Genetic and Molecular Analysis of Region 88E9;88F2 in *Drosophila melanogaster*, Including the *ear* Gene Related to Human Factors Involved in Lineage-Specific Leukemias

Claudia B. Zraly,[1] Yun Feng[1,2] and Andrew K. Dingwall[3]

Department of Biology, Syracuse University, Syracuse, New York 13244-1270

Manuscript received September 13, 2001
Accepted for publication December 12, 2001

ABSTRACT

We identified and characterized the Drosophila gene *ear* (***E**NL/**A**F9-**r**elated*), which is closely related to mammalian genes that have been implicated in the onset of acute lymphoblastic and myelogenous leukemias when their products are fused as chimeras with those of human *HRX*, a homolog of Drosophila *trithorax*. The *ear* gene product is present in all early embryonic cells, but becomes restricted to specific tissues in late embryogenesis. We mapped the *ear* gene to cytological region 88E11-13, near *easter*, and showed that it is deleted by $Df(3R)ea^{5022rx1}$, a small, cytologically invisible deletion. Annotation of the completed Drosophila genome sequence suggests that this region might contain as many as 26 genes, most of which, including *ear*, are not represented by mutant alleles. We carried out a large-scale noncomplementation screen using $Df(3R)ea^{5022rx1}$ and chemical (EMS) mutagenesis from which we identified seven novel multi-allele recessive lethal complementation groups in this region. An overlapping deficiency, $Df(3R)Po^{4}$, allowed us to map several of these groups to either the proximal or the distal regions of $Df(3R)ea^{5022rx1}$. One of these complementation groups likely corresponds to the *ear* gene as judged by map location, terminal phenotype, and reduction of EAR protein levels.

MANY human acute leukemias [acute lymphoblastic (ALL) and acute myelogenous leukemias] have been associated with chromosome disruptions that affect a gene residing at the 11q23 locus. The disrupted gene encodes a protein with limited, though significant, homology to the Drosophila trithorax (trx) protein and was thus named *HRX* (*H*uman tritho*R*a*X*). The HRX gene (also known as *ALL-1* and *MLL*) is rearranged in nearly 20% of all acute leukemias and >75% of infant acute leukemias (reviewed in Bernard and Berger 1995; Waring and Cleary 1997), as well as 85% of secondary leukemias resulting from treatment of neoplastic diseases with Topoisomerase II inhibitors (Super *et al.* 1993). Various chromosomal aberrations affecting *HRX* have been described, including internal duplications, deletions clustered around exon 8, and chimeric fusions with as many as 30 different genes on other chromosomes (Tkachuk *et al.* 1992; Ford *et al.* 1993; Rowley 1993, 1995, 1998; Ridge and Wiedemann 1994; Schichman *et al.* 1994; Lochner *et al.* 1996; Rubnitz *et al.* 1996; Waring and Cleary 1997; Saha *et al.* 1998).

HRX, like Drosophila *trx*, is widely expressed during embryogenesis and its transcripts have been observed in a variety of cell lines, including those derived from lymphoid, myeloid, epithelial, hepatic, and neural cell lineages. On the basis of its physical similarity to *trx*, it has been suggested that *HRX* encodes a DNA-binding transcription factor and is involved in the regulation of genes controlling human development and/or differentiation (Hanson *et al.* 1999). The *trx* gene is required for the maintenance, but not initiation, of homeotic (*Hox*) gene expression in flies (Mazo *et al.* 1990; Breen and Harte 1993; Breen *et al.* 1995; Ingham 1998). Similarly, *HRX* knockout mice were embryonic lethal and displayed skeletal and hematopoietic defects due to a failure to sustain the expression of the mammalian *Hox* genes, including *HoxA7* and *HoxC9* (Yu *et al.* 1995, 1998). *In vitro* differentiation assays of *HRX* +/+, +/−, and −/− yolk sac progenitor cells showed that *HRX* is required for myeloid and macrophage differentiation of early hematopoietic progenitors (Hess *et al.* 1997).

A surprising discovery was that many of the disruptions of *HRX* were actually reciprocal translocations that fused the HRX protein to as many as 30 different partner proteins on other chromosomes, in every known case creating hybrid proteins (reviewed in Cimino *et al.* 1998). Stabilization of the truncated HRX protein by fusion to a variety of partners may alone be sufficient for tumorigenesis (Dobson *et al.* 2000). However, mounting evidence has implicated two of the more common HRX fusion partners, the ENL and AF9 proteins (which are highly similar to each other), as serving a critical role in the leukemogenic potential of the chimeric proteins (Nakamura *et al.* 1993; Rubnitz *et al.* 1994).

[1]These authors contributed equally to this work.

[2]*Present address:* Genome Therapies, Waltham, MA 02453.

[3]*Corresponding author:* Department of Biology, 108 College Place, Lyman 422A, Syracuse University, Syracuse, NY 13244.
E-mail: akdingwa@syr.edu

In particular, the rapid rate at which *HRX-AF9* chimeric mice develop tumors and gain-of-function features of *HRX-ENL* and *-AF9* fusions (Slany *et al.* 1998; Adler *et al.* 1999) suggests that these *HRX* partners may have important biological roles in tumorigenesis. In contrast to wild-type HRX, expression of HRX-ENL and HRX-AF9 fusions in cell lines can abrogate the apoptotic effects mediated through the GADD34 protein following ionizing radiation, suggesting that the fusions function to negatively regulate cell death induced by DNA damage (Adler *et al.* 1999).

Several lines of evidence suggest that the ENL and AF9 proteins have roles in myeloid and/or lymphoid cell lineage determination as positive and/or negative transcription factors (Rubnitz *et al.* 1994; Corral *et al.* 1996; Lavau *et al.* 1997; Slany *et al.* 1998; Dobson *et al.* 1999; Garcia-Cuellar *et al.* 2000, 2001). ENL and AF9 are highly related to each other at their amino and carboxy termini (>90% identity). Interestingly, the conserved C termini of both ENL and AF9 can form strong protein contacts with a novel member of the human Polycomb protein family, hPc3, that functions as a transcriptional repressor (Garcia-Cuellar *et al.* 2001). In flies and humans, members of the Polycomb group of proteins are responsible for maintenance of repression of target loci through effects on chromatin (reviewed in Gebuhr *et al.* 2000; Brock and van Lohuizen 2001). The ENL protein has an intrinsic transcriptional activation capability *in vivo* that is mediated through the hydrophobic carboxy-terminal region and this same region is both necessary and sufficient for HRX-ENL leukemogenic function (Rubnitz *et al.* 1994). ENL and AF9 also share significant although limited homology with the yeast protein TFG3/TAF30/ANC1 (Rubnitz *et al.* 1994). The distantly related yeast protein is present in several transcription-promoting complexes, including TFIID, TFIIF, and the SWI/SNF chromatin-remodeling complex (Cairns *et al.* 1996). Further, TFG3 can form specific associations with the SNF5 subunit of the SWI/SNF complex, leading to the hypothesis that chromatin remodeling might be involved in acute leukemias. Also consistent with this, HRX-ENL fusion proteins can associate with human SNF5/INI1 (Adler *et al.* 1999). This hypothesis seemed especially compelling because the human homolog of *SNF5*, *INI1/SMARCB1*, is disrupted in almost all cases of aggressive childhood rhabdoid cancers (Sevenet *et al.* 1999) and the fly homolog of *SNF5*, known as *snr1*, is essential for normal development (Dingwall *et al.* 1995).

Although the structure-function studies summarized above provide compelling evidence that the HRX fusion partners AF9 and ENL are responsible for aspects of the leukemic potential, there are no reports of any *ENL* or *AF9* mutation independent of the *HRX* fusions, nor are there any "knock-out" studies on *ENL* or *AF9* in any mammalian system. To ascertain the biological functions of *ENL* and *AF9*, and to determine whether they interact functionally with factors required for cell fate control, we sought first to determine whether *ENL* and *AF9* were conserved. Here we report our identification and characterization of a Drosophila homolog of both *ENL* and *AF9*. We have named the fly gene *ear* (***E**NL/**A**F9-**r**elated*), which was previously referred to as *ENL/AF9* (Aoyagi and Wassarman 2000). We mapped the Drosophila *ear* gene to the 88E11-13 cytological region of the polytene chromosomes near the *easter* (*ea*) gene that encodes a serine protease. Subsequently, annotation of the completed fly genome sequence confirmed our mapping results. Similar to the mammalian *ENL* and *AF9*, there are no reported mutations within the *ear* gene. In addition to understanding the potentially vital function of *ear* in development, analysis of loss-of-function mutations in the gene should provide insight into the molecular mechanisms underlying the oncogenic potential of the mammalian genes. We used a small 60-kb deficiency that removed both *ea* and *ear* to carry out a noncomplementation screen to identify specific mutant alleles of *ear*. Although our putative *ear* mutant alleles did not exhibit significant genetic interactions with genes involved in growth control or transcriptional regulation, they did suggest that *ear* was essential for normal development.

MATERIALS AND METHODS

Fly strains: Flies were raised on yeast/cornmeal/dextrose medium at 25° in a humidified incubator (50–70% relative humidity) unless otherwise noted. The mutations and chromosome aberrations used in this study are summarized in Table 1. All markers and special chromosomes used that are not listed in Table 1 are described in Lindsley and Zimm (1992) or in FlyBase (http://flybase.bio.indiana.edu). The $Df(3R)ea^{5022rx1}$ deletion is an X-ray revertant of a dominant gain-of-function *easter* mutant, ea^{5022} (Erdelyi and Szabad 1989). The $Df(3R)ea^{5022rx1}$, *mwh, e* strain was kindly provided by K. V. Anderson (Memorial Sloan Kettering Cancer Center, New York) and the $Tm2^{J8}$ and $Tm2^{S2}$ strains by A. Kreuz (Villa Julie College, Stevenson, MD). Additional strains used in our study were obtained from the Bloomington Stock Center or the Umeå Stock Center.

The third chromosome tester strain used for the EMS mutagenesis screen, carrying both the $Df(3R)ea^{5022rx1}$ and $snr1^{R3}$ mutations but lacking the *ebony* (*e*) allele, was generated by recombination. The double-mutant chromosome was verified by the loss of the *e* mutation and failure to complement both the $snr1^{R3}$ allele and $Df(3R)Po^{4}$ that overlaps with the $Df(3R)ea^{5022rx1}$ deletion. The deletion of *ea* and *ear* on the tester chromosome was verified by quantitative Southern blot analysis (see Figure 5; data not shown). Approximately 0.3% (1 out of 330) of the tested recombinant chromosomes were of the appropriate genotype.

A strain isogenic for the third chromosome that carried appropriate recessive visible markers *red* and *e* and no recessive lethal mutations was generated as described in Ashburner (1989). A third chromosome balancer carrying a ubiquitously expressed green fluorescent protein (GFP) transgene ($w; Sb^{1}/TM3, Act\text{-}GFP, Ser^{1}$) obtained from the Bloomington Stock Center was used to identify homozygous mutant embryos and larvae.

