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Non-Random Distribution of Four Chloroplastic Isozymes and the Corresponding Cytosolic Isozymes with respect to DNA in the Pea Leaf Nucleus

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Class I aldolases (EC 4.1.2.13) catalyze the aldol condensation of dihydroxyacetone-P with glyceraldehyde-3-P to form fructosebisphosphate, and the reverse reaction, the aldol cleavage of fructosebisphosphate to the two triose phosphates. Fructose bisphosphatase (E.C. 3.1.3.11) removes the phosphate from carbon-1 of fructose-1,6-bisphosphate to generate fructose-6-P. P-glycerate kinases (EC 2.7.2.3) catalyze the transfer of phosphate from ATP to P-glycerate and the reverse reaction, the transfer of phosphate from 1,3-P_2-glycerate to ADP. The chloroplastic and cytosolic aldolase, fructose bisphosphatase and P-glycerate kinase isozymes are present in the nucleus in the pea (*Pisum sativum*) leaf [1-3]. Glyceraldehyde-3-P dehydrogenase catalyzes the pyridine nucleotide-dependent reduction of 1,3-P_2-glycerate to glyceraldehyde-3-P, and the reverse reaction, the oxidation of glyceraldehyde-3-P to 1,3-P_2-glycerate. The NAD-linked cytosolic (EC 1.2.1.12) and the NADP-linked chloroplastic subunit B (EC 1.2.1.13) glyceraldehyde-3-P dehydrogenase isozymes, but not the chloroplastic A subunit isozyme, are also present in the pea leaf nucleus [4].

Analysis of double immunolabeling experiments indicates that both aldolase isozymes [5], both fructose bisphosphatase isozymes (Figs. 1,2) and the cytosolic and chloroplastic subunit B glyceraldehyde-3-P dehydrogenase isozymes (not shown) are distributed non-randomly with respect to DNA, and therefore co-localized with DNA, in the nucleus, and that the chloroplast isozymes are distributed non-randomly with respect to DNA, in the chloroplast. In contrast, the P-glycerate kinase isozymes are co-localized with DNA in the nucleus (not shown), but the chloroplastic isozyme is not co-localized with DNA in the chloroplast. It seems possible that the chloroplast and cytosolic isoforms of these enzymes in the nucleus might function as sensors that link sugar metabolism in the cytoplasm to gene expression. Likewise it seems possible that the chloroplastic aldolase, fructose bisphosphatase and subunit B glyceraldehyde-3-P dehydrogenase isozymes might be involved in gene expression within the chloroplast.

P-glycerate kinases appear to act as primer recognition proteins for DNA polymerase-α in animals [6] and in plants [7]. Co-localization of the P-glycerate kinase isozymes with DNA is consistent with this secondary nuclear function.

Thin sections were prepared from pea leaf tissue fixed in 1% acrolein, 0.1% glutaraldehyde and embedded in LR White resin. The grids were floated on solution containing the antibodies directed against DNA and antibodies directed against one of the isozymes overnight. Exposure to the gold labeled secondary antibodies was for 4 hours the following morning. We used the method of J.B. Anderson et al. [8] for analysis of nearest neighbor distances on the micrographs from the double labeling experiments. For a population of two different non-interacting species the expression $n/N = 1 - \exp(-\pi r^2 \rho)$ gives the fraction n/N corresponding to position in an ordered list of samples with increasing nearest-neighbor distance r, where n is the number of the measurement in rank order, N is the total number of measurements, r is the distance between nearest neighbors, and ρ is the species

density. A plot of $-\ln(1-n/N)$ versus r^2 produces a straight line, if the two species are distributed randomly. Where there is positive interaction the initial data points will be displaced towards the $-\ln(1-n/N)$ axis and the curve will balloon out toward that axis. We measured the distance from the center of each large gold particle to the center of the nearest small gold particle using Scion Image (Scion Corporation, Frederick, MD) and plotted $-\ln(1-n/N)$ against r^2.

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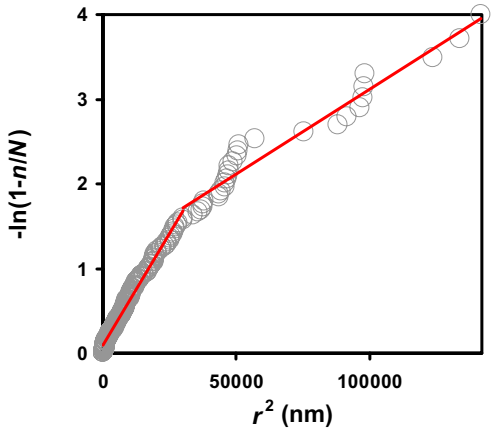

Fig. 1. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking cytosolic fructose bisphosphatase and gold particles marking ssDNA. The biphasic curve indicates co-localization.

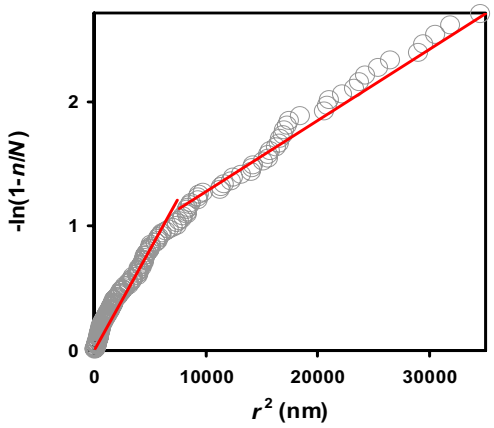

Fig. 2. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking chloroplastic fructose bisphosphatase and gold particles marking ssDNA. The biphasic curve indicates co-localization.

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