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Heat shock proteins in three related *Drosophila* species belonging to the *obscura* group

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Abstract. The effect of heat shock on protein synthesis in three related *Drosophila* species belonging to the *obscura* group was analyzed on SDS-acrylamide gels. Four major heat shock proteins (hsps) were found in these species, in which synthesis reaches a maximum at 34 °C. Although the higher molecular weight proteins are conserved, differences in size were found for the small hsps in these species. By means of in situ hybridization using *D. melanogaster* probes for the small hsp genes, it was inferred that the small hsp genes of the *obscura* group species are clustered at the 27A locus in all three species.

Key words. Drosophila-related species; *obscura* group; heat shock; hsps.

All organisms respond to temperature shock by inducing the synthesis of a specific set of proteins called heat shock proteins, hsps (reviewed elsewhere[1–5]). Three major types of hsps are found in most organisms: a hsp with a molecular weight of 70 kDa (hsp70), a larger hsp of 80–90 kDa, and a group of small hsps with MW between 15 kDa and 30 kDa. Comparing the hsps in *Drosophila* a high degree of evolutionary conservation is found in the high MW proteins. Hsps of 82,000 M_r and 70,000 M_r have been reported in all species studied[6–9]. Small hsps are recognized as a heterogeneous group where the size and number of the proteins vary between species. In order to check whether the small hsp family shows a different pattern even in related species, we compared the heat shock patterns of protein synthesis in three sibling *Drosophila* species of the *obscura* group: *Drosophila subobscura*, *D. guanche* and *D. madeirensis*.

The strains used in this study were H 271 of *D. subobscura*, TF 2 of *D. guanche*, and Md 1 of *D. madeirensis*. All three are isofemale strains deriving from individuals captured in Finland, the Canary Archipelago and Madeira Island, respectively. Individuals of the three species synchronized at the beginning of prepupa formation and kept at 19 ± 1 °C, were exposed to temperatures of 31 °C, 34 °C or 37 °C over a period of 30 min. Controls were obtained from synchronized prepupae kept at 19 °C during the treatment period. Experimental design was that described in Pascual and de Frutos[10]. Samples were electrophoresed in 9–12% SDS-acrylamide gels according to Laemmli's method[11] and fluorography of the gels was carried out following the Bonner and Laskey's procedure[12].

The effect of heat shock on protein synthesis was similar in the three species analyzed. Synthesis reached a maximum at 34 °C (fig. 1a). A dramatic decrease in protein synthesis was found at 37 °C which is the optimum temperature to switch on the heat shock machinery in *Drosophila melanogaster*. As can be seen in this figure, all three species produced four particularly abundant hsps that were not synthesized in significant amounts under control conditions. The induced high MW proteins show identical electrophoretic mobility in all three species, and correspond to hsp82 and hsp70 found in *D. melanogaster*. Hsp70 is also the main product synthesized after heat shock in the *obscura* group species. The table summarizes the results obtained in these species and compares them with that of *D. melanogaster*.

Four of the small hsps have been described in *D. melanogaster*[6,7], and can be separated into the doublets 28–26 kDa and 23–22 kDa by electrophoresis in low concentration acrylamide gels (fig. 1b). *D. subobscura*, *D. guanche* and *D. madeirensis* always showed two small hsps under the same experimental conditions. The two small proteins found in *D. subobscura* were previously reported by Pascual and de Frutos[10]. As can be seen in the table, the sizes of the small proteins were not the same in the three related species. Comparison of the amino acid sequences of the *D. melanogaster* small hsps indicates a large region with a high degree of sequence homology and two heterologous regions which account for the variable size of these proteins (reviewed in Craig[13]). The size differences in the *obscura* small hsps may be for similar reasons.

In situ hybridization using *D. melanogaster* probes for the four small hsp genes (88.5, 88.6)[14], gave positive signals with both probes at the 27A locus on the J polytene chromosome of the three *obscura* group species. The 88.5 and 88.6 probes contain, respectively, the hsp28–hsp23 and hsp26–hsps22 genes from the 67B locus of *D. melanogaster*. As an example, figure 2 shows the in situ results using the 88.5 probe. Chromosomes of the three species were prepared following Atherton

