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The Heat Shock Response of Carrot[1]

Protein Variations between Cultured Cell Lines

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ABSTRACT

We have defined several parameters surrounding the heat shock response of cultured cells of carrot (*Daucus carota* L.) and have found that these cells exhibit a typical "higher plant" heat shock response. In particular, the resolution of the heat shock proteins (hsps) by two-dimensional polyacrylamide gel electrophoresis (PAGE) has revealed a pattern of proteins very similar to the hsps from soybean; specifically, the low molecular weight class is composed of approximately 15 to 20 different polypeptides which likely represent different members of a small gene family. In addition, we have compared the (2-D) PAGE profiles of hsps isolated from several different cultured cell lines currently maintained in our laboratory and have found notable differences in the low molecular weight hsps between cell lines. Some of the differences appear to be quantitative, while others may be qualitative. Each of the cell lines was derived from a different seedling of the same seed stock of the same cultivar; thus, genetic differences should be minimized. In addition, two of the cell lines, which show clear differences, were initially derived from a single parental line, and thus arose from a single genetic stock. Possible explanations for the cell line differences observed here are either partial aneuploidy or modified gene regulation resulting from molecular changes during the time in culture (*i.e.* somaclonal variation). These observations serve to highlight the potential for variation that exists in cells in culture even for such a highly conserved response and gene set as the heat shock genes.

All organisms respond to significantly elevated temperatures by inducing a very characteristic cellular reaction which has been termed the 'heat shock response.' Although the precise molecular details of the heat shock response vary among organisms, in general it is characterized by the specific induction of a small set of new genes, the heat shock genes, culminating in the production of the hsps.[2] The regulation of heat shock gene expression appears generally to be both transcriptional and translational, although the stringency of specific translational selection of heat shock mRNAs varies considerably. In addition, there is significant precedent for subsets of the hsp gene set being expressed without heat shock either in response to developmental signals (5, 15, 25, 26) or perhaps as indicators of other types of cellular stress (4, 20).

Among higher plants, the heat shock response has been most extensively characterized in soybean seedlings (1, 7). Although the heat shock response of higher plants is basically similar to that of animals, one characteristic of the plant response is the existence of a very complex class of small molecular mass hsps ranging from approximately 16 to 22 kD (14). The genes encoding many of these proteins are interrelated as determined by *in vitro* translation of hybrid-selected mRNAs (23). The complexity of these small hsps can only be revealed by two-dimensional PAGE since the pattern seen by SDS-PAGE is much simpler (7, 14).

Most of the analyses of heat shock in higher plants have been conducted on whole plants (1–4, 7, 8); relatively few studies have focused on the response of plant cells, growing in cell or tissue culture, to elevated temperatures (6). One relevant exception is the analysis of Pitto *et al.* (19), in which the production of heat shock proteins by carrot cells and somatic embryos was analyzed by SDS-PAGE. This study revealed that carrot cells and somatic embryos were capable of displaying a typical heat shock response, although the complexity of the small hsp class could not be assessed since proteins were only separated on the basis of size. To extend these observations and establish the groundwork for subsequent molecular analyses, we have analyzed the hsps of carrot suspension cells by two-dimensional gel electrophoresis. Moreover, we have performed comparative analyses of hsps from several different (yet genetically related) cell culture lines which have been independently maintained for 2 to 3 years in our laboratory. That is, we have evaluated the variation which exists in the hsp pattern between cell lines and which may have arisen as a function of prolonged time in tissue culture. We have found stable, observable differences in the hsps of several related cell culture lines.

MATERIALS AND METHODS

Plant Material

Callus suspension cell cultures were initiated from single hypocotyls derived from sterilized seeds of *Daucus carota* L. (cv Danvers Half-Long). Cells were grown in Murishage and Skoog's (MS) medium (16) supplemented with 5 μM 2,4-D and 3% sucrose and were transferred weekly (or biweekly for slower growing lines). The time in culture from initiation to obtaining a larger scale culture was generally approximately 8 months.

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[2] Abbreviations: hsp, heat shock protein; IEF, isoelectric focusing.

Genetic Relationships of Cultured Cell Lines

All lines were initially derived from individual seeds of a single seed lot of the cultivar "Danvers Half-Long." Although this is a 'cultivar' and not an isogenic line, it is, by definition, an inbred line; thus, genetic variability should be minimized to the extent possible in an outcrossing species. Two of the cell lines, designated as GB-4 and OB-3, were initially derived as subcultures from the same parental line (21), and hence began as closely related, genetically, as possible. The four lines have been in suspension culture for approximately 2 years.

