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Allele Report

The Generation and Analysis of Deficiencies Within a Small Genomic Region on the X Chromosome of *Drosophila melanogaster* Containing Two Genes, *enhancer of rudimentary* and *CG15352*

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KEY WORDS

enhancer of rudimentary, *CG15352*, P element mutagenesis

ABBREVIATIONS

e(r)	*enhancer of rudimentary*
r	*rudimentary*

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ABSTRACT

The *enhancer of rudimentary* gene, *e(r)*, encodes a 104-amino-acid, highly conserved transcription cofactor. Hypomorphic mutations of *e(r)* show an enhancement of a hypomorphic *rudimentary* mutant wing phenotype. These mutants in a wild-type background are viable, fertile, and morphologically wild-type. Since the only mutant alleles were hypomorphic, it was important to isolate null mutations to determine if any other phenotypes might be associated with a loss-of-function of *e(r)*. We utilized a marked P element, *P{SUPor-P, y^+}*, located 895 bp upstream of the start of transcription of *e(r)* to generate nineteen deficiencies in the region. Deficiencies of *e(r)* enhance the mutant wing phenotype of a hypomorphic *rudimentary* allele, r^{hd1}. In a wild-type background, the deficiencies of *e(r)*, unlike the hypomorphic alleles, have a low viability and females have low fertility. The expression of *e(r)* in the nurse cells of the ovary is consistent with the low fertility, and suggests an ovarian function for *e(r)*. Deficiencies of *CG15352*, the gene directly upstream of *e(r)*, are not associated with any obvious mutant phenotypes and present the possibility that it encodes a nonvital or redundant function.

INTRODUCTION

The *enhancer of rudimentary* gene, *e(r)*, was isolated as a recessive enhancer of the mutant wing phenotype of *rudimentary*, *r*, mutants.[1,2] The gene was cloned and shown to encode a 104-amino-acid protein, ER.[2] Subsequent studies revealed that the gene is highly conserved, present in species as diverse as insects, vertebrates, higher plants, and slime molds.[3] Current BLAST[4] searches have also identified the gene in protists, nematodes, flatworms, but not yeasts (S. I. Tsubota, unpublished data). Studies in Xenopus revealed that ER is nuclearly localized and binds to the transcription factor, DCoH/PCD (dimerization cofactor of HNF1/pterin-4α-carbinolamine dehydratase), and thus may be acting as a transcription cofactor of the HNF1 homeobox transcription factor.[5] Further studies have shown that ER is also nuclearly localized in human and Drosophila cells,[6] consistent with its proposed role as a transcription cofactor.

The first *e(r)* mutation was an insertion of an unmarked P element into the first exon upstream of the start of translation. This mutation, $e(r)^{p1}$, reduces the levels of the *e(r)* transcripts, and genetically behaves like a hypomorphic mutation.[2] Two additional insertions of unmarked P elements have been identified in the exact same position.[7] All three mutations enhance the mutant wing phenotypes of hypomorphic *r* alleles in a similar fashion and, in the presence of a wild-type *r* allele, do not display any mutant phenotypes and can be maintained as homozygous stocks. Given the high conservation of the gene, it was surprising that these mutations did not display a more severe phenotype and it led us to hypothesize that null mutations might exhibit mutant phenotypes not seen in the hypomorphic mutants. To examine this possibility we performed a reverse-genetic screen for *e(r)* deficiencies. The screen took advantage of a marked P element, *P{SUPor-P, y^+}*,[8] inserted 895 bp upstream of the start of transcription of *e(r)* and within the first exon of *CG15352*. By selecting for the loss of this element, we isolated a set of deficiencies, some of which removed *e(r)*. This paper describes the results of the screen and the phenotypic analyses of the deficiencies.

MATERIALS AND METHODS

Drosophila strains and transgenes. The marked P-element stock, *y^1 P{SUPor-P, y^+}KG01830*, was obtained from the Bloomington Drosophila Stock Center. *TM3,*

