Single Amino Acid Exchanges in Separate Domains of the Drosophila serendipity δ Zinc Finger Protein Cause Embryonic and Sex Biased Lethality

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ABSTRACT

The Drosophila serendipity (*sry*) delta (δ) zinc finger protein is a sequence-specific DNA binding protein, maternally inherited by the embryo and present in nuclei of transcriptionally active cells throughout fly development. We report here the isolation and characterization of four ethyl methanesulfate-induced zygotic lethal mutations of different strengths in the *sry* δ gene. For the stronger allele, all of the lethality occurs during late embryogenesis or the first larval instar. In the cases of the three weaker alleles, most of the lethality occurs during pupation; moreover, those adult escapers that emerge are sterile males lacking partially or completely in spermatozoa bundles. Genetic analysis of *sry* δ thus indicates that it is an essential gene, whose continued expression throughout the life cycle, notably during embryogenesis and pupal stage, is required for viability. Phenotypic analysis of *sry* δ hemizygote escaper males further suggests that *sry* δ may be involved in regulation of two different sets of genes: genes required for viability and genes involved in gonadal development. All four *sry* δ alleles are fully rescued by a wild-type copy of *sry* δ, but not by an additional copy of the *sry* β gene, reinforcing the view that, although structurally related, these two genes exert distinct functions. Molecular characterization of the four *sry* δ mutations revealed that these mutations correspond to single amino acid replacements in the *sry* δ protein. Three of these replacements map to the same (third out of seven) zinc finger in the carboxy-terminal DNA binding domain; interestingly, none affects the zinc finger consensus residues. The fourth mutation is located in the NH_2-proximal part of the protein, in a domain proposed to be involved in specific protein-protein interactions.

THE Cys_2/His_2 "zinc finger," a DNA-binding motif, was initially identified by molecular analysis of TFIIIA, a Xenopus transcription factor specific for the 5S RNA genes (MILLER, MACLACHLAN and KLUG 1985; BROWN, SANDER and ARGOS 1985). This motif has now been described in a large number of eukaryotic transcriptional regulatory proteins (BERG 1990a). The 28–29-amino acid "finger" is postulated to exist in a folded conformation stabilized by the tetrahedral coordination of a zinc ion by two cysteine and two histidine residues located at invariant positions in each finger (MILLER, MACLACHLAN and KLUG 1985; KLUG and RHODES 1987). One proposed structure (BERG 1988), supported by two-dimensional NMR studies of a single "model" zinc finger consists of a two-stranded antiparallel β sheet containing the two cysteine residues and the histidine/cysteine (H/C) link, and an α-helical domain containing the two histidine residues (LEE *et al.* 1989; NEUHAUS *et al.* 1990). The α-helical region is thought to bind to the major groove of the target DNA helix and to be responsible for the specificity of interaction of the finger with DNA [see BERG (1990b) for review]. The structure of the complex formed between a three finger fragment of the protein *Zif268* and its consensus DNA-binding site recently provided an atomic level view of DNA recognition by Cys_2/His_2 zinc fingers (PAVLETICH and PABO 1991) and confirmed that the sequence-specific contacts have characteristic simplicity and modularity.

The identification of genes encoding Cys_2/His_2 zinc finger proteins in yeast and Drosophila, organisms with powerful genetic tools, means that it is now possible to correlate the effect of alterations in individual residues of such proteins with cellular and developmental defects (BLUMBERG *et al.* 1987; REDEMANN, GAUL and JÄCKLE 1988; GAUL, REDEMANN and JÄCKLE 1989).

Within the Cys_2/His_2 finger protein gene family, the Drosophila *sry* β and δ genes present an interesting problem in the evolution and diversification of structure and function (PAYRE and VINCENT 1988). The *sry* β and δ genes map to a single chromosomal region and probably arose as a result of a duplication event. Comparison of their respective protein products shows that an extensively conserved region is the

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DNA-binding domain which includes six contiguous fingers (VINCENT, COLOT and ROSBASH 1985; PAYRE *et al.* 1990). The *sry* β and δ proteins display partly overlapping, 13 bp long, *in vitro* DNA recognition sites and distinct patterns of *in vivo* binding sites on polytene chromosomes (PAYRE *et al.* 1990; PAYRE and VINCENT 1991; NOSELLI, PAYRE and VINCENT 1992). Both proteins are maternally inherited by the embryo but are also zygotically expressed at different levels throughout the rest of the fly's life cycle (PAYRE, YANISCOSTAS and VINCENT 1989; PAYRE *et al.* 1990). The presence of these proteins in embryonic nuclei before the onset of zygotic transcription and in nuclei of transcriptionally active cells throughout development suggested that *sry* β and δ are transcription factors involved in zygotic activation and maintenance of expression of general cellular functions (PAYRE, YANICOSTAS and VINCENT 1989; PAYRE *et al.* 1990).

We report here the characterization of four *sry* δ zygotic lethal alleles, showing that expression of the *sry* δ gene is essential for normal development. The rescue of viability and fertility of these mutants by *sry* δ, but not by *sry* β DNA indicates that, despite their homology, the function of the *sry* δ and β genes has diverged to the extent that β cannot substitute for *sry* δ. Sequencing of four of the alleles shows that the mutations cause single amino acid changes in two separate domains of the *sry* δ protein, which both have previously been shown to contribute to the specificity of *in vivo* interactions of *sry* δ with chromatin (NOSELLI, PAYRE and VINCENT 1992).

MATERIALS AND METHODS

Drosophila stocks: The *ry506* strain used in the transformation experiments was obtained from W. BENDER, the balanced stock *CyO^TM3, Sb, Ser, ry^rk/ap^xa* from J. DEUTSCH, the MKRS, *Sb/TM2, Ubx* stock (HILLIKER *et al.* 1980) from J. O'TUSA and the lethal alleles *SF1* and *SF2* and the Canton S parental strain (WARMKE, KREUZ and FALKENTHAL 1989) from S. FALKENTHAL. Third chromosome balancers used were *In(3R)Ubx^L Mcp^R*, which carries a duplication for the *99D* region (KONGSUWAN, DELLAVALLE and MERIAM 1986); *In(3L)P+(3LR)HR33+ (3R)Hu+ C+ (3)M6, Hu, Tb*; *In(3LR)TM3, ri p^p sep Su(HW)^2 Sb bx^34e e*; and *In(3LR)TM2, Ubx^130 e^s* (LINDSLEY and GRELL 1968); these chromosomes are referred to in the text as *DpUbxMcp, TM6B, TM3* and *TM2*, respectively. The synthetic deficiency *DfB81Dp67A* is a combination of the chromosomes *Df(3R)B81* and *Dp(3;1)67A*; the proximal and distal breakpoints of the deficiency are in 99D1,2 and 99D9,E1, respectively (KONGSUWAN, DELLAVALLE and MERIAM 1986). The *p[rp49, ry^+]* transformant chromosomes carry the *cp20.1-RP49* plasmid; this plasmid contains one copy of the ribosomal protein gene *49*, and rescues the *Minute* phenotype caused by hemizygosity of the *99D* region (KONGSUWAN *et al.* 1985).

