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Translational Control of The Heat Shock Gene Expression in Carrot

Cheol Ho HWANG
Department of Agronomy, Dankook University, Cheonan, 330-714

당근의 열충격 유전자 발현의 번역과정에서의 조절

황철호
단국대학교 농과대학 농학과

Carrot cultured cells are able to respond to a temperature increase by inducing a set of new proteins, heat shock proteins (HSP). Such an induction of the HS gene was known to be achieved mainly at the level of transcription. Howerever there has been an increasing number of evidences showing that a translational control was involved in the regulation of the HS gene expression. A comparison of HSP synthesized in vivo to in vitro (represent for mRNA level since the amount of the proteins produced by in vitro translation system will be proportional to an amount of the corresponding mRNA) showed no correlation between the amount of HS mRNA and the amount of the corresponding HSP at 30°C. It appears that a translational control may exert a major role in the expression of HS gene in carrot callus cells at 30°C.

Key words: callus, carrot, *Daucus carota*

Heat Shock Protein (HSP) was known to be produced in response to a temperature increase in almost all organisms so far studied. Carrot cultured-cells are able to respond to HS conditions in a manner silmilar to other plant cells and tissues (Mansfield and Key, 1987: Scharf and Nover, 1982), by inducing a set of new proteins, the heat shock proteins, and by repressing the synthesis of the proteins produced under non-HS condition (Hwang and Zimmerman, 1989). The synthesis of the HSP was primarily dependent on an active transcription of heat shock genes. Such a thermo-inducible transcription of HS genes has been shown to be due to the presence of both a cis-element called the "heat shock element" as a cis-acting factor and an activation of a heat shock transcription factor as a trans-acting factor (Perisic et al., 1989: Parker and Topol, 1984: Gurley and Key, 1991). In addition, there have been some reports indicating that a post-transcriptional control was also exerted in a thermo-induction of HS genes. During HS conditions, translational machinery selects only HS messages, which leads to preferential production of HSPs (Scharf and Nover, 1982). The globular embryos of carrot in particular was known to adapt a translational control as a major control mechanism in HS gene expression (Apuya and Zimmerman, 1992). There will be other evidence reported in this paper to show an involvement of translational control of HS gene expression in carrot cultured callus cells at 30°C, the lowest temperature inducing the synthesis of HSP.

MATERIALS AND METHODS

Plant Material

Callus suspension cell lines were initiated from 0.25 to 0.5 cm long section of the hypocotyl of carrot (*Daucus carota* L. cv. Danvers Half-Long) seedlings germinated from sterilized seeds and were cultured in Murashige and Skoog's medium supplemented with 5 g/ml 2,4-dichlorophenoxyacetic acid (2,4-D). and 3% (w/v) sucrose and transferred every other week.

Preparation and Analysis of Proteins from Carrot Tissues

Proteins were labeled by suspending 50 μL of gravity packed cells in 500 μL fresh MS^+ medium in the presence of 50 μCi of [^{35}S]-methionine (1,000 Ci/mM, NEN) and shaking was continued for an appropriate period. Labeled cells were collected by centrifugation and washed free of [^{35}S]-methionine with MS^+ medium, and frozen in liquid nitrogen. Proteins were extraced by homogenization in NP-40 buffer [9.0 M urea, 4.0% NP-40, 1.0% ampholine (LKB, 0.8% pH 5-8, 0.2% pH 3-10), 2.0% β-mercaptoethanol]. Debris was removed by centrifugation at 12,000 xg for 15 min. The supernatant, containing soluble proteins, was used as a protein extract. Proteins were resolved in two dimensions as described by O'Farrell (1975) using 15% (w/v) acrylamide gels for the second dimension. Proteins were fixed in 7% (v/v) acetic acid, and were visualized by soaking the gels in Fluorohance (Research Products International Corp.) followed by drying and exposing to Kodak X-AR film.

