Short Communications

Increased Transfer Ribonucleic Acid Methylase Activity in Tumours Induced in the Mouse Colon by the Administration of 1,2-Dimethylhydrazine

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It has been suggested that methylation of nucleic acids by enzymes within the cell may play an important role in carcinogenesis (Srinivasan & Borek, 1964, 1966). Although no direct evidence links such methylation of tRNA with a change in the properties of the molecule that could account for the deranged metabolism of tumour cells, there is an increased content of methylated bases in tRNA isolated from some tumours (Bergquist & Matthews, 1962; Mandel, Srinivasan & Borek, 1966; Burdon, 1966; Viale, Restelli & Viale, 1967; Craddock, 1969). Also, extracts prepared from transplantable tumours and from the tissues of animals infected with an oncogenic virus have been reported to have increased activities of tRNA methylases (Tsutsui, Srinivasan & Borek, 1966; Hancock, 1967; Silber, Goldstein, Berman, Decter & Friend, 1967; Mittleman, Hall, Yohn & Grace, 1967; Baguley & Staehelin, 1968; Hacker & Mandel, 1969; Riddle & Turkington, 1970). The choice of the appropriate source of control enzymes is difficult in many of these experiments, since the tumours may have been derived from a particular cell type that does not form a major proportion of the control tissue. Also, since the activity of tRNA methylases is markedly affected by compounds of low molecular weight such as polyamines (Leboy, 1970; Pegg, 1971), which are present in many cells, results obtained with undialysed tissue homogenates may reflect the amount of this material present rather than the actual amount of tRNA methylases. It is of considerable interest to determine whether an increase in tRNA methylases is a general phenomenon common to all tumours, including those induced by chemical carcinogens.

Administration of 1,2-dimethylhydrazine to rats and mice leads to the formation in the colon of multiple nodular tumours that can be readily dissected out from the surrounding tissue (Druckrey, Preussmann, Matzkies & Ivankovic, 1967; Weibcke, Lohrs, Gimmy & Eder, 1969). It is shown here that extracts prepared from such neoplastic nodules have a greatly increased ability to catalyse the incorporation of methyl groups from *S*-adenosyl-L-methionine into bacterial tRNA.

N.M.R.I. mice purchased from the Medical Research Council Laboratory Animal Centre, Carshalton, Surrey, U.K., were bred in this Institute. 1,2-Dimethylhydrazine hydrochloride was administered subcutaneously as a 0.35% solution with respect to the base in aq. 0.9% sodium chloride. This solution also contained 1.5mg of EDTA/100ml and the pH was adjusted to 6.5 with 0.25M-sodium hydroxide. The LD₅₀ of 1,2-dimethylhydrazine in N.M.R.I. mice was found to be 12mg/kg body wt. for males and 22mg/kg body wt. for females by the method of Weil (1952). Male mice (25g) and female mice (20g) were given weekly injections of 10mg and 15mg of 1,2-dimethylhydrazine/kg body wt. respectively until the appearance of the first nodule at 24 weeks. (Control animals were injected with the saline-EDTA solution alone.) Two types of tumour were observed: squamous-cell carcinomas arising from the anal margin and adenomas and adenocarcinomas of various degrees of differentiation arising from the mucosa of the descending colon and rectum. The latter were used in the experiments described below. Of the animals surviving to 24 weeks after the first injection, 19 out of 22 males and 22 of 28 females produced these nodules. (Post-mortems were not done on three animals.)

Approx. 200–300mg of tissue, either tumour nodules or normal tissue, was removed, rapidly frozen in liquid N₂ and stored at –20°C until required. The tissue was then homogenized in 2ml of medium containing (final concentrations) 0.25M-sucrose, 10mM-tris-HCl buffer, pH 7.8, 1mM-2-mercaptoethanol and 0.1mM-EDTA. The homogenate was centrifuged at 120000g for 2h (type 50 rotor in the Spinco model L centrifuge). The supernatant fluid was then removed, dialysed for 18h against 4 litres of medium containing (final concentrations) 10mM-tris-HCl buffer, pH 7.8, 1mM-2-mercaptoethanol and 1mM-EDTA and used directly as a source of enzyme. Methylation of tRNA from *Escherichia coli* catalysed by these dialysed ultracentrifuged extracts was assayed as described in Table 1. Some methyl groups were incorporated into protein present in the extracts, but there was no methylation of endogenous tRNA. Under the