Protein Labeling and Isolation

Hsps were labeled by suspending 50 μL of gravity packed cells in 500 μL fresh MS medium (containing 2, 4-D and sucrose), and preincubating the cells at the heat shock temperature (generally 38°C) for 1 h with constant aeration. Fifty μCi of [^{35}S]methionine (about 1,000 Ci/mmol, New England Nuclear) was then added, and shaking was continued at the heat shock temperature for two additional hours. Nonheat shock proteins were labeled identically, except that both preincubation and incubation temperatures were 23 to 25°C.

Labeled cells were collected by centrifugation, washed free of [^{35}S]methionine, and frozen in liquid nitrogen. Proteins to be analyzed by SDS-PAGE were extracted by homogenization in 0.25 M Tris (pH 6.8), 2.0% SDS, 5% β-mercaptoethanol, and 10% glycerol. Proteins to be analyzed by two-dimensional IEF/SDS-PAGE were extracted by homogenization in 9.0 M Urea, 4.0% NP-40 (w/v, Sigma), 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*g* for 15 min. The supernatant, containing soluble proteins, was aliquoted and frozen at −70°C until analyzed.

Electrophoresis

Standard SDS-PAGE was performed according to Laemmli (9) using 15% (w/v) acrylamide gels. Proteins were resolved in two dimensions as described by O'Farrell (18) using 15% (w/v) acrylamide gels for the second dimension. The effective pH range of the isoelectric focusing gels was from pH 6 to pH 9. 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 exposure to Kodak X-AR film. In all cases, equivalent numbers of acid-insoluble counts were loaded on each gel.

RESULTS

Parameters of Hsp Induction in Cultured Carrot Cells

To establish optimal conditions for the induction of hsps in cultured carrot cells, we examined the effects of varying temperature and time of exposure to heat shock conditions on the accumulation of newly synthesized proteins. Figure 1 demonstrates the SDS-PAGE profile of proteins synthesized during the last 2 h of a 3 h incubation of line 1 cells at increasing temperatures; all four lines showed a similar temperature induction pattern (data not shown). The major high and medium molecular mass hsps (84, 70, and 39 kD) begin

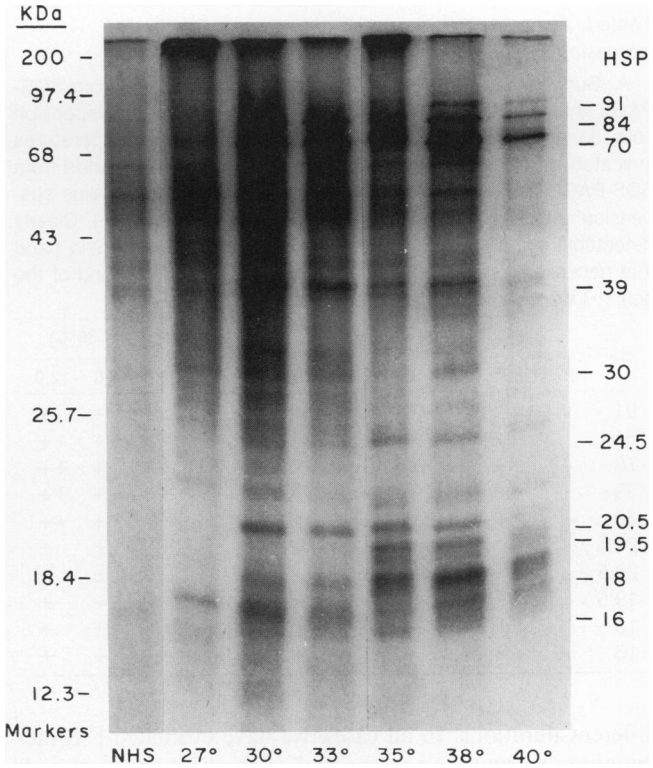

Figure 1. Synthesis of hsps as a function of increasing temperature. Total proteins, extracted from carrot suspension cells incubated in the presence of [^{35}S]methionine for the last 2 h of a 3 h incubation at the temperatures indicated, were resolved through a 15% polyacrylamide gel. The sample from a nonheat shocked cells (NHS lane) was incubated at room temperature (approximately 23°C) for 6 h to accumulate equivalent radioactivity. Molecular masses (kD) of protein standards are indicated on the left, and the positions of the major hsps are indicated on the right. Equivalent amounts of total protein were loaded in all but the 40° lane which contained approximately twice as much total protein.