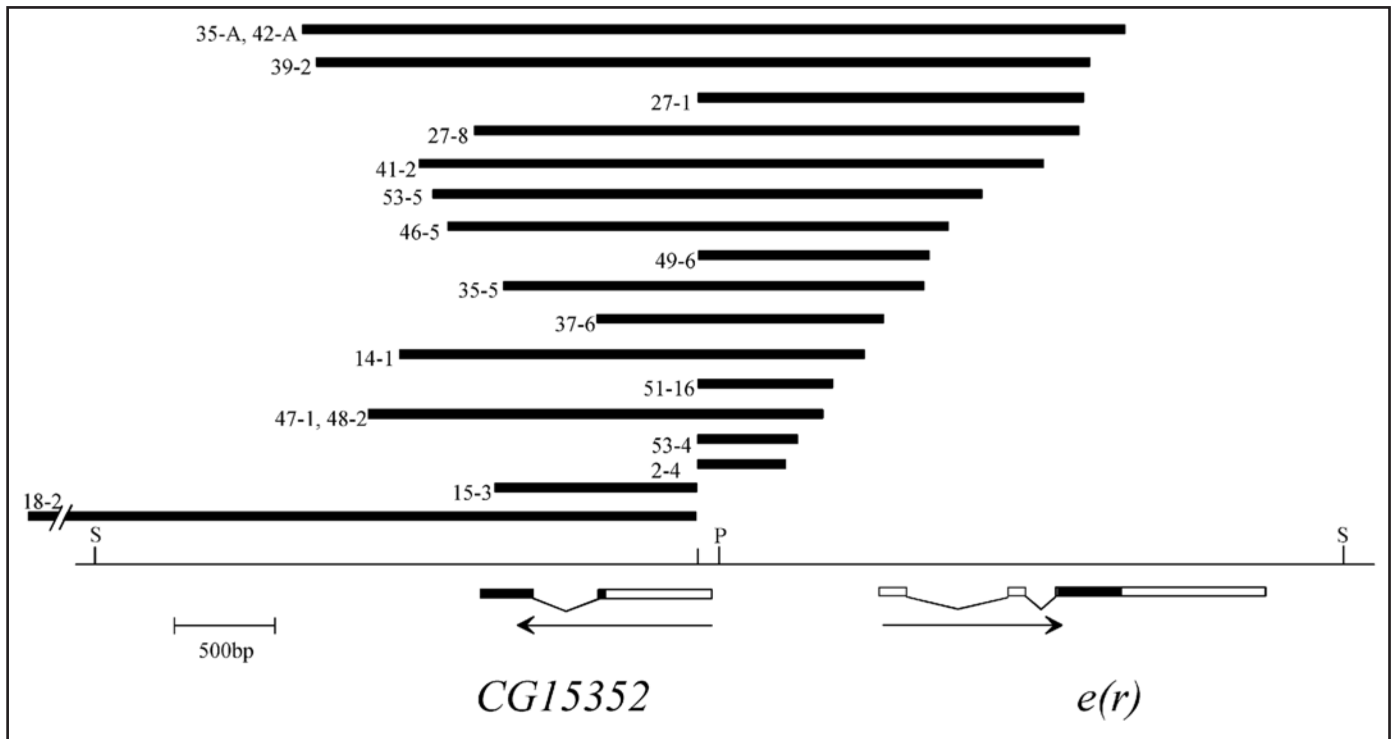

Figure 1. Deficiencies generated in the *CG15352-e(r)* region. The region is bounded by two *Sal*I sites (S) that define a 6.1-kb fragment that was previously shown to contain the *e(r)* gene in functional-rescue assays.[2] A *Pvu*II site (P) splits this region in two. The *Pvu*II-SalI fragment that contains the *e(r)* gene was used to make the *e(r)* transgene in *Tr[w⁺ e(r)⁺]^PS^*. The short vertical line just to the left of the *Pvu*II site designates the position of the starting P element, *P{SUPor-P, y^+}KG01830*. The endpoints of the deficiencies were determined by DNA sequencing (Table 1) and the extent of each deficiency is indicated by the length and position of the horizontal black bars. Deficiency *Df(1)e(r)18-2* extends beyond the region covered in this figure. The directions of transcription of *CG15352* and *e(r)* are indicated with arrows. Directly above the arrows, the exons of each gene are indicated as boxes and the amino-acid coding regions are designated as black boxes. The approximate 5′ end of *CG15352* is defined by two cDNAs (EL871517.1 and AA440305.2).

ry^{RK} Sb^1 Ser^1 P{Δ2-3}99B /Df(3R)C7, ry^{506} was used as a source of transposase for the mobilization of the P element. *$Tr[w^+\ e(r)^+]^{PS}$* is a transformation construct derived from *pP{CaSpeR-4}* and located on the second chromosome. It is marked with the mini w^+ gene of *pP{CaSpeR-4}*. *$Tr[w^+\ e(r)^+]^{PS}$* contains a 3063-bp, *Pvu*II-*Sal*I fragment that encompasses the *e(r)* transcribed region. It extends from -787 to 390 base pairs 3' of the last *e(r)* exon (Fig. 1). By all assays described in this paper, it confers wild-type *e(r)* activity. Transformants of this construct were produced by Genetic Services (Sudbury, MA). All Drosophila mutations and stocks, other than those generated in this study, are described in FlyBase.[9]