Isolation of recessive lethal mutations: Two mutagenesis schemes were used to identify lethal mutations in the chromosomal region around the *sry* gene. In both schemes, mutagenized chromosomes were tested for lethality over the synthetic deficiency *DfB81Dp67A*. In scheme I (Figure 1a), the *Minute* phenotype caused by the hemizygosity of the

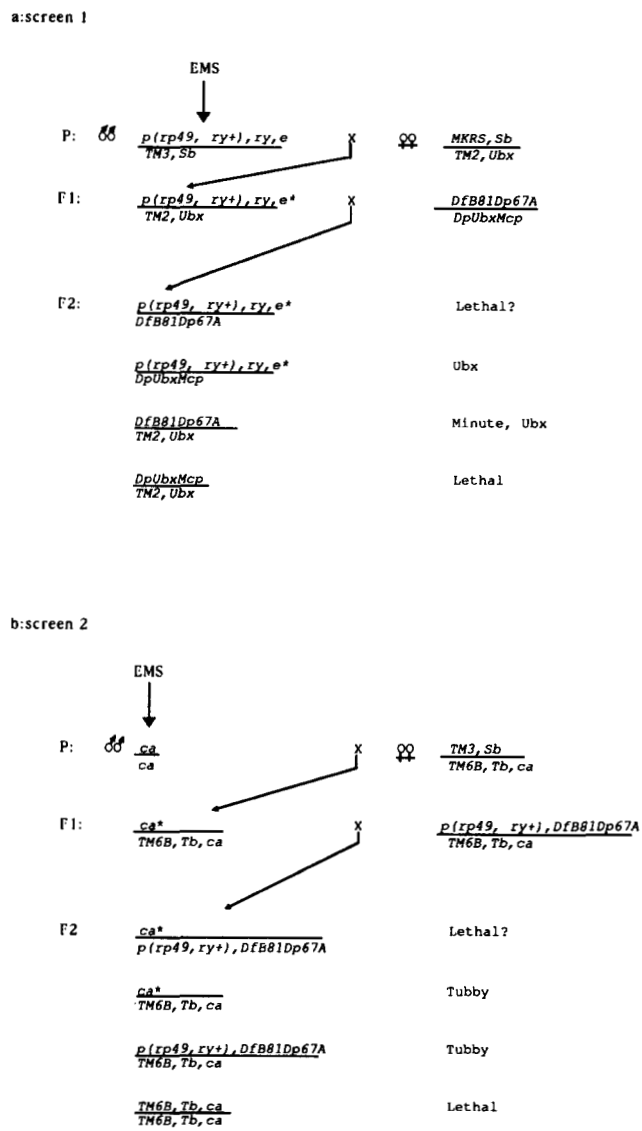

FIGURE 1.—Mutagenesis screens to induce and recover recessive lethal mutations in the *99D* region. For screen 1, the *A3* transformant, in which the *p(rp49, ry^+)* insert maps to *84F* (KONGSUWAN *et al.* 1985), was crossed onto a *ry, e* chromosome; for screen 2, this same insert was crossed onto the *DfB81Dp67A* chromosome. * indicates the mutagenized chromosome. Each possible F_2 genotype is given. If a lethal mutation was induced that was uncovered by the *DfB81Dp67A* deficiency, no F_2 progeny of the F_1 cross would be wild type.

99D region was rescued by the *p[rp49, ry^+] A3* insert at *84F* (KONGSUWAN *et al.* 1985) on the mutagenized chromosome, while in scheme II (Figure 1b), the *p[rp49, ry^+]* F_2 transformant insert, mapping to *80A* (KONGSUWAN *et al.* 1985) was crossed onto the *DfB81* chromosome. The scheme described in Figure 1a was carried out with both ethyl methanesulfate (EMS) and with X-ray mutagenesis.

For EMS mutagenesis, 3–5-day-old males were treated with 0.025 M EMS (Sigma) according to the procedure of LEWIS and BACHER (1968). For X-ray mutagenesis, males aged 3–5 days were treated with 3000 rad. Following mutagenesis, males were mated *en masse* to virgin females in half-pint bottles and transferred every 2 days; after 6 days the adults were discarded. The F_1 progeny from this cross were individually mated to the *DfB81Dp67A* flies according

to the schemes outlined in Figure 1. The resulting F_2 progeny were scored for the absence of the class carrying the mutagenized chromosome over the deficiency, as indicated in Figure 1. If this class was lethal, a stock was established from siblings bearing the mutagenized chromosome/balancer (TM3 or TM6B). Using scheme I, 4926 EMS-treated chromosomes were screened and 20 lethals were identified. Of these lethals, three were rescued when in *trans* to the *DfB81Dp67A* chromosome onto which the *p[CHs, ry⁺]* insert (see below) had been introduced by recombination and were therefore designated sry^{11}, sry^{12} and sry^{13}. Using scheme II, 2560 EMS-treated chromosomes were screened and 17 lethals were identified. Of these lethals, one was rescued by *p[CHs, ry⁺]* and was therefore designated sry^{14}.

An X-ray mutagenesis was carried out using scheme I; 1463 chromosomes were screened and four lethal mutations obtained. None of the X-ray-induced mutations was rescued by *p[CHs, ry⁺]*. One of the X-ray-induced lethal mutations was found to uncover the *99D* region, with breakpoints at 99D1,2 and 99E1,2; this mutation was designated *Df(3R)X3F* (referred to as *DfX3F*), and was used in *P* element rescue experiments described below. Note that the *DfX3F* chromosome provides a simple deficiency which uncovers the 99D region but, because it carries the *p[rp49, ry⁺]* plasmid, is not *Minute*. All screens and complementation tests were performed at 25°.

Plasmid constructions: The *p[CHs, ry⁺]* and *p[CHs-2, ry⁺]* plasmids, constructed by insertion of different genomic DNA fragments into the Carnegie plasmid cp20-1, are described in VINCENT, COLOT and ROSBASH (1986). The *p[CHs, ry⁺]* plasmid contains the *sry α, β and δ*, and *Jan A* and *B* genes, while the *p[CHs-2, ry⁺]* plasmid contains the complete *sry δ* gene and an internally deleted *sry α* gene (Figure 2). The *p[sry δ, ry⁺]* plasmid was constructed by insertion of a 3.4-kb *Xba*I-*Xho*I fragment containing the entire *sry δ* gene (including 1.7 kb of DNA upstream of the *sry δ* transcription start site) into the [ry⁺] *P* element transformation vector pDm23 (MISMER and RUBIN 1987) (Figure 2). The *p[sry β, ry⁺]* plasmid was constructed by insertion of a 2.5-kb *Bam*HI-*Xba*I fragment containing the *sry β* gene into pDm23 (Figure 2). These plasmids were used to transform ry^{506} flies according to standard methods (RUBIN and SPRADLING 1982). Chromosomal linkages of the inserted transposons were determined by segregation of the [ry⁺] eye color phenotype with respect to markers on balancer chromosomes.

***P* element rescue:** To test for rescue with different portions of the *sry* gene cluster, a variety of *P* element constructs were prepared and used for germ-line transformation. The transformant lines CHs and CHs-2 contain the *p[CHs, ry⁺]* and *p[CHS-2, ry⁺]* transposons, respectively (Figure 2). To test the ability of these transposons to rescue putative *sry* alleles from the mutant screens, the transposon insert was crossed onto the *Df(3R)B81* chromosome. The transformant lines CHs-δ_1 and CHs-β_1 carry the *p[sry δ, ry⁺]* and the *p[sry β, ry⁺]* element on the *X* chromosome, respectively. Rescue experiments of the δ^{14} mutation with *sry δ* and *sry β* DNA were repeated with a second transformant line for each construct (CHs-δ_2 and CHs-β_2, which have the transposon inserted on the second chromosome).

Dissection of gonads: Testes and ovaries were dissected in 0.7% NaCl. The ovaries were incubated 10 min in 1 volume of 16 mM KH_2PO_4/K_2HPO_4 (pH 6.8), 75 mM KCl, 25 mM NaCl, 3 mM $MgCl_2$, 6% formaldehyde and 6 volumes of heptane, then fixed 10 min in 1% glutaraldehyde in 1 × phosphate-buffered saline (PBS) and washed in 1 × PBS, prior to labeling of nuclei with 4′,6-diamidino-2-phenylindole (DAPI) and observation under the microscope.

Cloning of mutant genes: Female flies (*p[sry δ, ry⁺]/X; sry δ/DfX3F*) carrying a wild-type copy of the *sry δ* gene on the *X* chromosome and a hemizygous mutant copy on the third chromosome were used to prepare genomic DNA. DNA was digested with *Hin*dIII and *Eco*RI restriction enzymes, and the 3-kb and 5-kb DNA fragments containing the wild-type and mutant copies, respectively, were separated by electrophoresis on agarose gels. The 5-kb fragments were gel purified and subcloned into the pTZ18R plasmid (MEAD, SZCZESNA-SKORUPA and KEMPER 1986) cut with *Hin*dIII and *Eco*RI. Positive clones were isolated using a colony hybridization procedure (GRUNSTEIN and HOGNESS 1975).

