**Research Communication**

**Brinker is a sequence-specific transcriptional repressor in the Drosophila embryo**

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A Dpp activity gradient specifies multiple thresholds of gene expression in the dorsal ectoderm of the early embryo. Some of these thresholds depend on a putative repressor, Brinker, which is expressed in the neurogenic ectoderm (Frank and Rushlow 1996; Cubadda et al. 1997; Jazwinska et al. 1999b), whereas low levels directly or indirectly activate pannier and tolloid throughout the dorsal ectoderm (Ramain et al. 1993; Winick et al. 1993; Kirov et al. 1994; Jazwinska et al. 1999b; Ashe et al. 2000). Some of these thresholds depend on a putative repressor, Brinker, which is expressed in lateral stripes within the neurogenic ectoderm just outside the limits of the dorsal ectoderm (Jazwinska et al. 1999b; Ashe et al. 2000). Here we present evidence that Brinker functions as a sequence-specific transcriptional repressor.

Results

Many sequence-specific repressors interact with one of two ubiquitous corepressor proteins in the early embryo, dCtBP and Groucho ([e.g., Nibu et al. 1998b; Poortinga et al. 1998]). Hucklebein, Hairy, Goosecoid, and Engrailed are among the repressors that interact with Groucho (Paroush et al. 1994; Goldstein et al. 1999), whereas Kruppel, Knirps, and Snail interact with dCtBP ([Nibu et al. 1998a]). These corepressors interact with two distinct sequence motifs, Groucho interacts with W/F-RP-W/Y and FxLxxIL, dCtBP interacts with PxDLSxR/K/H. The 704-amino-acid Brinker protein contains potential dCtBP and Groucho motifs: PMDLSLG at position 377 and FKPY at position 461 (Campbell and Tomlinson 1999; Jazwinska et al. 1999a).

**Groucho is required for Brinker-mediated repression**

A GST–Brinker fusion protein was mixed with 35S-labeled dCtBP and Groucho proteins that were synthesized with a rabbit reticulocyte lysate. The fusion protein contains the entire Brinker sequence, amino acid residues 1–704. It weakly binds dCtBP (Fig. 1A, asterisk), but strongly interacts with Groucho (Fig. 1A, arrow). At least 20% of the total input Groucho protein specifically binds to the fusion protein. The conversion of the FKPY motif into AAGA results in a ~10-fold reduction in binding (Fig. 1B).

Brinker was misexpressed in transgenic embryos using the eve stripe 2 enhancer (Kosman and Small 1997; Fig. 2, cf. B with A). Normal and mutant versions of the gene containing AAGA in place of FKPY were examined. The anterior portion of the pannier expression pattern is repressed in embryos carrying the wild-type stripe2-Brinker transgene (Fig. 2C, arrowhead; Ashe et al. 2000).

In contrast, the mutant transgene lacking the FKPY motif fails to repress pannier (Fig. 2D). This transgene me-
dCtBP (CtBP) or 35S-Groucho. The bound protein was eluted and attached to glutathione-agarose beads and mixed with 35S-protein containing the full-length Brinker (Brk) sequence was 20% of the total amount of 35S-labeled protein used in the binding reactions. Aliquots containing Groucho binds strongly to the fusion protein (arrow, lane 6 Brinker sequences (lanes 2, 3, 4, and 5). There is no binding of either CtBP or Groucho to the GST moiety lacking Brinker sequences (lanes 2 and 5). Labeled dCtBP binds weakly to the GST–Brinker fusion protein (asterisk, lane 3), whereas Groucho binds strongly to the fusion protein (arrow, lane 6). [8] 35S-labeled Groucho protein was incubated with GST, the wild-type GST–Brinker fusion protein, and a mutant version of the fusion protein containing AAGA in place of the FKPY motif (GST–BrkFKPY). Groucho binds the wild-type fusion protein (lane 3) but exhibits only residual binding to the mutant protein (lane 4). There is no binding to the GST moiety (lane 2). Lane 1 contains 20% of the total amount of 35S-labeled Groucho protein used in the binding reactions.

di mates weak repression of dpp (Fig. 2F, arrowhead), although the wild-type transgene produces a more substantial gap in the dpp pattern (Ashe et al. 2000, Fig. 2E, arrowhead).

