Genetic Resistance to Viral Infection: The Molecular Cloning of a Drosophila Gene That Restricts Infection by the Rhabdovirus Sigma

D. Contamine,* A.-M. Petitjean* and M. Ashburner†

*Laboratoire de Génétique des Virus, C.N.R.S., Gif-sur-Yvette, 91190 France, and †Department of Genetics, University of Cambridge, Cambridge, England

Manuscript received February 3, 1989 Accepted for publication July 27, 1989

ABSTRACT

The ref(2)P gene of Drosophila melanogaster has two common alleles, ref(2)P⁺ which permits the infection of flies by the rhabdovirus sigma (σ), and ref(2)P⁰ which is restrictive for σ infection. This gene has been cloned by P element tagging and shown to code for two RNAs in adult flies. These RNAs are expressed in both males and females, but only the larger is expressed in ovaries. Both transcripts are shorter, by about 50 nucleotides, in flies carrying the ref(2)P⁺ allele than in those carrying ref(2)P⁰. The dominance relationships of these two alleles, and the fact that ref(2)P⁰ allele are permissive to σ infection, suggest that the ref(2)P⁺ product is antimorphic to that of the ref(2)P⁰ allele.

VIRUSES are cellular parasites: their replication demands interactions between their own macromolecules and those of their host. These interactions can include the use, for viral replication, of host cell proteins in novel ways. A classic example is that three of the four polypeptides of the replicase of the bacteriophage Qβ are encoded by the host cell. These three host proteins are the S1 ribosomal protein and the Tu and Tf elongation factors required for host protein synthesis. After viral infection these proteins are “hijacked” to function in viral RNA synthesis (Blumenthal, Landers and Weber 1972; Wahba et al. 1974).

Although the genetic analysis of viral-host function is relatively easy in the case of bacteriophage and their bacterial hosts this is not usually so for animal viruses. Nevertheless, genetic analysis of these interactions is important if we are to understand the role of host functions in the life cycles of particular viruses. One convenient model system for such an analysis is Drosophila melanogaster and its sigma virus (σ), a naturally occurring rhabdovirus that is widespread in natural populations of this fly (Brun and Plus 1980; Emeny and Lewis 1984).

The most dramatic consequence of sigma virus infection of D. melanogaster is that infected flies are very sensitive to the effects of carbon dioxide—in contrast to uninfected flies those carrying sigma virus are rapidly killed as a consequence of exposure to CO₂ (L’Heritier 1958). Natural populations of D. melanogaster are often polymorphic for alleles that confer resistance to sigma viral infection. These restrictive alleles map to five different loci (Gay 1978). Other than the differences seen on sigma infection, strains that are homozygous for the permissive or restrictive alleles at any of these five loci are indistinguishable. The specificity of the restrictive alleles is also shown by the occurrence of mutations of the virus that can overcome the consequences of host restriction (Contamine 1981; Coulon and Contamine 1982).

The best known of the loci at which genetic variation affects the ability of sigma virus to productively infect D. melanogaster is ref(2)P (Guillemain 1953). This gene maps in the cytogenetic region 37E3–37F3 on chromosome arm 2L, between the visible loci hooked and purple (Nakamura, Gay and Contamine 1986). Two classes of ref(2)P allele are common, ref(2)P⁺ that is permissive for sigma and ref(2)P⁰ that is restrictive. Heterozygotes show a reduced probability of viral infection (Nakamura 1978). In natural populations in France the frequency of the ref(2)P⁺ allele is between 0.15 and 0.4 (Fleuriet 1976). Loss-of-function alleles of ref(2)P, equivalent to a deletion of the gene, can be obtained after mutagenesis with X-rays (Nakamura 1978) and chemicals (D. Contamine and P. Gay, unpublished data).