DNA sequencing: Double-stranded DNA preparations were used as templates for sequencing by the chain termination method of SANGER, NICKLEN and COULSON 1977. Ten different 16-base oligonucleotides, scattered throughout the *sry δ* gene sequence, were used as sequencing primers. For each allele, the sequence of the entire mutant *sry δ* gene was determined on one isolated clone. The parental genes were cloned and the DNA sequence determined in the region where differences between mutant and wild-type DNA (VINCENT, COLOT and ROSBASH 1985) were identified. In each case two or three independent clones of parental DNA were sequenced. Differences between the parental and the mutant genes were then verified by sequencing the appropriate regions from three to four additional independent clones of mutant DNA.

RESULTS

Isolation of *sry δ* mutants: The *sry* gene cluster maps to *99D4-8* (ROARK *et al.* 1985). Because the *rp49* gene, which encodes a ribosomal protein, maps very close to *sry*, flies carrying deficiencies of this region have a strong *Minute* phenotype and low fertility (KONGSUWAN *et al.* 1985). In order to screen for lethal mutations over a deficiency uncovering the *sry* genes, it was therefore necessary to use a synthetic deficiency of the region and to provide a second copy of the *rp49* gene. A deficiency of the region 99D3 to 99D9, E1 was provided by the synthetic deficiency *Df-B81Dp67A* (described by KONGSUWAN, DELLAVALLE and MERRIAM 1986; see MATERIALS AND METHODS). A *Minute*-rescuing copy of the *rp49* gene was provided by using the *P* element construct *p[rp49, ry⁺]* (KONGSUWAN *et al.* 1985), which was present either on the mutagenized chromosome or crossed onto the synthetic deficiency (see MATERIALS AND METHODS). Two different protocols were used to screen for lethal mutations in the *99D* region. In screen I, a *(rp49,ry⁺), ry, e* chromosome was mutagenized (with EMS in one experiment and X-rays in another experiment); the mutagenized chromosomes were then tested over the *DfB81Dp67A* synthetic deficiency (Figure 1a). From this screen, 4926 EMS- and 1463 X-ray-treated chromosomes were tested, and 20 and 4 lethal alleles, respectively, were obtained. For screen II, a *ca* chromosome was mutagenized with EMS and the mutagenized chromosomes were tested over the *Df-B81Dp67A* synthetic deficiency chromosome carrying

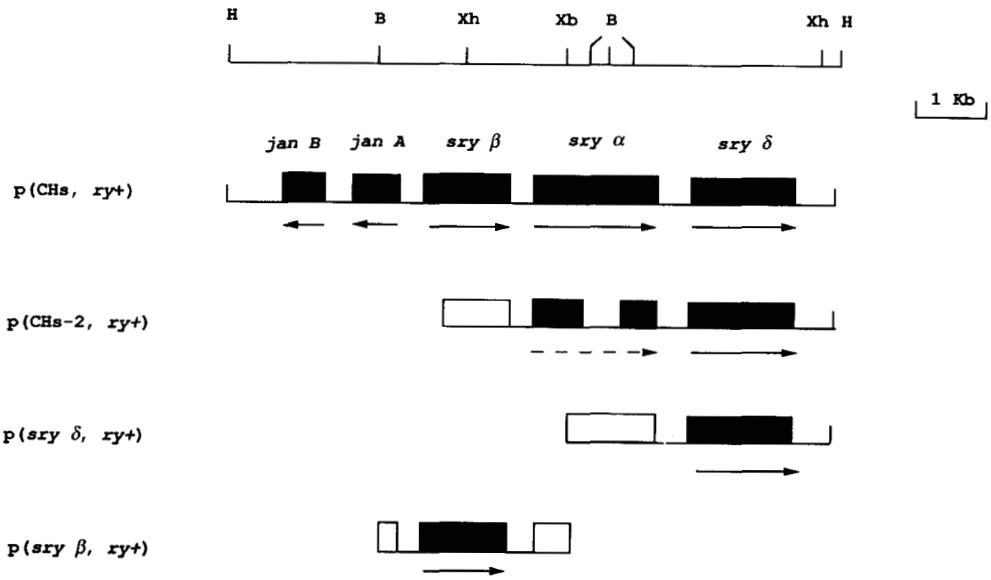

FIGURE 2.—*P* element constructs used for transformation rescue. The top line is a simplified restriction map (H = *Hin*dIII, B = *Bam*HI, Xh = *Xho* I and Xb = *Xba* I) of the genomic DNA around the *sry* gene cluster (VINCENT, COLOT and ROSBASH 1985). Below are shown the four different constructs subcloned into a *P* element transformation vector (see MATERIALS AND METHODS) and used to generate transformant lines. The transcription units carried in these constructs are shown as black boxes, with the direction of transcription indicated by an arrow. The open boxes indicate fragments of coding regions that are not transcribed, due to lack of a promoter.

a *p(rp49, ry*$^+$*)* transformant insert (Figure 1b). In this screen, 2560 chromosomes were tested and 17 lethal alleles obtained.

To determine which of the 41 lethal alleles obtained mapped to the *sry* region, we tested which alleles, in hemizygote combination, could be rescued by complementation with a chromosome carrying the construct *p[CHs, ry*$^+$*]*, which contains the *sry* α, β and δ genes and the closely linked *Jan A* and *B* genes (VINCENT, COLOT and ROSBASH 1985; YANICOSTAS, VINCENT and LEPESANT 1989; see Figure 2). Three lethal alleles (on the *e*-bearing chromosome) from the EMS-treated chromosomes of screen I and one lethal allele (on the *ca*-bearing chromosome) from the EMS-treated chromosomes of screen II were rescued. To more precisely define the transcription unit to which these lethal alleles could belong, they were tested for rescue by complementation with a chromosome carrying the *p(CHs-2, ry*$^+$*)* insert which contains, in addition to an internally deleted and presumably nonfunctional *sry* α gene, a functional *sry* δ gene (VINCENT, COLOT and ROSBASH 1986; see Figure 2). All four alleles that were rescued by the *p[CHs, ry*$^+$*]* insert were also rescued by the *p[CHs-2, ry*$^+$*]* insert, suggesting that all were in the *sry* δ gene (further confirmation of this is presented in experiments described below). These four EMS-induced alleles were designated *sry* δ^{11}, *sry* δ^{12}, *sry* δ^{13} and *sry* δ^{14}, respectively (LINDSLEY and ZIMM 1990). From the X-ray mutagenized chromosomes in screen I (see MATERIALS AND METHODS) a simple deficiency, *DfX3F*, which deletes *99D1,2-99E1*, was recovered; *DfX3F* uncovers all of the *sry* δ alleles. Crosses inter se revealed that the *sry* δ^{14} allele fails to complement the *sry* δ^{11}, *sry* δ^{12} and *sry* δ^{13} alleles, consistent with the notion that all four alleles are in the same gene.

An independent screen of 9,000 EMS-mutagenized chromosomes produced 44 recessive lethal alleles defining 12 complementation groups in the *99D3-99E2,3* interval (WARMKE, KREUZ and FALKENTHAL 1989). Complementation tests between the *sry* δ^{14} allele and lethal alleles identified in the WARMKE *et al.* screen, generously carried out by SCOTT FALKENTHAL, revealed a failure to complement the mutants SF1 and SF2. These latter alleles are therefore additional *sry* δ alleles which we refer to as *sry* δ^{SF1} and δ^{SF2}. Thus three screens, in which a total of 16,500 EMS-mutagenized chromosomes were examined, yielded 6 lethal alleles of *sry* δ.

Mutant phenotype: Because they displayed a range of phenotypic severities and complementation behaviors (see below), we concentrated on the analysis of the four alleles *sry* δ^{12}, *sry* δ^{14}, *sry* δ^{SF1} and *sry* δ^{SF2}, which all displayed partial embryonic lethality when in *trans* to *DfX3F*. The level of adult lethality was assessed in crosses between the mutant and the DfX3F stocks balanced with TM3. For each allele the percentage of the viable hemizygotes (*sry* δ*/DfX3F*) was determined. The TM3/TM3 homozygotes are 100% embryonic lethal (data not shown). Thus, while close to 100% adult female lethality is observed for each of the four *sry* δ alleles, approximately 30% of δ^{SF1}*/DfX3F* and 80% of δ^{SF2}*/DfX3F* males survive to adulthood (Table 1).

