DNA TURNOVER: EVIDENCE FROM STUDIES OF STEADY-STATE BACTERIAL POPULATIONS*

D. E. Contois and W. F. K. Seymour**

Department of Microbiology, University of Hawaii
Honolulu, Hawaii

Received April 21, 1964

Since the work of Hershey (1954) most biologists have regarded DNA pools of bacterial cells as stable or conservative. Nonetheless, turnover was invoked as a possible explanation of mutation in non-replicating bacterial cells (Ryan, 1949) and in spheroplasts (Ryan et al., 1963). It also could account for observations of mutation rates independent of growth rates in slowly growing cultures of Escherichia coli (Novick & Szilard, 1950; Fox, 1955). Results of recent studies suggest DNA turnover in cultures of primary human amnion cells (Chang & Vetrovs, 1963), mouse fibroblasts (Thomson et al., 1957), and bacteria (Theil & Zamenhof, 1963). We have obtained evidence of DNA turnover from studies of carbon-flux through steady-state cultures of Aerobacter aerogenes under conditions of nitrogen limitation.

METHODS

Steady-state populations were grown in a continuous culture apparatus utilizing a minimal medium consisting of a mineral salts base, glucose (11.1 mM) as a carbon source, and ammonium chloride (3.75 mM)

*Contribution No. 12 from the Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii. The investigation was supported in part by a research grant (No. E-3588) from the National Institutes of Health, U.S. Public Health Service.

**Present address: Department of Physiology, Duke University, Durham, North Carolina.

as a limiting nitrogen source (Contois, 1959). Culture volumes were between 25 and 30 ml, and the incubation temperature was 30°C. After a culture was maintained in a steady-state for at least 48 hours, as evidenced by constant population density (about $1x10^9$ organisms/ml) and flow-rate, approximately 4 X 10^6 cpm of uniformly labeled glucose-C^{14} (specific activity: 4.9 mc/mM) were added. Periodically, one ml samples were withdrawn from the culture and the cells harvested on a Millipore filter and washed with 3-4 volumes of phosphate buffer (pH 6.8). Each filter was loosely rolled and placed in a test tube, two ml of cold 5 percent TCA were added, and the tube was vigorously shaken at 0-4°C for thirty minutes. (Previous experience showed a recovery of more than 96 percent of the cells from the filter surface by this technique.) As carrier cells, eight ml of a cold suspension containing 3-4 mgm dry weight of non-radioactive cells per ml in 1.9 percent TCA were added and the shaking was continued for at least an additional 30 minutes.

After all samples of a particular series--usually eight in number--were processed in this manner, eight ml portions were removed from each tube and centrifuged at 10,000 rpm (approximately 12,800 g) at 0-4°C. The supernatant from each was carefully removed and stored as the 'cold TCA soluble fraction. Other fractions were obtained by sequential chemical extractions. The procedure was essentially the same as that of Schmidt and Thannhauser (1945).

Radioactivities of samples from the various fractions were then measured, corrected for self-absorption, and adjusted to give the activity per ml of the initial suspension of carrier and radioactive cells.

RESULTS AND DISCUSSION

Results from typical experiments (Table I) show pulses of activity moving through the DNA pools and, for comparison, the 'protein' pools. (The latter consist of material remaining after extraction of the other pools and are composed of cell wall material and 70-80% of the cell

TABLE I

FLUX OF C^{14} THROUGH THE DNA AND PROTEIN POOLS OF STEADY-STATE POPULATIONS OF A. aerogenes

Experiment A			Experiment B		
Time (hours)	DNA (Log_e cpm)	Protein (Log_e cpm)	Time (hours)	DNA (Log_e cpm)	Protein (Log_e cpm)
0.17	7.18	7.75	0.08	6.16	6.92
0.33	7.04	7.66	0.20	6.19	6.77
0.52	6.77	7.75	0.35	5.67	7.12
0.73	6.61	7.85	0.53	4.68	7.22
0.98	6.03	7.71	0.75	5.51	7.33
1.31	5.77	7.76	1.00	4.86	7.23
1.56	6.28	7.63	1.25	5.68	7.17
2.01	5.99	7.53	1.67	4.19	7.23

Time is in hours after the addition of approximately 4×10^6 cpm of glucose-U-C^{14}. In Exp. A the dilution rate of the culture was $-0.26\ hr^{-1}$. In Exp. B it was $-0.31\ hr^{-1}$.

protein.) Because of the kinetics of the system, the specific rate of change in activity of a pool (i.e., the rate of change in log_e of activity) is equal to the specific rate at which activity enters a pool less the specific rate at which it is lost; and the activity of a pool will reach a maximum when the two rates are equal.