to be apparent at 30°C and are synthesized at all higher temperatures tested. The low molecular mass class shows more variable synthesis as a function of temperature. At 30°C (and 33°C), bands corresponding to 20.5, 18, and 16.7 kD are obvious, but a set of bands corresponding to 19.5 kD is not yet synthesized. At 35°C, additional bands can be seen corresponding to proteins of 24.5 and 19.5 kD, and there is an increase in abundance of the 18 kD protein set. Incubation at 38°C elicits the synthesis of the entire set of hsps, with new bands being visible corresponding to approximately 91 and 30 kD. Cells incubated at higher temperatures (*i.e.* >40°C) show significantly decreased levels of total protein synthesis (the lane corresponding to 40°C represents twice the amount of total protein loaded in any of the other lanes), and the hsps are essentially the only proteins synthesized in substantial quantities. Table IA summarizes the synthesis patterns of the hsps as a function of temperature. In all subsequent experiments, 38°C was used as the optimal heat shock temperature.

We also determined the effect of increasing time of exposure to the optimal temperature (38°C). Table IB summarizes the analysis of proteins synthesized after exposure to 38°C for

Table I. *Noncoordinate Induction of Hsps as a Function of Increasing Incubation Temperature and Time of Exposure*

A. Summary of protein synthesis patterns evaluated from SDS-PAGE analysis of [^{35}S]methionine incorporation by callus suspension cells during the last 2 h of a 3 h exposure at the temperatures indicated. B. Summary of protein synthesis patterns evaluated from SDS-PAGE analysis of [^{35}S]methionine incorporation by callus suspension cells exposed to 38°C for the times indicated. (+), Clearly detectable signal; (++), maximal signal for any given protein band (not necessarily indicating amount relative to any other band of the gel); (±), barely detectable signal.

HSP	A. Temperature (°C)						B. Time (Hours at 38°C)				
	27°	30°	33°	35°	38°	40°	0.5	1.5	3.0	8.0	12.0
91					+	+	+	++	+	+	+
84		++	++	++	++	++	+	++	++	++	++
70		++	++	++	++	++	+	++	++	++	++
39		++	++	++	++	+	+	+	++	++	++
30		+	+	±	++	±		+	++	++	++
24.5				+	+	+		+	++	±	±
20.5		++	++	++	++	+		+	++	±	±
19.5				++	++	+		+	++	+	+
18		+		++	++	++		+	++	±	+
16	+	++	++	+	+	+		+	++	+	+

different durations. In all cases, we have evaluated [^{35}S]methionine incorporation at the end of the heat shock period. The high and medium molecular mass proteins are clearly visible by 30 min of heat shock. By 1.5 hr, all hsps are induced, and this pattern is essentially unchanged at 3 h. After prolonged exposure to heat shock (*i.e.* 8 and 12 h), the synthesis of hsp 84 and the low mol wt hsps is diminished relative to hsp 70.

Having established the optimal conditions for the induction of hsp synthesis (38°C, 3 h), we examined the stability of the hsps in the carrot cells. Suspension cells were preincubated for 1 h at 38°C (preshocked), labeled with [^{35}S]methionine at 38°C for 2 h, washed free of [^{35}S]methionine, and postincubated in the presence of cold methionine either at 38°C (heat shock) or 23°C (nonheat shock) for 2 and 8 h. The results are shown in Figure 2. Lanes 1 and 2 represent the NHS and HS control lanes, respectively. Lanes 3 to 6 represent heat shock proteins which were synthesized the second and third hours of a 3 h heat shock and were still present in the cells after several hours of incubation at either heat shock or nonheat shock conditions. The protein profiles are identical in all lanes, indicating that the hsps are stable proteins that persist in the cells for many hours even after the heat shock condition is removed. Similar observations have been made in soybean seedlings (8).