To analyze more precisely the lethality period associated with the *sry* δ mutations we determined the

TABLE 1

Percent viability of *sry* δ heteroallelic combinations

Strains	Strains	f non-Sb	m non-Sb	Σ	Non-Sb	Sb	Sb/4	V_{af} %	V_{am} %
DfX3f	δ^{14}	0	3	2720	3	2717	679	0	0.4
DfX3f	δ^{12}	0	3	2477	3	2474	618	0	0.5
DfX3F	δ^{SF1}	2	158	2239	160	2079	520	0.4	30
DfX3f	δ^{SF2}	13	693	4190	706	3484	871	1.5	80
δ^{14}	δ^{12}	0	7	681	7	674	168	0	4
δ^{14}	δ^{SF1}	0	23	945	23	922	230	0	10
δ^{14}	δ^{SF2}	0	31	302	31	271	68	0	46
δ^{12}	δ^{SF1}	8	59	351	67	284	71	11	83
δ^{12}	δ^{SF2}	14	45	285	59	226	56	25	80
δ^{SF1}	δ^{SF2}	64	62	496	126	370	92	69	67

Summed results of reciprocal crosses between *sry* δ mutants and DfX3F (deficiency) strains balanced with TM3. Among the offspring, 50% of the embryos are *sry* δ/TM3 or DfX3F/TM3, 25% are *sry* δ/DfX3F or *sry* δ/*sry* δ and 25% are TM3/TM3. TM3/TM3 is 100% embryonic lethal; *sry* δ/TM3 and DfX3F/TM3 shows normal viability (data not shown). Among the offspring, phenotypically Stubble (noted Sb) and non-Stubble (non-Sb) flies (if 100% viable) were expected to segregate in a 2:1 ratio. The total number of adults scored (Sb and non-Sb) for each combination is indicated (Σ). Assuming a sex ratio of 1:1 as was the case for Stubble flies, the viability indices for each allele (denoted V_{af} and V_{am} for females (f) and males (m), respectively) were calculated from the following equations: $V_{am} = \frac{\text{non-Sb males}}{\text{Sb/4}} \times 100$, $V_{af} = \frac{\text{non-Sb females}}{\text{Sb/4}} \times 100$.

TABLE 2

Percent viability of *sry* δ/*DfX3F* hemizygous embryos

Strains	Strains	Hatched embryos	Total embryos	Total embryos/4	Hatched *sry* δ/*DfX3F*	V_a %
DfX3F	δ^{14}	681	1128	282	117	42
DfX3F	δ^{12}	2306	3489	872	562	64
DfX3F	δ^{SF1}	1863	2813	703	457	65
DfX3F	δ^{SF2}	1150	1732	433	284	66

Summed results of reciprocal crosses between *sry* δ mutants and DfX3F (deficiency) strains balanced with TM3, data presented as described in Table 1. Of the total embryos 50% are *sry* δ/TM3 or DfX3F/TM3 (viable), 25% are TM3/TM3 (embryonic lethal), 25% are *sry* δ/DfX3F. The viability (V_a) of the *sry* δ/DfX3F hemizygous embryos was calculated as following:

$$V_a = \left[\left(\text{hatched embryos} - \frac{\text{total embryos}}{2}\right) \Big/ \left(\frac{\text{total embryos}}{4}\right)\right] \times 100.$$

percentage of lethality associated with the hemizygotes at the embryonic and pupal stages. The extent of embryonic lethality was assessed in crosses between mutant and DfX3F stocks balanced by TM3, in which 75% of the progeny could potentially have hatched; the lethal embryos in excess of 25% (those homozygous for the TM3 balancer) were presumed to be the hemizygotes (see legend of Table 2). The lethality level associated to DfX3F/TM3 or *sry* δ/TM3 is not different from the background level which is about 4% (data not shown). Thus approximately 40% of *sry* δ^{14}/*DfX3F* embryos survived, compared to 60–70% for *sry* δ^{12}, *sry* δ^{SF1} or *sry* δ^{SF2}/*DfX3F* (Table 2).

The lethal hemizygous embryos generally developed to the point of making cuticle. While some of the hemizygous *sry* δ^{14}/*DfX3F* cuticles appeared normal, others showed a defect in head involution (Figure 3). No other cuticular defects were observed (denticle belts and anal pads were examined). Furthermore, no internal defects in the peripheral nervous system (PNS) were detected in embryos in which the nervous system was stained with anti-HRP antibody (data not shown). All *sry* δ^{14}/*DfX3F* hemizygotes that do not die as embryos die as first instar larvae, while in the case of the *sry* δ^{12}/*DfX3F*, δ^{SF1}/*DfX3F* and δ^{SF2}/*DfX3F* hemizygotes, all the hatched larvae develop to give pupae (data not shown). Progeny from crosses of DfX3F and *sry* δ chromosomes balanced over TM6B were counted to determine the relative numbers of *sry* δ/*DfX3F* pupae (Tb^+) compared to the two classes of balanced heterozygous pupae (Tb). This affords a measure of the lethality of each *sry* δ allele prior and during the pupal stage (approximately 1000 pupae were counted for each allele, data not shown). About 0%, 26% and 65% of the *sry* δ^{12}, δ^{SF1} and δ^{SF2} hemizygous pupae, respectively, eclose to give adults which are nearly exclusively males (data not shown).

In all the allelic combinations in which they were obtained, hemizygous "escapers" were significantly smaller and took (at 25°) about 25% longer than their wild-type siblings to progress from the embryonic to pupal stage. Some of the escaper adult males showed rough eyes, extra humeral bristles (a mild *humeral* phenotype); and some escaper males lacked thoracic machrochaetes, especially the presutural bristles (data not shown). While the penetrance observed for each of these subtle defects was incomplete, 100% of the male escapers were sterile in crosses to wild-type females.

Gonadal defects: Examination of adult dissected testes from δ^{SF1}/*DfX3F* showed that the gonads have a much reduced size and contain very few spermatozoa bundles, compared to wild type, while the associated tissues, *i.e.*, the seminal vesicles and ejaculatory bulb, appear normal (Figure 4). Compared to δ^{SF1}/*DfX3F* males, δ^{SF2}/*DfX3F* escaper males show a more pronounced atrophy of the gonads; the associated tissues, however, are still normal if one takes into account the small size of these flies. Observations on squashes of dissected testes showed the complete absence of spermatozoa in the δ^{SF2}/*DfX3F* hemizygous males (Figure 4). The δ^{SF1}/*DfX3F* female escapers (two out of an expected 520) that emerged had seemingly normal ovaries (dissected postmortem), but these females were not tested for fertility because of their very short life span, a trait not observed for males of the same genotype. The few δ^{SF2}/*DfX3F* female escapers (13 out of an expected 871) had a normal life span and dis-