Identification of Brinker binding sites

Brinker contains a potential helix–turn–helix motif, suggesting that it might bind specific DNA sequences. SELEX assays were done with random oligonucleotides and a GST–Brinker fusion protein that contains the putative helix–turn–helix motif (amino acid residues 44–99). After four rounds of selection, a total of 12 random clones were selected and sequenced (Fig. 3A). Alignment of these sequences reveals a tightly conserved core motif, TGGCG. Eleven of the 12 sequences contain a T at position 1, whereas all 12 contain G, G, C, and G at positions 2, 3, 4, and 5, respectively. In addition, all 12 sequences contain either C or T at positions 6 and 7. Thus, the SELEX assays identify the following consensus sequence: T-G-G-C-G-T/c-t/c.

Previous studies have identified several potential target genes that are regulated by the Brinker repressor, including tolloid, pannier, and tail-up (Jazwinska et al. 1999b, Ashe et al. 2000). Among these genes, tolloid has been studied in the most detail (Kirov et al. 1994; see below). The tolloid 5′ regulatory region contains an 800-bp enhancer that has the potential to direct gene expression throughout precellular embryos (Kirov et al. 1994). It is kept off in ventral and lateral regions by the maternal Dorsal gradient. After cellularization, the enhancer directs restricted expression within the dorsal ectoderm in direct or indirect response to Dpp signaling. This pattern expands into lateral regions in Brinker−mutant embryos (Jazwinska et al. 1999b; see below).

Inspection of the 800-bp enhancer sequence identifies three potential Brinker binding sites (Fig. 3B). Gel shift assays suggest that only one of these, site 2, binds Brinker (Fig. 3C, lanes 2,3). This site contains a perfect match to the consensus sequence. In contrast, sites 1 and 3 exhibit little or no binding (Fig. 3C, lanes 7,8,10,11) and contain 6 of 7 matches to the consensus recognition sequence. Additional binding assays were done with a larger DNA fragment containing both sites 1 and 2 (Fig. 3C, lanes 13,14). There is no evidence for cooperative DNA-binding interactions between sites 1 and 2 because a single protein–DNA complex is observed, similar to the Brinker−site 2 complex. Point mutations were introduced into the core TGGCG motif within the site 2 recognition sequence (Fig. 3B). The mutagenized binding site essentially fails to bind the GST–Brinker fusion protein (Fig. 3C, lanes 16,17).

**Brinker functions as a sequence-specific repressor in vivo**

By the onset of gastrulation, tolloid expression is restricted to the dorsal ectoderm and excluded from the neurogenic ectoderm in lateral regions (e.g., Fig. 4A, arrowheads). There is extensive modulation of the tolloid staining pattern along the anterior-posterior axis. Male embryos hemzygous for a null mutation in the Brinker gene, brkM68, exhibit altered patterns of tolloid expression, including lateral expansion into the neurogenic ectoderm (Fig. 4B, arrowheads, Jazwinska et al. 1999b).

To determine whether Brinker−tolloid interactions are direct, site 2 was mutagenized in the context of an otherwise normal tolloid/lacZ transgene (see diagrams beneath Fig. 4C,D). The wild-type enhancer directs lacZ expression throughout the dorsal ectoderm; the pattern is quite similar to that observed for the endogenous gene (Fig. 4, cf. C with A). At this stage in development (mid-nuclear cleavage 14), expression is no longer under the control of the maternal Dorsal gradient but, instead, depends on Dpp signaling. Staining is excluded from the lateral neurogenic ectoderm where Brinker is expressed.