TABLE 1

Drosophila strains used in this study

Genotype	Recessive lethal	Relevant phenotypes	Cytological position[a]
Deficiencies			
Df(3R)ea^{5022rx1}, mwh, e/TM3, Sb, Ser	+	Dominant flightless	88F2-88F7
Df(3R)Po^{4}/TM6B	+	Dominant flightless	88F7; 89A11-13
Df(3R)Po^{2}/TM6B	+		89A1-2; 89A11-13
P-element insertions			
mwh^{1} red^{1} P{hs-neo}137 e^{1}, l(3)/TM3, Sb^{1} Ser^{1}	+		88F
ry^{506} P{ry$^{+t7.2}$=PZ}Tm1^{02299}/TM3, ry^{RK} Sb^{1} Ser^{1}	+		88F1-2
y^{1} w^{1118}; P{w^{+mC}=lacW}l(3)j6A6^{j6A6}/TM6B, Tb^{1}	+		88F7-8
ry^{506} P{ry$^{+t7.2}$=PZ}l(3)06490^{06490}/TM3, ry^{RK} Sb^{1} Ser^{1}	+		88F7-8
ry^{506} P{ry$^{+t7.2}$=PZ}l(3)05279^{05279}/TM3, ry^{RK} Sb^{1} Ser^{1}	+		89A1-2
mwh^{1} red^{1} P{hs-neo}l(3)neo43^{1} e^{1}/TM3, ry^{RK} Sb^{1} Ser^{1}	+		88E9-10
y^{1} w^{1118}; P{w^{+mC}=lacW}l(3)j6A3^{j6A3}/TM3, Sb^{1}	+		88E11-12
ry^{506} P{ry$^{+t7.2}$=PZ}Hsc70-4^{03550}/TM3, ry^{RK} Sb^{1} Ser^{1}	+		88E8-9
Gene mutations			
Tm2^{l8}/TM3, Sb, e	+	Dominant flightless	88F5 (*Tm2*)
Tm2^{S2}/TM3, Sb, e	+	Dominant flightless	88F5 (*Tm2*)
st^{1} snk^{1} sr^{1} ea^{1} e^{s} ca^{1}/TM3, Sb^{1}	Maternal effect		88F2 (*ea*)
ru^{1} st^{1} ea^{14} spz^{3} ca^{1}/TM1	Maternal effect		88F2 (*ea*)
w^{1118}; snr1^{SR21}/TM6B	+		83A (*snr1*)
w^{1118}; snr1^{R3}/TM6B	+		83A (*snr1*)
w^{1118}; snr1^{R3}, Df(3R)ea^{5022rx1}/TM6B	+	Dominant flightless	
w^{1118}; red, e (isogenic)	−		

[a] Available cytological positions of indicated loci were obtained from the BDGP database except for the breakpoints of *Df(3R)ea^{5022rx1}*, which were based on our estimation from this study.

Molecular analysis of the *ear* gene: An *ENL* cDNA (Rubnitz *et al.* 1994) was labeled by random priming and used to screen a Drosophila λgt11 embryonic cDNA library (Clontech, Palo Alto, CA) using hybridization conditions as described previously (Dingwall *et al.* 1995). Positive hybridizing clones were isolated and sequenced. Only partial clones containing the 5′ end of the *ear* gene were obtained in this screen. Additional portions of the gene, including the 3′ end, were obtained by screening a Canton-S embryonic cDNA library constructed in the Uni-ZAP XR vector (Stratagene, La Jolla, CA) using the partial *ear* cDNA. A 3.56-kb full-length *ear* cDNA clone (CZ1-11) was generated by combining two partial cDNAs in the vector pZErO-2 (Invitrogen, Carlsbad, CA). The cDNA was used to isolate Drosophila genomic phage clones (*gp5* and *gp9*) that spanned the *ear* locus from an isogenic strain library constructed in λEMBL3 (Tamkun *et al.* 1991). The full sequences of both the cDNA and genomic DNA spanning the *ear* locus were obtained on both strands using specific primers. Sequence alignments and database searches were performed using the BLAST program.

RNA and protein analyses: Developmentally staged total RNA as well as 0- to 20-hr embryonic poly(A)$^{+}$ RNA were prepared as described (Tamkun *et al.* 1992; Dingwall *et al.* 1995). Random-primed genomic DNA and cDNA probes were used for the Northern blot hybridizations using standard conditions (Sambrook *et al.* 1989).

Polyclonal antibodies directed against the N-terminal portion of the EAR protein [amino acids (aa) 32–198] were produced in rats using an *Eco*RI-linkered 502-bp *Bgl*II-*Bam*HI fragment fused to glutathione-S-transferase in the vector pGEX2TK (Amersham Pharmacia, UK). Overnight collections of Oregon-R embryos were fixed and immunostained for localization of the EAR protein using whole rat antisera as described previously (Dingwall *et al.* 1995).

Antibodies directed against the C-terminal portion of the EAR protein (aa 715–931) were produced using a *Xho*I-*Eco*RI fragment fused to a poly-histidine moiety in the vector pTrcHis (Invitrogen). C-terminal specific antibodies produced in rats were partially purified by ammonium sulfate precipitation to remove endogenous serum proteins and used for Western blot analyses. Native protein extracts were prepared from control (Oregon-R), *Df(3R)ea^{5022rx1}/TM6B*, and EMS mutant strains (balanced over *TM6B*) for quantitative Western blots. Overnight embryo collections (0–16 hr) were dechorionated and native extracts prepared as described previously (Dingwall *et al.* 1995). Determinations of protein concentration were made using the BioRad Protein Assay reagent (Bio-Rad, Hercules, CA) according to the procedure of Bradford (1976) with bovine serum albumin as a standard. Extract concentrations and quality were verified by staining SDS-PAGE separated proteins with Coomassie Brilliant Blue. Determinations of EAR protein levels were assessed by quantitative Western blots. Protein extracts (100 μg each) were subjected to 10% SDS-PAGE, followed by electrophoretic transfer to nitrocellulose. The upper and lower portions of the Western blot were probed with antibodies to the EAR and SNR1 proteins (Dingwall *et al.* 1995), respectively, followed by detection with appropriate HRP-conjugated secondary antibodies (Jackson Immuno Research Labs, West Grove, PA) and chemiluminescent detection (Supersignal, Pierce Chemical, Rockford, IL). Following application of the chemiluminescent substrate, blots were first briefly exposed to X-ray film and then transferred to a Kodak/NEN 440CF Digital Imaging Station for direct capture of the luminescent signals. Quantification and analyses were performed using the Kodak Digital Science 1D Image Analysis software (Kodak, Rochester, NY).

EMS mutagenesis and F_2 screening: EMS mutagenesis of *red, e* flies was carried out as described by Ashburner (1989).

TABLE 2

Summary results of the EMS screen

	No. of lines	% of lines tested
F_1 males tested	18,664	
Male sterile lines	602	3.2
Male fertile lines	18,062	96.8
Recessive lethal lines recovered	44	0.24
Recessive lethal lines in multiple allele complementation groups	38	86.4[a]
Recessive lethal lines in single allele complementation groups	6	13.6[a]
Temperature-sensitive mutants	8	
Mutants related to *snr1*	4	
Total complementation groups	14	
Groups with multiple alleles	8	
Groups with single allele	6	

[a] Percentages are based on a total of 44 recessive lethal mutations identified in this screen.

Flies were allowed to recover for 24 hr prior to mating with *w⁻; TM3, Sb, Ser/TM6B, Hu, Tb* virgin females in a bottle (ratio of three females per one male). For maximal reproduction, the flies were turned into fresh bottles every 2–3 days. After 4–5 days, mutagenized males were discarded to ensure that only postmeiotically treated chromosomes were tested. The above crosses were carried out at 25°.

Single *red, e/TM6B, Hu, Tb* male progeny (each potentially representing one mutagenized third chromosome) were mated with two to three virgin females of the tester line $snr1^{R3}$, $Df(3R)ea^{5022rx1}$*/TM6B, Hu, Tb* at 29°. The crosses were visually examined after 2–3 days and, if necessary, additional virgin females were added. The crosses were checked 11 or 12 days afterward by visually examining the pupae on the side of the vials. A total of 44 independent rounds of mutagenesis (EMS feeding) were performed (summarized in Table 2).

New mutant lines were established by crossing siblings from the above F_2 cross. Temperature sensitivity was determined by carrying out complementation crosses with the deficiency tester chromosome at 18°, 25°, and 29°. The appearance of nonbalanced progeny at 18°, but not at 29°, indicated the isolation of a potential temperature-sensitive mutation. To determine whether the observed lethality was due to the loss of *snr1* function, each mutant line was tested for complementation of two independent null alleles of *snr1* ($snr1^{R3}$ and $snr1^{SR21}$; A. Dingwall and S. Domville, unpublished results) and the nonrecombinant parental $Df(3R)ea^{5022rx1}$*, mwh, e* strain.

Complementation groups were identified among the recessive lethal mutations by *inter se* crosses performed at 29°. Flies from each complementation group were crossed to existing mutations in genes mapped to the *ea* region to test for potential allelism. Mutant lines tested included at least the following: $snr1^{R3}$, $Tropomyosin\ 1\ (Tm1)^{02299}$, $Tm2^{I8}$, $Tm2^{S2}$, and an overlapping deficiency $Df(3R)Po^{4}$. At least 50 progeny were scored from each cross. The nomenclature used for assignment of gene names (complementation group) conforms to Lindsley and Zimm (1992). Genes were designated *l(3)88EF (b–h)* on the basis of their inclusion within the $Df(3R)ea^{5022rx1}$ that removes a region spanning from within *Tm1* (mapped to 88E9-12) to distal of *Anon-Becker 2* at 88F1-2. A previously reported recessive lethal *P* insertion that included a portion of an *engrailed* enhancer was mapped by *in situ* hybridization to the 88EF region and was designated *l(3)88EFa* (Kassis *et al.* 1991). This insertion mutation is no longer extant (J. Kassis, personal communication).

The developmental period at which lethality first occurs was determined as follows. Flies from each mutant line (*e*/TM6B*) and $Df(3R)ea^{5022rx1}$*/TM6B* were first crossed to a w^{1118} fly stock. Progeny that did not carry marked chromosomes [*e*/+* and $Df(3R)ea^{5022rx1}$*/+*] were crossed to each other for 24 hr on standard cornmeal/dextrose-agar food and then at least 100 eggs were collected on molasses-agar plates and examined for embryonic lethality. If >10% of the embryos failed to hatch after 36–48 hr, the mutant was scored as an embryonic lethal. Lethality during larval or pupal stages was determined by crossing each mutant (*e*/TM6B*) to the *ea* deficiency line [$Df(3R)ea^{5022rx1}$*/TM6B*] at 29° and periodically examining the vials. The developmental stage at which nontubby progeny were last apparent was defined as the lethal phase in those cases (Table 3).

Larval cuticles were prepared for examination by light microscopy using standard procedures (Stern and Sucena 2000). Embryos (0–8 hr after egg laying) were collected and aged for an additional 20 hr at 25°, fixed, and mounted in Hoyer's medium. Cuticles were examined using darkfield optics at ×100 magnification on an Olympus BX-60 microscope.

