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Cytokinin activity of transfer RNA during chick embryogenesis

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A CLASS of N^6-substituted adenines, the cytokinins, promote proliferation and differentiation of plant tissues[1] and may have analogous effects on animal cells.[2] Nucleosides, corresponding to these bases, such as N^6-(Δ^2-isopentenyl)adenosine (2iPA), are constituents of bacterial, yeast, rat liver as well as plant *t*RNA (see ref. 1 for review). In *Escherichia coli*,[3] yeast[4] and *Lactobacillus acidophilus*,[5] cytokinin activity is present only in *t*RNA species that respond to codons containing *U* as the initial base. It is not known whether the promoting effect of the cytokinins on cellular growth is related to their presence in *t*RNA. This relationship might be reflected in a greater cytokinin content of *t*RNA derived from rapidly growing cells as has been shown in *E. coli*.[6] To investigate this possibility in animal cells, the cytokinin activity of *t*RNA was examined at stages of chick embryogenesis characterized by rapid growth and differentiation. The amount of cytokinin activity in total *t*RNA and the amount and chromatographic distribution of isoaccepting serine *t*RNA species corresponding to codons containing *U* as the initial base, were determined.

Transfer RNA was prepared from 4-, 8-, 12- and 19-day chick embryos by a procedure that included a DNase treatment as previously described.[7] Using $E^{0.1\%}_{260}$ (1 cm) for *t*RNA of 22, the yields were 71 (4-day), 71 (8-day), 45 (12-day) and 17 (19-day) mg *t*RNA per 100 g of tissue (wet wt.). The A_{260}/A_{280} ratios of the *t*RNA's ranged from 1·91 to 1·93. The *t*RNA was hydrolyzed and the hydrolysates were bioassayed for cytokinin activity by measuring their ability to promote growth of tobacco callus *in vitro* as described by Skoog *et al.*[8] Each *t*RNA preparation was assayed at several concentrations, with four replicate cultures for each concentration (Table 1). Activity of the preparation was expressed in kinetin equivalents (KE), i.e. micrograms of kinetin (N^6-furfuryladenine) required to give the identical growth response as determined by reference to a kinetin standard curve obtained from parallel tests. For comparison, values of cytokinin activity obtained from a sample each of yeast and *E. coli* *t*RNA assayed in the same manner are included.

TABLE 1. CYTOKININ ACTIVITY OF CHICK EMBRYO TRANSFER RNA'S

*t*RNA	No. of *t*RNA concentrations tested	Cytokinin activity in *t*RNA	
		KE/mg *t*RNA ± S.E.M.	KE/100 g of tissue
Chick embryo (4-day)	4	0·13 ± 0·02	9·2
Chick embryo (8-day)	7	0·12 ± 0·02	8·5
Chick embryo (12-day)	7	0·14 ± 0·02	6·3
Chick embryo (19-day)	8	0·17 ± 0·02	2·9
E. coli	3	0·23 ± 0·08	
Yeast	3	0·95 ± 0·15	

The results, summarized in Table 1, indicate that the cytokinin activity of all chick embryo *t*RNA preparations was similar. However, the younger embryos yielded more *t*RNA on a weight basis than the older embryos, resulting in greater total cytokinin activity in early embryonic tissues. The cyto-