The mutagenized tolloid enhancer directs a consistently expanded staining pattern (arrowheads, Fig. 4, cf. D with C). This altered pattern is similar to, but perhaps not quite as severe as, the expansion observed for the endogenous tolloid gene in Brinker−embryos (Fig. 4, cf. D with B). These results suggest that Brinker directly represses tolloid transcription in the neurogenic ectoderm.
Brinker represses pannier and dpp. Embryos were collected from transgenic strains containing either wild-type (C, E) or mutant (D, F) stripe2–Brinker transgenes. The embryos in A and B were hybridized with a Brinker antisense RNA probe, those in C and D were hybridized with a pannier probe, and the embryos in E and F were hybridized with a dpp probe. All embryos are undergoing cellularization and are oriented with anterior to the left. (A) Wild-type embryo. Brinker is expressed in two broad lateral stripes within the presumptive neurogenic ectoderm. (B) Same as A except that the embryo contains a copy of the wild-type stripe2–Brinker transgene [see diagram]. The FRT–STOP–FRT cassette was removed by introducing the transgene into a parental male that expresses the FLP recombinase under the control of a sperm-specific tubulin promoter. The transgenic embryo exhibits both lateral neurogenic stripes and an ectopic stripe2–Brinker pattern. A similar staining pattern is observed for a mutant form of the stripe2–Brinker transgene lacking the FKPY Groucho interaction motif. A normal pannier expression pattern is observed. In particular, stripe 1 is restored in anterior regions [arrowhead; cf. with C]. (E) The wild-type stripe2–Brinker transgene produces a broad gap in the dpp expression pattern [arrowhead]. (F) The mutant transgene retains some repression activity, although the gap in the pattern is not as pronounced as compared with that obtained with the wild-type transgene.

Discussion

Brinker is the fourth sequence-specific repressor that has been shown to interact with Groucho through the tetrapeptide motif, aromatic-basic-pro-aromatic. The first version of this motif that was identified is WRPW, located at the carboxyl terminus of the Hairy repressor (Paroush et al. 1994; Fisher et al. 1996). The related WRPY motif was subsequently shown to mediate Runx–Groucho interactions (Aronson et al. 1997; Levanon et al. 1998), and FRPW permits Huckebein to bind Groucho (Goldstein et al. 1999). The Brinker repression domain identified in this study, FKPY, conforms to the other three Groucho motifs except for the lysine residue at position 2.

Genetic studies are consistent with the occurrence of Brinker–Groucho interactions in the early embryo. The tail-up and pannier expression patterns appear to expand into lateral regions of embryos derived from grouch germ-line clones [H. Ashe, unpubl.]. It is conceivable that Brinker mediates both Groucho-dependent and Groucho-independent transcriptional repression because the removal of the FKPY motif does not abolish the ability of an otherwise normal stripe2–Brinker transgene to repress dpp expression [see Fig. 2]. The residual activity of the mutagenized transgene might be mediated by cryptic Groucho interaction motifs in Brinker (see Fig. 1B). Alternatively, Brinker might repress certain target enhancers via competition between Smad activators and the Brinker repressor to overlapping DNA-binding sites. A similar situation has been described for the Kruppel and Knirps repressors. They require the dCtBP corepressor to regulate some, but perhaps not all, target genes (Nibu et al. 1998a; La Rosee-Borggreve et al. 1999; Keller et al. 2000). The Groucho and dCtBP corepressors might be required only when activators and repressors bind to distinct, nonoverlapping sites within a target enhancer.

Dpp–Brinker interactions represent a particularly vivid example of how sequence-specific transcriptional repressors can limit inductive interactions by extracellular signaling molecules. Brinker helps promote neurogenesis by blocking Dpp signaling in the neurogenic ectoderm (Jazwinska et al. 1999b; Ashe et al. 2000). It might also work as a gradient repressor to subdivide the dorsal ectoderm into dorsal epidermis and amnioserosa. There are other examples of repressors limiting signaling pathways. High levels of the Spaetzle ligand lead to optimal activation of the Toll–Dorsal signaling pathway and the induction of the Snail repressor in the presumptive mesoderm of early embryos (for reviews, see Belvin and Anderson 1996; Rusch and Levine 1996). Snail prevents high levels of Spaetzle from activating neurogenic genes (e.g., Brinker, acaete-scute, and thromboid) in the mesoderm.
The interplay between extracellular signaling molecules and localized transcriptional repressors is reminiscent of the segmentation pathway in the early Drosophila embryo. Pair-rule stripes of gene expression are established by broadly distributed transcriptional activators such as Bicoid and dStat. The stripe borders are formed by localized gap repressors, including Hunchback, Kruppel, and Knirps (e.g., Small et al. 1991; Yan et al. 1996). Similarly, the activation of tolloid and pannier might depend on broadly distributed Smad proteins, whereas the lateral limits of the expression patterns are established by the localized Brinker repressor. It is likely that vertebrates also employ one or more transcriptional repressors to restrict TGF-β signaling interactions.

