



Characterisation of the *dead ringer* gene of
Drosophila melanogaster

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by

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Abstract

Interest in the mechanisms of homeo domain specificity led to a screen that identified *Drosophila* proteins able to bind a consensus homeo domain site. One clone isolated in this screen produced no homeo domain and was selected for further characterisation as a protein with an unknown DNA binding domain and the potential to interact with homeo domain proteins on the DNA. This thesis describes the characterisation of the *Drosophila* gene *dead ringer* (*dri*) corresponding to this clone.

Isolation of overlapping cDNA clones and sequence analysis allowed the identification of a complete open reading frame in the *dri* message that gave a predicted protein of 901 amino acids. Database searches and multiple sequence alignment revealed a widely conserved motif in the Dri sequence that is found in proteins from organisms as diverse as yeast, nematodes, flies and humans.

Biochemical analysis of the properties of this conserved motif revealed that it could function as a DNA binding domain when expressed in a fusion protein. The in vitro specificity of the Dri DNA binding domain was determined by selection and sequencing of target sites. The Dri consensus site obtained was strikingly similar to that of the Q₅₀ class of homeo domains, although the sequence and predicted secondary structure of the Dri DNA binding domain do not resemble a homeo domain.

Analysis of the developmental expression pattern of *dri* showed a ubiquitous maternal deposit gradually refined to localisation in the mesoderm at germ band extension, then further restriction to a diverse set of tissues including the salivary gland ducts, parts of the gut and a subset of the central nervous system. The phenotype of P-element insertion and deletion mutations of *dri* were identified as causing embryonic lethality preceded by a disruption of the hindgut and loss of Dri expression in the ring gland.

The identification of the novel, conserved DNA-binding domain in Dead ringer offers an explanation for the regulatory activity of several important related proteins and presents an opportunity to use the advantages of the *Drosophila* model system to clarify the role of these proteins in transcriptional control.

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