Molecular Analysis of Recombination Events in Drosophila

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ABSTRACT

The locations of crossover junctions and gene conversion tracts, isolated in the rosy gene of Drosophila melanogaster, were determined using DNA sequencing and denaturing gradient gel electrophoresis. Frequent DNA sequence polymorphisms between the parental genes served as unselected genetic markers. All conversion tracts were continuous, and half of the reciprocal crossover events had conversion tracts at the crossover junction. These experiments have also identified the sequence polymorphisms responsible for altered gene expression in two naturally occurring rosy variants.

MATERIALS AND METHODS

Genetic procedures: The isolation of the recombinant chromosomes and their genetic characterization has been previously described (CLARK et al. 1984). Molecular cloning: Genomic DNA was isolated from stocks carrying a recombinant chromosome over a chromosome with a deficiency for the rosy gene, Df(3R)ry*. DNA was prepared by a rapid "miniprep" method (BENDER, SPIERER and HOGNESS 1983) with the addition of a phenol/chloroform extraction step, and was digested to completion with HindIII. The digested DNA was size fractionated by velocity centrifugation through 5–30% sucrose gradients, ligated into HindIII cut, phosphatase treated, pEMBL 9 plasmid vector DNA (DENTE, CESARANT and CORTESE 1983), and transformed into competent bacteria (HANAHAN 1983) of strain KH802. Colony lifts were probed with gel purified 32P-labeled rosy HindIII fragment. To verify that no errors had been made in cloning, we compared denaturing gradient gel patterns of the genomic and cloned DNA for each rosy gene cloned.

DNA sequencing: Plasmids carrying rosy inserts were transformed into bacterial strain JM105 and single stranded template DNA was prepared by superinfection with helper phage strain M13K07 (VIEIRA and MESSING 1987). Sequencing was carried out by the dideoxy chain termination method of SANGER, NICKLEN and COULSEN (1977), using the Sequenase protocol (U.S. Biochemical Corp.), and T7 DNA polymerase kindly provided by Dr. Stan Tabor. The template DNA was prepared by superinfection with helper phage strain M13K07 (VIEIRA and MESSING 1987). Sequencing was carried out by the dideoxy chain termination method of SANGER, NICKLEN and COULSEN (1977), using the Sequenase protocol (U.S. Biochemical Corp.), and T7 DNA polymerase kindly provided by Dr. Stan Tabor. The template DNA was prepared by superinfection with helper phage strain M13K07 (VIEIRA and MESSING 1987). Sequencing was carried out by the dideoxy chain termination method of SANGER, NICKLEN and COULSEN (1977), using the Sequenase protocol (U.S. Biochemical Corp.), and T7 DNA polymerase kindly provided by Dr. Stan Tabor. The template DNA was prepared by superinfection with helper phage strain M13K07 (VIEIRA and MESSING 1987). Sequencing was carried out by the dideoxy chain termination method of SANGER, NICKLEN and COULSEN (1977), using the Sequenase protocol (U.S. Biochemical Corp.), and T7 DNA polymerase kindly provided by Dr. Stan Tabor.

Denaturing gradient gel electrophoresis: The procedures were based on those of FISCHER and LERMAN (1983), and will be presented in detail elsewhere (M. GRAY and W. BENDER, unpublished results). The gels used were approxi-
mately 15 x 17 cm in size and 0.75 mm thick, and consisted of 6.5% acrylamide in 1 x TAE buffer (40 mM Tris, 20 mM NaOAc, 1 mM EDTA, pH 7.4), with a gradient of urea and formamide concentration increasing from top to bottom. Two solutions were prepared, an "80% denaturant" solution (6.5% acrylamide, 1 x TAE, 32% (v/v) formamide, 5.6 M urea), and a "20% denaturant" solution (6.5% acrylamide, 1 x TAE, 8% (v/v) formamide, 1.4 M urea). The gels were poured using these solutions and a gradient maker to produce a linear gradient from 20% to 80% denaturant concentration. Genomic DNA samples were digested to completion with the restriction enzymes HaelIII, Alul, HhaI, MpiI or Rsal. The samples (5 μg/lane) were electrophoresed at 75 V for 17.5 h, with the gels submerged in a buffer tank maintained at 60°C with buffer circulation. After electrophoresis, the gels were treated with 0.5 M NaOH for 5 min, followed by 0.5 M Tris (pH 8.0) for 5 min, and then allowed to equilibrate for 10 min in transfer buffer (20 mm Tris (pH 8.0), 1 mM EDTA). The DNA was then electrotransferred to nylon membrane (Nytran, Schleicher & Schuell), and the blots were hybridized with radiolabeled rosy DNA probes.

