J. Biosci., Vol. 21, Number 2, April 1996, pp 207-219. © Printed in India.

hsp 83 mutation is a dominant enhancer of lethality associated with absence of the non-protein coding *hsrω* locus in *Drosophila melanogaster*

S C LAKHOTIA* and PRITHA RAY

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221005, India

MS received 24 November 1995; revised 15 January 1996

Abstract. The *hrsω* or the 93D heat shock locus of *Drosophila melanogaster*, which does not code for any protein, has an important role in development since nullosomy of this locus in trans-heterozygotes for two overlapping deficiencies, viz., *Df(3R)e*^{Gp4} (*eGp4*) and *Df(3R)GC14* (*GC14*), is known to cause a high (~ 80%) mortality with the small number of escapee nullosomic flies being sterile, weak and surviving for only a few days. We now show that a majority of the *hsrω*-nulosomics die as embryo and that the 20% escapee embryos develop slower compared to their sibs carrying either one or two copies of the *hsrω* locus but after hatching survive to pupal/imago stage. Most interestingly, we further show that when one *hsp83* mutant allele (*hsp83*^{e44}) is introduced in *eGp4/GC14* trans-heterozygotes, practically none of the *hsrω*-nullosomic embryos develop beyond the 1st instar larval stage. The specificity of this interaction between *hsp83* and *hsrω* genes was further confirmed by examining the effect of the *hsp83* mutant allele on other mutations in the 93D cytogenetic region. Therefore, we conclude that the *hsp83* mutation acts as a dominant enhancer of the lethality associated with nullosomy for the *hsrω* gene. The observed genetic interaction between these two members of the heat shock gene family during normal embryonic development of *Drosophila* reveals novel aspects of their biological functions.

Keywords. Heat shock; G-proteins; Sevenless; 93D locus.

1. Introduction

The heat shock response is a universal mechanism through which cells protect themselves against the thermal and other stress-induced damages: this response is most commonly identified through the elevated synthesis of a conserved set of heat shock proteins (HSP) following the stress. The different HSPs are grouped into several families, viz., HSP100, HSP90, HSP70, HSP60 and HSP30 (or low molecular weight HSPs), depending upon their molecular sizes and other characteristics (see Morimoto *et al* 1994 and other papers in this issue). Among the nine puffs induced by heat shock in polytene nuclei of *Drosophila melanogaster* (Ashburner 1970; Mukherjee and Lakhotia 1979), the 93D locus is unique in not coding for a typical protein product (Lakhotia and Mukherjee 1982; Fini *et al* 1989), being selectively inducible by drugs like colchicine, benzamide etc., and in several other features of its heat shock inducibility (Lakhotia 1987, 1989; Lakhotia and Sharma 1996; Lakhotia and Mutsuddi 1996). The multiple transcripts of the 93D locus (also called the *hsrω* locus, Bendena *et al* 1989), are widely expressed in all tissue types during all stages of development (Bendena *et al* 1989, 1991; Lakhotia 1989; Mutsuddi and Lakhotia 1995). These transcripts, although not coding for a protein, have important roles during

*Corresponding author (Fax, + 91 542 312584, 312059)

development of *Drosophila* since very few (only about 20%) of the zygotes that are deficient for both copies of the *hsrω* gene survive to pupal/imago stage; the few escapee flies are very weak, sterile and die within a few days (Mohler and Pardue 1984); *hsrω* nullosomic larvae are also thermosensitive and have poor thermotolerance (Lakhotia 1987).

After heat shock, only the 93D puff has been found to be associated with cyclic GMP (Spruill *et al* 1978). A recent report by Morcillo *et al* (1993) showed that under heat shock conditions, HSP83 is specifically present at the 93D puff on polytene chromosomes of *Drosophila melanogaster* and on its homologue in other species of *Drosophila* and *Chironomus* (also see Morcillo and Diez 1996). Synthesis of HSP83, a member of the HSP90 family, is enhanced by heat shock. However, this protein is ubiquitously present at high levels at all stages of development of the fly even in the absence of heat shock (Mason *et al* 1984; Xiao and Lis 1989; Ding *et al* 1993). Although the HSP90 family proteins are mainly found in cytoplasm (Jakob and Buchner 1994), nuclear localization of HSP90 is also reported in avian (Collier and Schlesinger 1986) and mammalian cells (Akner *et al* 1992). In the polytene nuclei of *Drosophila,* HSP83 has been seen to be associated with intra- and extra-chromosomal RNP fibrils (Carbajal *et al* 1990) and during heat shock, with the 93D puff (Morcillo *et al* 1993).