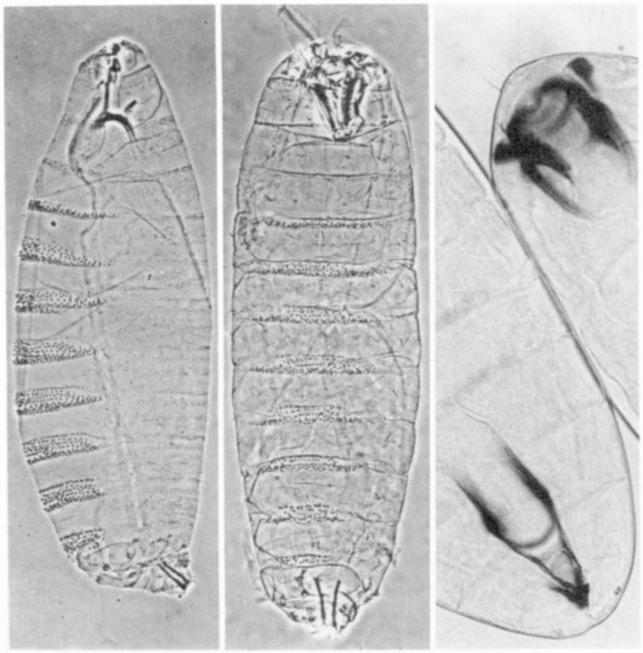

FIGURE 3.—Phenotypes of *sry δ* mutant embryos. Cuticles were prepared as described elsewhere (WIESCHAUS and NÜSSLEIN-VOLHARD 1986). In this case *sry* δ^{14}/*DfX3F* embryos were obtained by crossing *sry* δ^{14}/*DfX3F* and *DfX3F/TM6B* strains. *TM3/TM6B* is embryonic viable (data not shown). (a) Wild-type embryo, note involuted head and cephalopharyngeal skeleton. (b) *sry* δ^{14}/*DfX3F* hemizygous embryo. Head involution is defective, but remainder of embryo appears normal. (c) Enlargement of a wild-type (bottom) and a mutant (top) cephalopharyngeal skeleton. A similar phenotype is observed for embryos of each *sry δ* hemizygote combination (data not shown).

played normal appearing ovaries at the light microscopic level, but did not lay any eggs.

Complementation results: In complementation assays involving pairwise combinations of the four *sry δ* alleles, essentially no complementation between *sry* δ^{14} and the other *sry δ* alleles was found. These results were very similar to those obtained for hemizygotes suggesting that the *sry* δ^{14} allele is amorphic or nearly so (Table 1). Low levels of complementation (escaper adults) were found in crosses between δ^{12} and the δ^{SF1} and δ^{SF2} alleles (Table 1). As was seen for the same alleles in hemizygous combination with *DfX3F*, the escaper double mutant flies δ^{SF1}/δ^{12} or δ^{SF2}/δ^{12} were largely males (Table 1). Complementation between *sry* δ^{SF1} and δ^{SF2} was relatively strong: more than 60% of double mutant males and females eclosed; this was the only case in which equal numbers of males and females escaper were obtained (Table 1). In all cases, however, escapers took longer to emerge than wild type flies and all, both females and males were sterile. Observation on squashes of dissected testes of transheterozygote escaper males showed a phenotype comparable to that of δ^{SF1}/DfX3F hemizygotes (Figure 4 and data not shown). The complementation results, together with the different levels of embryonic lethality, allow the four *sry δ* alleles to be arranged in allelic series: the *sry* δ^{14} allele is the strongest, as it behaves like an amorph in complementation tests. The *sry* δ^{12} allele is weaker than *sry* δ^{14}; the *sry SF1* and *SF2* alleles are weaker, with *SF2* the weakest by a small margin, at least in terms of penetrance for lethality (see DISCUSSION).

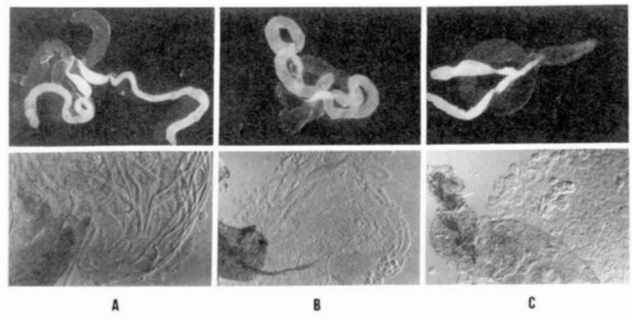

FIGURE 4.—Phenotypes of the testes from the *sry δ* mutant male escapers. The gonads and seminal vesicles are shown on top, squashes of dissected testes at the bottom. A, Wild-type testes, note the large amount of spermatozoa. B, *Sry* δ^{SF1}/*DfX3F* testes, few spermatozoa bundles are detected. C, *Sry* δ^{SF2}/*DfX3F* testes, note the complete absence of spermatozoa bundles.

Rescue of the adult viability and fertility of mutant alleles by *sry δ*, but not *sry β* DNA: Since there was a small possibility that the truncated *sry α* gene in the *p[CHs-2, ry*$^+$*]* insert might be providing some *sry α* function and thus rescuing some of the lethal alleles obtained in the screen, we carried out rescue experiments with a chromosome carrying an insert with only the *sry δ* DNA. That the four alleles described above are in the *sry δ* gene was shown unambigously by full rescue of *sry δ/DfX3F* hemizygotes by the transformed insert *p[sry δ, ry*$^+$*]*, which contains only the *sry δ* gene (see Figure 2). This *p[sry δ, ry*$^+$*]* insert on the *X* chromosome was able to suppress the *sry δ* mutant lethality and sterility: for each *sry δ* allele, the number of non-Sb (*sry δ/DfX3F*) females (in this cross, only the hemizygote females carry the transformed DNA insert) was that expected for 100% rescue by the transposed DNA (Table 3). Mating of the rescued females (*p[sry δ, ry*$^+$*]/X; sry δ/DfX3F*) to wild-type males showed that these females were fully fertile. Similar results were obtained using a *p[sry δ, ry*$^+$*]* insert on the second chromosome and tested with the strongest *sry δ* allele, δ^{14} (data not shown).

Transgenic flies carrying an extra copy of the *sry β* gene (construct *p[sry β, ry*$^+$*]*), see Figure 2) on the *X* chromosome were also constructed. This *p[sry β, ry*$^+$*]* -bearing *X* chromosome was introduced by crosses into *sry δ/DfX3F* flies. The non-*Sb* (*sry δ/DfX3F*) females carried the *p[sry β, ry*$^+$*]* insert while males did not. For all four *sry δ* alleles, there were very few progeny of either type (Table 3). The small number

TABLE 3

Rescue of *sry δ* alleles in presence of transformed *sry δ* or *sry β* DNA

Strains	Strains	f non-Sb	m non-Sb	Σ	Non-Sb	Sb	Sb/4	V_{af} %	V_{am} %
Sry δ[a]									
DfX3f	δ^{14}	257	1	1288	258	1030	257	100	0.4
DfX3f	δ^{12}	290	1	1353	291	1062	265	109	0.4
DfX3f	δ^{SF1}	218	71	1125	289	836	209	104	34
DfX3f	δ^{SF2}	600	460	3332	1060	2272	568	106	81
Sry β[b]									
DfX3f	δ^{14}	0	5	1247	5	1243	311	0	1.6
DfX3f	δ^{12}	0	10	1331	10	1321	330	0	3
DfX3f	δ^{SF1}	1	70	866	71	795	199	0.5	35
DfX3f	δ^{SF2}	26	249	1593	275	1318	329	8	76

To introduce a *p* transposon *p*(*sry*, *ry*$^+$) containing either *sry δ* or *sry β* DNA into a *sry δ* hemizygous background, we used the following cross:

$$♀♀ \frac{X}{X}; \frac{sry\ \delta}{TM3,\ Sb} \times \frac{p[sry,\ ry^+]}{Y}; \frac{DfX3F,\ e}{TM3,\ Sb}\ ♂♂$$

In this cross, males do not receive the *p*(*sry*, *ry*$^+$), transposon. Therefore the non-Sb males (designated as m non-Sb) are escapers, (see text and Table 1). All females receive one copy of either the *sry δ* or *sry β* transposon. Non-Sb females (noted as f non-Sb) (*sry δ*/DfX3F) that emerge are rescued adults and escapers (see Table 1). Viability indexes (noted V_{af} and V_{am} for males and females, respectively) were calculated, using the same equation given in Table 1.

[a] The transposon used was *p*[*sry δ*, *ry*$^+$].

[b] The transposon is *p*[*sry β*, *ry*$^+$]; both transposons are described in Figure 2.

of non-*Sb* flies observed for the *SF1* and *SF2* alleles was that expected from the occurrence of "escaper" males among these hemizygotes (see Table 1). Consistent with this interpretation, the non-*Sb* flies obtained from this cross were sterile, in contrast to the fertility of the progeny rescued by the *sry δ* DNA. Failure of an extra copy of the *sry β* gene to rescue *sry δ* mutations was confirmed using the *p*[*sry β*, *ry*$^+$] insert on the second chromosome and the δ^{14} allele (data not shown). We conclude that *sry β* DNA does not seem to rescue adult viability and fertility of *sry δ/DfX3F* hemizygotes and therefore that an extra copy of *sry β* gene does not seem to compensate for the loss of *sry δ* function.

