Distinct Variant DNA-Binding Sites Determine Cell-Specific Autoregulated Expression of the *Drosophila* POU Domain Transcription Factor Drifter in Midline Glia or Trachea

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Transcriptional regulators utilizing the POU domain DNA-binding motif have been shown to form multi-protein complexes dependent on the POU domain itself and its flexible recognition of various octamer sequence elements. We have identified two variant POU domain recognition elements, DFRE1 and DFRE2, which are found within a 514-bp autoregulatory enhancer of the *Drosophila melanogaster* POU domain gene *drifter* (*dfr*). Both elements are capable of binding bacterially produced full-length DFR protein with high affinity, although they differ in the 5'-to-3' orientation of POU-specific and POU homeodomain subelements. When placed in *dfr* loss-of-function genetic backgrounds, all expression of *dfr-lacZ* fusion genes under control of the autoregulatory enhancer is dependent on DFR activity levels. However, the complete enhancer sequence directs β -galactosidase expression in only a subset of cells which normally express the endogenous DFR protein, including the middle pair of midline glia of the ventral nerve cord, the oenocyte clusters, and all tracheal cells. In addition, DFRE1 and DFRE2 exhibit separable tissue-specific functions when independently disrupted or deleted. Disruption of DFRE1 function specifically abolishes β -galactosidase expression in the middle pair of midline glia. Deletion of DFRE2 causes a specific loss of tracheal expression, leaving oenocyte and midline glia expression intact. These results suggest that *dfr* cell-specific autoregulation is determined by the context of DFR POU domain binding within the enhancer, which is possibly mediated by the formation of recognition element-specific heteromultimeric complexes containing additional tissue-specific factors.

Molecular and developmental analyses of the *Drosophila melanogaster drifter* (*dfr*) gene have shown that DFR protein is expressed under tight tissue-specific control in the middle pair of midline glia (MGM) and selected neurons of the ventral nerve cord as well as in all tracheal cells and oenocytes during embryogenesis. Maintenance of this restricted pattern of expression is required for tracheal and midline glial cell differentiation and migration (2). The DFR protein contains an evolutionarily conserved DNA-binding domain, the POU domain, which is composed of the POU-specific (POU_{SP}) domain and the POU homeodomain (POU_{HD}) connected by a flexible linker (16). This bipartite structure allows the POU domain to display a broad range of specificity for DNA recognition elements (9, 26) and immense potential for tissue-specific interactions with coregulators (16). Such functionally adaptable characteristics result in a family of highly versatile regulators of developmental gene expression identified in numerous species ranging from nematodes to humans (34, 35).

Although POU factor-associated tissue-specific amino- and carboxy-terminal activation domains have been identified elsewhere (41–43), a great deal of functional specificity appears to be mediated by the POU domain itself, which is capable of binding a variety of diverse sequence elements based on the consensus octamer element (ATGCAAAT). Structural analysis of the DNA-bound OCT-1 POU domain has identified specific contacts between the POU_{SP} domain and the ATG subelement, while the POU_{HD} simultaneously binds the adjacent AAAT or TAAT sequence (23, 25). The flexible linker connecting the POU_{SP} and POU_{HD} subdomains considerably

reduces restrictions in relative spatial orientations normally seen between covalently linked protein domains. As a result, the POU domain is capable of recognizing sequence elements in which the ATG and AAAT subelements have been reversed in 5'-to-3' orientation or are separated by a variable number of nucleotides (9, 26). Class III POU domain factors such as BRN-2, SCIP, and DFR have been shown to be significantly more flexible in DNA site recognition than class IV factors such as BRN-3 (26). In agreement with this characteristic, the *dfr* gene was originally cloned on the basis of the ability of DFR protein to bind to such a diverged octamer element, CATAAAT, which functions as a neuron-specific regulatory element of the *dopa decarboxylase* (*Ddc*) gene (21, 22).

The variety of possible three-dimensional conformations resulting from interaction with variant recognition sequences may also contribute to the selective formation of protein-protein contacts between the POU domain and various promoter-specific coregulators (9). The mammalian POU domain factor OCT-1 recognizes the octamer motif ATGCAAAT as well as two promoter elements of the herpes simplex virus (HSV) immediate-early genes known as (OCTA⁺)TAATGARAT and (OCTA⁻)TAATGARAT (for a review, see reference 16). Both TAATGARAT elements are targets for formation of a multiprotein complex, the VP16-induced complex, involving the HSV transcriptional activator, VP16, the OCT-1 protein, and a cellular factor known as HCF (24, 49). The OCT-1 POU_{SP} domain influences the promotion of VP16-induced complex formation differently, depending on whether the complex is being constructed on the (OCTA⁺)TAATGARAT or (OCTA⁻)TAATGARAT forms of the VP16-responsive element (9). Similar site-dependent interactions between the mammalian PIT-1 POU protein and the estrogen receptor

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bound to the prolactin and growth hormone promoters have been demonstrated (18). PIT-1 bound as a monomer within the prolactin enhancer utilizes a tyrosine-dependent synergy domain which is unnecessary when PIT-1 binds as a dimer to a variant site within the growth hormone regulatory sequence. Distinctive target sequence-dependent protein-protein contacts are presumed to result from differences in the relative orientations of the POU_{SP} and POU_{HD} subdomains when bound to each respective recognition element, causing site-specific alterations in the solvent-accessible surface of the DNA-bound POU domain.

POU domain factors have been shown to interact with a number of coactivators or coregulators essential for tissue- and cell-specific transactivation (16). In addition to the VP16 activator, one of the best characterized is a B-cell-specific protein variously identified as OCA-B (28, 29), OBF-1 (39), or BOB1 (12), which is necessary for the activation of immunoglobulin promoters by OCT-1. The formation of B-cell-specific OCT-1/OCA-B complexes is essential for restricted transactivation by the ubiquitously expressed OCT-1 POU factor. It is likely that this general mechanism of coregulator-restricted function is utilized in other tissues and by other members of the POU domain family.

We have been interested in the tissue-specific function of the *Drosophila* POU domain transcription factor DFR and in whether protein-protein interactions with coregulators or coactivators might mediate its function in diverse tissues during development. Here, we report evidence demonstrating that cell-specific *dfr* autoactivation is determined by binding to two distinct variant octamer recognition sequences present in a *dfr* autoregulatory enhancer. Both sequence elements bind DFR protein with high affinity but appear to be responsible for separate tissue-specific components of autoregulated *dfr* expression. The two DFR recognition sequences differ in the 5'-to-3' orientations of POU_{SP} and POU_{HD} subelements as well as the number of nucleotides separating the subelements. We suggest that this recognition element-dependent cell specificity may result from corresponding differences in DNA-bound POU domain conformation which restricts protein-protein interactions with essential coregulators and/or coactivators.

MATERIALS AND METHODS

***Drosophila* stocks.** Flies were raised on standard cornmeal-yeast-agar medium. All stocks and balancer chromosomes not specifically mentioned in the text are described by Lindsley and Zimm (27). All genetic crosses were performed at 25°C unless otherwise specified.

