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The 93D (*hsr-omega*) locus of *Drosophila:* non-coding gene with house-keeping functions

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Abstract

The 93D, or *hsr-omega* (heat-shock RNA-omega), locus of *Drosophila melanogaster* and other species of *Drosophila*, besides being induced as a member of the heat shock gene family, is also selectively and singularly inducible by a variety of agents, notably benzamide, colchicine and vitamin B_6 (in species other than *D. melanogaster*). The genomic structure of this locus is highly conserved in all species, although the primary base sequence has diverged rapidly between species. Three transcripts (two nuclear and one cytoplasmic) are produced by this locus but none of them has any significant protein coding capacity. The profile of the three transcripts varies in a developmental and inducer-specific manner. This locus is developmentally active in nearly all cell types and is essential for viability of flies. Its induction during heat shock is independent of the other members of the heat shock gene family. The other selective inducers act on this locus through separate response elements. *hsr-omega* activity has a characterstic effect on transcription/turnover of the heat shock induced hsp70 and the alpha-beta transcripts in *D. melanogaster*. It appears that the *hsr-omega* locus has important house-keeping functions in transport and turnover of some transcripts and in monitoring the 'health' of the translational machinery of the cell.

Introduction

Polytene chromosomes of dipteran larvae have contributed immensely to our understanding of gene expression and regulation in eukaryotes. They have been used not only for analyzing the developmental programming of gene activity, but through experimental manipulations also for understanding the steps involved in activation of genes, the act of transcription and subsequent processing and turnover of the nascent transcript. Such studies at the cytological level in the '60s and '70s in fact laid the foundation for much of the subsequent molecular studies on gene expression and regulation. The experimental modulation of specific gene activity in polytene chromosomes, visible under the microscope as induction or regression of specific puffs, has been a powerful tool and has permitted establishment of a number of paradigms for global studies in different pro- and eukaryotes. A most notable example is the discovery of heat shock puffs in polytene chromosomes of *Drosophila* species by Ritossa in early '60s (Ritossa, 1962). One of the heat shock loci in *Drosophila melanogaster* which has evoked considerable interest due to its other unique inducible properties is the 93D puff. That the 93D locus has some interesting properties was first noticed by Lakhotia and Mukherjee (1970). In connection with the doctoral dissertation on transcriptional activity of the X-chromosome in relation to dosage compensation (Mukherjee & Beermann, 1965), Lakhotia (1970) used benzamide (Jacob, Birnsteil & Sirlin, 1964) to specifically inhibit chromosomal transcription in polytene chromosomes of *D. melanogaster*. As expected, benzamide did inhibit most of the chromosomal transcription without much effect on nucleolar activity. However, a totally unexpected but very interesting effect of benzamide treatment on the 93D region of the polytene chromosomes was also noted: the ^{3}H-uridine incorporation at the

93D site was increased several fold following the benzamide treatment of glands, in spite of the severe inhibition of the rest of the chromosomal transcriptions (Lakhotia & Mukherjee, 1970; Lakhotia, 1971). It may be mentioned that in the beginning of the seventies a direct correlation between puffs and specific protein products was not yet established. Therefore, in those days this singular and specific induction of the 93D puff by benzamide appeared to be a very convenient and promising system for exploring the relationship between puffing, transcription and the protein product. Our laboratory thus undertook a long-term study of this locus in detail (Lakhotia, 1971). Although the subsequent studies failed to fulfil this initial objective, these have helped unravel the existence of a different class of eukaryotic genes which apparently work without a typical translational product. Subsequent studies (Bonner & Pardue, 1976; Mukherjee & Lakhotia, 1979; Lakhotia & Mukherjee, 1980) which followed the brief report by Lakhotia & Mukherjee (1970) on the effect of benzamide on 93D inducibility made it clear that activity of 93D was regulated independently of the other heat shock loci. This and its other unusual and intriguing properties have made this locus increasingly interesting. The present review aims to provide the status of our current understanding of this enigmatic gene locus.

