Direct control of transcription of the *Drosophila* morphogen bicoid by the Serendipity δ zinc finger protein, as revealed by in vivo analysis of a finger swap

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Determination of the anterior structures of the *Drosophila* embryo is under control of the maternal gene product Bicoid (bcd), which specifies distinct domains of embryonic gene expression in a concentration-dependent manner. We show here that bcd transcription is controlled by serendipity δ (sry δ), a zygotic-lethal zinc finger protein gene. This sry δ germ-line function was revealed by transgenic expression of a modified Sry 8 protein, Sry DB56, carrying a two-finger swap. Although it almost fully rescues sry δ lethality, Sry DB56 does not substitute for the wild-type protein in activating bcd transcription. Two overlapping sites binding the Sry δ protein were identified in the bcd promoter region, a few base pairs upstream of the putative TATA box. Mutating one site impairs bcd transcription in vivo, indicating that Sry δ acts directly upstream of bcd. The specific requirement of sry δ for bcd transcription in the female germ line constitutes an unexpected link between a zygotic gene with pleiotropic functions and the establishment of coordinates of the *Drosophila* egg. It highlights the fundamental role of ubiquitous transcription factors in bringing about a specific developmental program.

[Key Words: *Drosophila*; bicoid; serendipity; transcription; zinc finger]

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In *Drosophila*, combined genetic and molecular analyses have defined an elaborate cascade of transcription factors that controls the basic organization and polarity of the two major axes, anteroposterior and dorsoventral, of the embryo (for review, see St Johnson and Nüsslein-Volhard 1992; Hoch and Jäckle 1993). Elaboration of the body plan in the head and thoracic regions is largely controlled by the anterior patterning system (Nüsslein-Volhard et al. 1987). Four maternal-effect genes, *bicoid* (bcd), *exuperentia* (exu), *swallow* (swa) and *staufen* (stau), are genetically identified components of this anterior system (Schüpbach and Wieschaus 1986; Frohnhöfer and Nüsslein-Volhard 1987; St Johnson et al. 1989), with bcd being the decisive component [Frohnhöfer and Nüsslein-Volhard 1986; Driever et al. 1990]. The relative severity of cuticular phenotypes associated with different bcd mutations defines an allelic series. Weak alleles affect the anterior-most structures of the head, the labral derivatives, whereas strong alleles develop neither head nor thorax and carry a duplication of the posterior-most structure, the telson, at the anterior end. Activity of exu, sww and stau is required to localize bcd RNA to the anterior pole of the egg during oogenesis. Localized bcd RNA is translated after fertilization to give rise to an anteroposterior gradient of the protein that peaks at the anterior pole (Driever and Nüsslein-Volhard 1988a). Bicoid has all the properties of a morphogen, that is, it determines cell fate along the anteroposterior axis of the embryo in a concentration-dependent manner (Driever and Nüsslein-Volhard 1988b). The Bcd protein contains a homeo domain and is thought to primarily activate transcription of different zygotic head and thoracic segmentation genes in specific domains of the embryonic head and trunk regions [Tautz 1988; Driever and Nüsslein-Volhard 1989; Struhl et al. 1989; Finkelstein and Perrimon 1990; Hoch et al. 1991; Pignoni et al. 1992; for review, see Driever 1992]. Down-regulation of Bcd activity at the anterior pole has recently been shown to be under the control of the *torso* receptor-mediated signaling pathway [Ronchi et al. 1993].

A series of elegant experiments by Driever and Nüsslein-Volhard [1988b] and Struhl et al. [1989] has demonstrated that 1) for the anterior pattern of segmenta-
tion, cell position information is largely determined by the local concentration of Bcd protein, and [2] the maximum concentration of Bcd protein is roughly proportional to the number of bcd gene copies. Together, these data suggest that the level of Bcd activity in early embryos reflects the level of bcd transcription. However, nothing is known of the transcriptional control of bcd.