RESULTS

Molecular analysis of the *ear* gene: An *ENL* cDNA was used to screen several Drosophila embryonic cDNA libraries. Positive hybridizing clones were selected and purified and cDNA fragments were sequenced. Our sequencing results revealed that all of the clones derived from the same gene. As none of the isolated cDNAs carried the complete gene, we generated a full-length 3562-bp cDNA from several partial clones (CZ1-11) and sequenced to verify the open reading frame (ORF; Figure 1A). The deduced ORF could encode a protein of 931 aa with a predicted molecular mass of 100 kD. Stop codons upstream of the predicted AUG suggested that no other potential initiation codon contained in the cDNA could be used to initiate the open reading frame. Several potential polyadenylation signals are present downstream of the predicted stop codon with an ~450-bp 3′ untranslated region preceding the poly(A) tail. A BLAST analysis of the complete cDNA sequence showed the highest degree of similarity with both the human *ENL* and *AF9* genes ($P < 10^{-34}$). On the basis of these similarities, we have named the Drosophila gene *ear*, for ***E**NL/**A**F9-**r**elated*. The highest degree of conservation was observed at the N and C termini between EAR and ENL or AF9, with 73% similarity (50% identity) among the first 139 aa and 73% similarity (50% identity) among the C-terminal 60 aa (Figure 1B). These same regions in ENL and AF9 are nearly 92% similar (Rubnitz *et al.* 1994). Although none of the proteins share any significant similarity within the large central portion of their coding regions, the ENL and AF9 proteins contain a large region enriched with serine and proline residues, whereas the EAR protein contains a similar, although larger region, with a high proportion of serine (11%) and lysine (17%) residues. The strong conservation

TABLE 3

Characterization of multi-allele lethal complementation groups in 88EF

Group	No. of alleles (temperature-sensitive alleles)	Complementation of:		Lethal phase
		Df(3R)ea^{5022rx1}	*Df(3R)Po*^{4}	
l(3)88EFb	7 (0)	No	No	Late embryonic/larval[a]
l(3)88EFc	5 (0)	No	Yes	Larval
l(3)88EFd	4 (1)	No	No[b]	Late larval/pupal
l(3)88EFe	6 (1)	No	No	Larval
l(3)88EFf	5 (3)	No	No	Late larval/pupal
l(3)88EFg	5 (1)	No	No	Pupal
l(3)88EFh	2 (0)	No	Yes	Embryonic

[a] Two members of this group [*l(3)88EFb-2* and *l(3)88EFb-5*] were embryonic lethal, while the remainder were lethal during the first larval instar stage.

[b] Members of the *l(3)88EFd* group showed mixed complementation (see text), suggesting this group affects a gene that resides at or near the proximal breakpoint of *Df(3R)Po*^{4}.

among these proteins suggests that they are highly related, possibly functional homologs. To determine whether there were other genes closely related to *ENL/AF9* in flies, we carried out reduced stringency Southern blots using both the *ENL* cDNA and the *ear* cDNA. No other hybridizing restriction fragments were detected with either probe under a variety of conditions (data not shown). Hybridization of the *ear* cDNA to the salivary gland polytene chromosomes detected only a single locus that mapped to the cytological position 88EF on the right arm of the third chromosome (data not shown). A BLAST database search of the completed Drosophila genomic sequence revealed no other closely related sequences, further suggesting that *ear* was the only likely homolog of *ENL* and *AF9* in flies. Thus, *ear* appears to be a single copy gene and the only *bona fide* homolog of *ENL* and *AF9* in *Drosophila melanogaster*.

In addition to the strong homology between EAR and ENL/AF9, significant homology (~63% similarity) was observed at the N terminus of all three proteins with the small (29-kD) subunit of TFIIF in *Schizosaccharomyces pombe*. This deduced protein is highly related to the TFG3/TAF30/ANC1 protein of *Saccharomyces cerevisiae*, which is found among three transcription activation

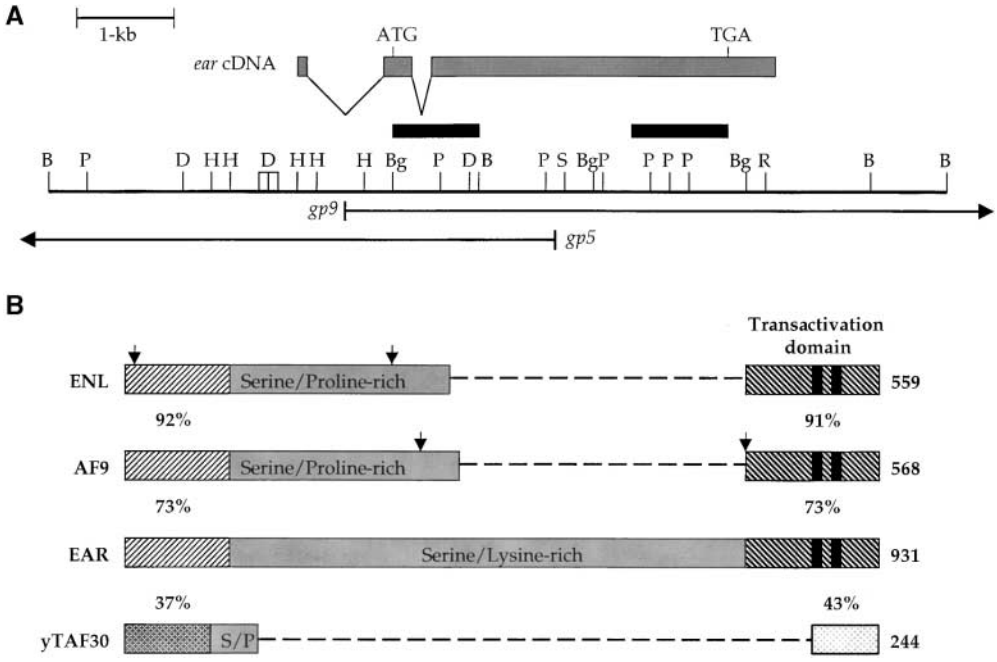


FIGURE 1.—Molecular structure and conservation of the Drosophila *ear* gene. (A) Molecular structure of the *ear* gene. The deduced intron/exon structure of the *ear* transcript is shown above the genomic DNA partial restriction map. The solid bars represent the regions within the N and C termini used to generate polyclonal antibodies. Shown below the restriction map are the relative positions of two overlapping genomic DNA phage clones (*gp5* and *gp9*) that span ~35 kb around the *ear* locus. Restriction sites are designated as follows: B, *Bam*HI; P, *Pst*I; D, *Dra*I; H, *Hin*dIII; Bg, *Bgl*II; S, *Sal*I; R, *Eco*RI. (B) Schematic alignment between Drosophila EAR, human ENL and AF9, and yeast TFG3/TAF30/ANC1 proteins. There is strong similarity between the human and fly proteins, indicated by the hatched areas at the N and C termini, and moderate similarity between EAR and the yeast protein TFG3. The carboxy terminus contains a region (~85 aa) shown to be necessary and sufficient for the transactivation properties and oncogenic potential of ENL and AF9. Solid boxes show the relative positions of two predicted helical structures. The arrows indicate the reported breakpoint positions in the ENL and AF9 proteins that were found fused to HRX in different leukemias.

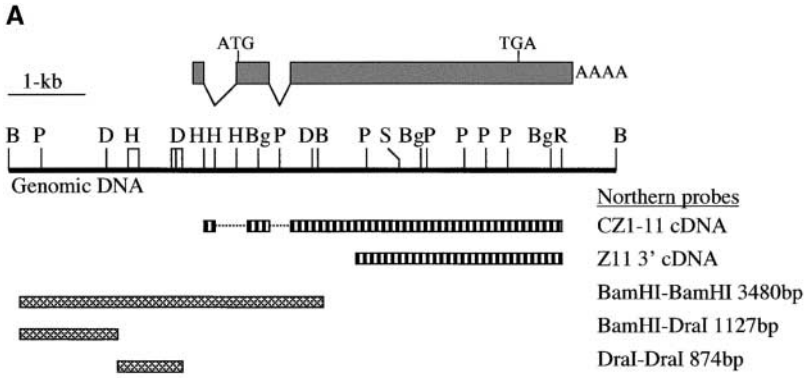
complexes, including TFIIF, TFIID, and the SWI/SNF chromatin-remodeling complex (Cairns *et al.* 1996).

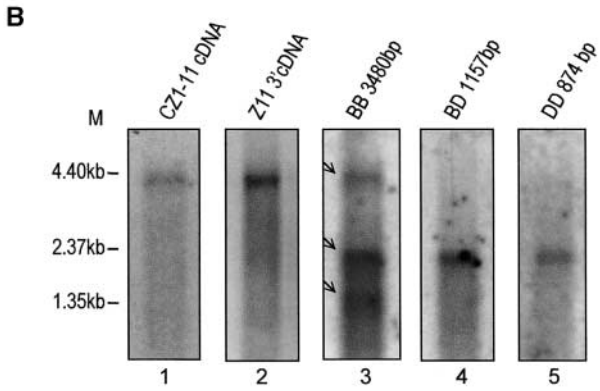
Analysis of *ear* transcripts: We mapped the locations of introns within the *ear* transcript by comparison of PCR products obtained from both genomic and cDNA clones and direct sequencing of the *ear* genomic region. To carry out this analysis we isolated overlapping genomic phage clones generated from an isogenic strain using the *ear* cDNA as a probe (Figure 1). Two introns were detected by PCR analysis and confirmed by direct sequencing of the genomic DNA. The first intron of 465 bp occurs 5′ to the initiating AUG codon, while the second intron is much smaller (71 bp) and is also located near the 5′ end. No other introns or alternative splice variants were detected (data not shown). Therefore, as depicted in Figure 1, the *ear* gene mRNA contains three exons, with the initiating AUG residing within the second exon. Using either the full-length CZ1-11 cDNA clone or the 3′ *ear* cDNA clone (Z11) as probe on a Northern blot, only one ~4-kb transcript was detected in fly embryo poly(A)$^+$ RNA that closely correlates with the size of the complete cDNA (Figure 2). As reported in the Berkeley *Drosophila* Genome Project (BDGP) database, five partial embryonic expressed sequence tag (EST) sequences contain the 5′ end of *ear* (data not shown). Among these ESTs, only one contains a sequence extension of 30 bases 5′ relative to our longest cDNA (CZ1-11), and several other ESTs start downstream of CZ1-11. Thus, the *ear* cDNA clone CZ1-11 most likely comprises a nearly full-length *ear* transcript, possibly missing only a very short noncoding sequence at the 5′ end. Long exposure of the Northern blots probed with the cDNAs did not reveal the presence of any additional transcripts.

We examined other transcripts in the immediate vicinity of *ear* to better define the genes residing in the region and to determine the proximity of other genes relative to the deduced 5′ end of the *ear* transcript. Genomic fragments obtained from the region 5′ to *ear* were used to probe Northern blots of embryonic poly(A)$^+$ RNA (Figure 2). A 1357-bp *Dra*I-*Dra*I genomic restriction fragment contained entirely within *ear* detected only the 4-kb transcript (data not shown), while both the 1157-bp *Bam*HI-*Dra*I and the 874-bp *Dra*I-*Dra*I restriction fragments that contain sequences upstream of the *ear* gene detected a second transcript of ~2 kb. Through database searching (BDGP) several ESTs were found with a transcript orientation opposite to *ear* located in the upstream region, suggesting a possible transcript originating nearby. The closest EST upstream of *ear* is ~1 kb away from the CZ1-11 start. It is not clear if these ESTs and the 2-kb transcript we observed on the Northern blots using genomic DNA probes are of the same origin. In addition, a TBLASTX search revealed that the sequence of those ESTs and the surrounding genomic DNA in this region do not correspond to any known genes (data not shown). A third 1.4-kb transcript of uncertain origin was seen only with a *Bam*HI-*Bam*HI genomic probe that included portions of both *ear* and the nearby transcript. This putative 1.4-kb transcript likely represents a degradation product of one of the larger transcripts as it was not consistently observed.

