Chicken Liver Transfer Ribonucleic Acid: Heterologous Interaction of Alanine Transfer Ribonucleic Acid with Yeast

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We are attempting to determine the nature of specific RNA–protein interactions by study of tRNA charging by homologous and heterologous enzymes. $tRNA^{Ala}_{chicken}$ and $tRNA^{Ala}_{yeast}$ were prepared by phenoxyacetylation of alanyl-tRNA and separation on benzoylated DEAE-cellulose (Gillam, Blew, Warrington, von Tigerstrom & Tener, 1968).

A partially purified alanyl-tRNA synthetase from yeast forms alanyl-$tRNA^{Ala}_{yeast}$ and alanyl-$tRNA^{Ala}_{chicken}$ equally well. In contrast, a similar alanyl-tRNA synthetase from chicken liver forms the heterologous alanyl-$tRNA^{Ala}_{yeast}$ less easily than the homologous alanyl-$tRNA^{Ala}_{chicken}$. A comparison of the kinetics of charging the two species of RNA, by using a partially purified alanyl-tRNA synthetase from chicken liver, showed that the heterologous system has a K_m for $tRNA^{Ala}_{yeast}$ that is ten to 20 times larger, and a $V_{max.}$ three to four times smaller, than is found for the homologous $tRNA^{Ala}_{chicken}$. These results are supported by the fact that a large excess of yeast tRNA will inhibit the rate at which the chicken enzyme will form alanyl-tRNA with smaller amounts of chicken tRNA.

Preliminary results on the binding of phenoxyacetylalanyl-tRNA preparations to chicken liver enzyme indicate that the heterologous adduct is kinetically more stable than the homologous one at neutral pH. Thus it seems that with the heterologous RNA the rates of association and dissociation are both decreased.

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Cytoplasmic Deoxyribonucleic Acid in Roots of *Pisum sativum*

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There have been several reports of cytoplasmic or microsome-associated DNA in animal cells (Bell, 1969; Schneider & Kuff, 1969; Bond, Copper, Courington & Wood, 1969; Williamson, 1970). It has been suggested that cytoplasmic DNA is involved in the transfer of information from the nucleus to the cytoplasm (Bell, 1969), but this idea has been contested (Williamson, 1970). The present report describes studies on cytoplasmic DNA isolated from roots of the pea (*Pisum sativum*).

Peas were surface-sterilized with 2% (v/v) peracetic acid containing sodium alkylarylsulphonate (0.16%,w/v) and germinated aseptically in a flexible-film animal isolator. Sterility was checked by inoculation of samples of pea tissue into a wide range of culture media. The seedlings were supplied with [2-^{14}C]thymidine or with [*Me*-^{3}H]thymidine for 4h; the roots were then harvested and homogenized in 0.1M-citrate buffer, pH 5.2. The homogenate was filtered through 25μm nylon mesh and the filtrate was centrifuged at 5100*g* for 15min. Small samples of the precipitate were removed for examination with the electron microscope. DNA was extracted from the precipitate and from the supernatant by a detergent–chloroform method. As expected, the bulk of the DNA was shown to occur in the 5100*g* precipitate. A small quantity of DNA, amounting to 5–10% of the total, was detected in the cytoplasmic fraction.

Partial characterization of the cytoplasmic DNA was carried out. Centrifugation of the cytoplasmic DNA in caesium chloride led to the formation of a single band; the band was very diffuse, suggesting that the DNA is of low molecular weight. Centrifugation in caesium chloride of ^{3}H-labelled cytoplasmic DNA together with ^{14}C-labelled DNA from the 5100*g* precipitate showed that the two DNA fractions have the same buoyant density. Further, examination of the 5100*g* precipitate with the electron microscope showed that, although the mitochondria and pro-plastids remain intact, almost all of the nuclei are broken during homogenization of the tissue. These results suggest that the cytoplasmic DNA is in fact nuclear DNA, released from the nuclei as low-molecular-weight fragments during homogenization.

The results are in agreement with those of Williamson (1970), who suggested that the cytoplasmic DNA of animal cells consists of low-molecular-weight DNA fragments lost from the nuclei.

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