The dominance relationships between ref(2)P⁺, ref(2)P⁰ and ref(2)P⁰ allele are not straightforward. ref(2)P⁺ homoygotes, ref(2)P⁺/Df and ref(2)P⁺/ref(2)P⁰ heterozygotes are all equally sensitive to sigma virus. ref(2)P⁺/ref(2)P⁺ heterozygotes are less sensitive than these three genotypes yet more sensitive than ref(2)P⁺/Df or ref(2)P⁺/ref(2)P⁰. ref(2)P⁺ homozygotes are the least sensitive genotype. Thus, in terms of decreasing sensitivity to sigma virus infection, the genotypes can be ranked in the following order: P⁺/P⁺ = P⁺/P⁰ = P⁺/Df > P⁺/P⁰ > P⁺/P⁰ = P⁺/P⁺ > P⁺/Df > P⁺/P⁰ > P⁺/P⁺. The intermediate pheno-

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
type of ref(2)(P)/ref(2)(P) argues for the codominance of these alleles; yet ref(2)(P), but not ref(2)(P), is haplo-insufficient. The equal sensitivity of ref(2)(P) and ref(2)(P) genotypes (e.g., when either allele is heterozygous with a deletion) points to a lack of function of ref(2)(P) yet this cannot be so, since ref(2)(P)/ref(2)(P) is a far more sensitive genotype than ref(2)(P)/Df or ref(2)(P)/ref(2)(P) (NAKAMURA 1978). These considerations make the molecular analysis of ref(2)(P) and of its gene products of particular interest.

We describe the molecular cloning of the ref(2)(P) gene by transposon tagging with the P element. Mutations of ref(2)(P) to alleles conferring resistance to sigma infection were recovered from a PM hybrid dysgenesis screen. These were found to result from the insertion of a P element into the ref(2)(P) gene.

**MATERIALS AND METHODS**

**Stocks:** The chromosomes used in this work are described in Table 1. The deletions were kept balanced over In(2L)R, Cy and the duplications as stocks of the type Dp(2;Y)/Df(2L)E55/In(2L)R0, Cy. The deficiencies and duplications all derive from chromosomes that carried the permissive, ref(2)(P), allele. The second chromosomes of the Canton-S and x2 stocks also carry this allele. Two second chromosomes carrying the restrictive allele, ref(2)(P), were used: one was unmarked (ref(2)(P)) and the other was ref(2)(P) Bl. The OM wild-type strain was used as the reference ref(2)(P) strain. The O and E chromosomes were the base chromosomes on which the OD and E series of deletions were respectively induced. The l(2)37Ea, l(2)37Fa and l(2)37Fb lethal complementation groups were represented by their alleles, E124, O692 and E146, respectively.

**Assay for sigma virus sensitivity:** A line of a CyO, ref(2)(P)/In(2L)Rpm, ref(2)(P) stock stabilized for an infection with a virus (that is to say, a stock in which the germ-line was infected with, and would transmit, the virus) was selected for transmission of sigma to all progeny. The ref(2)(P) Bl chromosome was then introduced and ref(2)(P) Bl/CyO that were sensitive to CO2 and would transmit sigma to all progeny were selected. With the σA3 strain of virus (GAY 1978) all ref(2)(P) Bl/Bl and ref(2)(P) Bl/ref(2)(P) progeny of this strain are resistant to CO2 (15 min exposure at 12°C). They recover from paralysis within 15 min of exposure to air following treatment with CO2. ref(2)(P) Bl/ref(2)(P) progeny of this strain are CO2 sensitive under the same test conditions; they remain permanently paralyzed by the CO2 treatment and, eventually, die.

The symbols [σ] and [M] or [P] are used to describe a stabilized infection by sigma and cytotype with respect to the PM system of dysgenesis. Where appropriate the genotype of the σ virus is indicated, where it is not, σA3 may be assumed.

**Molecular techniques:** The general source for molecular methods and recipes for buffers, etc., was MANIATIS, FRITSCH and SAMBROOK (1982).

For the extraction of total nucleic acids and RNA, flies were homogenized in lysis buffer (THIREOS, GRIFFEN-SHEA and KAFATOS 1980), using 25 flies per 0.5 ml. After two extractions with phenol (non-neutralized) and chloroform, ethanol precipitation the DNA was dissolved in 5 M CO3H for 20 min and then transferred to nitrocellulose, in a 1.5% agarose-6 M urea gel, as described by BLONDEL et al. (1988).