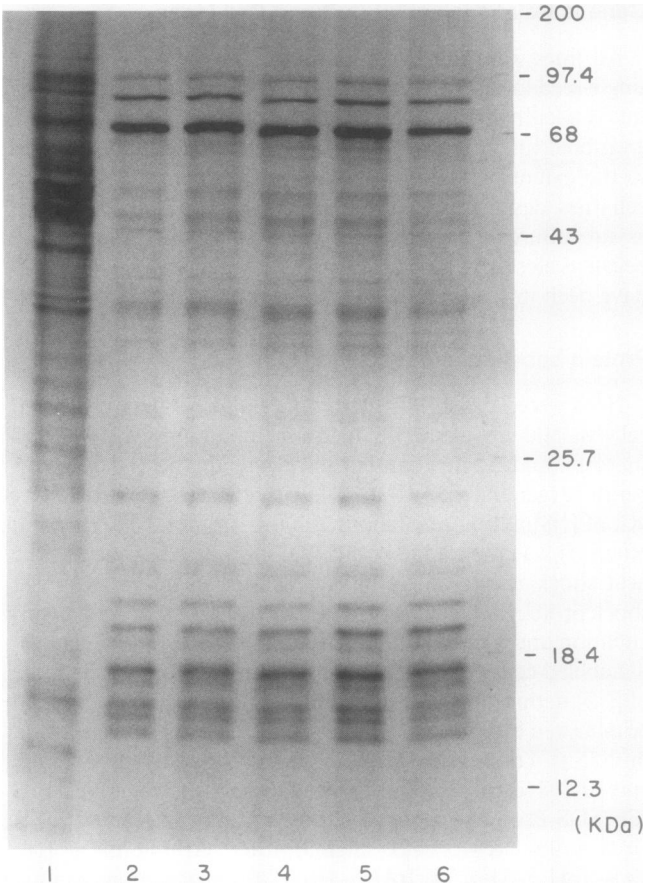

Figure 2. Stability of hsps in the presence and absence of continuous heat shock. Carrot suspension cells were incubated for 3 h at 23°C (lane 1) and 38°C (lanes 2–6), and were exposed to [^{35}S]methionine (50 μCi) at the same temperature for the final 2 h. The unincorporated [^{35}S]methionine was removed, and cells were extracted immediately (lanes 1 and 2) or postincubated in the presence of cold methionine for 2 h and 8 h at 23°C (lanes 3 and 4, respectively) or at 38°C (lanes 5 and 6, respectively). Samples were resolved through a 15% polyacrylamide gel and bands were visualized by fluorography (1.1×10^5 cpm/lane).

Two-Dimensional PAGE Resolution of Proteins from Line 28

A comparison of the two-dimensional IEF-PAGE profiles of proteins isolated from cultured cells of a particular line (line 28) before and after heat shock is presented in Figure 3 A and B, respectively. Visual inspection of these two panels reveals many clear differences. The most notable differences exist in the high (60–90 kD) and low (22–16 kD) molecular mass regions of the two gels. The low molecular mass hsps (hsp 22–16; Fig. 3B) are clearly lacking before heat shock (with the possible exception of the 22 and 19.7B proteins). Synthesis of the medium mol weight hsps (hsp 42–35) is, at the least, greatly enhanced by heat shock. The high molecular weight hsps (hsp 70, in particular) may be synthesized before heat shock and are greatly induced at 38°C. The hsp 84 spot(s) is difficult to assess in the NHS panel since there are so many proteins present in this region of the gel; it is possible that hsp 84 is synthesized, at a low level, prior to heat shock.

A comparison of the hsp profiles generated by SDS-PAGE *versus* two-dimensional PAGE resolution methods reveals that there are many more heat shock proteins identifiable after two-dimensional PAGE. This is particularly apparent for the low molecular mass group of hsps which is composed of approximately 25 different proteins. The proteins fall into three general clusters containing landmark proteins of hsp 19.7A (cluster 1), hsp 17.5A (cluster 2), and hsp 19 (cluster

