Inhibition of Chicken Myeloblastosis RNA Polymerase II Activity by Adriamycin†

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ABSTRACT: In vitro RNA synthesis by isolated RNA polymerase II of chicken myeloblastosis cells was shown to be highly sensitive to adriamycin inhibition. The template activity of the single-stranded DNA, purified by chromatography of denatured calf thymus DNA through hydroxylapatite columns, was found to be equally as sensitive to the inhibition as denatured calf thymus DNA. However, contrary to denatured DNA, the single-stranded DNA thus purified showed no significant binding to adriamycin as analyzed by cosedimentation of the drug and DNA through a sucrose gradient. This indicated that inhibition of RNA synthesis on a single-stranded DNA template might involve a mechanism other than DNA intercalation. Kinetic studies of the inhibition showed that the inhibition of RNA synthesis by adriamycin could not be reversed by increasing the concentrations of RNA polymerase and four nucleoside triphosphates, but it could be reversed by increasing DNA concentrations. Analysis of the size of RNA synthesized indicated that the ultimate size of the product RNA was not altered by adriamycin, suggesting that the drug may inhibit RNA synthesis by reducing RNA chain initiation.

The anthracycline antibiotic adriamycin is a 14-hydroxy derivative of daunorubicin (Figure 1). This structural modification has resulted in improved therapeutic effectiveness for adriamycin over daunorubicin, despite the fact that cellular drug uptake for daunorubicin is approximately twice that of adriamycin (Di Marco, 1975; Noel et al., 1978). Adriamycin has a wide spectrum of antineoplastic activity that induces objective tumor responses in a number of solid tumors as well as in acute leukemias and malignant lymphomas, whereas daunorubicin has a narrow therapeutic index that has shown maximal activity only toward acute leukemia (Blum & Carter, 1974). The antitumor activity of these drugs has been attributed to their ability to bind tightly with DNA and thereby to inhibit DNA and RNA synthesis (Calendi et al., 1965; Goodman et al., 1974; Zunino et al., 1974, 1975a; Mizuno et al., 1975). It has been proposed that intercalation occurs between the adjacent base pairs of a DNA helix (Ward et al., 1965; Pigram et al., 1972; Calabresi & Parks, 1975).

Previous studies (Zunino et al., 1975a) have shown that adriamycin inhibits *Escherichia coli* RNA polymerase activity and RNA synthesis of whole rat liver nuclei. In either of these cases, native or double-stranded DNA was the active template for the enzyme. Our studies on chicken myeloblastosis leukemic cells revealed that more than 97% of RNA polymerase activity of the cells belongs to the class II (or B) eukaryotic RNA polymerase (Chuang et al., 1975). This class of RNA polymerase has the general property of using denatured DNA as a preferential template for transcription (Roeder & Rutter, 1969; Chuang et al., 1975). Inasmuch as denatured DNA would be expected to provide much less opportunity for drug intercalation, we asked whether the activity of chicken leukemic RNA polymerase could be affected by adriamycin. This report describes the inhibition of chicken leukemic RNA polymerase II activity by adriamycin and the possible biochemical mechanism of the inhibition.

Materials and Methods

Adriamycin (doxorubicin hydrochloride) was manufactured by Farmitalia SPA (Italy) and supplied by Adria Laboratories (Wilmington, DE), or it was obtained from Aldrich Chemical Co. (Milwaukee, WI). Daunorubicin (NSC-82151, daunomycin hydrochloride) was obtained from the Drug Research and Development Branch, National Cancer Institute (Bethesda, MD). Solutions of the drugs were freshly prepared immediately before use. Hydroxylapatite was purchased from Bio-Rad Laboratories (Richmond, CA). Unlabeled nucleoside triphosphates and dithiothreitol (DTT[1]) were purchased from P-L Biochemicals (Milwaukee, WI). [^{3}H]UTP and [^{3}H]CTP were obtained from New England Nuclear (Boston, MA). *Escherichia coli* RNA polymerase (fraction IV) was purchased from Grand Island Biological Co. (Santa Clara, CA). Calf thymus DNA and chicken blood DNA were from Calbiochem (Los Angeles, CA). Denatured DNA was prepared by heating the native DNA at 100 °C for 6 min and quick-cooling in an acetone–dry ice bath.