DNA manipulation. Restriction mapping, subcloning, and other DNA manipulations were performed as described by Sambrook et al. (34a), except as modified here. Restriction enzymes were purchased from New England Biolabs or Boehringer Mannheim Biochemicals and were used as suggested by the suppliers. DNA sequences were determined by single-stranded dideoxy nucleotide sequencing techniques with Sequenase (U.S. Biochemicals). All sequence was verified by sequencing both sense and antisense strands. Regions with high GC content were also sequenced with dITP to overcome compression artifacts. Sequence data were manipulated and analyzed with DNA Strider and Gene Jockey.

Genomic clones encoding the *dfr* gene were isolated by screening a *Drosophila* genomic library constructed in λEMBL3 by Ron Blackman (5) by using the original *dfr* λgt11 cDNA insert (21) as a probe. *dfr-lacZ* fusion genes were constructed by cloning selected fragments of *dfr* regulatory DNA into pHS, a pUC18 plasmid containing a truncated *Hsp70* promoter previously used as a heterologous basal promoter (10, 17). The *dfr-HS* fusions were removed as an *EcoRI*-*Bam*HI fragment and cloned into pCaSpeR AUG-βgal, a P-element transformation vector containing a wild-type copy of the *white* (*w*) gene as a dominant marker of transformation as well as an *alcohol dehydrogenase* (*Adh*) translation start site to initiate translation of *lacZ* coding sequences (44).

Progressive deletions of the *dfr* *Hinc*II-*Xba*I fragment (kb -5.75 to -4.5) were constructed by PCR and with a series of synthetic oligonucleotide primers corresponding to sequences at evenly spaced intervals 5' of the *Xba*I site. PCR products were subcloned, sequenced to verify correct amplification, and cloned

into the pHS and pCaSpeR AUG-βgal vectors as described above. Deletions constructed by PCR amplification are designated by the number of nucleotides remaining from the deletion endpoint to the *Xba*I restriction site.

Oligonucleotides used for amplifications were designed to introduce synthetic *EcoRI* restriction sites for subsequent cloning. The 5' oligonucleotides were as follows: 1047dfr-lacZ, 5'-GTGCAATTCGCGATTGCAATTTCTG-3'; 889dfr-lacZ, 5'-GTGCAATTCCTACCTGGTAGACTGAA-3'; 650dfr-lacZ, 5'-GTCGAATTCCTATAAGAACCGAAA-3'; 587dfr-lacZ, 5'-GTGCAATTCCTTGATGTGCCAGGAG-3'; and 514dfr-lacZ, 5'-GTGCAATTCCTCGTAA TGATATGCAC-3'. For all amplifications, the 3' oligonucleotide was a modified T7 primer containing the *EcoRI* restriction site 5'-GTGCAATTCATACG ACTCACTATAG-3'.

mutDFRE1 was synthesized by using a previously described PCR-based in vitro mutagenesis protocol (4). The mutDFRE1 mutagenic primer was designed to disrupt DFRE1 by introducing a unique *Pvu*II site which could also be used for diagnostic digestions to select mutant clones. The sequence of the mutDFRE1 oligonucleotide is as follows, with the mutated nucleotides indicated in boldface and DFRE1 underlined: 5'-CCCAGGGGTG**CGCATCAGCT**CGGAGAATT CG-3'. Mutant clones were completely sequenced before incorporation into pHS and pCaSpeR AUG-βgal as described above.

Primer extension. Primer extension analysis was performed with a ³²P-end-labeled 17-nucleotide synthetic DNA primer that hybridizes to *dfr* sequences from 55 to 71 bp downstream from the 5' end of the largest cDNA insert. The protocols used were modified from those previously described (15). Poly(A)⁺ mRNA isolated from 8- to 22-h embryos was ethanol precipitated with ~50 fmol of ³²P-end-labeled single-stranded primer. The dried pellet was resuspended in 10 μl of primer extension buffer (0.25 M KCl, 2 mM Tris-HCl [pH 7.9], 0.2 mM EDTA), and the suspension was incubated at 55°C for 90 min to anneal the primer. Twenty-three microliters of reverse transcriptase mix (10 mM MgCl₂; 5 mM dithiothreitol; 100 μg of actinomycin D per ml; 0.33 mM [each] dATP, dGTP, dCTP, and dTTP; 20 mM Tris-HCl [pH 8.3]) containing 10 U of reverse transcriptase was added, and the mixture was incubated at 37°C for 60 min to extend the primer. Extension products were ethanol precipitated, resuspended in a mixture of 2 μl of 0.1 N NaOH and 4 μl of formamide-dyes, and analyzed on an 8% sequencing gel.

P-element transformation. Methods for P-element integration were essentially as described previously (2, 38). The *dfr-lacZ* fusion genes in the pCaSpeR AUG-βgal transformation vector were injected into *y ac w; Sb ry⁵⁰⁶ P[ry⁺Δ2-3](99B)/TM6, Ubx* embryos. Transformant flies were identified by screening for the appearance of *w⁺* eye color in the progeny of injectees crossed to *w¹¹¹⁸* adults. Three to five independent transformant strains were established for each fusion construct. Strains were confirmed to contain single-copy inserts of the appropriate P-element vector by Southern blot analysis of genomic DNA.

Immunohistochemistry. Staged *Drosophila* embryos were labeled by a modification of protocols previously described (20, 31). β-Galactosidase (β-Gal) was detected by using either a rabbit polyclonal antiserum (Cappel) or a mouse monoclonal antibody (Promega), both at final dilutions of 1:500. After repeated washes with PBT (1× phosphate-buffered saline [PBS], 0.5% bovine serum albumin, 0.2% Triton X-100) at room temperature, primary antibody was detected by the Vectastain ABC kit (Vector Laboratories) and horseradish peroxidase immunohistochemistry according to protocols suggested by the manufacturer. Embryos were either whole mounted or dissected in 70% glycerol-1× PBS by using electrolytically sharpened tungsten needles to slit labeled embryos lengthwise down the dorsal surface to prepare dorsal file-mounted embryos. After removal of mesodermal contents, lateral sides were laid flat to expose the dorsal surface of the nerve cord. Mounted embryos were examined and photographed on a Nikon Optiphot microscope with Nomarski optics. Homozygous embryos were identified by the absence of the *fz*-derived zebra stripe expression pattern produced from the TM3 *fz*-lacZ third-chromosome balancer.

DNase I protection assays. A full-length DFR protein expression vector was produced by cloning *dfr* cDNA coding sequence into the pOTSNeo12 bacterial expression vector (36) as an *Nco*I-*Xba*I fragment placing DFR expression under control of the P_L promoter. The synthetic *Nco*I site was created at the *dfr* translation start site by in vitro mutagenesis (Amersham). Full-length DFR protein was produced in *Escherichia coli* by growing cells at 30°C until A₆₀₀ = 0.6, when DFR protein expression was induced by incubation at 42°C for 80 min. Bacterial cell lysate was produced from induced cultures by protocols described previously (37). DNase I protection assays were performed as previously described (21, 22) with bacterial cell lysates containing full-length DFR protein. Control lysates prepared from noninduced cells did not contain DFR binding activity. Footprinting probes were single-end labeled with [γ-³²P]ATP and polynucleotide kinase and were isolated by polyacrylamide gel electrophoresis. DNase I digestion fragments were separated on 7% sequencing gels, dried, and exposed to X-Omat AR X-ray film to generate autoradiograms.