Genomic DNA for Southern analysis was prepared by a similar method to that described above, except that neutralized phenol containing 0.1% hydroxyquinoline was used and all mixings of solutions were done by gentle inversion of tubes, to avoid hydrodynamic shearing of the DNA. After ethanol precipitation the DNA was dissolved in 5 μl TE buffer containing ribonuclease (at 20 μg/ml) per fly equivalent and then incubated at 37°C for 1 hr. This material was then used directly for enzymatic digestion. Restriction enzyme digestions were all done overnight in the presence of bovine serum albumin (at 100 μg/ml) and spermidine (at 4 mm). Digested DNA was electrophoresed on 1% agarose gels in Tris-acetate buffer. The gels were treated with 0.25 N HCl for 20 min and then transferred to nitrocellulose, essentially as described by SOUTHERN (1975). The filters

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Cytology</th>
<th>l(2)37Ea</th>
<th>ref(2)(P)</th>
<th>l(2)37Fa and l(2)37Fb</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(2L)TW158</td>
<td>37B2-8;37E2-F4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>Df(2L)E55</td>
<td>37D2-E1;37F5-38A1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1</td>
</tr>
<tr>
<td>Df(2L)OOP5</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2</td>
</tr>
<tr>
<td>Df(2L)Opl21</td>
<td>37E3-F1;38C6-10</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-3</td>
</tr>
<tr>
<td>Df(2L)OD12</td>
<td></td>
<td>+</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Df(2L)TW12</td>
<td>37E2-F4;39C2-D1</td>
<td>+</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dp(2;Y)G</td>
<td>36B5-C1;40F;Y</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Dp(2;Y)G-M15*</td>
<td>37EF</td>
<td>(++)</td>
<td>-</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Dp(2;Y)G-H1*</td>
<td>37F4-38A1;39C2-39D1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Dp(2;Y)G-H3*</td>
<td>37E2-F1;40B-F</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-5</td>
</tr>
</tbody>
</table>

* - included within deletion. +, not included within deletion.

References: (1) WRIGHT, HODGETTS and SHERALD (1976); (2) P. GAY and D. CONTAMINE (unpublished data); (3) BRITT-NACHER and GANETSKY (1983); (4) NAKAMURA, GAY and CONTAMINE (1986); (5) HODGETTS (1980).

Deletion derivatives of Dp(2;Y)G.

An X-ray induced derivative of Dp(2;Y)G with the new order Y[(36C1-57EF)]/[(37EF-40F)]Y, the lethal l(2)37Ea is present but subject to position effect inactivation.
were rinsed in 3 × SSC, air dried, baked at 82° for 4 hr.

For the cloning of genomic DNA in plasmid vectors DNA was prepared as described above but, after ethanol precipitation, dissolved in 20 μl TE buffer, per fly equivalent, re-precipitated with ethanol, washed with 70% ethanol and then re-dissolved in 3 μl TE buffer containing ribonuclease (20 μg/ml per fly equivalent). After complete digestion with EcoRI the DNA was electrophoresed in 1% low melting point agarose (Bethesda Research Laboratories Inc.) and a band corresponding to fragments of molecular weights 2.2–2.8 kb was removed, melted at 65° and its DNA extracted with phenol.

The purified DNA fragments, within the size range 2.2–2.8 kb, were ligated with 0.25 μg of pUC9 plasmid DNA (in 180 μl of ligation mix) that had been cut with EcoRI and alkaline phosphatase treated. After ligation, competent Escherichia coli DH1 cells (prepared by the protocol of Hanahan 1985) were transformed (54 μl ligation suspension with 600 μl of cell suspension) and plated onto ten plates. Replica colonies were lifted onto nitrocellulose filters and screened as described by Mason and Williams (1985), with slight modifications.

A library of *D. melanaster* Oregon-R genomic DNA cloned in the phage vector λEMBL4 was kindly made available to us by M.-C. Mariol. DNA was prepared from phage as described by Mariol, Prent and Limbourg-Bouchon (1987).

Nitrocellulose filters were prehybridized, for at least 6 hr at 45°, in a mixture of 50% formamide (deionized), 5 × Denhardt’s solution, 0.5% SDS, 3 × SSCP (0.36 M NaCl, 75 mM Na-citrate, 100 mM Na-phosphate, pH 7.2) and 0.1 mg/ml denatured salmon sperm DNA. Filters were hybridized with radiolabeled probes overnight at 45° in the prehybridization solution plus 10 mM EDTA, 10% (w/v) dextran sulfate and 10 mg/ml nick-translated probe (usually 2 × 10° cpm/μg). The filters were then washed with agitation for six 20-min periods and various stringency conditions—the lowest stringency used was that in 1 × SSC, 0.5% SDS at 65° (for Southern blots) or at 55° (for Northern blots). Filters were autoradiographed with X-ray film without pre-flashing. For reuse the filters were de-hybridized by incubation for at least 4 hr at 75° in TE buffer containing 0.2% SDS.

