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TEMPERATURE ADAPTATION OF *DROSOPHILA* POPULATIONS. THE HEAT SHOCK PROTEINS SYSTEM

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Abstract—1. The heat shock proteins (hsps) system has been studied in two replicate cage populations (1D, 1C) of *Drosophila melanogaster*, which were maintained under different environmental conditions (temperature and relative humidity) for approximately 7 yr and exhibited different survival when they were subjected to temperature shock.

2. The kinetics of protein synthesis in ovaries from D and C flies (and their reciprocal hybrids DC and CD) were subjected to heat stress, and the electrophoretic patterns of heat shock proteins (especially of the hsp 70 K), are correlated with the survival of the fly.

3. These results confirm already obtained results using different stocks which were subjected to different type of selection (indirect selection; Stephanou *et al.*, 1982), which represents good evidence for the biological significance of the hsps.

4. The capacity of protein synthesis (and especially of hsp 70 K) following temperature shock is higher in ovaries from D (25°C) or DC flies as compared with those from C (14°C) or CD ones.

5. It is proposed that the regulation of hsps synthesis can be considered as a major target of temperature-induced selection, and an effective molecular mechanism for temperature compensation.

INTRODUCTION

Temperature change is a major environmental variable for poikilothermic animals, such as insects and results in various interactions of their genetic and biochemical machinery (e.g. changes of protein conformation and the transport properties of membranes; Hochachka & Somero, 1973; Alexandrov, 1977; Alahiotis, 1980). However, the understanding of the capacity of a living organism to be adapted to a temperature change is still a complex problem. Species-specific temperature preferences have been described (e.g. Hochachka & Somero, 1973; Parsons, 1979; Malogolowkin-Cohen *et al.*, 1979), and much done to correlate the ability of the animal to survive under different temperatures with allozymic genotypes (e.g. Johnson & Powell, 1974; Schenfeld & McKehnie 1979; Trippa *et al.*, 1980). Emphasis is also given to reveal convergence between the distribution of *Drosophila* and the temperature-dependent catalytic ability of homologous enzymes or allozymes (Miller *et al.*, 1975; Alahiotis *et al.*, 1977; Alahiotis & Berger, 1978; Alahiotis, 1979). It has been shown from these investigations that as other poikilotherms, insects, can compensate temperature fluctuations through the positive and negative thermal modulation model of Hochachka & Somero (1973). While, this mechanism can account for a variety of species, we do not know how these kinetic differences translate into physiological differences *in vivo*.

The ability of *Drosophila melanogaster* to survive temperature stress has been studied recently (Stephanou & Alahiotis, 1982). By utilizing two strains, one sensitive (S_1) to heat shock, and another resistant (R_1), constructed through indirect selection, we have shown that the heat sensitivity (or heat resistance) of the fly is controlled by nucleocytoplasmic interactions, in a quantitative sense, and responds to selection (Stephanou & Alahiotis, 1982). Two replicate cage populations (1C and 1D) established in 1973, were subjected to temperature effect for more than 7 yr since they were maintained at different temperatures (14–25°C respectively). Survival experiments have shown that flies from 1C population are more sensitive to heat shock (40°C/25 min) as compared to flies from 1D population. The genetics of the heat sensitivity character studied in these populations is similar to that found for the S_1 and R_1 strains of *Drosophila melanogaster* (Stephanou & Alahiotis, 1982). On the other hand we have found that the survival of the R_1 and S_1 strains is correlated with the regulation of heat shock proteins (hsps) synthesis, the R_1 strain synthesizing more hsps compared with the S_1 strain, which may be considered as evidence for the adaptive significance of these proteins.

The heat shock proteins system has been recently discovered (Tissieres *et al.*, 1974; Lewis *et al.*, 1975; McKenzie *et al.*, 1975). These proteins are a reflection of the genetic machinery to temperature stress, and are synthesized in a few minutes soon after the stress (Storti *et al.*, 1980). Although the function of the hsps is still largely unknown, our previous investigations (Stephanou & Alahiotis, 1982; Stephanou *et al.*, 1982) indicated that the hsps could be a major target of temperature-induced selection. If this is true then it can be considered as another important molecular mechanism through which insects respond to temperature elevation.

