POLYRIBOSOMES IN LEAVES

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Received October 14, 1963

For several mammalian systems it has recently been shown that during protein synthesis a number of ribosomes become associated with messenger RNA to form polyribosomes (e.g. Warner et al., 1962; Gierer, 1963; Wettstein et al, 1963). We have developed a method for the isolation and temporary preservation of polyribosomes from leaf tissue, and have studied some of the factors affecting polyribosome levels in leaves. The principal difficulty in studying polyribosomes is their high susceptibility to nuclease action. Nucleases appear to be particularly active in leaves, and the method described below exploits polyvinyl sulphate as a nuclease inhibitor (Fellig and Wiley, 1959).

Chinese cabbage plants (Brassica pekinensis, Rupr., var. Wong Bok.) were grown in pots in the glasshouse. To prepare extracts containing polyribosomes excised leaves were immediately plunged into ice-water, transferred to the cold room (2°), blotted to remove water, deribbed, weighed, and ground in a pestle and mortar with an equal weight of the following medium:- polyvinyl sulphate (prepared by the method of Liquori et al., 1959) 10 mg/ml; 0.1 M sodium acetate buffer, pH 6.0; and 0.02 M magnesium chloride. This concentration of Mg^{++} is required in the presence of the high level of polyvinyl sulphate. Sand was added before

grinding (about 0.3 gm./gm. of leaf). The extract was expressed through muslin, centrifuged at 200 G for one minute, and examined in the Spinco model E analytical centrifuge at 39,460 r.p.m. using Schlieren optics. This procedure gives extracts in which the polyribosome pattern remains apparently stable for about 30 minutes at 2°, but after 60 minutes some degradation of the higher polymers has taken place. We have not been able to isolate polyribosomes in good yield by density gradient or differential centrifugation. All such preparations have given patterns indicating marked degradation of the higher polymers. Approximate S_{20W} values for various components were calculated by reference to an added standard material (turnip yellow mosaic virus, S_{20W}=114). Areas under the Schlieren curves were measured, and converted to concentration in mg./ml. by a factor empirically determined using a purified preparation of 83 S ribosomes. Using the above procedure, extracts from young leaves harvested following a period of daylight show a high proportion of the ribosomes in polyribosome form. The polyribosomes move away from the meniscus as a single but rapidly spreading component. For this mixed peak, S values ranged from 175 to 201 with a mean of 190. The mixed polyribosome peak rapidly splits into at least 4 - 6 small peaks, the four slowest of which have the following approximate S values:- 125, 159, 186 and 202 S. These figures are in fair agreement with those for polyribosomes from reticulocytes (Gierer, 1963). The ribosome monomer has an S $\simeq$ 83. In most samples low concentrations of a series of slower moving components could be observed with S values $\simeq$ 26, 37, 44 and 60. To assess the total ribosome level in a leaf sample we allowed leaf

nucleases to degrade the polyribosomes to 83 S ribosomes by grinding the leaf at room temperature in an equal weight of a medium containing only 0.1 M sodium acetate buffer, pH 6.0. In such material three components were observed:- a small 60 S, a large 83 S, and a small 125 S peak. The uncharacterised components with S $<$ 60 were rapidly destroyed under these conditions.

The concentration of total ribosomes in leaf laminae falls steadily from about 5.0 mg./gm. fresh weight in leaves weighing 0.16 gm. to about 0.5 mg./gm. in fully expanded but still active leaves (about 3.0 gm.). However, ribosome production in the leaf continues until leaves are about half their final fresh weight. Beyond this stage the weight of ribosomes per leaf begins to fall. The proportion of ribosomes in active polyribosome form, in daily samples taken during the middle period of the day, rises steadily during the first half of the expansion period to a maximum of about 90%, and then tends to fall. The white parts of the Chinese cabbage plant (petiole and midrib, stem and roots) contain only about one tenth the ribosome concentration (per unit fresh weight) found in leaf laminae. Considering the plant as a whole, 80-90% of the ribosomes are in the green part of the leaves.

