeled RNA transcribed from liver chromatin activated by tumor nonhistone proteins. The amount of each chromatin was equivalent to 1.5 mg DNA.

chromatin in the presence of Walker tumor nonhistone protein fraction), and tumor RNA (RNA transcribed from Walker tumor chromatin). As can be seen, annealing of the filter with $^3$H-labeled liver RNA did not result in additional hybrid formation, demonstrating that the DNA filters were saturated with unlabeled liver RNA. Annealing of the filters with tumor protein-activated RNA resulted in 1% hybrid formation, while hybridization of the filters with tumor RNA gave additional 2% hybrid formation.

If the tumor nonhistone fraction alters transcription of liver chromatin to that characteristic of the tumor, *i.e.*, a specificity in the activation by nonhistone proteins, the activated transcript should compete with tumor RNA. However, since the additional hybrid formed between activated RNA and liver RNA-saturated DNA is 1%, the competition should be partial. It follows, then, that the expected additional hybrid formed by annealing DNA saturated with tumor protein-activated RNA to tumor RNA should be less than 2%. As shown in Chart 2, when liver DNA double saturated with unlabeled liver RNA and tumor protein-activated RNA was further annealed with tumor RNA, the additional hybrid formation was lowered from 2 to 1.1%. These results indicate that part of the activated transcript is, by the criteria of DNA-RNA hybridization, indistinguishable from tumor RNA and that this RNA is transcribed from liver chromatin as a result of activation by tumor nonhistone protein fraction, previously absent from liver chromatin transcript.

**Specificity of Nonhistone Protein Fraction in the Activation of Transcription of Chromatin.** If, as the foregoing data suggest, the tumor nonhistone proteins are capable of augmenting DNA sequences in liver chromatin specified by the tumor protein fraction, similar experiments with Walker tumor chromatin and rat liver nonhistone proteins should also exhibit an altered transcription of tumor chromatin partially characterized by normal liver transcription. Charts 3 and 4 summarize such results. As shown in Chart 3, the control curve demonstrates that Walker tumor DNA saturated with unlabeled tumor RNA did not allow hybrid formation with $^3$H-labeled tumor RNA. Annealing of tumor DNA that had been saturated with tumor RNA to $^3$H-labeled liver RNA yielded an additional 6% hybrid formation, agreeing with previously reported data (14). When the tumor RNA-saturated DNA filters were annealed with $^3$H-labeled liver

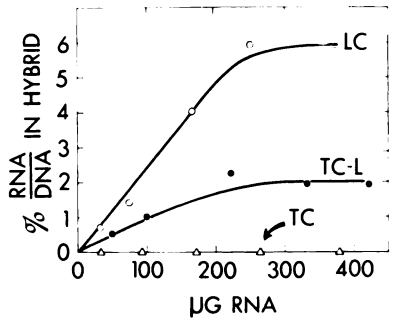


Chart 3. RNA-$^3$H synthesized from Walker tumor chromatin (*TC*), from Walker tumor chromatin activated by 2.5 mg of rat liver nonhistone protein fraction (*TC-L*), and from rat liver chromatin (*LC*) were annealed to 1 μg of tumor DNA saturated with unlabeled tumor chromatin transcript. Each chromatin used was equivalent to 1.5 mg DNA.

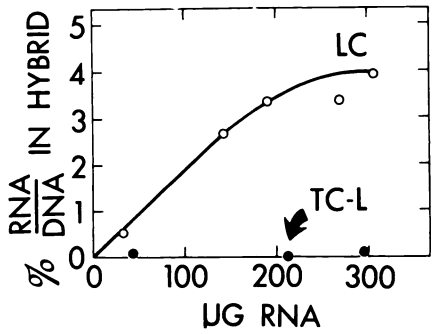


Chart 4. RNA-$^3$H synthesized from Walker tumor chromatin activated by 2.5 mg of liver nonhistone protein fraction (*TC-L*) and from rat liver chromatin (*LC*) were annealed to Walker tumor DNA saturated with unlabeled transcript of tumor chromatin activated by liver nonhistone proteins. The chromatin used in each case was equivalent to 1.5 mg DNA.