Inducibility of 93D

The 93D locus, so named because of its location in the 93D region of the cytological maps of polytene chromosomes of *D. melanogaster* (Bridges, 1935), is one of the several loci that are induced as puffs by heat shock (HS), carbon dioxide, recovery from anoxia, 2-4-dinitrophenol, arsenic compounds, etc. (see Ashburner & Bonner, 1979; Lakhotia, 1987, 1989). However, brief *in vitro* treatment of salivary glands of late third instar larvae of *D. melanogaster* with benzamide (BM) or colchicine (COL) results in increased ^{3}H-uridine incorporation and puffing only at the 93D region (Lakhotia & Mukherjee, 1980, 1984). These observations made it clear that not all the heat shock loci in *D. melanogaster* were co-ordinately regulated. Bonner and Pardue (1976) found that upon heat shock in aged Graces' medium, the inducibility of 93D was much higher than that of the other heat shock loci in larval tissues like salivary glands, imaginal disks, etc. Mukherjee and Lakhotia (1979) found that even during a routine heat shock, the level of incorporation of ^{3}H-uridine at the 93D puff varied, independent of the incorporation at the other heat shock loci, which were active in more or less co-ordinated fashion. Compton and McCarthy (1978), using an *in vitro* system, observed that the 93D was relatively less puffed than the other heat shock sites when isolated polytene nuclei were incubated in the cytoplasm extracted from heat-shocked Kc cells. On the other hand, Mukherjee and Lakhotia (1981, see also Singh & Lakhotia, 1984) found that incubation of intact salivary glands in the homogenate of heat shocked salivary glands or brain ganglia resulted in an enhanced incorporation of ^{3}H-uridine only at the 93D puff site, while the other HS loci remained unaffected. Thiamphenicol (Behnel, 1982) and paracetamol (Srivastava & Bangia, 1985) have also been reported to selectively induce the 93D puff. Ghosh and Mukherjee (1988) reported that the level of induction of the 93D locus by BM treatment was affected by salt concentration in the medium. 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 overlapping' deficiencies (*Df(3R)e*^{Gp4} and *Df(3R)GC14*, for details see Lindslay & Zimm, 1992) that overlap only in the 93D6-7 band region (Mohler & Pardue, 1982, 1984; Burma & 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 becomes transcriptionally less active than even in control cells. The first such situation was noted by Lakhotia and Mukherjee (1980) when salivary glands of late third instar larvae of *D. melanogaster* were exposed to combinations of BM and HS treatments; although individually either of these treatments leads to a strong transcriptional induction of the 93D puff, when applied in combination the 93D site was actually inhibited, although the other heat shock puffs were induced (Lakhotia & Mukherjee, 1980). A comparable inhibition of the 93D site was seen when salivary glands were allowed to recover from anoxia at 37°C rather than at 24°C (Mukherjee & Lakhotia, 1982). Likewise, COL and HS treatments together resulted in regression of the 93D puff while the other HS puffs were induced (Lakhotia & Mukherjee, 1984). When *D. melanogaster* larvae were reared at 10°C and their salivary glands subsequently exposed to 24°C, the 93D site, but not the other HS loci, showed increased ^{3}H-uridine incorporation; interestingly, when salivary glands from 10°C grown larvae were heat shocked

at 37°C the 93D site regressed, although the other HS puffs were induced (Lakhotia & Singh, 1985). A common fact emerging from these results is that heat shock in combination with other inducers of 93D results in non-induction or regression of the 93D site. More recently, while examining position effect variegation at the 93D locus (see Lakhotia, 1989; Lakhotia, Karchowhuri & Burma, 1990), an intriguing influence of the levels of beta-alanine on HS inducibility of the 93D site was discovered in our laboratory. These studies were initiated with a rare chromosome rearrangement, *T(1;3)e*^{H2} (Henikoff, 1980), that causes position effect variegation at the *ebony* locus. Since the *ebony* locus is very close and proximal to the 93D heat shock locus, it was expected that the 93D site would also variegate in this chromosome. During the course of these studies (Lakhotia, Kar Chowdhuri & Burma, 1990), an unexpected revelation was that the 93D site in salivary glands of larvae hetero- or homozygous for a mutant allele at the *ebony* locus failed to respond to HS although its inducibility by BM or COL was not affected. Since a mutation at the *ebony* locus results in elevated levels of beta-alanine (see Wright, 1987), the effect of excess beta-alanine on the HS inducibility of the 93D locus in wild type salivary glands was also examined; as expected, the 93D locus failed to respond to HS in this case too (Lakhotia, Kar Chowdhuri & Burma, 1990). Interestingly, a mutation at the *black* locus, which leads to reduction in beta-alanine levels (see Wright, 1987), also caused the 93D locus to be refractory to HS induction although it continued to be selectively inducible with BM or COL (Lakhotia, Kar Chowhuri & Burma, 1990).