Developmental expression of *ear*: The preponderance of early onset lineage-specific leukemias associated with both *ENL* and *AF9* fusions suggested that their gene products might be present in early development and possibly restricted to certain tissues. We examined this issue in flies using both the *ear* mRNA and gene product to determine when and where the Drosophila gene was expressed. A developmentally staged RNA blot was probed with the *ear* cDNA. We observed a single ~4-kb transcript that was present at high levels in unfertilized eggs and during early embryogenesis (Figure 3). The accumulation of the transcript was significantly diminished by midembryogenesis and there was little detectable *ear* transcript in larvae. There was, however, an increase in detectable transcript during pupal development, although little in adult males. The same blot was reprobed with another gene, *snr1*, that showed a similar, although not identical, pattern of transcript accumulation (data not shown; see Dingwall *et al.* 1995). Therefore, the vast majority of *ear* transcript is present maternally; however, there is late zygotic expression. The absence of detectable *ear* transcript at multiple stages and in adult males suggests that *ear* is not essential for general cell viability.

We generated specific antibodies to the most conserved regions of the EAR protein at both the N and C termini (see Figure 1). Both antisera recognized a single protein species of ~120 kD in wild-type embryo extracts (see Figure 7 and data not shown). The size of the observed protein on Western blots was generally consistent with predictions based on ORF analysis of the *ear* cDNA, and the ~20-kD discrepancy most likely reflects unknown post-translational modifications. We used these antibodies to examine the distribution of EAR protein at various stages during embryonic development using whole-mount immunolocalization (Figure 4). As shown in Figure 4A, antibodies directed against the EAR N terminus indicated that the EAR protein was present in all nuclei during syncytial blastoderm (Figure 4A) and following cellularization (Figure 4B). The EAR protein was still uniformly distributed along the anterior-posterior axis at the extended germband stage (stage 11, Figure 4C), although the protein was present at a slightly higher level in the developing mesoderm. At later stages of embryonic development (stage 13) the EAR protein accumulated in specific tissues: Uniform levels were observed in the abdomen region (segments A1–A8), while being generally excluded from the thoracic region (Figure 4D). In addition, EAR protein was found in bilateral regions within the head primordium. Unlike the Drosophila Brahma (Brm) complex proteins





C

Transcript	CZ1-11 cDNA	Z11 3'cDNA	BB 3480bp	BD 1157bp	DD 874bp
~4kb	+++	+++	++	-	-
~2kb	-	-	+++	+++	+++
~1.4kb	-	-	+	-	-

FIGURE 2.—Analysis of transcripts in the vicinity of *ear*. (A) Schematic of the *ear* gene structure and relative positions of selective restriction enzyme sites in the genomic DNA. Shown below the restriction map is a schematic of the cDNA or genomic DNA fragments used as probes of poly(A)$^+$ RNA. Restriction enzyme sites are as in Figure 1. (B) Northern blot hybridization. Random-primed DNA probes shown in A were used to detect transcripts using 3 μg poly(A)$^+$ per lane. RNA size standards are shown on the left. The positions of all three transcripts are indicated by arrows in lane 3. (C) Summary of Northern blot analyses, indicating the probes and transcripts detected.

(the counterpart of the yeast SWI/SNF chromatin-remodeling complex) that are ubiquitous in early embryos and then later localized to the nervous system, the highest level of EAR protein is found in restricted ectoderm and mesoderm tissues and little, if any, is found in the central nervous system (CNS; DINGWALL *et al.* 1995; ELFRING *et al.* 1998). Thus, unlike the yeast TFG3/TAF30/ANC1 protein (to which EAR is related), the EAR protein is unlikely to be a constitutive component of Drosophila Brm (SWI/SNF) complexes in all tissues even at substoichiometric amounts.

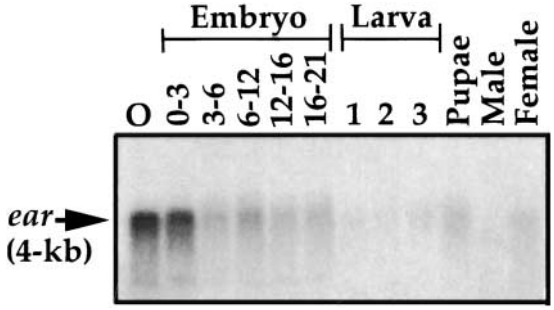


FIGURE 3.—Developmental expression of *ear* mRNA. A blot containing RNA isolated from oocytes (O), embryos (0–3, 3–6, 6–12, 12–16, and 16–21 hr), larvae (L1, L2, L3), pupae, and adult males and females was probed with a random-primed *ear* cDNA (CZ1-11). The *ear* mRNA appears as a 4-kb transcript.

Molecular analysis of the *easter* region: The *ear* gene was mapped cytologically to the 88E11-13 region of the salivary gland polytene chromosomes by *in situ* hybridization near the *ea* gene at 88F1-2 (data not shown). The genomic region surrounding *ea* has been sequenced by the Drosophila Genome Project (http://www.fruitfly.org/). Analysis of the sequence confirmed our mapping studies and showed that the *ear* gene resided very close to *ea*. Thus far, no *P*-element transposon insertions have been located within 10 kb of *ea* and there are few, if any, known recessive lethal mutations mapped within the region (http://flybase.bio.indiana.edu/). To carry out a genetic screen to identify mutations within the *ear* gene, deficiencies that potentially removed both *ear* and *ea* were characterized. Three known deficiencies map to the 88F-89A region, *Df(3R)ea*^{5022rx1}, *Df(3R)Po*^{4}, and *Df(3R)Po*^{2}. The proximal breakpoint of *Df(3R)Po*^{4} is re-

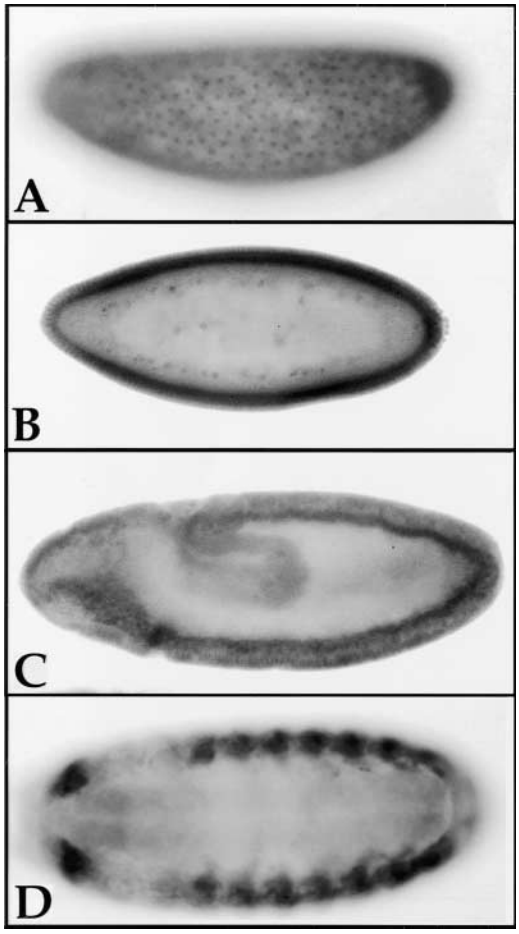


Figure 4.—Distribution of EAR protein during embryonic development. Oregon-R embryos were fixed and immunostained with rat polyclonal antibody directed against the conserved N terminus (aa 32–198) of EAR. Embryos are oriented with anterior to the left. (A) The EAR protein localizes to nuclei in early development and shows uniform distribution in all nuclei at the syncytial blastoderm stage (stages 3 and 4). (B) At cellular blastoderm (stage 5) most of the EAR protein is associated with cells at the periphery, while some protein is still associated with yolk nuclei. The EAR protein is uniformly distributed and present in the pole cells at the posterior. (C) Stage 11 embryo (at full germ-band extension) showing EAR protein uniformly distributed along the A-P axis. (D) Dorsal view of stage 13 embryo showing high expression of EAR in the epidermis and mesoderm cells. Expression is restricted to cells in the abdomen and the cephalic region, with low-level expression in the thoracic region and CNS.

ported as 88F7 with a distal breakpoint at 89A11-13 (Nelson and Szauter 1992). The reported breakpoints for *Df(3R)Po*^{2} are 89A1-2 and 89A11-13. Quantitative Southern hybridization of genomic DNA from these strains and Oregon-R (wild-type) flies indicated that *Df(3R)ea*^{5022rx1} deletes both the *ear* and *ea* genes, while *Df(3R)Po*^{4} and *Df(3R)Po*^{2} do not (data not shown). Similar hybridization analyses placed the *ear* gene proximal to *ea* and distal of *Tm2* (data not shown). In confirmation of the molecular mapping data, genomic sequence data obtained from the BDGP database revealed that the *ear* gene resided 5.6 kb distal to *Tm2* and 3.8 kb proximal to *ea*. To map approximate breakpoints of the *Df(3R)ea*^{5022rx1} and *Df(3R)Po*^{4} deletions, genomic probes of *ea*, *Surf4*, and *Anon-becker2* were generated by PCR amplification using genomic DNA extracted from adult flies, labeled, and hybridized to Southern blots of genomic DNA obtained from deficiency strains. Hybridization results revealed that the *Df(3R)Po*^{4} proximal breakpoint resided ~6 kb distal to the *Surf4* gene (see Figure 5; data not shown). On the basis of molecular mapping and genetic complementation tests, the distal breakpoint of *Df(3R)ea*^{5022rx1} lies between the *Anon-Becker2* gene and a lethal *P*-element insertion [identified on the physical map as *l(3)j6A6*] that was mapped between *ea* and *Act88F*.

The relative cytogenetic and molecular positions of the deficiencies, genes, and *P*-element insertions in the 88EF region are presented in Figure 6. We have refined the molecular locations on the basis of quantitative Southern blots, complementation tests (data not shown), and available information. Genetic complementation tests indicated that *Df(3R)ea*^{5022rx1} and *Df(3R)Po*^{4} overlap, as do *Df(3R)Po*^{4} and *Df(3R)Po*^{2}. However, *Df(3R)ea*^{5022rx1} and *Df(3R)Po*^{2} complement each other, indicating that their deficiency regions do not overlap. The *Tm1*, *Tm2*, and *ea* genes are all removed by *Df(3R)ea*^{5022rx1} (Kreuz *et al.* 1996) but not by *Df(3R)Po*^{4}, while the *Actin 88F* (*Act88F*) gene is deleted in *Df(3R)Po*^{4} but not in *Df(3R)ea*^{5022rx1}. Therefore, the proximal breakpoint of *Df(3R)Po*^{4} and the distal breakpoint of *Df(3R)ea*^{5022rx1} reside in the interval between *ea* and *Act88F*.

