HYBRIDIZATION OF BEAN LEAF LEUCYL-tRNA WITH NUCLEAR DNA AND WITH CHLOROPLAST DNA

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SUMMARY

DNA-RNA hybridization studies were carried out between leucyl-tRNA, prepared from a system isolated from bean leaves, and DNA from the nuclei or the chloroplasts of similar cells. Significant levels of hybridization were observed with both sources of DNA. However, annealing of leucyl-tRNA with chloroplast DNA is more than twice that with the same level of nuclear DNA. Approximately 0.025% of the chloroplast DNA forms ribonuclease-resistant hybrids with green leaf leucyl-tRNA whereas less than 0.011% of the nuclear DNA is involved.

Chloroplasts of higher plant cells contain all macromolecular features required for the several steps of protein synthesis (1,2,3). In addition to the ability of these organelles to incorporate amino acids into peptide structures (1,2) and the existence of unique chloroplast ribosomes (4,5), the affiliation of tRNA and an amino acid activating system with chloroplasts has been noted (6,7). The mere presence of tRNA molecules in the chloroplast, however, fails to establish the site of their synthesis and the involvement of the chloroplast genome in this process. In the investigation described below, we have only attempted to answer the question of whether or not chloroplast DNA can serve as a template for certain species of tRNA isolated from mature leaf cells. The technique of DNA-RNA hybridization was employed as a means to demonstrate complementarity between leucyl-tRNA molecules and DNA preparations which originated from the nuclei or chloroplasts of similar green leaf cells.

MATERIALS AND METHODS

Preparation of [^{3}H] Leucyl-tRNA. Pruified leucyl-tRNA synthetase and tRNA from bush bean (Phaseolus vulgaris, L.) leaves were incubated under appropriate conditions with [^{3}H] leucine (spec. act. = 58.2 Ci/millimole) and the [^{3}H] leucyl-tRNA product was purified extensively by phenol extraction and DEAE-cellulose column chromatography. (Techniques for purification of tRNA and the synthetase from this source as well as those for the formation and isolation of the resultant leucyl-tRNA will be described in a separate communication). The final product contained 1.76×10^4 counts/min per ug sRNA. Other measurements (reported in detail elsewhere) indicate that about 5.4% of the sRNA preparation from mature bean leaves is $tRNA^{Leu}$.

Isolation of Chloroplast and Nuclear DNA's. DNA fractions were isolated from green bean leaf cells by unpublished techniques routinely used in this laboratory. Earlier characterization studies showed no demonstrable cross-contamination between the chloroplast and nuclear DNA's as evidenced by banding profiles in CsCl in the analytical ultracentrifuge. Also, renaturation data support the contention that such cross-contamination, if it existed, was relatively low (Crandall, G.D., unpubl. Ph.D. thesis).

DNA-RNA Hybridizations. DNA filters were prepared by the technique of Gillespie and Spiegelman (8) with minor modifications. Hybridizations and subsequent treatment of the resultant hybrids, including incubation with T-1 ribonuclease, were carried out under the conditions described by Weiss et al. (9). Background filters (with no DNA) were included in each incubation vessel. The dried filters were counted and all data reported herein have been corrected for the background levels observed. Generally, the corrections employed were less than 10% to 15% of the observed counts; the background levels were highly uniform in all cases.

RESULTS

The data in Table I show the specificity of bean leaf tRNA hybridization for chloroplast and nuclear DNA from the same organism. Levels reported for calf thymus and salmon sperm DNA are the highest obtained with those DNA sources. "Observed Counts" are reported here and the adjustments to values expected at a DNA level of 20 ug per filter are given. Similar adjustments were made in all subsequent data.

TABLE I

HYBRIDIZATION OF BEAN LEAF [^{3}H] LEUCYL-tRNA WITH VARIOUS DNA'S

Data are expressed as counts/min of [^{3}H] leucyl-tRNA hybridized per filter and are also normalized to the levels expected with 20 ug of DNA. The reaction vessels contained 13.2 ug sRNA in a final volume of 2.5 ml which was 2xSSC (pH 4.3) and 50% (v/v) formamide. Filters were incubated 3 hr at 33°.