Ghosh and Mukherjee (1990) reported an effect of trisomy of either the left arm of chromosome 2 (T2L) or of the left arm of chromosome 3 (T3L) on puffing at the 93D locus. The 93D locus was transcriptionally more active in the polytene nuclei of T2L or T3L salivary glands at 22°C than in wild type glands, but a heat shock to salivary glands of T2L or T3L larvae actually caused a slight inhibition of ^{3}H-uridine uptake at the 93D site. In view of the above-noted effects of beta-alanine, it is likely that the non-inducibility of the 93D locus by heat shock in such trisomics is related to altered beta-alanine levels in these larvae due either to a mutant allele at the *ebony* locus, in the case of T3L genotype, or to the three copies of *black*$^{+}$ locus in the T2L genotype.

An interesting consequence of a deletion of the 93D site on one homolog on the wild type copy on the other homolog was also noted: the single copy of the 93D site in deletion heterozygotes (*Df(3R)e*^{gp4}/+) or Df(3R)GC14/+) was transcriptionally as active as the two copies in wild type cells (Burma & Lakhotia, 1986). Intriguingly, the above-noted effect of a mutant allele at the *ebony* locus on HS inducibility of the 93D puff was not seen in deletion heterozygotes carrying only one copy of the 93D locus (unpublished observations in our laboratory; see also Mohler & Pardue, 1982, 1984).

The heat shock induced 93D puff accumulates much more cyclic GMP than the other heat shock puffs (Spruill *et al.* 1978). Dangli *et al.* (1983) reported monoclonal antibodies against chromosomal proteins that bind specifically to the 93D site of *D. melanogaster*. Lakomek *et al.* (1984) also reported specific binding of sera from patients suffering from ankylosing spondylitis with the heat shock induced 93D puff. Morcillo *et al.* (1993) found a specific association of the 83kD heat shock protein (hsp83) with the heat shock induced 93D puff, but not with the BM induced puff.

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 independently of the other HS loci. Vazquez, Pauli and Tissieres (1993) reported the kinetics of turnover of the 93D transcripts to be different than those of the other HS loci in *D. melanogaster*. Our own recent studies (Lakhotia & Sharma, 1995) have shown a complex pattern of processing and accumulation of the different transcripts at this locus in a treatment-specific manner (see below). Another intriguing feature is the near absence of active heat shock transcription factor on this locus after HS (Westwood, Clos & Wu, 1991). It appears that the absence of active HSTF on the 93D locus after a 25 min heat shock (Westwood, Clos & Wu, 1991) is related to the fact that the 93D locus is the first to be induced by heat shock and also the first to regress: thus by 25 min, the HSTF may already have lost its binding with the 93D locus. The above-noted different effects of its other inducers (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 & Lakhotia, 1993); it is interesting to note that the distal breakpoint of the *Df(3R)e*^{P}

deletion is at least 15–20 kb upstream of the 93D locus (unpublished). This suggests that the BM and COL response elements of the 93D locus are likely to be located at least 15–20 kb upstream of the transcription start point. P-element mediated germline transformation of *D. melanogaster* for assaying promoter activity using the *lacZ* reporter gene (Thummel, Boulet & Lipshitz, 1988) revealed that a sequence about 900bp upstream of the 93D locus includes promoters for HS inducibility and developmental expression of the 93D but not for the BM or COL inducibility (Mutsuddi & Lakhotia, 1994). It is likely that BM and COL also act on this locus through different upstream regulatory sites since they result in different profiles of transcripts of this locus (see later).