Drosophila melanogaster alleles, chromosomes, and transformation constructs used in this study:

y^1 P{SUPor-P, y^+}KG01830, Stock 14167: FBst0014167
P{SUPor-P, y^+}KG01830: FBti0023365
TM3, ry^{RK} Sb^1 Ser^1 P{Δ2-3}99B /Df(3R)C7, ry^{506}: FBst0305182
FM7c y^{31d} sc^8 w^a sn^{X2} v^{Of} g^4 B: FBba0000009
r^{hd1}: FBal0032541
Canton-S strain: FBst0000001
pP{CaSpeR-4}: FBmc0000178
$Tr[w^+\ e(r)^+]^{PS}$

Screen for deficiencies of *e(r)* induced by P-element excision. The screen utilized a P-element insertion, *P{SUPor-P, y^+}*.[8] This element, designated *P{SUPor-P, y^+}KG01830*, is inserted 895 bp upstream of the start of transcription of *e(r)* and within the noncoding region of the first exon of the gene *CG15352* and, importantly for purposes of the screen, is marked with y^+. The P element was mobilized in hemizygous males and putative deficiencies were isolated as yellow-body females. Since *e(r)* is on the X chromosome, excisions were isolated as heterozygous females in case the deficiencies were recessive lethal or sterile. The X-chromosome balancer, *FM7c y^{31d} sc^8 w^a sn^{X2} v^{Of} g^4 B*, was used in maintaining all of the mutations generated in this screen. Each yellow-body female was individually mated to *FM7c* males to establish balanced stocks. In generation one, homozygous *P{SUPor-P, y^+}KG01830* females were crossed to *TM3, ry^{RK} Sb^1 Ser^1 P{Δ2-3}99B /Df(3R)C7, ry^{506}* males en masse to introduce the P element *Δ2-3* as a source of transposase activity. In the second generation, *y P{SUPor-P, y^+}KG01830*; *TM3, ry^{RK} Sb^1 Ser^1 P{Δ2-3}99B /+* males were collected. In these males, the P-element *Δ2-3* induced the mobilization of *P{SUPor-P, y^+}KG01830*. Vials were set up containing 3–5 of these males and 5–10 females heterozygous for the X-chromosome balancer, *FM7c*. In generation three, the heterozygous *FM7c* females were scored for body color. Excisions were identified by the loss of y^+ as yellow-body females. Each yellow-body female was crossed to *FM7c* males to establish a stock. To ensure that independent mutational events were identified, the vial number of each mutant was recorded.

PCR and DNA sequence analyses of excisions. The majority of the excisions were male viable. The few that were recessive lethal were not examined further. DNA from representative males was isolated and used as templates for a number of different PCR primers to identify deficiencies and to determine their approximate endpoints. Once deficiencies were identified, flanking primers were used to amplify the region containing the deficiency. The amplified fragments were

Table 1 **Deficiencies showing breakpoints**

Deficiency	Left-Hand Breakpoint	Right-Hand Breakpoint
Df(1)e(r)2-4	-887	-453
Df(1)e(r)14-1	-2344	-68
Df(1)e(r)15-3	-1886	-896
Df(1)e(r)18-2	-18,685	-896
Df(1)e(r)27-1	-887	+1001
Df(1)e(r)27-8	-1982	+979
Df(1)e(r)35-5	-1837	+220
Df(1)e(r)35-A	-2821	+1201
Df(1)e(r)37-6	-1381	+25
Df(1)e(r)39-2	-2379	+1039
Df(1)e(r)41-2	-2252	+1002
Df(1)e(r)42-A	-2821	+1201
Df(1)e(r)46-5	-1204	+346
Df(1)e(r)47-1	-2495	-268
Df(1)e(r)48-2	-2495	-268
Df(1)e(r)49-6	-887	+240
Df(1)e(r)51-16	-887	-223
Df(1)e(r)53-4	-887	-398
Df(1)e(r)53-5	-2173	+511

The positions of the breakpoints were determined by DNA sequencing and numbered with respect to the start of transcription of *e(r)*.

isolated using a QIAquick PCR Purification Kit (QIAGEN, Inc.) and their DNA sequences determined using BigDye Terminator Cycle Sequencing (Applied Biosystems). These results gave the exact extent of each deficiency.

