HSR-Omega: An Unusual Non-protein Coding Heat Shock Gene of Drosophila Having Important Developmental Roles

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Polytene chromosomes of dipteran larvae, by virtue of their distinct visibility under microscope at a stage when they are transcriptionally active, have contributed immensely to our understanding of gene expression and regulation in eukaryotes. A most notable example of such a contribution is the discovery of heat shock puffs in polytene chromosomes of *Drosophila* species by Ritossa in early sixties (Ritossa 1962). This seminal observation, however, remained confined to *Drosophila* and some other dipterans (like *Chironomus*) where polytene chromosomes could be studied for induced puffing. The demonstration of heat shock induced protein synthesis in *Drosophila* by Tissieres et al. (1974) opened a new chapter in the heat shock paradigm since now any organism and/or cell type could be studied with respect to the induction of synthesis of the heat shock proteins. The revelations in recent years that the heat shock proteins and their constitutively expressed cognates function as molecular chaperones that help in protein-folding and their translocation across membranes have provided newer insights into cell structure and function. The heat shock proteins have also become very important in human genetics and diseases due to their involvement in a number of pathological conditions and in the immune response.

Unlike the other heat shock genes, one member of the heat shock responsive gene family in *Drosophila melanogaster* has evoked considerable interest due to its non-protein coding and other unique inducible properties. This is the *93D* or the *hsrw* locus which my laboratory has been studying for a long time. Some of the salient features of this enigmatic locus will be briefly reviewed here (for more detailed reviews, see Lakhotia 1987, 1989; Lakhotia and Sharma 1996).

Inducibility of 93D

The *93D* locus, located in the 93D6-7 bands of the cytological maps of polytene chromosomes of *D melanogaster*, is one of the major puffs induced by heat shock (HS), carbon dioxide, recovery from anoxia, 2-4-dinitrophenol, arsenic compounds,

etc. (see Ashburner and Bonner 1979; Lakhotia 1987 1989). However, brief in vitro treatment of late third instar larval salivary glands of *D. melanogaster* with benzamide (BM) or colchicine (COL) results in increased ^{3}H-uridine incorporation and puffing only at the 93D region (Lakhotia and Mukherjee 1980, 1984). Thiamphenicol (Behnel et al. 1982) and paracetamol (Srivastava and Bangia 1985) have also been reported to selectively induce the 93D puff. Very recent studies in our laboratory (Tapadia and Lakhotia 1996) have revealed that a number of different amides (3-amino-benzamide, formamide, acetamide and nicotinamide) induce the 93D puff in much the same way as benzamide and colchicine. Since colchicine (or colcemid) is also an amide, it appears that this locus is sensitive to amide treatment. That the different inducers of a puff in the 93D region of polytene chromosomes act on the same genetic locus was confirmed through the use of two small deficiencies, viz., *Df(3R)e^{Gp4}* and *Df(3R)GC14*, respectively, that overlap only in the 93D6-7 band region (Mohler and Pardue 1982, Burma and Lakhotia 1986).

In contrast to the above noted specific activation of the 93D puff by a variety of agents, several studies from our laboratory revealed that under certain conditions of HS, the 93D puff is not only not induced but transcriptionally becomes less active than even in control cells. One set of conditions included application of heat shock in conjunction with another inducer of the 93D puff while the other conditions were those in which heat shock was applied under altered levels of b-alanine (see reviews by Lakhotia 1987, 1989, Lakhotia and Sharma 1996).

The heat shock induced 93D puff specifically accumulates much more of cyclic GMP than the other heat shock puffs (Spruill et al. 1978) and also shows specific binding of HSP83 after heat shock but not benzamide induction (Morcillo et al. 1993). Certain monoclonal antibodies against chromosomal proteins (Dangli et al. 1983) and sera from some patients suffering from ankylosing spondylitis (Lakomek et al. 1984) were shown to bind specifically to heat shock induced 93D site of *D. melanogaster*.

All these observations suggest that this locus is extremely sensitive to a variety of perturbations in the cell. These also point to a complex regulation of the 93D locus. The HS inducibility of this locus is regulated independent of the other HS loci. Our recent studies (Lakhotia and Sharma 1995) have shown a complex pattern of processing and accumulation of the different transcripts at this locus in a treatment-specific manner. The above noted different effects of its other inducers, viz., BM, COL, etc., suggest that these agents exert their influence through pathways other than the HS (see Lakhotia 1989). More direct evidence for these independent paths of regulation of this locus has been obtained in recent studies in our laboratory. A small deletion from 93B6-7 to 93D3-5 bands on polytene chromosome (*Df(3R)e^{P}*), generated during P-element mobilization, has lost BM and COL inducibility of the 93D locus without any effect on its HS inducibility (Tapadia and Lakhotia 1993); it is interesting to note that the distal breakpoint of the *Df(3R)e^{P}* deletion is about 20

kb upstream of the 93D locus (Tapadia and Lakhotia 1996). This suggests that the BM and COL response elements of the 93D locus are likely to be located at least 20 kb upstream of the transcription start point. On the other hand, P-element mediated germline transformation of *D. melanogaster* for assaying promoter activity using the *lacZ* reporter gene revealed that only about 840 bp of upstream sequence of the 93D locus is sufficient for conferring HS inducibility and developmental expression to the reporter gene (Misted and Lakhotia 1995, Lakhotia and Mutsuddi 1996).