Preparation of Chicken Myeloblastosis RNA Polymerase II. Chicken myeloblastosis (leukemic) cells were generously provided by Drs. A. J. Langlois and D. P. Bolognesi, Duke University Medical Center, Durham, NC. DNA-dependent RNA polymerase was solubilized from the leukemic cells, fractionated through DEAE-Sephadex column chromatography, and further purified by glycerol gradient centrifugation. RNA polymerase II (subspecies IIa and IIb) thus purified (Chuang et al., 1975) was assayed in the presence of adriamycin and it was later found that enzymes IIa and IIb were equally sensitive to adriamycin inhibition. RNA polymerase IIa, which represents the major species of RNA polymerase II in chicken myeloblastosis cells, was therefore used for the present studies.

Assay for RNA Polymerase Activity. The standard assay system (0.1 mL) contained 50 mM Tris-HCl (pH 7.9), 2 mg of pyruvate kinase, 4 mM phosphoenolpyruvate, 1 mM $MnCl_2$, 10 mM KCl, 1 mM DTT, 0.2 mM each of ATP, GTP, and CTP, 0.04 mM UTP and [^{3}H]UTP (600–700 cpm/pmol), 40 μg of denatured calf thymus DNA or denatured chicken blood DNA and chicken myeloblastosis RNA polymerase (20 μg/mL). After incubation for 45 min at 37 °C, the reactions were stopped by adding 0.1 mL of cold 0.1 M sodium pyrophosphate (pH adjusted to 7.0) containing RNA, 2 mg/mL, bovine serum albumin, 2 mg/mL, 5 mM UTP, and 30% trichloro-

† From the Department of Biochemistry, Schools of Medicine and Dentistry, Oral Roberts University, Tulsa, Oklahoma 74171. *Received December 27, 1978.* This work was supported in part by Grant CA 21982 from the National Institutes of Health. A portion of this work was done at the University of California, Davis, CA, and presented at the annual meeting of Federation of American Societies for Experimental Biology, Chicago, IL [(1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36*, 3218].

[1] Abbreviations used: DTT, dithiothreitol; AD 32, *N*-trifluoroacetyladriamycin 14-valerate.

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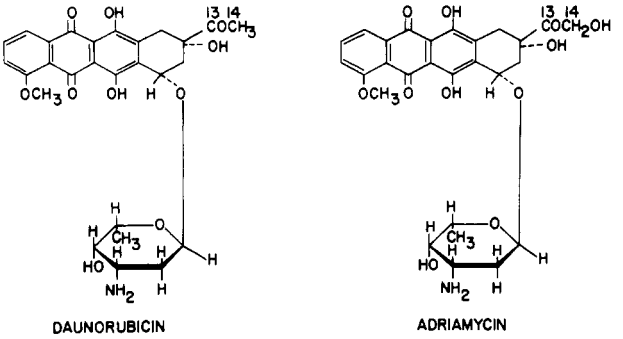

FIGURE 1: Structural relationship of adriamycin and daunorubicin.

acetic acid, 0.5 mL. Acid-precipitable radioactivity was collected on Whatman GF/C filters and washed more than ten times with 5% trichloroacetic acid. Filters were then dried and counted in a scintillation counter.

Preparation of Single-Stranded Calf Thymus DNA. To prepare single-stranded DNA, hydroxylapatite column chromatography was used. Single- and double-stranded DNAs have different affinities for hydroxylapatite (Kohne & Britten, 1971). Adsorption of DNAs to hydroxylapatite was controlled by phosphate ion concentration. Isolation of single-stranded DNA from denatured DNA (which contained portions of double strandedness) was done as follows: 5 mg of denatured calf thymus DNA was loaded onto a 1.6 × 8.5 cm (60 °C) hydroxylapatite column and the column was washed with 0.01 M phosphate buffer, pH 6.8. Stepwise elution of the column with 0.14 and 0.4 M phosphate buffer, pH 6.8, resolved single-stranded DNA (0.14 M) from double-stranded DNA (0.4 M). The single-stranded DNA was dialyzed against 2 L of 0.005 M Tris-HCl, pH 7.9 at 4 °C for 24 h. After dialysis, NaCl was added to 0.4 M and the DNA was precipitated with 2 volumes of cold ethanol and kept at −20 °C. After centrifugation at 12000 rpm in a Sorval centrifuge for 60 min, the supernatant fraction was discarded and the precipitate was resuspended in H_2O. Approximately 3.2 mg of single-stranded DNA was resolved.

