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The Breathless FGF receptor homolog, a downstream target of *Drosophila* C/EBP in the developmental control of cell migration

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SUMMARY

To investigate the molecular mechanisms responsible for the temporal and spatial control of cell movements during development, we have been studying the migration of a small group of follicle cells, called the border cells, in the *Drosophila* ovary. Timely initiation of border cell migration requires the product of the *slow border cells* (*slbo*) locus, which encodes the *Drosophila* homolog of the transcription factor C/EBP. Here we report evidence that one target of C/EBP in the control of border cell migration is the FGF receptor homolog encoded by the *breathless* (*btl*) locus. *btl* expression in the ovary was border cell-specific, beginning just prior to the migration, and this expression was reduced in *slbo* mutants. *btl* mutations dominantly enhanced the border cell migration defects found in weak *slbo* alleles. Furthermore, C/EBP-independent *btl* expression was able to rescue the migration defects of hypomorphic *slbo* alleles. Purified *Drosophila* C/EBP bound eight sites in the *btl* 5′ flanking region by DNAse I footprinting. Taken together these results suggest that *btl* is a key, direct target for C/EBP in the regulation of border cell migration.

Key words: cell migration, C/EBP, FGF receptor, *Drosophila*, border cells, *breathless*

INTRODUCTION

During embryonic development of multicellular organisms, a variety of cell types undergo temporally and spatially controlled cell migrations. Although cell motility is believed to involve dynamic changes in cell adhesion and rearrangement of the cytoskeleton, the molecular mechanisms regulating these activities are not well understood. In addition, the factors controlling the coordination of migratory behavior with cell fate determination and differentiation during embryonic development remain to be elucidated. Transcription factors would be expected to play a major role in these events and several transcription factors have been shown to be required for specific cell movements during development (Montell et al., 1992; Salser and Kenyon, 1992; Miller et al., 1992; Garriga et al., 1993; Klambt et al., 1993; Anderson, 1995). However the downstream targets mediating these effects are not known.

We have been studying the migration of 6-10 follicle cells, known as the border cells, in the *Drosophila* ovary as a model system to address these questions. Egg chambers in the ovary comprise a cluster of sixteen germline cells (fifteen nurse cells and 1 oocyte) surrounded by a monolayer epithelium of somatic follicle cells (Fig. 1). During stage 9 of oogenesis (see Spradling, 1993, for review of oogenesis), the majority of the 1100 follicle cells begin a posteriorward displacement so that eventually more than 95% will stack up to form a columnar epithelium in contact with the oocyte (Fig. 1). Of the remaining 5%, most will stretch to cover the large nurse cell cluster. However a group of 6-10 cells remains rounded at the anterior tip of the egg chamber until mid stage 9 (Fig. 1) when they extend processes in between the nurse cells and migrate through the nurse cell cluster to the oocyte border. These so-called border cells serve at least 2 essential functions in oogenesis: they participate in formation of the eggshell structure known as the micropyle, maintaining an opening through which the sperm enters at fertilization (Montell et al., 1992); and they secrete the Torso-like protein, which is an essential patterning signal (Savant-Bhonsale and Montell, 1993).

Previously we have shown that initiation of border cell migration is controlled by the *slow border cells* (*slbo*) locus (Montell et al., 1992). Weak *slbo* mutations cause delayed border cell migration whereas stronger alleles result in complete failure of migration (Montell, 1992; Fig. 1) causing female sterility; null mutations are embryonic lethal. The *slbo* locus encodes the *Drosophila* homolog of C/EBP, a basic region/leucine zipper transcription factor. In vertebrates C/EBPα is a terminal differentiation factor required for expression of cell-type specific products (Friedman et al., 1989; Christy et al., 1989; Lin and Lane, 1992; Umek et al., 1991). *Drosophila* C/EBP has been shown to be a DNA binding protein (Rørth and Montell, 1992) and a transcriptional activator in vitro (Rørth, 1994). Therefore to elucidate the