RESULTS AND DISCUSSION

Genetic mapping experiment: Many ry+ isoalleles have been isolated from laboratory strains of Drosophila, and among these, two show a quantitative difference in the production of the enzyme xanthine dehydrogenase (XDH), the product of the rosy gene. ry+4 produces twofold more, and ry4+10 twofold less XDH protein and poly(A)+ mRNA than other wild-type strains such as ry4+, ry2, and ry+5 (CHOVNICK et al. 1976; McCARRON et al. 1979; CLARK et al. 1984). The ry4+ overproduction is due primarily to increased synthesis of XDH in one tissue, the fat body, while in ry4+10 the underproduction is common to at least two tissues, the fat body and the Malpighian tubules (CLARK et al. 1984). Sites responsible for these differences have been defined by genetic mapping; the site in ry4+ is called i409H (High), and the site in ry4+10 is called i1005L (Low). The corresponding alleles in strains producing normal levels of XDH are designated N.

A half-tetrad recombination experiment was designed in order to map the sites responsible for differential expression of these two alleles. The construction of the stocks, the selection protocol, and the recovery of the recombinants has been described (CLARK et al. 1984). Figure 1a diagrams the right arm of the compound third chromosome used in the selection experiment. Flies with this genotype produce a low amount of XDH (25% of wild type) and die on purine-supplemented medium. However, rare recombination events within the rosy gene will generate flies that produce more XDH, and consequently survive the selection. One such recombinant, a reciprocal crossover between i1005L and i409H, is illustrated in Figure 1b. Other possibilities include crossovers between i409H and ry4+10, conversions of i1005L to i1005N, conversions of i409N to i409H, and conversions of ry4+10 to ry4+.

Crossover and conversion events were operationally distinguished by the behavior of the closely linked flanking markers. ry+ recombinants were classified as crossovers when accompanied by the exchange of flanking markers, while those without associated flanking marker exchange were classified as gene convertants. Gene conversion is a nonreciprocal process, by which information on one chromatid is lost and replaced by the corresponding information on another chromatid. To demonstrate this phenomenon unequivocally requires tetrad analysis, where conversion is observed as nonmendelian segregation during meiosis: a heterozygote for a marker gene (+/-) gives rise to progeny with the marker ratio 3+:1- or 1+:3-, rather than the expected 2+:2-.

Figure 1.—a, Schematic representation of the compound 3R chromosome used in the genetic recombination experiment. Both right arms of the third chromosome are attached to the same centromere. The arm deriving from the ry4+ strain is colored white, and the arm deriving from ry+ is black. ry4+10 is a null rosy point mutation on the ry+ background. Relative positions of the markers are not drawn to scale; the rosy gene spans about 0.005 cm, while the genetic distance between kar and Ace is 0.5 cm. b, An example of a recombinant between i1005L and i409H. A fly carrying this recombinant half-tetrad will survive the purine selection.

From a total of 1.75 x 10⁶ tested progeny, 20 recombinant survivors were recovered, including examples of each of the expected classes (CLARK et al. 1984). Nine of the 20 were reciprocal crossovers, while the other 11 were conversion events retaining the parental flanking marker configuration. The com-
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pound chromosomes were detached, and balanced stocks of the individual members of each pair were established. Some recombinant chromosomes carry a ry' gene, and for these the quantity of XDH protein produced was determined directly. For the chromosomes still carrying the ry"606 mutation, the amount of XDH synthesized by the recombinant transcriptional control region could not be assayed. A ry' protein coding region was therefore reintroduced into some of these through a second round of recombination against ry"606 (CLARK et al. 1984). Two chromosomes derived from this second experiment, labeled #2 and #6, were included in our sample. We describe here the molecular analysis of 18 of the 20 original recombinant half-tetrads.