DNA FILTERS		BEAN LEAF [^{3}H] LEU-tRNA HYBRIDIZED	
DNA SOURCE	DNA LEVEL (ug/filter)	OBSERVED (cpm/filter)	ADJUSTED (cpm/20 ug DNA)
CHLOROPLAST (BEAN LEAF)	16.75	385.3	460.0
NUCLEAR (BEAN LEAF)	53.48	555.1	207.5
CALF THYMUS	56.47	10.0	3.5
SALMON SPERM	19.90	15.1	15.2

Effects of varying the leucyl-tRNA input level are shown in Table II. Weiss et al. (9) noted that the conditions employed in order to preserve the acyl bond (namely, the use of formamide in place of high temperatures and the relatively brief annealing period), result in incomplete hybridization in the sense that not all the DNA is saturated. These data show that the process may be hastened by raising the RNA level. Kinetic treat-

TABLE II

HYBRIDIZATION OF BEAN LEAF [^{3}H] LEUCYL-tRNA WITH CHLOROPLAST DNA AND LEAF NUCLEAR DNA AT VARYING RNA INPUT LEVELS

Data are adjusted as noted in Table I and are expressed as counts/min of [^{3}H] leucyl-tRNA hybridized per 20 ug of DNA. Reaction conditions are as described in Table I except as noted below.

DNA SOURCE	DNA LEVEL (ug/filter)	sRNA INPUT (ug/2.5 ml)	[^{3}H] LEU-tRNA HYBRID FORMATION	
			ADJUSTED LEVEL (cpm/20 ug DNA)	CHLOROPLAST: NUCLEAR RATIO
NUCLEAR	23.57	166.7	607.3	
NUCLEAR	50.02	"	593.0	2.25
CHLOROPLAST	19.07	"	1,323.1	
CHLOROPLAST	19.66	"	1,378.0	
CHLOROPLAST	10.24	41.7	930.3	
CHLOROPLAST	19.54	"	938.4	
NUCLEAR	56.60	10.4	141.6	
NUCLEAR	24.60	"	167.7	2.22
CHLOROPLAST	19.33	"	342.9	

ment of these and other data yielded maximum potential hybrid levels of 1677 counts/min (or 5.12×10^{-3} ug $tRNA^{Leu}$) for 20 ug of chloroplast DNA and 714 counts/min (or 2.18×10^{-3} ug $tRNA^{Leu}$) for 20 ug of nuclear DNA. One-half maximal levels of hybridization were observed in both cases after three hrs at 33° with an sRNA input of 33 ug per 2.5 ml. The ratios between chloroplast and nuclear DNA hybridization at sRNA input levels ranging from 4.2 ug to 166.7 ug were all between 2.22 and 2.34.

The incubation period was extended from three to six hrs in an effort to determine whether the relative difference between the two DNA sources was one of kinetics rather than a reflection of a real difference between

the two with respect to the per cent of DNA available for hybridization with leaf leucyl-tRNA. Under conditions that led to about one-half maximal hybridization in six hrs, the hybridization ratio of chloroplast: nuclear DNA was greater than 2:1.

DISCUSSION

The above data show that, while salmon sperm DNA and calf thymus DNA contain no significant levels of complementary sequences with bean leaf leucyl-tRNA, approximately 0.025% of the bean leaf chloroplast DNA appears to hybridize with that tRNA and almost 0.011% of the leaf nuclear DNA is similarly complementary. If the commonly-held notion that complementarity in DNA-RNA hybridization experiments is indicative of the potential template activity of the DNA is valid, it is clear, on the basis of our data, that chloroplast DNA can indeed serve as a template for the formation of at least some of the cellular $tRNA^{Leu}$.

Whether or not any tRNA is actually formed on the chloroplast DNA template and whether such tRNA, if formed, is unique in base composition is not clear from these data. We have established (unpublished observations) the existence of multiple isoacceptors for a number of tRNA species in green bean leaf cells and the possibility exists that one or more of these may be of chloroplast origin. Further, we find that only certain $tRNA^{Leu}$ isoacceptors are synthesized preferentially upon greening of etiolated leaves and those exhibiting accelerated synthesis during chloroplast development are acylated exclusively by a second leucyl-tRNA synthetase present in all of our crude enzyme preparations from leaves. We now expect to examine these unique leucyl-tRNA isoacceptors in an effort to determine whether or not they differ from the remainder of the leaf cell leucyl-tRNA in their ability to hybridize with chloroplast DNA.

If the amount of DNA per chloroplast is assumed to be 2×10^{-15} g, the number of leucyl-tRNA molecules hybridized for each chloroplast DNA

equivalent is about 10 to 12. Also, using the above data and an estimate of 1 x 10^{-12} g of DNA per bean leaf nucleus, approximately 2400 to 2600 molecules of leucyl-tRNA are hybridized per nucleus. This high level of repetition is not unprecendented. Tewari and Wildman (10) estimated that eight molecules of chloroplast ribosomal RNA hybridized with tobacco leaf chloroplast DNA, whereas they found about 2000 cistrons in the tobacco nucleus complementary to cytoplastmic ribosomal RNA. Further, repetitive sequences exceeding 2000 have been reported in the DNA of a number of higher organisms (11). We wish to emphasize, however, that these values calculated for levels of repetition in bean leaves are clearly based on certain estimates in addition to our data and should be accepted as rough approximations at best.

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