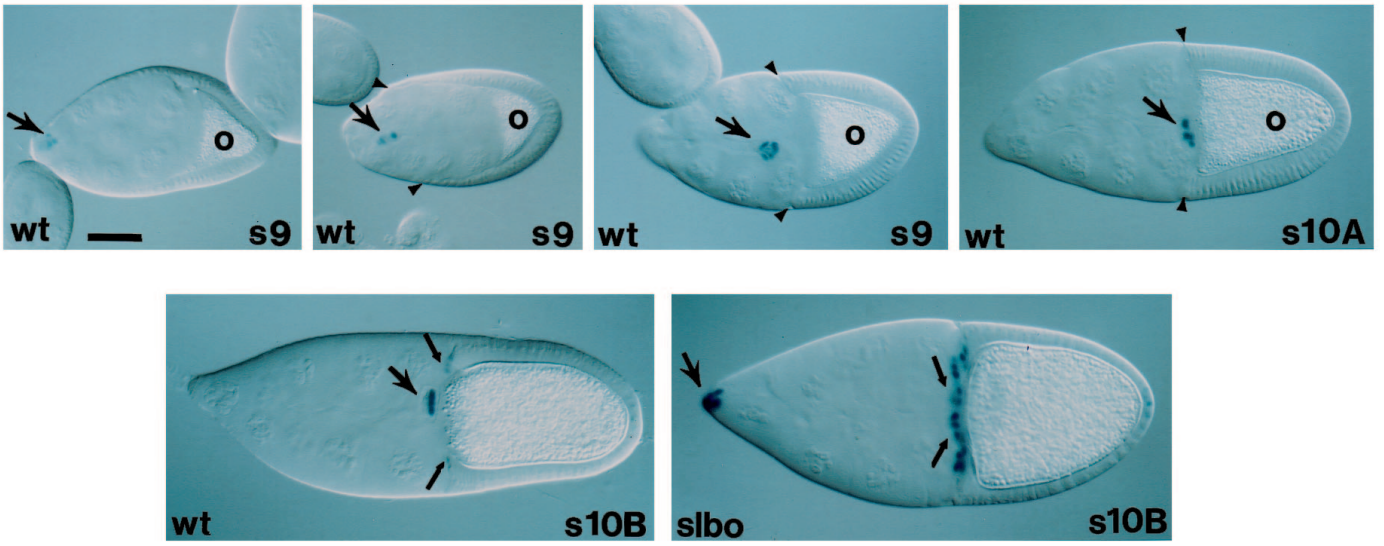

Fig. 1. Border cell migration in wild-type and *slbo* mutant egg chambers. Border cells are stained for β-galactosidase activity (blue) in egg chambers heterozygous (wt) or homozygous (*slbo*) for the *slbo*^{1} enhancer trap line. Border cells migrate through the nurse cell cluster toward the oocyte (o) during stage 9 (s9). At the same time the outer follicle cells rearrange: those in the posterior of the egg chamber become more columnar and stack up in contact with the oocyte and those associated with the nurse cells become very flat. The border between columnar and flat cells (arrowheads) moves posteriorly at approximately the same rate as the border cells (large arrows). By stage 10A the border cells have reached the oocyte. Stage 10B (s10B) is characterized by centripetal cell migration of outer follicle cells (long arrows) at the oocyte/nurse cell junction. In *slbo*^{1} mutants border cells remain at the anterior tip of the egg chamber whereas centripetal cell migration occurs normally. Bar = 50 μm.

pathway by which C/EBP controls initiation of border cell migration, we set out to identify targets of C/EBP in the border cells.

We postulated that some gene products required for border cell migration might also be utilized for other cell migrations since cell migrations occur throughout development. One locus known to control embryonic cell migration is *breathless* (*btl*). The *btl* locus encodes a homolog of the vertebrate fibroblast growth factor receptor (FGFR), a membrane receptor tyrosine kinase (RTK) (Glazer et al., 1991). During embryonic development *btl* is expressed in cells of the tracheal system, in a subset of glial cells and in the salivary duct cells. Mutations in the *btl* locus cause failure of tracheal cell migration, without affecting their proliferation or differentiation, as well as defects in the migration of a pair of midline glia (Klambt et al., 1992) and ultimately lethality.

In this report we present evidence that *btl* is one key target for C/EBP in the regulation of border cell migration. We found border cell-specific *btl* expression in the ovary which was dependent on *slbo*$^{+}$ function. We also found that *btl* mutations were dominant enhancers of weak *slbo* alleles. Furthermore, *slbo*-independent *btl* expression was able to rescue the migration defects found in weak *slbo* mutants. The binding of purified *Drosophila* C/EBP protein to eight sites in the *btl* 5′ regulatory sequence suggested that C/EBP regulation of *btl* expression may be direct.

MATERIALS AND METHODS

Staining for β-galactosidase activity

Egg chambers were fixed for 10 minutes at room temperature in 100 μl of 15 mM KH_2PO_4 /K_2HPO_4 (pH6.8), 75 mM KCl, 25 mM NaCl, 3.3 mM $MgCl_2.6H_2O$, 6% formaldehyde. Following one rinse with 1 ml of PBS+0.1% Triton X-100 (PBS-Triton), chambers were stained at 37°C for 10 minutes to overnight, depending on the line (staining solution: 10 mM $NaH_2PO_4/Na_2HPO_4.2H_2O$ (pH7.2), 150 mM NaCl, 1 mM $MgCl_2.6H_2O$, 3 mM $K_4[Fe^{II}(CN)_6]$, 3 mM $K_3[Fe^{III}(CN)_6]$, 0.3% Triton X-100 with 0.2% X-gal. Chambers were rinsed with PBS/Triton, equilibrated in PBS:glycerol (1:1), and mounted on slides for viewing.

Cloning and sequencing

Plasmid rescue of DNA flanking the *btl*^{H82} insertion was performed as follows: 3 μg genomic DNA from P-element bearing flies was digested with *Eco*RI for 2 hours in a 5-fold excess of enzyme, phenol extracted and ethanol precipitated. Following resuspension in TE (10 mM Tris, 1 mM EDTA), the DNA was ligated in a 200 μl reaction with 1 unit of T4 DNA ligase, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, 1 mM ATP, 1 mM DTT. Following overnight incubation at 15°C, the DNA was ethanol precipitated, resuspended in 10 μl H_2O, and used for transformation of *E. coli* by electroporation. Ampicillin-resistant colonies were grown in liquid cultures and plasmids prepared according to standard protocols (Sambrook et al., 1989). 5.5 kb of flanking DNA including the entire *btl* coding region was recovered (see Klambt et al., 1992, for complete map of the gene). The proximal 3 kb *Bam*HI fragment was subcloned in bluescript and sequenced using the T7 primer from the *Bam*HI side and a primer from the 5′ end of the previously reported *btl* cDNA (Klambt et al., 1992) from the other end. The fragment was sequenced on both strands by the Johns Hopkins Genetic CORE facility. This fragment was further subconed into fragments ranging from 120 to 520 basepairs in length, for footprinting analysis (see below).

Heat shock constructs and germline transformation

C/EBP and FGFR1 genomic sequences (*Hpa*I to *Xho*I for C/EBP and *Bgl*II to *Eco*RI for Btl) including all of the coding regions, were cloned into the PCaSpeR-hs vector (a generous gift from Carl Thummel, University of Utah). DNAs were purified over cesium chloride/ethidium

