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Phosphoglycerate Kinase is a Near Neighbor of CF_1 in the Pea Leaf Chloroplast

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The Calvin cycle enzymes phosphoglycerate kinase (EC 2.7.2.3) and phosphoribulokinase (EC 2.7.1.19), ADP-glucose pyrophosphorylase (EC 2.7.7.27) which is the first enzyme in starch synthesis, and the protein Rubisco activase utilize ATP generated by the β subunit of the chloroplast ATP-synthase, CF_1. The purpose of the experiments described here was to determine whether the two kinases, ADP-glucose pyrophosphorylase and Rubisco activase are co-localized with CF_1, where they would have ready access to photosynthetically-generated ATP.

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 antibodies direct against the β subunit of CF_1 and antibodies raised against the different ATP-utilizing enzymes or Rubisco activase overnight. Exposure to gold labeled secondary antibodies was for 4 hours the following morning. We used the method of J.B. Anderson et al. [1] 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.

The results of the double immunogold labeling experiments with antibodies directed against CF_1 and antibodies directed against the phosphoglycerate kinase and phosphoribulokinase indicate that both kinases are co-localized with CF_1 (Figs. 1,2). Likewise ADP-glucose pyrophosphorylase appears to be co-localized with CF_1 (Fig. 3). These results suggest that the two kinases and the pyrophosphorylase have immediate access to ATP generated in the light in the chloroplast. This might be expected to enhance photosynthetic CO_2 fixation. Location near the source of ATP will also allow ready recycling of the adenine nucleotides between the kinases and the coupling factor. In contrast, when nearest neighbor distances are measured from gold particles marking CF_1 to gold particles marking Rubisco activase, the data plot indicates co-localization, but when measurements are made from gold particles marking to activase to gold particles marking CF_1, there is no indication of co-localization (Fig. 4). This suggests that most of the Rubisco activase protein is not located close to CF_1, although a significant fraction of the CF_1 population is located near Rubisco activase. Rubisco activase is co-localized with the CO_2 fixing enzyme Rubisco, and it is possible that it shuttles between the ATP source, CF_1 on the thylakoid membrane, and Rubisco, in the chloroplast stroma.

Reference

[1] J.B. Anderson et al. J. Structural Biol. 143 (2003) 95.

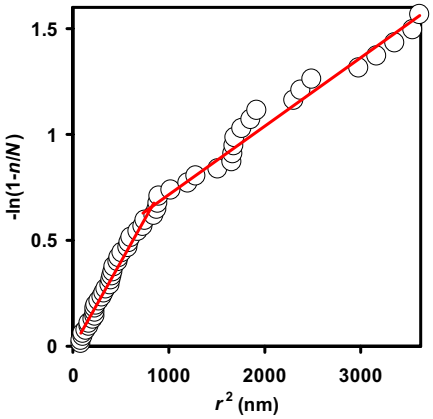

Fig. 1. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking $CF_1\beta$ and gold particles marking phosphoglycerate kinase. The biphasic curve indicates co-localization.

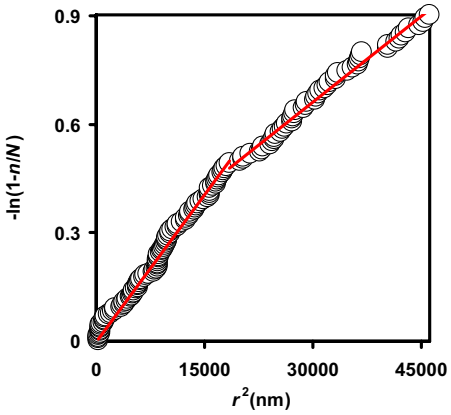

Fig. 2. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking $CF_1\beta$ and gold particles marking phosphoribulokinase. The biphasic curve indicates co-localization.

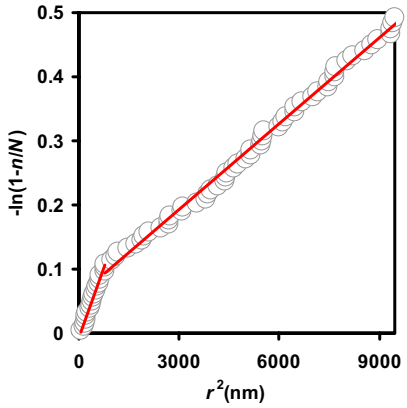

Fig. 3. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking $CF_1\beta$ and gold particles marking ADP-glucose pyrophosphorylase. The two enzymes appear to be co-localized.

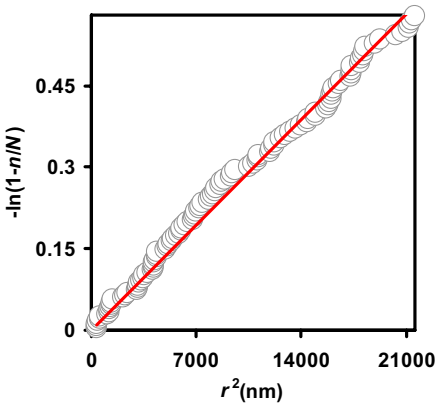

Fig. 4. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking $CF_1\beta$ and gold particles marking Rubisco activase. Most of the activase molecules are distributed randomly with respect to CF_1.

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