Molecular characterization of recombinant chromosomes: In order to define molecular positions for the genetic sites affecting XDH expression, we needed to sequence a subset of the recombinant rosy alleles. We cloned a 7.3-kb HindIII fragment containing the rosy gene from each of 23 individual recombinant chromosomes. All nine crossover half-tetrads were included. For eight of these we cloned both members of the half-tetrad (chromosomes #10-#25, Figure 2), and the ninth was represented by the chromosome #1 and two derivatives of its reciprocal arm, #2 and #6 (described above). For three gene conversion half-tetrads we cloned only the member of the compound pair which had been converted (#3-#5). We also included a recombinant recovered from a previous free stand recombination experiment. This recombinant (#26) is a conversion of i1005L to i1005N against a ry mutant chromosome derived from the ry"11 background (McCarron et al. 1979). We also isolated the HindIII fragment from the three parental chromosomes: kar7 ry"606 Ace126, ry+10, and ry+44. The latter two alleles had previously been recovered from bacteriophage lambda libraries (Lee et al. 1987, and our unpublished results). Using a set of oligonucleotide primers specific to the rosy gene, we sequenced from 2 to 5 kb from each of the 26 genes. Figure 2 illustrates the extents of the genes sequenced and the patterns of polymorphisms found in each. The sequence of the ry"+ allele (Lee et al. 1987, and this work) serves as the baseline, and only the nucleotide positions at which the alleles differ from each other or from the baseline sequence are indicated.

We examined both arms of an additional six half-tetrads (pairs #27-#32) carrying conversions of ry"606 to ry" (CLARK et al. 1984) using both southern blotting, as described (Clark, Hilliker and Chovnick 1988), and denaturing gradient gel electrophoresis (FISCHER and LERMAN 1983). The latter technique has recently been adapted for the purpose of detecting single nucleotide point mutations in genomic restriction digests of Drosophila DNA, and will be described in detail elsewhere (M. Gray and W. Bender, unpublished results). Because the melting point of a fragment depends on its nucleotide sequence, as little as a single base pair change in the sequence can shift the position of the fragment on the gradient gel. This technique allowed us to detect many more of the polymorphisms between the parental strains than was possible by scoring restriction fragment length polymorphisms on nondenaturing gels.

Figure 3a shows an example of a denaturing gradient gel of HhaI digested genomic DNA from five of these half-tetrad pairs and the two parental strains, electroblotted to nylon membrane, and probed with the 7.3-kb rosy HindIII fragment. Nearly every fragment shows a melting difference between the ry"606 and ry"10 parental strains. By comparing the fragment patterns of the recombinants to those of the parents, the lengths of conversion intervals were determined to within a few hundred nucleotides (Figure 3b). The analysis was repeated using four other enzymes: HaeIII, Alul, Rsal and MspI (not shown), and the extents of the six conversion tracts, based on the combined data, are shown (Figure 3d). The precision in measurement of conversion tract lengths by gradient gels approached that obtained by sequencing.

We are confident that we have analyzed the entire interval of each of the recombination events in the sample, and that each represents a single isolated event. This is based on the following: first, the genetically determined flanking marker configurations are consistent with the directions of exchanges that we see at the molecular level. Second, we see no evidence on denaturing gradient gels for additional exchange events within the 7.3-kb HindIII fragment for any of the recombinants. And third, the low overall frequency of recombination at rosy, coupled with the established phenomenon in Drosophila of positive interference, in which a crossover event inhibits additional crossovers in adjacent intervals on the same chromosome arm (Hilliker and Chovnick 1981), argues against the possibility of additional, incidental recombination in the interval.