**Materials and methods**

**GST pull-down assays**

Brinker coding sequences (codons 1–704 and 23–103) were cloned into the pGEX-5X-1 expression vector (Pharmacia). GST fusion proteins were induced in Escherichia coli strain BL21-DE3pLysS as described by Nibu et al. (1998b). The fusion proteins were immobilized on glutathione-agarose beads (Sigma), and aliquots containing 5 µg of GST or GST–Brinker (1–704) were mixed with 5 µL of 32S-labeled dCTP (800 Ci/mmol) or Groucho proteins. These proteins were synthesized in the TNT transcription/translation system (Promega) using phiLuescript plasmids. Labeled proteins were eluted and fractionated by SDS-PAGE as described by Zhang et al. (1996).

**SELEX assays**

A mixture of 76mer oligonucleotides containing 26 random residues was labeled with [γ-32P]dCTP (800 Ci/mmol), purchased from NEN) as described by Pollock (1996). Aliquots containing 1 µL of the labeled oligonucleotides (0.4 ng) were mixed with 0.65–1.3 µg of GST–Brinker (23–103), and protein–DNA complexes were fractionated on polyacrylamide gels as described by Catron et al. (1999). The shifted complexes were eluted from the gel using a buffer containing 0.5 M ammonium acetate and 1 mM EDTA (pH 8). The eluted oligonucleotides were amplified by PCR [Pollock (1996) and mixed with the GST–Brinker fusion protein, as described above. After four rounds of selection, the DNA was amplified by PCR using 5′ and 3′ primers containing BamHI and EcoRI restriction sites, respectively, and cloned into the phiLuescript SK (+) vector (Stratagene). Twelve randomly selected recombinant plasmids were sequenced.

**Gel shift assays**

The GST–Brinker (23–103) fusion protein was mixed with the following double-stranded oligonucleotides that contain the three best matches to the Brinker consensus sequence (boldface) within the 800-bp tolloid enhancer: 5′-CTTCTGCCTGGCGCTT-3′ (site 1); 5′-GCTATTCAAGAGCCAGTTTT-3′ (site 2); and 5′-CTCCTCATGACCA-3′ (site 3). Additional binding assays were done with both a wild-type double-stranded oligonucleotide containing sites 1 and 2, as well as a mutant version that contains single nucleotide substitutions within the core TGCC motif (underlined): 5′-AGAGCCATGGCCGCTTTCTCGGTCCGCGATG-3′ (site 1); 5′-CAAGGCCATGGCCGCTTTCTCGGTCCGCGATG-3′ (site 2); and 5′-CTTCTGCTGGCCGATG-3′ (site 3). The double-stranded oligonucleotides were labeled with [γ-32P]ATP (6000 Ci/mmol, NEN) at the 5′-end using T4 polynucleotide kinase (New England Biolabs). Aliquots containing 1 µL of labeled DNA were mixed with 0.25–0.5 µg of the GST–Brinker fusion protein and fractionated on 5% polyacrylamide gels.

**Transgenic assays**

Wild-type and mutant Brinker coding sequences were cloned into the P-element transformation vector 22FPE containing two tandem copies of the eve stripe 2 enhancer (MSE) and an FRT-Stop-FRT cassette (Kosman and Small 1997). The FPKY motif (ttt aag ccc tat) at codons 461–464 was mutated to AAGA (gca ggc ggc gca) using PCR and mutant oligos. The
scribed by Small et al. [1992]. The expression patterns of the
lacZ reporter gene and various endogenous genes (e.g., Brinker, pannier, dpp) were visualized by in situ hybridization using
digoxigenin-labeled antisense RNA probes [Jiang et al. 1991].

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Note added in proof

Sivasankaran et al. [2000] recently reported direct regulatory interactions between Brinker and omb in the Drosophila wing imaginal disk.