We report here that serendipity delta (sry δ), which encodes a transcriptional activator of the C2H2 zinc finger type, is required for bcd transcription. Sry δ was initially characterized as a zygotic lethal gene with pleiotropic mutation effects; single amino acid changes in different domains of the Sry δ protein caused embryonic and sex-biased lethality. Both somatic and germ-line defects are associated with sry δ mutations, the relative severity of which depends on the allele considered [Crozatier et al. 1992]. Whereas a single copy of wild-type sry δ fully suppresses sry β mutant lethality and sterility, increasing the copy number of sry β, a sry δ paralog (Vincent et al. 1985; Ferrer et al. 1994), does not rescue any of these phenotypes [Crozatier et al. 1992]. Both Sry β and Sry δ are sequence-specific DNA-binding proteins that bind in vivo to distinct sets of specific sites on polytene chromosomes [Payre and Vincent 1991; Noselli et al. 1992]. In vivo experiments in which the six contiguous zinc finger domains were reciprocally swapped between Sry β and Sry δ indicated that the specificity of chromosomal binding of each protein is largely, although not exclusively, dependent on DNA-specific recognition [Noselli et al. 1992]. To gain further insight into the contribution of separate zinc fingers to the functional difference between Sry β and Sry δ, as well as to identify some of their respective gene targets, we expressed modified Sry δ proteins containing chimeric Sry β/δ zinc finger domains. One construct, sry DB56, that encodes a Sry δ protein in which fingers 5 and 6 are replaced by the homologous fingers of Sry β, fully suppressed sry δ lethality but brought to light one maternal effect of sry δ mutations, the relative increase in the embryonic and sex-biased lethality of sry δ mutant lethality and sterility, whereas fingers 1, 5, and 6 are much more divergent [Chant et al. 1985; Payre et al. 1990]. Amino acid sequence comparison of the two proteins reveals that fingers 2, 3, and 4 are very similar, whereas fingers 1, 5, and 6 are much more divergent. To test for the specific contribution of fingers 5 and 6 in selective binding of Sry

### Results

**Swapping fingers 5 and 6 between the Sry δ and Sry β proteins; in vitro binding studies**

Previously, we reported the respective DNA recognition sequences of the closely related Drosophila Sry β and Sry δ C2H2 zinc finger proteins, 5'-YTAGAGATGGCAA-3' and 5'-YTAGAGATGGGRAA-3', respectively [Payre and Vincent 1991]. The Sry β and Sry δ consensus binding sites, therefore, differ at 4 of the 13 nucleotide positions (Fig. 1A), and these differences are sufficient for discriminatory binding of Sry β and Sry δ to their respective in vitro recognition sites [F. Payre, unpubl.]. The DNA-binding specificity of Sry β and Sry δ is dictated in vitro only by the domain of six adjacent zinc fingers [Noselli et al. 1992]. Amino acid sequence comparison of the two proteins reveals that fingers 2, 3, and 4 are very similar, whereas fingers 1, 5, and 6 are much more divergent [Chant et al. 1985; Payre et al. 1990]. To test for the specific contribution of fingers 5 and 6 in selective binding of Sry

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**Figure 1.** DNA-binding properties of the Sry DB56 protein. (A) Schematic representation of the Sry DB56 protein. Zinc fingers 5 and 6 of Sry δ, right (shaded boxes), have been replaced by the corresponding fingers of Sry β, left (open box). This selective finger swap results in 36 of 58 amino acid changes, with 7 of these changes being conservative (E-->D), (S-->T), (K-->R). The 13-bp consensus recognition sites of Sry β and Sry δ are aligned with nucleotide differences indicated by italics. B) EMSA of DNA recognition by the Sry DB56 protein. Purified proteins made in E. coli were incubated with oligonucleotides containing either a Sry β [B22] or Sry δ [D524] consensus DNA-binding site. Using moderate ionic strength conditions (150 mM KCl) Sry DB56 DNA-binding properties are indistinguishable from Sry δ, except for the relative migration of the formed complex. In lower ionic strength conditions (80 mM) Sry DB56 binds also to the oligonucleotide containing a Sry β consensus-binding site.
β and Sry δ to their respective recognition sequences, we examined the binding specificity of a chimeric Sry δ protein, Sry DB56, in which fingers 5 and 6 were exchanged for the corresponding domains of Sry β (Fig. 1A). The Sry β, Sry δ, and Sry DB56 proteins, purified from overproducing Escherichia coli strains, were assayed for specific binding to oligonucleotides containing either a Sry β (B22) or a Sry δ (D524) consensus binding site, in electrophoretic mobility shift assays (EMSAs). Compared with wild-type Sry δ, Sry DB56 does not display a major modification of its DNA recognition properties in vitro, as it binds efficiently to the Sry δ-binding site under all experimental conditions tested (Fig. 1B). The fact that at low ionic strength Sry DB56 also binds to the Sry p-bind-}

The chimeric SryBD56 protein reveals a maternal sterility effect of sry δ mutations

To test for the functional consequences in vivo of swapping fingers 5 and 6 between Sry p and Sry 8, we constructed transgenic lines expressing the Sry DB56 chimeric protein under the control of the sry δ promoter and assayed for its ability to rescue sry δ lethal mutations. When the p[sry DB56] transposon was introduced into sry δ− hemizygotes there was almost complete rescue of lethality, for each of the four sry δ alleles (Table 1). Similar results were obtained using two independent insertions on different chromosomes (Table 1; data not shown). In all cases, the rescued males were fully fertile. Strikingly, however, the rescued females mated to wild-type males do not give any viable progeny, although egg laying is normal. This correlates with the normal morphology of ovaries from rescued females upon visual inspection by light microscopy (data not shown). Therefore, sry DB56 behaves like a maternal effect-specific allele of sry δ, thus providing access to at least some sry δ maternal functions.

