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A genetic analysis of the cytological region 46C-F containing
the Drosophila melanogaster homolog of the jun proto-oncogene

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Abstract The cytogenetic region 46C-F on the right arm
of Drosophila chromosome 2, which contains the ho-
omolog of the human jun proto-oncogene, has been ge-
etically mapped and characterized. This project led to
the identification and characterization of a Jra (jun-
related antigen) mutation, which has been described in
detail elsewhere. Three mutagens, EMS, DEB and
gamma-rays, were used to isolate 126 lethal lines for this
interval. Complementation analysis of the 126 lethal
lines identified 29 lethal complementation groups in the
region; nine of which have now been correlated with
known genes or phenotypes. The region has been sub-
divided into ten intervals using various small deletions,
seven intervals in 46C/D and three intervals in 46E/F.
Sixteen P-element lines have been mapped to this inter-
val and are allelic to eight of our complementation
groups. The remaining unidentified complementation
groups have been analyzed for critical phase, which is
when the first observable defect arises and/or when
death occurs. There are twelve embryonic lethal groups
and seven larval lethal groups. Three lines show visible
abnormalities in gut and tracheal development prior to
death.

Keywords Drosophila · Jun oncogene · Mutagenesis ·
Complementation analysis

Introduction

The Drosophila melanogaster homolog of the proto-
oncogene jun was isolated by Zhang et al. (1990) and the
cDNA by Perkins et al. (1990). In vertebrates, the pro-
tein product of this gene functions as a dimer, usually as
a heterodimer with the product of the fos proto-onco-
gen, and is known as AP-1 (Activator Protein-1). AP-1
is a transcription factor that regulates genes in response
to signals from various signal transduction pathways.
These pathways have been thoroughly characterized
biochemically in vertebrates (see, e.g., Elion 1998). The
recent recovery of Jra mutations in the Drosophila jun
homolog (Hou et al. 1997; Köckel et al. 1997; Riesgo-
Escovar and Hafen 1997a) and fos mutations in the kay
(kayak) gene (Riesgo-Escovar and Hafen 1997b;
Zeitlinger et al. 1997) in Drosophila will allow for
comparative analysis.

The initial goal of this project was to isolate a lethal
Jra mutation in order to extend and complement the
existing data concerning the signal transduction path-
ways in Drosophila. The Jra mutation was initially
mapped to the right arm of the second chromosome at
46E. This region of chromosome 2 was not well char-
acterized and the only deletion available was the large
Df(2R)X1 (O’Brien et al. 1994). Only the gene even-
skipped (eve) had been characterized in 46C. The project
reported here led to the isolation and characterization of
a Jra mutation (Hou et al. 1997) and the genetic char-
acterization of the 46C-F region uncovered by
Df(2R)X1. We have identified 29 lethal complementa-
tion groups in the region. Nine of these, including Jra
(complementation group V) and eve (complementation
group F), have now been identified with known genes or
phenotypes, and characterized by various groups. We
present here the genetic analysis of the 46C-F region
uncovered by Df(2R)X1. This analysis complements
and extends a previous analysis of the 46C-D region (O’Brien et al. 1994). This report will serve as the basis for the integration of the genetic data presented here with genomic DNA sequence data (Adams et al. 2000) and cytological data.

Materials and methods

*Drosophila* stocks, mutagenesis and complementation testing

Fly stocks were raised at room temperature on standard medium containing propionic acid. Males from an *adl cn pr* strain were mutagenized with ethyl methane sulfonate (EMS), diepoxybutane (DEB) or gamma-rays under standard mutagenic conditions (Grigliati 1986). Due to the low recovery of lethal mutations with the standard concentration of DEB, a range of concentrations, from 6.5 μl to 9.0 μl/10 ml of sugar solution, was used. A concentration of 8.0 μl/10 ml was found to be most effective. The lines were tested for lethality uncovered by the deficiency *Df(2R)X1* (O’Brien et al. 1994) in a standard F2 screen and then balanced over *CyO*. Lethality is defined as the absence of homozygous mutant flies. No line has shown any leakiness. Flies containing lethal alleles were crossed inter se to determine patterns of complementation.