Conservation of the 93D locus in the genus *Drosophila*

A specific and singular induction of one of the heat shock puffs in polytene nuclei of *D. hydei* by vitamin B_6 was reported by Leenders *et al.* (1973). Since vitamin B_6 did not induce any puffs in polytene nuclei of *D. melanogaster* (see Ashburner & Bonner, 1979), the 2-48C puff was believed to be a unique feature of the *D. hydei* and other related species (Peters, Lubsen & Sondermeijer, 1980). However, a systematic study by Lakhotia and Singh (1982) of BM inducibility of puffs in polytene chromosomes of different species of *Drosophila* revealed that every species of *Drosophila* that was examined had a BM inducible puff which was also a member of the HS puff family (see also Burma & Lakhotia, 1984). The same puff was also induced in all species except *D. melanogaster* by vitamin B_6, and this puff in all the species was closely linked to the *ebony* locus; this same puff was also specifically inducible by COL in different species (Gubenko & Baricheva, 1979; Lakhotia & Mukherjee, 1984; Burma & 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 striatipennins* (Nath & 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* (Santa-Cruz, Morcillo & Diez, 1984; Carmona *et al.*, 1985; Nath & Lakhotia, 1991; Morcillo *et al.*, 1993). It may thus appear that a 93D-like locus is widely conserved.

Molecular organization of the 93D-like loci

The 93D locus from *D. melanogaster* and the 93D-like loci from *Drosophila hydei* (2-48B) and *D. pseudoobscura* (58C) have been cloned (Walldorf *et al.*, 1984; Garbe & Pardue, 1986; Garbe, Bendena & Pardue, 1986; Garbe, Bendena & Pardue, 1989; Peters *et al.*, 1984). Although the DNA sequences at 93D and equivalent loci in other *Drosophila* species are highly diverged, these loci share a unique molecular design and show small regions of conserved sequences at several places in the transcription unit in different species (see reviews by Lakhotia, 1987, 1989; Bendena *et al.*, 1989b; Pardue *et al.*, 1990; Pardue, Ballinger & Hogan, 1992). The genomic organization of the locus is remarkably similar in all species of *Drosophila* examined so far: the locus spans 15–20 kb with a stretch (10–15 kb) of short tandem repeats on its 3′ end; these short repeats are unique to this locus; the 5′ end of the transcription unit is comprised of two exons and an intron whose sizes are fairly constant in different species. The 93D locus of *D. melanogaster* and the 93D-like loci in other species were designated *hsr-omega* (*H*eat *S*hock *R*NA-*omega*; Bendena *et al.*, 1989a). In all species, the locus produces three transcripts: i) a large (>10 kb) transcript corresponding to the entire gene including the stretch of tandem repeats on the 3′ end; ii) a 1.9 to 2.0 kb transcript corresponding to the two exons and an intron on the 5′ end; and iii) a 1.2 kb transcript resulting from splicing of the 1.9 kb RNA. All three transcripts start at the same position but the larger (>10 kb) transcript is not a precursor to the 2.0 kb transcript, which results from an alternative termination point (Garbe & Pardue, 1986; Hovemann, Walldorf & Ryseck, 1986; Ryseck *et al.*, 1987). The first two transcripts are nuclear while the 1.2 kb RNA is seen in the cytoplasm, mostly associated with mono- and disomes (Fini, Bendena & Pardue, 1989). The three transcripts were initially (Bendena *et al.*, 1989a) designated as *hsr-omega1*, *hsr-omega2* and *hsr-omega3*, respectively, but more recently have been renamed (Hogan *et al.*, 1994) as *hsr-omega-n* (*n*uclear), *hsr-omega-pre-c* (*pre*cursor of the *c*ytoplasmic transcript) and *hsr-omega-c* (cytoplasmic) to indicate their location and precursor-product relationship. None of these transcripts have any appreciable open reading frames except the *omega-c* which carries, at a comparable position in different species, a short open reading frame (ORF-omega) coding for 23–29 amino acid residues (Fini, Bendena & Pardue, 1989); indirect evidence suggests that this ORF is translated (Fini, Bendena

& Pardue, 1989) and perhaps the translational product turns over very rapidly. Surprisingly, the amino acids coded by this ORF are not conserved in different species (Fini, Bendena & Pardue, 1989).