The deleted region in *Df(3R)ea*^{5022rx1} was previously estimated to be ~60 kb (Kreuz *et al.* 1996). Estimates of gene density in *D. melanogaster* suggest that, on average, there is 1 gene per 9 kb, yet substantial variation exists (Adams *et al.* 2000). On the basis of that assumption, as many as 6 or 7 genes may be located in the *Df(3R)ea*^{5022rx1} deficiency, including *ear*. Thus, in addition to *Tm1*, *Tm2*, and *ea*, there could be at least 3 to 4 unidentified genes removed by the *Df(3R)ea*^{5022rx1} deficiency. However, annotation of the completed genome sequence has revealed that up to 26 genes (on both strands) may reside within the 60-kb region removed by the *Df(3R)ea*^{5022rx1} deficiency (http://www.fruitfly.org/). Our hybridization data suggest that the overlap between *Df(3R)ea*^{5022rx1} and *Df(3R)Po*^{4} could encompass up to 25 kb (data not shown; see Figure 6); therefore, as many as 12 genes may reside in this region alone (http://www.fruitfly.org/).

EMS screen results: Our goal was to identify mutations within the *ear* gene. Transposon insertions previously mapped within the 88E-F cytological region were tested for inclusion within the *Df(3R)ea*^{5022rx1} deficiency by genetic complementation and for linkage with the *ear* gene by the presence of restriction fragment polymorphisms. Genetic analyses revealed that only two of the known transposon insertions, *P{PZ}Tm1*^{02299} and *P{lacW}L7160c*, both of which disrupt *Tm1* gene func-

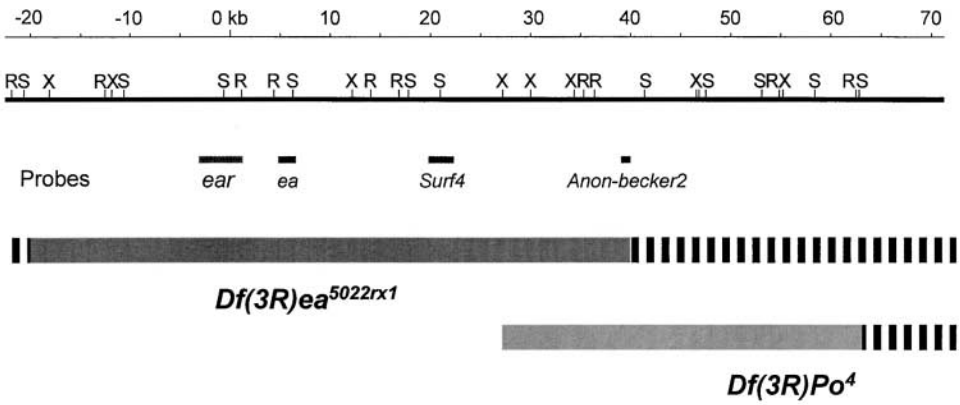


FIGURE 5.—Molecular mapping of genes and deficiency breakpoints. Quantitative Southern blots were used to localize genes on a physical map of the *easter* genomic region. Centromere proximal is to the left and distal to the right. The origin of the numbering is arbitrarily placed within the *ear* gene. The proximal breakpoint of $Df(3R)Po^4$ was mapped to within 6 kb of the *Surf4* gene. The distal breakpoint of $Df(3R)ea^{5022rx1}$ is approximate, based on Southern blot hybridization and genetic analyses as described in the text.

tion, failed to complement $Df(3R)ea^{5022rx1}$. Furthermore, Southern blot hybridization of genomic DNA, obtained from known transposon insertion lines broadly mapped to the *ea* region, failed to reveal any close linkage to *ear* using an *ear* cDNA probe. The results are summarized in Figure 6. We therefore carried out an F_2 recessive lethal noncomplementation screen to attempt to identify mutations within *ear*.

Following mutagenesis with EMS, third chromosomes from 18,062 fertile male progeny were individually tested for lethality *in trans* to the $snr1^{R3}$, $Df(3R)ea^{5022rx1}$ tester chromosome (Table 2). Complementation tests were carried out at 29°, as ~5–10% of EMS-induced mutations are temperature sensitive (GRIGLIATTI 1986). Forty-four third chromosomes that failed to complement the $snr1^{R3}$, $Df(3R)ea^{5022rx1}$ tester strain (Table 2) were recovered. Among the 44 third chromosomes recovered from the recessive lethal screen, 4 were found to be allelic to *snr1* and the remaining 40 mutant lines were then crossed to $Df(3R)Po^4$ and to $Df(3R)ea^{5022rx1}$. After these first two steps of classification, mutants within each class were test crossed to one another or *inter se* for complementation. Mutants that failed to complement each other were assigned to alleles of the same group.

Altogether, we identified 14 lethal complementation groups including the new *snr1* alleles (our unpublished results). Among the 44 mutants recovered, 38 fall into 8 complementation groups of multiple alleles, with an average of 4.75 alleles per group. Within each group, except for group *l(3)88EFb*, all members carried different batch numbers of EMS feeding, indicating that the mutations were induced independently. Among these groups, *l(3)88EFb*, *l(3)88EFe*, *l(3)88EFf*, and *l(3)88EFg* clearly failed to complement both $Df(3R)ea^{5022rx1}$ and $Df(3R)Po^4$, indicating that these groups affect genes located within the overlapping portion of the two deficiencies. The groups *l(3)88EFc* and *l(3)88EFh* failed to complement $Df(3R)ea^{5022rx1}$ but did complement $Df(3R)Po^4$; thus, these groups correspond to genes that reside in the more proximal portion of the $Df(3R)ea^{5022rx1}$ deficiency that does not overlap with $Df(3R)Po^4$. Group *l(3)88EFd* is more complicated, because two alleles complemented and two failed to complement $Df(3R)Po^4$. This group most likely represents a gene that resides at or near the proximal breakpoint of $Df(3R)Po^4$ (Figure 6). Among the six single allele mutants isolated in the screen (13.6% of all mutants), five were found to map outside the $Df(3R)ea^{5022rx1}$ region.

We attempted to assign individual mutant groups to specific genes on the basis of genetic complementation. Among the genes identified within the $Df(3R)ea^{5022rx1}$ region, only three have any reported mutant alleles, including *Tm1* (TETZLAFF *et al.* 1996), *Tm2* (MOGAMI and HOTTA 1981; KREUZ *et al.* 1996), and *ea* (reviewed in ANDERSON 1998). Both recessive loss-of-function and dominant gain-of-function *ea* mutants are maternal effect lethal and female sterile (ANDERSON and NUSSLEIN-VOLHARD 1984; ANDERSON 1998). *Trans*-heterozygous combinations of $Df(3R)ea^{5022rx1}$ over a strong amorphic allele of *ea* (ea^1) survive (data not shown), but females are sterile due to the depletion of maternal easter protein (ANDERSON and NUSSLEIN-VOLHARD 1984). New *ea* mutations were not expected as the screen was designed to isolate only F_2 recessive lethal mutations. At least two members from each EMS mutant group and all of the single allele mutants were tested for complementation with existing mutant alleles of *Tm1* and *Tm2*. A single temperature-sensitive mutant [*l(3)88EFi*] showed partial complementation with a strong loss-of-function *Tm1* mutant [$P\{PZ\}Tm1^{02299}$] at 29° (data not shown). Complementation tests using two loss-of-function *Tm2* alleles, $Tm2^{J8}$ and $Tm2^{S2}$ (KREUZ *et al.* 1996), indicated that none of the EMS mutants isolated in the screen were new alleles of the *Tm2* gene. Therefore, all of the multi-allele mutant groups isolated in our screen represent mutations in genes not previously associated with any specific genetic lesion.

Characterization of EMS mutants: All of the mutations identified in the screen were isolated as recessive lethals when placed *in trans* to the deficiency [$Df(3R)ea^{5022rx1}$]. The lethal phase of each mutant within a multi-allele group was determined as described in MATERIALS AND METH-

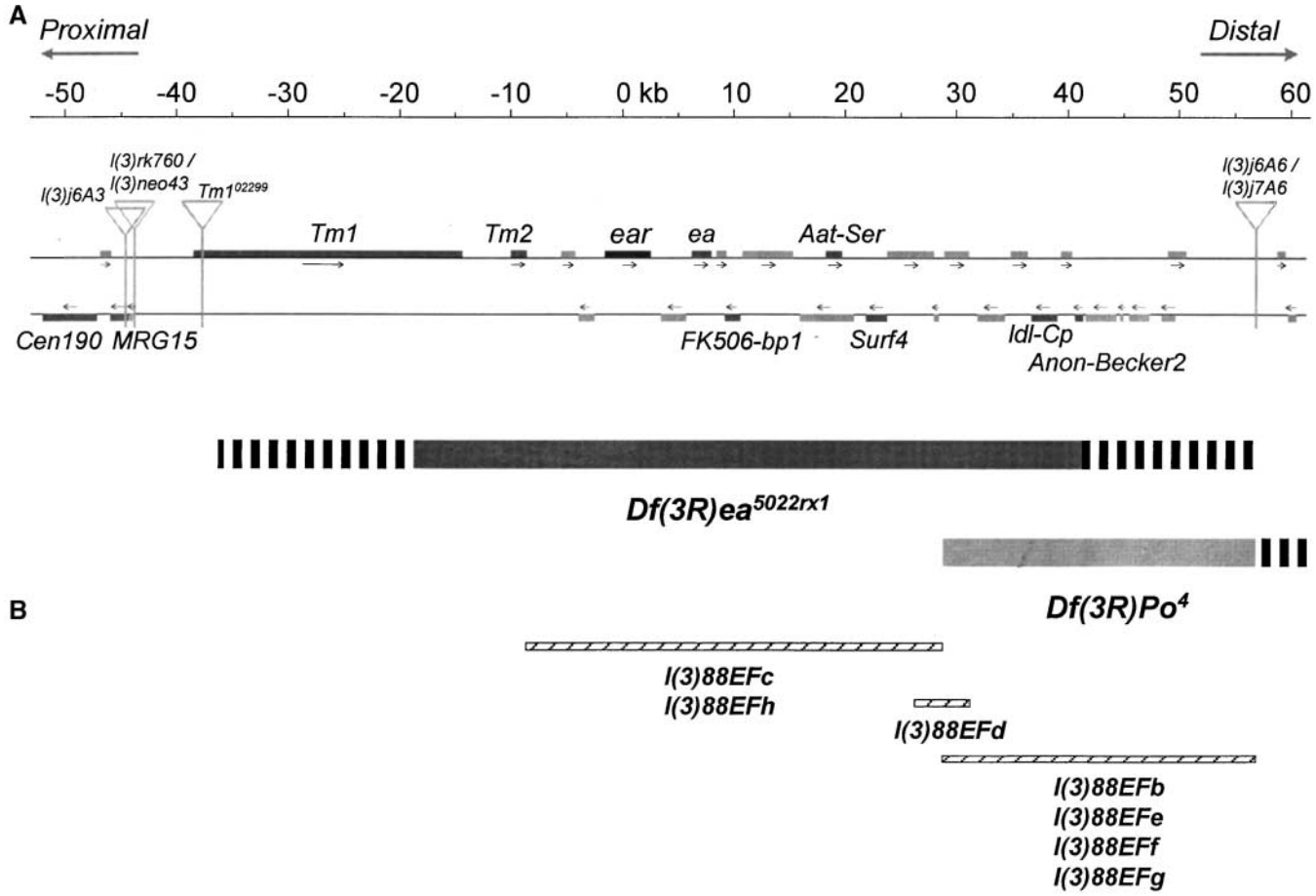


FIGURE 6.—Gene annotation in the *easter* genomic region. (A) The ~110-kb genomic region adjacent to *ear* is diagrammed. The top scale numbers reflect distances from the origin (arbitrarily placed within the *ear* gene) in kilobases. The two lines in the middle represent both DNA strands. Annotated genes (predicted or confirmed by the BDGP) on the upper strand are transcribed in the proximal-to-distal direction, while genes on the lower strand are transcribed in the distal-to-proximal direction. Molecularly identified genes are depicted as solid boxes with name and arrows to indicate their transcription directions. Other BDGP annotated transcripts are depicted with shaded boxes. Lethal *P* insertions that have been molecularly mapped close to *ear* are indicated by triangles with vertical lines. Locations and approximate breakpoints of the deficiencies $Df(3R)ea^{5022rx1}$ and $Df(3R)Po^{4}$ are shown with shaded bars. (B) Possible locations of complementation groups identified from our screen are indicated.