Information about functions of the HSP90 family chaperone proteins has been obtained from their association with various cellular proteins. Among the various proteins with which HSP90 is associated, some important ones are the different oncogenic receptor tyrosine kinases, tubulin, actin, eukaryotic initiation factor 2α-kinase, Raf and different members of the steroid receptor family (Prat 1993; Jakob and Buchner 1994; Cutforth and Rubin 1994; Pal *et al* 1996). Several mutant alleles of *hsp83* gene have been identified in

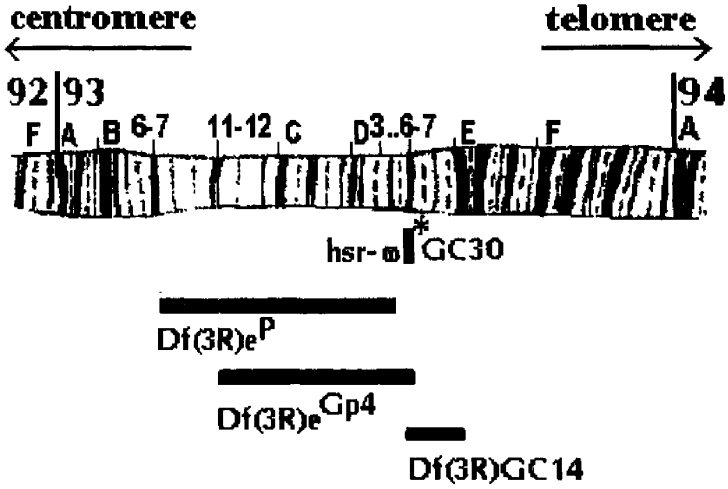

Figure 1. Diagrammatic representation of the different deletions and the point mutation in the 93D cytogenetic region used in this study. The standard banding pattern (Bridges 1935) of the segment of larval salivary gland polytene chromosomes from region 93A to 93F is shown on top and the solid horizontal bars in the lower part depict the extent of chromosomal deletion in the *Df(3R)e*^{Gp4} (***eGp4***), *Df(3R)e*^{P} (***eP***) and *Df(3R)GC14* (***GC14***) chromosomes, respectively. The location of the *hsrω* locus is shown as a solid vertical bar corresponding to the region of overlap (bands 93D6–7) in the *eGp*4 and *GC14* deficiency chromosomes. The *GC30* point mutation (*) maps immediately after (towards the telomeric end) the *hsω* locus.

Drosophila by their properties of enhancing the Sevenless phenotype (Cutforth and Rubin 1994) in adult eye. The Sevenless signaling pathway is one of the most well studied receptor tyrosine kinase mediated signal transduction pathway in *Drosophila.* In addition, *hsp83* mutations also suppress a gain-of-function mutation in the Torso receptor protein tyrosine kinase (PTK) suggesting that HSP90 proteins may regulate the activity of other receptor PTKs also (Doyle and Bishop 1993).

The presence of HSP83 and of cyclic GMP at the 93D puff site after heat shock and the varied roles of HSP83 prompted us to further examine the interaction of HSP83 with the *hsrω* gene or its products during normal development of *D. melanogaster*. A convenient approach to study the interaction between two genes is to examine effects of mutation in one gene on the phenotype due to mutation in the other gene. It is this kind of approach that allowed identification of the involvement of HSP83 in the Torso and Sevenless signaling pathways (Doyle and Bishop 1993; Cutforth and Rubin 1994). Therefore, we have made use of the mutations at the *hsp83* and *hsrω* gene loci to see if they interact during normal development. Our results reveal that these two heat shock genes interact during the course of normal development as well, since a recessive mutation at the *hsp83* locus was found to act as a dominant enhancer of the lethality associated with nullosomy of the *hsrω* gene.

2. Materials and methods

2.1 *Fly stocks*

In addition to a wild type (Oregon R^+) stock of *D. melanogaster,* the following stocks bearing mutations/small deficiencies in and around the 93D cytogenetic region (see figure 1) and at the *hsp83* gene were used (for a detailed description of the mutants, see Lindsley and Zimm 1992 and other references as noted).

2.1a *Df*(*3R*)e^{Gp4}/*TM6B:* As shown in figure 1, *Df*(*3R*)e^{Gp4} is a small deletion (abbreviated as *eGp4* in the following) in the third chromosome spanning from band 93B11–13 to band 93D6–7 (Mohler and Pardue 1984) with its distal breakpoint being just beyond the *hsrω* transcription unit. The *TM6B* chromosome is a balancer which carries the dominant *Tb* marker that allows larvae, pupae and flies carrying this chromosome to be distinguished by their tubby phenotype. The *Df*(*3R*)e^{Gp4} as well as the *TM6B* chromosomes are embryonic lethal when homozygous.

2..1b *Df*(*3R*)*GC14*/*TM6B* and *Df*(*3R*)*GC14*/*ln*(*3R*)*C, e Sb l*(*3*)*e: Df*(*3R*)*GC14* (abbreviated as *GC14* in the following), described by Mohler and Pardue (1984), is also a small deletion in the right arm of chromosome 3 spanning from bands 93D6–7 to band 93D10 (figure 1). The proximal breakpoint in the *GC14* chromosome is about 10 kb upstream of the *hsrω* transcription start site (M L Pardue, personal communication). This deficiency is homozygous lethal and is maintained against the *TM6B* or the *ln*(*3R*)*C* balancer chromosome. The *GC14* and the *eGp4* deficiencies do not complement each other; the region of overlap of these two deletions actually defines the 93D heat shock gene location (Mohler and Pardue 1984).