*In situ* hybridization: *In situ* hybridization to polytene chromosomes was done as described by Gubb et al. (1984).

**RESULTS**

**P element induced *ref(2)P* mutations:** PM dysgenic males homozygous for *ref(2)P* were cross to *ref(2)P* Bl/CyO females that were stabilized for *σ* virus infection (Figure 1a). All the progeny of this cross will be infected by *σ*, due to the maternal inheritance of the virus, and all will be sensitive to CO₂ unless heterozygous for *ref(2)P* and either a newly induced *ref(2)P* allele or a deletion of the *ref(2)P* gene (Nakamura 1978; Nakamura, Gay and Contamine 1986). Of approximately 30,000 *ref(2)P* Bl/*ref(2)P* progeny (where *ref(2)P* indicates the *ref(2)P* allele subjected to dysgenesis) 75% CO₂ resistant flies were recovered. These were backcrossed to *ref(2)P* Bl/CyO *σ*, and subjected to a second screen with carbon dioxide. After the second screen three independent mutations of *ref(2)P* were retained (*ref(2)P*d, *ref(2)P*hd and *ref(2)P*hd2). Two of these (*hd1* and *hd3*) appeared as clusters, of six and seven flies, respectively, in the first screen.

The PM induced alleles of *ref(2)P* have similar genetic properties to *ref(2)P*null alleles (Nakamura 1978): thus, *ref(2)P* / *ref(2)P*, like *ref(2)P*null / *ref(2)P*, are restrictive to *σ* and *ref(2)P* pm* / ref(2)P*, like *ref(2)P*null / *ref(2)P*, are permissive to *σ* infection. All three dysgenic alleles are viable when heterozygous with a deletion for *ref(2)P*, Df(2L)E55. However, *ref(2)P* / Df(2L)E55, like *ref(2)P*null / Df(2L)E55, are all male sterile. These males possess sperm, but these are amotile. The male sterility of *ref(2)P*null alleles is conditional: on some genetic backgrounds *ref(2)P*null / Df(2L)E55 males are fertile. This has been found to be due to a dominant suppressor of their sterility that maps just proximal to *scarlet* on chromosome arm 3L. Laboratory stocks, and natural populations, are polymorphic for dominant suppressor and recessive nonsuppressor alleles of this gene (P. Gay, unpublished data).
If the resistance to \( \sigma \) shown by the flies heterozygous for \( \text{ref}(2)P' \) is due to the insertion of a \( P \) element into the \( \text{ref}(2)P \) gene, then the mutant alleles should revert to \( \text{CO}_2 \) sensitivity in the presence of \( \sigma \), by further dysgenesis. Dysgenic \( \text{ref}(2)P'/\text{CyO} \) males were obtained from a cross of \( \text{ref}(2)P'/\text{CyO} [P] \) males to \( \text{Df}(2L)E55/\text{CyO} [M] \) females and backcrossed to \( \text{Df}(2L)E55/\text{CyO} \) females (Figure 1b). The male \( \text{ref}(2)P'/\text{CyO} \) progeny were tested for their fertility and the \( \text{ref}(2)P'/\text{CyO} \) progeny tested for lethals mapping within \( \text{Df}(2L)E55 \). From approximately 1500 individual males 12 independent revertants of \( \text{ref}(2)P' \) and three revertants of \( \text{ref}(2)P'' \) were recovered, by the criterion of the fertility of males heterozygous with \( \text{Df}(2L)E55 \). All of these revertants also recovered the full characteristics of a \( \text{ref}(2)P' \) allele, that is to say they were all active and permissive for \( \sigma \) virus. One lethal revertant of \( \text{ref}(2)P'' \) was recovered—this chromosome carries a deletion, \( \text{Df}(2L)CPP2R (= \text{Df}(2L)37F4;38A5.6 \text{inclusive}) \) that overlaps \( \text{Df}(2L)E55 \). This deletion does not include \( \text{ref}(2)P' \), but does include the two lethal complementation groups \( [l(2)37Fa \text{ and } l(2)37Fb] \) that map immediately proximal to this gene.

\( \text{ref}(2)P'' \) has a \( P \) element in 37F: One of the three mutations, \( \text{ref}(2)P' \), and one of its revertants, \( \text{ref}(2)P'' \), were selected for further study. Using a cloned \( P \) element as a probe this mutation was shown to have a \( P \) element inserted into region 37F, as well as at about 30 other sites. Its revertant lacked the insertion at 37F (data not shown).