Table 1. *Methylation of tRNA and protein by extracts derived from mouse colon and colonic tumours*

The assay medium contained 15 μ mol of tris-HCl buffer, pH 8.8, 0.1 μ mol of 2-mercaptoethanol, 5 nmol of *S*-adenosyl-L-[Me-¹⁴C]methionine (65 c.p.m./pmol), 4 μ mol of putrescine (Expt. 1) or 0.8 μ mol of spermidine (Expt. 2), and 0.03–0.215 mg of protein from the tissue extract in a total volume of 0.15 ml. Methylation of tRNA was measured in the presence of 0.05 mg of tRNA from *E. coli* K 12. After incubation of the assay tubes at 37°C for various times up to 1 h incorporation of methyl groups into material insoluble in 5% (w/v) trichloroacetic acid was determined (Pegg, 1971). The results are given as means $\pm$ s.e.m. of determinations with the number of animals for each group given in parenthesis. The differences between the methylation of tRNA by extracts of tumours compared with the normal colon are highly significant ($P < 0.001$), but the differences in protein methylation are not significant when tested by Student's *t* test.

	Tissue extract	Sex of mouse	Methylation of tRNA (pmol of methyl group incorporated/15 min per mg of protein)	Methylation of protein (pmol of methyl group incorporated/15 min per mg of protein)
Expt. 1	Normal colon	Male	75 $\pm$ 9 (6)	30 $\pm$ 3 (6)
	Normal colon	Female	79 $\pm$ 12 (6)	36 $\pm$ 4 (6)
	Colonic tumour	Male	214 $\pm$ 18 (6)	30 $\pm$ 6 (6)
	Colonic tumour	Female	228 $\pm$ 36 (6)	24 $\pm$ 6 (6)
Expt. 2	Normal colon	Male	60 $\pm$ 9 (6)	21 $\pm$ 5 (6)
	Colonic tumour	Male	219 $\pm$ 33 (5)	24 $\pm$ 7 (5)
	Colonic tumour	Female	252 $\pm$ 39 (5)	42 $\pm$ 10 (5)

conditions used in these experiments the methylation of bacterial tRNA was proportional both to the amount of protein added and to the time of incubation up to 1 h.

Results given in Table 1 show that extracts from colonic tumours methylated tRNA from *E. coli* at a rate three- to four-fold greater than that by extracts from comparable sections of normal colon. (Extracts prepared from pieces of colon from mice treated with 1,2-dimethylhydrazine but free of tumour nodules also had tRNA methylase activities similar to those obtained from untreated controls.) There was no significant difference in tRNA methylase activities between extracts prepared from male and female mice. Methylation of protein was not significantly different in extracts from normal tissue and tumours (Table 1). However, no additional substrate for protein methylation was added to the medium, and the activity measured in this assay may be determined by the number of available sites for methylation present in the tissue extract rather than by the amount of enzyme(s) present.

The methylation of tRNA *in vitro* by mammalian enzymes is markedly stimulated by the addition of certain amines (Leboy, 1970; Pegg, 1971). This requirement can be satisfied by the diamines 1,3-diaminopropane, putrescine and cadaverine or by considerably lower concentrations of the polyamines spermine and spermidine. Similar tRNA methylase activities were observed in the presence of 28 mM-putrescine (Table 1, Expt. 1) and 5.5 mM-spermidine (Expt. 2), and in both cases there was a similar increase in the activity of tumour extracts when compared with the normal colon.

