ployed Flow Cytofluorometry to define its role in the cell cycle of eukaryotic cells, since this approach permits rapid analysis of the DNA content of intact cells.

Zinc is essential to specific steps of the cell cycle, i.e. G_1, S and the $G_2 \rightarrow M$ transition[5], and its deficiency can block each of them, followed by growth arrest. Such studies with E. gracilis have provided basic information which allows investigation of the role of this and other metals in the metabolism of malignant cells in general, and leukemic cells, in particular. In this manner metal dependent steps pertinent to the $G_1 \rightarrow S$ transition, and S itself have been defined in a human leukemic cell line.

The involvement of the element in the premitotic events of the cell cycle is consistent with earlier observations of its importance to nucleic acid metabolism. Zinc, among other metals, stabilizes the structures of RNA and DNA and presumably affects their metabolism[6]. Moreover, it plays an important role in nucleic acid synthesis and is indispensable to the function of DNA-dependent DNA and RNA polymerases of E. coli[7,8]. The discovery of the RNA dependent DNA polymerase–reverse transcriptase–in Type C oncogenic RNA viruses[9] has stimulated study of the initiation, biochemical basis and maintenance of malignant transformations and of the manner by which a DNA copy is transcribed from viral RNA. The above indications of the importance of zinc in normal and leukemic leukocyte metabolism have prompted us to examine the RNA dependent DNA polymerases from avian myeloblastosis virus and mammalian Type C RNA tumor viruses.

1,1-O-Phenanthroline (OP) reversibly inhibits all of the RNA dependent DNA polymerases from avian myeloblastosis[10], murine, simian, feline and RD-114 RNA tumor viruses[11]. The pK_{OP} varies only from 4.7 to 4.9 and the number of moles of inhibitor bound to the metal, $\bar{n}$, from 1.3 to 2.2, values very close to those previously reported for known zinc enzymes. Combined use of microwave-induced emission spectrometry and micro gel exclusion chromatography has demonstrated stoichiometric amounts of zinc essential to the function of the enzymes from avian myeloblastosis, murine leukemia and woolly monkey Type C viruses[10,11]. Most recently we have shown further that the DNA dependent RNA polymerase II from E. gracilis also is a zinc metalloenzyme. The implications of these findings to normal and abnormal growth will be examined.

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Comparison of transfer ribonucleic acid aminoacylation by various regions of chicken brain

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Summary. Aminoacylation of tRNA with valine was greater in the cerebrum of chicken than the cerebellum or optic lobes. This greater aminoacylation was due to both the activity of the cerebral valyl-tRNA synthetases and the accepting activity of the cerebral $tRNA^{val}$.

Studies comparing protein synthesis by various regions of brain have shown regional differences[1–3]. Recently Liu et al.[4] reported that the greater cell-free protein synthetic activity of the post-mitochondrial supernatant of the cerebrum of chicken brain compared to the cerebellum or optic lobes was partially due to soluble factors in the post-microsomal supernatant (cell sap), possibly factors responsible for the aminoacylation of tRNA. Takahashi and Abe[5] found that the aminoacylation activity of cell sap from the cerebral cortex of rabbit brain was higher, as determined by ^{32}P-pyrophosphate exchange, than the activity from the cerebellum, medulla, pons, thalamus or hypothalamus. However, Liu et al.[4] found in chicken brain that the cell sap from the cerebellum had a greater ability to attach radioactively labeled amino acids onto tRNA than the cerebrum or optic lobes. Because the cell sap contains both components required for aminoacylation, aminoacyl-tRNA synthetases and tRNAs, it is necessary to study the aminoacylation activity of specific aminoacyl-tRNA synthetases and tRNA when comparing the aminoacylation activities of various brain regions. This study is the first report comparing the aminoacylation activity of tRNA synthetases and the charging capacity of tRNA isolated from different brain regions.

Materials and methods. The post-mitochondrial supernatant and cell sap of the cerebrum (cbr), cerebellum (cbl), and optic lobes (opl) were obtained from 12-18-month-old leghorn hens according to the methods published by Liu et al[4]. Aminoacyl-tRNA synthetases were obtained from the cell sap by adding ammonium sulfate to the cell sap to 51% saturation. The resulting suspension was centrifuged at low speed and the precipitate collected and dissolved in a phosphate buffer pH 7.5 (15% glycerol, 10 mM KH_2PO_4, 5 mM KCl, 1 mM $MgCl_2$, and 20 mM 2-mercaptoethanol). The solutions were dialyzed against the phosphate buffer overnight, passed through a 1.8×20 cm Sephadex G-100 column, eluted with phosphate buffer, and fractions containing protein were pooled and used as the source of aminoacyl-tRNA synthetases.

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RNA was isolated from the cell sap by the method of Solymosy et al.[6]. The tRNA was recovered from the RNA by precipitating rRNA with 1 M NaCl[6]. Amino acids attached to the tRNA were discharged from the tRNA as described by Chou and Johnson[7]. Aminoacylation was performed by incubating the aminoacyl-tRNA synthetases and tRNA in a buffer (pH 7.5) containing 50 mM Tris, 100 mM KCl, 3 mM ATP, 2 mM glutathione, 9 mM $MgCl_2$, and 2 μCi/ml ^{14}C-L-valine (252 mCi/mmole) at 37 °C for 20 min. The radioactivity precipitated by cold 5% trichloroacetic acid but hydrolyzed by incubation at 100 °C in 5% trichloroacetic acid represents the ^{14}C-L-valine specifically attached to tRNA.