Cosedimentation of DNA and Adriamycin through Sucrose Gradient. DNA (0.3 mg) was incubated with adriamycin (0.1 mg) in 0.02 M phosphate buffer, pH 8.0, at 37 °C for 10 min. After incubation, the mixture was chilled at 0 °C and loaded on a 5–20% sucrose gradient containing 0.2 M phosphate, pH 8.0, and 1 mM EDTA. The gradient was centrifuged at 49000 rpm for 3.5 h in an SW 50.1 rotor. Fractions were collected from the bottom of the gradient. H_2O (0.4 mL) was added to each fraction and absorbencies at 480 nm (due to adriamycin) and at 260 nm (due to DNA and adriamycin) were determined. A_{260} readings plotted in Figure 6 were values contributed by DNA only (the A_{260} readings contributed by adriamycin were calculated from the A_{480} readings and have been subtracted).

Formaldehyde/Sucrose Gradient Centrifugation. The size of RNA synthesized in an RNA polymerase reaction was analyzed by the formaldehyde/sucrose gradient centrifugation procedure as we previously described (Chuang & Chuang, 1975). Samples (0.2 mL) containing RNA were added to 20 μL of 5% sodium dodecyl sulfate. After incubation for 3 min at 37 °C, the samples were made up to 0.3 mL in a solution containing 16 mM sodium phosphate buffer (pH 7.7) and 1.1 M formaldehyde and incubated for 15 min at 65 °C. The chilled, formaldehyde-treated sample was layered directly on a 5-mL 10–30% linear sucrose gradient containing 0.1 M sodium phosphate buffer (pH 7.7) and 1.1 M formaldehyde. The sucrose was pretreated with diethyl pyrocarbonate

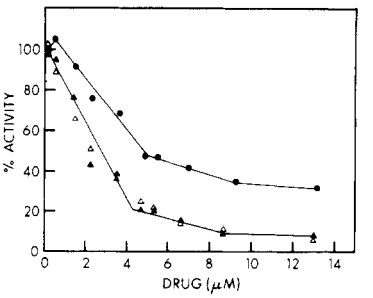

FIGURE 2: Effect of adriamycin and daunorubicin on chicken myeloblastosis RNA polymerase activity. Chicken myeloblastosis RNA polymerase II was assayed for activity in the presence of various concentrations of adriamycin (▲) or daunorubicin (●) as indicated. The reaction mixture and assay conditions were as described under Materials and Methods, except that 0.5 μg of denatured chicken blood DNA was used per each assay. Control (100%) activity was 10.8 pmol. For comparison, effect of adriamycin on *Escherichia coli* RNA synthesis (△) was also studied. The reaction mixture for *E. coli* enzyme was similar to those described above, except that it contained *E. coli* RNA polymerase (0.25 unit), 4 mM $MgCl_2$, 5 mM DTT, and native *E. coli* B DNA (Sigma), and the control activity was 15.1 pmol.

(Solymosy et al., 1968) to inactivate any nuclease present. After centrifugation at 35000 rpm in an SW50.1 rotor at 16 °C for 18 h, the gradients were fractionated by dripping the solution from the bottom of the tube. Fractions were collected in tubes containing 0.2 mg of bovine serum albumin and 0.2 mg of yeast RNA and precipitated by adding 0.5 mL of 30% trichloroacetic acid. The precipitates were collected and washed with 5% trichloroacetic acid, and their radioactivity was determined. 4S and 18S marker RNAs were processed like the samples and centrifuged in a parallel gradient. Absorbance at 260 nm of the fractions collected from the marker gradients was read in a spectrophotometer. 4S tRNA was prepared from *Bacillus subtilis* by a procedure described previously (Chuang & Doi, 1972). The 18S RNA was a byproduct of hemoglobin mRNA preparation (Aviv & Leder, 1972).