RNA Preparation and Analysis

Total RNA was isolated from carrot cells using the method described by Glisin et al. (1974). Oligo (dT) cellulose (Boehringer Mannheim) was used to isolate poly (A^+) RNA from total RNA using the procedure of Aviv and Leder (1972). RNA was analyzed by loading 10 μg of poly (A^+) RNA onto a 1.2% agarose/formaldehyde gel using the method described by Lehrach et al. (1977). RNA was transferred onto Gene Screen (DuPont-NEN) by capillary transfer, and the resulting blot was hybridized according to the manufacture's instruction. DNA probes were prepared by random priming of a purified DNA fragment from a agarose gel following a procedure described by Feinberg and Vogelstein (1983). The resulting autoradiograms were scanned by a video densitometer (Bio-Rad model 629) with the background subtracted, and the area of peak absorbance was integrated by computer.

In Vitro Translation

In vitro translation was performed with the rabbit reticulocyte lysates (Promega) following the instruction of the manufacturer based on the method described by Pelham and Jackson (1976). Right after translation in vitro, the proteins were collected by adding nine times volume of ice-cold acetone and then precipitating the proteins at -20°C overnight. The resulting pellet, after centrifugation at 12,000 xg for 10 min, was resuspended in NP-40 buffer.

RESULTS AND DISCUSSION

Induction of HSP and HS Message Syntheses as a Function of Increasing Temperature

To define the optiomal temperature for induction of HSPs in carrot, callus cells, grown for seven days after subcloning, were incubated at increasing temperatures. The result of 2D-IEF/SDS-PAGE, shown in Figure 1, indicated that the synthesis of HSP started at 30°C and HSP was synthesized maximally at 38°C. It appears that both HSPs and non-HSPs were synthesized at 30°C. The majority of HSPs were induced at 30°C and accumulated maximally at 38°C. At 40 °C, overall protein synthesis decreased so that the entire set of HSPs appeared to be reduced (the data not shown).

In order to see whether the induction of the heat shock proteins shown above correlates with the induction of the corresponding message, the accumulation of the HSP17 (denoting a HSP17 gene family) and HSP70 transcripts was analyzed as a function of increasing temperature. Northern analysis of poly (A^+) RNAs isolated from callus cells incubated at different temperatures is shown in Figure 2. A typical temperature-induced accumulation of the transcripts was shown. Quantitation of the autoradiogram by densitometry reveals that the accumulation of HSP17 message was induced 84 fold and the accumulation of HSP70 message was induced 6 fold as the cells were shifted from 23°C to 38°C, The dramatic induction of HSP17

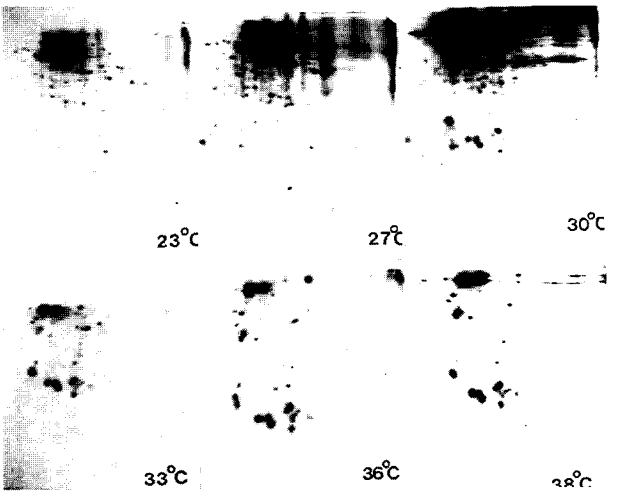

Figure 1. 2D-IEF/SDS-PAGE anglysis of the proteins synthesized in carrot cells as a function of increasing temperature. Equivalent amounts of TCA precipitable counts (6.3×10^6 cm) per protein sample were loaded.