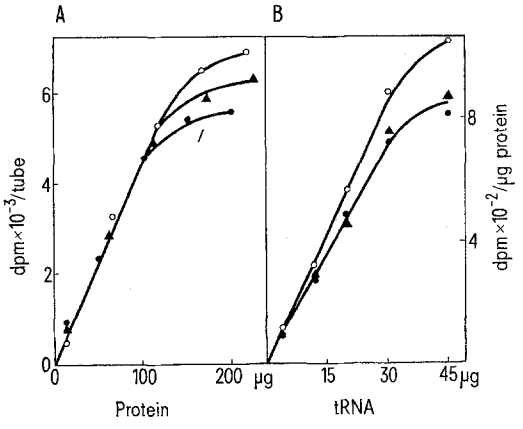

Fig. 1. Aminoacylation of tRNA with valine by aminoacyl-tRNA synthetases isolated from the 3 regions of chicken brain. Aminoacyl-tRNA synthetases were isolated from the cbr (○), cbl (▲) and opl (●) of chicken brain, and their aminoacylation activities with yeast tRNA (*A*) and cbr tRNA (*B*) were measured. In *A* the concentration of yeast tRNA in each reaction mixture was maintained at 125 μg RNA/ml, whereas the amount of aminoacyl-tRNA synthetase protein was varied from 75 to 1150 μg/ml. In *B* 46 μg/ml of aminoacyl-tRNA synthetase protein was added to the indicated amounts of cbr tRNA (7–75 μg RNA/ml). The charging of tRNA with radioactive valine was determined as described in 'methods'.

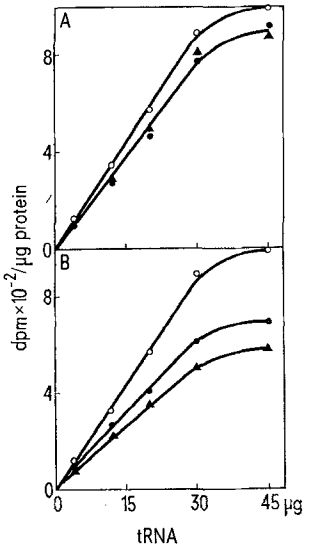

Fig. 2. Valine accepting activity of tRNA isolated from the 3 regions of chicken brain. Transfer RNA was prepared from the cbr (○), cbl (▲) and opl (●) of chicken brain, and the valine accepting activity determined with aminoacyl-tRNA synthetases prepared from the cbr (*A*) or from the cbr, cbl, and opl (*B*). In *A* each reaction sample contained 43 μg/ml of cbr aminoacyl-tRNA synthetase protein to which various amounts of tRNA (7–75 μg RNA/ml) were added. In *B* tRNA (7–75 μg RNA/ml) from the cbr, cbl or opl were incubated with 45 μg/ml of aminoacyl-tRNA synthetase protein obtained from the same brain region as the tRNA. The charging of tRNA with radioactive valine was determined as described in 'methods'.

Results and discussion. Figure 1 shows the ability of various concentrations of aminoacyl-tRNA synthetases from the cbr, cbl or opl to attach radioactive valine onto either yeast tRNA (purchased from Sigma Chemical Company) or the tRNA isolated from the cbr of chicken brain. At low concentrations of the aminoacyl-tRNA synthetases the aminoacylation of yeast tRNA with radioactive valine was similar for aminoacyl-tRNA synthetases from the 3 regions of chicken brain; however, at saturating levels of the aminoacyl-tRNA synthetase preparations the aminoacylation by the valyl-tRNA synthetases from the cbr was about 10% or 20% higher than valyl-tRNA synthetases from the cbl or opl respectively (figure 1A). When yeast tRNA was replaced with tRNA isolated from the cbr, similar differences in the activities of valyl-tRNA synthetases from the 3 brain regions were observed. Again the attachment of radioactive valine onto tRNA was 20% higher by the aminoacyl-tRNA synthetase preparation from the cbr than the preparations from the cbl or opl. Similar results were observed when cbl or opl tRNA was used in place of cbr tRNA. There was no specificity of aminoacyl-tRNA synthetases from a particular region of the brain for tRNA from that same region. Therefore, the valyl-tRNA synthetases from the cbr have a greater capacity than valyl-tRNA synthetases from the cbl or opl to aminoacylate either brain or yeast tRNA. Next a comparison of the valine accepting ability of tRNA from the 3 regions of chicken brain was performed using aminoacyl-tRNA synthetases from the cbr (figure 2A). The valine accepting ability of opl and cbl tRNA preparations were 10–15% less than that observed with cbr tRNA. Rimilar results were observed when cbr aminoacyl-tRNA synthetase preparations were replaced with either cbr or opl preparations.

Figure 2B shows the aminoacylation activity of various concentrations of cbr, cbl and opl tRNA with aminoacyl-tRNA synthetase preparations from the same brain region as the tRNA. The synthetase-tRNA system from the cbl or opl was 50–60% less active in the attachment of valine onto tRNA than the system from the cbr. Therefore, our results clearly show that there are regional differences in the ability of different regions of the chicken brain to aminoacylate tRNA. This difference is due to both the activities of the valyl-tRNA synthetases and the accepting activity of $tRNA^{val}$. The fact that our laboratory[4] has shown that the aminoacylation activity of various regions of chicken brain with phenylalanine were similar emphasizes the importance of studying the aminoacylation of tRNA with a specific amino acid rather than gross acylation activities when comparing different regions of the brain.

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