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EVIDENCE FOR CHANGES IN MESSENGER RNA CONTENT RELATED TO TOMATO FRUIT RIPENING

NITHIYA RATTANAPANONE*, JAMES SPEIRS† and DONALD GRIERSON
University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, LE12 5RD, Leicestershire, U.K.

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; messenger RNA; *in vitro* protein synthesis; fruit ripening.

Abstract—Poly(A)-containing *m*RNA was purified from tomato fruits and translated in a wheat germ *in vitro* protein-synthesizing system. Comparison of the protein products produced in response to *m*RNA samples from unripe and ripening fruits provides evidence for changes in the amounts of *m*RNA coding for specific proteins during ripening.

INTRODUCTION

There is considerable circumstantial evidence that changes in gene expression are involved in fruit ripening. Several enzymes have been shown to increase in activity [1–3], increased incorporation of radioactive amino acids and nucleic acid precursors into protein and RNA occurs just before or during the respiratory climacteric [4–11, 13] and cycloheximide, an inhibitor of protein synthesis on 80 S ribosomes, prevents ripening [4, 12, 13]. However, there is no firm evidence as to whether enzyme activation or *de novo* synthesis is responsible for the increase in enzyme activity. Similarly, although a translational control mechanism involving regulation of specific *t*RNAs has been suggested [14], there is no evidence as to whether transcription of new *m*RNAs occurs during ripening or whether stored *m*RNA is utilized. Recently it was shown that Poly(A)-containing *m*RNA could be purified from developing tomato fruit and translated in a wheat germ *in vitro* protein synthesizing system [15]. In the present work the results are given of a preliminary characterization of the protein products synthesized in response to *m*RNA prepared from tomato fruits. The results provide evidence for the synthesis of new *m*RNA during or just prior to ripening.

RESULTS AND DISCUSSION

Poly(A)-containing *m*RNA from tomato fruit was translated in a wheat germ *in vitro* protein synthesizing system. The stimulation of incorporation of methionine-[^{35}S] into protein by added *m*RNA was between 14 and 20 times higher than the endogenous level of incorporation. TMV RNA stimulated a 50 fold increase in incorporation with respect to the endogenous level. The lower level of activity of the tomato fruit *m*RNA may be due in part to the presence of inhibitory substances such as carbohydrates, which co-purity with the *m*RNA.

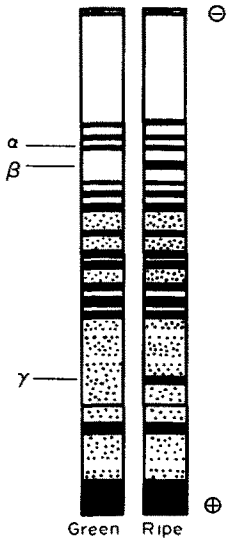

Fig. 1. Comparison of *in vitro* translation products of *m*RNAs from green and ripening fruit of the GCR 145 variety of tomato. Samples were denatured in 2% SDS, fractionated in 15% polyacrylamide slab gels and the radioactive proteins detected by fluorography.

*m*RNA samples from both green and ripe fruits were active in stimulating protein synthesis *in vitro*. The radioactive protein products synthesized in response to *m*RNA from mature green and ripe fruits were fractionated by polyacrylamide gel electrophoresis under denaturing conditions and the protein bands visualized by fluorography. Figure 1 shows the translation products of *m*RNA from green and ripe fruits of the GCR 145 variety. Most of the proteins were synthesized in similar proportions in response to each of the *m*RNA samples. However, the bands marked β and γ are found in the translation products of *m*RNA from ripening fruits of the GCR 145 variety but are present in much smaller amounts or are absent using *m*RNA from mature green

Present addresses: * Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand; † CSIRO Division of Food Research, Plant Physiology Unit, School of Biological Sciences, MacQuarie University, P.O. Box 52, North Ryde, N.S.W., Australia.