ods. To avoid potential confusion in the analysis by the possible presence of other recessive lethal mutations induced on the same chromosome, the lethal phase of each mutant was examined when heterozygous with the deficiency (Table 3). Among the larval lethals were *l(3)88EFc*, *l(3)88EFd*, *l(3)88EFe*, and *l(3)88EFf*. Members of the group *l(3)88EFg* die late, during pupal development. Both members of the *l(3)88EFh* group were found to be embryonic lethal, while some members of the *l(3)88EFb* group die as late embryos and others are lethal during the first larval instar stage.

The identification of two multi-allele lethal complementation groups within the proximal portion of the $Df(3R)ea^{5022rx1}$ deficiency where the *ear* gene resides raised the possibility that one of these groups represented alleles of *ear*. PCR and Southern blot analyses of genomic DNA from each of the mutant lines in *l(3)88EFh* and *l(3)88EFb* failed to reveal any obvious restriction fragment length polymorphism (RFLP) associated with the *ear* gene (data not shown). Embryonic native protein extracts were prepared from Oregon-R (wild-type) flies and from each of the heterozygous balanced mutant lines that mapped to the proximal region of the $Df(3R)ea^{5022rx1}$. Equivalent amounts of each protein extract were probed on Western blots with polyclonal antibodies produced against the highly conserved carboxyl-terminal region of EAR. As a control, the lower portions of the same blots were probed with antibodies to the SNR1 protein, whose expression level should not be affected in these EMS mutant lines as each was shown to fully complement a *snr1* null allele (see MATERIALS AND METHODS). Antibodies to EAR recognize a single protein species of ~120 kD, consistent with its predicted size (Figure 7). Comparison between EAR detection signals obtained from Oregon-R (wild-type) embryo extracts *vs.* extracts prepared from a heterozygous *ear* deficiency line [$Df(3R)ea^{5022rx1}$] shows a significantly lower amount of EAR protein in the deficiency line (Figure

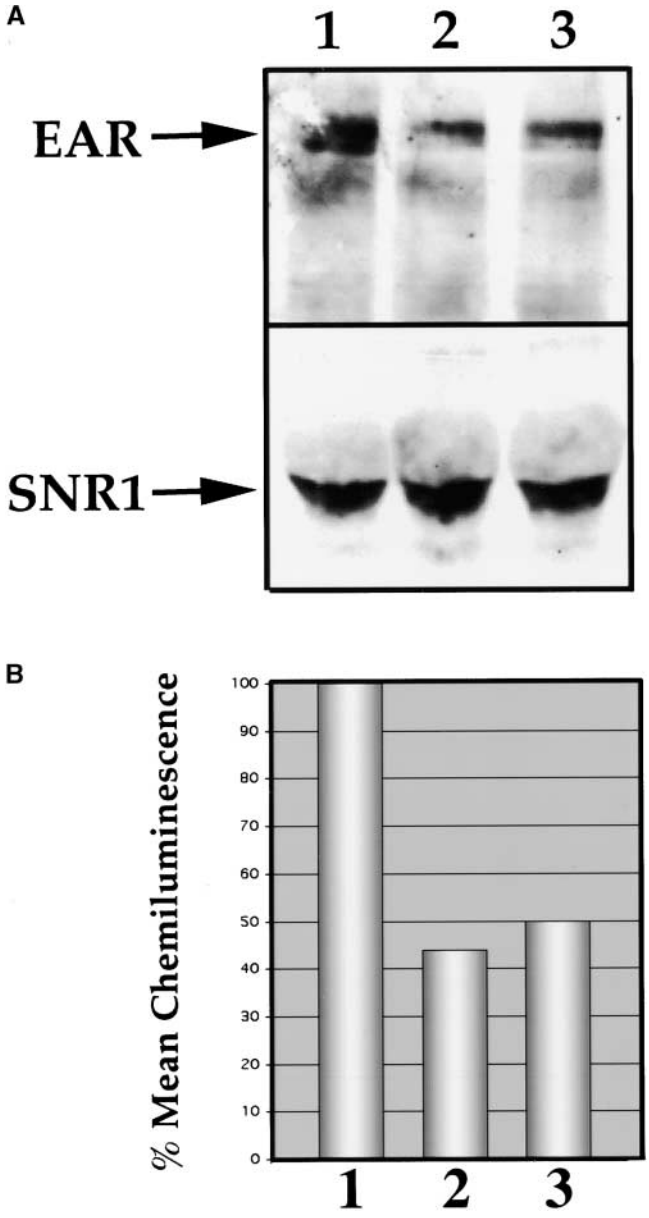


FIGURE 7.—Quantitative Western blots of EAR protein. Native embryonic protein extracts were prepared from Oregon-R, *ea* deficiency, and *l(3)88EFh-2* mutant strains. Extracts (100 μg each) were probed on Western blots with antibodies to the EAR and SNR1 proteins. Chemiluminescence signals were quantitated by direct capture using a Kodak/NEN 440CF Imaging system. (A) Western blot probed with antibodies to EAR (top) revealing a protein of ~115 kD and with antibodies to SNR1 (bottom). Lane 1, Oregon-R; lane 2, *Df(3R)ea*^{5022rx1}/*TM6B*; lane 3, *l(3)88EFh-2/TM6B*. (B) Quantitation of the EAR chemiluminescent signals shown in A. Values are expressed as a percentage of the mean chemiluminescence for each sample compared with the value obtained for Oregon-R.

7A, top; compare lanes 1 and 2). The amount of EAR protein was also diminished in one of the lethal lines obtained from the screen *l(3)88EFh-2* (lane 3). All other tested mutant lines, including another member of the same complementation group [*l(3)88EFh-1*], showed approximately wild-type levels of the protein (data not shown). Probing the lower portions of these same blots with antibodies to SNR1 revealed that equivalent amounts of protein were present in each lane (Figure 7A, bottom). Quantification of the relative amount of EAR protein present in each extract was performed by direct digital capture of the luminescent antibody detection signal (Figure 7B). The mean relative fluorescence signals indicated that there was ~44% of the wild-type level of the EAR protein in the heterozygous *Df(3R)ea*^{5022rx1} deficiency strain and ~50% in the heterozygous *l(3)88EFh-2* strain. These results are consistent with a loss of one functional copy of the *ear* gene in the mutant strains. Combinations of *l(3)88EFh-1*, *l(3)88EFh-2*, and *Df(3R)ea*^{5022rx1} cause embryonic lethality, while balanced heterozygotes appear to be completely normal. Thus, the lethal complementation group *l(3)88EFh* likely represents loss-of-function or hypomorphic mutations within the *ear* gene.

The *l(3)88EFh-2* mutation was balanced with a chromosome carrying a GFP marker. Approximately 4 kb of genomic DNA encompassing the *ear* gene in the *l(3)88EFh-2* strain was PCR amplified with specific primers from homozygous (non-GFP) embryos. Primers were used to directly sequence the entire gene on both strands and no detectable changes were observed in the open reading frame for *ear*. Thus, the lesion that produced the lowered EAR protein levels likely affects sequences that regulate the expression of *ear*.

Larval cuticles were prepared and examined from both of the *l(3)88EFh* mutant strains (Figure 8). While the larval cuticles from heterozygotes appeared completely normal, cuticles from homozygous *l(3)88EFh-1* and *l(3)88EFh-2* larvae exhibited defects in head development and shortened bodies. The patterning of the abdominal cuticle segments was relatively normal with the exception of the A8 segment that often displayed denticle pattern defects. This phenotype was consistent with the expression of EAR protein in wild-type embryos.

DISCUSSION

We report here the identification and characterization of the *ear* gene of *D. melanogaster*. The *ear* gene is closely related to the human *ENL* and *AF9* genes that were identified on the basis of their involvement in a large number of acute leukemias as fusion partners with *HRX*, a human counterpart of the Drosophila *trithorax* gene. The *ear* gene appears to be the only fly relative of both *ENL* and *AF9*, with nearly equal identity to each, suggesting that the human genes might derive from a common ancestor. The *ear* gene encodes a protein of 931 amino acids, considerably larger than either ENL or AF9, although all three deduced proteins are very highly related at the N and C termini. Database searches revealed the likely presence of related genes in *Caenorhabditis elegans* and *Arabidopsis thaliana*, further sug-

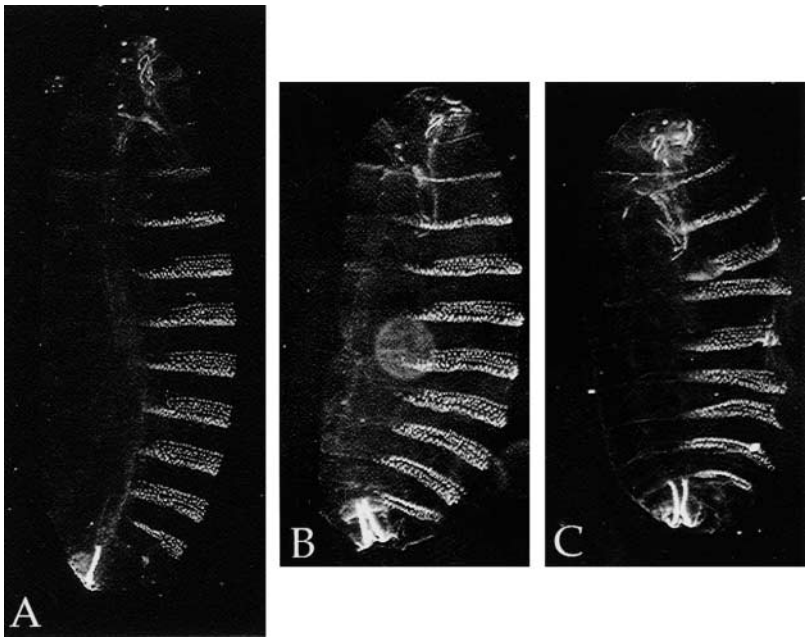


FIGURE 8.—Head patterning defects associated with *l(3)88EFh*. Cuticle preparations showing a ventrolateral view of the denticle belts. (A) Phenotypically wild-type cuticle of a heterozygous *l(3)88EFh-1* first instar larva. (B and C) Defective head development observed in larval cuticles associated with *l(3)88EFh-1* (B) and *l(3)88EFh-2* (C). Although the mutants are smaller than the heterozygous sibling, the patterning of the abdominal cuticle segments is relatively normal with the exception of the A8 segment that shows denticle pattern defects.

gesting functional evolutionary conservation. In addition, the metazoan proteins share significant homology with a yeast protein identified as the small subunit (TFG3/TAF30/ANC1) of the transcription initiation complex TFIIF and as a core component of the TFIID and SWI/SNF complexes that are also essential for transcriptional activation (HENRY *et al.* 1994; WELCH and DRUBIN 1994; CAIRNS *et al.* 1996). It is important to note, however, that the highest degree of conservation was observed between the fly and human proteins.