Maternal phenotype of sry δ: bcd-like

Embryos laid by sry δ mutant females rescued by introduction of p[sry DB56] develop to the point of making cuticle. The cuticular pattern of the embryos (Fig. 2) shows reductions and/or deletions of distinct anterior structures, depending on the sry δ allele. In the case of the sry δ8SF1 allele, only the anterior-most head structure, the labrum, is affected. In the case of sry δ12, all derivatives of the head are affected. In the case of the sry δ8SF2 or sry δ14 alleles, thoracic segments are missing and elements of the posterior telson are duplicated at the anterior (Fig. 2). In the strongest sry δ allele, sry δ14 (Crozatier et al. 1992), segmentation defects are also observed frequently in the anterior abdomen, most often in the abdominal segments A2, A3, or A4. The fact that similar results are obtained with the p[sry BD56] transposon inserted on the two different chromosomes confirms that the observed phenotype is not attributable to an insertion position effect (Table 1; data not shown). The phenotype of embryos laid by the p[sry BD56] rescued sry δ− females is clearly reminiscent of that described for a bcd hypomorphic series (Fronhöfer and Nüsslein-Volhard 1987). These results raised the possibility that wild-type sry δ function is necessary for normal levels of bcd activity. This hypothesis was supported by the progressively stronger shift toward the anterior in the positions of the cephalic furrow and the anterior margin of Krüppel expression that is observed in embryos, when going from weak to strong sry δ mutant alleles (data not shown). Such a shift in the fate map of the early embryo is diagnostic of a deviation from the normal dose of bcd− activity (Driever and Nüsslein-Volhard 1988). That the bcd-like phenotype is not attributable to a dominant-negative effect of the chimeric gene was further confirmed by examining embryos laid by the very few transallelic female escapers that emerge in the sry δ8SF1/sry δ8SF2 combination. The cuticular phenotype of these embryos is indistinguishable from that of strong bcd mutant embryos (Fig. 2). Together, these results suggest that

### Table 1. Rescue of sry δ alleles by one copy of the chimeric sry DB56 gene

| sry δ alleles | δ12 | δ14 | δ8SF1 | δ8SF2 |
|---------------|-----|-----|-------|-------|
| X | 1367 | 837 | 1217  | 1550  |
| Sb/4* | 256  | 146 | 194   | 254   |
| F non-Sb | 207  | 138 | 237   | 287   |
| M non-Sb | 184  | 143 | 240   | 295   |
| Va% | 81   | 95  | 122   | 113   |
| F | 0    | 0   | (0.4) | (1.5) |
| VaM% | 72   | 98  | 124   | 116   |
| M | (0.4) | (0.5) | (30) | (80) |

Data are for a transgenic line with the p[sry DB56;ry+*] transposon inserted on the X chromosome and introduced into sry δ hemizygous backgrounds using the following cross:

\[
\delta \delta \times X; sry b^* \rightarrow \delta \delta \delta \rightarrow \delta \nonumber
\]

Non-Stubble (Sb, a dominant marker of bristles) females and males have received one copy of the transposon and correspond to rescued adults. Viability indexes (noted VaF% and VaM% for females and males, respectively) were calculated as follows:

\[
Va% = \frac{\text{non-Sb}}{\text{Sb/4*}} \times 100.
\]

Below, and in parenthesis, is the percentage of viable escaper adults observed in the same crosses but in the absence of the p[sry DB56] transposon (Crozatier et al. 1992). Viability obtained with the p[sry DB56;ry+*] transposon on the second chromosome was 100% in the case of the sry δ8SF1 and δ14 alleles, 87% and 100% for females and males, respectively, for the sry δ13 allele, and not determined for the sry δ8SF2 allele.

The viability of Sb flies is lower than wild-type flies. This difference is taken into account in our calculations by introducing a correction coefficient of 1.05, as determined in control experiments (data not shown).
one maternal function of \( sry \delta \) is to positively regulate the level of \( bcd \) activity.