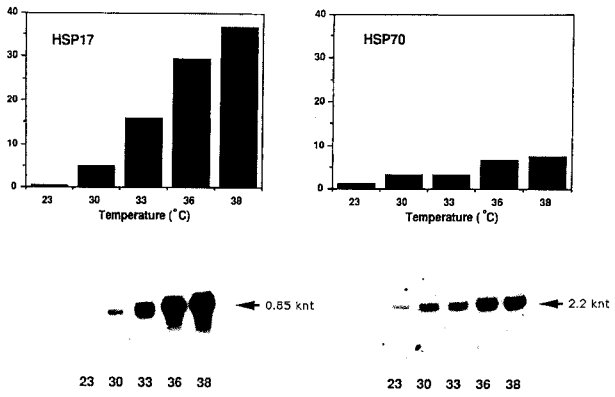

Figure 2. Accumulation of the HSP17 and HSP70 RNAs in carrot cells at increasing temperatures. Poly(A^+) RNAs were isolated from carrot callus cells incubated at increasing temperatures for 3 hours. 12 μg of poly(A^+) RNAs per sample was separated in 1.2% agarose/formaldehyde gel and blots were hybridized with radioactively labeled DNAs from plasmids containing carrot DChsp17.7 and Maize HSP70 cDNA. The level of the accumulated transcripts was measured by densitometry (Model 620, BioRad).

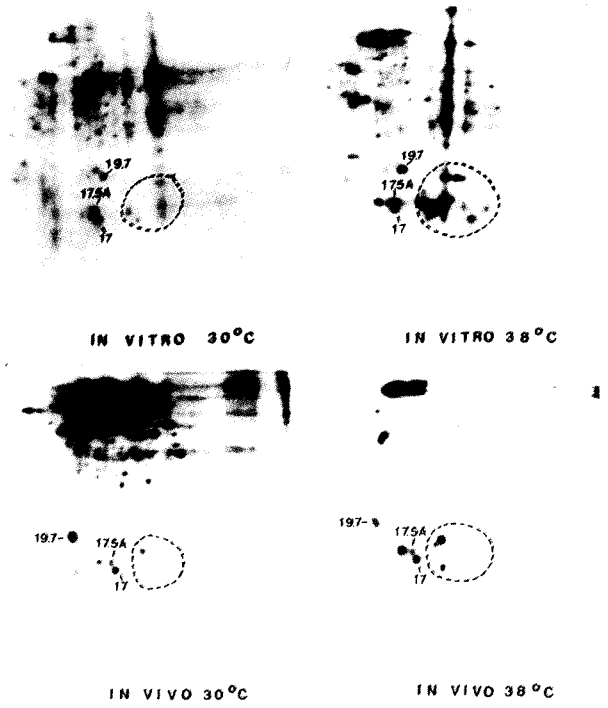

Figure 3. Comparison of heat shock proteins synthesizing in vivo and in vitro at 30° C and 38° C. For in vivo labeling of HSP, carrot callus cells were incubated in the presence of 50 μCi of [^{35}S]-methionine for the last two hours of a three hour incubation at a given temperature. In order to synthesize and label HSP in vitro, poly(A^+) RNAs isolated from carrot callus cells heat shocked at 30° C and 38° C were translated in vitro in the presence of [^{35}S]-methionine (50 μCi) using a rabbit reticulocyte lysate (Promega). An Equivalent amount of TCA-precipitable count (6.3 × 10^6 cpm) of each protein sample was analyzed.

message at 38° C paralleled the induction of the corresponding protein. In addition, this analysis revealed that HSP17 and HSP70 messages are present in callus cells under non-HS conditions, though at a low level. The accumulation of HSP70 message under non-HS conditions was expected given the accumulation of the HSP70 protein as shown in Figure 1, and the role of HSP70 in the non-HS condition as revealed in other systems (Linquist and Craig, 1988). The observation of the presence of HSP17 messages under non-HS conditions may also imply an involvement of Low molecular weight (LMW) HSP of mRNAs in normal cellular activity or in the regulation of HS gene expression at non-HS condition as shown with HSP70 in many other systems.