The extent to which the survival of *Drosophila melanogaster* is affected by the hsps and the possibility

for the regulation of hsps synthesis to be affected by temperature selective pressure was studied, in this paper, by utilizing the above referred cage population and their hybrids. Our findings are analogous to those obtained when simple strains (S_1 and R_1) were used, upon which a different type of temperature selection was performed (indirect selection); they are also in good agreement with recent discoveries which deal with the physiological significance of the hsps on the protection from heating (in yeasts, McAlister & Finkelstein, 1980; in sea urchins, Roccheri *et al.*, 1981).

MATERIALS AND METHODS

The 1C *and* 1D *cage populations*

The flies utilized in this study were obtained from two cage populations (1C, 1D) which originated from a common population (1B) established by the progeny of 100 isofemale lines captured in Cephalonia, Greece in the summer of 1973 (Alahiotis, 1976). Populations 1C and 1D were maintained under different environmental conditions. 1C was kept at 18 ± 0.5°C for the first 2 yr and at 14 ± 0.5°C since 1975, and 43 ± 4% r.h. Population 1D was kept at 25 ± 0.5°C and 90 ± 0.5% r.h. Flies were reared on cornmeal–sugar–agar medium (Alahiotis, 1976).

Labeling

Ovaries from adult females 3–5 days old were removed and placed in 30 μl Robb's saline (Robb, 1969) at 25°C in depression slides. The preparations (each containing 6 ovaries) were covered with coverslips, sealed with a glue (Gloria rubber solution) and submerged in the water bath (37°C) for 30 min. Controls were kept in the incubation medium, under the same conditions, but at 25°C. Soon after the heat shock, the Robb's saline was removed and replaced by Grace's medium (lacking cold methionine) containing 250 μCi/ml of ^{35}S-methionine (Amersham) of specific activity 1000 Ci/mM. Afterwards, the ovaries were incubated at 25°C for 50 min. Subsequently, they were washed three times in Robb's saline, dried and were dissolved in 60 μl sample buffer by heating 3 min in boiling water. The rate of protein synthesis was measured by precipitating 10 μl aliquots of the homogenized ovaries with 10% TCA on whatman GF/C glass fiber filters as described by Mans & Novelli (1961). The radioactivity was counted in a Beckman LS-7000 liquid scintillation counter. In parallel, dissolved samples (40 μl each) were loaded on 12.5% acrylamide-SDS gels (1% bismethylene acrylamide) with a 5% acrylamide stacking gel and run in the discontinuous buffer system of Laemmli (1970). The gels were run at 25 mA for 5 hr. Fluorography of the dried gel slabs was performed according to Laskey & Mills (1975). Kodak XR-5 film was for autoradiography of the dried gels.

RESULTS AND DISCUSSION

Previous studies have shown that populations 1C and 1D subjected to different environmental conditions (temperature and relative humidity) have been differentiated genetically (Alahiotis & Pelecanos, 1980). These populations exhibit strong sexual isolation which is under nuclear and cytoplasmic control (Kilias & Alahiotis, 1982). Our *Drosophila* populations were useful for the study of the quantitative character "heat sensitivity" (Stephanou & Alahiotis, 1982). Flies obtained from the 1C population are more sensitive to heat shock as compared with flies obtained from population 1D, which quite possibly reflects a selective effect of the temperature as regards with the factor(s) responsible for the ability of *Drosophila melanogaster* to survive under extreme high temperatures (Stephanou & Alahiotis, 1982). The effect of the cytoplasm on the survival was to be dramatic; e.g. flies carrying D cytoplasm and C nucleus exhibit survival value which resembles that of D flies and vice versa (Stephanou & Alahiotis, 1982).