\( sry \delta \) controls the level of \( bcd \) mRNA accumulation in oocytes

To test whether the reduction of \( bcd \) activity in \( sry \delta \) mutant embryos is attributable to a decreased level of \( bcd \) transcription, we prepared RNA from ovaries of \( sry \delta \) mutant females rescued by the presence of the \( p[sry DB56] \) transposon, \( sry^{8SF2} \) homozygous females (only for this allele, sterile adult females emerge), and some rare \( sry^{8SF1}/sry^{8SF2} \) and \( sry^{8SF2}/sry^{8SF2} \) transheterozygote females. Northern blot analysis shows that mutations in \( sry \delta \) drastically decrease the level of \( bcd \) RNA accumulation, which is undetectable in the strong \( sry \delta \) allelic combinations [Fig. 3A]. This reduction of mRNA level appears to be specific to \( bcd \), as other genes tested, either strictly maternal such as (Wang and Lehmann 1991) or housekeeping [oskar (Ephrussi et al. 1991)] and normal localization of oskar RNA (Ephrussi et al. 1991) in ovaries of \( p[sry DB56] \)-rescued \( sry^{8^{14}} \) mutant females show that the absence of detectable \( bcd \) mRNA is not attributable to a general effect of \( sry \delta \) mutations on synthesis, transport, and/or localization of mRNA in oocytes via, for example, general defects of membrane or cytoskeletal elements (Theurkauf et al. 1992). Therefore, we conclude that \( sry \delta \) is specifically required for \( bcd \) transcription during oogenesis.

Wild-type \( sry \delta \) activity in germ-line cells is required for proper oogenesis in addition to \( bcd \) transcription

Whereas \( bcd \) transcription is restricted in the ovary to the germ line, the \( Sry \) protein is present in nuclei of both the germ line [nurse cells] and somatic (follicle cells) components of the ovary (Payre et al. 1989). To test the germ-line effect of \( sry \delta \) mutations, we generated homozygous \( sry \delta \) germ-line clones by X-ray-induced mitotic recombination in heterozygous \( sry \delta^{-} \) females carrying, in trans, a transposon \( p[ovo^{D1-8}] \) (Mevel-Ninio et al. 1994; see Materials and methods). Because of the dominant effect of \( ovo^{D1} \), a block of oogenesis at previtellogenic stages, developed ovarioles found in irradiated \( sry \delta^{-}/[p[ovo^{D1-8}]] \) females derive only from \( sry \delta \) homozgyous mutant germ-line cells generated by recombination. Examination of these ovarioles, using differential interference contrast (DIC) optics and fluorescent probes for actin and DNA, revealed profound alterations in the cellular morphology of \( sry \delta^{-} \) ovarioles, starting at mid-oogenesis (data not shown). In no case did we observe significant egg deposition. Furthermore, no Bcd protein is detected in the few embryos derived from \( sry \delta^{-} \) homozgyous germ-line clones [data not shown], confirming the requirement of \( Sry \delta \) for \( bcd \) transcription.
Figure 3. Defective accumulation of bcd mRNA in sry 8 mutant ovaries. [A] Northern analysis of bcd expression in ovaries of sry 8 mutant females. Fifteen micrograms of total RNA extracted from hand-dissected ovaries was run in each lane of a formaldehyde-agarose gel, transferred to a nitrocellulose membrane, and probed with labeled bcd cDNA (Berleth et al. 1988) together with rp49 (O’Connell and Rosbash 1984). A single bcd transcript of ~2.6 kb was detected. The relative level of bcd mRNA accumulation in ovaries of wild-type flies, flies containing four copies of bcd (Struhl et al. 1989), and p[sry BD56] containing flies in the context of different sry 8 mutant combinations was calculated using rp49 and rpL17A (Noselli and Vincent 1992) as internal standards for quantitation of deposited RNA, and is indicated below each lane. Wild-type levels of bcd mRNA are observed with a single copy of sry 8, in the presence or absence of p[sry BD56]. Low amounts of bcd mRNA are detected in ovaries of sry 8^11 and sry 8^12 hemizygous or sry 8^S1/sry 8^S2 transheterozygote females, and no signal can be observed in ovaries from sry 8^S2 and sry 8^14 hemizygous or sry 8^S2 homozygous flies or sry 8^S2/sry 8^12 transheterozygote escapee females. [B] Detection of bcd mRNA in whole-mount egg chambers by in situ hybridization using a digoxigenin-labeled probe. [a] p[sry DB56], DfX3F/+ egg chambers. Similar results were obtained with wild-type ovaries. [b] p[sry DB56], DfX3F/sry 8^S1; [c] sry 8^13, and [d] sry 8^14 egg chambers. [d] Small amounts of an oskar DNA probe (Ephrussi et al. 1991) have been added to the hybridization mixture. In all panels, anterior is to the left and posterior to the right. Arrowheads indicate the normal anterior localization of the low amounts of bcd mRNA detected in sry 8^S1 and sry 8^13 mutant ovaries at stage 10A of oogenesis. No signal corresponding to bcd mRNA can be detected at any stage in sry 8^14 mutant egg chambers.