Variant strain regulatory sites: The recombinant chromosomes that proved useful in locating the sites of i1005L and i409H are diagrammed in Figure 2b. Chromosome pairs #1/#2 and #24/#25 were isolated as crossover half-tetrads. #2 also carries an independent conversion of ry"606 to ry", recovered in the second round of recombination against ry"606. #3, #4, #5, #6 and #26 are simple conversion events. The conversion tract in #4 extends beyond the end of the cloned HindIII fragment, but we determined by denaturing gradient gels that the conversion tract ends less than 1 kb to the left of the HindIII site (not shown).

Based on their XDH overproduction phenotypes (Figure 2d) (CLARK et al. 1984), #2, #25, #3 and #6 each carry i409H. The only ry"-specific polymorphism common to all four of these recombinants is
Figure 2.—Sequence polymorphisms in the background strains and the recombinants. a. The ry" sequence provides a baseline for comparison, and polymorphisms in the strains used in this experiment are indicated by the position and nature of the change. The positions of the polymorphisms with respect to the intron/exon structure of rosy are indicated along the top of the figure. "Intergenic region" refers to the space between rosy and the next gene to the left (centromere-proximal), il(3)S12. A dot indicates that a sequence is identical to the baseline sequence at a position, a letter indicates a nucleotide substitution, and a number or a dash indicates an insertion (V) or deletion (A) of the indicated number of base pairs. Polymorphisms indicated by numbers at positions -2366, -2030 and -590 are length differences.
the single guanine to cytosine transversion at -1145, located in the first intron of the gene (Figure 2). We conclude that this single nucleotide change is i1005L, and that it is sufficient to bring about the increase in XDH production found in the fat body of ry" animals. This polymorphism is located within the limits of the 0.5-kb deletion of intron sequences in a rosy mutant sd[32226-547] (DANIELS et al. 1985), which reduces fat body expression of XDH (DUTTON and CHOVNICK 1988).

Two recombinants, #2 and #3, express XDH at a level greater than normal, but less than the overproducer ry"", and are genotypically i1005L i409H (CLARK et al. 1984). Four other recombinants, the #24/#25 pair, #4, #5, and #26 are convertant for i1005L to i1005N. Correlating the ry"" specific polymorphisms with the phenotypes of these recombinants, we can restrict the sites for i1005L to only two possibilities, nucleotides -1701 and -1619 (Figure 2). The start site for rosy transcription is at approximately -1645, based on primer extension data and cDNA 5' endpoints (our unpublished results). This places the -1701 polymorphism just 5' of the transcription start site, and the -1619 polymorphism just within the transcribed region.

We sequenced this region from several other wild-type alleles which had been characterized (CHOVNICK et al. 1976) as normal level XDH producers. The sequence polymorphisms for 1.6 kb of one of these, ry"", are given in Figure 2a. ry"" (and also two other normal level XDH producing strains, ry+2 and Okanogon #31, data not shown), shares the -1619 polymorphism with ry"", but it does not share the -1701 polymorphism. This suggests that i1005L consists of the single polymorphism at -1701.

In summary, at most two nucleotides at the 5' end of the gene are responsible for the general underexpression found in the ry"" strain, and probably only one of them, the thymidine to cytosine transition at -1701, corresponds to i1005L. Although the rosy gene does not have any easily recognized promoter sequence motifs, such as the TATA box (BUCHER and TRIFONOV 1986), which would be altered by this polymorphism, we expect that i1005L might influence the rate of transcriptional initiation at the rosy promoter. Further in vitro mutagenesis experiments will investigate the full sizes and functions of the cis-acting sequences identified by the i1005L and i409H polymorphisms.

**Product of recombination:** The large number of polymorphisms serving as unselected markers in these experiments allows us to observe the nature and size of conversion tracts, and to see conversion tracts associated with crossover junctions. In fungi, complex recombination events are sometimes observed. These include independent or partial mismatch repair in a single heteroduplex tract, crossover points separated from converted markers by unconverted markers, and events involving three or four chromatids (FOGEL et al. 1979; KALOGEROPOULOS and ROSSIGNOL 1980; BORTS and HABER 1987; SYMINGTON and PETES 1988). However, none of the events in this Drosophila sample were complex. We have examined ten gene conversion events at the sequence level, and an additional six using denaturing gradient gels. Fourteen of these conversion tracts include three or more markers, and all 14 are completely continuous. If conversion results from the repair of heteroduplex DNA mismatches, we do not see "patchy" repair.