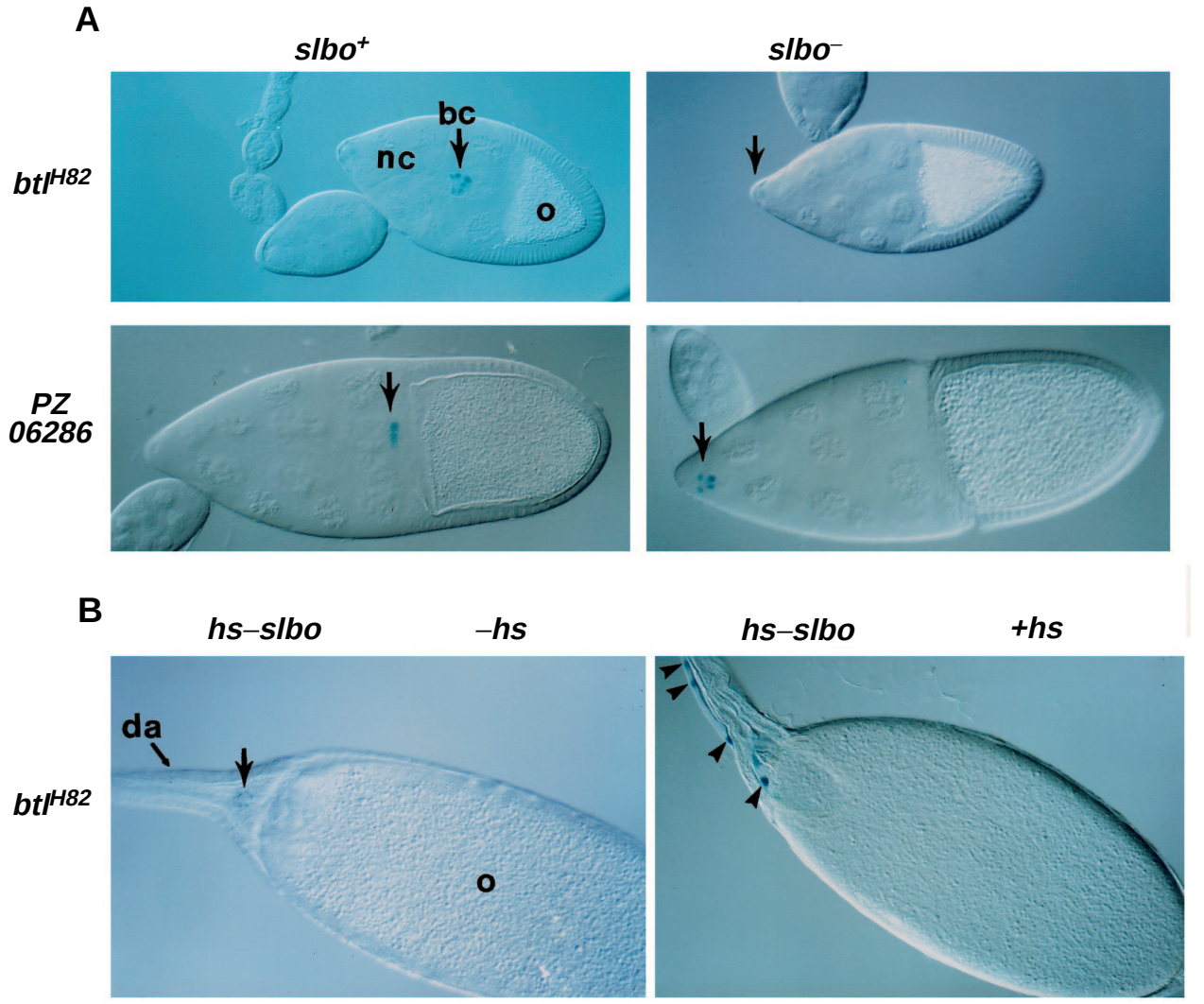

Fig. 2. *btl* expression in wild-type and *slbo* mutant egg chambers. (A) Comparison of the effect of the *slbo* mutation on expression from two different enhancer trap lines: btl^{H82} and PZ06286. Both lines display border cell-specific expression in wild-type egg chambers (left panels). Only btl^{H82} displays a reduction in staining in *slbo* mutant egg chambers (right panels). Note that the cells have not migrated because of the *slbo* mutation. (B) Effects of ubiquitous C/EBP expression on btl^{H82} expression. Border cell-specific expression is detected in the absence of heat shock (–hs) whereas expression in dorsal appendage (da) associated follicle cells can be detected following heat shock (+hs). o, oocyte; nc, nurse cell cluster; bc, border cells.

bromide gradients. Purified DNA (200 μg/ml) was mixed with wings-clipped DNA (50 μg/ml) and injected into 0-1 hour *white* mutant embryos following standard techniques. Transformants were mapped to a chromosome by segregation from dominant markers, balanced and, if possible, made homozygous.

DNAse I footprinting

DNAse I footprinting was performed as described (Rørth et al., 1992). DNA fragments were digested with restriction endonucleases, phosphatase treated, 5′ end labeled with [γ-^{32}P]ATP and digested with a second enzyme to release the fragment. Purified labeled DNA fragment and protein (*Drosophila* C/EBP and/or BSA) were incubated at room temperature in 20 μl of 25 mM Hepes (pH 7.9), 75 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM DTT, 0.2 mM PMSF, 12% glycerol and 1 μg of poly d(I-C) for 15-20 minutes. After an additional 2 minutes of incubation in the presence of 2.5 mM $CaCl_2$ and 0.1 ng of DNase I (Worthington Diagnostics), the incubation was stopped by adding EGTA to a concentration of 10 mM and SDS to 0.1%, followed by phenol-chloroform extraction and ethanol precipitation. The products were separated on denaturing polyacrylamide gels alongside a Maxam-Gilbert G+A sequencing lane of the same labeled fragment.

RESULTS

slbo-dependent *btl* expression in the border cells

To test the hypothesis that *btl* might be involved in border cell migration, we examined the ovary for *btl* expression, using the *btl* P-element *lacZ* enhancer trap line btl^{H82}, which has previously been shown to reflect accurately the normal pattern of *btl* gene expression (Klambt et al., 1992). Ovarian expression of β-galactosidase in this line was border cell specific (Fig. 2) and was first detected during stage 9, just prior to the migration. To test whether *btl* expression might be regulated by *Drosophila* C/EBP, *btl* expression was compared in wild-type