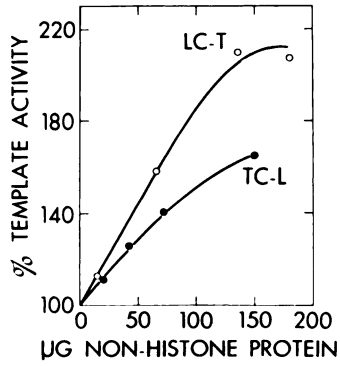


Chart 5. Chromatin-templated RNA synthesis activated by heterologous nonhistone proteins with the use of homologous RNA polymerase. The assay mixture and conditions were as described in "Materials and Methods," except that 0.1 unit of Walker tumor RNA polymerase (•) and 0.2 unit of rat liver RNA polymerase (○) were used. *LC-T*, template activity of rat liver chromatin activated by Walker tumor nonhistone proteins; *TC-L*, template activity of Walker tumor chromatin activated by rat liver nonhistone proteins. *Abscissa*, amounts of each nonhistone protein used. Controls in which either rat liver chromatin or Walker tumor chromatin was used without the addition of nonhistone proteins are taken as 100% template activity.

protein-activated RNA (RNA transcribed from Walker tumor chromatin activated by rat liver nonhistone protein fraction), there was an additional 2% hybrid formation. By similar reasoning, if the liver nonhistone proteins alter transcription of tumor chromatin to that characteristic of rat liver, the 2% hybrid formed by liver protein-activated RNA with tumor RNA-saturated tumor DNA should contain RNA species of the liver transcript. It should then partially compete with liver RNA, resulting in less than 6% hybrid formation. The result shown in Chart 4 of tumor DNA that had been double saturated with tumor RNA and liver protein-activated RNA, when annealed with liver RNA, shows only 4% additional hybrid, as was expected.

**Template Activities of Tumor and Liver Chromatins with Homologous RNA Polymerase.** The activated transcription of chromatin by heterologous nonhistone fractions described above and reported previously (15, 27) has been observed, irrespective of whether *M. luteus* RNA polymerase or homologous enzyme is used. In homologous RNA polymerase

Table 1
*Effect of RNA isolated from nonhistone protein fractions in chromatin-templated RNA polymerase reaction*

| Source of RNA polymerase[a] | Template[b] | Source of RNA | RNA added (μg) | UMP-$^3$H incorporated (cpm) |
|---|---|---|---|---|
| Walker tumor | Walker tumor chromatin | Rat liver nonhistone protein fraction | 0.0 | 1357 |
| | | | 0.1 | 1357 |
| | | | 2.0 | 1320 |
| | | | 4.0 | 1325 |
| | | | 10.0 | 1289 |
| Rat liver | Rat liver chromatin | Walker tumor nonhistone protein fraction | 0.0 | 2008 |
| | | | 0.1 | 2019 |
| | | | 2.0 | 2007 |
| | | | 4.0 | 1988 |
| | | | 10.0 | 1955 |

[a] Enzyme 12 μg, was used; assay was as described in "Materials and Methods."
[b] Chromatin equivalent to 20 μg DNA was used.

reaction with either Walker tumor chromatin or rat liver chromatin as the template, it is shown (Chart 5) that nonhistone fraction stimulates template activities of the chromatin. This result, in conjunction with that of the differential activation by nonhistone proteins (15, 27), indicate specificity of the nonhistone proteins.

**The Effect of Nonhistone Fraction RNA on Template Activity of Chromatin.** In describing the above results, the term "nonhistone protein fraction" was used to denote a chromosomal protein fraction which also contains RNA (15, 26). Bulk RNA isolated from unfractionated rat liver and Walker tumor nonhistone proteins does not stimulate transcription *in vitro* (15, 26). However, in view of the report that a RNA fraction obtained from chick liver chromatin stimulates chromatin-templated RNA synthesis *in vitro* (11), the RNA of the nonhistone fraction was investigated as to its effect on chromatin transcription. As shown in Table 1, RNA isolated from the nonhistone fraction does not stimulate chromatin-templated RNA synthesis *in vitro*. This result thus rules out the possibility that the RNA alone is the causal agent in activation of transcription.