2.1c *Df*(*3R*)e^{p}/*TM6B*: *Df*(*3R*)e^{p} (abbreviated as *eP* in the following) chromosome carries a small deletion in the right arm of chromosome 3 from 93B6–7 to 93D3–5

bands (figure 1, Tapadia and Lakhotia 1993). The *eP* deficiency complements the *GC14* deficiency since the distal breakpoint in the *eP* chromosome is about 20 kb upstream of the *hsrω* transcription start point (Tapadia and Lakhotia 1993 and in preparation). This is also homozygous lethal and therefore, is maintained against the *TM6B* balancer.

2.1d *GC30/TM6B:* The *GC30* point mutation complements the *Df(3R)e*^{Gp4} deletion and maps immediately downstream of the 93D heat shock locus (figure 1, Mohler and Pardue 1984). This mutation is semilethal in homozygous condition and is also maintained against the *TM6B* balancer.

2.1e *hsp83*^{e4A}*/TM6B:* The *hsp83e*^{4A} (abbreviated as *e4A* in the following) is a point mutation in the coding region of *hsp83* gene on chromosome 3, resulting in Inactivation of the HSP83 protein (Cutforth and Rubin 1994). This mutation is a recessive lethal, with the only known visible effects being a dominant enhancement of the Sevenless phenotype and suppression of a gain of function mutation of the Torso gene (Cutforth and Rubin 1994; Doyle and Bishop 1993). It is maintained against the *TM6B* balancer chromosome.

2.1f *hsp83*^{e4A} *Df(3R)e*^{Gp4}*/TM6B:* A stock generated by a series of crossings, stalling with the stocks a and e above, and selecting appropriate recombinant progeny carrying the *e4A* point mutation and the *eGp4* deficiency on the same homologue of chromosome 3.

2.1g *hsp83*^{e4A} *Df(3R)e*^{p}*/TM6B:* This stock was also generated by a series of crossings, starting with the stocks c and e above, and selecting the appropriate recombinant progeny carrying the *e4A* point mutation and the *eP* deficiency on the same homologue of chromosome 3.

All the above stocks show a good healthy growth. All flies and larvae were reared at 22 ± 1°C on the standard *Drosophila* food under uncrowded conditions.

For generating hybrid progeny carrying deletion or mutation in the 93D region in combination with wild type or mutated *hsp83* gene, *e4A + eGpA/TM6B* or *e4A eGp4/TM6B* or *e4A + eP/TM6B* or *e4A eP/TM6B* virgin females were mass mated with males of the desired genotype (see table 1 for the crosses) and the viability of embryos and pupae in the next generation was examined. For embryonic viability, eggs were collected at hourly intervals on agar plates (0·6% Bacto-agar and 4% sugar) and the eggs that failed to hatch after 24 h and 48 h of egg laying were counted. To identify embryos that lacked both copies of the *hsrω* locus, DNA-DNA *in situ* hybridization was also carried out in some cases (see below). For assessing pupal viability, mass matings of flies of the desired genotypes were set up in milk bottles with the parental flies being transferred to fresh bottles every day. After 10 days, when all larvae in the bottle had pupated, the numbers of pupae of different genotypes, identified on the basis of the colour and shape of pupal case, were counted. To determine the larval period up to which the dying genotypes survived (see § 3), dead larvae were picked up from the Petri dishes at regular intervals and mounted on slides and examined for the number of teeth on their mandibular hooks to stage the larvae as 1st, 2nd or 3rd instar (Bodenstein 1950).

2.2 *DNA-DNA in situ hybridization in whole embryos*

Eggs from the desired crosses (see § 3) were collected on agar plates and allowed to grow for different time intervals varying from 13 to 30 h at 22°C. After the desired period of

Table 1. Survival of pupae carrying mutations in the 93D region on both homologues in the presence of wild type or mutated *hsp83* allele