RESULTS

Molecular characterization of the *drifter* transcription unit. Three overlapping λEMBL3 bacteriophage clones containing *Drosophila* genomic DNA inserts from the *dfr* locus were isolated by using the original *dfr* λgt11 cDNA insert (21) as a

FIG. 1. Molecular characterization of the *dfr* transcription unit. (A) Schematic representation of the *dfr* genomic restriction map derived from genomic phage inserts. Top thick line, genomic DNA with indicated restriction sites relative to the *dfr* transcription start site (arrow) and the *dfr* transcript (open box) and coding sequence (stippled box); thin lines at bottom, genomic regions recovered from phage inserts. R, *Eco*RI; X, *Xba*I; S, *Sal*I; B, *Bam*HI; H, *Hind*III. (B) Identification of the *dfr* transcription start site by primer extension analysis. Lanes: 3 to 6, dideoxy sequencing reactions; 1 and 2, duplicate primer extension reactions. The single primer extension product is indicated by an arrow. The derived sequence from the region surrounding the start site is bracketed at the right. (C) Nucleotide sequence surrounding the *dfr* transcription start site including the *Drosophila* consensus start site sequence and downstream element (both underlined). (D) Genomic Southern blot analysis of *dfr*^{E82}. A Southern blot of *Bam*HI-SacI-digested genomic DNA was hybridized with a ³²P-labeled, 2.5-kb *Bam*HI-*Eco*RI fragment from within *dfr* 5'-untranslated sequence. Lanes: 1, *mwh red e*; 2, *mwh Df(3L)XBB70 red e/TM6B, Tb e ca*; 3, *mwh dfr*^{E82} *red e/TM6B, Tb e ca*. The deleted *Bam*HI-SacI restriction fragment derived from *dfr*^{E82} is indicated by the arrow. (E) Schematic representation of the *dfr*^{E82} deletion deduced from nucleotide sequence of PCR products amplified from genomic DNA. The 553-bp deletion from +20 to +572 is indicated by brackets.

³²P-labeled probe (Fig. 1A). The three inserts cover a total of 24 kb of genomic DNA including the *dfr* transcript as well as 14 kb of 5'-flanking and 6.5 kb of 3'-flanking sequence. The single *dfr* transcription start site was identified by primer extension analysis (Fig. 1B and C) and was shown to correspond exactly to the most 5' nucleotide of *dfr* cDNA clones (2). The *dfr* transcription unit contains no introns, as determined by correlation of restriction mapping and sequence analysis of genomic DNA clones with identical results from full-length *dfr* cDNAs. The absence of introns is consistent with placement into the POUIII subclass on the basis of structural characteristics of the DFR POU domain (21).

An analysis of proximal promoter sequence revealed that the *dfr* gene utilizes a TATA-less promoter with highly conserved consensus transcription initiation sequences and a downstream CGTG element at bp +33 (Fig. 1C). Approximately one-half of known *Drosophila* promoters are classified as TATA-less and contain essential downstream sequence elements located between bp +20 and +35 (3). Molecular and functional characterization of the ethyl methanesulfonate (EMS)-induced *dfr*^{E82} allele (2) demonstrated that the *dfr* downstream CGTG element is essential for full promoter function. Compared with the parental chromosome on which the

mutation was induced, *dfr*^{E82} genomic DNA contains a unique restriction fragment hybridizing with a ³²P-labeled probe from the 5' end of the *dfr* transcript on genomic Southern blots (Fig. 1D). DNA fragments corresponding to the *dfr* transcript were PCR amplified from *dfr*^{E82}/TM6, *Tb e* genomic DNA by using selective oligonucleotide primers. Amplification products were cloned and sequenced to identify inserts derived from the *dfr*^{E82} chromosome by distinctive polymorphisms characteristic of the multiply marked parental chromosome *mwh red e*. Analysis of DNA sequence showed that *dfr*^{E82} encodes a wild-type copy of the DFR protein but carries a deletion of 5' untranslated sequences between bp +21 and +575 (Fig. 1D). This deletion leaves the *dfr* transcription start site intact but deletes the downstream CGTG element at bp +33. Homozygous *dfr*^{E82} embryos show barely detectable levels of DFR protein (2) and mRNA transcripts (data not shown), suggesting that the mutation disrupts transcription initiation. Although it is possible that other sequences removed by the *dfr*^{E82} deletion are responsible for the loss of *dfr* transcription, this result is consistent with an essential function for the downstream CGTG element.

Identification of a drifter trachea- and MGM-specific enhancer. Cell-specific regulatory elements were identified in *dfr* 5'-flanking sequence by using *dfr-lacZ* fusion genes expressed from P-element transposons in transgenic embryos. Genomic DNA fragments derived from the λG2 phage insert (Fig. 1A) were placed into the pCasper AUGβgal P-element vector (44) and used for the generation of germ line transformant strains to be examined by whole-mount immunolabeling with β-Gal antibody. Analysis of three progressive 5' deletions with the endogenous *dfr* transcription start site (Fig. 2) demonstrates that 7.0 kb of *dfr* 5'-flanking sequence is capable of directing β-Gal expression in a subset of cells normally expressing DFR protein. The 7.0Rdfr-lacZ transposon expresses high levels of β-Gal in all tracheal cells, the MGM in the ventral nerve cord, and the oenocyte clusters of abdominal segments A1 to A7. Failure of the deleted 4.5Xdfr-lacZ and 0.8Rdfr-lacZ transposons to express β-Gal suggests that essential regulatory elements are localized within a 2.5-kb *Eco*RI-*Xba*I restriction fragment between kb -4.5 and -7.0 upstream of the *dfr* transcription start site (Fig. 2). This was confirmed by results from

FIG. 2. Identification of a tissue-specific enhancer with *dfr-lacZ* transposons. Genomic DNA including the *dfr* transcription unit is represented by the thick line at the top with the indicated restriction sites, *dfr* transcription start site (arrow), *dfr* transcript (lightly stippled box), and coding sequence (darkly stippled box). *dfr-lacZ* fusion constructs are aligned below to indicate corresponding regions of 5'-flanking DNA included in each fusion and the resulting tissue-specific expression in trachea, oenocytes, and MGM as assayed in transgenic strains expressing the fusion gene. Since β-Gal levels detected by immunohistochemistry did not vary significantly among expressing lines, the detection of tissue-specific expression is simply represented by plus signs. Minus signs indicate no detectable β-Gal expression. Note that the top three *dfr-lacZ* fusion genes, 7.0Rdfr-lacZ, 4.5Xdfr-lacZ, and 0.8Rdfr-lacZ, utilize the endogenous *dfr* transcription start site and that the XRdfr-lacZ and RXdfr-lacZ fusions utilize a truncated *Hsp70* basal promoter. R, *Eco*RI; X, *Xba*I; S, *Sal*I; B, *Bam*HI; H, *Hind*III.