In the case of a non-conservative pool, the specific rate at which activity is lost is equal to the specific rate of turnover plus the dilution rate. The latter is defined (Herbert et al., 1956) as the flow rate per unit volume of culture. In the case of a conservative pool, the specific rate of loss of activity is equal only to the dilution rate; and, as a result, the specific change in activity after reaching

a maximum is less than the dilution rate since radioactive precursors are still present.

From Fig. 1 it is clear that the loss of activity from DNA is greater than that due to dilution, indicating loss due to turnover. The decrease in the $\log_e$ of the DNA activity is estimated by the regression coefficient to be -0.69 hr^{-1} after peak activity. The dilution rate is calculated from the flow rate (7.0 ml/hr) and culture volume (27 ml) to be -0.26 hr^{-1}. From the difference, -0.43 hr^{-1}, and by assuming the flux into the pool is essentially zero, a half life for DNA can be estimated. In this instance the value is a little less than two hours; i.e., 0.69/0.43 hr^{-1}. Using the data of Table I-B, similar calculations give an estimate of slightly over one hour for the half life.

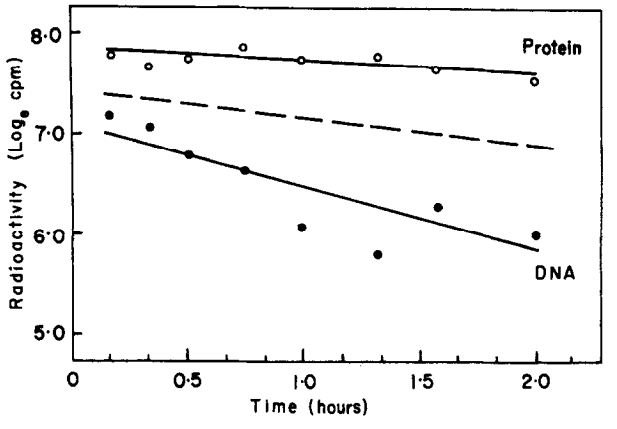

Figure 1. Regression of $\log_e$ of activities of DNA and of protein pools on time. Data of Table I-A fitted by the method of least squares. The dashed line represents the loss of activity due to the dilution rate of -0.26 hr^{-1}.

The large values for turnover encountered in this study may be due to a number of factors, such as growth of the test organism under nutrient limitation and at relatively low specific growth rates. The fact that thymine--a possible product of turnover--is not a precursor to DNA and was found by us to be rapidly metabolized by the organism might have had the effect of reducing or eliminating cryptic turnover.

REFERENCES

Chang, R. S., and Vetrovs, H., Science 139, 1211 (1963).

Contois, D. E., J. Gen. Micro. 21, 40 (1959).

Fox, M. S., J. Gen. Physiol. 39, 267 (1955).

Herbert, D., Elsworth, R., and Telling, R. C., J. Gen. Micro. 14, 601 (1956).

Hershey, A. D., J. Gen. Physiol. 38, 145 (1954).

Novick, A., and Szilard, L., Proc. Nat. Acad. Sci. U. S. 36, 708 (1950).

Ryan, F. J., J. Gen. Micro. 21, 530 (1959).

Ryan, F. J., Okada, T., and Nagata, T., J. Gen. Micro. 30, 193 (1963).

Schmidt, G., and Thannhauser, S. J., J. Biol. Chem. 161, 83 (1945).

Theil, E. C., and Zamenhof, S., Nature 199, 599 (1963).

Thomson, R. Y., Paul, J., and Davidson, J. N., Biochem. J. 69, 553 (1957).