We examined both arms of the half-tetrads for eight simple conversions using denaturing gradient gels (half-tetrads #27-#32, #4 and #5; Figure 3, and not shown). Although in these events the reciprocal arm was only one of two potential nonsister chromatid donors, we saw no changes in the reciprocal arms. We thus see no evidence for symmetric heteroduplex in generating these events, since symmetric heteroduplex formation, followed by independent repair of the
Figure 3.—Mapping of conversion intervals using denaturing gradient gel electrophoresis. a, A denaturing gradient gel of genomic DNA digested with Hha1, blotted to nylon membrane, and probed with the 7.3-kb HindIII fragment. Each pair, for example #28 and #28A, are the members of an original recombinant half-tetrad characterized as a conversion of ry^406 to ry^+. The A denotes the unconverted, ry^406/Ace^26 members of the half-tetrads. Bands are labeled by the letter designations indicated on the restriction map in part c. All of the bands on the blot except E, G, and L (G and L ran together here) show melting point differences between the two parental strains, ry^406 and ry^+. The band labeled K* is present in ry^406, instead of K, due to the gain of the restriction site indicated by * in part c. b, Schematic representation of the results of the blot shown in a. An “X” under the 4 or 10 column indicates that a chromosome exhibits melting behavior of the ry^406 or ry^+ parental chromosome, respectively, for that fragment. A “y” shows that the conversion chromosome exhibits a non-parental melting type, indicating that the endpoint of the conversion tract lies between polymorphisms within the fragment. Shaded boxes represent the ~ry^406 parental chromosome strand, and white boxes ry^+. Only one member of each half-tetrad is diagrammed, since none of the other members (labeled A in part a) show evidence of being changed in the event. c, The rosy transcriptional unit is shown relative to the restriction map. The locations of the i1005L, i409H, and ry^406 sites (Figure 2) are indicated. Hha1 sites are indicated by unlabeled vertical bars, and the Hha1 fragments visible on the gel in part a are lettered. Fragments C and D are visible upon longer exposure of the same blot. d, Extents of the conversion tracts in the six events in which ry^406 was converted to ry^+. Endpoints were determined from denaturing gradient gel analysis repeated with a total of five enzymes. Black bars indicate the minimum conversion tract length, and the hatched regions extend to the maximum length.

two tracts, could have led to reciprocal conversions.

Nine crossover half-tetrads were sequenced; in the four cases in which a conversion tract was associated with the crossover, the tracts were not separated from the crossover. In one experiment in Drosophila, putative convertant classes that might have resulted from complex events at rosy have been reported (CHOVNICK et al. 1970; BALLANTYNE and CHOVNICK 1971). This stands in contrast to the large number of experiments which have monitored several markers within the rosy gene, as well as flanking markers, and which have provided no evidence for multiple events (Mc-
TABLE 1