Cross	Observed no. of pupae: 93D mutant* (genotype and no.)	Observed no. of pupae: Others	Expected no. of 93D mutant pupae**	Survival of 93D mutant pupae (%)
1. *e4A*$^+$ *eGp4/TM6B* females × *e4A*$^+$ *GC14/ln(3R)C* males	*e4A*$^+$ *eGp4/ e4A*$^+$ *GC14* 35	779	260	13·46
2. *e4A eGp4/TM6B* females × *e4A*$^+$ *GC14/ln(3R)C* males	*e4A eGp4/ e4A*$^+$ *GC14* 03	519	173	1·73
3. *e4A*$^+$ *eGp4/TM6B* females × *e4A*$^+$ *GC14/TM6B* males	*e4A*$^+$ *eGp4/ e4A*$^+$ *GC14* 67	651	325	20·62
4. *e4A eGp4/TM6B* females × *e4A*$^+$ *GC14/TM6B* males	*e4A eGp4/ e4A*$^+$ *GC14* 27	2128	1064	2·54
5. *e4A*$^+$ *eGp4/TM6B* females × *e4A*$^+$ *GC30/TM6B* males	*e4A*$^+$ *eGp4/ e4A*$^+$ *GC30* 50	88	44	113·64
6. *e4A eGp4/TM6B* females × *e4A*$^+$ *GC30/TM6B* males	*e4A eGp4/ e4A*$^+$ *GC30* 64	125	62·5	102·4
7. *e4A*$^+$ *eP/TM6B* females × *e4A*$^+$ *GC14/TM6B* males	*e4A*$^+$ *eP/ e4A*$^+$ *GC14* 132	202	101	130·69
8. *e4A eP/TM6B* females × *e4A*$^+$ *GC14/TM6B* males	*e4A eP/ e4A*$^+$ *GC14* 79	189	94·5	83·6

*The 93D mutant class refers to genotypes: *eGp4/GC14* (crosses 1–4) or *eGp4/GC30* (crosses 5–6) or *eP/GCl4* (crosses 7–8); in crosses 2, 4, 6 and 8 they were also heterozygous for the *e4A* mutant allele; in all the crosses, the 93D-mutant class of pupae could be easily identified by being non-ebony and non-tubby while the others were either ebony tubby or ebony non-tubby or non-ebony tubby.

**Expected number of the93D mutant pupae (column 4) in crosses 1 and 2 was calculated as 1/3 of the total number of observed pupae of the other genotypes since in these crosses, 4 classes of pupae were expected; in the remaining crosses (nos. 3 to 8), this was calculated as 1/2 of the total number of observed pupae of the other genotypes since in these crosses involving *GC14/TM6B* or *GC30/TM6B* males, only 3 classes of viable pupae were expected (*TM6B/TM6B* embryos die).

development, they were removed from the agar plates onto a nylon mesh for a thorough washing with distilled water to remove the adhering particles etc. The embryos were dechorionated by immersing in 5% bleach solution following which they were washed several times with distilled water and finally were transferred to an eppendorf tube in heptane. The heptane was soon replaced by 1:1 heptane: methanol mixture and embryos were vigorously shaken for a few seconds. The dechorionated embryos that settled down were collected and washed several times in methanol and then in a series of methanol:buffer A (15mM HEPES, pH 7·0, 80mM KCl, 20mM NaCl, 0·5 mM EGTA, 2 mM EDTA, 0·5 mM spermidine, 0·2 mM spermine, 0·1 % 2-β mercaptoethanol) mixtures (75%, 50%, 25%, 10% of methanol) and finally stored at –20°C in buffer A till further processing.

The *Hind*III genomic fragment (~ 19 kb), which includes the entire transcription unit and 5' and 3'flanking sequences, of the *hsrω* gene was gel purified from the clone *pDRM102* (kindly provided by Prof. M L Pardue), further digested with Alu1 and Rsal to generate smaller fragments for efficient entry into the whole embryos and was

labelled with digoxigenin (DIG)-dUTP by random priming as instructed by the manufacturer (Boehringer and Mannheim) for use as a probe for *in situ* hybridization *In situ* hybridization to chromosomal DNA in whole embryos was done following Hiraoka *et al* (1993). After denaturing the embryos at 70°C for 15 min in the hybridization mix (5×SSC with 50% formamide, 0·1% Tween 20), hybridization (50 ng probe/100 ml) was allowed at 37°C for 15–18 h. After hydridization, the embryos were washed at room temperature for 20 min each in a series of 50%, 40%, 30%, 20%, 10% formamide solutions in 4 × SSC, 0, 1% Tween 20. After two final washes in 4 × SSC, the embryos were processed for colorimetric detection of hybridization using anti-DIG-alkaline phosphatase conjugated antibody as instructed by the manufacturer (Boehringer and Mannheim). Finally, the embryos were washed repeatedly in 1 × PBS (130 mM NaCl, 7 mM NaH_2 PO_4, 3mMNa_2 HPO_4pH 7·2) and mounted in 80% glycerol for microscopic detection of the hybridization signal.

2.3 *Cuticular preparations of embryos*

In certain cases (see §3), the embryos that did not hatch after 24 h or 48 h of development were directly mounted in a mixture of 2:1:1 Hoyer's medium:lactic acid:water (Wieschaus and Nusslein-Volhard 1986). After overnight incubation at 60°C, they were examined under microscope.

