SHORT COMMUNICATION

Metabolic Integrity of Deoxyribonucleohistones during Enzyme Induction

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SUMMARY

Previous work has shown that after individual labeling of histone and DNA of various normal and neoplastic tissues of the rat, the first-order decay rates of their respective specific activities were approximately similar.

The present studies indicate that the normal turnover of histones in liver parenchymal cells is not altered during periods of alternate enzyme induction and repression resulting from dietary changes or drug injections.

Although the idea that histones may act as "gene-suppressors" was proposed by Stedman and Stedman as early as 1950 (1), the biological function of these nuclear proteins still remains in doubt. Recently much evidence has accumulated which indicates that complex formation of DNA with these basic proteins greatly reduces its effectiveness to serve as primer for RNA polymerase (2–6), which would seem to support the original hypothesis of the Stedmans.

To gain some insight into the function of histones, experiments were carried out in this laboratory, in which the decay rates of histones and DNA isolated from deoxyribonucleoproteins of rapidly and slowly growing rat tissues and transplantable rat tumors were compared. The results of these studies indicated that the half-lives of histones and DNA were similar in all cases, which would appear to be evidence for metabolic integrity of the deoxyribonucleoprotein complex as a whole (7). If histones have a dynamic function in the sense of the repressor substances of Jacob and Monod (8), one would expect to find large differences in turnover rates. It seems therefore that if histones do influence information transfer from DNA, it is probably due to the manner in which they are built into the deoxyribonucleohistone complex, although we have to recognize the alternative possibility that the regulatory function is associated with such a small fraction of the total histone that it would not affect its estimated half-life. It appeared therefore of interest to see whether the relative turnover rate of histones as compared to that of DNA can be altered, when substantial changes take place in the information being transcribed from the DNA.

As enzyme induction in the liver has been found to be an actinomycin D-sensitive process (9–14) and is dependent on synthesis of new template RNA (15), it seems reasonable to assume that it is based on changes in the information available for transcription. In the present experiments we tried therefore to obtain such changes by alternate induction and repression of en-

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zymes in rat liver. Histone turnover in hepatocytes was estimated under these conditions, according to a previously described experimental design (7). Fifty microcuries of L-lysine-U-^{14}C/kg were injected intraperitoneally into rats 3–4 weeks old, in order to obtain high levels of labeling in parenchymal liver cells of these young, rapidly growing animals. Interference from the more rapidly turning over liver cell population was avoided by allowing the specific activity of the nucleoproteins of these cells to decay for a sufficient period of time. The first batch of animals was therefore killed 3 weeks after the administration of the tracer, at which time the specific activities of histones in the nonhepatocyte cell population had dwindled to negligible values. At this point the remaining animals were divided into two groups, and three rats in each group were killed at weekly intervals. One of the groups was used as control and remained untreated, whereas the other received two doses of 75 mg/kg phenobarbital with an interval of 12 hr on the same day each week. Pilot experiments carried out in this laboratory showed that this treatment resulted in nearly maximal induction of liver aminopyrine demethylase in approximately 3 days, and that the enzyme activity returned to very low or normal levels at 6–7 days after the phenobarbital injection. It was assumed that the other phenobarbital-inducible drug-metabolizing liver enzymes were also subject to alternate inductive and repressive changes. In a second experiment 4-week-old rats were injected with lysine-^{14}C and 3 days later 5 animals were killed. At this point one half of the animals were put on weekly alternating protein-rich and carbohydrate-rich diets, a treatment that causes alternate induction and repression of enzymes involved in amino acid metabolism, as has been shown by Pitot *et al.* (16). The controls were fed Purina chow ad libitum. Groups of 5 animals of each group were killed at weekly intervals, up to 7 weeks after the administration of labeled lysine.

