Biochemical Pharmacology, Vol. 20, pp. 1029–1033. Pergamon Press, 1971. Printed in Great Britain

STUDIES ON THE EFFECTS OF CHEMICALS ON THE PROCESSING OF NUCLEAR RNA

SOME POSSIBLE IMPLICATIONS WITH RESPECT TO CARCINOGENESIS

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Abstract—Many chemical carcinogens are known to cause segregation of the granular and fibrillar components of the nucleolus, with associated rapid loss of nuclear RNA. The loss of RNA from the nucleus may result from an enhancement of nuclear RNA degradation, as well as an inhibition of nuclear RNA synthesis. Chemical agents such as actinomycin D apparently do not directly enhance the activity of the nuclear exoribonuclease which is believed to be responsible for degradation of newly synthesized nuclear RNA. However, it is suggested that such agents may interfere with the normal mechanisms for processing newly synthesized RNA in the nucleus, so that a proper amount of newly synthesized RNA is not stabilized against degradation. A general hypothesis that a molecular defect in the stabilization of nuclear RNA may be involved in the development of the malignant state is also discussed.

WHEN actinomycin D, aflatoxin B_1, *N*-hydroxy-acetylaminofluorene* or 3′-methyl-4-dimethylaminoazobenzene are given to a rat, a distinctive morphological change rapidly occurs in the nucleolus of liver hepatocytes.[1–4] This ultrastructural change involves separation of the granular and fibrillar components of the nucleolus and has been termed segregation. In this discussion I would like to ask if there is some biochemical process which is common to the phenomenon of nucleolar segregation produced by these chemical agents.

One of the simplest measurements which can be made on isolated nuclei is their total RNA content, and it has been shown in our laboratory that actinomycin D,[5] aflatoxin B_1,[6] *N*-hydroxy-acetylaminofluorene[7] and 3′-methyl-4-dimethylaminoazobenzene[7] all cause rapid depletion of RNA from the liver cell nucleus after intraperitoneal injection.

The mechanism of this phenomenon of nuclear RNA depletion is of great interest, and two principal explanations need to be considered. The first, and most popular current explanation, is that these agents, all of which have been shown to bind to DNA either *in vivo* or *in vitro*,[5,6,8–10] all inhibit RNA synthesis because of their capacity to bind to DNA, and there is indeed good experimental evidence, particularly with actinomycin D[5,8] and aflatoxin B_1,[11] that these agents inhibit RNA polymerase in rat liver. A second possibility, often mentioned, but rarely dealt with experimentally until the recent studies of Farber and his colleagues,[12] is that agents such as actinomycin accelerate the degradation of newly-synthesized RNA in the cell nucleus. Farber and co-workers have indeed recently presented extensive evidence that actinomycin D, but not ethionine, accelerates the degradation of newly-synthesized RNA in the nucleus. Thus, the inhibition of synthesis of RNA in the nucleus, combined with an acceleration of degradation of newly-synthesized RNA, would cause rapid depletion of nuclear RNA.

* T. KAKEFUDA and M. B. SPORN, unpublished observations.

If we wish to understand this entire process further, we must now turn to a consideration of the mechanism whereby newly-synthesized RNA is degraded in the nucleus itself. First of all, it is now apparent from the work of many investigators[13-17] that most of the newly-synthesized RNA of the cell nucleus is destroyed in the nucleus itself, very shortly after synthesis. As much as 90 per cent of the newly-synthesized RNA may be destroyed, and its half-life may be of the order of only a few minutes.[14]