Comparison of the HSP Synthesized in Vivo Versus in Vitro

The levels of the transcripts for individual HSP were measured by analyzing their protein products in vitro with the assumption that the amount of protein translated in vitro should be proportional to the level of its corresponding mRNA. Figure 3 shows the in vitro translation products of poly(A^+) RNAs isolated from callus cells at 30° C and 38° C. When in vitro translation products of mRNAs produced at 30° C and 38° C were compared, many of the LMW HSPs were missing at 30° C. Only a subset of LMW HSPs appeared at 30° C in vitro compared to the LMW HSPs at 38° C in vitro. This indicates that many LMW HS transcripts were not accumulated to a detectable level in callus cells at 30° C. When the amounts of individual LMW HSP synthesized at 30° C in vitro and in vivo were compared, the relative abundance of the transcripts as reflected by the abundance of protein synthesized in vitro, does not correlate with the relative abundance of the protein synthesized in vivo. Specifically, the level of the transcript for HSP17.5A was the most abundant at 30° C as shown in in vitro translation, but the amount of HSP17.5A produced in vivo was not more than HSP17. This implies that the amount of protein synthesized in vivo was not proportional to the level of the message. This may further suggest that there is some mechanism to enhance the in vivo translation to achieve a specific level of individual LMW HSPs at 30° C. An analogous phenomenon was observed with globular embryos that responded to heat shock in an unusual way by accumulating a very reduced amount of HSP17 mRNAs, 6.5X less for HSP17 and 1.5X less for HSP70, compared to

callus cells (Zimmerman et al., 1989). Even with the reduced synthesis of LMW HS mRNAs, globular embryos could synthesize the corresponding proteins in a relatively similar amount to that of callus cells. This enhanced synthesis of LMW HSPs was achieved at the level of translation, based on polysomal analysis (Apuya and Zimmerman, 1992). These results strongly indicate that translational control may exert an important regulatory role in the expression of heat shock genes at 30°C as shown with globular embryos.

In addition, the comparison of HSPs synthesized at 38°C in vitro and in vivo also suggests the involvement of post-translational modification of LMW HSPs, especially for HSP19.7 (which is shifted in isoelectric point) and most of the LMW HSPs within the circle, since there were obvious differences in their constellation between the in vivo and in vitro protein profiles. There were some evidences for an involvement of post-translational modification such as phosphorylation and/or methylation to increase the diversity in some HSPs (Wang et al., 1981: Nover and Scharf, 1984). Moreover, many non-HS proteins were shown to be synthesized in vitro at 38°C, which may reflect the translation of non-HS messages released from sequestration during RNA extraction.

적 요

당근의 현탁배양 캘러스 세포가 고온에 노출되었을때 다른 식물에서 알려진 것과 유사한 열충격반응을 나타낸다. 이는 기존의 유전자의 발현을 억제하고 새로운 열충격단백질을 생산하기 위한 열충격유전자의 발현을 촉진한다. 이러한 열충격유전자의 고온조건에서의 발현은 주로 전사과정에서 이루어지는 것으로 알려져 있다. 그러나 몇가지의 경우에서 이들 열충격 유전자의 발현이 번역과정에서 조절되는 것으로 보고된 바 있다.

본 고에서는 열충격과정과는 무관한 토끼의 적혈구로 만들어진 시험관 내 번역시스템과 2차원단백질분리 시스템을 이용하여 생성된 단백질의 양을 통해, 번역에 사용된 mRNA의 양을 추정하였다. 이를 생체내에서 같은 조건에서 만들어진 단백질의 양과 비교할 때에 당근세포내의 특정 mRNA의 양과 해당 열충격단백질의 양이 30°C에서 불일치함을 확인하였다. 이를 통해 당근의 캘러스 세포가 열충격반응을 나타내는 가장 낮은 온도인 30°C에서 mRNA의 양과는 다소 무관하게 해당 열충격단백질을 번역을 촉진하는 과정이 있음을 추정할 수 있었다.

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