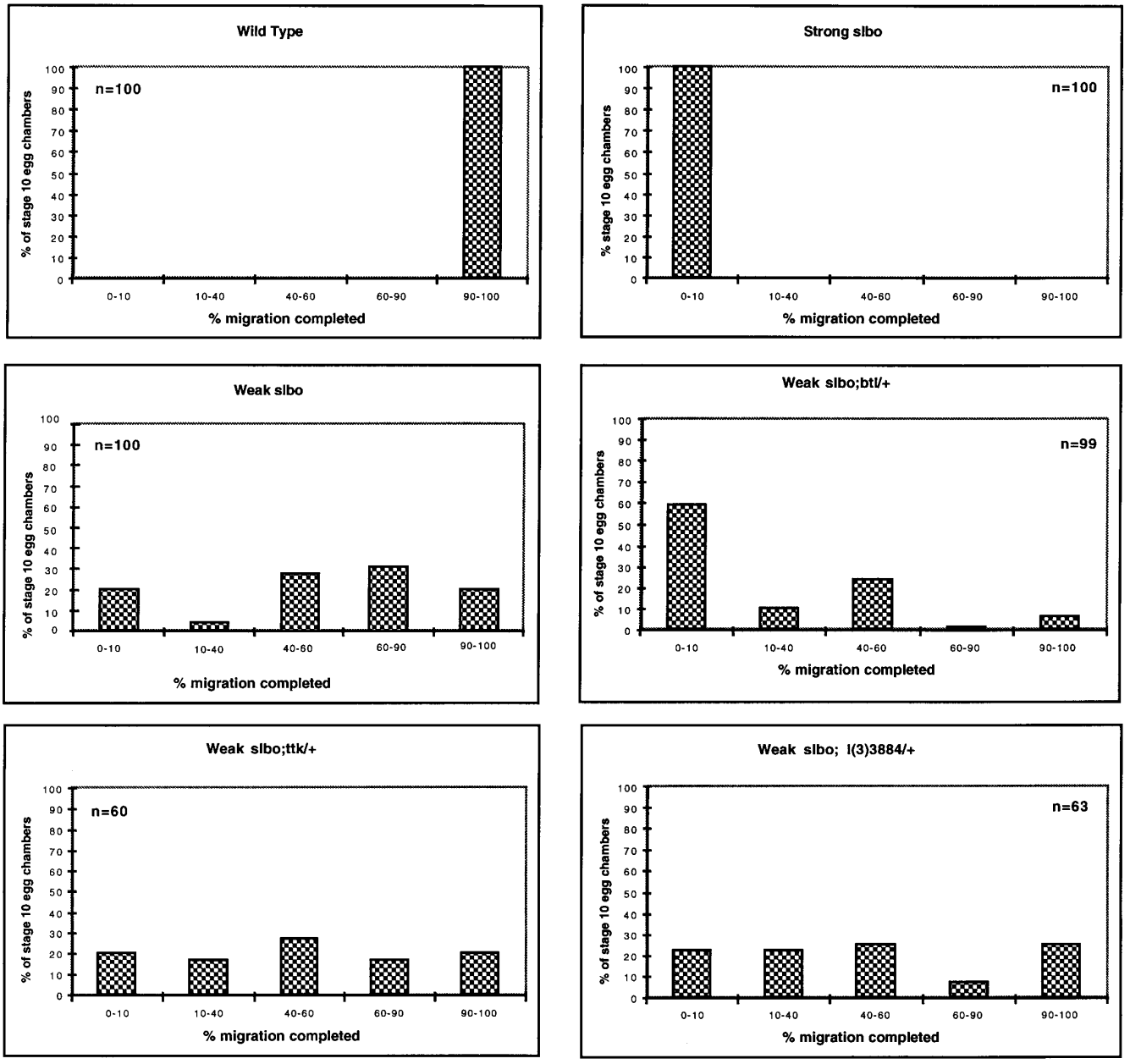

Fig. 3. Dominant enhancement of weak *slbo* alleles by *btl* mutations. Histograms showing extent of border cell migration in stage 10 egg chambers from females of various genotypes. X-axis indicates position of border cells within the egg chamber. Strong slbo = $slbo^{e2b}/slbo^{e7b}$; weak slbo = $slbo^{e14a}/slbo^{e7b}$; ttk = $tramtrak^{1}$ (Xiong and Montell, 1993). Both $btl^{H82\Delta3}$ and the null allele btl^{LG19} had similar effects.

and *slbo* mutant ovaries. We found that in egg chambers from *slbo* mutant females, btl^{H82} expression was reduced to a level that was barely detectable (Fig. 2) after 4 hours of staining. This was not due to absence of the cells or to a general reduction in gene expression in the border cells because in a more general screen of border cell staining enhancer traps, 18 out of 20, including PZ06286, showed no reduction in *slbo* mutants (Fig. 2).

To examine whether ectopic C/EBP could cause ectopic *btl* expression, transgenic flies were generated in which the *slbo* gene was expressed under the control of the hsp70 heat-inducible promoter (See Methods for details). In the absence of heat shock, *slbo* and *btl* expression were indistinguishable from wild-type (not shown). However, following heat shock, high levels of C/EBP expression were induced in follicle cells at all stages (not shown) and ectopic *btl* reporter gene expression was found in dorsal appendage-associated follicle cells at stage 13 (Fig. 2B). Thus, although heat shock induced high levels of *slbo* in all follicle cells, ectopic *btl* expression was limited to dorsal follicle cells at late stages of oogenesis. This observation suggested that C/EBP expression was not sufficient to induce *btl* expression in all cells. Heat shock alone, in the absence of the hs-*slbo* transgene, did not cause any ectopic expression of *btl* (not shown).

Dominant enhancement of weak *slbo* mutations by lethal *btl* mutations

To determine whether lethal *btl* mutations had any effect on border cell migration, we first examined whether lethal *btl* mutations could act as dominant enhancers of the border cell migration delay which characterizes weak *slbo* alleles. In wild-type stage 10 egg chambers the border cells have invariably completed their migration and are found at the oocyte-nurse cell border (King, 1970; Montell et al., 1992; Figs 1, 3A). Strong *slbo* mutations cause complete failure of border cell migration; therefore at stage 10, the border cells are invariably found at the extreme anterior end of the egg chamber (Montell et al., 1992) and (Figs 1, 2 and 3). In weak *slbo* mutants, the stage 10 border cells display incomplete migration and can be found anywhere along the migration pathway (Fig. 3). When females are heterozygous for either of two *btl* alleles in a weak *slbo* background, migration was significantly further delayed (Fig. 3). Thus *btl* mutations acted as dominant enhancers of *slbo*. This effect was not observed when another lethal mutation *tramtrack* (*ttk*) was crossed into the same *slbo* background (Fig. 3), nor with another lethal enhancer trap line with border cell staining, PZ3884, in the same background (Fig. 3).