Results

Inhibition of RNA Polymerase II Activity by Adriamycin. To study whether RNA synthesis on a denatured DNA template could be affected by adriamycin, chicken leukemic DNA-dependent RNA polymerase II was assayed in the presence of various concentrations of adriamycin. It was found that in vitro RNA synthesis by RNA polymerase II was indeed sensitive to adriamycin inhibition (Figure 2). In addition, adriamycin inhibited RNA polymerase II activity on denatured DNA template to the same extent as it inhibited *Escherichia coli* RNA polymerase activity on a native DNA template. Daunorubicin was less potent in inhibiting leukemic RNA polymerase II than adriamycin (Figure 2).

Kinetics of the Inhibition. Studies on the kinetic nature of the inhibition indicated that adriamycin inhibition of RNA polymerase II activity could not be reversed by increasing either the concentrations of four ribonucleoside triphosphates (Figure 3) or the concentration of enzyme (data not shown). However, similar to the effect of daunorubicin inhibition of RNA synthesis (Zunino et al., 1974), it could be reversed by increasing the concentration of template DNA (Figure 4).

Effect of Adriamycin on Single-Stranded DNA Template Activity for RNA Synthesis. To determine whether the inhibition by adriamycin of RNA polymerase II activity on a denatured template was simply a result of insertion of the drug molecule into reannealed double-stranded regions of the denatured DNA, we tested the inhibitory activity of adria-

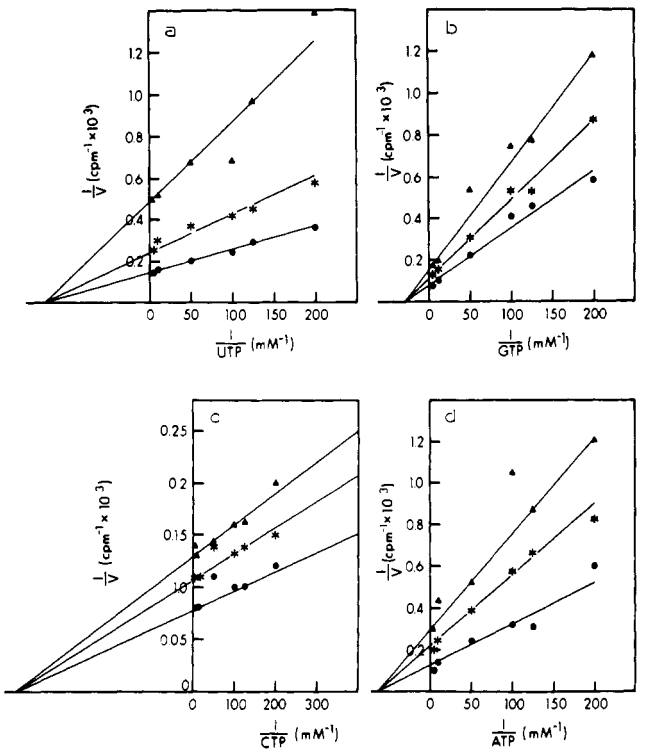

FIGURE 3: Effect of increasing substrate concentration on the inhibition of RNA synthesis by adriamycin (Lineweaver–Burk plot). The reaction mixture was similar to that described under Materials and Methods, except that the four ribonucleoside triphosphates used were varied as follows: (a) 0.2 mM each of ATP and GTP, 0.04 mM CTP and [^{3}H]CTP (8 μCi) and varied UTP concentration; (b) 0.2 mM each of ATP and CTP, 0.04 mM UTP and [^{3}H]UTP (12 μCi) and varied GTP concentration; (c) 0.2 mM each of ATP and GTP, 0.04 mM UTP and [^{3}H]UTP (12 μCi) and varied CTP concentration; (d) 0.2 mM each of GTP and CTP, 0.04 mM UTP and [^{3}H]UTP (12 μCi) and varied ATP concentration. Adriamycin was added in each reaction mixture as follows. [a and d] (●) No drug; (*) 52 μM; (▲) 86 μM. [b and c] (●) No drug; (*) 34 μM; (▲) 69 μM. Denatured chicken blood DNA (40 μg) was used per each assay and incubation was at 37 °C for 15 min. The reaction was terminated and the RNA precipitated, filtered, and counted as described under Materials and Methods. The Lineweaver–Burk plot of the data indicates noncompetition between adriamycin and the four ribonucleoside triphosphates.