References

Aronson, B.D., Fisher, A.L., Blechman, K., Caudy, M., and Gergen, J.P. 1997. Groucho-dependent and -independent repression activities of Runt domain proteins. Mol. Cell. Biol. 17: 5581–5587.

Ashe, H.L. and Levine, M. 1999. Local inhibition and long-range enhancement of Dpp signal transduction by Sog. Nature 398: 427–431.

Ashe, H.L., Mannervik, M., and Levine, M. 2000. Dpp signaling thresholds in the dorsal ectoderm of the Drosophila embryo. Development 127: 3303–3312.

Belvin, M.P. and Anderson, K.V. 1996. A conserved signaling pathway: The Drosophila toll-dorsal pathway. Annu. Rev. Cell Dev. Biol. 12: 393–416.

Cai, H. and Levine, M. 1995. Modification of enhancer-promoter interactions by insulators in the Drosophila embryo. Nature 376: 533–536.

Campbell, G. and Tomlinson, A. 1999. Transducing the Dpp morphogen gradient in the wing of Drosophila: Regulation of Dpp targets by brinker. Cell 96: 553–562.

Catron, K.M., Iler, N., and Abate, C. 1993. Nucleotides flanking a conserved TAAT core dictate the DNA binding specificity of three murine homeodomain proteins. Mol. Cell. Biol. 13: 2354–2365.

Cubadda, Y., Heitzler, P., Ray, R.P., Bourrous, M., Ramain, P., Gelhart, W., Simpson, P., and Haenlin, M. 1997. U-shaped encodes a zinc finger protein that regulates the proneural genes achaete and scute during the formation of bristles in Drosophila. Genes & Dev. 11: 3083–3095.

Ferguson, E.L. and Anderson, K.V. 1992. Decapentaplegic acts as a morphogen to organize dorsal-ventral pattern in the Drosophila embryo. Cell 71: 451–461.

Fisher, A.L., Obsako, S., and Caudy, M. 1986. The WRPW motif of the hairy-related basic helix-loop-helix repressor proteins acts as a 4-amino-acid transcription repression and protein–protein interaction domain. Mol. Cell. Biol. 16: 2670–2677.

Francois, V., Solloway, M., O'Neill, J.W., Emery, J., and Bier, E. 1994. Dorsal-ventral patterning of the Drosophila embryo depends on a putative negative growth factor encoded by the short gastrulation gene. Genes & Dev. 8: 2602–2616.

Frank, L.H. and Rushlow, C.A. 1996. A group of genes required for maintenance of the amnioserosa tissue in Drosophila. Development 122: 1343–1352.

Goldstein, R.E., Jimenez, G., Cook, O., Gur, D., and Paroush, Z. 1999. Hubeckin repressor activity in Drosophila terminal patterning is mediated by Groucho. Development 126: 3747–3755.

Holley, S.A., Jackson, P.D., Sasai, Y., Lu, Y.B., De Robertis, E.M., Hoffmann, F.M., and Ferguson, E.L. 1989. A conserved system for dorsal-

Figure 4. Expression of tolloid/lacZ transgenes. Embryos were collected from wild type, Brinker mutant [brkM68], and transgenic strains that contain either a wild-type or mutant version of the tolloid/lacZ fusion gene (see diagrams beneath panels C and D). Embryos have completed cellularization and are oriented with anterior to the left and dorsal up. They were hybridized with either a tolloid [A,B] or lacZ [C,D] antisense RNA. [A] Wild-type embryo hybridized with a tolloid probe. Staining is restricted to the dorsal ectoderm. The pattern has been resolved into a series of stripes and bands along the anterior-posterior axis (e.g., arrowheads). [B] Mutant embryo derived from brkM68 heterozygous female. The tolloid staining pattern is expanded and extends into the lateral neurogenic ectoderm (e.g., arrowheads). [C] Transgenic embryo that expresses the wild-type tolloid/lacZ transgene. The lacZ staining pattern is restricted to the dorsal ectoderm and includes various stripes and bands along the anterior-posterior axis. Two of the stripes are indicated by arrowheads. The overall lacZ pattern is very similar to the expression pattern of the endogenous tolloid gene [A,D]. Same as C except that the embryo contains a mutant version of the tolloid enhancer with nucleotide subtitutions in the core TGGCG motif. The lacZ staining pattern is somewhat broader than the normal pattern. In particular, the two stripes indicated by arrowheads expand into lateral regions. This expansion is similar to the one observed for the endogenous tolloid gene in brkM68 mutants [B].