Rescue of *slbo* migration defects by C/EBP-independent *btl* expression

If the lack of *btl* expression were the primary defect in *slbo* mutants, we postulated that it might be possible to rescue *slbo* defects by providing C/EBP-independent *btl* expression. To test this possibility, the *btl* gene was cloned behind a heat-inducible promoter and transgenic flies were generated (see Methods for details). To confirm expression of the wild-type Btl protein, we determined whether this transgene could rescue the tracheal migration defects of *btl* mutants as described by Reichman-Fried et al. (1994). A single heat shock at 4 hours after egg laying was sufficient to rescue tracheal morphology to a wild-type pattern, as detected by staining with the monclonal antibody 2A12 (data not shown). After crossing the hs-btl transgene into a *slbo* mutant background, we found that the heat inducible *btl* gene could rescue the border cell migration defects of $slbo^{1}$ homozygotes (Fig. 4). $slbo^{1}$ is a relatively strong hypomorphic allele (Montell et al., 1992), since border cell migration fails in 95% of egg chambers from $slbo^{1}$ homozygotes and those cells that initiate migration do so in stage 10. Following a 20 minute or 1 hour heat shock at 37°C and 20 hours of recovery at 18°C, border cells were found to have initiated migration in 90% of late stage 9 egg chambers, and migration was complete or nearly so in 20% (Fig. 5). No border cell migration was observed in the absence of heat shock. Heat shock alone, in the absence of the hs-btl transgene, did not induce migration (not shown). In addition, the fertility of the $slbo^{1}$ mutant females was improved following heat shock breathless expression (data not shown). Ectopic expression of *btl* in all of the follicle cells as a result of heat shock did not appear to cause any deleterious effects on oogenesis as egg chambers of all stages appeared healthy and no abnormalities were observed (not shown).

C/EBP binding sites in btl 5′ flanking region

To determine whether the regulation of *btl* by C/EBP might be direct, we sequenced 2.5 kilobases (kb) of 5′ flanking DNA from the *btl* gene and examined it for potential C/EBP binding sites (Fig. 6). Fragments ranging from 120 to 520 basepairs in length and corresponding to all of the DNA between the *Bam*HI site at −2705 and the *Apo*I site at −251 were sublconed into bluescript and tested for binding to purified *Drosophila* C/EBP protein in DNAse I footprinting experiments (Rørth et al., 1992). Three sites within 600 basepairs of the H82 P-element insertion site were found to footprint with 200 ng of purified protein (Fig. 7). Five additional sites bound with similar affinity. Three lower affinity sites required 500 ng of protein to footprint effectively (open circles in Fig. 7). An alignment of the footprinted sequences is shown in Fig. 8. A consensus sequence could be derived from this alignment which is shown below and compared to the optimal binding sequence for vertebrate C/EBP. We further examined the *btl* 5′ flanking DNA for sequences that matched the consensus but failed to bind in the footprinting assays. No such matches were found.

We were able to investigate the importance of some of the C/EBP binding sites further by examining *btl* alleles with alterations in the 5′ regulatory sequences. The mutant allele $btl^{H82\Delta3}$ was created by excision of the H82 P-element (Klambt et al., 1992). The *lacZ* gene remains (Klambt et al., 1992), however we found by genomic Southern blotting that approximately 500 basepairs of *btl* 5′ flanking DNA was deleted, in addition to the rest of the P-element. The deleted region included three of the high affinity C/EBP binding sites (shown schematically in Fig. 7). This deletion eliminated border cell expression (not shown), although embryonic expression of β-gal is intact (Klambt et al., 1992, and our unpublished observations). In addition, we sequenced the insertion site of enhancer trap insertion $btl^{6\text{-}81}$. The P-element was found to be inserted very close to (35 basepairs from) the distalmost C/EBP binding site (Fig. 7). Flies carrying this insertion did not display border cell expression of β-gal (not shown), although the embryonic β-gal expression from $btl^{6\text{-}81}$ is nearly identical to that in btl^{H82} (Klambt et al., 1992).

To examine whether there was a requirement for *btl* in an otherwise wild-type background we generated follicle cell mosaics (Xu and Rubin, 1994) using two different alleles: $btl^{H82\Delta3}$ and btl^{LG19}, both of which display a strong phenotype in the embryo (Klambt et al., 1992). The LG19 allele is an EMS induced mutation and presumed to be null (Klambt et al., 1992). The FLP/FRT system was employed, in which the wild-type chromosome is marked with a heat inducible myc-tagged protein (Xu and Rubin 1993). Therefore, homozygous mutant clones are detected by the absence of myc staining. Since the border cells are deeply embedded in the nurse cells, they are more difficult to stain reliably than other cells of the egg chamber (Montell et al., 1992). Therefore we double labeled egg chambers with fasciclin III monoclonal antibody, which stains two of the border cells (Ruohola et al., 1991), as a positive control for adequate penetration of the antibodies to the border cells. In addition we stained with DAPI, to visualize all nuclei. Border cells were identified by DAPI staining and scored as btl^{-} only if they were negative for myc staining and two of them were positive for FasIII staining. After observing hundreds of egg chambers with various follicle cell clones, we found five stage 10 egg chambers in which all of the border cells appeared to be btl^{-}. No defects in border cell migration or in any other aspect of oogenesis were detected. However, this