To reduce the number of \( P \) elements in the \( \text{ref}(2)P'' \) and \( \text{ref}(2)P''/\text{CyO} \) stocks the X and third chromosomes were replaced with those of an M strain. In addition recombination between these second chromosomes and those from an M strain, using \( pr \) as a closely linked marker, was used to reduce the numbers of \( P \) elements in these stocks. Several independent \( \text{ref}(2)P'' \) and \( \text{ref}(2)P''/\text{CyO} \) recombinant chromosomes were retained for molecular analysis.

Cloning strategy: Genomic DNA was extracted from several independent recombinant lines of \( \text{ref}(2)P' \) and, after digestion with the restriction enzyme EcoRI, electrophoresis and Southern blotting, this was probed with a labeled probe prepared from the 540-bp HindIII-PvuII fragment of the \( P \) element. As is evident from the data shown in Figure 2 all the lines show many EcoRI fragments with homology to the \( P \) element. Nevertheless all of the \( \text{ref}(2)P'' \) lines, but none of the \( \text{ref}(2)P''/\text{CyO} \) lines, show hybridization of the probe to an approximately 2.5-kb EcoRI fragment. This is the pattern of hybridization expected to a restriction fragment from the \( \text{ref}(2)P \) gene carrying an inserted \( P \) element. These results were confirmed by probing EcoRI-SalI double digests of DNA from these stocks (data not shown).

DNA from one of the \( \text{ref}(2)P'' \) recombinant lines was digested to completion with EcoRI and fragments in the size range of 2.2–2.8 kb were isolated by gel purification on a preparative agarose gel. These were cloned into the EcoRI site of the plasmid pUC9. From approximately 20,000 colonies of \( E. \text{coli} \) transformed with these constructs and probed with \( P \) element sequences one positive clone was recovered (P2). This clone hybridized to a 2.5-kb EcoRI fragment from \( \text{ref}(2)P''/\text{CyO} \) DNA but to a 1.3-kb EcoRI fragment from \( \text{ref}(2)P''/\text{CyO} \) DNA (data not shown). DNA from two different \( \text{ref}(2)P''/\text{CyO} \) genotypes, \( \text{ref}(2)P''/\text{Df}(2L)E55 \) and \( \text{ref}(2)P''/\text{Df}(2L)ODP5 \), digested with EcoRI and probed with P2 shows only the 2.5-kb band. DNA from \( \text{ref}(2)P''/\text{CyO} \) genotypes shows both the 2.5-kb and 1.3-kb bands while DNA from control genotypes shows only the 1.3-kb band (Figure 3). These data indicate that the 2.5-kb EcoRI fragment includes both DNA from the \( \text{ref}(2)P \) region and approximately 1.2-kb of \( P \) element DNA: the element had, in the \( \text{ref}(2)P'' \) mutation, inserted into an approximately 1.3-kb genomic EcoRI fragment.

Using probes prepared from the P2 plasmid nine \( \lambda \) phage were selected from a library of Oregon-R DNA cloned in the vector \( \Lambda \text{EMBL}4 \). These phage include about 34-kb of genomic DNA from which EcoRI subclones in pUC9 were made for further characterization of the \( \text{ref}(2)P \) region (Figure 4).

Mapping breakpoints in the \( \text{ref}(2)P \) region: Genetically \( \text{ref}(2)P \) maps between the proximal limit of \( \text{Df}(2L)TW158 \) and the distal limits of \( \text{Df}(2L)CPP2R \) and the deletion associated with \( \text{Dp}(Y;2)G-H3 \). A breakpoint of \( \text{Dp}(Y;2)G-M15 \) is associated with a
that the ref(2)P gene includes sequences within the 1.3-kb EcoRI fragment that spans coordinate 0.

Identification of ref(2)P transcripts: Northern hybridization to total RNA, extracted from ref(2)P (the OM strain) and ref(2)P

and other aberrations in this chromosome region, as identified here, is unique in the genome and that both RNAs are smaller, by about 50 nucleotides, in ref(2)P females of the larger is expressed (at any abundance) in ovaries. Figure 7 is shown the result of hybridizing the 2E probe to poly(A)+ selected RNA from various ref(2)P genotypes. These data confirm that flies carrying the dysgenic loss-of-function alleles have much less of the 2.3-kb RNA than ref(2)P or ref(2)P. These data also illustrate allele specific novel transcripts associated with the dysgenic alleles.

