The heat shock protein hsp70 binds in vivo to subregions 2-48BC and 3-58D of the polytene chromosomes of *Drosophila hydei*

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Abstract. Multiple interactions of members of the hsp70 family with cellular components have already been described. We present, however, the first evidence that upon heat shock treatment hsp70 molecules interact with specific chromosomal subdivisions of the polytene chromosomes of *Drosophila hydei*. After a heat shock treatment of 20 min the protein binds to subdivision 3-58D₁ and to the heat shock inducible subdivisions 2-48B₃₋₆ and 2-48C₁₋₂. Hsp70 molecules were also observed in subdivision 3-58D₁ during recovery at 25°C but not in subdivisions 2-48B₃₋₆ and 2-48C₁₋₂. Our data suggest that this interaction is stress specific. DNase and RNase experiments suggest, moreover, that the hsp70 molecules bind to RNA from ribonucleoproteins (RNPs) in subdivisions 2-48B₃₋₆ and 2-48C₁₋₂ and to DNA in subdivision 3-58D₁. The DNA sequences in subdivision 3-58D₁ seem to have the potential to adopt the Z-DNA conformation.

Introduction

When prokaryotic and eukaryotic cells are exposed to a temperature a few degrees above their normal growth conditions, they exhibit a typical physiological reaction, called the heat shock response. As a result of the heat shock the cells shut down normal protein synthesis and a small set of proteins, known as the heat shock proteins (hsp), are induced. These proteins constitute the most highly conserved genetic system known, induced not only by heat but by many other environmental stresses (Ashburner and Bonner 1979). In eukaryotes hsp90, hsp70 and hsp60 appear to be involved in assembly and disassembly of protein complexes and members of the hsp70 family are also involved in translocation of certain proteins through intracellular membranes (for references see Cheng et al. 1989). Recently it has also been suggested that the stress proteins may be involved in infection and immune surveillance (Young and Elliot 1989).

By far the best studied of the heat shock proteins is hsp70. It has been shown that hsp70 molecules occur mostly in the nucleus of stressed *Drosophila* salivary gland cells but that they move to the cytoplasm during recovery (Velazquez and Lindquist 1984). Moreover, the protein accelerates the recovery of nucleolar morphology (Pelham 1984), its accumulation appears to repress its own transcription (DiDomenico et al. 1982) and it restores the RNA processing interrupted during heat shock (Yost and Lindquist 1986). However, the precise function of the heat shock protein during heat shock and recovery remains largely unknown. Another way to investigate the function of hsp70 is to identify molecules or structures with which it interacts. Thus, it has been found that in addition to the general association of the hsp70 with RNA (Storti et al. 1980; DiDomenico et al. 1982), the protein interacts with heterogeneous nuclear RNA (hnRNA) and ribonucleoproteins (RNPs) (Kloetzel and Bautz 1983; Burdon 1986; Welch and Mizzen 1988) and DNA replication complexes (Neidhardt et al. 1984). There are no data, however, on whether hsp70 associates with specific chromosomal loci which would suggest a possible genetic function of the protein.

Our results indicate that hsp70 interacts with the DNA of subdivision 3-58D₁ and with the RNase removable fraction of subdivisions 2-48B₃₋₆ and 2-48C₁₋₂ of the polytene chromosomes of *Drosophila hydei*.

Materials and methods

A wild-type strain of *D. hydei* from our laboratory collection was used in this study.

Immunoblotting. Protein extracts were prepared by homogenization of late third instar larvae in a lysis buffer containing 150 mM NaCl, 10 mM Tris-HCl, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 1% Triton X-100. Heat shock samples were obtained from larvae incubated for 1 h at 37°C. Protein samples (200 μg) were...
fractionated on 10% SDS-polyacrylamide preparative gels (Laemmli 1970), and electrothermically transferred onto nitrocellulose paper (Amersham). The blots were incubated either with a rat monoclonal anti-hsp70 antibody (dilution 1:40), raised against the D. melanogaster hsp70, or a rabbit mono-specific anti-hsp70 antibody (dilution 1:200). The rabbit antibodies were obtained by immunizing a conjugate containing a synthetic peptide of sequence ANDQGNRTTPSY, a highly conserved sequence located at positions 30 to 41 of the reported Trypanosoma cruzi hsp70 sequence (Requena et al. 1989). The antibody was detected by 125I-labelled protein A from Staphylococcus aureus (30 mCi/mg) at a concentration of 0.1 μCi/ml.

Results

Figure 1 shows the specificity of the monoclonal antibody anti-hsp70 from Drosophila melanogaster against D. hydei hsp70. Blots containing 200 μg protein from third instar larvae grown at 25°C (lane A) or incubated for 1 h at 37°C (lanes B and C) were incubated with the monoclonal anti-hsp70 antibody (lanes A and B) or rabbit mono-specific anti-hsp70 (lane C). Only an Mr=70,000 band is detected by both antibodies in heat shock protein extracts against a rabbit mono-specific anti-hsp70 raised against a peptide from the T. cruzi hsp70. No other protein bands were labelled by any of the antibodies.

The specific association of hsp70 molecules with chromosomal subdivisions 2-48B3-6, 2-48C1-2 and 3-58D; 30 min after a heat shock at 37°C is shown in Fig. 2. Subdivision 3-58D, 3-58D1 is not heat inducible but subdivisions 2-48B3-6 and 2-48C1-2 are induced by a variety of stress conditions including heat (Leenders and Berendes 1972) forming a large puff which accumulates small and large RNPs and transcribes a 3-5S RNA (Berendes et al. 1973; Lubsen et al. 1978), a cytoplasmic polysomal RNA of about 9S (Sondermeijer and Lubsen 1979) and a 38S non-polyadenylated RNA (Bisseling et al. 1976). Hsp70 molecules did not show association with other heat shock inducible puffs. The hsp70 protein was observed associated with nucleoli of early third instar larvae but it did not generally show interaction with nucleoli from salivary glands of late third instar larvae. Also, as indicated for D. melanogaster polytenic chromosomes (Velazquez and Lindquist 1984), low and diffuse anti-hsp70 labelling was observed over decondensed regions of the chromosomes but this varied from preparation to preparation.

Following the time course of interaction of hsp70 with the chromosomes indicated that the protein asso-