Enhancement of the mutant wing phenotype of r^{hd1}. Alleles of *e(r)* were previously isolated based on their ability to enhance the mutant wing phenotype of hypomorphic *rudimentary* alleles.[1,2,7] To test the ability of the deficiencies to enhance *rudimentary*, each was recombined onto an X chromosome containing the hypomorphic *r* allele, r^{hd1}. The measure of enhancement was based on a previously published scale for *r* mutants.[10] As controls, the original insertion, *P{SUPor-P, y^+}KG01830*, and an imprecise excision, 36-14, were also combined with r^{hd1}. The excision, 36-14, is technically an insertion of 23 bp from the P element and the 8-bp site duplication.

Viability of deficiency mutants. To test the viability of the deficiency mutants, crosses were performed to compare the viability of deficiency males with their heterozygous sisters. If there is no effect on viability, the number of males should equal the number of females.

Rescue of low viability of an *e(r)* null mutant. *y w Df(1)e(r)27-1* males, heterozygous for *Tr[w^+ $e(r)^+$]^{PS}*, were crossed to *y w Df(1)e(r)27-1/FM7c* females. The male and female progeny were counted and the presence of the *e(r)*$^+$ transgene was monitored by the presence of w^+. Rescue of low viability was measured by comparing the number of w^+ flies to *w* flies for each genotype.

Fertility tests of female. To test the fertility of each stock, ten females from each stock were mated to ten wild-type males in vials. Each set of flies was transferred to new vials as necessary. The females were allowed to lay eggs for ten days, after which the vials were cleared. The resulting progeny from each cross were scored.

In situ hybridization to *e(r)* and *r* transcripts. Whole-mount in situ hybridization was performed on Drosophila embryos or ovaries (Canton S strain) with digoxigenin-labeled *e(r)* cDNA probes (Roche Applied Science) for *e(r)* transcripts essentially as described by Tautz and Pfeifle,[11] with the modifications of Bodmer et al.[12]

RESULTS

Isolation of deficiencies in the *e(r)* region. It has previously been shown that P elements can be mobilized to create localized deficiencies.[1,13] This approach was used to generate deficiencies in the *e(r)* region. The starting P element, *P{SUPor-P, y^+}*, is located 895 bp upstream of the start of transcription of *e(r)* and within the first exon of *CG15352* (Fig. 1). Even though it is inserted within *CG15352*, it is not associated with any obvious mutant phenotypes. The stock is homozygous viable and fertile, and has no morphological mutant phenotypes. Importantly, it does not display any of the mutant phenotypes associated with *e(r)* mutations. Losses of this P element were identified by the loss of the dominant marker y^+. Among approximately 7000 heterozygous females in generation three, 393 yellow-body flies were obtained. Of these, six carried a recessive lethal on the X, while the remaining 387 are male viable. This study is restricted to these viable strains. PCR analyses of these excisions revealed that the large majority of them are precise or nearly precise excisions of *P{SUPor-P, y^+}KG01830*. The remaining 19 mutations are deficiencies, 11 of which extend into the *e(r)* transcribed region. The exact breakpoints of these deficiencies were determined by DNA sequencing. The extent of each deficiency with respect to the start of *e(r)* coding region is presented in tabular (Table 1) and graphic (Fig. 1) form. Each deficiency was given the designation *Df(1)e(r)* followed by a number designation, e.g., *Df(1)e(r)14-1*. In the body of the text each deficiency is identified by its number.

By the nature of the screen, all of the deficiencies extend out from the position of *P{SUPor-P, y^+}KG01830*, a point upstream of the 5' end of *e(r)*, and remove part of *CG15352* (Fig. 1). Eight of the deficiencies leave the transcribed region of *e(r)* intact—2-4, 14-1, 15-3, 18-2, 47-1, 48-2, 51-16, 53-4. Two of these, 15-3 and 18-2, leave the start of transcription of CG15352 intact, while six deficiencies remove the start of transcription - 2-4, 14-1, 47-1, 48-2, 51-16, 53-4. The remaining eleven deficiencies extend into the e(r) transcribed region - 27-1, 27-8, 35-A, 35-5, 37-6, 39-2, 41-2, 42-A, 46-5, 49-6, 53-5.

The eleven deficiencies that extend into the *e(r)* transcribed region range from 37-6 with a right-hand breakpoint at +25 to 35-A and 42-A with right-hand breakpoints at +1201 (Table 1). These last two deficiencies remove the entire *e(r)* amino acid coding region and by definition are *e(r)* null mutations. Data that will be discussed later indicate that all of the deficiencies extending into the *e(r)* transcribed region are phenotypically similar. Thus, all of them behave as null mutations. This is not too surprising, since they all remove the start of transcription of the *e(r)* gene.