During the past 3 years in my laboratory, we have isolated and purified the nuclear enzyme which we believe is the principal agent responsible for degradation of newly-synthesized nuclear RNA in animal cells.[18,19] The enzyme is an exoribonuclease, which preferentially attacks single-stranded, non-helical, rapidly-labelled RNA, in contrast to ribosomal or transfer RNA. The products of degradation are always 5′-mononucleotides, never 3′-mononucleotides or oligonucleotides. We have done many studies on various aspects of the mechanism whereby this enzyme attacks single-stranded RNA, but I would like to emphasize just one major point with respect to the mechanism of the degradation process: in contrast to many enzyme–substrate systems, in this system, one has to consider the chemical state of both the enzyme *and* its substrate, when one considers how efficiently the enzyme functions as a destroyer of single-stranded RNA. This is particularly well illustrated in Table 1, which shows the ability of a *fixed amount* of enzyme to degrade a *fixed amount* of polyadenylic acid, under standard conditions. The only variable in the experiment is the nature of the end-groups at the 3′-end of the potential substrate molecule, polyadenylic acid. Poly A normally has 3′-hydroxyl end-groups and is readily degraded by exoribonuclease (Table 1, Expt. 1). However, the presence of 3′-phosphate end-groups on an otherwise susceptible substrate molecule converts such a molecule to a species that is highly resistant to exonucleolytic attack. The pretreatment of poly A with very small amounts of micrococcal nuclease is an excellent way to introduce such 3′-phosphate end-groups, and it can be seen that poly A can be made into a totally resistant form (Table 1, Expts. 2 and 3). There is as yet no evidence that this particular mechanism does in fact exist in a physiological sense in the cell itself, and one can easily think of several other

TABLE 1. EFFECT OF 3′-HYDROXYL AND 3′-PHOSPHATE END-GROUPS ON DEGRADATION OF POLY A BY EXORIBONUCLEASE

Experiment	mμmoles of AMP Residues liberated/hr
1. Exonuclease + poly A, untreated	184
2. Exonuclease + poly A, pretreated with 0·01 μg of micrococcal nuclease	11
3. Exonuclease + poly A, pretreated with 0·1 μg of micrococcal nuclease	0

Details of enzyme assay and pretreatment of poly A are given in ref. 19. In all three experiments, the amounts of exonuclease and poly A are constant; the only variable is the nature of the 3′-end groups of poly A.

plausible ways to make newly-synthesized nuclear RNA resistant to degradation. The data are shown merely to demonstrate that in this system one must consider the state of *both* the enzyme and its substrate with respect to the process of RNA degradation.

What I do want to emphasize with respect to the problem of nuclear RNA turnover are the following three points:

(1) First, there is an extremely large and rapid turnover of newly-synthesized RNA in the nucleus, in many different cell types.

(2) Second, this turnover involves an interaction between a particular enzyme, and a substrate molecule which must be in a susceptible state in order to be degraded.

(3) Third, the data in the literature also strongly suggest that something special has happened to the newly-synthesized, rapidly-labeled nuclear RNA that is *not* destroyed by the exoribonuclease, that something has been done to stabilize it against degradation. To put it in Henry Harris' terms,[20] "The limiting step in the transfer of information from the genes to the cytoplasm is not the synthesis of the template, but its incorporation into a structure which resists intranuclear degradation".

Does this information on the mechanism of nuclear RNA degradation shed any further light on the mechanism whereby actinomycin D or aflatoxin B_1 cause such a rapid loss of nuclear RNA? I would first like to present some further data on the effects of actinomycin D and other antibiotics which bind to DNA on the activity of the nuclear exoribonuclease which destroys single-stranded or rapidly-labeled RNA. Table 2 shows the effects of actinomycin D and three other antibiotics which bind to DNA, namely daunomycin,[21] mithramycin,[21] and anthramycin,[22] on the activity of the exoribonuclease in intact Ehrlich ascites tumor cell nuclei. As can be seen, none of the antibiotics, at either 2 μg/ml or 20 μg/ml, significantly affected the rate at which nuclear exoribonuclease destroyed the single-stranded RNA substrate. We also did essentially the same experiment, at even higher antibiotic concentrations, namely 50 μg/ml, but used endogenous rapidly-labeled RNA of the nucleus as substrate for the enzyme, and found that actinomycin D, daunomycin, and mithramycin at these extremely high concentrations had only minimal inhibitory effects on the degradation of rapidly-labeled RNA *in isolated nuclei.* Certainly there is no evidence from the