3. Results

3.1 *Lethality due to nullosomy for hsrω locus is enhanced by heterozygosity for hsp83 mutation*

Earlier studies by Mohler and Pardue (1984) had shown that the 93D heat shock locus or the hsr*ω* gene of *D. melanogaster* is located in the band 93D6–7 as defined by the overlapping distal and proximal breakpoints of the *eGp4* and *GC14* deficiencies (see figure 1), respectively, and that only about 20 to 30% of the *hsrω*-nullosomics (*eG4*/*GC14* trans-heterozygotes) developed to imago stage with all such escapee files dying within a few days of eclosion. To examine if the presence of *hsp83* mutant allele in the *hsrω*-deficient individuals affected their survival, a series of crosses were made and the viability of progeny was examined. The different crosses that were set up and the survival of pupae carrying different combinations of mutations in the 93D region (*eGp4, GC14, eP* or *GC30*) in the two homologues in presence of either wild type (homozygous) or mutant (heterozygous) *hsp83* allele was compared (see table 1).

In agreement with earlier results (Mohler and Pardue 1984), the *hsrω*-nullosomics with two wild type *hsp83* alleles had a poor viability since only 13 to 20% of the expected numbers of pupae of this genotype were actually seen (cross 1 and 3 in table1); almost all of these pupae developed to adult flies which, although morphologically nearly normal, were very weak and died within a few days. Interestingly, when one copy of a recessive lethal mutant allele of *hsp83* (*e4A*) was introduced, only 1 or 2% of the *hsrω*-deficient zygotes survived to pupal stage (crosses 2 and 4 in table 1) and at least 50% of these rare surviving pupae did not develop further. It may be noted that $e4A^{+}$/*e4A* heterozygotes by themselves have more or less normal viability (Cutforth and Rubin 1994 and our own observations). To confirm that this lethal enhancer effect

of the *e4A* mutation was specifically due to absence of the *hsrω* gene copies, additional crosses were made with other mutations that map immediately upstream (*Df*(*3R*)e^{p}) or downstream (*GC30*) of the *hsrω* locus. As seen from the results of crosses 5–6 in table 1, heterozygosity for a mutation al the *hsp83* gene (*e4A*) did not have any effect on the *eGp4*/*GC30* trans-heterozygotes. The *e4A* mutation had an interesting effect on the viability of *eP*/*GC14* trans-heterozygotes (crosses 7–8): the viability of such trans-heterozygotes when carrying two wild type *hsp83* alleles appeared better than expected (about 131 %); this apparently better survival of *eP*/*GC14* trans-heterozygotes actually seems to be due to a relatively poorer viability of the balancer chromosome (*TM6B*) carrying sibs. In the presence of a mutant *hsp83* allele, the viability of *eP*/*GC14* trans-heterozygotes to pupal stage was reduced: if we presume all the three surviving genotypes in this cross to be equally viable, the surviving *e4A eP*/*e4A*$^{+}$ *GC14* trans-heterozygotes were only about 84% of expected (table 1). However, if the viability of *eP*/*GC14* trans-heterozygotes carrying two wild type *hsp83* alleles was directly compared with those carrying one mutant *hsp83* allele, the viability of the latter was only about 63 % of the former.

3.2 *Enhanced lethality of hsrω nullosomics due to the hsp83 mutation is post-embryonic*

To investigate if the drastically reduced number of pupae that were nullosomic for *hsrω* gene and heterozygous for *hsp83* mutant allele, was due to a greater lethality at embryonic stage, the viability of embryos was also examined. The data are presented in table 2. As expected, there were hardly any wild type embryos that did not hatch by 24 h after egg laying. However, about 5% of the eggs laid by *e4A*$^{+}$ *eGp4*/*TM6B* females mated with *e4A*$^{+}$ *GC14*/*ln*(*3R*)*C* males as well as those laid by *e4A eGp4*/*TM6B* females mated with *e4A*$^{+}$ *GC14*/*ln*(*3R*)*C* males were delayed in hatching and hatched between 24 h and 48 h after oviposition while another 20 % or so did not hatch even after 48 h (see rows 2 and 3 in table 2). It is interesting to note that the sum of unhatched and delayed hatching embryos in these crosses agreed with the proportion of eggs (25 %) that were expected to be trans-heterozygotes for *eGp4* and *GC14* deficiencies and thus without any copy of the *hsrω* heat shock locus.

3.3 *The surviving hsrω-nullosomics are delayed in embryonic development.*

To confirm if the eggs that were either delayed in hatching or did not hatch at all were indeed without any copy of the *hsrω* locus, another batch of embryos from these two

Table 2. Viability and hatching time of embryos in different crosses.

Cross	Total eggs examined	Unhatched (% of total)		Delayed* (% of total)
		at 24 h	at 48 h**	
Wild type	1296	0·39	0·39	0·0
e4A^{+} *eGp4/TM6B* × *e4A*$^{+}$ *GC14/ln(3R)C*	777	25·87	20·08	5·79
e4A eGp4/TM6B × *e4A*$^{+}$ *GC14/ln(3R)C*	304	22·37	17·11	5·26

*Those that hatched between 24 h and 48 h after egg laying were classified as delayed.
**Those that did not hatch at 48 h after egg laying were dead embryos.