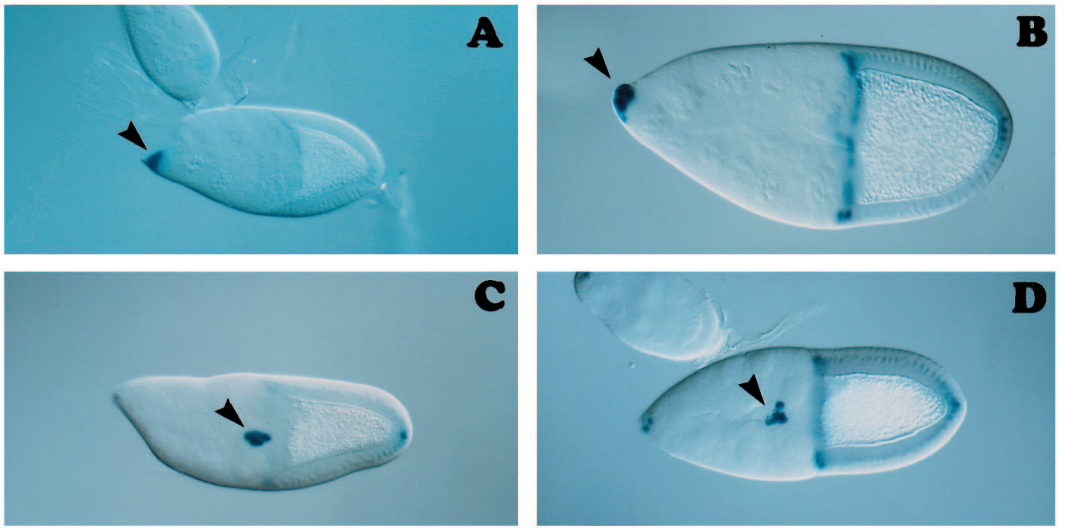

Fig. 4. Rescue of *slbo* border cell migration defects by hs-btl. Egg chambers stained for β-galactosidase activity from the *slbo* enhancer trap line *slbo*^{1} as described by Montell et al. (1992), to show extent of border cell migration. (A) *slbo*^{1}/*slbo*^{1}, stage 9; (B) *slbo*^{1}/*slbo*^{1};hs-btl without heat shock, stage 10; (C,D) *slbo*^{1}/*slbo*^{1};hs-btl after heat shock, stage 9 (C) and stage 10 (D).

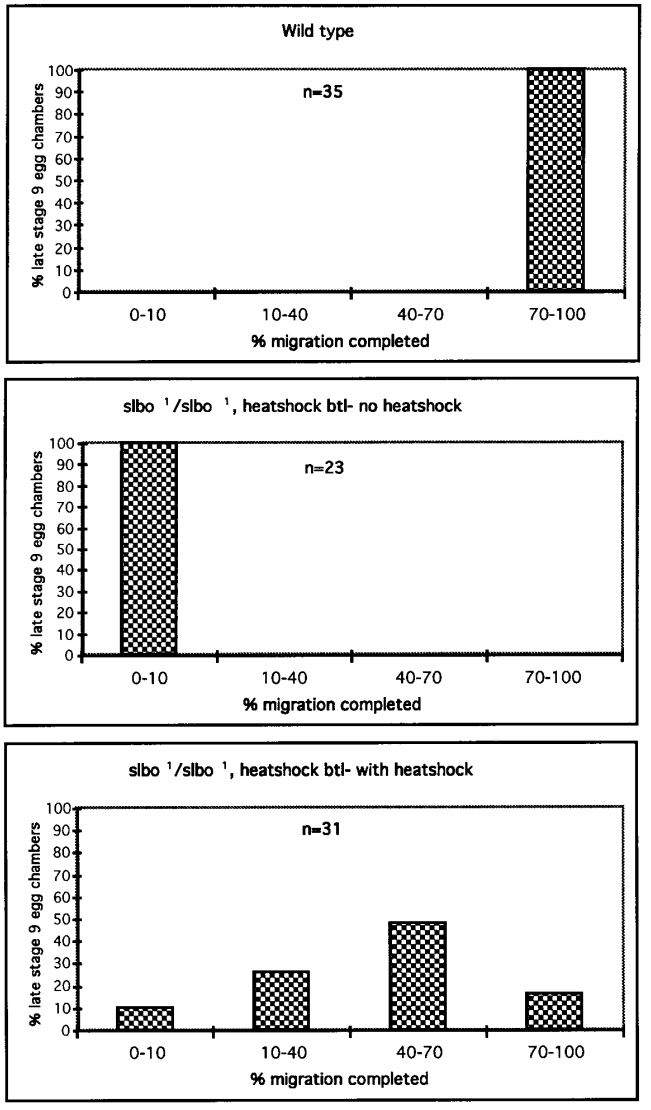

Fig. 5. Summary of *slbo* rescue by hs-btl. Histograms representing the extent of border cell migration in late stage 9 egg chambers in the genotypes shown. Overall 90% of egg chambers showed some rescue.