Figure 4.
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Drosophila dorsal morphogen repression involves the tolloid gene by interacting with a silencer element. Mol. Cell. Biol. 14: 713–722.

Kosman, D. and Small, S. 1997. Concentration-dependent patterning by an ectopic expression domain of the Drosophila gap gene knirps. Development 124: 1343–1354.

La Rocce-Borggreve, A., Hader, T., Wainwright, D., Sauer, F., and Jackle, H. 1999. hairy stripe 7 element mediates activation and repression in response to different domains and levels of Kruppel in the Drosophila embryo. Mech. Dev. 89: 133–140.

Levanon, D., Goldstein, R.E., Bernstein, Y., Tang, H., Goldenberg, D., Stifani, S., Paroush, Z., and Groner, Y. 1998. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Proc. Natl. Acad. Sci. 95: 11590–11595.

Marques, G., Musacchio, M., Shimell, M.J., Wunnemngen-Stapleton, K., Cho, K.W., and O’Connor, M.B. 1997. Production of a DPP activity gradient in the early Drosophila embryo through the opposing actions of the SOG and TLD proteins. Cell 91: 417–426.

Nibu, Y., Zhang, H., Barolo, S., Small, S., and Levine, M. 1998a. dCtBP mediates transcriptional repression by Kruppel and Small in the Drosophila embryo. EMBO J. 17: 7009–7020.

Nibu, Y., Zhang, H., and Levine, M. 1998b. Interaction of short-range repressors with Drosophila CtBP in the embryo. Science 280: 101–104.

Paroush, Z., Finley Jr., R.L., Kidd, T., Wainwright, S.M., Ingham, P.W., Brent, R., and Ish-Horowicz, D. 1994. Groucho is required for Drosophila neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. Cell 79: 805–815.

Podos, S.D. and Ferguson, E.L. 1999. Morphogen gradients: New insights from DPP. Trends Genet. 15: 396–402.

Pollock, R.M. 1996. Determination of protein-DNA sequence specificity by PCR-assisted binding-site selection. In Current protocols in molecular biology (ed. F.M. Ausubel et al.), pp. 12.11.1–12.11.11. Wiley.

Poortinga, G., Watanabe, M., and Parkhurst, S.M. 1998. Drosophila CtBP: A Hairy-interacting protein required for embryonic segmentation and hairy-mediated transcriptional repression. EMBO J. 17: 2067–2078.

Ramain, P., Heitzler, P., Haenlin, M., and Simpson, P. 1993. Pannier, a negative regulator of achaete and scute in Drosophila, encodes a zinc-finger protein with homology to the vertebrate factor GATA-1. Development 119: 1277–1291.

Rusch, J. and Levine, M. 1996. Threshold responses to the dorsal regulatory gradient and the subdivision of primary tissue territories in the Drosophila embryo. Curr. Opin. Genet. Dev. 6: 416–423.

—. 1997. Regulation of a dpp target gene in the Drosophila embryo. Development 124: 303–311.

Sanicola, M., Secelsky, J., Elson, S., and Gelbart, W.M. 1995. Drawing a stripe in Drosophila imaginal disks: Negative regulation of decapentaplegic and patched expression by engrailed. Genetics 139: 745–756.

Sivasankaran, R., Vigano, M.A., Muller, B., Affolter, M., Basler, K. 2000. Direct transcriptional control of the dpp target omb by the DNA binding protein brinker. EMBO J. 19: 6162–6172.

Small, S., Kraut, R., Dogter, T., and Levine, M. 1991. Transcriptional regulation of a pair-rule stripe in Drosophila. Genes & Dev. 5: 827–839.

Small, S., Blair, A., and Levine, M. 1992. Regulation of even-skipped stripe 2 in the Drosophila embryo. EMBO J. 11: 4047–4057.

St. Johnston, R.D. and Gelbart, W.M. 1987. Decapentaplegic transcripts are localized along the dorsal–ventral axis of the Drosophila embryo.
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