The major short-term variation in the level of polyribosomes in Chinese cabbage leaves was found to follow a diurnal cycle in which polyribosome levels are highest during daylight and generally lowest at the end of the dark period (Figs. 1 and 2). Light intensity and duration appear to be the major factors involved in this cycle although temperature plays some part. At the end of the dark period 50-80% of the ribosomes (83 S or greater) may be in 83 S

form. From first light onwards this proportion falls. Within 2-4 hours of sunrise on a sunny day about 90% may be in forms greater than 83 S. This state is maintained until near sunset unless there is a prolonged dull period. The proportion in forms greater than 83 S falls during the night. If the night period is artificially prolonged by 2-3 hours polyribosomes fall to a very low level. When such plants are then exposed to as little as 4 minutes of bright sunlight their ribosome pattern shows a drop in 83 S and an increase in polyribosomes.

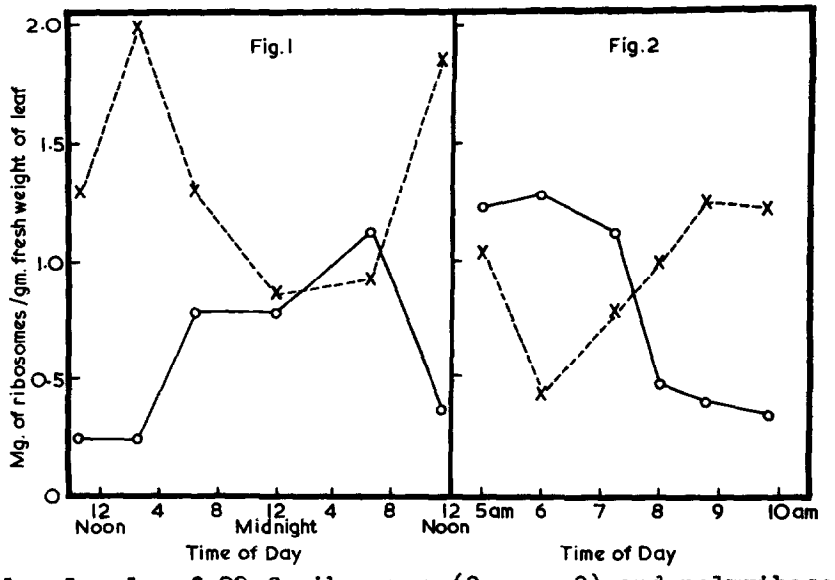

Fig. 1. Levels of 83 S ribosomes (0 —— 0) and polyribosomes (X --- X). Samples of 3 Chinese cabbage leaves, weighing about 0.8 gm. fresh weight/leaf, harvested at various times. Plants were held in a glasshouse at 21° ± 1°. Sunset at 5.30 pm. Sunrise at 7.05 am.

Fig. 2. Levels of 83 S ribosomes (0 —— 0) and polyribosomes (X --- X). Conditions as for experiment of Fig. 1. First light at 6.10 am. Sunrise at 7.00 am, followed by bright sunlight.

Our measurements of polyribosomes in leaf extracts presumably indicate the proportion of the protein

synthesising machinery which is active at a given time. The diurnal variation we have found is consistent with a number of earlier observations made by other methods indicating that protein synthesis in leaves is greater in light (e.g. Chibnall, 1924; Parthier, 1961). Different plant species and genotypes within a species may vary widely in their growth responses to different environmental conditions. A study of ribosome and polyribosome levels may provide a closer insight into these effects. For example, the very rapid response in polyribosome level that can be detected in leaves exposed to light after an artificially prolonged night period, suggests a new experimental approach to the problem of how day length controls development in plants. This will be of particular interest if it can be shown that the rise in polyribosome level is associated with a light-dependent synthesis of new messenger RNA.

This investigation was supported in part by U.S. Public Health Service Research Grant AI-04973-01.

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