Abbreviations: Poly(A), polyadenylic acid: oligo (dT)-cellulose, cellulose with oligo deoxythymidylic acid covalently attached; TMV, tobacco mosaic virus.

fruits. Comparable changes were found with other varieties. In the GCR 118 variety band β was present only with *m*RNA from ripe fruits whereas band γ was synthesized with *m*RNA from both ripe and green fruit. In the Minipopella and GCR 141 varieties bands similar but not identical to β and γ were found (β' and γ'). In these varieties there was no detectable change in the amounts of the β' band comparing *m*RNA from green and ripe fruit but the γ' band was much more prominent with *m*RNA from ripening fruit. In addition, whereas in the GCR 145 variety approximately equal quantities of the band marked α (Fig. 1) were found in the translation products of *m*RNA from green and ripe fruit, in the GCR 118 and Minipopella varieties the α band was found in *m*RNA products from green fruit but it was reduced or absent when *m*RNA from ripening fruit was translated.

We have made the assumption that the detection of an *in vitro* protein product is due to the presence of a particular *m*RNA in the assay. It has been shown that, when stimulation of synthesis in the wheat germ system is low, a significant contribution to the protein products is made by the endogenous wheat germ *m*RNAs [16]. In our experiments it is clear that the precise nature of the products is determined by the tomato variety used and that there are consistent differences between the *m*RNA from unripe and ripening fruit. This suggests that there is a genuine change in the amount of *m*RNA coding for the α, β or γ proteins during ripening. The approximate MWs of these proteins are as follows: α 56000, β 46000, (β' 38000), γ 20000, (γ' 21500). Although there is no evidence that these *m*RNAs are correctly translated to produce polypeptides occurring *in vivo*, the results strongly suggest that changes in coding properties of the *m*RNA population occur during ripening. The possibility that these results are due to changes in polyadenylation of the *m*RNAs for the α, β and γ proteins just prior to ripening is considered unlikely. Translation of poly(A)-containing and poly(A)-lacking RNA from tomato fruits gives a largely similar spectrum of proteins [17].

The identity of the α, β and γ proteins is unknown. There is no evidence that they control ripening and there is no conclusive evidence that they are synthesized *in vivo*. Nevertheless the appearance or disappearance of *m*RNA sequences in ripening fruit giving rise to those proteins when translated *in vitro* gives the first evidence for changes in the amount of specific *m*RNAs associated with fruit ripening.

EXPERIMENTAL

Tomato plants (*Lycopersicum esculentum* Mill) of the GCR 145, GCR 118 and Minipopella (slow ripening) varieties were grown in soil in a heated greenhouse at temps between 15 and 25°. The ages of the fruits at the onset of ripening varied from variety to variety (cf. [15]). In the text unripe refers to fruits 6–8 weeks old which have not yet started to synthesize lycopene. Ripening refers to fruit 1–2 weeks older in which lycopene synthesis is well established.

Extraction and purification of nucleic acids. Nucleic acids were extracted from outer pericarp tissue as previously described [15]. The nucleic acids, dissolved in 50 mM Tris/HCl pH 7.4, 2 mM Mg acetate, were incubated with DNase (Sigma) at 100 μg/ml for 30 min at 0°. The soln was made up to 100 mM KCl and 10 mM Mg acetate and further purification was carried out by incubation at 10° for 30 min with 200 μg/ml Proteinase K (Boehringer). After incubation the vol. was increased 2–3 fold by the addition of 50 mM Tris/HCl pH 7.4 containing 10 mM Mg acetate and the soln deproteinised ×3 by shaking with an equal vol. of $CHCl_3$–PhOH (1:1) [18] followed by centrifugation. The supernatant was made 2% with NaOAc pH 5 and the RNA precipitated for 18 hr at −20° by the addition of 2.5 vols EtOH

Poly(A)-containing RNA was purified by oligo (dT)-cellulose chromatography [18].

Nucleic acid concentration was estimated by measuring the A_{260} nm. A 40 μg/ml soln was assumed to have an $A_{260} = 1$ using a 1 cm light path.

In vitro *protein synthesis.* Synthesis was carried out using an 80S ribosome system prepared from wheat germ (obtained from Bar-Rav Mill, Tel Aviv, Israel). Preparation and use of the wheat germ S.30 system followed the procedure of ref. [19]. The radioactive amino acid was methionine-[^{35}S].

Acrylamide gel electrophoresis. In vitro synthesized proteins were fractionated by electrophoresis on 15% slab gels (20) containing 0.2% SDS and 8 M urea. Detection of methionine-[^{35}S] proteins was by fluorography [21].

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