BamHI

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GGATCCTAGCAAACATTATCGCAGGGAACCACAGATACCCACGATACCCACTCGTTTCGTATCGGATCGT   70
ATTGGATCGGTTCGGTTCGATCGGCACGTTCGGATCGTTCGTATCGTACCGCGTAAAAGTTTCTTCCGTT  140
TCGATCGTTACTTTCAGTTGTTTGCGCACATTCAAGCCAATCGGTTGGCTTCGCTCGATTCCTTCCACAA  210
ACTTTACGGATTACGGTTAGTGCAACGGAAGTGACCCCCCCCCCCCCCCCACCAACACCAGCTACCCATCA  280
CATATTTCTTTTCATTTATGCCTGGTAAAGGGAAATAAAAGTTGGCAACGAAGCGGAAATGCAAGGCGCC  350
TTCCGCTGCCCTCTTGCCGAGTCTTAAGATTTTTATCCAAGCCCCAACCGCGATTGACAGCCGAATAAAC  420
ATAGTGACAATGTTTTTTGCAATCTTATTTGCCTTCTCCGGCTCCTCGAAAATAAAAGACAAAGAACACG  490
ATTTTTTTGGTCAAAGATTTGGGGTTGTGGCATGGCAGAAAAGAATTGTGTTCCCACTGCGAACTGACAA  560
GTAGCAGTGAAACCAAAATAAAGCAAAAAATAATTTAAGAGTGAATTGTTCCAGCAGTAAAATAAATACA  630
AATCTTTCCAAGTGCAGCCAAAAGATCATAAACCACAGATAAATAAAAACAAAAAGTCTGCAAAGTAACC  700
AAATGTTAAAAATAAAATTTTAATTGAGACAAAGAAACGTGATAAAACTGATTCATACATAATTAAATAA  770
ACACCAATGAAATAAATAAGAAAATAAAATTAAAGTGATGTCAATTGTATGAATGAATAATTTATGTCTT  840
GATCCAAACATTCCTTTTACATTAAGTCAATGTTTGCAGAAAGAAAACTGAATGAGGAGTTAATACCCAG  910
GGTTCCAAATAAAGAACTTATTCTATTCTCGATATAGCTGATTGACCAAGTTGAAATATCAAATCGTTCA  980
TGCATTAGATTTATACGCTCGATAATAATTCACAAGATAAAAAGGTGTGAGCTAAACGGTTTTGGCCATG 1050
TGCGCTTGTGGCTTAAAATCGAATCGAATAATATTGAATGAATGACAAACTGAGAAAAAAATTAAATAAA 1120
AATAGCAATAATACATACTTCAAATACAGCGATCGACTCGTATTTGGCAACAAATGAGAAAAAAAGGAAT 1190
TGAATAAATTAAAACCTGCGACGCGTGTGCATACATACATAAATCGGGCCTGAAGTCAAACGGCTAGCGG 1260
CTAGCGGCAATATCGGCTAAAACAACAACAACAATTACTGGCAGCACTAGCACCATATTAACAACAACAA 1330
TGACAATCGCAACGGTTGTCCCAAGCCTTACAAACAATAAAATACTTTTCATTAAACAGCACAGTGGTCA 1400
AAACGATTTTATAAAAGTTAAAAATTTTTTAATTTTCTGATAGAAAAAATAGTCGAACTATGCTATGCCC 1470
CATTATTATCAATATTTTGCTAAACTTAGTTTAAAACTTTACATTTTCTACAAAGCTTACATTCGCACAC 1540
AACAAGTTTGGGAATTTACCCTATCATCATTAATCATTGAACTAAAAATTGTTTATTTTTATTTGGCAAA 1610
CATTTGGCGAACCCAAGTAGATGGAATAACCACTGTCCTATACCCTTTACGTGATTATACTAAAAATTAA 1680
TTTTGAAATAATTATCTGCTATTAGGATTTTCGAGCAGTGTAATTCAGTGTTTAAAGCATAACTTTATTA 1750
TAAATATACCTTTTAATTGATTAAAATGCTGAGCTCATATTGCACAGAGAATTTGAATGTTAATTAGTTG 1820
CTATTTCAGATTGTGGCAAATCATTTAGGTAATTCTCGATTTGCTGTTGCAATTCTCTAGCACCTTGTCT 1890
GTTAGTTTTGTAAGTGCAAGTATAGTACTTGGGAAATAACTGAAAAGTGTGACACCACTGTAACAACTGA 1960
TTTCAACAAATAGCTAAGTGATTTTCTAACTGTTCGGCATTTAGTTGAATGGAAAACGAGGAGCGCAGGC 2030
CGCAGCAACAAAAGCATTTGGCGAACGTCGAATATAGACAAGTTATAATGGAGATCCCCTTCGGTAGTGG 2100
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ATTCATATATTTAATCCTTCCTTTAGCTGGAGAACGCTCGATTTGAAATCGAGGAACTCGAATGAAGATA 2590
CCCACAGGAAGTGATATTATTCGACCGCAACTGTAACCCATATGTACACGATCGTGCCCATCCAGATACG 2660
AGATCTTGGTGGCTGTTGAACTCAAGAGAATCCACTTGAATCATGGCAAAAGTGCCGATCACGCTGGTGA 2730
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Fig. 6. Nucleotide sequence of *btl* 5′ flanking region. C/EBP binding sites (as determined by DNAse I footprinting, see Fig. 6) are underlined. High affinity sites are underlined in bold; a potential site which has not been tested is underlined with a broken line. The transcription start site (Klambt et al., 1992) is indicated by an arrow. The *btl*$^{6\text{-}81}$ insertion site is indicated by the triangle.

experiment would not have detected either mild delay in the border cell migration or a low frequency of border cell migration defects. Thus, reduction of *btl* function had a discernible effect on border cell migration in a compromised genetic background (weak *slbo* mutant), but no effect of removing *btl* was detected in a wild-type background.

DISCUSSION

The role of C/EBP in border cells

C/EBP is a transcription factor that appears to control terminal differentiation in a variety of cell types and organisms (Friedman et al., 1989; Christy et al., 1989; Lin and Lane, 1992; Umek et al., 1991). In addition, regulated C/EBP expression has recently been implicated in mediating the changes in gene expression that are essential for long-term learning and memory in *Aplysia* (Kundras et al., 1994). We have previously described the identification of a *Drosophila* homolog of C/EBP and the phenotypes associated with female sterile and embryonic lethal mutations in the gene (Montell et al., 1992; Rørth et al., 1992). In both the embryo and the ovary, C/EBP expression initiates late in the differentiation of various epithelial cell types, much like vertebrate C/EBPα. In the border cells of the *Drosophila* ovary C/EBP expression is essential for initiation of border cell migration. In this report we present evidence that the FGF receptor gene encoded by the *btl* locus is one of the key targets for transcriptional regulation by *Drosophila* C/EBP. Thus C/EBP appears to link the differentiation of the border cells with their ability to migrate.

Role of C/EBP in the regulation of *btl* expression

Several lines of evidence presented in this paper suggest that, in the ovary, *btl* expression levels are enhanced by *Drosophila* C/EBP. The observation of eight high affinity C/EBP binding sites within the *btl* control region suggests that this enhancement may be direct. C/EBP expression does not appear to be sufficient to cause ectopic *btl* expression in most cells. Thus in this system as well as in several well-characterized vertebrate cell types, C/EBP appears not to specify patterns of gene expression; rather this transcription factor carries out the program of gene expression specified by other factors. Therefore C/EBP can be deployed for very different purposes in different cell types: e.g., fat metabolism in adipocytes, long term memory in *Aplysia* neurons and cell migration in the border cells.