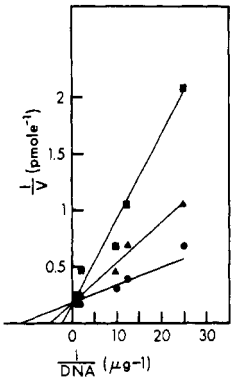

FIGURE 4: Effect of increasing DNA concentration on the inhibition of RNA synthesis by adriamycin (Lineweaver–Burk plot). The reaction mixture was as described under Materials and Methods, except that RNA polymerase was assayed in the presence of various concentrations of denatured chicken blood DNA, with or without the addition of adriamycin. The specific activity for [^{3}H]UTP was 1654 cpm/pmol and $1/v$ shown on the ordinates is the reciprocal of pmol of UMP incorporated into RNA per 0.1 mL of reaction mixture per 15 min. (●) No drug added; (▲) 1.30 μM adriamycin; (■) 2.15 μM adriamycin. The Lineweaver–Burk plot of the data indicates competition between adriamycin and DNA.

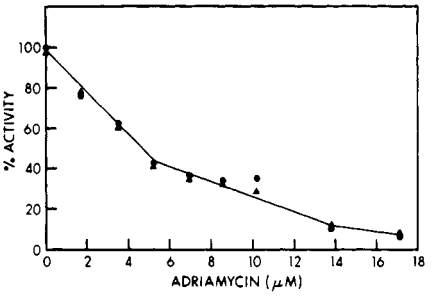

FIGURE 5: Effect of adriamycin on single-stranded calf thymus DNA template activity. RNA polymerase II was assayed in the presence of various concentrations of adriamycin when single-stranded calf thymus DNA (●) or denatured calf thymus (▲) was used as template. Single-stranded calf thymus DNA was prepared by purifying the denatured DNA through two consecutive hydroxylapatite columns. The assay conditions were similar to those described under Materials and Methods, except that DNA used was 1 μg/assay. The control (100%) activity at 1 μg of single-stranded DNA or denatured DNA was 9.09 pmol.

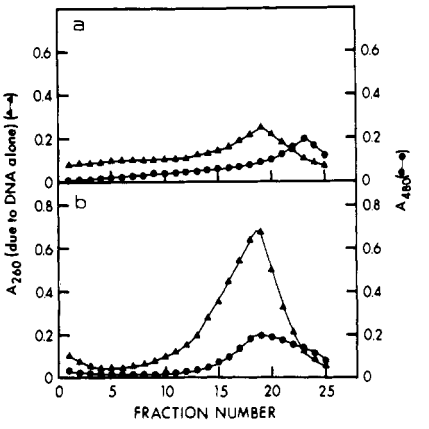

FIGURE 6: Binding of DNA with adriamycin. (a) Single-stranded calf thymus DNA and adriamycin; (b) denatured calf thymus DNA and adriamycin. The experimental procedures were as described under Materials and Methods.

mycin on a single-stranded DNA template. Single-stranded calf thymus DNA was prepared by purification of denatured calf thymus DNA through a hydroxylapatite column as described under Materials and Methods. The DNA obtained from the single-stranded region of the hydroxylapatite column, which represented 87% of total recovered DNA, was further purified by chromatography on a second hydroxylapatite column. DNA resolved from the single-stranded region of the second hydroxylapatite column represented 96% of total recovered DNA from the column. The single-stranded DNA thus obtained from the second hydroxylapatite column contained no detectable double strandedness as determined by a third hydroxylapatite column chromatography and was used for RNA polymerase assay. It was found that the single-stranded DNA served equally as well as denatured DNA as a template for leukemic RNA polymerase II: the DNA saturation curves for the enzyme activity were the same for the two DNA templates and the amounts of RNA synthesized at various concentrations of DNA were also the same for these two DNAs. Furthermore, as shown in Figure 5, both single-stranded DNA and denatured DNA were equally sensitive to adriamycin inhibition. However, adriamycin showed little, if any, binding to single-stranded DNA (Figure 6a) when analyzed by centrifugation through a 5–20% sucrose gradient, although it was found to bind to denatured DNA possibly by intercalation (Figure 6b). This finding was further substantiated by cochromatography of adriamycin with DNA on