Functions of *hsr-omega* loci

The strong conservation of the common inducible properties of the *hsr-omega* locus in different species of *Drosophila* and the highly conserved design of its genomic organization suggests important roles for this locus in the life of organisms. However, the fact that this locus does not code for any typical polypeptide (Lakhotia & Mukherjee, 1982; Ryseck *et al.*, 1987; Garbe & Pardue, 1986; Fini, Bendena & Pardue, 1989) and the unusually rapid divergence of base sequence (Peters, Lubsen & Sondermeijer, 1980; Peters *et al.*, Bendena & Pardue, 1984; Garbe, 1986; Hovemann, 1986) at this heat shock locus make this functional conservation paradoxical. A clear function for this locus is still not known. However, an analysis of its activity in normal development and under the various stress conditions may indicate the nature of its role in the cell.

Activity of *hsr-omega* in normal development

The 93D and its homologs in other species are known to puff during the late third instar stage soon after the release of ecdysone (Ashburner, 1967; Lakhotia & Singh, 1982). Bonner and Pardue (1976) noted that the RNA extracted from imaginal disks of late third instar larvae was rich in 93D transcripts. Hovemann, Walldorf and Ryseck (1986) and Garbe, Bendena and Pardue (1986) showed by Northern analysis that the different *hsr-omega* transcripts were detectable during normal embryonic and larval stages. Bendena *et al.* (1991) used *in situ* hybridization to cellular RNA to detect hsr-omega transcripts in non-stressed cells at various stages of development of *D. melanogaster*: except for the pre-blastoderm stage embryos and the primary spermatocytes, these transcripts were detectable in all cell types examined. Recent research in our laboratory (Mutsuddi & Lakhotia, 1994) using *in situ* hybridization as well as a reporter gene assay confirmed a widespread 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. The *hsr-omega* transcripts are also present in cultured Kc cells and the level of these transcripts increases following ecdysone exposure (Bendena *et al.*, 1991). In salivary glands of late third instar larvae of *D. melanogaster*, the 93D puffing parallels the increase in ecdysone titre and this locus develops a puff earlier than the 67B site which harbors the genes for the low molecular weight heat shock protein family (Corces *et al.*, 1980) and which is also ecdysone inducible. However, it is not known if the response of the *hsr-omega* locus to ecdysone is primary or secondary.

Mohler and Pardue (1982) found that *Df(3R)e*^{gp4}*/Df(3R)GC14* trans-heterozygotes that lacked the 93D6-7 band (the site of the *hsr-omega* locus) on both homologs were very poorly viable; only 20–25% of such embryos hatched, the majority of which failed to pupate, and of those that did nearly all failed to develop further. The few that emerged as adults were very weak and died shortly. An unpublished study in our laboratory (M. Sandhu and Lakhotia, see Lakhotia, 1987) showed that the imaginal disks from late third instar larvae that lacked the *hsr-omega* locus in both homologs (*Df(3R)e*^{gp4}*/Df(3R)GC14*) failed to respond to ecdysone *in vitro*. Although these studies do not reveal the specific role of transcripts of this locus in a cell, it is likely, especially in view of the wide-spread activity in nearly all cell types of *Drosophila*, that the *hsr-omega* locus has important house-keeping functions. The pattern of lethality of the *Df(3R)e*^{gp4}*/Df(3R)GC14* trans-heterozygotes suggests that the house-keeping function of this locus is critical at certain specific stages of development and if the organism is able to escape one critical stage, it can continue to develop until the next critical stage is encountered. It is known that for several functions there are redundant genetic pathways, so that mutation in one gene may not necessarily give rise to a distinct phenotype (Brookfield, 1992). Such redundancy may explain why some of the *hsr-omega* deficient individuals escape a critical lethal phase and this could also be a major cause for the failure to identify specific functions for the *hsr-omega* locus.