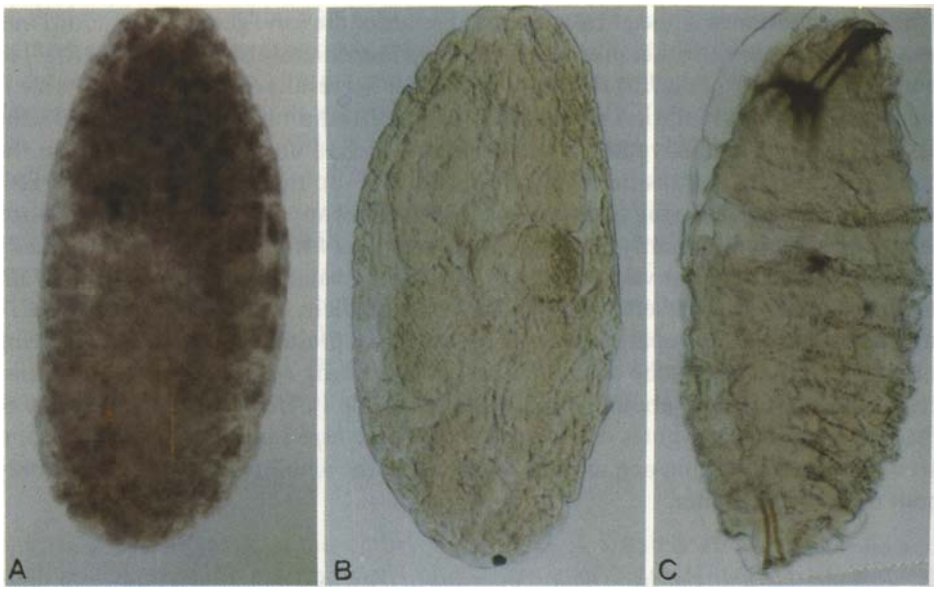

Figure 2. Representative examples of *in situ* hybridization of DIG-labelled *hsrω*-specific probe to embryos from the cross *e4A eGp4*/*TM6B* × *e4A*$^{+}$ *GC14*/*ln*(*3R*)*C*. 13–17h old embryos are shown in (**A**) and (**B**) and a nearly 30h old embryo is shown in (**C**). While the embryo in (**A**) shows a positive hybridization signal, those in (**B**) and (**C**) are without any hybridization with the *hsrω* probe and therefore, classified as *eGp4*/*GC14* trans-heterozygotes.

crosses were hybridized *in situ* to a DIG-labelled probe that hybridizes specifically to the *hsrω* gene and thus would allow identification of the *hsrω*-nullosomic eggs by absence of any signal (see figure 2). Fifty eggs from the cross *e4A*$^{+}$ *eGp4*/*TM6B* × *e4A*$^{+}$ *GC14*/*ln*(*3R*)*C* and 66 eggs from the cross *e4A eGp4*/*TM6B* × *e4A*$^{+}$ *GC14*/*ln*(*3R*)*C* were examined for hybridization with the *hsrω*-specific probe. Examination of these embryos after *in situ* hybridization clearly showed that nearly all of the eggs that did not hatch till 27-30h after egg laying in either of the crosses also did not show any hybridization signal with the probe (figure 2C) and thus were trans-heterozygotes for the *eGp4* and *GC14* deficiencies.

Taken together, these results showed that nearly 80% of the *hsrω*-nullosomic embryos (i.e., about 20% of total embryos) did not hatch at all while the rest (i.e., about 5 % of total) that survived, were delayed in development and hatched much later than their sibs; the presence of an *hsp83* mutant allele in heterozygous condition did not have any effect on the hatchability of *hsrω*.-deficient embryos.

3.4 *hsp83 mutant homozygotes die as 2nd instar larvae but the rare surviving hsrω-nullosomics heterozygous for the hsp83 mutation die as 1st instar larvae*

The *hsrω*-deficient enbryos that failed to hatch were processed for examination of their cuticular patterns. This examination revealed that in terms of their cuticular structures, these dead embryos had developed to almost the hatching stage (see figure 2).

The above results showed that the *hsrω*-nullosomics (with or without a *hsp83* mutant allele) undergo a more or less complete embryonic development and a few of them also

Table 3. Time of death of *hsp83* mutant homozygotes and of *hsrω* nullosmics heterozygous for the *hsp83* mutation

Cross	Total eggs examined	Numbers of dead larvae	
		1st instar	2nd instar*
e4A/TM6B × *e4A/TM6B*	519	7	34
e4A eGp4/TM6B × *e4A*$^{+}$ *GC14/TM6B***	701	33	3

*A11 the dead 2nd instar larvae in both the crosses were unambiguously identified as non-tubby and, therefore, were *e4A/e4A* in cross 1 and nullosomic for *hsrω* (*e4A eGp4/e4A*$^{+}$ *GC14*) in cross 2.
**All the dead larvae in cross 2 were from eggs that hatched after 24h and, therefore, were identified as nullosomic for *hsrω*.