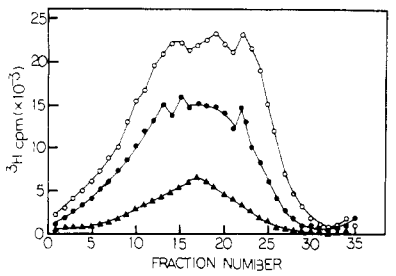

FIGURE 7: Effect of adriamycin on RNA chain length (formaldehyde/sucrose gradient centrifugation). The reaction mixture was as described under Materials and Methods, except that the assay volume was scaled up to 0.2 mL and the specific activity of [^{3}H]UTP was scaled up to 13 974 cpm/pmol. Denatured calf thymus DNA was used as template and the size of RNA synthesized by RNA polymerase II as shown in the figure was between 4 S and 18 S. (○) No drug; (●) 51 μM adriamycin; (▲) 103 μM adriamycin.

a hydroxylapatite column, which showed that the drug could be eluted off the column in conjunction with either native DNA or denatured DNA (with much less drug/DNA ratio) but not with purified single-stranded DNA (data not shown). These observations correlate with the report of Tsou & Yip (1976) that single-stranded synthetic polynucleotide had little effect on the fluorescence of adriamycin. Therefore, these data suggest that the inhibition of RNA polymerase II activity by adriamycin probably involves mechanisms other than direct drug–DNA interaction.

Size of RNA Synthesized in the Presence of Adriamycin. The size of the RNA chains, synthesized in the presence of adriamycin, was determined by sucrose gradient sedimentation in formaldehyde. The results (Figure 7) showed that the size of RNA synthesized by RNA polymerase II is between 4 S and 18 S, and this chain length of RNA was not influenced by the inclusion of various concentrations of adriamycin. The data suggest the possibility that adriamycin reduces frequency of initiation of RNA chains rather than affects RNA polymer length.

Discussion

The above studies demonstrated that leukemic RNA synthesis is highly sensitive to adriamycin inhibition. Zunino et al. (1975a) have shown that adriamycin inhibited the activity of *Escherichia coli* RNA polymerase which used native DNA as template. Our studies revealed that adriamycin inhibited leukemic RNA polymerase II activity on denatured DNA template to the same extent as it inhibited *Escherichia coli* RNA polymerase on a native DNA template. The observation that daunorubicin had a less inhibitory effect on RNA polymerase II than adriamycin correlated with the clinical results of a greater therapeutic index for adriamycin. Adriamycin was reported to inhibit the activities of DNA polymerases (Goodman et al., 1974; Zunino et al., 1975b) and RNA polymerases (Zunino et al., 1975a), presumably by the mechanism of insertion of the drug molecule into the double helix of DNA, as has been demonstrated for daunorubicin (Ward et al., 1965; Pigram et al., 1972; Phillips et al., 1978). Our studies showed that single-stranded calf thymus DNA had significantly less binding with adriamycin as compared with denatured calf thymus DNA, yet that it had the same sensitivity as denatured calf thymus DNA to adriamycin inhibition. These data suggest that inhibition of eukaryotic RNA polymerase II activity in vitro by adriamycin may be resulting from a mechanism other than DNA intercalation.

N-Trifluoroacetyladriamycin 14-valerate (AD 32), an analogue of adriamycin, has been reported to have antitumor activity significantly greater than adriamycin (Israel et al., 1975; Krishan et al., 1976; Parker et al., 1978; Vecchi et al., 1978). AD 32 differs from adriamycin in having a 5-carbon straight-chain ester function at the 14-carbinol position and trifluoroacetyl substitution on the glycosidic amino group. AD 32 was found not to bind to calf thymus DNA in vitro due to the trifluoroacetyl substitution of amino group of glucosamine which has been demonstrated to be essential for DNA intercalation (Krishan et al., 1976). It appears that either AD 32 is metabolized to a DNA-binding metabolite, adriamycin, or its cytotoxic effect is not a consequence of DNA binding. Recent studies that analyzed the metabolism of AD 32 by thin-layer chromatography–fluorometry and high-performance liquid chromatography indicated that adriamycin was not a metabolite of AD 32 (Israel et al., 1978). These studies, along with ours, indicate that the tetracycline ring structure of the anthracycline compounds, and mechanisms other than DNA binding, may also contribute to the cytotoxic activities of the drugs.