To study the biological function of *ear*, we searched for specific mutations that would allow us to examine potential loss-of-function phenotypes. Given the significance of ENL and AF9 in lineage-specific leukemias and the potential role of these proteins in transcriptional regulation, we reasoned that mutations in the *ear* gene might reveal an essential role for the gene in normal development. The only identifiable existing lesion affecting *ear* was a 60-kb chromosomal deletion [$Df(3R)ea^{5022rx1}$] that removed up to 25 other genes that had also not previously been genetically characterized. Our results from an EMS mutagenesis screen identified seven "new" vital, nonredundant complementation groups within this region, a number that is consistent with the expectation that one in three genes in Drosophila may be essential for viability. This expectation is based on estimates of 3600–5000 essential loci (BRIZUELA *et al.* 1994; SPRADLING *et al.* 1995, 1999) together with a prediction of ~13,600 genes by computational methods (ADAMS *et al.* 2000). Four of the new complemetation groups were found to reside within the distal ~30 kb of the deficiency that overlaps with $Df(3R)Po^4$, one group resides at or near the proximal breakpoint of $Df(3R)Po^4$, and two groups reside within the proximal region of the *ea* deficiency, where the *ear* gene resides. Our EMS-induced mutations and cytological characterization of the 88EF region should prove useful for investigations on the functions of the corresponding genes. The $Df(3R)ea^{5022rx1}$ deficiency has a dominant flightless phenotype. None of the complementation groups identified displayed such a phenotype, suggesting that we had not induced mutations in genes that are required for flight muscle function.

Our finding of a Drosophila homolog of *ENL/AF9* suggested that flies might be a useful model system to apply whole-organism genetic tests to understand *ENL/AF9* function. Although nothing is known about the normal biological functions of *ENL* and *AF9*, as fusions to *HRX* their only obvious effects are to provide lineage-specific oncogenic potential through a gain-of-function mechanism. ENL can function as a *bona fide* transcription factor as it has been shown to transactivate certain promoters *in vivo* (RUBNITZ *et al.* 1994). This property suggests that the native ENL and AF9 proteins might function to regulate developmental processes in a tissue-specific manner. Our developmental expression studies of the fly *ear* gene support this view. The *ear* gene is expressed maternally and is present in all nuclei in early embryos. Later, the protein is found in a restricted set of mesoderm cells within the abdomen and cephalic regions, while it is present at only very low levels in the thoracic region and the CNS. The RNA and protein expression profile of *ear* is consistent with it potentially serving to regulate the early development of specific mesodermal tissues.

As a result of our mutagenesis screen, two lethal complementation groups that affect genes within the proximal portion of the $Df(3R)ea^{5022rx1}$ deficiency where *ear* resides were identified. Although there is no discernible change in the genomic DNA detectable by RFLP analysis in any of the mutants from either group, quantitative

Western blots revealed that one group, *l(3)88EFh*, might represent mutant alleles of *ear*. The EAR antibodies used recognize only the most conserved carboxy-terminal amino acids; thus nonsense or frameshift mutations, or mutations that affect the regulatory region, are most likely the cause of the diminished EAR protein. The latter explanation seems most likely as sequencing the genomic DNA of this particular mutant allele did not reveal any obvious changes within the open reading frame of *ear*. The importance of the C-terminal region in both ENL and AF9 function is unequivocal: The C terminus is critical for transactivation properties (Rubnitz *et al.* 1994; Lavau *et al.* 1997; Schreiner *et al.* 1999) and for both the oncogenic and transforming potential (Corral *et al.* 1996; Slany *et al.* 1998; Dobson *et al.* 1999) as well as anti-apoptotic effects (Adler *et al.* 1999) of the HRX/MLL fusions. The C-terminal regions of ENL and AF9 can also specifically associate with the human Polycomb group protein hPc3 and may thus either help to recruit repressor complexes to specific loci or assist in corepression of gene activity in cooperation with the Polycomb repressor complexes (Garcia-Cuellar *et al.* 2001). Although a functional link between ENL and hPc3 may occur in a regulated fashion, we did not detect any significant interaction between several dominant alleles of the *Polycomb* (*Pc*) gene and either the deficiency or our putative *ear* mutant alleles (data not shown).

The yeast protein TFG3/ANC1/TAF30, weakly conserved with EAR, ENL, and AF9, has been shown to be a component of several transcription regulatory complexes, including the SWI/SNF chromatin-remodeling complex, TFIID, TFIIF, and the NuA3 histone acetyltransferase (HAT) complex (John *et al.* 2000). However, deletion of the yeast *TAF30* gene (*taf30*Δ) does not result in any loss of SWI/SNF function (integrity of the complex or catalytic function) and shows only modest decreases in NuA3 HAT activity, and the gene is not required for viability (Cairns *et al.* 1996). Therefore, the precise role of the yeast protein is not well understood and it may serve in nonessential functions, such as possibly helping to target the various complexes to chromatin or to the transcriptional machinery. Furthermore, while the TFIIF and TFIID protein complexes are functionally conserved in higher eukaryotes, counterparts of the TFG3/TAF30/ANC1 protein are not found as a core component in either of these complexes (Dynlacht *et al.* 1991; Kokubo *et al.* 1994; Aoyagi and Wassarman 2000).

As a component of the SWI/SNF ATP-dependent chromatin-remodeling complex, the TFG3 protein can form direct protein contacts with the SNF5 subunit (Cairns *et al.* 1996). It was therefore hypothesized that ENL or AF9 might be a component of the mammalian SWI/SNF complex, thus linking the onset of acute leukemias to defects in chromatin remodeling. Although the fly and vertebrate SWI/SNF complexes (Brm complex in flies, BRG1 and HBRM complexes in vertebrates) do not appear to contain EAR, ENL, or AF9, they do contain SNF5-related proteins (Dingwall *et al.* 1995; Wang *et al.* 1996; Papoulas *et al.* 1998). One critical question is whether ENL/AF9 or EAR is capable of forming stable protein interactions with the human or Drosophila SNF5-related proteins or genetically interacts with Polycomb group or Brm complex genes (such as *snr1*, the *SNF5* homolog in flies) to regulate cell fates and proliferation in higher eukaryotes. We performed coimmunoprecipitation experiments using antibodies to SNR1, but failed to detect coprecipitation of EAR (data not shown). Also, yeast two-hybrid analyses using fusions of EAR and SNR1 failed to detect any significant direct protein contacts between the two proteins (data not shown). These results do not rule out the possibility that SNR1 and EAR may interact in a restricted fashion in some tissues or that their potential interaction might be mediated by another protein such as a GADD34 homolog (Adler *et al.* 1999). We also did not detect any significant genetic interaction between *snr1* and *ear*. There was no discernible phenotype associated with the tester chromosome used for the genetic screen, as this chromosome carried both a strong loss-of-function allele of *snr1* and a deletion of *ear*. We did, however, observe a modest synthetic lethality among certain *trans*-heterozygous allelic combinations of *snr1* together with the deficiency. This result should be interpreted cautiously, as most mutant alleles of *snr1* are not dosage sensitive (Dingwall *et al.* 1995) and the putative *ear* mutant alleles are fully recessive.

The embryonic lethality and cuticle phenotypes associated with *l(3)88EFh* are consistent with the expression pattern of *ear* and suggest that the gene is likely to be involved in regulating essential patterning functions during embryogenesis. Additional genetic and biochemical tests will be required to further clarify any potential interactions and to understand the functions of these conserved and critically important genes in the development of multicellular organisms.

We thank Nicole Levin, Dan Marenda, Shauna Haygood, Runjhun Nanchal, and Kevin Fabrizio for assistance with the genetic screen and lethal phase analyses. We thank John Belote and Eleanor Maine for advice and for critically reading the manuscript, Sarah Ritchie for keeping up with the fly food, and Andrew Kreuz, Bob Levis, Kathryn Anderson, and the Bloomington, Indiana, Drosophila Stock Center for fly strains. We also thank Matthew P. Scott at Stanford University and the Howard Hughes Medical Institute for support during the initial stages of this investigation. This work was supported by awards from the Ruth Meyer Undergraduate Research Fund (Syracuse University) and a Basil O'Connor Starter Scholar Research Award from the March of Dimes Birth Defects Foundation to A.K.D.

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. Science **287:** 2185–2195.

Adler, H. T., R. Chinery, D. Y. Wu, S. J. Kussick, J. M. Payne *et al.*, 1999 Leukemic HRX fusion proteins inhibit GADD34-induced apoptosis and associate with the GADD34 and hSNF5/INI1 proteins. Mol. Cell. Biol. **19:** 7050–7060.

Anderson, K. V., 1998 Pinning down positional information: dorsal-ventral polarity in the Drosophila embryo. Cell **95:** 439–442.

Anderson, K. V., and C. Nusslein-Volhard, 1984 Information for the dorsal-ventral pattern of the Drosophila embryo is stored as maternal mRNA. Nature **311:** 223–227.

Aoyagi, N., and D. A. Wassarman, 2000 Genes encoding *Drosophila melanogaster* RNA Polymerase II general transcription factors: Diversity in TFIIA and TFIID components contributes to gene-specific transcriptional regulation. J. Cell Biol. **150:** 45F–50.

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bernard, O. A., and R. Berger, 1995 Molecular basis of 11q23 rearrangements in hematopoietic malignant proliferations. Genes Chromosomes Cancer **13:** 75–85.

Bradford, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

Breen, T. R., and P. J. Harte, 1993 *Trithorax* regulates multiple homeotic genes in the bithorax and Antennapedia complexes and exerts different tissue-specific, parasegment-specific and promoter-specific effects on each. Development **117:** 119–134.

Breen, T. R., V. Chinwalla and P. J. Harte, 1995 *Trithorax* is required to maintain engrailed expression in a subset of engrailed-expressing cells. Mech. Dev. **52:** 89–98.

Brizuela, B. J., L. Elfring, J. Ballard, J. W. Tamkun and J. A. Kennison, 1994 Genetic analysis of the *brahma* gene of *Drosophila melanogaster* and polytene chromosome subdivisions 72AB. Genetics **137:** 803–813.

Brock, H. W., and M. van Lohuizen, 2001 The Polycomb group—No longer an exclusive club? Curr. Opin. Genet. Dev. **11:** 175–181.

Cairns, B. R., N. L. Henry and R. D. Kornberg, 1996 TFG/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. Mol. Cell. Biol. **16:** 3308–3316.