Cloning and sequence analysis of four *sry δ* alleles: We took advantage of the fact that the *p*[*sry δ*, *ry*$^+$] transposon and the genomic *sry δ* gene yield *Hin*dIII and *Eco*RI restriction fragments of different sizes to isolate and clone the mutant *sry δ* DNA from *p*[*sry δ*, *ry*$^+$]/*X*; *sry δ/DfX3F* lines (see MATERIALS AND METHODS). The DNA fragments containing the *sry* δ^{14}, *sry* δ^{12}, *sry* δ^{SF1} and *sry* δ^{SF2} mutant alleles were cloned into the pTZ18R plasmid, and the entire *sry δ* transcribed region sequenced and compared to wild type [Figure 5; for the full nucleotide and protein sequence of the wild type *sry δ* gene, see VINCENT, COLOT and ROSBASH (1985) and PAYRE *et al.* (1990)]. The four *sry δ* alleles sequenced are point mutations due to G to A (C to T) transitions, consistent with the mode of action of the EMS mutagen (WILLIAMS and SHAW 1987). At the amino acid level, *sry* δ^{14} results in a cysteine (residue position 7) to tyrosine replacement in the NH_2-terminal part of the protein. The three other mutations are localised in the third zinc finger. *Sry* δ^{12} causes a methionine (residue position 271) to isoleucine replacement in the His-His α-helical domain of the finger, *sry* δ^{SF2} results in an arginine (residue position 263) to cysteine replacement and *sry* δ^{SF1} causes a glutamic acid (residue position 251) to lysine replacement (Figure 5). DNA cloned from the *sry* δ^{SF1} mutation also has an additional A to G transition which results in an isoleucine to valine replacement at residue position 144. This conservative replacement is located within a segment of the sry δ protein showing high primary sequence divergence during evolution as opposed to the third finger (P. FERRER, N. CROZATIER and A. VINCENT, manuscript in preparation). Differences between the parental and the mutant genes were verified by cloning the *sry δ* genes from the parental strains used to isolate the mutations, and sequencing the appropriate regions from two or three independent clones. None of the base exchanges identified in the mutants has been found in the corresponding parental gene indicating that the modification found in each clone is the relevant mutation.

DISCUSSION

Generation of lethal alleles in the *sry δ* gene: By providing a transformed copy of the *rp49* gene to rescue the *99D* hemizygous *Minute* phenotype, we were able to screen over a deficiency for lethal alleles of the *sry* genes, and identified six alleles of the *sry δ* gene. From screens of 7500 chromosomes (Figure 1), in which four *sry δ* alleles were identified, no lethal alleles in the other genes carried by the *p*[*CHs*, *ry*$^+$] insert (which contains *sry β*, *sry α*, *Jan A* and *Jan B* in addition to *sry δ*; see VINCENT, COLOT and ROSBASH 1986) were recovered. These other genes might be less mutable, have homologs elsewhere in the genome able to replace their function, and/or are not required for viability. An additional possibility is that the screen was not saturating (we did not test for frequency of lethal hits for other loci in the *99D* region). Although the *sry α* gene has been shown to be required for normal cellularization at the blastoderm stage, it is not known whether lack of this gene results in lethality (SCHWEISGUTH, LEPESANT and VINCENT 1990). From the screen of 9000 mutagenized chromosomes obtained in S. FALKENTHAL's laboratory, 26 lethal mutations were recovered and mapped into 12 complementation groups (WARMKE, KREUZ and FALKENTHAL 1989). One allele of each complementation group (kindly provided by A. KREUZ) was tested for rescue

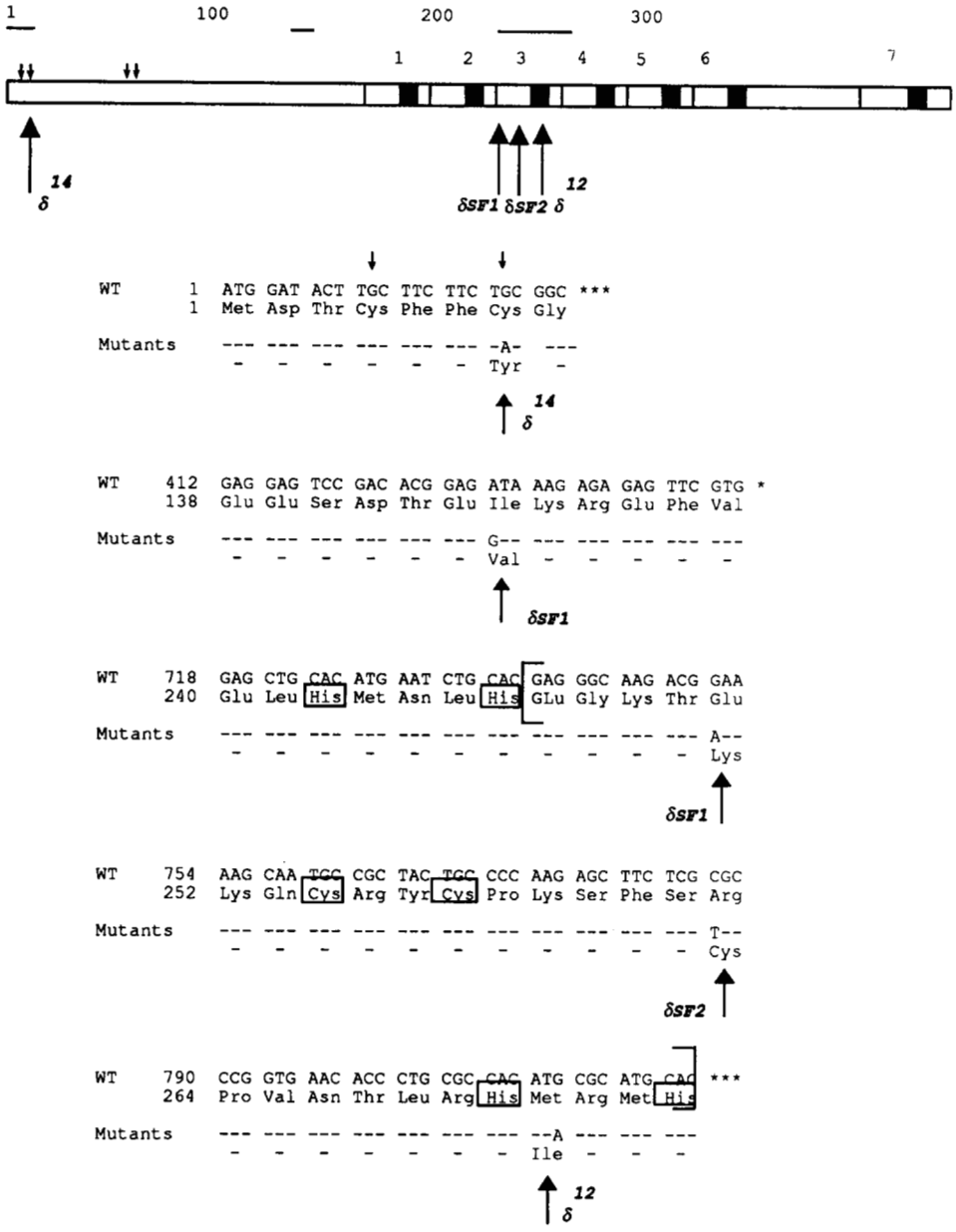

FIGURE 5.—Sequence comparison of the sry δ wild-type (WT) and the four sry δ alleles. For orientation, the complete structure and the putative DNA-binding domain of the *sry* δ wild-type protein are schematically drawn on the top line: the position of each finger (numbered from 1 to 7) is represented by an open box, with the shaded area corresponding to the predicted His-His α-helical domain (see BERG 1988). Horizontal bars above correspond to regions whose nucleotide sequence was determined on both the parental and mutant chromosomes. The corresponding nucleotide and predicted protein sequences are given, whith the third finger being bracketed. Mutations were found at nucleotide positions 20 (cysteine position 7 replaced by tyrosine) in *sry* δ^{14}, position 751 (glutamic acid position 251 replaced by lysine) in *sry* δ^{SF1}; position 787 (arginine position 263 replaced by cysteine) in *sry* δ^{SF2} and position 813 (methionine position 271 replaced by isoleucine) in *sry* δ^{12}. In *sry* δ^{SF1} DNA, an additional nucleotide substitution is found at position 430 (isoleucine position 144 replaced by a valine). In both the diagram at the top and in the sequence, the *sry* δ mutations are indicated by upward pointing arrows. In the sequence, the cysteine and histidine residues of fingers are boxed, while cysteine residues in the Cys-X_2-Cys motifs present in the NH_2-terminal domain are indicated by downward pointing arrows.

by the *p(sryβ, ry⁺)* containing the *sry β* gene, but none seems to correspond to a *sry β* mutant.