FIG. 3. Tissue-specific expression of *dfr-lacZ* transposons. (A to C) Lateral views of RXdfr-lacZ embryos whole-mount labeled with β -Gal antiserum and horseradish peroxidase-based immunohistochemistry. Left, anterior; top, dorsal. The abdominal segment A2 oenocyte cluster is indicated by the arrow. Bar, 50 μ m. (A) Stage-12 RXdfr-lacZ embryo displaying β -Gal expression in the oenocyte clusters and the tracheal pits; (B) stage-13 RXdfr-lacZ embryo at completion of germ band retraction showing migration of tracheal cells; (C) stage-14 RXdfr-lacZ embryo showing continued β -Gal expression, dorsal trunk fusion, and extensive migration of tracheal branches; (D) dissected stage-15 ventral nerve cord from an RXdfr-lacZ embryo shown at high magnification under Nomarski optics (top, anterior) to visualize the unlabeled anterior (ac) and posterior (pc) commissures and the longitudinal connectives (lc). β -Gal expression in the MGM and MGA is indicated by the filled and open thick arrows, respectively. Lateral cells expressing β -Gal (arrowhead) are derived from the ganglionic branches of the tracheal tree which have migrated into the nerve cord by this stage. Bar, 10 μ m.

the RXdfr-lacZ transposon containing the 2.5-kb *EcoRI-XbaI* fragment placed upstream of a truncated *Hsp70* basal promoter. RXdfr-lacZ directs β -Gal expression in a tissue- and cell-specific pattern identical to that of the complete 7.0Rdfr-lacZ transposon beginning in stage-11 germ band extended embryos and continuing until late embryogenesis (Fig. 3). The function of RXdfr-lacZ with a heterologous basal promoter also demonstrates that sequences associated with the *dfr* proximal promoter including the transcription start site and downstream element are unnecessary for trachea- and MGM-specific expression.

We have further delimited essential sequences within the 2.5-kb *EcoRI-XbaI* fragment by analyzing β -Gal expression directed from a series of smaller deletion fragments constructed with convenient restriction sites and selective PCR amplifications (Fig. 4; see Materials and Methods for details concerning cloning and PCR primers used). Using these results, we have identified a minimal 514-bp trachea- and MGM-specific enhancer lying approximately 4.5 kb upstream of the *dfr* transcription start site (Fig. 4). The presence and integrity

of nonexpressing *dfr-lacZ* transposons were confirmed by genomic Southern blotting.

We have also observed the unexpected expression of fully functional *dfr-lacZ* fusion genes in a pair of cells associated with the anterior commissure of each neuromere (Fig. 3D) corresponding to the anterior pair of midline glia (MGA). Although we have not previously detected DFR protein expression in the MGA using DFR antiserum (2), expression could be reflective of undetectably low levels of DFR expression which are enhanced by the increased stability of the β -Gal protein relative to DFR. Alternatively, this may represent a characteristic artifact associated with this series of fusion genes. We have not yet distinguished between these two possibilities.

Autoregulation of cell-specific *drifter* expression. Placement of the RXdfr-lacZ transposon in a homozygous *dfr* mutant background (Fig. 5) suggests that RXdfr-lacZ expression is dependent on DFR protein function and that the 514-bp cell-specific enhancer may serve an autoregulatory function. Expression of RXdfr-lacZ is undetectable in both *dfr*^{B129} and

FIG. 4. Delimitation of the *dfr* autoregulatory enhancer. The *dfr* EcoRI-XbaI genomic DNA fragment (kb -4.5 to -7.0) is represented by the thick line at the top, with the indicated restriction sites. Note the difference in scale relative to constructs shown in Fig. 2. *dfr-lacZ* fusion constructs are aligned below to indicate corresponding regions of genomic DNA included in each fusion and the resulting tissue-specific expression in trachea, oenocytes, and MGM as assayed in transgenic strains expressing the fusion gene. The presence or absence of β-Gal expression detected by immunohistochemistry is indicated by plus or minus signs, respectively, as described in the legend to Fig. 2.

Df(3L)XBB70 homozygous embryos (Fig. 5BC). The *dfr*^{B129} allele, isolated in an EMS-induced lethal screen for additional *dfr* mutations, behaves genetically with a severity similar to that of *Df(3L)XBB70*, a small deficiency removing the *dfr* locus along with two other lethal loci (2). A full description of the lethal screen and the characterization of new *dfr* mutant alleles will be published elsewhere. In contrast to these severe alleles, homozygous *dfr*^{E82} embryos, which provide a low level of wild-type DFR protein, express RXdfr-lacZ at levels sufficient for use as a trachea- and MGM-specific marker (2). Thus, when expressed in genetic backgrounds ranging from a null deficiency allele to various hypomorphic *dfr* mutations, RXdfr-lacZ expression levels are entirely dependent on and proportional to levels of DFR activity.

Since DFR activity is essential for tracheal development (2), loss of RXdfr-lacZ expression in severe *dfr* mutant backgrounds could be due to the complete absence of tracheal tissue. We have ruled out this possibility by labeling embryos with the trachea-specific monoclonal antibody, 2A12, to detect the presence of tracheal structures (Fig. 5D). These results indicate that although tracheal structures are defective because of the absence of *dfr* activity, they are present in *dfr*^{B129} embryos and are capable of expressing an independent tracheal marker. These results demonstrate that RXdfr-lacZ expression and, therefore, the maintenance of endogenous *dfr* gene expression are dependent on the presence of autoregulatory DFR protein.

Discrepancies between the onset of endogenous DFR protein expression in the tracheal placodes of stage-10 embryos (2) and the delayed appearance of RXdfr-lacZ expression until after formation of the tracheal pits in stage 11 (Fig. 3A) are also consistent with an autoregulatory mechanism. Delayed RXdfr-lacZ expression would then reflect a temporal switch point between two mechanisms mediating the initiation and subsequent autoregulated maintenance of *dfr* expression. These results suggest that early *dfr* expression in the tracheal placodes is initiated by a transient, not yet identified pattern

formation signal. Once DFR protein has accumulated to levels sufficient for autoactivation, *dfr* expression becomes self-sustaining.

Characterization of cell-specific variant Drifter recognition elements. Nucleotide sequence from the 514-bp *dfr* autoregulatory enhancer (Fig. 6) contains two potential DFR protein recognition elements, DFRE1 (TAATGATATGC) and DFRE2 (ATGCAAAT), containing the AAAT/TAAT POU_{HD} recognition site and the ATG sequence previously shown to bind the POU_{SP} domain (9, 26). DFRE2 is a perfect consensus octamer element known to bind various POU domain proteins. However, DFRE1 is a variant octamer sequence differing both in the relative 5'-to-3' orientations of POU_{SP} and POU_{HD} subelements and in the numbers of nucleotides separating the subelements. The bipartite POU domain structures of other well-characterized proteins such as OCT-1 have previously been shown to display pronounced flexibility in their recognition of sequence elements in which the POU_{SP} and POU_{HD} subelements are reversed (9). In addition, in the previously identified DFR protein recognition element (CATAAAT) found in the *Ddc* neuron-specific enhancer (21, 22) the POU_{SP} and POU_{HD} recognition elements are not separated by intervening nucleotides and are also reversed in 5'-to-3' orientation, suggesting that the DFR POU domain is also capable of recognizing variant octamer elements.