Cimino, G., M. C. Rapanotti, T. Sprovieri and L. Elia, 1998 *ALL1* gene alterations in acute leukemia: biological and clinical aspects. Haematologica **83:** 350–357.

Corral, J., I. Lavenir, H. Impey, A. J. Warren, A. Forster *et al.*, 1996 An *MLL-AF9* fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell **85:** 853–861.

Dingwall, A. K., S. J. Beek, C. M. McCallum, J. W. Tamkun, G. V. Kalpana *et al.*, 1995 The Drosophila snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. Mol. Biol. Cell **6:** 777–791.

Dobson, C. L., A. J. Warren, R. Pannell, A. Forster, I. Lavenir *et al.*, 1999 The *MLL-AF9* gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. EMBO J. **18:** 3564–3574.

Dobson, C. L., A. J. Warren, R. Pannell, A. Forster and T. H. Rabbitts, 2000 Tumorigenesis in mice with a fusion of the leukaemia oncogene *Mll* and the bacterial *lacZ* gene. EMBO J. **19:** 843–851.

Dynlacht, B. D., T. Hoey and R. Tjian, 1991 Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66:** 563–576.

Elfring, L. K., C. Daniel, O. Papoulas, R. Deuring, M. Sarte *et al.*, 1998 Genetic analysis of *brahma*: the Drosophila homolog of the yeast chromatin remodeling factor *SWI2/SNF2*. Genetics **148:** 251–265.

Erdelyi, M., and J. Szabad, 1989 Isolation and characterization of dominant female sterile mutations of *Drosophila melanogaster*. I. Mutations on the third chromosome. Genetics **122:** 111–127.

Ford, A. M., S. A. Ridge, M. E. Cabrera, H. Mahmoud, C. M. Steel *et al.*, 1993 In utero rearrangements in the trithorax-related oncogene in infant leukaemias. Nature **363:** 358–360.

Garcia-Cuellar, M. P., S. A. Schreiner, M. Birke, M. Hamacher, G. H. Fey *et al.*, 2000 ENL, the MLL fusion partner in t(11;19), binds to the c-Abl interactor protein 1 (ABI1) that is fused to MLL in t(10;11)+. Oncogene **19:** 1744–1751.

Garcia-Cuellar, M. P., O. Zilles, S. A. Schreiner, M. Birke, T. H. Winkler *et al.*, 2001 The ENL moiety of the childhood leukemia-associated MLL-ENL oncoprotein recruits human Polycomb 3. Oncogene **20:** 411–419.

Gebuhr, T. C., S. J. Bultman and T. Magnuson, 2000 Pc-G/trx-G and the SWI/SNF connection: developmental gene regulation through chromatin remodeling. Genesis **26:** 189–197.

Grigliatti, T., 1986 Mutagenesis, pp. 39–58 in *Drosophila: A Practical Approach*, edited by D. Roberts. IRL Press, Oxford.

Hanson, R. D., J. L. Hess, B. D. Yu, P. Ernst, M. van Lohuizen *et al.*, 1999 Mammalian Trithorax and polycomb-group homologues are antagonistic regulators of homeotic development. Proc. Natl. Acad. Sci. USA **96:** 14372–14377.

Henry, N. L., A. M. Campbell, W. J. Feaver, D. Poon, P. A. Weil *et al.*, 1994 TFIIF-TAF-RNA polymerase II connection. Genes Dev. **8:** 2868–2878.

Hess, J. L., B. D. Yu, B. Li, R. Hanson and S. J. Korsmeyer, 1997 Defects in yolk sac hematopoiesis in *Mll*-null embryos. Blood **90:** 1799–1806.

Ingham, P. W., 1998 *trithorax* and the regulation of homeotic gene expression in Drosophila: a historical perspective. Int. J. Dev. Biol. **42:** 423–429.

John, S., L. Howe, S. T. Tafrov, P. A. Grant, R. Sternglanz *et al.*, 2000 The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. Genes Dev. **14:** 1196–1208.

Kassis, J. A., E. P. VanSickle and S. M. Sensabaugh, 1991 A fragment of *engrailed* regulatory DNA can mediate transvection of the *white* gene in Drosophila. Genetics **128:** 751–761.

Kokubo, T., D. W. Gong, J. C. Wootton, M. Horikoshi, R. G. Roeder *et al.*, 1994 Molecular cloning of Drosophila TFIID subunits. Nature **367:** 484–487.

Kreuz, A. J., A. Simcox and D. Maughan, 1996 Alterations in flight muscle ultrastructure and function in Drosophila tropomyosin mutants. J. Cell Biol. **135:** 673–687.

Lavau, C., S. J. Szilvassy, R. Slany and M. L. Cleary, 1997 Immortalization and leukemic transformation of a myelomonocytic precursor by retrovirally transduced HRX-ENL. EMBO J. **16:** 4226–4237.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Lochner, K., G. Siegler, M. Fuhrer, J. Greil, J. D. Beck *et al.*, 1996 A specific deletion in the breakpoint cluster region of the *ALL-1* gene is associated with acute lymphoblastic T-cell leukemias. Cancer Res. **56:** 2171–2177.

Mazo, A. M., D. H. Huang, B. A. Mozer and I. B. Dawid, 1990 The *trithorax* gene, a trans-acting regulator of the bithorax complex in Drosophila, encodes a protein with zinc-binding domains. Proc. Natl. Acad. Sci. USA **87:** 2112–2116.

Mogami, K., and Y. Hotta, 1981 Isolation of Drosophila flightless mutants which affect myofibrillar proteins of indirect flight muscle. Mol. Gen. Genet. **183:** 409–417.

Nakamura, T., H. Alder, Y. Gu, R. Prasad, O. Canaani *et al.*, 1993 Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. Proc. Natl. Acad. Sci. USA **90:** 4631–4635.

Nelson, C. R., and P. Szauter, 1992 Cytogenetic analysis of chromosome region 89A of *Drosophila melanogaster*: isolation of deficiencies and mapping of *Po, Aldox-1* and transposon insertions. Mol. Gen. Genet. **235:** 11–21.

Papoulas, O., S. J. Beek, S. L. Moseley, C. M. McCallum, M. Sarte *et al.*, 1998 The Drosophila trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development **125:** 3955–3966.

Ridge, S. A., and L. M. Wiedemann, 1994 Chromosome 11q23 abnormalities in leukaemia. Leuk. Lymphoma **14:** 11–17.

Rowley, J. D., 1993 Rearrangements involving chromosome band 11q23 in acute leukaemia. Semin. Cancer Biol. **4:** 377–385.

Rowley, J. D., 1995 Chromosome translocations: good genes gone wrong. Pathol. Biol. **43:** 197–201.

Rowley, J. D., 1998 The critical role of chromosome translocations in human leukemias. Annu. Rev. Genet. **32:** 495–519.

Rubnitz, J. E., J. Morrissey, P. A. Savage and M. L. Cleary, 1994 *ENL*, the gene fused with *HRX* in t(11;19) leukemias, encodes a nuclear protein with transcriptional activation potential in lymphoid and myeloid cells. Blood **84:** 1747–1752.

Rubnitz, J. E., F. G. Behm and J. R. Downing, 1996 11q23 rearrangements in acute leukemia. Leukemia **10:** 74–82.

Saha, V., B. D. Young and P. S. Freemont, 1998 Translocations,

fusion genes, and acute leukemia. J. Cell. Biochem. (Suppl.): 264–276.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schichman, S. A., M. A. Caligiuri, Y. Gu, M. P. Strout, E. Canaani *et al.*, 1994 *ALL-1* partial duplication in acute leukemia. Proc. Natl. Acad. Sci. USA **91:** 6236–6239.

Schreiner, S. A., M. P. Garcia-Cuellar, G. H. Fey and R. K. Slany, 1999 The leukemogenic fusion of MLL with ENL creates a novel transcriptional transactivator. Leukemia **13:** 1525–1533.

Sevenet, N., A. Lellouch-Tubiana, D. Schofield, K. Hoang-Xuan, M. Gessler *et al.*, 1999 Spectrum of *hSNF5/INI1* somatic mutations in human cancer and genotype-phenotype correlations. Hum. Mol. Genet. **8:** 2359–2368.

Slany, R. K., C. Lavau and M. L. Cleary, 1998 The oncogenic capacity of HRX-ENL requires the transcriptional transactivation activity of ENL and the DNA binding motifs of HRX. Mol. Cell. Biol. **18:** 122–129.

Spradling, A., D. Stern, I. Kiss, J. Roote, T. Laverty *et al.*, 1995 Gene disruptions using *P* transposable elements: an integral component of the Drosophila genome project. Proc. Natl. Acad. Sci. USA **92:** 10824–10830.

Spradling, A. C., D. Stern, A. Beaton, E. J. Rhem, T. Laverty *et al.*, 1999 The Berkeley Drosophila Genome Project gene disruption project: single *P*-element insertions mutating 25% of vital Drosophila genes. Genetics **153:** 135–177.

Stern, D. L., and E. Sucena, 2000 Preparation of larval and adult cuticles for light microscopy, pp. 601–615 in *Drosophila Protocols*, edited by W. Sullivan, M. Ashburner and R. S. Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Super, H. J., N. R. McCabe, M. J. Thirman, R. A. Larson, M. M. LeBeau *et al.*, 1993 Rearrangements of the MLL gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood **82:** 3705–3711.

Tamkun, J. W., R. A. Kahn, M. Kissinger, B. J. Brizuela, C. Rulka *et al.*, 1991 The *arflike* gene encodes an essential GTP-binding protein in Drosophila. Proc. Natl. Acad. Sci. USA **88:** 3120–3124.

Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci *et al.*, 1992 *brahma*: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:** 561–572.

Tetzlaff, M. T., H. Jackle and M. J. Pankratz, 1996 Lack of Drosophila cytoskeletal tropomyosin affects head morphogenesis and the accumulation of *oskar* mRNA required for germ cell formation. EMBO J. **15:** 1247–1254.

Tkachuk, D. C., S. Kohler and M. L. Cleary, 1992 Involvement of a homolog of Drosophila *trithorax* by 11q23 chromosomal translocations in acute leukemias. Cell **71:** 691–700.

Wang, W., J. Cote, Y. Xue, S. Zhou, P. A. Khavari *et al.*, 1996 Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J. **15:** 5370–5382.

Waring, P. M., and M. L. Cleary, 1997 Disruption of a homolog of *trithorax* by 11q23 translocations: leukemogenic and transcriptional implications. Curr. Top. Microbiol. Immunol. **220:** 1–23.

Welch, M. D., and D. G. Drubin, 1994 A nuclear protein with sequence similarity to proteins implicated in human acute leukemias is important for cellular morphogenesis and actin cytoskeletal function in *Saccharomyces cerevisiae*. Mol. Biol. Cell **5:** 617–632.

Yu, B. D., J. L. Hess, S. E. Horning, G. A. Brown and S. J. Korsmeyer, 1995 Altered Hox expression and segmental identity in *Mll*-mutant mice. Nature **378:** 505–508.

Yu, B. D., R. D. Hanson, J. L. Hess, S. E. Horning and S. J. Korsmeyer, 1998 *MLL*, a mammalian trithorax-group gene, functions as a transcriptional maintenance factor in morphogenesis. Proc. Natl. Acad. Sci. USA **95:** 10632–10636.

Communicating editor: A. J. Lopez