At present, the mechanism by which adriamycin inhibits RNA polymerase II activity in vitro without its binding to DNA template is not clear. In analyzing the size of RNA synthesized, it was found that, contrary to cytosine arabinoside inhibition of RNA synthesis (Chuang & Chuang, 1976a) in which the drug caused a premature termination of RNA chains (Chuang & Chuang, 1976b), adriamycin at various concentrations did not change the sedimentation profile of product RNA, suggesting the possibility that it reduces RNA chain initiation without affecting RNA chain length. The competitive nature of the inhibition with regard to DNA leads us to speculate that adriamycin competes with DNA for a DNA-binding site (Krakow et al., 1976) on the enzyme (RNA polymerase II) molecule and subsequently interferes with proper RNA chain initiation. Under this hypothesis, as long as the DNA/drug ratio remains constant in an assay, increasing concentrations of either the four ribonucleoside triphosphates or the enzyme itself will, as we have observed, have no effect on the inhibition. These in vitro studies suggest that adriamycin need not function solely as a DNA-intercalation agent in vivo.

Acknowledgments

We thank Drs. A. J. Langlois and D. P. Bolognesi for generously supplying myeloblastosis cells and Dr. R. H. Doi for helpful discussions.

References

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Blum, R. H., & Carter, S. K. (1974) *Ann. Intern. Med. 80*, 249–259.

Calabresi, P., & Parks, R. E., Jr. (1975) in *The Pharmacological Basis of Therapeutics* (Goodman, L. S., & Gilman, A., Eds.) pp 1288–1290, Macmillan, New York.

Calendi, E., Di Marco, A., Reggiani, M., Scarpinato, B., & Valentini, L. (1965) *Biochim. Biophys. Acta 103*, 25–49.

Chuang, R. Y., & Doi, R. H. (1972) *J. Biol. Chem. 247*, 3476–3484.

Chuang, R. Y., & Chuang, L. F. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2935–2939.

Chuang, R. Y., & Chuang, L. F. (1976a) *Nature (London) 260*, 549–550.

Chuang, R. Y., & Chuang, L. F. (1976b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35*, 1572.

Chuang, R. Y., Chuang, L. F., & Laszlo, J. (1975) *Cancer Res. 35*, 687–693.

Di Marco, A. (1975) *Cancer Chemother. Rep. 59*, 91–105.

Goodman, M. F., Bessman, M. J., & Bachur, N. R. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1193–1196.
Israel, M., Modest, E. J., & Frei, E., III (1975) *Cancer Res. 35*, 1365–1368.
Israel, M., Wilkinson, P. M., Pegg, W. J., & Frei, E., III (1978) *Cancer Res. 38*, 365–370.
Kohne, D. E., & Britten, R. J. (1971) *Proc. Nucleic Acid Res. 2*, 500–523.
Krakow, J. S., Rhodes, G., & Jovin, T. M. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 127–157, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Krishan, A., Israel, M., Modest, E. J., & Frei, E., III (1976) *Cancer Res. 36*, 2108–2110.
Mizuno, N. S., Zakis, B., & Decker, R. W. (1975) *Cancer Res. 35*, 1542–1546.
Noel, G., Peterson, C., Trouet, A., & Tulkens, P. (1978) *Eur. J. Cancer 14*, 363–368.
Parker, L. M., Hirst, M., & Israel, M. (1978) *Cancer Treat. Rep. 62*, 119–127.
Phillips, D. R., Di Marco, A., & Zunino, F. (1978) *Eur. J. Biochem. 85*, 487–492.
Pigram, W. J., Fuller, W., & Hamilton, L. D. (1972) *Nature (London), New Biol. 235*, 17–19.
Roeder, R. G., & Rutter, W. J. (1969) *Nature (London) 224*, 234–237.
Solymosy, F., Fedorcsák, I., Gulyás, A., Farkas, G. L., & Ehrenberg, L. (1968) *Eur. J. Biochem. 5*, 520–527.
Tsou, K. C., & Yip, K. F. (1976) *Cancer Res. 36*, 3367–3373.
Vecchi, A., Cairo, M., Mantovani, A., Sironi, M., & Spreafico, F. (1978) *Cancer Treat. Rep. 62*, 111–117.
Ward, D. C., Reich, E., & Goldberg, I. H. (1965) *Science 149*, 1259–1263.
Zunino, F., Di Marco, A., Zaccara, A., & Luoni, G. (1974) *Chem. Biol. Interact. 9*, 25–36.
Zunino, F., Gambetta, R., & Di Marco, A. (1975a) *Biochem. Pharmacol. 24*, 309–311.
Zunino, F., Gambetta, R., Di Marco, A., Zaccara, A., & Luoni, G. (1975b) *Cancer Res. 35*, 754–760.