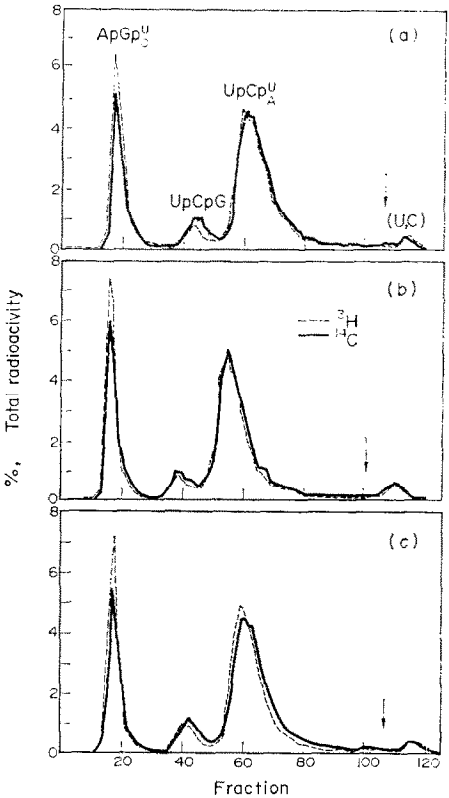

FIG. 1. Cochromatography on benzoylated DEAE–cellulose of (a) 8-day, (b) 12-day and (c) 19-day chick embryo ^{3}H-seryl-*t*RNA's (– – –) and rooster liver ^{14}C-seryl-*t*RNA (——). Liver aminoacyl-*t*RNA ligase preparation was used for all acylations.[7] The total amount of *t*RNA applied to each column was adjusted to 2 mg by the addition of liver *t*RNA. Columns (0·9 × 20 cm) were eluted at room temperature with a linear NaCl gradient (400 ml: 0·6–1·0 M, containing 0·01 M $MgCl_2$ and 0·005 M sodium acetate, pH 4·43), followed (arrow) by a linear NaCl and ethanol gradient (80 ml: 1·0–1·5 M NaCl, 14% ethanol containing 0·005 M sodium acetate, pH 4·43). Aliquots of each fraction were prepared for scintillation counting as previously described.[7] Codon assignments of the liver *t*RNA^{Ser} fractions were made as described elsewhere.[12]

kinin activity of *E. coli* *t*RNA was slightly greater, and that of yeast *t*RNA was approximately 5-fold greater than that of the embryonic *t*RNA's.

Although there is a similar cytokinin activity per mg of *t*RNA at each embryonic age, there may be variations in the amounts of isoaccepting *t*RNA species containing cytokinin-active bases. Cytokinin activity has been detected in *E. coli*,[3] yeast,[9] rat liver[10] and *Staphylococcus epidermidis*[11] $tRNA^{Ser}$ species. Transfer RNA^{Ser} from rooster liver is resolved into four species by benzoylated DEAE-cellulose chromatography.[7] Three of these species bind to ribosomes in response to $(U,C)_n$ or triplet codons containing *U* as the 5'-nucleoside[12] and by analogy with *E. coli* $tRNA^{Ser}$ [6], probably contain cytokinin-active bases. One species binds with AGU and AGC and the analogous $tRNA^{Ser}$ species in *E. coli* contains a nucleoside adjacent to the anticodon, 6-(2-threoninecarbamoyl)-9-β-D-ribofuranosylpurine,[13] the synthetic base of which does not show cytokinin activity in the tobacco bioassay.[3] To determine whether there are developmental differences in cytokinin content of isoaccepting species of $tRNA^{Ser}$ *t*RNA from 8-, 12- and 19-day chick embryos was acylated with ^{3}H-serine and their chromatographic profiles were compared with rooster liver ^{14}C-seryl-*t*RNA by benzoylated DEAE-cellulose chromatography. Acylations were performed with a partially purified rooster liver aminoacyl-*t*RNA ligase preparation under conditions in which the synthesis of seryl-*t*RNA was proportional to *t*RNA concentration.[7] Serine acceptance (mole serine/mole *t*RNA × 100 was 0·65% (8-day), 0·98% (12-day), 1·2% (19-day) and 1·2% for rooster liver *t*RNA. Chromatographic analyses revealed that the relative amount of each of the four seryl-*t*RNA species was identical at all embryonic stages, and equivalent to the relative amounts in adult liver (Fig. 1). Thus, although there was an increase in total seryl-*t*RNA with embryonic age, the relative amount of each isoaccepting seryl-*t*RNA remained constant.