TABLE 2. EFFECT OF ANTIBIOTICS WHICH BIND TO DNA ON DEGRADATION OF POLYADENYLIC ACID BY ISOLATED EHRLICH ASCITES TUMOR CELL NUCLEI

Antibiotic	μmoles AMP per hr per 5×10^6 nuclei
None	1·00
Actinomycin D, 20 μg/ml	1·04
Actinomycin D, 2 μg/ml	1·02
Daunomycin, 20 μg/ml	1·00
Mithramycin, 20 μg/ml	1·06
Mithramycin, 2 μg/ml	1·05
Anthramycin, 20 μg/ml	1·05
Anthramycin, 2 μg/ml	0·98

Nuclei were isolated by a detergent procedure[18] and assayed for their ability to degrade poly A under standard reaction conditions.[18]

above two experiments that an agent such as actinomycin D enhances the activity of the exoribonuclease which destroys rapidly-labeled RNA in the nucleus.

We are thus left with a problem. Farber's work of the past several years strongly suggests that *in the intact cell* actinomycin D accelerates the destruction of rapidly-labeled nuclear RNA. Our own work demonstrates that *in the isolated nucleus* actinomycin does *not* accelerate the activity of the enzyme which destroys rapidly-labeled nuclear RNA. If one allows that the above two conclusions are correct, then there would seem to be only one simple explanation that can be made of the problem, namely, that *in the intact cell*, actinomycin D changes the susceptibility of nuclear RNA to degradation, so that a greater percentage of the rapidly-labeled, newly-synthesized RNA is now susceptible to degradation—or to put it another way, actinomycin D interferes with the normal mechanism for processing newly-synthesized RNA in the nucleus, so that the proper percentage of this material is not stabilized against degradation.

The following sequence of events is thus suggested as a tentative explanation of what is occurring chemically in the segregated nucleolus of the hepatocyte exposed to actinomycin D or aflatoxin B_1. First: there has been a failure of the proper mechanisms for stabilizing newly-synthesized RNA. Second: since a proper percentage of RNA molecules have not been properly stabilized against degradation, they are more susceptible to attack by nuclear nucleases, particularly the exoribonuclease. In such a situation there is therefore an enhanced degradation of newly-synthesized nuclear RNA, with the resultant rapid depletion of RNA from the nucleus. Perhaps the most succinct description of the entire process at work has been given by Henry Harris, as follows, "The ability of a cell to transfer RNA of high molecular weight from nucleus to cytoplasm, and hence to express genetic information, is linked in some way to the development of a nucleolus.[23] The absence of normal nucleoli is associated with defective transfer of the RNA synthesized on the chromatin to the cytoplasm and with intranuclear degradation of this RNA."[20]

Thus, the role of the nucleolus in the processing of nuclear RNA emerges as an extremely vital problem for the biochemical pathologist. Although some of the enzymatic mechanisms for destruction of newly-synthesized RNA have been established, the mechanisms for selective stabilization of other species of newly-synthesized nuclear RNA are not yet known. It is apparent that chemical agents such as actinomycin D and aflatoxin B_1 can exert profound effects on the processing of nuclear RNA. However, there are still many unsolved questions relating to the processing of nuclear RNA. The elucidation of the normal biochemical mechanisms for processing nuclear RNA, and the mechanisms whereby chemical (and perhaps viral) agents might interfere with this process is a problem in need of intensive investigation.