## DISCUSSION

Thus far, in studies on tissue specificity of the nonhistone proteins (8, 15, 27), on activation of transcription of chromatin, and on hybridization of the activated RNA (10, 15, 26, 27) *M. luteus* RNA polymerase has been used. Expediency for using a bacterial enzyme is apparent in that sufficient RNA synthesis is required for DNA-RNA hybridization studies. Although the specific and differential activation of chromatin transcription by nonhistone proteins (5, 27) rules out the possibility that the enzyme used is the contributing factor, one could, nevertheless, ask whether such gene activation by nonhistone proteins can be duplicated in a homologous RNA polymerase reaction. With the available procedure of Roeder and Rutter (21), partially purified RNA polymerase from rat liver and Walker tumor, while insufficient for synthesizing RNA for hybridization study, has been used to ascertain the activation of template activity of chromatin.

The ineffectiveness of the RNA moiety of the nonhistone protein fraction on template activity of chromatin clearly shows that "gene activation" by the acidic chromosomal proteins is not the effect of its RNA but of the nonhistone proteins. Since the nonhistone protein fraction is heterogeneous, the only other possibility that the RNA could be involved in effecting the transcription would be in the form of ribonucleoprotein. Whether this chromosomal RNA in association with protein(s) plays a role in transcription remains to be explored by future experiments.

The present data showing that RNA's transcribed from chromatins activated by heterologous nonhistone proteins contain different RNA species than found in normal transcript of chromatin agree with our previous findings (15, 27). The results indicate that the nonhistone proteins can modify the transcriptional characteristics of chromatin to that of the "modifier" tissue from which they were isolated. These results and those reported previously (15, 27) thus show that the nonhistone proteins are regulatory molecules involved in the control of gene expression.

Tissue specificity of nonhistone proteins has been demonstrated in normal organs (8, 12, 15, 25, 27) and, in this work, Walker 256 carcinosarcoma. Results presented in this paper indicate tissue specificity for the nonhistone proteins on the transcriptional level. The partial effect of the nonhistone proteins on transcriptional transformation in Walker tumor reflects specific fractional changes of the total characteristics of the tumor. It is therefore not indicative of total transformation of neoplasm to normal tissue, or *vice versa*. In addition, the complexity of the nonhistone proteins and the lack of a fractionation procedure yielding biologically active components of relative simplicity make such a study difficult in determining the regulatory mechanism. However, the present results demonstrate a role for the nonhistone proteins in one of the control mechanisms in gene expression.

## REFERENCES

1. Allfrey, V. G., Littau, G., and Mirsky, A. E. On the Role of Histones in Regulating Ribonucleic Acid Synthesis in the Cell Nucleus. Proc. Natl. Acad. Sci. U. S., *49:* 414–421, 1963.
2. Burton, K. A. A Study on the Conditions and Mechanism of the