Additional stocks in the region were obtained from M. O’Brien and P. Taghert, including *Df(2R)X3, Df(2R) 520* and *Df(eve)27* (O’Brien et al. 1994), from Schwarz and Burgess [*Df(2R)J2* and from Hama [*Df(2R) Stan 2*]. P-element induced lethal stocks were obtained from the Berkeley *Drosophila* Genome Project and the Bloomington Stock Center.

Analysis of lethal phase

Eggs were collected and examined for abnormal development, with the rearing medium, in microtiter plate wells, which were half filled with agar and some yeast paste. Typically, lines were outcrossed to a wild-type Canton S stock to eliminate the *CyO* balancer chromosome. Alternatively, the *adl cn pr* chromosome was used to replace the *CyO* chromosome.

Results and discussion

Mutagenesis and complementation testing

The initial goal of this project was to isolate a mutation in the *Drosophila* homolog of the mammalian proto-oncogene *jun*. The smallest deletion uncovering this region available at the time this project began was *Df(2R)X1* (O’Brien et al. 1994), which spans the region from 46C to 46F. To isolate mutations in the *Jra* gene, multiple rounds of mutagenesis were performed with three different mutagenic agents. EMS (a point mutagen) mutagenesis yielded 74 lethal lines from approximately 15,000 progeny. DEB was then used for mutagenesis, because of its reported propensity for creating small deletions; this screen yielded 21 lethal lines from approximately 18,000 progeny. Gamma-ray mutagenesis was then used with the expectation of creating a set of larger deletions, and yielded 31 lethal lines from approximately 24,000 progeny. In total, 126 lines were established and balanced over *CyO*.

Lines were then crossed inter se to group them into complementation groups in such a way as to minimize the number of crosses necessary. These results are summarized in Table 1. Complementation testing revealed 29 independent complementation groups, labeled A–Z and then (since there turned out to be more than 26) AA–CC in approximate order of the number of alleles in each group. There are four large complementation groups with 11–20 alleles, four medium-sized groups with 5–9 alleles and 21 small groups with 1–2 alleles. A number of lethal lines failed to complement multiple complementation groups. Several of these we believe to carry independent double mutations in two complementation groups. Others we believe to be small deletions and these will be discussed further below.

Two different visible dominant mutations were recovered multiple times. A dominant curly-wing mutation was found in two lines that failed to complement each other. These two lines uniquely make up the BB complementation group. The 22–40 line has an ultra curly phenotype when balanced over *CyO*, while the 58–3 line does not. We do not know if the lethality and curly-wing phenotype are associated with the same gene. The second is a dominant withered-wing phenotype. Three lines show this effect, two of them make up the AA complementation group and the third represents the CC complementation group. Again, we do not know if the lethality and withered-wing phenotypes are associated with the same gene.

From our sets of lethal lines, nine complementation groups have now been identified with known genes or phenotypes. This total includes seven new ones including the *Jra* gene identified by us (Hou et al. 1997). The A complementation group corresponds to the *Phosphofructokinase (Pfk)* gene (Currie and Sullivan 1994); the C complementation group to the *Dme2* gene, which encodes a transcription factor (Bour et al. 1995; Lilly et al. 1995); the D complementation group is *Hr46*, which encodes an orphan nuclear receptor (Carney et al. 1997); the I complementation group is *ter94*, which encodes a membrane fusion protein located in the fission that is required for mRNA localization (Leon and McKearin 1999; Ruden et al. 2000); the J complementation group is *synaptobrevin (syb)*, which encodes a membrane SNARE protein (R. Burgess and C. O’Kane, personal communication); and the K complementation group is the *leonardo* gene which encodes the 14-3-3 zeta adapter protein. In addition, the Z complementation group has been functionally identified as an enhancer of *chickadee (chic)*, the profilin gene (S. Doberstein, personal communication). A number of other groups are using sets of these lethal lines in attempts to correlate their phenotypes with our complementation groups.

An interesting problem arose concerning the identification of the *Pfk* gene. Currie and Sullivan (1994) identified the B complementation group with *Pfk* based on enzymatic analysis of extracts from wild-type and heterozygous mutant flies. Subsequently, a P-element insert was identified in the A complementation group,