I would like to close this discussion by raising the following questions: "What would be the overall cellular result of a permanent molecular lesion in the process for stabilizing newly-synthesized RNA? Are there in fact disease states whose essential nature is characterized by such a molecular lesion in the processing of nuclear RNA?" Although we now know of many diseases (such as the classical in-born errors of metabolism) in which there is a primary defect in structural genes, that is in DNA itself; we do not as yet know of any disease states in which the primary biochemical pathology is a defect in the processing of RNA within the nucleus. However, it is not unreasonable to imagine that such molecular lesions do in fact exist in nature. Whether or not

such a molecular lesion would have any relevance to the phenomenon of malignant cell growth is entirely unknown at present. However, the data that have been discussed, which indicate that some carcinogens may exert some of their cellular effects on the processing of nuclear RNA, would seem to offer an optimistic note for future investigation in this area. Thus, the approach we have just discussed might lead one to consider the general hypothesis that a molecular defect in the stabilization of nuclear RNA may be involved in the development of the malignant state. Pitot[24] has previously suggested that altered stability of the template RNA in the cytoplasm may be a characteristic of malignancy. The profound effects of some carcinogenic chemicals on nuclear structure and function strongly suggest that nuclear mechanisms of RNA stabilization must also be considered in any comprehensive theory of carcinogenesis.

REFERENCES

1. R. C. Reynolds, P. O. Montgomery and B. Hughes, *Cancer Res.* **24**, 1269 (1964).
2. W. Bernhard, C. Frayssinet, C. Laparge and E. Le Breton, *C. r. Acad. Sci. Paris* **261**, 1785 (1965).
3. D. Svoboda and J. Higginson, *Cancer Res.* **28**, 1703 (1968).
4. P. J. Goldblatt, R. J. Sullivan and E. Farber, *Cancer Res.* **29**, 124 (1969).
5. C. W. Dingman and M. B. Sporn, *Science* **149**, 1251 (1965).
6. M. B. Sporn, C. W. Dingman, H. L. Phelps and G. N. Wogan, *Science* **151**, 1539 (1966).
7. M. B. Sporn and C. W. Dingman, *Cancer Res.* **26**, 2488 (1966).
8. E. Reich and I. H. Goldberg, *Prog. Nucleic Acid. Res. Mol. Biol.* **3**, 183 (1964).
9. J. I. Clifford and K. R. Rees, *Nature, Lond.* **209**, 312 (1966).
10. C. W. Dingman and M. B. Sporn, *Cancer Res.* **27**, 938 (1967).
11. H. V. Gelboin, J. S. Wortham, R. G. Wilson, M. Friedman and G. N. Wogan, *Science* **154**, 1205 (1966).
12. G. A. Stewart and E. Farber, *J. biol. Chem.* **243**, 4479 (1968).
13. H. Harris, H. W. Fisher, A. Rodgers, T. Spencer and J. W. Watts, *Proc. R. Soc. (London) B* **157**, 177 (1963).
14. R. Soeiro, M. H. Vaughan, J. R. Warner and J. E. Darnell, *J. cell Biol.* **39**, 112 (1968).
15. G. Attardi, H. Parnas, M. H. Hwang and B. Attardi, *J. molec. Biol.* **20**, 145 (1966).
16. K. Scherrer and L. Marcaud, *J. cell Physiol.* **72**, *Suppl.* **1**, 181 (1968).
17. W. K. Roberts, *Biochim. biophys. Acta* **108**, 474 (1965).
18. H. M. Lazarus and M. B. Sporn, *Proc. natn. Acad. Sci. U.S.A.* **57**, 1386 (1967).
19. M. B. Sporn, H. M. Lazarus, J. M. Smith and W. R. Henderson, *Biochemistry* **8**, 1698 (1969).
20. H. Harris, *Nucleus and Cytoplasm*, Oxford University Press, London (1968).
21. W. Kersten, H. Kersten and W. Szybalski, *Biochemistry* **5**, 236 (1966).
22. K. W. Kohn, V. H. Bono and H. E. Kann, *Biochim. biophys. Acta* **155**, 121 (1968).
23. H. Harris, E. Sidebottom, D. M. Grace and M. E. Bramwell, *J. Cell Sci.* **4**, 499 (1969).
24. H. C. Pitot, *Perspectives Biol. Med.* **8**, 50 (1964).