These data suggest that the cytokinin activity of unfractionated *t*RNA and the relative amounts of the $tRNA^{Ser}$ species thought to contain cytokinin-active bases, are similar at all embryonic stages studied. The decrease in cytokinin activity per unit weight of tissue with increasing embryo age possibly reflects a greater amount of *t*RNA in the younger, more rapidly growing cells.

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Synthesis of catecholamines in the locus coeruleus from ^{3}H-tyrosine *in vivo*

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HISTOCHEMICAL fluorescent methods indicate that the locus coeruleus (LC) is made up of catecholamine-containing cell bodies.[1] Indirect evidence, such as the immounhistochemical demonstration of the presence of dopamine-β-hydroxylase[2] and the use of dopamine-β-hydroxylase inhibitors to show decreased histochemical fluorescence intensity in cells in rats pretreated with these drugs,[3] suggests that these cells are noradrenergic. However, direct analysis of extracts of the LC in the rabbit and the cow by thin-layer chromatography showed that the LC contained more dopamine (DA) and its metabolite, dihydroxyphenylacetic acid, than norepinephrine (NE).[4] These latter results may suggest that the LC contains both noradrenergic and dopaminergic cells. To investigate further the biochemical nature of the LC in the rat, we have examined the synthesis of catecholamines and their metabolites in the LC after intraventricular injection of tritiated tyrosine. The methods utilized were sensitive enough to measure newly synthesized NE and DA in the locus coerulei in a single animal.

Charles River male rats (175–225 g), anesthetized with 8% chloral hydrate (0·5 ml/100 g), were injected intraventricularly with 25 μ L-tyrosine-3,5-^{3}H (44 c/m-mole, New England Nuclear Corp., Boston, Mass.). The animals were decapitated at various times after the injection and their brains rapidly removed to a dish of ice-cold saline. The locus coerulei were removed from their lateral positions in the brainstem of each animal in two small blocks of tissue (each less than 2 mg in weight) which were immediately homogenized in 1 ml of ice-cold 10% trichloroacetic acid, containng 25 μg tyrosine, DA and NE for carrier purposes. After removing insoluble material by centriifugation, radioactivity was measured in a small portion of the supernatant fluid which was designated "total tissue extract". After the pH was raised to 8·4, the total tissue extracts were passed over an alumina column to retain catechols, which were subsequently eluted from the columns with weak acid. Radioactivity was measured in a portion of the eluate which was designated "catechol fraction" and the remainder of the catechol fraction was applied to an Amberlite-CG120 column to yield three subfractions containing deaminated metabolites, DA and NE.[5] The deaminated metabolites were contained in the water washes of the Amberlite columns. DA and NE were eluted from the columns with an HCl (0·25–2 N) gradient; about twenty-five 2-ml fractions were collected and 1 ml of each fraction was counted in 20 ml of Triton X-100 phosphor (5·5 g 2,5-diphenyloxazole, 300 mg dimethyl-1,4-bis-2-(5-phenyloxazolyl), 2 l. toluene, 1 l. Triton X-100). There was no overlap of the NE and DA fractions. The levels of radioactivity in the NE fractions were at least eight times background levels. All catechol fractions were counted for a long enough period to provide a 2·5 per cent standard deviation. The recovery of total radioactivity from the Amberlite-CG120 columns was 76·4 $\pm$ 8 per cent (mean $\pm$ S.D., n = 10). The recovery of NE and DA in this procedure was at least 90 per cent. In some experiments, blocks of tissue from the brainstem central gray area that did not contain catecholamine cells and also portions of the caudate nucleus were carried through the analysis. The dissection procedure for the LC was worked out in conjunction with the use of the histochemical fluorescence technique to localize the LC.[6]

The animals were decapitated at 7·5, 15, 30 and 60 min after intraventricular injection of radioactive tyrosine. The total tritium in the tissue extracts was highest at 7·5 min, reduced by more than