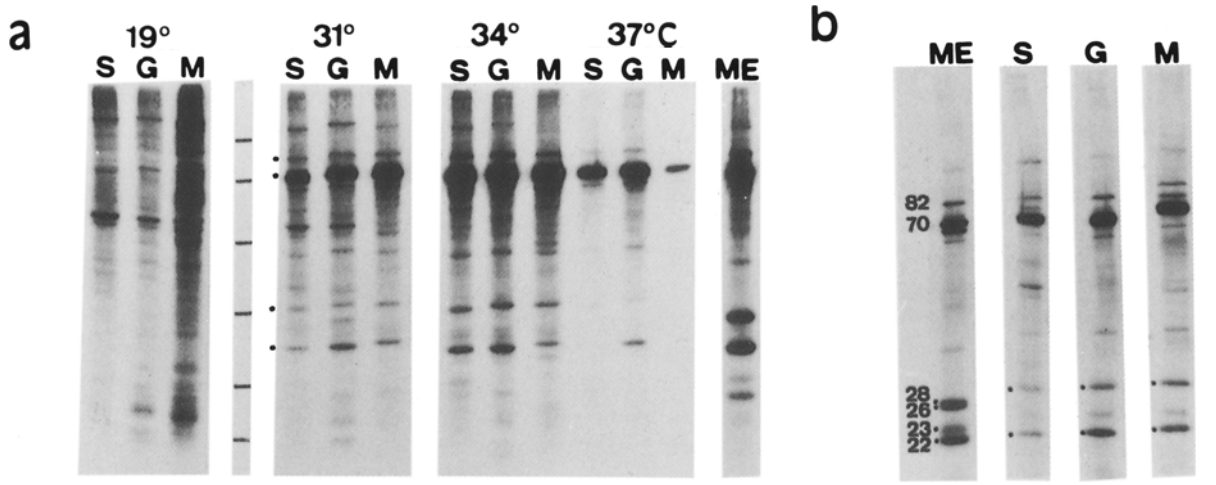

Figure 1. Heat shock proteins in three *obscura* group *Drosophila* species. *a* Proteins were analyzed before heat shock at control temperature, and after heat treatments in *D. subobscura* (S), *D. guanche* (G) and *D. madeirensis* (M). ME, *D. melanogaster* hsp pattern at 37 °C. Molecular weights are indicated by short black lines which correspond to 92,500, 66,200, 45,000, 31,000, 21,500 and 14,500 daltons. *b* Electrophoretic distribution on 9% polyacrylamide gels of hsps in these species. The *D. melanogaster* hsp pattern is indicated in kDa.

and Gall's procedure[15]. Probes were radiolabeled with ^{3}H-dCTP (1′,2′,5-^{3}H dCTP, Amersham, sp. act. 68 Ci/mmol) following the oligolabeling method described by Feinberg and Vogelstein[16]. Prehybridization, hybridization and subsequent washes were performed at high temperature as described by Pardue[17].

From the in situ results, it was inferred that, as in *D. melanogaster*, genes encoding the small hsps are organized in a closed linkage group in all three *obscura* group species and probably came from one ancestral gene. During the evolution of *Drosophila* species, fewer changes must have taken place in the genes encoding hsps of high MW than in the genes of the small hsps, which show a wide range of size and number of proteins among the different species found today.

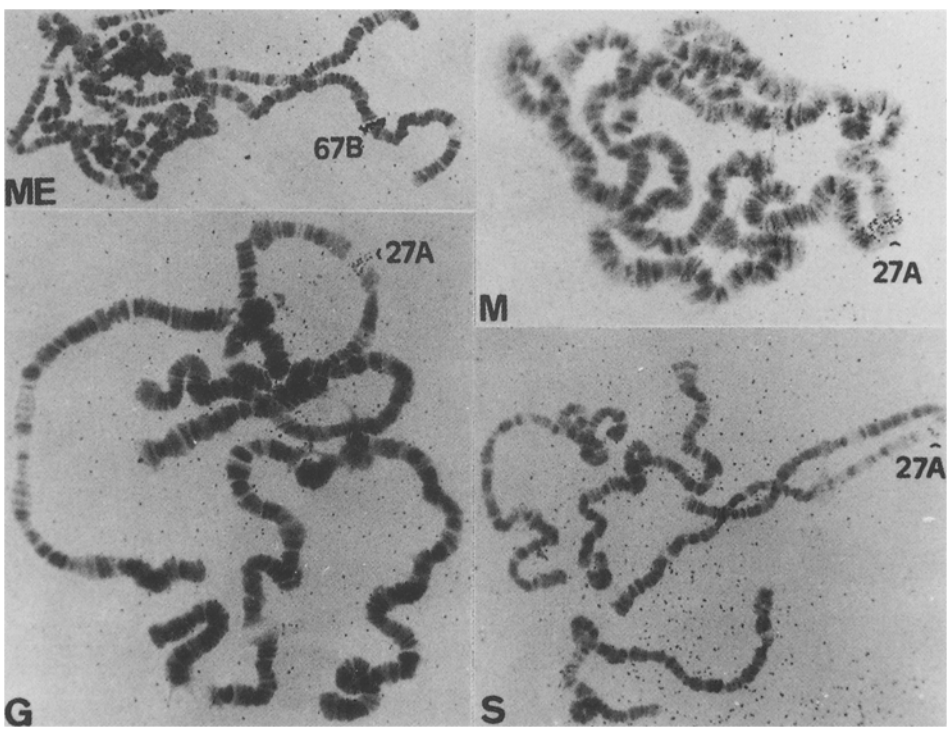

Figure 2. Hybridization of *D. melanogaster* hsp28–hsp23 probe from 67B locus of this species, to *obscura* group species polytene chromosomes. It shows the uniqueness of the hybridization site at the 27A locus on the J chromosome of these species. ME, *D. melanogaster*; G, *D. guanche*; M, *D. madeirensis*; S, *D. subobscura*.

Comparison between hsp patterns of the *obscura* group species with that of *D. melanogaster*. The small hsp apparent MW of the *obscura* group species are indicated in daltons

hsp family	*D. melanogaster*	*D. subobscura*	*D. guanche*	*D. madeirensis*
hsp82	+	+	+	+
hsp70	+	+	+	+
small hsps				
hsp32	−	+ (32,000)	+ (33,000)	+ (33,000)
hsp28	+	−	−	−
hsp26	+	−	−	−
hsp23	+	+ (23,500)	+ (23,400)	+ (24,200)
hsp22	+	−	−	−

+, presence; −, absence.

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