The livers of the animals were excised, and nuclei were prepared from them by homogenization in 0.25 M sucrose and centrifugation for 10 min at 600 *g*. The sediments were resuspended in 2.1 M sucrose and spun at 40,000 *g* for 30 min. Lysine-rich histones and the combined other histone fractions were prepared from the resulting purified nuclei according to a previously described procedure (7). Protein concentrations were determined according to Lowry *et al.* (17), and radioactivity by plating at infinite thinness and counting in a Nuclear Chicago gas flow counter. Half-lives of the two histone fractions were calculated from the first-order decay curves, plotted by the method of least squares. The half-lives of lysine-rich and other histones were approximately 25 and 26 days, respectively, in the phenobarbital-induction experiment. In the diet-induction experiment these values were approximately 11 and 15 days, due to the fact that the animals were very young and therefore rapidly growing. The results recorded in Table 1 show that there was no appreciable difference between treated animals and controls with respect to the half-lives of either histone fraction studied, indicating that metabolic integrity of deoxyribonucleohistones is maintained under conditions that presumably induce substantial changes in the flow of information from liver DNA. Although it appears therefore that structural changes in deoxyribonucleohistone, responsible for regulation of information transfer from

TABLE 1
Ratios of the half-lives of histones from control and treated animals

Induction	$t_{1/2}$[a] Histones, controls: treated	
	Lysine-rich histones	Other histones
By phenobarbital injections	0.89	0.91
By alternating protein and carbohydrate-rich diets	0.98	0.83

[a] Twelve experimental values obtained from the same number of animals were used for the calculation of each half-life. Application of the *t* test according to Hald (18) showed that none of the ratios differed significantly from unity at the $\alpha = 0.05$ level.

DNA, do not involve turnover of the whole histone molecule, they could be effected by changes in histone structure *in situ*. An example of such alterations in structure are those resulting from acetylation of the N-terminal end (19, 20). Preliminary data obtained in this laboratory indicate that the turnover of acetyl N-terminal groups is indeed much faster than that of the whole histone molecule.

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REFERENCES

1. E. Stedman and E. Stedman, *Nature* **166**, 780 (1950).
2. V. G. Allfrey and A. E. Mirsky, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 247 (1963).
3. J. Hindley, *Biochem. Biophys. Res. Commun.* **12**, 175 (1963).
4. G. C. Barr and J. A. V. Butler, *Nature* **199**, 1170 (1963).
5. R. C. Huang, J. Bonner and K. Murray, *J. Mol. Biol.* **8**, 54 (1964).
6. M. C. Liau, L. S. Hnilica and R. B. Hurlbert, *Proc. Natl. Acad. Sci. US.* **53**, 626 (1965).
7. P. Byvoet, *J. Mol. Biol.* **17**, 311 (1966).
8. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
9. H. V. Gelboin and N. Blackburn, *Biochim. Biophys. Acta* **72**, 657 (1963).
10. H. V. Gelboin and N. Blackburn, *Cancer Res.* **24**, 356 (1964).
11. H. M. Dyer, P. M. Gullino and H. P. Morris, *Cancer Res.* **24**, 97 (1964).
12. H. C. Pitot and C. Peraino, *J. Biol. Chem.* **239**, 1783 (1964).
13. S. Orrenius, J. L. E. Ericsson and L. Ernster, *J. Cell. Biol.* **25**, 627 (1965).
14. D. O. Foster, P. D. Ray and H. A. Lardy, *Biochemistry* **5**, 555 (1966).
15. A. M. Nemeth, *in* "Advances in Enzyme Regulation" (G. Weber, ed.), Vol. I, 57 (1963).
16. H. C. Pitot, V. R. Potter and H. P. Morris, *Cancer Res.* **21**, 1001 (1961).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1945).
18. A. Hald, "Statistical Theory with Engineering Applications." Wiley, New York, 1957.
19. V. G. Allfrey, R. Faulkner and A. E. Mirsky, *Proc. Natl. Acad. Sci. US.* **51**, 786 (1964).
20. V. G. Allfrey, *Cancer Res.* **26**, 2026 (1966).