The fourth chromosome: targeting heterochromatin formation in Drosophila

DNA packaging domains

- **Euchromatin**
  - Less condensed
  - Chromosome arms
  - Unique sequences; gene rich
  - Replicated throughout S
  - Recombination during meiosis

- **Heterochromatin**
  - Highly condensed
  - Centromeres and telomeres
  - Repetitious sequences; gene poor
  - Replicated in late S
  - No meiotic recombination

But-
- the banded 1.2 Mb of the dot chromosome contains 82 genes - a normal gene density for the Drosophila chromosome arms
- and a 10-fold higher concentration of repetitious elements
- suggesting interspersed euchromatin and heterochromatin
HP1: a banded pattern on chromosome 4
HP1 distribution is coincident with H3-mK9 but opposed to H4-acK8

\[ T_{-1917} hsp26-plant hsp70-white +490 \]

\( P \) element transposon: variegating phenotype
Chromosome four has interspersed heterochromatic and euchromatic domains

*Many variegating reporters lie within genes.

- This variegation is suppressed by loss of HP1.
Mobilization of the P element

Local transposition

Local deletion

Local duplication

Local Deletions Produce a Switch in Eye Phenotype
Local deletions induce a change in chromatin structure

A shift in histone acetylation correlates with shift in eye phenotype
Heterochromatin vs euchromatic domains

- Heterochromatin
  - HP1 complex
  - Methylated histone H3 tail
- Euchromatin
  - HATs
  - Transcriptional activators
  - Acetylated histone tail

Duplications also cause a switch in eye phenotype
Local duplications change the distance between the $P$ element and 1360 remnants

Variegating $P$ inserts lie within 10 kb of a copy of element 1360
Model: element 1360 (hoppel) as an initiator of heterochromatin

A Model for Targeting Heterochromatin Formation
Conclusions

• The fourth chromosome of *D. melanogaster* is largely heterochromatic
  – But 82 genes in 1.2 Mb- normal gene density
  – Ten-fold higher levels of repetitious sequences

• Incomplete transposition of the *P* element on the fourth chromosome
  – Results in local deletions and duplications
  – Can cause a switch in phenotype
  – Argues against a fixed boundary
  – Supports an equilibrium model
  – Suggests competition between alternative packaging states, summed
    by nucleosome modification
  – Proximity to a 1360 associated with heterochromatin formation

• A role for RNAi?
  - mutations in RNAi machinery impact silencing, levels of H3-mK9
  - observe 22 bp dsRNA from 1360
  - suggests RNAi may target assembly of HP1-associated heterochromatin
Our research goal:
To compare finished sequence from the dot chromosomes of *D. melanogaster* with *D. virilis*
Selection of target genes

- Small (dot) chromosome of many Drosophila species is known to have several of the same genes (Podemski, 2001*)
- Sequencing has recently been completed for *D. pseudoobscura*, a species 25-30 my diverged from *D. melanogaster*
- Spring 2003, Rachel Shevchek did a BLAST comparison using cDNA sequences from all the genes from the dot chromosome of *D. melanogaster* to the genomic sequence of *D. pseudoobscura* from the Baylor website
- She looked for >200bp chunks of genes that were very highly conserved (>80%) and designed PCR primers using Primer3 (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi)
- Library screened summer 2004 by Elmer Kellman & Libby Slawson; identified fosmids used in Bio 4342 in spring 2004.


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In situ hybridizations

- Ten fosmids confirmed by *in situ* hybridization to the polytene chromosomes of *D. virilis*
- Done in the lab of Dr. Mary-Lou Pardue at MIT
- Will not tell us what gene the fosmid contains, but will tell us where some of the DNA from the fosmid is localized

*example *in situ*, M-L Pardue
Comparison of repeat densities in *D. virilis* (Dv) and *D. melanogaster* (Dm)

- **Dv**
  - Simple Repeats
  - DNA Transposons
  - Retrotransposons

- **Dm**
  - Simple Repeats
  - DNA Transposons
  - Retrotransposons

Percentage of Repetitious DNA (%)

- Dot Chromosomes
- Long Arms
Project Participants

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Big Questions

• As we complete more of the D. virilis dot chromosome, will the same conclusions hold?
  – Gene identity, synteny, evidence of rearrangements?
  – Size of genes, gene density?
  – Levels and kinds of repetitious sequences?

• Given sequence data from other Drosophila species, can we do a better job in defining genes? What about patterns of repetitious sequences?
  – Previous- primarily identified coding regions
  – Start sites for transcription? Regulatory motifs?
  – Will D. mohavaensis look more like D. virilis than D. melanogaster?

• Other features?
  - Should we look for conserved non-coding regions?
  - How does our finished sequence compare to unfinished strain?
  - Other questions?