Diphenylamine Reaction for the Colorimetric Estimation of Deoxyribonucleic Acid. Biochem. J., *62:* 315–323, 1956.
3. Busch, H., and Steele, W. J. Nuclear Proteins of Neoplastic Cells. Advan. Cancer Res., *8:* 45–47, 1964.
4. Chauveau, J., Moulé, Y., and Roueller, C. Isolation of Pure and Unaltered Liver Nuclei. Morphology and Biochemical Composition. Exptl. Cell Res., *11:* 317–321, 1956.
5. Dastugue, B., Tichonicky, L., Penit-Soria, J., and Kruh, J. Comparaison des Protéines de la Chromatine, du Noyau et du Cytoplasme, et des RNA de Trois Types de Cellules Differenciées. Bull. Soc. Chim. Biol., *52:* 391–403, 1970.
6. Georgiev, G. P. Histones and the Control of Gene Action. Ann. Rev. Genet., *3:* 155–180, 1969.
7. Gillespie, D., and Spiegelman, S. A Quantitative Assay for DNA-RNA Hybrids with DNA Immobilized on a Membrane. J. Mol. Biol., *12:* 829–842, 1965.
8. Gilmour, R. S., and Paul, J. Role of Non-histone Components in Determining Organ Specificity of Rabbit Chromatins. Federation European Biochem. Soc. Letters, *9:* 242–244, 1970.
9. Huang, R. C. C., and Bonner, J. Histone, a Suppressor of Chromosomal RNA Synthesis. Proc. Natl. Acad. Sci. U. S., *48:* 1216–1222, 1962.
10. Kamiyama, M., and Wang, T. Y. Activated Transcription from Rat Liver Chromatin by Non-histone Proteins. Biochim. Biophys. Acta, *228:* 563–576, 1971.
11. Kanehisa, T., Fujitani, H., Sano, M., and Tanaka, T. Studies on Low Molecular Weight RNA of Chromatin. Effect on Template Activity of Chick Liver Chromatin. Biochim. Biophys. Acta, *240:* 46–55, 1971.
12. Kleinsmith, L. J., Heidema, J., and Carroll, A. Specific Binding of Rat Liver Nuclear Proteins to DNA. Nature, *226:* 1025–1026, 1970.
13. Kostraba, N. C., and Wang, T. Y. Tissue Variations of Acidic Nuclear Proteins and Their Biosynthesis during Liver Regeneration. Intern. J. Biochem., *1:* 327–334, 1970.
14. Kostraba, N. C., and Wang, T. Y. Transcription of Walker 256 Carcinosarcoma Chromatin. Cancer Res., *31:* 1663–1668, 1971.
15. Kostraba, N. C., and Wang, T. Y. Differential Effect of Chromatin Transcription by Non-histone Fractions. Biochim. Biophys. Acta, *262:* 169–180, 1972.
16. Langan, T. A. A Phosphoprotein Preparation from Liver Nuclei and Its Effect on the Inhibition of RNA Synthesis by Histones. *In:* V. V. Koningsberger and L. Bosch (eds.), Regulation of Nucleic Acid and Protein Synthesis, pp. 233–241. Amsterdam: Elsevier Publishing Co., 1967.
17. Loeb, J. E., and Creuzet, C. Electrophoretic Comparison of Acidic Proteins of Chromatin from Different Animal Tissues. Federation European Biochem. Soc. Letters, *5:* 37–40, 1969.
18. Melli, N., and Bishop, J. Hybridization between Rat Liver DNA and Complementary RNA. J. Mol. Biol., *40:* 117–136, 1969.
19. Nakamoto, T., Fox, F. C., and Weiss, S. B. Enzymatic Synthesis of Ribonucleic Acid. I. Preparation of Ribonucleic Acid Polymerase from Extracts of *Micrococcus lysodeikticus.* J. Biol. Chem., *239:* 169–174, 1964.
20. Platz, R. D., Kish, V. M., and Kleinsmith, L. J. Tissue Specificity of Non-histone Chromatin Phosphoproteins. Federation European Biochem. Soc. Letters, *12:* 38–40, 1970.
21. Roeder, R. G., and Rutter, W. J. Multiple Forms of DNA-dependent RNA Polymerase in Eukaryotic Organisms. Nature, *224:* 234–237, 1969.
22. Seligy, V., and Miyagi, M. Studies of Template Activity of Chromatin Isolated from Metabolically Active and Inactive Cells. Exptl. Cell Res., *58:* 27–34, 1969.
23. Stedman, E., and Stedman, E. Probable Function of Histones as a Regulator of Mitosis. Nature, *152:* 556–557, 1943.
24. Tan, C. H., and Miyagi, M. Specificity of Transcription of Chromatin *in vitro.* J. Mol. Biol., *50:* 641–653, 1970.
25. Teng, C. S., Teng, C. T., and Allfrey, V. G. Studies of Nuclear Acidic Proteins. J. Biol. Chem., *246:* 3597–3609, 1971.
26. Wang, T. Y. Activation of Transcription *in vitro* from Chromatin by Non-histone Proteins. Exptl. Cell Res., *61:* 455–457, 1970.
27. Wang, T. Y. Tissue Specificity of the Non-histone Proteins. Exptl. Cell Res., *69:* 217–219, 1971.