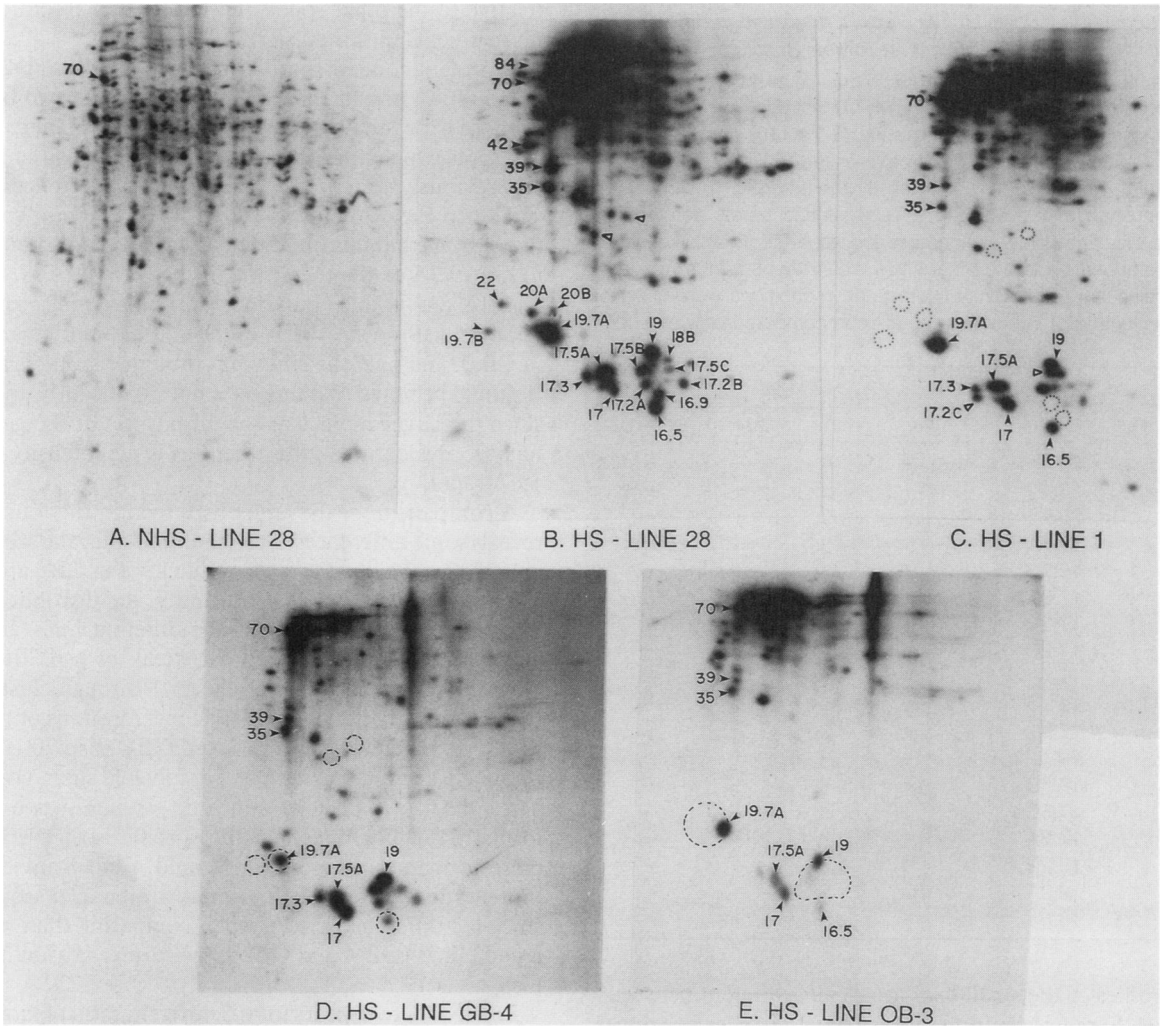

Figure 3. Two-dimensional separation of total protein from control and heat shocked carrot suspension cells from several independent cell culture lines. Total protein, extracted from carrot suspension cells, incubated in the presence of [^{35}S]methionine for the final 2 h of a 3 h exposure to 23°C (panel A) or 38°C (panels B–E) were resolved by two-dimensional IEF/SDS-PAGE. All gels were visualized by fluorography (6.3×10^5 cpm/gel). Numbers on photographs reflect the apparent molecular masses (kD) of the major heat-inducible proteins. Open arrows indicate protein spots unique to a given sample. Areas enclosed by dashed lines highlight positions where obvious changes in protein pattern can be seen. The analysis was performed on cells from line 28 (panels A and B), line 1 (panel C), line GB-4 (panel D), and line OB-3 (panel E). The pH gradient ranged from approximately pH 6.9 to pH 9.