Conversion tract lengths

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Lengths (bp)</th>
<th>Midpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Conversions without crossover, unrestricted:</td>
<td>Min - Max</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1100 - 1684</td>
<td>1592</td>
</tr>
<tr>
<td>28</td>
<td>2468 - 3679</td>
<td>3674</td>
</tr>
<tr>
<td>29</td>
<td>450 - 1622</td>
<td>1036</td>
</tr>
<tr>
<td>30</td>
<td>1 - 448</td>
<td>224</td>
</tr>
<tr>
<td>31</td>
<td>1119 - 1832</td>
<td>1476</td>
</tr>
<tr>
<td>32</td>
<td>213 - 1106</td>
<td>660</td>
</tr>
<tr>
<td>b. Conversions without crossover:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>778 - 1521</td>
<td>1150</td>
</tr>
<tr>
<td>3</td>
<td>322 - 677</td>
<td>500</td>
</tr>
<tr>
<td>4</td>
<td>1512 - 2782</td>
<td>2147</td>
</tr>
<tr>
<td>5</td>
<td>238 - 638</td>
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<td>727 - 1583</td>
<td>1155</td>
</tr>
<tr>
<td>26</td>
<td>788 - 1697</td>
<td>1243</td>
</tr>
<tr>
<td>c. Conversions associated with crossovers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>1 - 473</td>
<td>237</td>
</tr>
<tr>
<td>14/15</td>
<td>12 - 657</td>
<td>335</td>
</tr>
<tr>
<td>22/23</td>
<td>97 - 278</td>
<td>188</td>
</tr>
<tr>
<td>24/25</td>
<td>512 - 710</td>
<td>611</td>
</tr>
<tr>
<td>d. Crossover without associated conversion:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/11</td>
<td>0 - 78</td>
<td></td>
</tr>
<tr>
<td>12/13</td>
<td>0 - 86</td>
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<td>16/17</td>
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<td>0 - 86</td>
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</tr>
<tr>
<td>20/21</td>
<td>0 - 149</td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>343</td>
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tracts resulting from repair of the heteroduplex, or gapped, DNA should be independent of how the Holliday junction is resolved. A length difference such as suggested by our data could imply a mechanistic distinction between recombination events resolved as crossovers versus those resolved as conversions, as has been suggested in other contexts (Carpenter 1984, 1987; Smithies and Powers 1986). It will be important to confirm this suggestion, and a larger sample size, as well as a variety of parental allele combinations will be necessary to determine if tract lengths in Drosophila are consistently shorter when associated with crossing over. Our results clearly do not support the hypothesis that only longer heteroduplex tracts can be substrates for crossing over.

Previous genetic results in Drosophila (Smith, Finnerty and Chovnick 1970; Chovnick, Ballantyne and Holm 1971; Clark et al. 1984) led us to anticipate conversion at crossover sites, and we did see conversion of polymorphisms adjacent to the crossover in four of nine pairs sequenced. There are at least two possible reasons for the lack of conversion in the other five cases. One is that there was no heteroduplex tract, or gap generated during the event which could have resulted in conversion, or there was such a tract but it was too short to detect. The distances between informative polymorphisms flanking these five crossovers range from 78 to 170 bp (Table 1d), and conversion tracts shorter than this would be invisible. Alternatively, a region of heteroduplex was generated, but it was restored back to the parental configuration. If all crossovers are in fact associated with the generation of heteroduplex DNA, and if there is no bias in the direction of repair of mismatches, then 50% of the heteroduplexes should be repaired back to the parental arrangement. This is consistent with the frequency that we find in our sample, and with results from studies on yeast (Borts and Haber 1987, Symington and Petes 1988). Alternative models have been proposed. Carpenter (1987) postulates separate pathways and separate roles in meiosis for gene conversion and crossing over, based on electron microscope studies of recombination nodules, and on mutations which affect the spectrum of recombination events observed at rosy. Rossignol et al. (1984), in their genetic studies of recombination in Ascosolus, note that crossing over may arise by two pathways. Crossover events with certain allele combinations apparently occur in the absence of conversion or heteroduplex DNA formation.

The principal evidence requiring the inclusion of heteroduplex DNA formation in all models of recombination is the phenomenon of postmeiotic segregation (PMS). Although common for certain alleles and at certain genetic loci in the fungi (White, Lusnak and Fogel 1985; reviewed in Orr-Weaver and Szostak 1985; Hastings 1987), PMS is normally very rare in Drosophila (Chovnick, Ballantyne and Holm 1971). However, in the presence of recombination-defective mutations at the mei-9 locus, PMS is frequently observed at rosy in conjunction with both conversions and crossovers (Carpenter 1982). This result suggests that a heteroduplex DNA intermediate is normally involved in recombination in Drosophila, and the mei-9 mutants are defective in the recognition and repair of mismatched bases (Carpenter 1982).

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LITERATURE CITED


Chovnick, A., G. H. Ballantyne, D. L. Bailie and D. G. Holm, 1970 Gene conversion in higher organisms: Half-tetrad analy-


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