Using in vitro DNase I protection assays, we have demonstrated binding of bacterially produced full-length DFR protein to both DFRE1 and DFRE2 (Fig. 7). Protected regions cover approximately 19 and 16 bp for DFRE1 and DFRE2, respectively. In addition, both elements bind DFR protein with comparable affinity and are associated with DNase I-hypersensitive bands (Fig. 7). These fundamental similarities might suggest that DFRE1 and DFRE2 make interchangeable contributions to the *dfr* autoregulatory mechanism. However, results seen when the two elements are separately disrupted or deleted (Fig. 8) imply that these two variant elements may

FIG. 5. DFR-dependent expression of RXdfr-lacZ. Lateral views of stage-14 whole-mount embryos labeled with β -Gal antiserum and horseradish peroxidase immunohistochemistry are shown. Embryos are shown with anterior to the left and dorsal to the top. Homozygous mutant embryos were identified by the absence of *fz* stripe labeling derived from the marked balancer chromosome *TM3, Sb fzh-lacZ*. The RXdfr-lacZ transposon was present as a homozygous insertion on the second chromosome. (A) RXdfr-lacZ wild-type embryo displaying the normal high levels of β -Gal expression throughout the tracheal tree and oenocyte clusters; (B) homozygous mutant *dfr*^{B129} embryo carrying the RXdfr-lacZ transposon (β -Gal expression is undetectable); (C) homozygous Df(3L)XBB70 embryo carrying the RXdfr-lacZ transposon (no β -Gal expression is detectable); (D) homozygous mutant *dfr*^{B129} embryo labeled with the trachea-specific monoclonal antibody 2A12. Although defective, tracheal labeling is apparent, demonstrating the presence of tracheal tissue. Although not visible in these lateral views, RXdfr-lacZ expression in midline glia was also undetectable in these mutant backgrounds.

independently mediate selective tissue-specific *dfr* transactivation in MGM and trachea.

Disruption of DFRE1 with clustered point mutations introduced by in vitro mutagenesis completely blocks DFR protein

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1  TCCTGTAATGATATGCGCCCTGGGCTAAAGGCCACGACCCCTTGAATGCGATGATACTGTTTGACG
   DFRE1 EcoRV
71  CAATCGTTTTTTTTTTCAGTTTTCTGTTTTTCGAAGGAAAGCAACCAATGCGTGGCGTAGTTGTGGCC
141 ATAGTTTGCAAGTTTGGGCACGTGTTGAAGTCCAGTCACCTGGGCACAGGAAAAGAGAGTCGGAGAA
211 CGCGTTGCCCTTTTGACTGCATATGCAAAATGTTCCATGAAAAGAGATTTTATTGACATATGTTTTC
   DFRE2
281 CTTTGCCGCCCGGCTGTAGGTATTCGTTGAATTCCTGTCGCCACTTCGGTGGGTGTGAACAGAGTTT
351 TAATTTTCATACATTTTCCATTTTCCAGCCATCATTTGGGTGGGTTTTAGTGACTGAGTGCTTTGG
421 TTTGCATTTCAAGCCGATTGAAAGTGAGGACAGCACACAAAAGTGAAGTTTATGGAGATTAAACGCTT
491 TAATGAATTATTTATTTTCTAGA
      XbaI

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FIG. 6. *dfr* autoregulatory enhancer nucleotide sequence. Nucleotide residues corresponding to the 514-bp minimal enhancer sequence are numbered from the 5' end of the minimal enhancer for reference. Variant octamer elements DFRE1 and DFRE2 are indicated by boxes. *EcoRV* and *XbaI* restriction sites are also indicated.

binding (Fig. 7). When the same point mutations are included in the mutDFRE1-514dfr-lacZ transposon, which retains a functional DFRE2, resulting transgenic embryos express β -Gal at normal high levels in the trachea and oenocyte clusters but show no detectable MGM expression (Fig. 8B). In contrast, the 198RVdfr-lacZ transposon, containing a 198-bp enhancer fragment including only DFRE1, is expressed in the oenocyte clusters and MGM but shows no tracheal expression (Fig. 8C). On the basis of these results, we can assign separate tissue-specific transactivation potentials to DFR protein bound to DFRE1 or DFRE2, respectively. When bound to DFRE1, DFR protein is capable of specific transactivation in MGM, while trachea-specific transactivation requires an additional autoregulatory element assumed to be DFRE2. The DFRE2 consensus octamer element is the only sequence bound by full-length DFR protein within the 458-bp fragment necessary for trachea-specific expression (Fig. 7 and 8). Oenocyte expression can apparently utilize both elements interchangeably. The separable functions of DFRE1 and DFRE2 could be due to the proximity of each element to distinct tissue-specific *cis*-regulatory elements within the *dfr* autoregulatory enhancer. Alternatively, considering the remarkable conformational flexibility of the POU domain, we suggest that this distinction could result from conformational differences induced in the DFR POU domain when bound to each respective element. Distinguishable alterations in the solvent-accessible surface of the DFR POU domain caused by flexible recognition of the variant elements DFRE1 and DFRE2 may influence interactions between DNA-bound DFR protein and tissue-specific coactivators or coregulators. Additional experiments will attempt to distinguish between these two possibilities.

DISCUSSION

One of the first steps of organogenesis is the irreversible commitment of embryonic precursor cells to a defined pathway of differentiation. This is normally necessary both to remove cells from the cell cycle (13, 32) and to initiate a transcriptional program specific for progression toward the ultimate cellular phenotype, whether it be tracheal, neuronal, or epidermal. The results presented here demonstrate that cell-specific expression of the *Drosophila* POU domain transcription factor Drifter in MGM, oenocytes, and tracheal cells is maintained by a positive autoregulatory loop. Expression of *dfr-lacZ* transgenes containing *dfr* autoregulatory enhancer sequences is completely dependent on in vivo levels of DFR protein activity. Thus, the RXdfr-lacZ transgene serves as a sensitive assay of in vivo DFR activity. Our previous genetic analysis has shown

FIG. 7. Binding of DFR protein to sequence elements within the *dfr* autoregulatory enhancer. (Top) Schematic representation of the *dfr* transcription unit with the enlarged autoregulatory enhancer. The two DFR protein recognition elements, DFRE1 and DFRE2, are indicated by boxes labeled to show sequences thought to bind the DFR POU_{HD} and POU_{SP} domains, respectively. (Bottom) DNase I protection assays of DFR protein binding sites with increasing concentrations of bacterial cell lysate containing full-length DFR protein. Amounts of total protein added per lane were 0.2 (lanes 3, 9, and 15), 0.6 (lanes 4, 10, and 16), 1.0 (lanes 5, 11, and 17), and 1.4 (lanes 6, 12, and 18) μ g. Lanes: 1 to 6, wild-type DFRE1 probe; 7 to 12, mutDFRE1 containing a 5-bp clustered point mutation disrupting DFRE1; 13 to 18, wild-type DFRE2 probe. Lanes 1, 7, and 13, Maxam-Gilbert purine cleavage fragments; lanes 2, 8, and 14, no protein controls. Both the DFRE1 and mutDFRE1 probes were prepared from the corresponding *EcoRI*-*XbaI* fragment derived from the 587dfr-lacZ fusion gene which was single-end labeled at the synthetic *EcoRI* site with [γ -³²P]ATP and polynucleotide kinase. The DFRE2 probe is the 455-bp *EcoRV*-*XbaI* fragment labeled at the *EcoRV* site. Protected regions surrounding DFRE1 and DFRE2 are marked by brackets; flanking hypersensitive sites are indicated by arrows. Nucleotide residues corresponding to DFRE1 and DFRE2 are indicated to the left of each protected region. Nucleotide residues mutated in mutDFRE1 are marked by asterisks in the DFRE1 sequence. See Materials and Methods for the exact mutant sequence.