3). This complexity of the small hsp class is quite characteristic of the heat shock response of higher plants (8), and is not generally seen in animal systems. In contrast to this complexity, the high and medium molecular mass hsps show a simpler pattern, with hsp 84 and hsp 70 resolving into two or three major spots, and the other size classes showing only single spots (*i.e.* hsp 42, 39, and 35).

Hsps Vary in Different Cultured Cell Lines of the Same Cultivar

A comparison of hsps isolated from different cell lines of carrot (all derived from the same seed lot of the same cultivar, Danvers Half-Long) reveals a surprising number of differences, primarily within the low molecular mass class. These are illustrated in Figure 3, B to E, and are summarized in Table II. Hsps isolated from line 28 *versus* line 1 are shown in Figure 3, B and C, respectively. It must be noted that the patterns shown in Figure 3, B to E, have been consistently observed in several independent protein labelings and analyses (three in the case of OB-3 and GB-4, and in excess of 10 in the case of lines 1 and 28). Moreover, the hsp patterns for any given line persists during the development of somatic embryos (*e.g.* for line 1, GB-4 and OB-3; line 28 is not embryogenic) and over considerable periods of time (OB-3 shows the same hsp pattern 1 year after the initial observation was made; CH Hwang, unpublished data). Finally, the hsp pattern of each line does not change when the proteins are isolated either in the presence or absence of the potent protease-inhibitor, PMSF (0.5 mM [22], data not shown), and thus protein degradation does not appear to be a factor in this analysis.

Table II. *Summary of Small Mol Wt Hsps Represented in Different Carrot Cell Culture Lines after Exposure to Heat Shock*

[^{35}S]Methionine incorporation into individual small mol wt hsps was evaluated from the distribution of newly synthesized proteins after two-dimensional PAGE and fluorography (6.3×10^5 cpm/gel). The intensity of each designated spot is expressed relative to the landmark protein of each cluster (denoted by the asterisk, *) with +++ representing maximal levels and ± representing barely detectable minimal levels. The value assigned to individual spots also reflects their intensity relative to the other small mol wt hsps from the same gel (and cell culture line). Since fluorography conditions were essentially equivalent, spot intensities can also be compared between cell lines.

Cluster	Molecular Mass	Cell Line			
		28	1	GB4	OB3
I	22	+	−	−	−
	20A	+	±	+	±
	20B	+	±	+	±
	19.7A*	+++	+++	+++	+++
	19.7B	+	−	−	−
II	17.5A*	+++	+++	+++	+
	17.3	+	+	+	+
	17.2C	−	+	−	−
	17	+	+	+	+
III	19*	++	++	++	++
	18A	−	+	−	−
	18B	+	+	±	±
	17.5B	+	+	+	−
	17.5C	+	±	+	−
	17.2A	+	−	+	−
	17.2B	+	±	+	−
	16.9	+	−	−	−
	16.5	++	++	+	+

The profiles of the small hsps are clearly different between these lines. Some of the differences reflect changes in abundance (relative to the landmark hsp 19.7, 17.5A, and 19). In line 1, hsps 20A, 20B, 22, and 19.7B of cluster 1 are all decreased in amount, and some are undetectable. In cluster 2, the smear below hsp 17.5A appears to be missing from line 1, and there is a new spot in line 1 directly below hsp 17.3 (indicated by the open arrow, Fig. 3C). In cluster 3, hsp 17.2A and 16.9 are missing from line 1, and there appears to be one or two new spots resolved below hsp 19 in line 1. These two new spots could be different molecular weight forms of the missing line 28 hsps 17.2A and 16.9, since their isoelectric points are equivalent to those proteins; perhaps they represent preprocessing forms of the proteins.

An examination of the hsps of line GB-4 reveals that this line shows a low molecular weight hsp pattern which closely resembles that of line 28 (cf. Fig. 3, D and B). In cluster 1, the 19.7A spot is evident in GB-4, although reduced in intensity, as is a spot corresponding to hsp 20A. Hsp 20B is not seen in GB-4, but there is a larger molecular mass spot above 19.7A. Hsps 22 and 19.7B are not detectable in GB-4. In cluster 2, the pattern is essentially the same for GB-4 and line 28. Cluster 3 proteins of GB-4 appear to be a subset of those of line 28. Hsps 19, 17.5B, 17.2A, 17.2B, and 17.5C are identical between GB-4 and 28. Hsp 16.5 is greatly reduced relative to the other spots, and hsp 16.9 is absent. There are no new spots in this cluster.

