Journey from extra-nuclear DNA to non-coding transcripts

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Extra-nuclear DNA in a Myxosporidian parasite (1966-67)

Significance?

Learn GENETICS

Dosage compensation in Drosophila

First encounter with “Fly Genetics”

Dosage compensation in Drosophila is cell autonomous (1969)

"In a very original study, Lakhotia and Mukherjee (1969) show that the hyperactivity of the male X is cell autonomous." (M. Ashburner, 1972)

The single X chromosome in polytene cells of male Drosophila completes replication earlier than autosomes (1970)

Further research interests originating from studies on dosage compensation in Drosophila

1. Replication in Drosophila chromosomes
2. Heat shock response in Drosophila and other insects
3. Non-coding hsr-omega gene in Drosophila
Replication in Drosophila chromosomes

This stemmed from the observations on transcription and replication patterns in X chromosome of male and female Drosophila

Dr. Mahesh Kumar
Dr. Sabita Roy
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Dr. Pradip Sinha
Dr. Arati Mishra
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Dr. Sujata Roy

Temporal order of replication of specific bands in Drosophila cells is affected by specific developmental conditions

Cell division cycles in brain ganglia show certain unusual features including independent replication cycles in hetero- and eu-chromatin regions in mitotic cells (1978-1995)

DNA-Fibre autoradiography established existence of at least two kinds of active replications in Drosophila cells (1983)

Heterochromatin in Drosophila

- Active and inactive chromatin regions replicate at different times
- Heterochromatin content varies in different species
- Review on heterochromatin

A Rat, Rattus blanfordi, has unusual chromosomes and abundant centromeric and sex-chromosomal heterochromatin

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Contrary to common belief, heterochromatin in *Drosophila* is transcriptionally active (1973-74)

Heterochromatin in *Drosophila nasuta* is comprised of asymmetric A-T rich sequences (1979)

Heterochromatin in *Drosophila melanogaster* species group differs in repetitive sequences (1980)

Heat shock response in *Drosophila*

Interest originated from the observation in 1969 that the benzamide-inducible 93D puff was a member of heat shock gene family

The RNA synthesis and turnover of hsp70 and αβ transcripts (non-coding) at the 87A and 87C heat shock puffs is affected by the state of activity of the hura or the 93D gene (1980-1995)
Heat shock induces the hsp70 genes of 87A and 87C clusters very differently in different cell types (2002)

Inducibility of the different heat shock genes in Chironomus varies in summer and winter months in relation to the ambient temperature (1989)

The Malpighian tubules of Drosophila respond to heat shock in a manner very different from the other tissues (1989)

The heat shock induced 64 kDa polypeptide in Malpighian tubules is a member of the Hsp60 family (1998)

Malpighian tubule cells display a complex pattern of transcription, translation and stability of hsp70 and hsp64 transcripts following heat shock and during recovery (2002)

Non-coding 93D or hsrω gene in Drosophila

Inhibition of chromosomal transcription with benzamide in salivary glands revealed unique and singular induction of the 93D puff (1969)
Could the 93D puff provide a good model system to examine the relation between puffs, gene activity and proteins?

In the early 1970s, this relationship was still not well established.

The 93D puff is singularly induced by benzamide (1970, 1980).


A homologue of the 93D puff is present in all species of Drosophila (1982).

The 93D puff in Drosophila is unlikely to code for a protein (1982).

Heat shock induced activity of the 93D puff is not coordinated with the other heat shock puffs (1979).

Transgenic flies used for analysing promoter of the hsp 70 gene (1995).

Spatial Expression of the hsp 70 (hsp 70) Gene in Different Tissues of Drosophila melanogaster and Identification of Promoter Elements Controlling Its Developmental Expression

Research Laboratory of Genetics, Department of Zoology, Boston University, Boston, Mass.