FIG. 8. Cell-specific expression mediated by DFRE1 and DFRE2. Stage-14 embryos labeled with β -Gal antiserum were prepared as flattened filets to present tracheal and nerve cord labeling in the same focal plane. Oenocyte expression is also visible but is slightly out of focus in this focal plane. The filets were prepared by slitting lengthwise down the dorsal surface of the embryo with electrolytically sharpened tungsten needles. This is a dorsal view of the nerve cord (anterior at the top). (A) β -Gal expression from the minimal 514dfr-lacZ transposon showing strong expression in trachea (white arrowhead), oenocytes (open white arrow), and MGM (thin black arrow); (B) mutDFRE1-514dfr-lacZ embryo showing strong expression in oenocytes and trachea but no detectable expression in MGM; (C) 198RVdfr-lacZ embryo with only DFRE1 showing expression in MGM and oenocytes but showing no detectable expression in tracheal cells. Note that the remaining β -Gal labeling visible in the mutDFRE1-514dfr-lacZ nerve cord corresponds to the ganglionic branches of the tracheal tree which have migrated into the nerve cord by this point in development.

that DFR protein is essential for correct MGM and tracheal development (2), supporting an overall model in which commitment to these differentiation pathways is mediated at least in part by the establishment of maintained *dfr* expression via a positive feedback loop.

Autoregulated maintenance of expression may also be a highly conserved characteristic of the POU domain family of transcription factors. The extensively characterized mammalian POU domain protein PIT-1 positively activates its own expression through multiple *cis*-active promoter elements (8, 30, 33). The PIT-1 protein plays an essential role in the determination of pituitary cell lineages (1), suggesting that a similar fundamental mechanism of cell commitment may be shared

among POU domain family members in *Drosophila* species and higher vertebrates.

We have previously shown that expression of wild-type DFR protein under control of the *dfr* autoregulatory enhancer is sufficient for rescue of the *dfr*^{ES2} loss-of-function mutant tracheal phenotype (2). Molecular characterization of *dfr*^{ES2} has revealed an EMS-induced 553-bp deletion of 5' untranslated sequence removing a conserved downstream element important for transcription initiation. As a result, *dfr*^{ES2} produces a low level of wild-type *dfr* mRNA and protein that is apparently insufficient for normal regulation of endogenous downstream genes but is capable of maintaining DFR expression via high-affinity DFR protein recognition elements within the autoreg-

FIG. 9. DFR recognition elements. The three sequence elements known to bind DFR protein with high affinity are aligned to show the relative orientations of POU_{SP} and POU_{HD} subelements. Black and gray boxes, the POU_{HD} binding sequences and POU_{SP} sequences, respectively.

ulatory enhancer. Demonstration of phenotypic rescue mediated solely by autoregulated DFR expression suggests that this feedback loop is a primary source of functional DFR protein during normal tracheal development.

Although *in vivo* DFR protein activity is clearly essential for transcriptional activation via the *dfr* autoregulatory enhancer, our results imply that it is not by itself sufficient. This is suggested by the failure of *dfr-lacZ* transgenes containing *dfr* autoregulatory enhancer sequences to function in all cells or tissues expressing endogenous DFR protein. None of our current *dfr-lacZ* fusion transposons recovers central nervous system expression in the mediolateral and lateral cell clusters in the ventral nerve cord, including the serotonergic neurons or RP3 motoneurons which normally express DFR protein (2) (Fig. 3D). Endogenous DFR protein is also present at high levels in the epidermis, as detected with a specific antiserum (2), but does not support epidermal expression of autoregulated *dfr-lacZ* transgenes. This lack of expression could be due to subthreshold levels of DFR protein in these cells. This seems unlikely, however, since tracheal expression of RXdfr-lacZ is activated by barely detectable levels of wild-type DFR protein in homozygous *dfr^{ES2}* embryos. In addition, we have tested RXdfr-lacZ expression in embryos ubiquitously expressing wild-type DFR protein at high levels under the control of the *Hsp70* promoter (7). These embryos express RXdfr-lacZ in the pattern that is seen in a wild-type background, with expression limited to the trachea, oenocytes, and MGM, suggesting that DNA-bound DFR protein is not by itself sufficient for transactivation. This implies that tissue-specific DFR function might then require interaction with additional coregulators or coactivators.

We have identified two endogenous sequence elements, DFRE1 and DFRE2, within the *dfr* autoregulatory enhancer, both of which bind DFR protein but which mediate separate tissue-specific programs of transactivation *in vivo*. Our results indicate that DFRE1 and DFRE2 are necessary for MGM- and trachea-specific expression, respectively. Autoactivation of *dfr-lacZ* expression in the oenocyte clusters is seen with either element, suggesting a nonselective activation in these cells. Comparison of these newly identified DFR recognition sites with the known DFR binding element from the neuron-specific enhancer of the *Ddc* gene, Element C (21, 22) (Fig. 9), shows that the DFR protein is capable of profound flexibility in sequence recognition. The degrees of flexibility in site recognition appear to vary somewhat among members of the POU domain subclasses POU-I through POU-VI (14, 16). Members of the POU-III class, which includes the DFR protein, are of significance because of their markedly broad selectivity for target elements. Of particular interest is their ability to recognize sequence elements in which the relative 5'-to-3' orientations of the POU_{SP} and POU_{HD} subelements are reversed or

the subelements are separated by 0, 1, 2, or 3 nucleotides (16, 26). The three DFR target elements differ as to the numbers of nucleotides separating the POU_{SP} and POU_{HD} subelements, with spacings ranging from 0 nucleotides in *Ddc* Element C to 3 nucleotides in DFRE1. In addition, the POU_{SP} subelements of *Ddc* Element C and DFRE1 are reversed in 5'-to-3' orientation relative to the POU_{HD} recognition elements. A detailed sequence comparison reveals that DFRE1 closely resembles an (OCTA⁻)TAATGARAT element previously identified only in the regulatory sequences of HSV immediate-early genes as a target for OCT-1/VP-16-mediated transactivation (for a review, see reference 16) (Fig. 9). However, this promiscuity in DFR site selection is not unlimited, since a closely related potential recognition element (AAATGCGATGG) located at nucleotides 47 to 56 of the 514-bp minimal enhancer fragment (Fig. 6) does not bind DFR protein *in vitro* and is not necessary for enhancer function *in vivo* (data not shown).

The results from conformational analysis of the DNA-bound POU domains of other POU factors (18, 26) would predict that pronounced differences in subelement orientation between DFRE1 and DFRE2 could alter the relative conformations of the DFR POU_{SP} domain and POU_{HD} when bound to the respective variant sequences and thereby influence tissue-specific protein-protein contacts with potential coregulators or coactivators. Therefore, the diversity of POU domain recognition elements may contribute significantly to the restriction of tissue-specific transactivation in a site-dependent manner. The proposed site-dependent conformational differences in the DFR POU domain would be analogous to previously reported differences in OCT-1 POU_{SP} domain protein-protein contacts seen in the VP16-dependent complex bound to the HSV immediate-early gene (OCTA⁺)TAATGARAT and (OCTA⁻)TAATGARAT elements (9) as well as differential contacts made between PIT-1 and estrogen receptors when bound within prolactin and growth hormone regulatory sequences (18).