The 2.3-kb RNA from ref(2)P can be resolved into two RNA species by electrophoresis, these differ in their size by about 100 nucleotides (Figure 8). Both RNAs are seen in adult males and females but only the larger is expressed (at any abundance) in ovaries. That both RNA species are the products of the same gene is known from the fact that the ref(2)P region, as identified here, is unique in the genome and that both RNAs are smaller, by about 50 nucleotides, in ref(2)P flies than in ref(2)P flies (Figure 8).

DISCUSSION

The interest in the ref(2)P gene comes from the fact that its alleles differ in whether or not they permit a productive infection of D. melanogaster by the rhab-
Alleles are apparently loss-of-function, since they are readily induced by diepoxybutane. The virus reduce fecundity but distal to the right-hand limits of the virus and the male sterility of null alleles of one, ref(2)P, ref(2)PnL and Xhol (X) are shown over the entire walk. Only the sites for the restriction enzymes SalI (S), HindIII (H) and Xhol (X) are shown proximal to coordinate 0. Sites marked with an asterisk are only found on the Cy0 chromosome. The small insertion at about +16.5 is about 1 kb in size and is also only in the Cy0 chromosome. The two insertions are not drawn to scale. Beneath the map are shown the limits of the subcloned EcoRI fragments used to analyze both the DNA of mutant strains and the transcripts of this region. Subclone 41E is of a single EcoRI-EcoRI fragment; subclones 41EA and 41EB are the EcoRI-PvuII and PvuII-EcoRI regions of 41E, respectively. The order of EcoRI sites between coordinates +7 kb and +10 kb is uncertain, therefore the precise position of the 17E EcoRI subclone is ambiguous. The approximate regions from which transcripts were detected in adult flies are also indicated. The distal breakpoints of Df(2L)TW12 and Df(2L)OD12 were not found on the cloned DNA—they are presumably located to the right of the region cloned.

The ref(2)P gene maps to the chromosome region 37E3 to 37F3 on 2L. It is proximal to the left-hand end of Df(2L)TW158 but distal to the right-hand limits of Df(2L)CPP2R, Df(2L)pr21, Df(2L)OD12 and the deletion associated with Dp(2;Y)G-H3. The smallest interval (between the endpoints of Df(2L)TW158 and Df(2L)CPP2R) is about 25 kb. The entire region is included within a much longer deletion, Df(2LE)55. This deletion (of approximately 18 polytene chromosome bands) has been reasonably well "saturated" for EMS and diepoxybutane induced lethal mutations. The 145 mutations recovered map to 14 different lethal complementation groups (P. Gay and D. Contamine unpublished data). None of these 14 loci maps to the interval between the endpoints of Df(2L)TW158 and Df(2L)CPP2R. Yet, in addition to ref(2)P, this interval includes at least three different transcripts that can readily be detected in RNA from adult flies.

ref(2)P has been cloned by P element tagging, using the difference in CO2 sensitivity between ref(2)P/ ref(2)P and ref(2)PmW/ref(2)P genotypes (stably infected with σ) as the basis for the mutant screen. All three mutations show both the CO2 sensitivity and male sterility characteristic of ref(2)PmW alleles; both of these phenotypes revert when the P element is lost from 37EF. All three mutations lack, or have in a greatly reduced amount, transcripts of approximately 2.3 kb in size that originate from the genomic region into which the P elements have, in each case, inte-
The *ref(2)*P gene is transcribed into two RNAs that differ by only 100 nucleotides or so in their size. This difference is due to transcription initiating at two different sites, about 100 bp apart (S. DÉZÉLÉE and F. BRAS unpublished data). These two RNAs are of approximately equal abundance in both males and females. However transcription of *ref(2)*P does show tissue specificity, since only the longer of the RNAs is expressed in the ovaries. The ovarian expression of *ref(2)*P is expected on two grounds: one is that the gene is transcribed into two RNAs that are codominant, since heterozygotes are intermediate between either homozygote in their sensitivity to *σ* infection. This would argue that *ref(2)*P<sup>n</sup> is not a null allele. This is confirmed by the fact that the sensitivity to *σ* of *ref(2)*P<sup>n</sup> homozygotes is increased by the addition of a duplication carrying *ref(2)*P<sup>n</sup> (Nakamura 1978). The *ref(2)*P<sup>n</sup> allele displays haploinsufficiency, since when heterozygous with a deletion (or with a *ref(2)*P<sup>Psnull</sup> allele) its sensitivity to *σ* infection is greater than that when homozygous. The *ref(2)*P<sup>n</sup> allele is not dosage sensitive, *ref(2)*P<sup>n</sup> homozygotes and *ref(2)*P<sup>n</sup>/Df (or *ref(2)*P<sup>n</sup>/*ref(2)*P<sup>Psnull</sup>) heterozygotes have the same sensitivity to *σ* infection. Homozygous, or hemizygous, *ref(2)*P<sup>Psnull</sup> flies have the same sensitivity to *σ* as these genotypes.