A preliminary study in our laboratory, which appeared to suggest an effect of 93D deficiency on abdominal segmentation in embryos (Lakhotia, 1990), turned out to be an effect of the *hairy* gene mutation associated with the deficiency chromosome.

Mode of action of the various inducers of *hsr-omega*

Some idea of the function of the *hsr-omega* locus could possibly be obtained from a knowledge of how the various inducers of this locus affect the cell. However, there appears to be little in common between the various inducers (e.g., heat shock, benzamide, colchicine and vitamin-B_6) of the *hsr-omega* puffs. Benzamide is known to 1) delay or prevent pupation in *Drosophila* larvae by affecting ring gland metabolism (Abd-el-Wahab & Sirlin, 1959); 2) inhibit chromosomal but not nucleolar transcription in polytene nuclei (Jacob, Birnsteil & Sirlin, 1964; Lakhotia & Mukherjee, 1970); 3) affect the mitotic spindle functions and to act as a clastogenic agent in mammalian cells (Babu, Shah & Lakhotia, 1980); and 4) block poly-ADP-ribosylation in mammalian and other cells (see Cleaver, Milam & Morgan, 1985). Obviously, the above list of actions of benzamide fails to provide a common denominator for understanding the basis of its induction of 93D. Effect of benzamide on poly-ADP-ribosylation may look important, but a more potent inhibitor of poly-ADP-ribosylation, 3-aminobenzamide, did not induce 93D, although its effect on chromosomal transcription was comparable to that of benzamide (unpublished). The effect of BM on RNA metabolism in ring gland (Abd-el-Wahab & Sirlin, 1959) is interesting in view of the recent observation in our laboratory (Mutsuddi & Lakhotia, 1994) that the *hsr-omega* transcripts are highly abundant in prothoracic gland of *D. melongaster* larvae. This needs to be examined further.

The best known effect of colchicine is on polymerization of microtubules (Dustin, 1978). However, a variety of other microtubule poisons failed to induce the 93D puff in polytene nuclei (Singh & Lakhotia, 1984). Nevertheless, some relationship between cytoskeleton and *hsr-omega* activity appears likely in view of the fact that all the inducers of this locus, BM, COL and HS, have some effect on the cytoskeleton.

Vitamin-B_6 is a specific inducer of *hsr-omega* loci in other *Drosophila* species but the basis for its action is not clear. A correlation between increased tyrosine-amino-transferase (TAT) activity and induction of the 2-48C (*hsr-omega*) locus by vitamin B_6 in *D. hydei* is not known (Belew & Brady, 1981). However, since the *hsr-omega* locus is not a structural gene for TAT, the basis for this correlation is not understood.

Heat shock has a variety of cellular effects, including those on cellular transcription, translation and native protein structure (Nover, 1984; Schleisinger 1990). The role of the *hsr-omega* locus in heat shock response is not clear, since even in the absence of any copy of the 93D locus, the synthesis of the different hsps is not affected (Mohler & Pardue, 1982, 1984). Nevertheless, the 93D-deficient larvae are poorer in thermo-adaptation (see Lakhotia, 1987).

A common effect shared by all the inducers of *hsr-omega* is the inhibition of general chromosomal, but not nucleolar, transcription. This suggests that activity of the *hsr-omega* locus may be related to general transcriptional activity in the cell (see below).

A connection between *hsr-omega* activity and the translational machinery is suggested by the rapid accumulation, without new transcription, of the 1.2 kb transcript (*omega-c*) following inhibition of protein synthesis (Bendena *et al.*, 1989a). Since the 1.2 kb *omega-c* transcript associates with mono- and disomes, and its short ORF may be translated (Fini, Bendena & Pardue, 1989), it is suggested that inhibition of protein synthesis also stops the normal rapid turnover of this transcript, which results in its accumulation. It is likely that the purpose of the translation of *omega-c* is not to produce any functionally active protein but to link the process of protein synthesis in the cell with turnover of *omega-c* (Lakhotia, 1990; Pardue, Ballinger & Hogan, 1992) and the act of translation of *omega-c* allows the cell to monitor the rate of protein synthesis in cell at any particular time.