hatch as larvae but those heterozygous for the mutant *hsp83* allele (*e4A*) do not develop to pupal stage. Therefore, the larval period up to which these individuals survived was examined. Likewise since embryos homozygous for the *hsp83* mutation, but carrying functional *hsrω* gene copies, were found to hatch (data not presented) but not pupate, the larval stage up to which such individuals survived was also examined. For this purpose, Petri dishes carrying eggs from *e4A/TM6B* × *e4A/TM6B* and *e4A eGp4/TM6B* × *e4A*$^{+}$ *GC14/TM6B crosses* were periodically examined for dead larvae which were staged as described in § 2. In the case of *e4A/TM6B* × *e4A/TM6B* cross, all of the dead larvae were homozygous for the *hsp83* mutant allele (*e4A/e4A*) since they were non-tubby (i.e., not carrying the *TM6B* chromosome) and as the data presented in table 3 show most of them had developed to 2nd instar stage; however, no non-tubby larva was found to have developed to 3rd instar or later stages. On the other hand in the case of *e4A eGp4/TM6B* × *e4A*$^{+}$ *GC14/TM6B* cross, 33 out of the 36 dead larvae had not developed beyond the 1st instar stage (see table 3): all these dead larvae were presumed to be nullosomic for *hsrω* and heterozygous for the *hsp83* mutant allele since all of them had hatched 24 h after oviposition and also since all the 3 dead 2nd instar larvae were non-tubby.

4. Discussion

It is known that trans-heterozygotes for *eGp4* and *GC14* deficiencies, being nullosomic for the *hsrω* locus, are poorly viable since only about 20% of them develop to pupal/imago stage (Mohler and Pardue 1984). The present results further showed that absence of both copies of the *hsrω* locus was embryonic lethal in about 80% cases while the remaining *hsrω*-nullosomic eggs developed rather slowly but ultimately hatched as normal looking larvae, all of which could pupate. It is known that *hsrω* transcripts are not inherited maternally (Bendena *et al* 1991; Mutsuddi and Lakhotia 1995). Therefore, development of these *hsrω*-deficient zygotes to pupal/imago stage is rather surprising since the *hsrω* locus, when present, is developmentally expressed in almost all the embryonic and larval tissues (Bendena *et al* 1991; Mutsuddi and Lakhotia 1995). It appears, therefore, that the developmental expression of *hsrω*) in the various larval cell types is not crucial for larval life itself but has significance for development of the imago since the *hsrω* deficient flies that eclose are not normal (Mohler and Pardue

1984; Mutsuddi and Lakhotia 1995). It is also interesting to note that the *hsrω*-nullosomics that died as embryos or as 1st instar larvae, had more or less normal cuticular patterns suggesting that these animals suffer from some other subtle but crucial perturbations in cellular metabolism.

The most interesting result of present study was that heterozygosity for a mutation at the *hsp83* gene (*e4A* mutant allele, Cutforth and Rubin 1994) enhanced the lethality associated with nullosomy for the *hsrω* locus in *eGp4*/*GC14* trans-heterozygotes so that the 20% escapees also failed to develop beyond the 1st instar stage. Heterozygosity of the *e4A* mutant allele in combination with any one of the two deficiencies has no detectable deleterious effect on viability. Therefore, the near zero viability of *eGp4*/*GC14* trans-heterozygous larvae when also carrying a mutant allele at the *hsp83* locus is most likely due to a dominant enhancer effect of the *hsp83* mutation on lethality due to nullosomy of the *hsrω* locus. A good viability of the *e4A eGp4*/*e4A*$^{+}$ *GC30* pupae showed that the effect of *hsp83* mutation maps to within the limits of *eGp4* deletion. The significantly reduced viability of *eP*/*GC14* trans-heterozygotes when heterozygous for the *hsp83* mutant allele is remarkable since it has been seen in our laboratory that although the heat shock induced expression of the *hsrω* locus on the *eP* deficiency chromosome is not affected, its inducibility with benzamide or colchicine is completely abolished (Tapadia and Lakhotia 1993 and in preparation). Therefore, the reduced survival of *e4A eP*/*e4A*$^{+}$ *GC14* embryos to pupal stage also seems to be due to some dysfunction of the single copy of *hsrω* locus on the *eP* chromosome. The poorer viability of the very rare surviving *e4A eGp4*/*e4A*$^{+}$ *GC14* pupae compared to that of the *e4A*$^{+}$ *eGp4*/*e4A*$^{+}$ *GC14* pupae further reflects an effect of the *hsp83* gene mutation on the absence of *hsrω* gene. Therefore, we conclude that *hsp83* and *hsrω* genes interact during normal development and that *hsp83* (*e4A*) mutation has a dominant enhancer effect on lethality associated with nullosomy of the *hsrω* locus.