One obvious model is that *ref(2)*P<sup>n</sup> and *ref(2)*P<sup>n</sup> make different gene products, that compete for some target, the *ref(2)*P<sup>n</sup> being required for the nonpermissive state. It is of some considerable interest, therefore, that the transcripts from these genes differ in their size. This difference is due, at least in part, to a deletion within the coding region of the *ref(2)*P<sup>n</sup> allele (D. CONTAMINE, unpublished data).

On a competitive model we can envisage the product of *ref(2)*P<sup>n</sup> interacting, perhaps stoichiometrically (to account for this allele’s dosage sensitivity), with the
FIGURE 7.—Northern analysis of the ref(2)P transcript in poly(A)* selected RNA of wild-type and mutant strains. Poly(A)* selected RNA (10 μg/track) from ref(2)P (tracks 1–3), ref(2)P (tracks 4–6), ref(2)P (tracks 7–9) and ref(2)P (tracks 10–12). Tracks 1, 4, 7, and 10 are from females; tracks 2, 5, 8 and 11 from males; and tracks 3, 6, 9 and 12 from mixed sexes. Probed with clone 23E. In the lower part of the figure the same blot is shown, but after hybridization with a D. melanogaster Act5C gene probe, as a control for the loading of the tracks.

product of some other gene (perhaps the product of another ref gene or of the viral genome, see below). This interaction would be required for the establishment of the condition of being nonpermissive to σ infection. The product of the ref(2)P allele would

FIGURE 8.—The tissue specificity of ref(2)P transcripts. Poly(A)* selected RNA (1–3 μg) from hand-dissected ovaries (tracks 1 and 4), total adult females (tracks 2 and 5) and total adult males (tracks 3 and 6) from ref(2)P (tracks 1–3) and ref(2)P (tracks 4–6) strains, probed, after electrophoresis and blotting, with clone 23E.

compete for this interaction, and hence promote a permissive state. The ref(2)Pnull mutations would result in the permissive state simply because the absence of a gene product would prevent any opportunity for the interaction required for nonpermissiveness to occur. Since temperature sensitive mutations of σ that can infect ref(2)P/ref(2)P hosts can be selected (the haP mutations, CONTAMINE 1981), the viral genome, or its products, presumably interacts directly with a host product related to the activity of ref(2)P. Indeed
it may be just this interaction which is targeted by the products of the different ref(2)P alleles: if there is a viral gene product, V, that is required for viral replication then the complex of V with the ref(2)P product may be inactive, but V alone or V complexed with the ref(2)P product active. It is interesting that the restrictive effects of ref(2)P are stronger in the early stages of the viral cycle than after viral genome replication (D. CONTAMINE, unpublished data). Since replication is followed by an increase in transcription, then we would expect the concentration of V to increase as a consequence of replication. This would ameliorate the effects of a presumably constant concentration of the ref(2)P gene product.

If substantiated, this model would mean that the ref(2)P allele, that usually most common in natural populations, is an antimorph with respect to nonpermissiveness for σ infection. The product of this allele, or that of ref(2)P is, however, required for the normal development of sperm.

This work was supported in part by an SERC grant to M.A. We would like to thank C. SAVAKIS and S. MCGILL for their help in the early stages of this work. We also thank M.-C. MARIOL for the gift of the genomic library of Drosophila DNA, and P. LASKO for his comments on the manuscript.

LITERATURE CITED


HODGETTS, R. B., 1980 A cytogenetic description of three duplications in which portions of proximal 2L have been inserted into the Y-chromosome. Drosophila Inform. Serv. 55: 63.