An explanation for the failure to obtain sry β mutations specifically (as opposed to *sry* δ mutants) might be the difference in expression pattern between the two genes. Zygotic expression contributes only a minor fraction of the total *sry β* embryonic protein (PAYRE *et al.* 1990) and, further, *sry β* expression during later development is quite low (VINCENT, COLOT and ROSBASH 1985). Maternal expression of *sry β* might thus provide all of the *sry β* expression necessary for survival to adulthood, making it impossible to identify *sry β* alleles in a screen for zygotic lethals. Alternatively, it remains possible that *sry* δ may at least partly complement for *sry β* function, even though the reciprocal is not true.

The *sry δ* allelic series: The four *sry δ* alleles characterized in greater detail displayed varying penetrance for lethality. Hemizygotes for the strongest allele, *sry* δ^{14}, showed almost 100% lethality at either the embryonic or first instar larval stage. This phenotype shows that zygotic expression of *sry δ* is necessary for embryonic viability (PAYRE *et al.* 1990).

Large scale genetic screens have revealed that about 25% of all lethal mutations are embryonic lethal, while only about 3% affect severely the morphology of the mature embryonic cuticle (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; JÜRGENS *et al.* 1984). The head involution defect of the *sry* δ^{14} hemizygotes places the *sry δ* gene in small subclass of all embryonic lethal producing "subtle" phenotypes, including head defects (Jürgens *et al.* 1984). Two mutants with more seriously defective cuticles were identified in the *99* region, *kayak* and *shroud* (Jürgens *et al.* 1984); complementation tests with *DfX3F*, however, indicate that neither gene could be *sry* (data not shown). Since the *sry δ* gene is expressed in virtually all transcriptionally active nuclei of the embryo (PAYRE *et al.* 1990), it is probable that the head involution defect of the *sry δ* mutant embryos is not due to a specific role of *sry δ* in head involution, but to the cumulative effect of inadequate levels of expression of diverse genes involved in many different processes. Because of the large maternal contribution of *sry δ* RNA and protein, it is plausible that a more severe embryonic phenotype of the *sry δ* alleles might be observed if the maternal component were removed; this possibility is currently under investigation.

For the three other alleles δ^{12}, δ^{SF1} and δ^{SF2}, we observed survival of a substantial portion of hemizygotes to the pupal stage. As there is a relatively high level of maternal *sry δ* RNA (VINCENT, COLOT and ROSBASH 1985), it would not be surprising for embryos carrying weak alleles of *sry δ* to be able to survive embryogenesis and hatch. *sry δ* does not seem to be strictly required during larval stages, although larval development is considerably slowed, since all the δ^{12}, δ^{SF1} and δ^{SF2} hemizygous hatched embryos develop to pupae (data not shown). *sry δ* function is required during pupation, however, since 0%, 26% and 65% of δ^{12}, δ^{SF1} and δ^{SF2} hemizygous pupae, respectively, eclose as adults (data not shown). Altogether, these data indicate that the continued expression of *sry δ* at distinct stages of the life cycle, notably during embryogenesis and pupal stage, is required for viability.

Pairwise combinations of the four *sry δ* alleles gave varying degrees of viability (Table 1). Only δ^{14} failed to complement the other *sry δ* mutants, suggesting that it is close to an amorph. The numbers of escapers obtained for the alleles in *trans*-heterozygous combination are greater to the numbers obtained for the alleles as hemizygotes; this partial complementation suggests that the alleles *sry* δ^{12}, *SF1* and *SF2*, are hypomorphs, and is consistent with each of the mutations mapping to the same functional domain of the protein (see below).

Specificity of *sry δ* rescue: That a single copy of the *sry δ* gene restores both full viability and fertility indicates that both lethality and sterility displayed by the *sry* δ^{12}, δ^{14}, δ^{SF1} and δ^{SF2} hemizygotes are due to loss of *sry δ* function. Even the weakest *sry δ* alleles, which can be fully rescued by *sry δ* DNA (Table 3), cannot be rescued by the *sry β* DNA with its complete upstream control region. This result shows that two doses of the *sry β* gene cannot compensate for (an even partial) loss of *sry δ* function, and therefore that the *sry δ* and *β* genes exert separate functions. Other evidence that supports a distinct function of *sry δ* and *sry β* is that the encoded proteins differ in both their expression during embryogenesis and in their DNA binding specificities *in vitro* and *in vivo* (PAYRE *et al.* 1990; PAYRE and VINCENT 1991; NOSELLI, PAYRE and VINCENT 1992). Our results do not distinguish whether this failure to rescue viability and fertility of the hemizygotes is due to a functional difference between the *sry δ* and *β* proteins or whether it is due to a difference in the pattern of expression driven by the two promoters.

Adult somatic and germ-line defects are associated with *sry δ* mutation: Subtle but reproducible somatic phenotypes are shown by the *sry δ* hemizygote adult escapers (small body size, slow growth rate, rough eyes and short bristles). These defects are reminiscent of the phenotypes of strong *Minute* mutations. About 80 loci corresponding to *Minute* mutations have been cytologically mapped on chromosomes (review by ASHBURNER 1989). From molecular cloning studies, it is clear that at least some *Minute* mutations code for ribosomal proteins. The cytological positions of three ribosomal proteins, at 5D3, 58F and 62E-63A, could possibly coincide both with *Minute* mutations and chromosomal binding sites of the *sry δ* protein (ASHBURNER 1989; NOSELLI, PAYRE and VINCENT 1992). Whether the promoter region of any of these genes contains a *sry δ* binding site remains to be determined. Some specific defects of the *sry δ* mutant escapers, such as small rough eyes, are also reminiscent of the roughened eye phenotype that is associated with a dominant mutation of the *rap 1* gene, a gene expressed throughout development and a putative antagonist of *ras* action (HARIHARAN, CARTHEW and RUBIN 1991). A strong binding site of the *sry δ* protein at 67B7-12 coincides with the position of *rap 1*, raising the possibility of an interaction between *sry δ* and *rap 1*. Genetic experiments will be conducted to test for such an interaction.

In dissected testes of hemizygote "escaper" males,

which were all sterile, no (δ^{SF2}) or very few (δ^{SF1}) bundles of spermatozoa were seen (Figure 4). The few (δ^{SF2}) female escapers seemed to have anatomically normal ovaries but they did not lay any eggs. These fertility defects can be correlated with the expression of the *sry δ* gene in premeiotic spermatocytes as well as in both follicle cells and nurse cells during oogenesis (PAYRE, YANICOSTAS and VINCENT 1989). The reason for the presence of many fewer females than males among the escapers is not immediately obvious, but might be due to, an at least indirect, requirement for the *sry δ* protein in expression of genes involved in sex determination, or *X* chromosome dosage compensation. Both processes are regulated through a key *X*-linked control gene, *Sex-lethal* (*Sxl*), whose activity must be on in females and off in males (for review see CLINE 1985). Results from genetic crosses, introducing the *Sxl* dominant alleles Sxl^{M1} and Sxl^{M4} (MAINE *et al.* 1985) into the $sry\ \delta^{SF1}$ or $sry\ \delta^{SF2}/DfX3F$ hemizygous genomes failed to reveal any interaction between *sry δ* and *Sxl* (data not shown). The sex bias in *sry δ* mutant lethality is therefore probably due to interactions between *sry δ* and gene(s) downstream of *Sxl* in the sex differenciation regulatory pathway.