Sry 8 binds in vitro to bcd DNA, upstream of the putative TATA box

As a first step toward testing for a possible direct interaction between Sry 8 and bcd, we looked for in vitro binding sites of the Sry 8 protein within the bcd genomic region shown previously to fully rescue the bcd phenotype. Three plasmids containing bcd DNA were used for immunoprecipitation with the Sry 8 protein. A single fragment was precipitated with DNA cut with different restriction enzymes, locating the Sry 8-binding site close upstream of the bcd transcription start. Immunoprecipitation analysis performed in parallel using the Sry DB56 protein indicated that the chimeric protein is able to bind to the same DNA fragment, and with an efficiency similar to that of wild-type Sry 8 (Fig. 5B). Further mapping allowed the Sry 8-binding site to be located within a 70-bp fragment containing the putative bcd TATA box (Figs. 4 and 5A). The nucleotide sequence of this fragment revealed the presence of two partly overlapping motifs displaying extensive sequence homology with the Sry 8 consensus DNA-binding site (Fig. 5A; see Fig. 1A). Point mutations were introduced at either of these two sites. In both cases (N1 and N3 mutations respectively), binding of Sry 8 to the bcd promoter region is drastically decreased (Fig. 5B), whereas elimination of both sites, using a 12-bp deletion [NO mutation], fully abolishes Sry 8 binding. These results demonstrate that in vitro the Sry 8 protein binds specifically to two sites located between 20 and 40 bp upstream of the presumptive bcd TATA.
box. Together with the dependence of bcd transcription on wild-type sry δ activity, this finding supports the conclusion that Sry δ directly controls bcd transcription by binding to a bcd cis-regulatory element.

The Sry δ-binding site is required for wild-type levels of bcd expression

To test the importance of the Sry δ-binding sites for bcd transcription in vivo, we introduced the N1 mutation in an otherwise intact 8.7-kb bcd genomic fragment within a P-element transformation vector. The wild-type p[bcdGE] and mutated p[bcdGEN1] constructs were introduced into the fly genome, and transgenic Drosophila lines were established. We first stained embryos laid by transformant females with anti-Krüppel antibodies as a measure of bcd activity. Figure 6A shows that as the number of p[bcdGE] copies increases from one to two, the anterior boundary of Krüppel expression shifts to a progressively more posterior position. This reflects increased bcd activity in early embryos as a result of expression of p[bcdGE], as documented previously in similar experiments by Driever and Nüsslein-Volhard (1988). No shift could be detected in the presence of two copies of p[bcdGEN1] (tested with two independent transformed lines, Fig. 6A). These results indicate that the N1 mutation impairs the expression of the bcd transgene. To determine the severity of this reduction in bcd activity the mutated bcd transgene was tested for its ability to rescue the bcd phenotype. One or two copies of p[bcdGE] or p[bcdGEN1] were introduced in females homozygous for the strong bcd" recessive mutation. While one copy of p[bcdGE] rescues all of the alterations due to the bcd" mutation and allows embryos to develop into fertile adult flies, p[bcdGEN1] does not rescue the bcd phenotype. Even with two copies, we still observe a deletion of all the head and thoracic structures and, in most cases, the first or the first two abdominal segments (Fig. 6B). Therefore, there is no improvement of the bcd phenotype by the p[bcdGEN1] construct. Together, the results from both overexpression (Fig. 6A) and phenotypic rescue experiments (Fig. 6B) establish that alteration of one Sry δ-binding site in the bcd promoter region results in a strong decrease of bcd transcription to a level insufficient for significant bcd activity in embryos.

Discussion

Sry δ, a transcription factor upstream of bcd

A small number (~30) of coordinate genes that are spe-
DNA fragment (70 bp), with the two potential Sry 8-binding DNA; (p) precipitated DNA.