Examination of the endpoints of the deficiencies reveals a certain amount of nonrandomness (Table 1). Five of the deficiencies remove DNA only to the right of the original insertion sight and two deficiencies remove DNA just to the left. In each case these deficiencies leave one copy of the 8-bp site duplication, part of the 31-bp inverted repeat, and a sequence generated during the excision-repair process. Two pairs of independent, but identical deficiencies were obtained (35-A and 42-A and 47-1 and 48-2).

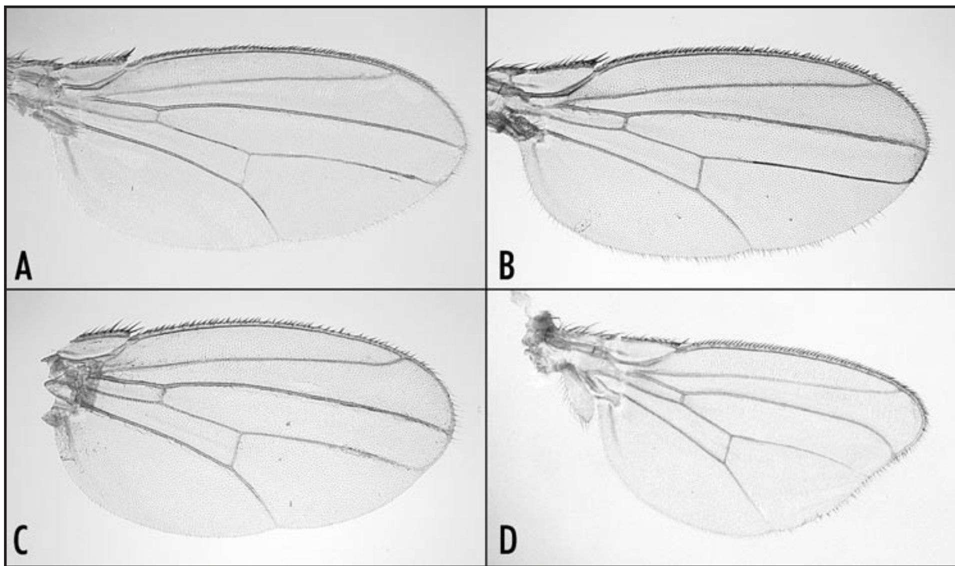

Figure 2. Wing phenotypes of various *e(r)* mutants. The effects of different *e(r)* alleles on the mutant wing phenotype of r^{hd1} are pictured. (A) $e(r)^{+}$ r^{hd1} male. In an $e(r)^{+}$ background, the wings of r^{hd1} are nearly wild-type in appearance. The posterior wing margin has a disorderly array of bristles, which is the easiest way to identify this mutant. (B) *P{SUPor-P,* y^{+}*}KG01830* r^{hd1} male. The P element insertion, *P{SUPor-P,* y^{+}*}KG01830*, does not have a noticeable effect on the r^{hd1} wing phenotype. The deficiencies *Df(1)e(r)18-2*, *Df(1)e(r)47-1*, *Df(1)e(r)48-2*, *Df(1)e(r)51-16*, and *Df(1)e(r)53-4* also do not enhance the mutant wing phenotype. (C) *Df(1)e(r)14-1* r^{hd1} male. Deficiency *Df(1)e(r)14-1* displays a very slight enhancement of the r^{hd1} wing phenotype. The wings are slightly more truncated than those of $e(r)^{+}$ r^{hd1}. (D) *Df(1)e(r)27-1* r^{hd1} male. Deficiency *Df(1)e(r)27-1*, which removes the 5′ end of the *e(r)* gene including part of the coding region, noticeably enhances the r^{hd1} wing phenotype. This enhancement is characteristic of the other *e(r)* deficiencies that remove part of the transcribed region, e.g., *Df(1)e(r)27-8*, *Df(1)e(r)35-5*, *Df(1)e(r)37-6*, *Df(1)e(r)39-2*, and *Df(1)e(r)41-2*.

The deficiencies were tested for a number of mutant phenotypes. When mutant phenotypes were detected, the ability of an $e(r)^{+}$ transgene, $Tr[w^{+}\ e(r)^{+}]^{PS}$, to rescue the mutant phenotypes was assayed. In all cases the mutant phenotypes associated with *e(r)* deletions could be rescued by $Tr[w^{+}\ e(r)^{+}]^{PS}$, verifying that the phenotypes were specific to deficiencies of *e(r)*.