Since we know the survival behavior of our populations an attempt was made to see whether convergence exists between survival and the heat-induced protein patterns. If the relationship is positive then we have evidence that hsps contributes to survival, and a strong argument on their biological significance. Moreover, the direct long-term effect of temperature on these populations can be considered as a major selective factor for the regulation of the hsps. Samples of eggs (in food vials) were obtained from the 1C and 1D populations and were allowed to develop until the adult stage at common conditions (25°C and 43 ± 4% r.h.). Virgin females of either flies were used to form reciprocal hybrids (♀ D × C♂ → DC and ♀ C × D♂ → CD). Ovaries from the D, C, DC and CD flies, developed at 25°C, were obtained, shocked (as it is described in the "Materials and Methods section) and the incorporation rate of ^{35}S-methionine into proteins was determined. Samples were also subjected to SDS-acrylamide gel electrophoresis. The kinetics of protein synthesis after temperature elevation in D, C, DC and CD flies studied, is shown in Table 1. The incorporation of the ^{35}S-methionine into proteins while lower for the D flies as compared with the C ones, in the control experiments (without heat shock), it is higher for the D flies, in comparison to the C after the heat shock. The C/E (Control/Experimental) ratio of incorporation is 2.43 for the C flies and 1.88 for the D. The reduction of ^{35}S-methionine incorporation into the total proteins (including hsps) after heat shock is 58.8% for the C flies and 46.7% for the D. The incorporation of ^{35}S-methionine into the total proteins of the heat-treated ovaries includes that into the heat shock proteins and that no corrections for possible variations of permeability to amino acids and of the pool have been made; these variations would, however, most probably affect to the same

Table 1. ^{35}S-methionine incorporation (cpm) into proteins of ovaries from C, D, CD and DC flies

Stocks	cpm
C_C	68,263
C_E	28,101
D_C	54,854
D_E	29,217
CD_C	93,129
CD_E	32,235
DC_C	82,868
DC_E	62,075

Ovaries from C, D, CD and DC stocks were incubated in parallel in the same experiment. E denotes shocked (37°C for 30 min) ovaries, and C refers to the control experiments (incorporation of ^{35}S-methionine at 25°C for 30 min).

extent ^{35}S-methionine incorporation into the total protein and into the heat shock proteins.

Table 1 shows that analogous disparity also holds true for the two reciprocal hydrids (ratio C/E = 2.89 for CD flies and C/E = 1.33 for DC ones). The reduction of ^{35}S-methionine incorporation into the total proteins after the heat shock is higher for the CD hybrids (65.4%) compared with the DC (25.1%). These observations show that the protein synthesis capacity after the heat shock is higher for the D and DC flies in comparison to C and CD ones, which agrees with their cytoplasm-dependent survival (Stephanou & Alahiotis, 1982). The survival values for the D and C flies are 52.02 and 30.67% respectively while for flies carrying D cytoplasm and C nucleus and vice versa the survival is 54.33 and 17.89% respectively (Stephanou & Alahiotis, 1982).

The studies of SDS-acrylamide gel patterns revealed clear cut differences between the stocks. Figure 1 shows the protein patterns for the D and C flies. Although, one can notice differences in terms of the intensity of labeling proteins, which are in agreement with the data of Table 1, the most striking differences appear in the heat shock protein 70 K which is weaker in the C flies as compared with the D (Fig. 1). Figure 2 shows the protein patterns obtained from ovaries of the reciprocal hybrids. Here, again, an assymetry was observed in terms of the heat-induced protein patterns. The intensity of labeling proteins is higher for the DC flies in comparison to CD ones (see also Table 1). Among the hsps the major differences appear again, for the 70 K band which is weaker for the CD flies as compared with the DC ones. The DC stock seems to behave similarly with the D stock and the CD with the C, as regards the intensity of labeling of the proteins synthesized after the heat shock, and especially of the heat shock protein 70 K. This observation reinforces the situation described earlier (Stephanou *et al.*, 1982) where analogous patterns were obtained for the S_1, R_1, S_1R_1 and R_1S_1 stocks. The effect of the cytoplasm in both, the survival and the regulation of hsps synthesis is clear (although the reciprocal hybrids used here bear D and C chromosomes, but D or C cytoplasm). The correlation revealed between the heat-induced protein patterns and the temperature under which the two replicate populations (1D, 1C) are maintained, suggests that some kind of temperature selection has taken place and results in the differential behavior of populations in both the survival and the hsps patterns.

The situation described in two different selective systems (strains S_1,R_1-populations C, D) indicates that the regulation of the hsps synthesis can be considered as a major target of temperature-induced selection and bears on their biological significance, regarding the fly's ability to survive under extreme temperature elevation. The existence of hsps polymorphism in natural populations (Peterson *et al.*, 1979) strengthens the significance of the system as a contributing factor for the temperature adaptation of *Drosophila* populations. Evidence for the biological significance of the hsps system has also been recently obtained for yeasts (McAlister & Finkelstein, 1980) and for the sea urchins (Rocheri *et al.*, 1981).