Figure 5. Mutational analysis of the Sry 8-binding site in the bcd promoter. (A) Nucleotide sequence of the TaqI-PstI bcd DNA fragment (70 bp), with the two potential Sry 8-binding sites (sdb1, sdb2) boxed and shaded. T/C → G point mutations were introduced in the core of either binding site [N1 and N3 mutations] while a 12-nucleotide deletion [NO mutation] removes most of the two binding sites. The presumptive bcd TATA box is boxed. (B) Immunoprecipitation analysis of Sry 8/bcd interaction. The wild-type Sry 8 or the Sry DB56 protein was incubated with restriction enzyme-digested plasmid DNA containing 1.8 kb of sequence upstream of the bcd transcription start, either wild type or mutated [N1, N3 or NO]; [i] Input DNA; [p] precipitated DNA.

Sry 8 directly controls bcd transcription

If Sry 8 directly controls bcd transcription, it should be possible to identify a bcd cis-acting regulatory sequence binding the Sry 8 protein and mimic the effect of sry 8 mutations by altering this sequence. Two in vitro Sry 8-binding sites are found in a single region that is located in the proximal bcd promoter, 20 bp upstream of the putative TATA box. The sequence of these two overlapping sites is almost identical to the Sry 8 consensus recognition site, as determined previously starting from whole Drosophila genomic DNA (Payre et al. 1991), and in the orientation conferring the most efficient transcriptional activation by Sry 8 in cell transfection assays [F. Payre, P. Buono, and A. Vincent, in prep.]. Alteration of one of these Sry 8-binding sites results in a drastic reduction in bcd activity, indicating that this site is critical for bcd expression. The clear phenotypic correlation between the effect of mutations in Sry 8, the alteration of the Sry 8-binding site in bcd DNA, and mutations in bcd itself strongly argues in favor of a positive and direct control of bcd transcription by Sry 8.

Double heterozygote embryos mutant for a strong bcd mutation (bcd8) and a null for sry 8 are wild type [data not shown]. Furthermore, the relative levels of bcd mRNA in flies carrying either two or four copies of bcd are not significantly changed by reducing the dose of wild-type sry 8 from two to one. Therefore, sry 8 activity in ovaries is in excess relative to that required for normal bcd transcription. Yet, bcd appears to be highly sensitive to mutations in sry 8, indicating that the cis-acting element binding Sry 8 in the bcd promoter is critical for bcd transcription, as subsequently confirmed by the in vivo effect of mutating this binding site.

Genetic experiments in which not only the overall level, but also the shape, of the Bcd gradient is modified have revealed that at least two different regions of the embryonic fate map are specified in a Bcd concentration-dependent manner [Driever and Nüsslein-Volhard 1988b, for review, see Driever 1992]. Low concentrations of Bcd autonomously define thorax identity, whereas high levels define head. Correlation between the calculated relative levels of bcd mRNA in various sry 8 mu-

Payre et al.
Figure 6. Effect of the N1 promoter mutation on bcd activity in early embryos. (A) Anti-Krüppel immunostaining of whole-mount embryos from females either wild type (w.t.) or carrying one or two copies of pbcdGE construct. Position of the anterior boundary of Krüppel expression was measured with camera lucida examination of ~55 blastoderm-stage embryos of each genotype. Wild-type position is at 58% egg length (EL), s.D. = 1.02; pbcdGE1X at 50.3% EL, s.D. = 1.07; pbcdGE2X at 47.7% EL, s.D. = 1.3; pbcdGEN12X at 57.9% EL, s.D. = 1.2. The same value (s.D. = 0.97) was obtained with a second pbcdGEN1 strain. (B) Cuticle preparations of embryos from either wild-type females or females homozygous for the bcd mutation with or without two copies of the pbcdGEN1 transgene. Note that whereas one copy of pbcdGE is able to restore normal segmentation and hatching (Berleth et al. 1988; data not shown) there is no significant rescue of the bcd phenotype by two copies of pbcdGEN1. Insertions on the first or second chromosome were crossed into a bcd mutant background to give stocks of the following genotype p[w+,bcdGE or bcdGEN1]/CyO or FM7,bcd+/TM2. These stocks were used to generate flies homozygous for bcd and either heterozygous (1X) or homozygous (2X) for the transposon.