hsr-omega transcripts following various treatments

Northern analysis by Bendena *et al.* (1989a) revealed that each of the different inducers of the 93D locus of *D. melanogaster* causes a different profile of the three *hsr-omega* transcripts in cell: HS increases levels of all the three transcripts, with the ratios between the three remaining the same as in control cells. BM and COL treatments, on the other hand, lead to a greater increase of the larger and nuclear *omega-n* transcript. However, these two treatments differ in their effects on the two smaller (omega-pre-c and *omega-c*) transcripts. We have recently quantified, *in situ*, the levels of the three *hsr-omega* transcripts at the 93D site on polytene chromosomes by *in situ* hybridization of anti-sense RNA probes to polytene chromosomes in variously treated larval salivary glands (Lakhotia & Sharma, 1995). These studies, in addition to confirming the above-noted inducer-specific profile of the various *hsr-omega* transcripts, revealed additional inter-

esting insights into the RNA metabolism at this site. Firstly, it was found that the splicing of *omega-pre-c* RNA takes place at the site of transcription itself and while *omega-c* is transported away, the spliced intron (termed *omega-free-intron, omega-fi*) accumulates at the 93D site. It is interesting to note that the rate of splicing of the *omega-pre-c* and, therefore, the level of *omega-fi* at the site varies in relation to the treatment applied to salivary glands (Lakhotia & Sharma, 1995). Although a combined treatment to salivary glands with HS and BM or HS and COL is known to inhibit puffing and ^{3}H-uridine incorporation at 93D (see above), these combination treatments resulted in a treatment-specific increase in *in situ* levels of the different *hsr-omega* transcripts at the 93D site (Lakhotia & Sharma, 1995). Since the increase in the *in situ* levels of these transcripts occurred in the absence of fresh transcription (little or no ^{3}H-uridine uptake at the site), the HS-BM or HS-COL combination treatments appear to reduce turnover of the transcripts from the 93D site. Considering the effects of HS, BM or COL treatments on the cellular transcriptional and translational activities and the effect of these treatments on the relative levels of the various *hsr-omega* transcripts, it has been suggested (Lakhotia & Sharma, 1995) that while the cytoplasmic *omega-c* RNA has a role in monitoring translational activities, the larger and nuclear localized *omega-n* transcript is involved in general transcription, RNA processing and/or turnover/transport of RNA.

Interaction of the 93D locus with the 87A and 87C heat shock puffs in *Drosophila melanogaster*

A series of earlier studies from this laboratory has shown that whenever the 93D site fails to puff in response to HS, the HS induced puffs at the 87A and 87C sites behave atypically (see Lakhotia, 1987, 1989, for review of earlier studies and also Lakhotia, Kar Chowdhuri & Burma, 1990; Ghosh & Mukherjee, 1990). During a typical heat shock to salivary glands, the 87A and 87C sites form equal-sized large puffs which also show nearly equal ^{3}H-uridine uptake (Ashburner, 1967; Mukherjee & Lakhotia, 1979). However, whenever, the 93D site fails to puff during HS, the 87A and the 87C sites puff unequally and incorporate ^{3}H-uridine to varying levels; it is interesting that depending upon the specific condition of HS, the 87A or the 87C puff is the larger of the two in a highly reproducible manner (Lakhotia, 1987, 1989; Lakhotia, Kar Chowduri & Burma, 1990). Genes coding for the hsp70 occur in multiple copies at the 87A (2 copies) and 87C (3 copies) loci in *D. melanogaster* (for review see Nover, 1984). The 87C site, in addition, also carries several tandemly repeated copies of heat inducible 'alpha-beta' sequences, which, like the 93D locus, do not seem to code for any protein (Lengyel & Graham, 1984). Using inter-specific hybrids between *D. melanogaster* and *D. simulans*, Kar Chowdhuri and Lakhotia (1986) showed that the non-induction of 93D by HS-BM or HS-COL combination treatment did not affect the 87A and 87C puffs on the *D. simulans* homolog, and this was correlated with the absence of the alpha-beta repeats at the 87C site of *D. simulans*. With a view to more specifically understanding the basis for such unequal puffing, Sharma and Lakhotia (1995) examined the *in situ* hybridization of hsp70 or alpha-beta anti-sense RNA probes to transcripts located at these two sites in polytene chromosomes. HS followed by BM treatment, which results in a larger puff at the 87A, caused an increase in the hsp70 transcripts per gene copy at 87A and a decrease at the 87C site without affecting the alpha-beta transcript level; HS in presence of COL, which causes the 87C puff to be larger than 87A, resulted in a decrease in the level of hsp70 RNA at the 87A site but an increase in the levels of hsp70 as well as the alpha-beta transcripts at the 87C site (Sharma & Lakhotia, 1995). Since these changes in the profiles of hsp70 and alpha-beta transcripts at the 87A and 87C sites occurred in relation to specific changes in the synthesis/turnover of the different transcripts at the 93D site, it has been suggested (Sharma & Lakhotia, 1995) that *hsr-omega* transcripts influence RNA metabolism at the 87A and 87C sites. Although the 5 copies of hsp70 genes located at the 87A and 87C sites produce nearly identical hsp70, it is possible that the differences in their 3′ untranslated transcribed regions (UTR) cause the mRNAs produced by the different hsp70 genes to localize in specific cellular compartments; subtle effects of the *hsr-omega* transcripts on synthesis/turnover of the hsp70 transcripts with different 3′ UTRs could be an important aspect on cellular response to different stress conditions (Sharma & Lakhotia, 1995). In recent years, it has become increasingly clear that the cytoskeleton has a major role in intra-cellular transport and targeting of mRNA (Singer, 1992). Since the activity of *hsr-omega* is affected by agents that act on some components of the cytoskeleton (see above), an involvement of this locus in RNA transport and turnover appears likely.

