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DNA Methylation as a Transcriptional Regulation in Plastids during Tomato Fruit Development

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The developmental maturation and ripening of a tomato (*Lycopersicon esculentum*) fruit is accompanied by a number of morphological and physiological changes, including the plastid differentiation from chloroplasts in green fruit to chromoplasts in ripened red fruit. Several investigators have reported that the levels of most transcripts for both plastid- and nuclear-encoded photosynthesis genes decline to marginally low levels in fully ripened tomato fruits.

We have employed the plastid run-on transcription assays to demonstrate whether or not the reductions of photosynthesis gene transcripts during tomato fruit ripening is ascribed to transcriptional regulation. Furthermore, the acting factors-mediating transcription was showed to be primarily excluded by the results ascribing regulatory step to DNA template activities in the run-off *in vitro* transcription assays. Since there is no rearrangment of plastid DNA during plastid transition from chloroplasts to chromoplasts, the modification of DNA would be most considered. The analysis of modified base composition, revealed the presence of a variety of methylated bases in chromoplast DNA, in which the amounts of individual methylated bases increased about two-fold during the conversion of chloroplasts to chromoplasts in ripening tomato fruits. In order to precisely determine the methylated loci on DNA, we employed the methyl-sensitive and -insensitive isoschizomeric endonucleases digestion and the subsequent Southern hybridization with the specific gene probes. The results revealed the presence of methylated DNA sequences in regions containing the lowly-expressed genes in chromoplast DNA, in contrast to the chloroplast DNA of tomato fruits and leaves (1). It is thus conceivable that tomato plastid DNA is selectively methylated during fruit ripening, resulting in the suppression of photosynthesis gene transcription.

Independently, we have found that DNA methylation is a likely causative factor for the suppression of transcriptional activity of photosynthesis genes in another non-green plastid-type, amyloplasts, of nonphotosyntetic cell line of sycamore (2). Similar mechanism was found to operate in the differential expression of photosynthesis genes in two-cell types of maize (3). Our present investigation appears to provide further evidence for a significant role of DNA methylation in the transcriptional regulation of photosynthesis genes in a naturally occurring nonphotosynthetic plastid-type, the chromoplasts in ripening tomato fruits.

(1) J. Ngernprasirtsiri, H. Kobayashi and T. Akazawa (1988) *Plant Physiol.* 88, 16-20.
(2) J. Ngernprasirtsiri, H. Kobayashi and T. Akazawa (1988) *Proc. Natl. Acad. Sci. USA.* 85, 4750-4754.
(3) J. Ngernprasirtsiri, R. Chollet, H. Kobayashi, T. Sugiyama and T. Akazawa (1989) *J. Biol. Chem.* 264, (in press).