Enhancement of the *rudimentary* mutant wing phenotype by *e(r)* deficiencies. The *e(r)* gene got its name because the original mutation was isolated as an enhancer of a hypomorphic *rudimentary*, *r*, mutant.[1,2] This allele and two subsequently isolated alleles are all P-element insertions into the same position in the first exon of *e(r)*, upstream of the start of translation.[2,7] To determine if this enhancement is a novel property of these P-element-associated alleles or is also a property of *e(r)* null mutations, the *e(r)* deficiencies were recombined onto the same chromosome as a hypomorphic *r* allele, r^{hd1} (Fig. 2A). All of the deficiencies that extend into the *e(r)* transcribed region enhance the mutant wing phenotype of r^{hd1}. Thus, a phenotype of null mutations of *e(r)* is an enhancement of the *rudimentary* mutant wing phenotype. This mutant enhancement is rescued by $Tr[w^{+}\ e(r)^{+}]^{PS}$ (data not shown). The phenotype of deficiency 27-1 is characteristic of this enhancement (Fig. 2D). This deficiency removes the 5' end of the *e(r)* gene including the coding region of the first 35 of the 104 amino acids. Deficiency 14-1 shows a very slight but reproducible enhancement of the wing phenotype of r^{hd1} and thus is acting as a hypomorphic *e(r)* allele (Fig. 2C). This deficiency has a right-hand breakpoint at -68, so it has probably removed sequences necessary for optimum expression of *e(r)*. On the other hand, *P{SUPor-P,* y^{+}*}KG01830* and the remaining deficiencies that end before the transcribed region of *e(r)* do not enhance the wing phenotype of r^{hd1} (Fig. 2B). Together these data indicate that *e(r)* null mutations enhance the *rudimentary* mutant wing phenotype and that this enhancement is not a unique property of the original P-element-associated alleles.

Low viability of *e(r)* null mutants. In handling the *e(r)* null stocks, we noticed that the *e(r)* null mutants had lower viability and that, unlike the hypomorphic mutants, it was impossible to establish homozygous stocks. In crosses to examine viability, hemizygous males have a much lower viability than their heterozygous sisters. In these crosses, the ratio of males to females ranged from 0.00 to 0.50 with an average of 0.39 (Table 2, top eight stocks). If there were no viability differences, then the ratio should have been 1.00. The remaining tested deficiencies showed near normal viability. The male-to-female ratio ranged from 0.93 to 1.13 with an average of 1.02. (Table 2). All of these deficiencies with normal viability have in common the fact that they do not extend into the *e(r)* transcribed region. Deletion 14-1, which in other tests showed reduced *e(r)* expression, nevertheless has good viability. This result is in agreement

Table 2 **Viability of *e(r)* Deficiencies**

Stock	Females	Hemizygous Males	Ratio: Males/ Females
Df(1)e(r)27-1/FM7c	734	243	0.33
Df(1)e(r)27-8/FM7c	219	18	0.08
Df(1)e(r)35-A/FM7c	575	272	0.47
Df(1)e(r)37-6/FM7c	1137	530	0.47
Df(1)e(r)39-2/FM7c	30	4	0.13
Df(1)e(r)41-2/FM7c	33	0	0.00
Df(1)e(r)42-A/FM7c	71	11	0.15
Df(1)e(r)46-5/FM7c	50	25	0.50
Df(1)e(r)14-1/FM7c	542	502	0.93
Df(1)e(r)18-2/FM7c	530	540	1.02
Df(1)e(r)47-1/FM7c	271	300	1.11
Df(1)e(r)51-16/FM7c	143	162	1.13
Df(1)e(r)53-4/FM7c	181	195	1.08
$e(r)^{p2}$*/FM7c*	344	333	0.97

Females from each tested stock were crossed to *FM7c* males. The number of heterozygous females and hemizygous males were counted. The ratio of the males to females is a measure of the viability of each tested genotype. The top eight stocks are deficiencies that extend into the *e(r)* transcribed region. The remaining five deficiencies do not extend into the *e(r)* transcribed region. The allele, $e(r)^{p2}$ is a P element insertion into the first exon of *e(r)*.

Table 3 **Rescue of decreased viability with *Tr[w⁺ e(r)⁺]^PS***

Females				Males		
Df(1)e(r)27-1/FM7c		*Df(1)e(r)27-1/Df(1)e(r)27-1*		*Df(1)e(r)27-1*		*FM7c*
e(r)⁺	+	*e(r)⁺*	+	*e(r)⁺*	+	
384	334	362	116	333	137	644

The females and males in this table were generated from the following cross: *y w Df(1)e(r)27-1/FM7c X y w Df(1)e(r)27-1/Y; Tr[w⁺ e(r)⁺]^PS/+*. This cross was designed to examine the viability of *e(r)* null females and males in the presence and the absence of an *e(r)⁺* transgene. The presence of the transgene was monitored by the presence of the w⁺ gene on the transformation construct. This cross showed that the low viability of *e(r)* null females and males can be rescued by an *e(r)⁺* transgene.