A novel translation product in relation to induced activity of the 93D puff in Drosophila melanogaster

S.C. Lakhota and T. Mukherjee

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The amide-response element on the hsr_ω gene is more than 21kb upstream of the transcription start point (Promoter mapping using chromosomal deletions) (1998)

Does a non-coding gene like the 93D have any function or is it “junk”?

A VARIETY OF hnRNPs AND RELATED NUCLEAR PROTEINS SPECIFICALLY BIND WITH THE 93D PUFF FOLLOWING HEAT SHOCK

Antibodies to the following proteins specifically bind with the 93D puff in heat shocked polytene nuclei of Drosophila melanogaster

- hrb57A (hnRNP A), hrb57F (hnRNP A2), hrb57T (hnRNP K), S5 (hnRNP M), Sxl

Under heat shock condition, all the hnRNPs get progressively sequestered at the hsr_ω gene site

hsr_ω transcripts essential for organizing OMEGA SPECKLES, which regulate the availability of hnRNPs for RNA processing (2000)

Omega species—a novel class of nuclear species containing hnRNPs transcribed within transcribing heat-activated RNA in Drosophila

The non-coding transcripts of hsr_omega gene in Drosophila: Do they regulate trafficking and availability of nuclear RNA-processing factors?

S.C. Lakhota*, Pavel Rejč, T.K. Kojzenta and K.V. Prasad

Under heat shock condition, all the hnRNPs get progressively sequestered at the hsr_ω gene site
hsrω RNA dynamically regulates the availability of hnRNPs in nucleus

The non-coding, developmentally active stress inducible hsrω gene of Drosophila melanogaster integrates post-transcriptional processing of other nuclear transcripts

Sahibul C. Lakhotia

Over-expression of hsrω-n transcripts in cyst cells of 05241 mutant testes excessively sequesters hnRNPs into the inactive compartment (clusters of omega speckles) and this affects processing of a variety of other nuclear transcripts

Compromise in cyst cell function prevents sperm maturation and individualization

(interesting parallel with the RNA-foci in DM1 and DM2 human disorders which sequester CUG-BP, involved in processing of several other transcripts)
MOST TISSUES, OTHER THAN GUT, IN hsr\(^{ww}\) pl10 MUTANT LARVAE ARE SMALLER AND LARVAL LIFE IS PROLONGED

-hsr\(^{ww}\) pl10 LARVAE
  - IMAGINAL DISCS ARE SMALL & DISORGANIZED
  - hsr\(^{ww}\) TRANSCRIPTS CLUSTERED AT THE GENE SITE IN MOST TISSUES
  - hsr\(^{ww}\) IS NOT EXPRESSED IN PROTHORACIC GLANDS OF MUTANT LARVAE
  - MUTANT LARVAE PUPATE AFTER 11-12 DAYS BUT ALL DIE

Sonali Sengupta & Lakhotia, unpublished

ABERRANT EXPRESSION OF hsr\(^{ww}\) IN hsr\(^{ww}\) pl10 IS ASSOCIATED WITH CLUSTERING OF hsr\(^{ww}\) TRANSCRIPTS AND hnRNPs IN NUCLEI

Excessive sequesteration of hnRNPs by hsr\(^{ww}\) transcripts at the hsr\(^{ww}\) gene site (>) and in clusters of omega speckles compromises processing of various mutant transcripts in affected cells, resulting in the various anomalies in pl10 larvae and their ultimate death

Sonali Sengupta & Lakhotia, unpublished

The Sxl Protein in hsr\(^{ww}\) nullisomic egg chambers moves in the oocyte

Confocal images (projection) of Malpighian tubule cells
RED - chromatin
GREEN - hsr\(^{www}\)-n RNA

Distribution of an hnRNP and Actin in wild type and 65241 mutant egg chambers

Distribution of an hnRNP and Actin in wild type and 65241 mutant egg chambers

The Bar gene, besides its role in ommatidial differentiation, acts as a proximal-distal selector gene in antennal and leg disks of Drosophila

S. Mandal & Lakhotia (1999)

Developmental Genetics

Current Science

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