A systematic study by Lakhotia and Singh (1982) of BM inducibility of puff/s in polytene chromosomes of different species of *Drosophila* revealed that in every species of *Drosophila,* one member of the HS puff family was also BM inducible (also see Burma and Lakhotia 1984); the same puff was also induced by vitamin B_6 and by COL in other species (Lakhotia and Mukherjee 1984, Burma and Lakhotia 1984). This established that a 93D-like locus is functionally conserved in all species of *Drosophila*. A search for a BM or COL inducible puff in *Anopheles stephensi* and *Chironomus striatipennis* (Nath and Lakhotia 1991) failed to reveal any specific site that responds to these agents. However, one of the HS puffs in *Chironomus thummi* displays a number of properties that are strongly reminiscent of the 93D locus of *D. melanogaster* (Nath and Lakhotia 1991, Morcillo et al. 1993, Morcillo and Diez 1996). It may thus appear that a 93D-like locus is widely conserved. These functionally homologous loci in different species have been named as *hsrw* (*h*eat *s*hock *R*NA-*omega*) loci (Bendena et al. 1989).

Functions of *hsr-omega* loci

Although the strong conservation of the inducible properties of the *hsr-omega* locus in different species of *Drosophila* and the highly conserved design of its genomic organization (Walldorf et al. 1984, Peters et al. 1984, Garbe and Pardue 1986) suggest important roles for this locus in the life of organisms, its inability to code for a typical polypeptide (Lakhotia and Mukherjee 1982, Ryseck et al. 1987, Garbe and Pardue 1986, Fini et al. 1989) and its unusually rapid divergence of base sequence (Peters et al. 1984, Garbe et al. 1986, Hovemannn et al. 1986) make this functional conservation paradoxical.

Puffing and northern analysis studies show that the *hsrw* locus is active during normal embryonic and larval stages (Ashburner 1967, Lakhotia and Singh 1982, Hovemann et al. 1986, Garbe et al. 1986). In situ hybridization and reporter gene assay studies in our laboratory (Mutsuddi and Lakhotia 1995) confirmed a wide-spread expression of 93D locus during normal development; in addition these studies also revealed a very strong expression of 93D transcripts in the prothoracic gland in all larval stages and in ovarian nurse cells.

Zygotes that are nullosomic for the *hsr-omega* locus (*Df(3R)e*^{Gp4}*/Df(3R)GC14* trans-heterozygotes, Mohler and Pardue 1982) are poorly viable since only about 20-25% of such embryos hatch; the few that emerge as adult flies are very weak,

sterile and die shortly. The ovarian development in the rare surviving *hsrw*-nullosomic females is seriously affected so that the nurse and follicle cells are poorly differentiated. The above noted wide expression of this gene in different stages of development and the deleterious effects of its nullosomy suggest important functions for this non-protein coding locus. It is very significant that if the *hsrw*-nullosomics are also made heterozygous for a recessive mutation at the *hsp83* locus, all of them die as embryos: this suggests that the HSP83 has very vital interaction with the *hsrw* locus or its transcripts during normal development (Lakhotia and Ray 1996).

The *hsrw* locus makes three transcripts which are of $\geq$ 12 kb, 1.9 kb and 1.2 kb sizes, respectively. The first two are nuclear while the 1.2 kb transcript is cytoplasmic and is a spliced product of the 1.9 kb species (see Lakhotia 1987, Lakhotia and Sharma 1996 for reviews). The 1.2 kb cytoplasmic transcript has a very short open reading frame (23-27 amino acids) which may be translated but the translated product appears to turnover very rapidly (Fini et al. 1989). Since the levels of the 1.2 kb transcript increase rapidly when protein synthesis is inhibited (Bendena et al. 1989), it has been suggested that one of the important functions of this RNA may be to monitor the "health" of the translational machinery (Lakhotia and Sharma 1996). Recent studies (Lakhotia and Sharma 1995, Sharma and Lakhotia 1995) in our laboratory have also shown that perturbations at the 93D puff site during heat shock conditions affect the synthesis and/or turnover of Hsp70 and the heat shock inducible $\alpha\beta$ repeat transcripts at the 87A and 87C puff sites. In view of these results, it has been suggested that the larger nuclear Hsrw transcript may have roles in regulating turnover/transport of other transcripts like the Hsp70. It is also interesting to note that the 1.9 kb transcript is processed at the site of synthesis and the spliced out intron (~700 bp) is relatively stable (Lakhotia and Sharma 1995). Significance of the stability and functions, if any, of this intronic RNA are not known.

Future prospects

Studies on the *hsr-omega* locus unravelled the existence of "genes" that function without a conventional translational product. In recent years, an increasing number of genes with non-coding transcripts have been known: some notable examples of such genes include the "fertility" genes on the Y-chromosome of *Drosophila* and the more recently discovered *Xist*, *H19* and other genes in mammals (for a review, see Lakhotia 1996). These genes have many properties, viz., rapid sequence divergence and very large intra-nuclear transcripts without any coding function, that remind of the *hsr-omega* loci. Although the specific ways in which the "fertility" genes of *Drosophila* or the *Xist* gene in mammals function are not known, but it is likely that their functions depend upon interactions with other macromolecular structures in the cell. Rapid sequence divergence in all these cases suggests that their functions are dependent not upon their precise base sequence but on the higher order structures that these transcripts attain.

Since the *hsrw* locus is highly conserved in the genus *Drosophila* and possibly in other insects like the *Chironomus*, it is tempting to speculate that genes with functional similarities to the *hsrw* may be present in other taxa as well and an active search will be rewarding.

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