Table 4 **Fertility of *e(r)* mutant females**

Tester Females	Females	Males
Df(1)e(r)27-1	206	92
Df(1)e(r)27-1	257	67
Df(1)e(r)14-1	1083	1057
Df(1)e(r)1-14	1169	1140
36-14	1400	1286
36-14	1115	1099
Df(1)e(r)27-1/FM7c, e(r)⁺	1164	777[a]
Df(1)e(r)27-1; Tr[w⁺ e(r)⁺]^PS/+	1081	873[b]

[a]This total consists of *Df(1)e(r)27-1* males (252) and *FM7c* males (525). [b]This total consists of *Df(1)e(r)27-1* males (241) and *Df(1)e(r)27-1; Tr[w⁺ e(r)⁺]^PS/+* males (632).

with the hypomorphic mutant, *e(r)^p2*, which also has good viability (Table 2). Deletions 14-1, 47-1, and 48-1 remove the start of transcription, the first two exons, and the majority of the protein-coding region of *CG15352*, while leaving *e(r)* intact. These deficiencies also have good viability indicating that a deficiency of *CG15352* does not affect the viability of the flies. In addition, these flies are fertile, allowing the deficiencies to be maintained as homozygous stocks.

Homozygous *e(r)* null females show a similar decrease in viability as was seen in hemizygous males (Table 3). In this cross, homozygous null females were approximately 35% as viable as their heterozygous sisters (116 vs. 334). This cross was designed to determine if the *e(r)⁺* transgene, *Tr[w⁺ e(r)⁺]^PS*, could rescue the decreased viability of null females and males. The *e(r)* null mutations are on an X chromosome marked with *white*, *w*, and the autosomal construct is marked with *w⁺*. This enabled the presence of the transgene to be monitored by the eye color of the flies. A zygotic copy of the transgene rescues the low viability of the null females (116 vs. 362) and the null males (137 vs. 333). As expected the transgene has little effect on the viability of the heterozygous females (334 vs. 384). This last result also serves as a control for the transmission of the chromosome containing the transgene. The data confirm that the decrease in viability maps to a loss-of-function of the *e(r)* gene and not to *CG15352* or an another mutation on the X chromosome.

Low fertility of *e(r)* null females. We were unable to establish homozygous stocks of *e(r)* null mutants, however the fertility of the hemizygous males was sufficient to maintain the stocks if heterozygous females were utilized (S. I. Tsubota, unpublished data). We decided to examine the fertility of the mutant females to determine if that was a factor in the low fecundity of the stock. The fertility of a null mutant (27-1), a hypomorphic mutant (14-1), an *e(r)⁺* strain (36-14), 27-1/*FM7c* heterozygous females, and 27-1 homozygous females with one copy of *Tr[w⁺ e(r)⁺]^PS* was measured (Table 4). Wild-type males were used in all of the fertility tests. The number of female progeny served as a measure of fertility since they all carried an *e(r)⁺* allele, and therefore should not have viability differences due to low *e(r)* expression. The fertility of *e(r)* null females was greatly reduced in comparison to that of the heterozygous strain. In two separate crosses the number of female progeny was substantially less than that of the heterozygous strain (206 and 257 vs. 1164). Females from the hypomorphic strain, 14-1, had good fertility, comparable to that of the heterozygous strain (1083 and 1169 vs. 1164). The *e(r)⁺* strain, 36-14, was isolated in the same screen that generated 27-1 and 14-1. It is actually an imprecise excision of the P element that has left behind 31 bp. In all of our tests, it behaves like an *e(r)⁺* allele. Females from this strain also had very good fertility (1400 and 1115 vs. 1164). These data clearly demonstrate that *e(r)* null females have reduced fertility. The reduced fertility of 27-1 homozygous females could be rescued by *Tr[w⁺ e(r)⁺]^PS*. These females had a fertility measurement similar to that of 27-1/*FM7c* females (1081 vs. 1164). These data also demonstrate that the reduced fertility of 27-1 homozygous females is caused by the deletion of the *e(r)* gene and not by a mutation in *CG15352* or another gene on the X chromosome.