The differential hsps response of the C and D populations cannot be elucidated from these experiments. Since D, C, DC and CD flies were not taken directly from the cage populations but were reared for one (C and D) or two (DC and CD) generations in common conditions (25°C, 43 ± 4% r.h.) before the heat shock, the differences revealed as regards the survival and the heat-induced protein patterns cannot be due to acclimation. It is known that during the heat shock only the heat shock mRNAs plus a small number of preexisting mRNAs are translated (Storti *et al.*, 1980). The temperature effect on the long-term populations

Fig. 1. The patterns of heat-induced protein synthesis in ovaries of C and D flies. Electrophoresis was carried out as it is described in the Materials and Methods section. Labeled proteins of ovaries from (a) C and (b) D flies. 1: protein patterns at 25°C. 2: protein patterns after 30 min heat shock (37°C).

could be the result of a differential selection of factors which affect the translation of the mRNAs of these *Drosophila* populations.

Although cytoplasmic effects can be due to many factors (e.g. organelle inheritance: Sager, 1977), one should consider the possibility that the heat shock phenomenon is related to viral effect(s) (Scott *et al.*, 1980). Analogous heat behaviour is true for the viral proteins (Scott *et al.*, 1980) which indicates that the viral mRNAs appears to share the structural features of heat-shock mRNAs which permit their translation in heat shocked cells. This convergence raises the possibility that the heat shock response itself may be due to the action of an integrated viral genome or to an unintegrated virus that stimulates synthesis of certain proteins when the cell is incapacitated by increased temperature. Some viruses (e.g. Sigma virus) are inherited in a stabilized state through the maternal germ line. Taking into consideration our data, in combination with the information given above, as well as the fact that 1D and 1C populations are kept for many years under different temperatures and relative humidities we could attribute the assymetry observed for the hsps and survival in these populations and their hybrids, to a differential viral incorporation and action. This possible selective viral incorporation could be due to different environmental conditions of the 1C and 1D populations. This situation is reinforced by the development of strong sexual isolation between the 1C and 1D populations, which is also affected by the cytoplasm (Kilias & Alahiotis, 1982).

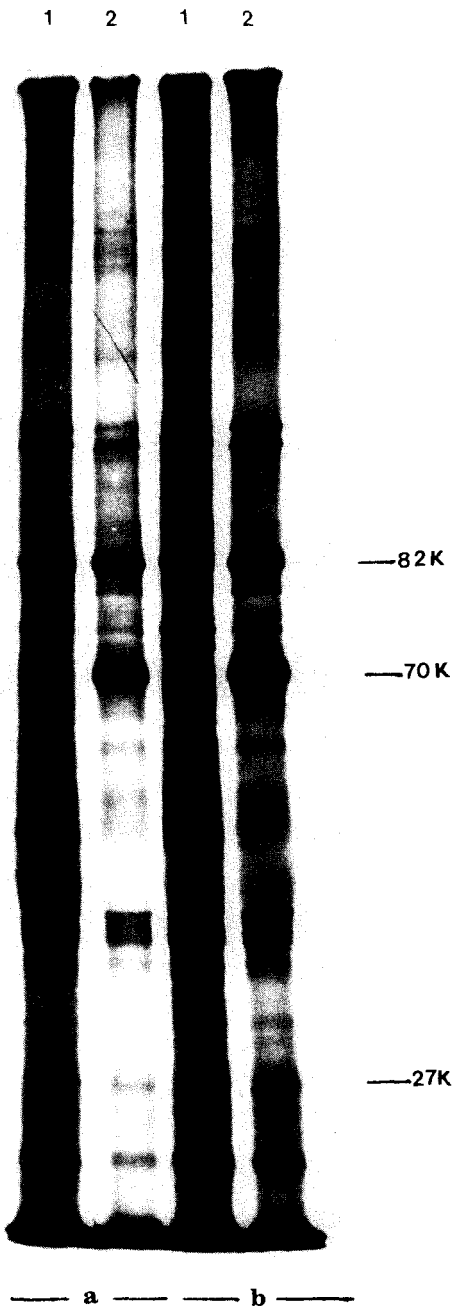

Fig. 2. The patterns of heat-induced protein synthesis in ovaries of CD and DC flies. Electrophoresis was carried out as it is described in the Materials and Methods section. Labeled proteins of ovaries from (a) CD and (b) DC flies. 1: protein patterns at 25°C. 2: protein patterns after 30 min heat shock (37°C).

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