The exact nature of DFR protein interactions with tissue-specific coregulators or coactivators could take a number of different forms, on the basis of previous results with other POU domain factors and DFR itself. Although the results presented here allow a clear designation of separate tissue-specific functions between DFRE1 and DFRE2, they do not allow us to distinguish whether those functions are inherent in the variant recognition elements themselves or are due to the positions of the individual elements within the autoregulatory enhancer and thereby to their proximity to other essential tissue-specific sequence elements with which they might interact. Experiments currently under way should shed light on this distinction, which is important in determining the type of protein-protein interactions responsible. Position-dependent tissue specificity suggests a scenario in which the DFR protein binds as part of a heteromeric complex with coactivators capable of binding to their own adjacent *cis*-regulatory sites. Analogous interactions have been demonstrated for both the OCT-1 protein (6, 19, 47, 48) and the *Caenorhabditis elegans* POU protein UNC-86 (50). However, if tissue specificity is a characteristic inherent in the variant elements themselves, it leads to an even more interesting possibility, i.e., that selectivity is dependent on site-specific differences in DNA-bound POU domain conformation which allows interaction with MGM- or trachea-specific coregulators. The DFR protein has also been previously shown to participate in formation of an inhibitory heterodimer with another *Drosophila* POU domain protein, I-POU (40, 45, 46). Therefore, the site-dependent formation of tissue-specific heterodimers is also a distinct possibility.

Elucidation of further details describing the regulation of DFR tissue-specific expression should provide information to explain possible mechanisms by which a single transcription factor is often utilized for multiple tissue-specific functions during development. On the basis of our initial results with the *dfr* autoregulatory mechanism, we suggest that a portion of that specificity may be derived from the formation of site-specific heteromeric protein complexes. On the basis of binding of the mammalian brain-specific POU domain factors BRN-2 and BRN-3.0 to sequence elements within the corticotropin-releasing hormone promoter, it has been previously proposed that POU domain sequence elements in which the POU_{SP} domain recognition motif ATG is reversed in orientation relative to those of the consensus motifs recognized by PIT-1, OCT-1, and OCT-2 may preferentially mediate neuron-specific function (11, 26). In agreement with this proposal, it should be noted that the DFR central nervous system-specific recognition elements *Ddc* Element C and DFRE1 both contain the POU_{SP} subelement in reverse orientation, while the consensus octamer element DFRE2 functions only in the trachea (Fig. 9).

Although it is possible that the results obtained in our experiments reflect unique interactions between the *dfr* autoregulatory enhancer and its proximal promoter, interchangeable use of the heterologous truncated Hsp70 promoter in *dfr-lacZ* fusion genes makes it more likely that similar, if not identical, interactions may mediate the tissue-specific transactivation of other *dfr* target genes as well as the targets of homologous POU domain transcription factors in other systems. The regulation of tissue-specific gene activation is of fundamental importance for controlled cell growth and differentiation. Therefore, the *dfr* autoregulatory enhancer will serve as a valuable tool for further investigations that apply molecular and genetic approaches to identify MGM- and/or trachea-specific coactivator/coregulator proteins.