A protein comparison between the two 'sibling lines,' GB-4 and OB-3, reveals that the OB-3 hsps appear to be a subset of those expressed by either GB-4 or line 28; there are many hsp spots which are either greatly reduced or absent in OB-3. As previously seen, most of the obvious differences lie in the small hsp class. In cluster 1 of OB-3, one major spot is seen (hsp 19.7A), but it is present in essentially equivalent quantity to hsp 19.7A of all other lines. In cluster 2 of OB-3, hsp 17.5A and 17 are visible, but both are substantially diminished relative to 19.7A. Very few of the cluster 3 proteins are visible in OB-3, and only the landmark protein, hsp 19, is abundant. It should be noted that this gel is not significantly underloaded since the larger molecular weight portion of the gel is essentially equivalent to all the others, as is the intensity of the hsp 19.7A spot.

A comparison of the larger hsps seen in the four lines also reveals some differences. The medium molecular weight spots, denoted by the open arrows in line 28 (Fig. 3B), are not seen in any other line. More significantly, the distribution of hsp 70 is clearly different among the different lines. In lines 28 and OB-3, there is a horizontal streak of hsp 70 extending from the relative position of cluster 1 through cluster 2, with two (or possibly three) apparent concentrations of proteins at these two positions. In lines 1 and GB-4, hsp 70 is primarily in the cluster 1 range with two resolvable spots visible there. Thus, the hsp 70 proteins appear in essentially two isoelectric forms in all four lines, but the specific isoelectric points of these forms are different; this could result from changes in protein modification or in primary amino acid sequence, but since the difference is seen when comparing the two clonally related lines (GB-4 and OB-3), the former possibility is more likely.

Finally, there appears to be a different ratio of large to small proteins between the lines. In lines 28, 1 and OB-3, hsp 70 is much more heavily labeled than hsp 19 or 17.5A, whereas in GB-4, the hsp 70 spot is substantially reduced relative to these landmark low mol wt hsps.

Thus, many variations exist in the hsp profile when proteins isolated from different tissue culture lines are analyzed by two-dimensional PAGE. To what extent these protein differences reflect gene differences is unknown.

DISCUSSION

Heat Shock Response of Carrot Cells

Carrot cells, growing in suspension culture, can be induced to synthesize abundant quantities of hsps which are typical with respect to their time and temperature requirements for induction. These hsps generally exhibit the same molecular mass and isoelectric points as seen for hsps from other higher plants (1, 3, 14). Moreover, the substantial complexity of the small hsp class resolved here by two-dimensional PAGE is very characteristic of higher plants.

Different members of the hsps of carrot cells accumulate differentially as a function of temperature; the large hsps are generally induced at lower temperatures and the small hsps require higher temperatures for induction. This pattern of hsp

induction showing an optimum of 36 to 38°C was constant for the four lines. In our culture lines, hsp 70 is quickly induced by even mild stress, and can often be visualized in the nonheat shock samples, but is not always present. This is not surprising since it is one of the first proteins to appear as the incubation temperature rises (*i.e.* it can be seen at 30°C). Thus, the appearance of hsp 70 in substantial quantity in these cells may serve as an indicator of a mild stress.

Variation among Hsps in Different Cell Lines

Both quantitative and qualitative differences were observed in comparing the hsp profiles from cells of four different cell lines. The hsp 70 pattern of lines 28 and OB-3 contains two or three major species which extend over a broad range in pH. The pattern is different in lines 1 and GB-4 where hsp 70 is primarily resolved over a narrower pH range, and is only composed of two major spots. One additional difference between cell lines is the relative synthesis of large and small molecular mass hsps. In this regard, GB-4 is unusual in that significantly less hsp 70 is synthesized relative to hsp 19.7A, 17.5A, and 19 (major landmark proteins of the small hsp family).

Even more dramatic differences are seen between cell lines when the small hsp class is compared. Line 28 can be considered to be the 'prototypic' hsp pattern, since the three other cell lines examined exhibit, for the most part, a subset of line 28 hsps. One possible exception is the two novel spots identified in line 1 (denoted by arrows in Fig. 3C); however, it is possible that these two spots correspond to different forms of proteins in line 28 but absent in line 1.