The bcd regulatory function of Sry δ revealed by a designed finger swap

The crystal structure of the five finger GLI-DNA complex has brought new insights on C2H2 zinc finger-DNA recognition by showing that individual fingers may contribute from 0 to 5 direct nucleotide contacts and/or make extensive protein interactions with other fingers (Pavletich and Pabo 1993). Furthermore, systematic disruption of each of the nine zinc fingers of TFIIIA has suggested structurally and functionally nonuniform interactions of zinc fingers with DNA (Del Rio et al. 1993; Rolling et al. 1993). The Sry DB56 protein was designed to test for the in vivo consequences of the selective exchange of a subset of fingers between Sry δ and its close paralog Sry β (Payre et al. 1990; Ferrer et al. 1994). This finger swap leads to only minor changes in DNA recognition properties in vitro, as Sry DB56 binds to Sry δ-binding sites, both consensus or within bcd, with an affinity similar to the wild-type protein. Sry DB56 is also able to substitute for Sry δ in most, if not all, of its functions throughout development, except bcd transcription. Therefore, and despite the fact that exchanging fingers 5 and 6 introduces 33 amino acid changes, Sry DB56 behaves as a sry δ site-specific allele. It contrasts with the pleiotropic, yet in part allele specific, phenotypes associated with different single amino acid changes in the third Sry δ finger (Crozatier et al. 1992). Specific failure of Sry DB56 to promote bcd transcription could be attributable to either inefficient binding to the bcd promoter in vivo or improper protein-protein interactions involved in bcd transcription — these two possibilities not being mutually exclusive. In this regard, it is interesting to note the peculiar twin structure of the Sry δ-binding sites in the bcd promoter, with the two sites partly overlapping. Our data on Sry DB56 illustrate further the functional versatility of C2H2 zinc finger motifs in promoter specificity and modulation of RNA polymerase II transcription.

Zygotic factors and maternal programs

Why was sry δ not identified in previous genetic screens for genes involved in establishing the coordinates of the embryo? While a number of arguments suggest that most maternal genes specifically involved in establishing positional information in the egg have been identified, the
screens have not been designed to identify maternal effect genes with zygotic functions. Systematic germ-line clonal analysis of X-linked zygotic lethal mutations has identified numerous additional genes that affect specific aspects of embryonic development (Perrimon et al. 1989). In this study 27% of larval–pupal lethal mutations on the X chromosome were found to be germ-line cell lethals. Germ-line clones for sry 8 mutations produce small eggs with severe defects ranging from an abnormal anterior end and fused dorsal appendages, to a more drastic open chorion phenotype (Schiopbach and Wieschaus 1991), and in no case do the few eggs laid develop (M. Crozatier, unpubl.). Although this phenotype still deserves a more thorough analysis, it already indicates that failure to express bcd correctly is only one specific consequence of the effect of sry 8 mutations, that could not be recognized in a classic germ-line clonal analysis. Requirement for sry 8 function at multiple developmental steps, including oogenesis, probably conceals its specific role in the control of bcd transcription. Whereas the various somatic and germ-line phenotypes associated with sry 8 mutations correlate with the widespread expression of Sry 8 throughout development, they contrast with the temporal restriction of bcd expression. Requirement of Sry 8 for bcd transcription underscores the fundamental role of ubiquitously expressed transcription factors in regulating the expression of specific genes and achieving specific developmental programs. It also sheds new light on the molecular dialog between zygotic factors and maternal genes in building a proper Drosophila egg.

Materials and methods

Fly strains

The four sry 8 ethylmethane sulfonate (EMS)-induced alleles sry 8^D2, sry 8^D4, sry 8^SFI and the DH(3R)X3F (DX3F) deficiency strains were described in Crozatier et al. (1992). The bcd^-B(3R) mutant strain was obtained from the Tubingen Stock Center (Tearel and Nüsslein-Volhard 1987). All mutant alleles were kept out as balanced stocks. P-element-mediated transformation was done as described in Spradling and Rubin (1982) using the helper plasmid pr25.7 w.c. as a source of transposase. The rr^-B(3R) strain was used as wild-type strain for RNA preparations, and for injection of the p[rr DB56, ry^-] rescue construct. The p[bcdGE] and p[bcdGEN1] constructs were injected into a white (w^-) strain. Flies were grown under standard conditions at 25°C, and eggs processed for the determination of the cuticular phenotypes as described (Wieschaus and Nüsslein-Volhard 1986).

Generation of sry 8^- homozygous germ-line clones

Crosses were conducted to produce flies trans-heterozygous for a sry 8 mutation and p[oovO17.8] (Mevel-Ninio et al. 1994). Because sry 8 is recessive, these flies are phenotypically sry 8^- but totally lack developed ovaries, owing to the effect of the ovo^O17.8 dominant female sterile mutation. Following X-ray treatment (dose 1000 rads) during the first-instar larval stage to induce mitotic recombination, high frequency germ-line clones of cells homozygous for sry 8 that lack the dominant female sterile mutation were obtained (in our hands, in ~20% of treated flies). Female flies were dissected between 3 and 20 days after eclosion, and ovarian morphology was examined using DIC optics and fluorescent probes for actin and DNA. The few laid embryos were collected and stained with anti-Bcd antibody.