Future prospects

Studies on the *hsr-omega* locus unravelled the existence of 'genes' that function without a conventional translational product. Some more examples of genes with similar properties have been known in recent years. The 'fertility' genes on the Y-chromosome of *Drosophila*, particularly those that form the so-called 'lampbrush loop' in primary spermatocytes (Hennig *et al.*, 1989) and the recently discovered 'Xist' gene in mammals, involved in inactivation of one of the X-chromosomes in somatic cells of females (see recent reviews in Rastan, 1994; Migeon, 1994), have many properties, such as rapid sequence divergence and very large intra-nuclear transcripts without any coding function, reminiscent 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, it is obvious that their functions depend upon their interaction 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. This will be an interesting aspect for further studies. The unusual stability and *in situ* persistence of the spliced out intron (*omega-fi*) also needs to be examined further for significance.

As noted above, recent studies on the *hsr-omega* locus in *D. melanogaster* suggest a role in monitoring the transcriptional and translational activities in a cell; in addition, this locus also appears to be involved in modulating transcription and/or transport of other RNAs, like the hsp70 and the alpha-beta transcripts. A possible connection of this locus with the cytoskeleton organization in the cell may also be viewed in light of transport/turnover of other transcripts since it is clear that components of cytoskeleton have important roles in this process. In this context, it is also notable that while in large polytene cells of the larval salivary glands, the *hsr-omega* locus becomes transcriptionally induced within a short time of BM or COL treatment (Lakhotia & Mukherjee, 1980, 1984), in the mitotically dividing cultured cells, a much longer treatment with these agents is required to detect the induced *omega* transcripts (Bendena *et al.*, 1989a). While in polytene cells, the BM or COL treatment severely inhibits chromosomal transcription, the same does not happen in mitotically dividing cells (unpublished observations). The reason for these differences in polytene and mitotic cells are not clear but may be related to the necessary differences in their architecture due to grossly different cell sizes. A very large sized cell (like the polytene cells in larval salivary glands) will have to have different strategies for intra-cellular transport of macromolecules and other smaller molecules than a smaller cell. The cytoskeleton organization is also expected to be different in these cells. It will be very useful to examine the possible connection of the *hsr-omega* locus with the cytoskeleton, and of both of these with intra-cellular transport/turnover of RNA. It is expected that within the next few years, the mode of action of genes like *hsr-omega*, *Xist* and the 'fertility' genes of *Drosophila*, which work without a translational product, will be understood.

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