Complexity of Poly(A)-Containing Heterogeneous Nuclear Ribonucleic Acid from Mouse Embryoid Bodies (OTT6050)†

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ABSTRACT: Poly(A+)-hnRNA was isolated from mouse embryoid bodies (OTT6050). We have investigated this RNA with respect to sequence complexity by using a tritiated cDNA probe to poly(A+)-hnRNA ([^{3}H]cDNA$_{hn}$). A comparison was also made with total poly(A+)-RNA from mouse embryoid bodies. Two classes of sequences were found in poly(A+)-hnRNA. They represented 50 and 46% of the sequences in the poly(A+)-hnRNA, which corresponds to 570 and 12 500 different hnRNA sequences, respectively, of an average length of 2700 nucleotides (NT) present on an average of 12 and 1 copies per cell, respectively. The total complexity in poly(A+)-hnRNA was 3.4×10^7 NT. Three classes of sequences could be detected in total poly(A+)-RNA (mainly cytoplasmic), representing 9, 61, and 25%, respectively, of the sequences in its [^{3}H]cDNA probe. This corresponds to 12 000, 620, and 16 copies per cell of poly(A+)-RNA with each class having 2, 140, and 2500 different sequences, respectively, of an average length of 1350 NT. The total complexity of the poly(A+)-RNA was 3.4×10^6 NT. This indicates that the poly(A+)-hnRNA is approximately 10 times more complex with respect to sequence information than the total poly(A+)-RNA. The vast majority of the total poly(A+)-RNA sequences are present in the poly(A+)-hnRNA but in much lower concentration. Total poly(A+)-RNA saturated 65% of the nuclear [^{3}H]cDNA$_{hn}$ probe. This suggests that there are poly(A+)-hnRNA sequences present in the nucleus which have no major detectable counterpart in the total poly(A+)-RNA [90–96% poly(A+)-containing cytoplasmic RNA]. Total poly(A+)-RNA from the more differentiated mouse neuroepithelial teratocarcinomas saturated only 37% of the nuclear [^{3}H]cDNA$_{hn}$ probe from mouse embryoid bodies compared to 65% for the total poly(A+)-RNA from embryoid bodies.

The flow of genetic information from the nucleus to the cytoplasm in eucaryotic cells involves several posttranscriptional events (Getz et al., 1975; Hough et al., 1975; Bantle & Hahn, 1976; Herman et al., 1976). These events include specific nucleolytic cleavage from what are presumed to be high molecular weight primary transcripts (Lee et al., 1971; Greenberg & Perry, 1972; Jelinek et al., 1973) and a posttranscriptional addition of a poly(A) tract at the 3′ end to the high molecular weight nuclear precursor (Darnell et al., 1971; Philipson et al., 1971).

By the use of RNA–DNA hybridization, the amount of genetic information in cytoplasmic and nuclear poly(A+)-RNA can be determined by synthesizing cDNA to the poly(A+)-RNA templates. From such studies of different tissues, poly(A+)-hnRNA seems to have a total sequence complexity which is 5–10 times greater than that found in the poly(A+)-mRNA population (Getz et al., 1975; Bantle & Hahn, 1976; Herman et al., 1976; Levy et al., 1976; Jacquet et al., 1978).

Recently, it has been shown that the genes for globin and ovalbumin contain intervening sequences (IV's) not present

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