General molecular techniques and DNA/protein-binding assays

RNA preparation from ovaries and Northern blot analysis were done according to Vincent et al. (1984), using random primed probes. Expression in E. coli and purification of the recombinant Sry β, Sry 8, and Sry DB56 proteins, EMSA, and immunoprecipitation procedures were as described in Payre and Vincent (1991). A SacI fragment [positions 4448–5504 from pXDB56, see below] was used to replace zinc fingers 5–6 of Sry 8 by those of Sry β in the E. coli purification vector pSDA1 (Payre et al. 1989). The genomic 8.7-kb EcoRI fragments containing the functional bcd gene (Berleth et al. 1988) and a fragment containing only the S^- and S^+ bcd nontranscribed regions were prepared from the P20 transformation vector [a gift from W. Driever, Massachusetts General Hospital, Boston] and the Casper bcd BglII [a gift from C. Desplan, Rockefeller University, NY], respectively, and cloned into the pGEM7ZF vector (Promega). psb6 was obtained from C. Desplan; pbd 342 was constructed by inserting the bcd genomic region [positions +869 to +1241] as a HindIII–PstI fragment in pGEM7ZF. For site-specific mutagenesis the procedure of Kunkel (1985) was used along with modification of some steps to optimize production of high quality uracylated single-stranded DNA. Sequences of the oligonucleotides used to introduce the N1, N3, and N0 mutations in the pbdel.8 subclone [1.8-kb BamHI–PstI (+1241) bcd fragment in pTZ 18R] are N1, 5'-CAATTTGTCATGTTGATACATCTTTGCG-3'; N3, 5'-CCATCTCTCATGGTGATACATCTTTGCGG-3'; and N0, 5'-GCCGCCAATTTGTCCCTGTCTACCC-3'. N1 and N3 oligonucleotides incorporated a NcoI restriction site (underlined) to facilitate mutant identification. The specific nature of the introduced mutations was then confirmed by sequencing.

Transformation constructs and transgenic fly strains

p[sry DB56] construct The DNA fragment encoding fingers 5 and 6 of Sry 8 was prepared from a sry 8 genomic clone as a Ndel–Smal fragment, position 1494–1663 (the numbering referring to the sry sequence in Vincent et al. 1985). The Ndel and Smal sites were created by site-directed mutagenesis using the oligonucleotides 5'-GGAAGCCTCATTATCGAGGTTGTCGTCC-3' and 5'-CCAGAAGCCGGGGGAGGGCACCTCGCC-3', respectively. This sry 8 fragment was then inserted into a genomic clone containing the functional sry 8 gene (from an Xbal site, position 2486 to an HindIII site, position 6074) (Crozatier et al. 1992) in replacement of the sry 8Ndel–Smal fragment [position 5171–5340]. The Smal site was created in the sry 8 sequence, using the 5'-GGGCAATTGTGGCGTTCGCTGATCCC-3' oligonucleotide. This fragment of genes in the sry 8 construct. The chimeric sry 8/sry 8^D2 (sry 8DB56) gene was then inserted into the [ry^-] P-element transformation vector pDm23.

p[bcdGE] and p[bcdGEN1] constructs After introduction of the N1 mutation, the 1.8-kb BamHI–PstI bcd genomic fragment was put back into p[bcdGE] [a w^- P-vector containing the 8.7-kb bcd genomic fragment obtained from C. Nüsslein-Volhard (Max Planck Institute, Tübingen, Germany) as CaspbcDE] to give the p[bcdGEN1] construct.
In situ hybridization and antibody staining

Whole-mount ovaries were dissected in EBR solution [130 mM NaCl, 5 mM KCl, 2 mM CaCl2, 10 mM HEPES at pH 6.9] and hybridized with digoxigenin-labeled DNA probes, prepared with the genius kit from Boehringer Mannheim, using the procedure described by Tautz and Pfeifle (1989), with minor modifications [Ephrussi et al. 1991]. Whole-mount immunodetection on early embryos was as described by Payre et al. (1990), using a polyclonal anti-Krüppel antibody raised in rabbits (a gift from M. Levine’s laboratory, University of California, San Diego), or a monoclonal anti-Bed antibody obtained from C. Nüsslein-Volhard.

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