The other changes in small hsps observed in comparing each of the cell lines to line 28 appear to result from the synthesis of a diminishing subset of the hsps. This is not simply a matter of decreasing visibility of the total small hsp set, as evidenced by the fact that the ratios of autoradiographic intensity change between the small hsps of the different lines. Rather, it appears that specific hsp members of each cluster are disproportionately decreased to some extent in line GB-4 and more dramatically in OB-3.

Generation of Hsp Variation in Cell Culture Lines

Although all the cell culture lines were derived from individual seeds of the same seed-lot of the same cultivar of domestic carrot (Danvers Half-Long), a certain level of hsp variation observed in this study can be attributed to genetic variation which existed at the individual seed level. Since the cell culture lines are clonal in their propagation, we have the opportunity to observe genetic differences between individuals, since these differences are, in essence, amplified rather than homogenized. This intrinsic seed-to-seed variation might possibly explain some (or all) of the differences seen between lines 28, 1, and GB-4/OB-3. However, this cannot be the explanation for the differences seen between GB-4 and OB-3, since these are different sublines of the same parental line (line 31.5, which is no longer viable). In this case, and also perhaps to some degree in the other lines, the differences which exist have arisen at some time in tissue culture after which they are stably expressed; that is, they are a product of somaclonal variation (10, 11). In the most extreme case of a change in hsp pattern (*i.e.* OB-3), the observed pattern has persisted for over a year since it was first observed (data not shown). Moreover, the hsp patterns of line 1, GB-4, and OB-3 were observed both in heat shocked callus suspension cells (Fig. 3) and developing somatic embryos (CH Hwang, unpublished data). Thus, we consider all of the changes to represent very stable, heritable, genetic alterations.

There are, unfortunately, no very clear patterns of hsp disappearance when the lines are compared. It does appear that similar molecular mass species may increase or decrease together, for example, hsp 17.5A, 17.5B, and 17.5C are all decreased in OB-3, and hsp 17.2A and 17.5B are generally of equal intensity within a given line (high in GB-4, low in OB-3). This could imply that hsps of similar molecular mass are encoded by members of small gene families which are coordinately regulated. It is clear in soybean (17, 23, 24), and from our own work (KEl-Darwish, JL Zimmerman, unpublished data) that such gene families do exist for the small hsps of plants. It is thus possible that what has changed in the different cell lines are molecules/factors which regulate the expression of some of these gene families. This is consistent with the fact that the greatest variation observed here is localized in the small hsp class as is the occurrence of small gene families encoding hsps. Most other heat shock genes are single copy (in other organisms, both animal and plant) with the exception of hsp 70, which may be present in a few copies, and which, coincidently, also shows some variation in this study.

Significance of the Variation in hsp Patterns between Different Cell Lines

Although the specific cellular function(s) of the hsps is largely unknown in eukaryotes, it does appear that at least some of the hsps function in a role of 'thermal-protection' (2, 12). It is also unclear why the small hsp class is so complex in higher plants, and whether there is overlapping function between some gene family members. All of the cell lines analyzed here are viable lines, and three of the four (lines 1, GB-4, and OB-3) can fully regenerate through somatic embryogenesis at room temperature (21; JL Zimmerman, unpublished data). The fourth line, line 28, had lost its embryogenic capability by the time this analysis was performed. Thus, it is clear that the reduced or absent hsps play no essential role in normal growth and development. It should be noted, however, that lines GB-4 and OB-3 are 'variant' lines which each exhibit a temperature-sensitive block in the process of somatic embryogenesis. Schnall *et al.* (21) have described these two lines with respect to their phenotype at the restrictive temperature and the time in development at which they exhibit temperature-sensitivity (the temperature-sensitive period). It is possible that the developmental defect(s) exhibited by these lines are, in some way, related to the hsp profiles we have observed. It has been suggested by Lo Schiavo *et al.* (13) that an alteration in the phosphorylation of some hsps of the medium molecular mass class might be correlated with a different temperature sensitive carrot cell line also blocked in somatic embryogenesis. It is possible that, in this case, the temperature sensitivity and hsp phosphorylation are not directly related in the variant TS-59 (13), since the lack

of hsp phosphorylation was constant through development and not correlated with the time frame of temperature sensitivity of the line. It may be that this is yet another example of cell line variation in hsp pattern. On the other hand, it is possible that there is a specific relationship between hsp change and temperature-sensitivity; we are currently investigating this possibility in lines GB-4 and OB-3 and other related temperature sensitive lines.

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