In situ hybridization to *e(r)* transcripts. It has previously been shown that *e(r)* encodes two transcripts of 1237 and 1016 bases in length.[2] This study also showed that the two transcripts are generated by alternative polyadenylation sites and that the longer of the two transcripts is primarily found in females. These results and the low fertility of an *e(r)* null mutation suggest that *e(r)* may be expressed in the ovaries of the female. To examine this possibility, in situ hybridization to the *e(r)* mRNA was performed on dissected ovaries. The *e(r)* mRNA clearly localizes to the nurse cells of the ovary (Fig. 3A). The maternal *e(r)* transcripts are deposited into the developing egg, shown by the even distribution of *e(r)* transcripts in the blastoderm embryo (Fig. 3B). This expression pattern indicates an ovarian function for *e(r)* and can explain the low fertility of *e(r)* null females.

DISCUSSION

Function of *e(r)*. The high conservation of *e(r)* suggests that it encodes a vital function,[3] however previous mutagenic analyses revealed that *e(r)* hypomorphic mutations were viable, fertile, and morphologically wild-type and provide no compelling argument for the high conservation of the gene.[2,7] Data from the current study indicate that in *Drosophila melanogaster*, deficiencies of the *e(r)* gene are viable and morphologically wild-type, but that the null males and females have a decreased viability and that the females have a decreased fertility. These decreases are not seen with the hypomorphic alleles. The combination of low viability and fertility makes it impossible to establish homozygous stocks of *e(r)* deficiencies, however the addition of an *e(r)⁺* transgene makes the establishment of homozygous stocks routine. This is in contrast to the hypomorphic alleles, which can be maintained as homozygous stocks. Thus, while *e(r)* null mutants are viable, in evolutionary terms their fitness level is essentially zero. This could explain the high conservation of the gene.

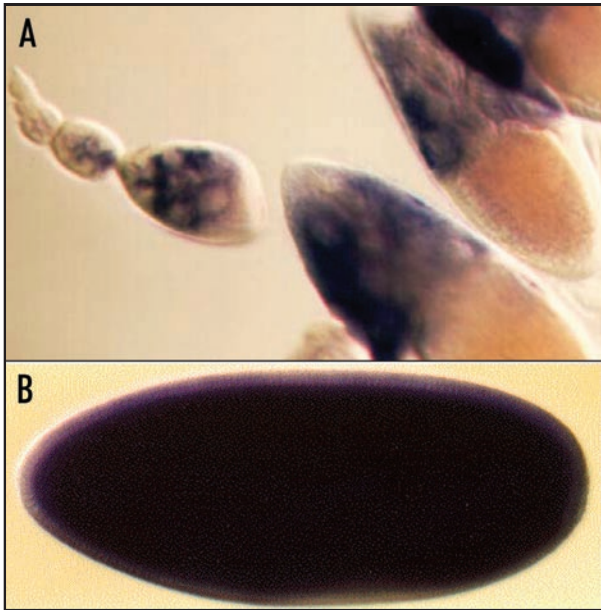

Figure 3. In situ hybridization to the *e(r)* transcripts. A full-length cDNA probe was used for these experiments. (A) Developing ovarioles. Expression is seen in the nurse cells of the ovaries. (B) Blastoderm embryo. The transcripts synthesized in the nurse cells are deposited into the developing egg so that an even distribution of maternal transcripts is seen in the early embryo.

We previously showed that *e(r)* encodes two transcripts of 1237 and 1016 bases in length.[2] This study also showed that the two transcripts are generated by alternative polyadenylation sites and that the longer of the two transcripts is primarily found in females. The present study demonstrates that *e(r)* is expressed in the nurse cells of the ovary and that the transcripts are maternally deposited into the egg. The fact that the longer *e(r)* transcript is primarily female-specific and that *e(r)* is expressed in the nurse cells suggests that the longer transcript is localized to the nurse cells. Indeed, the large majority of the longer transcript is synthesized in the ovaries of the female.[14] In addition, the alternative polyadenylation of the *e(r)* transcript in the ovaries is regulated by *Sex-lethal*.[14]

The pleiotropic effects of *e(r)* null mutations and their interaction with different genes point to the involvement of *e(r)* in a number of different pathways-pyrimidine biosynthesis (*rudimentary*[2]), nerve cell specification (*Notch*[15]), and ovarian development. The enhancement of the mutant phenotypes of the hypomorphic mutations of *rudimentary* and *Notch* also indicate that *e(r)* normally acts to activate these genes or the pathways that they are in. The Drosophila *e(r)* protein localizes to the nucleus[6] and the Xenopus *e(r)* protein has been implicated as a transcription cofactor.[5] We would argue that *e(r)* encodes a transcription coactivator with a necessary but not a vital function in a number of different pathways.

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