The maternally derived HSP83 in *Drosophila* embryos (Mason *et al* 1984; Xiao and Lis 1989; Ding *et al* 1993) may explain the survival of *e4A* homozygotes till 2nd instar stage (table 3) and also the absence of an aggravating effect of heterozygosity for *hsp83* mutation on embryonic lethality of the *hsrω*-nullosomics. However, it is interesting that while *hsp83* mutant homozygotes (carrying one or two functional *hsrω* gene copies) could develop up to the 2nd instar stage, almost none of the *hsrω*-nullosomics heterozygous for the *e4A* mutation developed beyond the 1st instar stage in spite of their carrying one functional *hsp83* gene copy. It may be expected that the levels of functional HSP83 would become limiting in the *e4A* heterozygotes. This limiting supply of normal HSP83 did not seem to have any seriously deleterious effect in embryos carrying at least one functional copy of the *hsrω* gene. But nearly all of those without any *hsrω* gene copies suffered strongly and were not able to develop-beyond the 1st instar stage. The survival of *e4A* homozygotes or heterozygotes having functional *hsrω* locus to 2nd instar stage or beyond, respectively, but the death of *e4A* heterozygotes nullosomic for *hsrω* locus at an earlier stage (1st instar larva) is yet another evidence of a crucial interaction between these two genes during normal development.

The survival of about 20% *hsrω*-nullosomic eggs to apparently normal pupal but aberrant adult stage suggests that the functions of the *hsrω* transcripts can, to a limited extent, be dispensable so that at least some *hsrω*-deficient embryos go through their embryonic and larval periods without any noticeable damage. This limited dispensability of *hsrω* gene functions is possibly due to the presence of redundant genetic pathways (see Brookfield 1992; Lakhotia and Sharma 1996). However, the simultaneous presence

of two such limiting factors, mutant HSP83 and the complete absence of the *hsrω* transcripts, perhaps cannot be compensated by the reduntant pathways and does not allow development beyond the 1st instar stage.

Since, besides their expression under conditions of stress, both the genes are also widely expressed during the course of normal development (Xiao and Lis 1989; Mutsuddi and Lakhotia 1995), their interactions are possible. We may note the situation in ovary as an example since *hsp83* as well as *hsrω* are abundantly expressed in ovarian nurse cells (Ding *et al* 1993; Mutsuddi and Lakhotia 1995). It is indeed interesting that the rare surviving *eGp4*/*GC14* trans-heterozygote female flies have poorly differentiated ovaries (Mutsuddi and Lakhotia 1995). On the other hand, *hsrω* transcripts are absent or less frequent in early stages of spermatogenesis (Bendena *et al* 1991; Mutsuddi and Lakhotia 1995) and, compared to ovaries, testes in *hsrω*-deficient flies appear much less affected. It is likely that the normal ovary differentiation requires a crucial interaction between the *hsp83* and *hsrω* genes. Interaction/s between these two genes, the absence of which lead to early lethality, remain to be identified.

The earlier finding of association of HSP83 with the heat shock induced *hsrω* gene (Morcillo *et al* 1993) and the present finding of an interaction between *hsp83* and *hsrω* during normal development strongly argue for an entirely different role of HSP83. While the functions of HSP83, in terms of their interaction with other proteins in the cell are better understood (see § 1), the present results provide the first evidence of interaction of this versatile chaperone protein with a non-protein coding RNA. Whether the HSP83 modulates activities of the *hsrω* transcripts or the *hsrω* transcripts affect HSP83 functions is not known but either are possible as considered in the following. In view of the complex metabolism of the *hsrω* transcripts at their site of synthesis (Lakhotia and Sharma 1995) and the presence of the HSP83 at this locus during heat shock (Morcillo *et al* 1993), their interaction may modulate the *in situ* processing and/or activity of the *hsrω* transcripts. HSP83 seems to have a function in regulation of initiation of protein synthesis (see Pal *et al* 1996) and the 1·2 kb *hsrω-c* transcript also has been implicated to have a role in monitoring protein synthesis (Lakhotia and Sharma 1996). Association of cGMP with the heat shock induced *hsrω* locus (Spruill *et al* 1978) and the involvement of HSP83 in G-protein signal transduction pathways (Cutforth and Rubin 1994) may suggest a role for the *hsrω*)transcripts in this pathway also. In recent years, the non-coding transcripts have been shown to have a variety of functions (reviewed in Lakhotia 1996) one of which is to modulate activities of certain proteins by binding (e.g., see Retallack and Friedman 1995). Thus it remains possible that *hsrω* transcripts may modulate some functions of the HSP83. Further studies on association of HSP83 with *hsrω* gene and/or its transcripts are necessary to unravel the significance of this novel interaction between a chaperone protein and non-coding transcripts.

Acknowledgements

This work was supported by a research grant from the Department of Science and Technology, New Delhi to SCL. PR was supported by a senior research fellowship from the Council of Scientific and Industrial Research, New Delhi. We thank Dr T Cutforth for the *hsp83* mutant stocks and Prof Mary Lou Pardue for the *pDRM102* clone. We also thank Dr Pradip Sinha, Indore, for his valuable comments on the manuscript.

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