Implications of amino acid replacements in *sry δ* alleles: The $sry\ \delta^{12}$, δ^{14}, δ^{SF1} and δ^{SF2} mutations are all due to single amino acid replacements in the *sry δ* protein product (Figure 5). The strongest allele $sry\ \delta^{14}$ causes substitution of the cysteine at position 7 by a tyrosine; this substituted cysteine is part of a Cys-X_2-Cys doublet that is repeated twice in the NH_2-terminal domain of both the *sry δ* and *β* proteins. Recent experiments making use of modified *sry δ* and *β* genes fused to *lacZ* and expressed in Drosophila transgenic lines suggest that the NH_2-terminal domain of the *sry δ* and *β* proteins may be involved in selective protein-protein contacts and may determine the specificity of *in vivo* interaction with chromatin (NOSELLI, PAYRE and VINCENT 1992).

The three other sequenced *sry δ* mutations are located within the DNA-binding domain, in the same (the third) zinc finger, but none affects the zinc finger consensus residues. In Drosophila, *Krüppel* is the only gene where similar mutations have already been reported (Figure 6). $Sry\ \delta^{12}$, a somewhat weaker allele than $sry\ \delta^{14}$, causes a methionine to isoleucine replacement in the His-His *α*-helical region; this region is thought to make sequence-specific contacts with DNA (BERG 1990b; PAVLETICH and PABO 1991). While methionine is found at this position in the second and third fingers of the *sry δ* and *β* proteins (and the second and fourth fingers in the *Krüppel* protein) (Figure 6), isoleucine is found at this same position in numerous other fingers (GIBSON *et al.* 1988), including the first finger in *sry δ* and *β* (Figure 6). Therefore, breaking of the *α*-helix would not seem to be the

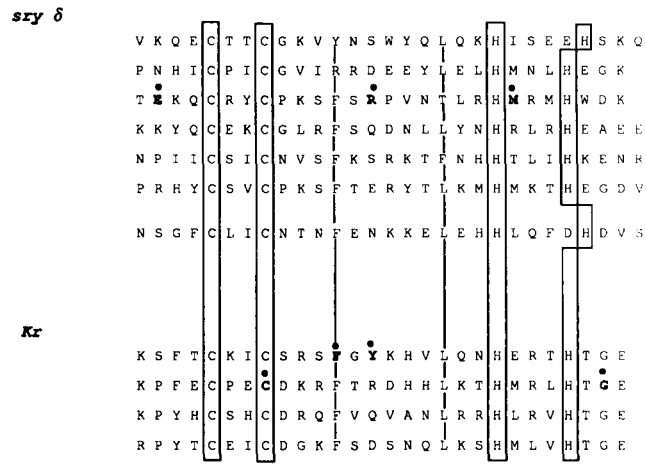

FIGURE 6.—Summary of point mutations localized in the finger domain of the *sry δ* and *Krüppel* proteins. Sequences of each individual finger from the two proteins are given in agreement to the representation used by KLEVIT (1991). The conserved cysteine and histidine residues are boxed, the other consensus positions of the fingers are indicated by vertical lines. Positions of point mutations localized in *sry δ* fingers and *Krüppel* are indicated in bold letters topped by black dots.

reason for the lethal effect of this mutation. It seems more likely that the methionine replacement in the third finger modifies specific contacts between DNA and the protein.

$sry\ \delta^{SF1}$ has two amino acid exchanges compared to the parental copy. The conservative replacement of an isoleucine (residue position 144) to valine is in a region displaying high primary sequence divergence during evolution (PAYRE *et al.* 1990; P. FERRER, M. CROZATIER and A. VINCENT, manuscript in preparation), suggests that this replacement might correspond to a silent mutation. The other modification found in $sry\ \delta^{SF1}$ is a glutamic acid to lysine replacement in the "finger link" separating the second and the third *sry δ* fingers. This exchange of negatively charged by a positively charged residue is likely to modify contacts between the *sry δ* protein and the phosphate DNA backbone. The Kr^1 mutation, a glycine to glutamic acid substitution, also maps in a "finger link" (GAUL, REDEMANN and JÄCKLE 1989; see Figure 6). The *Kr* TGEKP "finger links" (SCHUH *et al.* 1986) are all similar to each other, in contrast to those in *sry δ* which differ from each other and from those found in the *Kr* protein (ROSENBERG *et al.* 1986; VINCENT, COLOT and ROSBASH 1985). The lethality of both the $sry\ \delta^{SF1}$ and the Kr^1 alleles demonstrates that specific sequences of the "finger links" are critically important for the function of Cys_2/His_2 finger proteins.

$sry\ \delta^{SF2}$ causes an arginine to cysteine replacement (position 263) (Figure 6). This replacement occurs at the residue position "*X*" in the three- dimensional structure of the *Zif268* DNA complex proposed by PAVLETICH and PABO (1991), with *X* being one of the three residues involved in a direct base contact of each

finger with DNA. Each of the three *Zif268* fingers also contains an arginine at the *X* position that contacts a guanine of the DNA. It is therefore plausible that the δ^{SF2} mutation results in loss of one base contact between the *sry* δ protein and its recognition site (5′ *Y*TAGAGATGGRAA 3′; PAYRE and VINCENT 1991), resulting in a weakened binding affinity. *In vitro* experiments to determine the consequences of the δ^{SF1}, δ^{SF2} and δ^{14} mutations on the DNA recognition and binding properties of the *sry* δ protein are in progress.

That all three point mutations localized in the DNA-binding domain map to the third zinc finger indicates the crucial functional importance of this finger. Interestingly, it correlates with the fact that, out of the six contiguous *sry* δ fingers, only the third one could possibly make three direct contacts with bases (GAG) according to the pattern of recognition of DNA by Cys_2-His_2 zinc fingers proposed by KLEVIT (1991), based on amino acid positions *X*, *Y* and *Z* (PAVLETICH and PABO 1991). Even though these different amino acid replacements are in the same finger, they do not appear to be equivalent. As already pointed out, δ^{SF1} results in greater lethality than does δ^{SF2}, while δ^{SF2} results in a phenotype characterized by greater gonadal atrophy. Taken together with the strong complementation observed for $\delta^{SF1}/\delta^{SF2}$ *trans*-heterozygotes, these results suggest that the δ^{SF1} and δ^{SF2} mutations differentially affect the relative expression of two different sets of genes: genes coding for general cellular functions, *i.e.*, required for viability, whose expression is more disturbed in δ^{12}, and genes involved in gonadal development, whose expression is more disturbed in δ^{SF2} compared to δ^{SF1}. Characterization of *sry* δ target genes, currently in progress, should aid a detailed understanding of the role of *sry* δ in development.

We are grateful to the memory of our departed colleague, S. FALKENTHAL for carrying out complementation experiments using the *sry* δ^{14} allele and providing us with two new *sry* δ mutations. We also thank A. KREUZ for providing us with other mutants isolated from S. FALKENTHAL's mutagenesis, M. SNYDER for sharing unpublished data, M. STEINMANN-ZWICKY for mutant stocks, A. MALIGLIG and E. STEINGRIMSSON for preparing cuticles, D. CRIBBS, J. DEUTSH, F. MASCHAT and F. PAYRE for comments on the manuscript, E. BARBET and J. MAUREL for photographic and secretarial assistance, respectively, and all members of the Toulouse laboratory for helpful discussion. This work was supported by Centre National de la Recherche Scientifique, and Association pour la Recherche contre le cancer (ARC) grant 6457 to A.V., a National Science Foundation grant DCB10451 to J.A.L. and J.R.M, a National Institutes of Health grant HD09948 to J.A.L. M.C. was supported by a fellowship from ARC.

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