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REFERENCES

- Andersen, B., and M. G. Rosenfeld. 1994. Pit-1 determines cell types during development of the anterior pituitary gland. A model for transcriptional regulation of cell phenotypes in mammalian organogenesis. *J. Biol. Chem.* **269**:29335–29338.
- Anderson, M. G., G. L. Perkins, P. Chittick, R. J. Shrigley, and W. A. Johnson. 1995. Drifter, a *Drosophila* POU-domain transcription factor, is required for correct differentiation and migration of tracheal cells and midline glia. *Genes Dev.* **9**:123–137.
- Arkhipova, I. R. 1995. Promoter elements in *Drosophila melanogaster* revealed by sequence analysis. *Genetics* **139**:1359–1369.
- Barrettino, D., M. Feigenbutz, R. Valcarcel, and H. G. Stunnenberg. 1993. Improved method for PCR-mediated site-directed mutagenesis. *Nucleic Acids Res.* **22**:541–542.
- Blackman, R. K., R. Grimaila, M. M. D. Koehler, and W. M. Gelbart. 1987. Mobilization of hobo elements residing within the *decapentaplegic* gene complex: suggestion of a new hybrid dysgenesis system in *Drosophila melanogaster*. *Cell* **49**:497–505.
- Bruggemeier, U., M. Kalf, S. Franke, C. Scheidereit, and M. Beato. 1991. Ubiquitous transcription factor OTF-1 mediates induction of the MMTV promoter through synergistic interaction with hormone receptors. *Cell* **64**:565–572.
- Certel, S., and W. A. Johnson. Unpublished data.
- Chen, R., H. A. Ingraham, M. N. Treacy, V. R. Albert, L. Wilson, and M. G. Rosenfeld. 1990. Autoregulation of pit-1 gene expression mediated by two cis-active promoter elements. *Nature (London)* **346**:583–586.
- Cleary, M. A., and W. Herr. 1995. Mechanisms for flexibility in DNA sequence recognition and VP16-induced complex formation by the Oct-1 POU domain. *Mol. Cell. Biol.* **15**:2090–2100.
- Driever, W., G. Thoma, and C. Nüsslein-Volhard. 1989. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature (London)* **340**:363–367.
- Gerrero, M. R., R. J. McEvilly, E. Turner, C. R. Lin, S. O'Connell, K. J. Jenne, M. V. Hobbs, and M. G. Rosenfeld. 1993. Brn-3.0: a POU-domain protein expressed in the sensory, immune, and endocrine systems that functions on elements distinct from known octamer motifs. *Proc. Natl. Acad. Sci. USA* **90**:10841–10845.
- Gstaiger, M., L. Knoepfel, O. Georgiev, W. Schaffner, and C. M. Hovens. 1995. A B-cell coactivator of octamer-binding transcription factors. *Nature (London)* **373**:360–362.
- Haley, O., B. G. Novitch, D. B. Spicer, S. X. Skapek, J. Rhee, G. J. Hannon, D. Beach, and A. B. Lassar. 1995. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. *Science* **267**:1018–1021.
- He, X., M. N. Treacy, D. M. Simmons, H. A. Ingraham, L. W. Swanson, and M. G. Rosenfeld. 1989. Expression of a large family of POU-domain regulatory genes in mammalian brain development. *Nature (London)* **340**:35–42.
- Heberlein, U., and R. Tjian. 1988. Temporal pattern of *alcohol dehydrogenase* gene transcription reproduced by *Drosophila* stage-specific embryonic extracts. *Nature (London)* **331**:410–415.
- Herr, W., and M. A. Cleary. 1995. The POU domain: versatility in transcriptional regulation by a flexible two-in-one DNA-binding domain. *Genes Dev.* **9**:1679–1693.
- Hiroimi, Y., and W. J. Gehring. 1987. Regulation and function of the *Drosophila* segmentation gene *fushi tarazu*. *Cell* **50**:963–974.
- Holloway, J. M., D. P. Szeto, K. M. Scully, C. K. Glass, and M. G. Rosenfeld. 1995. Pit-1 binding to specific DNA sites as a monomer or dimer determines gene-specific use of a tyrosine-dependent synergy domain. *Genes Dev.* **9**:1992–2006.
- Janson, L., and U. Pettersson. 1990. Cooperative interactions between transcription factors Sp1 and OTF-1. *Proc. Natl. Acad. Sci. USA* **87**:4732–4736.
- Johnson, W. A. 1992. Characterization of neuron-specific transcription factors in *Drosophila melanogaster*. *Methods Neurosci.* **9**:362–380.
- Johnson, W. A., and J. Hirsh. 1990. Binding of a *Drosophila* POU-domain protein to a sequence element regulating gene expression in specific dopaminergic neurons. *Nature (London)* **343**:467–470.
- Johnson, W. A., C. A. McCormick, S. J. Bray, and J. Hirsh. 1989. A neuron-specific enhancer of the *Drosophila dopa decarboxylase* gene. *Genes Dev.* **3**:676–686.
- Klemm, J. D., M. A. Rould, R. Aurora, W. Herr, and C. O. Pabo. 1994. Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. *Cell* **77**:21–32.
- Kristie, T. M., J. L. Pomerantz, T. C. Twomey, S. A. Parent, and P. A. Sharp. 1995. The cellular C1 factor of the herpes simplex virus enhancer complex is a family of polypeptides. *J. Biol. Chem.* **270**:4387–4394.
- Kristie, T. M., and P. A. Sharp. 1990. Interactions of the Oct-1 POU subdomains with specific DNA sequences and with the HSV α -trans-activator protein. *Genes Dev.* **4**:2383–2396.
- Li, P., X. He, M. R. Gerrero, M. Mok, A. Aggarwal, and M. G. Rosenfeld. 1993. Spacing and orientation of bipartite DNA-binding motifs as potential functional determinants for POU domain factors. *Genes Dev.* **7**:2483–2496.
- Lindsley, D. L., and G. G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press, Inc., San Diego, Calif.
- Luo, Y., H. Fujii, T. Gerster, and R. G. Roeder. 1992. A novel B-cell-derived coactivator potentiates the activation of immunoglobulin promoters by octamer-binding transcription factors. *Cell* **71**:231–241.
- Luo, Y., and R. G. Roeder. 1995. Cloning, functional characterization, and mechanism of action of the B-cell-specific transcriptional coactivator OCA-B. *Mol. Cell. Biol.* **15**:4115–4124.
- McCormick, A., H. Brady, L. E. Theill, and M. Karin. 1990. Regulation of the pituitary-specific homeobox gene GHF1 by cell-autonomous and environmental cues. *Nature (London)* **345**:829–832.
- Mitchison, T. J., and J. Sedat. 1983. Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99**:261–264.
- Parker, S. B., G. Eichele, P. Zhang, A. Rawls, A. T. Sands, A. Bradley, E. N. Olson, J. W. Harper, and S. J. Elledge. 1995. p53-independent expression of p21Cip1 in muscle and other terminally differentiating cells. *Science* **267**:1024–1027.
- Rhodes, S. J., R. Chen, G. E. DiMattia, K. M. Scully, K. A. Kalla, S.-C. Lin, V. C. Yu, and M. G. Rosenfeld. 1993. A tissue-specific enhancer confers Pit-1-dependent morphogen inducibility and autoregulation on the pit-1 gene. *Genes Dev.* **7**:913–932.
- Rosenfeld, M. G. 1991. POU-domain transcription factors: pou-er-ful developmental regulators. *Genes Dev.* **5**:897–907.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

35. **Schöler, H. R.** 1991. Octamania: the POU factors in murine development. *Trends Genet.* **7**:323–329.
36. **Shatzman, A. R., and M. Rosenberg.** 1987. Expression, identification, and characterization of recombinant gene products in *Escherichia coli*. *Methods Enzymol.* **152**:661–673.
37. **Singh, H., R. G. Clerc, and J. H. LeBowitz.** 1989. Molecular cloning of sequence-specific DNA binding proteins using recognition site probes. *Bio-Techniques* **7**:252–261.
38. **Spradling, A. C.** 1986. P element-mediated transformation, p. 175–197. *In* D. B. Roberts (ed.), *Drosophila, a practical approach*. IRL Press, Oxford, England.
39. **Strubin, M., J. W. Newell, and P. Matthias.** 1995. OBF-1, a novel B cell-specific coactivator that stimulates immunoglobulin promoter activity through association with octamer-binding proteins. *Cell* **80**:497–506.
40. **Tabin, C. J.** 1992. Why we have (only) five fingers per hand: Hox genes and the evolution of paired limbs. *Development* **116**:289–296.
41. **Tanaka, M., and W. Herr.** 1990. Differential transcriptional activation by Oct-1 and Oct-2: interdependent activation domains induce Oct-2 phosphorylation. *Cell* **60**:375–386.
42. **Tanaka, M., J.-S. Lai, and W. Herr.** 1992. Promoter-selective activation domains in Oct-1 and Oct-2 direct differential activation of an snRNA and mRNA promoter. *Cell* **68**:755–767.
43. **Theill, L. E., J.-L. Castrillo, D. Wu, and M. Karin.** 1989. Dissection of functional domains of the pituitary-specific transcription factor GHF-1. *Nature (London)* **342**:945–948.
44. **Thummel, C. S., A. M. Boulet, and H. D. Lipshitz.** 1988. Vectors for *Drosophila* P-element-mediated transformation and tissue culture transfection. *Gene* **74**:445–456.
45. **Treacy, M. N., X. He, and M. G. Rosenfeld.** 1991. I-POU: a POU-domain protein that inhibits neuron-specific gene activation. *Nature (London)* **350**:577–584.
46. **Treacy, M. N., L. I. Neilson, E. E. Turner, X. He, and M. G. Rosenfeld.** 1992. Twin of I-POU: a two amino acid difference in the I-POU homeodomain distinguishes an activator from an inhibitor of transcription. *Cell* **68**:491–505.
47. **Ullman, K. S., W. M. Flanagan, C. A. Edwards, and G. R. Crabtree.** 1991. Activation of early gene expression in T lymphocytes by Oct-1 and an inducible protein, OAP40. *Science* **254**:558–562.
48. **Ullman, K. S., J. P. Northrop, A. Admon, and G. R. Crabtree.** 1993. Jun family members are controlled by a calcium-regulated, cyclosporin A-sensitive signaling pathway in activated T lymphocytes. *Genes Dev.* **7**:188–196.
49. **Wilson, A. C., K. LaMarco, M. G. Peterson, and W. Herr.** 1993. The VP16 accessory protein HCF is a family of polypeptides processed from a large precursor protein. *Cell* **74**:115–125.
50. **Xue, D., Y. Tu, and M. Chalfie.** 1993. Cooperative interactions between the *Caenorhabditis elegans* homeoproteins UNC-86 and MEC-3. *Science* **261**:1324–1328.