The measured tRNA methylase activity represents the sum of the reactions of a number of enzymes, each of which catalyses the incorporation of methyl groups into a particular nucleotide sequence present in the unfractionated tRNA from *E. coli* utilized as a substrate. The increase in this total activity seen in colonic tumours could be due to the induction of new methylases recognizing additional sites in the bacterial tRNA or to an increase in the activity of pre-existing tRNA methylases. Analysis of the methylated bases produced by the colonic extracts by paper chromatography after hydrolysis of the tRNA in 1M-hydrochloric acid (Pegg, 1971) revealed no marked differences between the products obtained when tumour cells were compared with normal tissue. Also, when an excess of enzyme extract was allowed to methylate tRNA under conditions where the amount of tRNA present limited the extent of the reaction, there was considerably less difference between the normal tissue extracts, which incorporated 8 pmol of methyl groups/ μ g of tRNA, and the tumours, which incorporated 11 pmol of methyl groups/ μ g of tRNA. Neither extract catalysed the methylation of tRNA isolated from rat liver. These results support the hypothesis that the tumour contains greater amounts of pre-existing tRNA methylases, but until more specific assays for the individual tRNA methylases are available this question must remain open.

The results reported here and recent observations of increased tRNA methylase activity in tumours of the rat kidney induced by dimethylnitrosamine (A. E. Pegg, unpublished work; Craddock, 1970) confirm that such increases do also occur in

tumours induced by the administration of chemical carcinogens. Since the colonic tumours are derived from the mucosal cells, which form about 70% of the normal colon, the three- to four-fold increase in tRNA methylase activity cannot be due entirely to a change in the relative proportions of epithelial to mesenchymal cells in the tumour and the control tissue. The relevance of this increase to carcinogenesis is, however, unclear, and elevated tRNA methylase activity may be only part of a generalized increase in the activities of enzymes involved in macromolecular syntheses in the rapidly growing tumour cells. The induction of well-defined tumours of the colon by 1,2-dimethylhydrazine in very high yield provides a useful system in which further studies of the significance of tRNA methylases and other enzymes in carcinogenesis can be investigated.

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Baguley, B. C. & Staehelin, M. (1968). *Eur. J. Biochem.* **6**, 1.
Bergquist, P. L. & Matthews, R. E. F. (1962). *Biochem. J.* **85**, 305.

Burdon, R. H. (1966). *Nature, Lond.*, **210**, 797.
Craddock, V. M. (1969). *Biochim. biophys. Acta*, **195**, 351.
Craddock, V. M. (1970). *Nature, Lond.*, **228**, 1264.
Druckrey, H., Preussmann, R., Matzkies, F. & Ivankovic, S. (1967). *Naturwissenschaften*, **54**, 285.
Hacker, B. & Mandel, L. R. (1969). *Biochim. biophys. Acta*, **190**, 38.
Hancock, R. L. (1967). *Cancer Res.* **27**, 646.
Leboy, P. (1970). *Biochemistry, Easton*, **7**, 1577.
McFarlane, E. S. & Shaw, G. J. (1968). *Can. J. Microbiol.* **14**, 499.
Mandel, L. R., Srinivasan, P. R. & Borek, E. (1966). *Nature, Lond.*, **209**, 586.
Mittleman, A., Hall, R. H., Yohn, D. S. & Grace, J. T. (1967). *Cancer Res.* **27**, 1409.
Pegg, A. E. (1971). *Biochim. biophys. Acta* (in the Press).
Riddle, M. & Turkington, R. W. (1970). *Cancer Res.* **30**, 650.
Silber, R., Goldstein, B., Berman, E., Decter, J. & Friend, G. (1967). *Cancer Res.* **27**, 1264.
Srinivasan, P. R. & Borek, E. (1964). *Science, N.Y.*, **145**, 548.
Srinivasan, P. R. & Borek, E. (1966). *Prog. nucleic Acid Res.* **5**, 157.
Tsutsui, E., Srinivasan, P. R. & Borek, E. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 1003.
Viale, G. L., Restelli, A. F. & Viale, E. (1967). *Tumori*, **53**, 533.
Weibeke, B., Lohrs, U., Gimmy, J. & Eder, M. (1969). *Z. ges. exp. Med.* **149**, 277.
Weil, C. S. (1952). *Biometrics*, **8**, 249.