C/EBP is unlikely to regulate *btl* expression during embryogenesis because C/EBP expression in the tracheal system does not begin until long after *btl* expression (Rørth et al., 1992). Recently however, a POU domain transcription factor, Drifter (Dfr), has been described which may enhance *btl* expression in tracheal cells. Dfr protein is expressed in tracheal cells near the time that *btl* expression initiates; the *dfr* mutant phenotype is similar to *btl*; and *dfr* expression is not altered in *btl* mutants (Anderson et al., 1995). Thus it is possible, even likely, that *dfr* regulates *btl* expression. Preliminary experiments suggest that *dfr* is not expressed in the border cells (W. Johnson and D. Montell, unpublished data). One interpretation then, is that Dfr may regulate *btl* in the embryo in much the same way that C/EBP does in the ovary.

Drosophila C/EBP was previously reported to have a similar DNA binding specificity as vertebrate C/EBP. This conclusion was reached by comparing the abilities of various oligonucleotides to compete for binding to purified *Drosophila* C/EBP in gel mobility shift assays (Rørth and Montell, 1992).

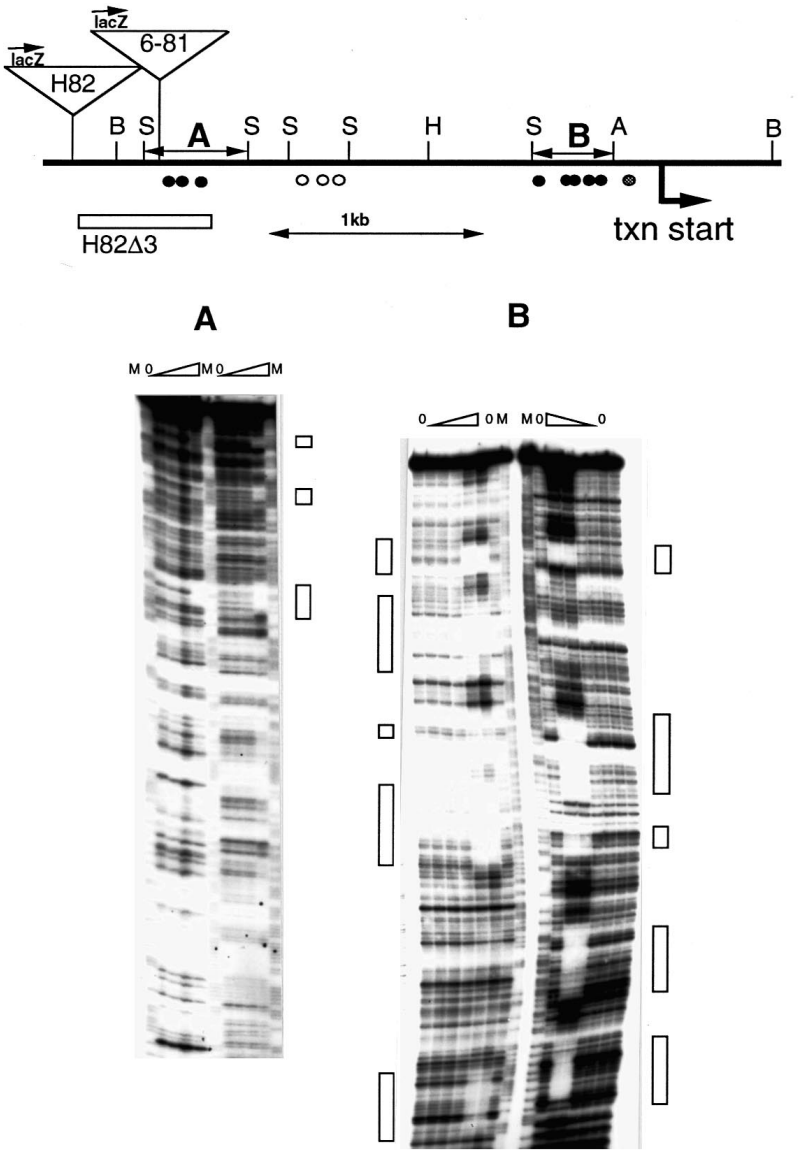

Fig. 7. DNAse I footprinting of C/EBP binding sites in the *btl* 5′ flanking DNA. (Top) Schematic representation of genomic DNA in the *btl* 5′ flank. Large triangles represent P-element enhancer trap insertions. Open box indicates DNA deleted in the excision line $btl^{H82\Delta3}$. Open circles indicate low affinity C/EBP binding sites. Closed circles represent high affinity binding sites. Hatched circle represents putative site which has not been tested. A, *Apo*I; H, *Hin*dIII; S, *Sau*3aI; B, *Bam*HI. (Bottom) DNAse I footprinting of fragments A and B indicated in schematic, with purified C/EBP protein. M, marker lane; 0, no C/EBP protein control lane; triangles indicate increasing protein added. For fragment A, 2, 20, or 200 ng of protein was used; for fragment B, reactions with 5, 50, 100, 200, and 500 ng of protein are shown. Footprinted sequences are indicated by the open boxes to the right of the gel. Both strands of each fragment are shown. For fragment A both strands are read in the same direction whereas for fragment B the strands are read in opposite directions.

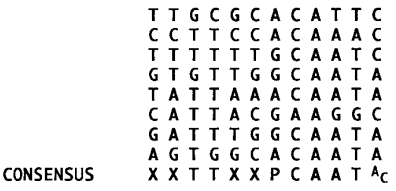

Fig. 8. Alignment of C/EBP binding sites from the *btl* promoter.

Oligonucleotides that differed from the sequence AGATTGCGCAATCT by four basepairs were found to compete less effectively than did cold oligonucleotide of the same sequence for binding to both the *Drosophila* and vertebrate proteins. In the present analysis, *Drosophila* C/EBP protein was mixed with complex DNA sequences from the *btl* regulatory region and sequences selected by their ability to bind. Therefore a less biased determination of binding specificity was possible. The consensus that emerged was $XX^{A}/_{T}TTXXP_{u}CAAT^{A}/_{C}{}^{A}/_{T}$. The optimal vertebrate sequence matches the *Drosophila* consensus at each of the conserved positions. Comparison of the oligonucleotides used in the competition assays described above indicates that each of the oligonucleotides that competed less well than the original had a change in at least one of the *Drosophila* consensus nucleotides. Thus our results confirm the similarity in binding specificities of vertebrate and *Drosophila* C/EBP.

The role of *btl* in border cell migration

The observation that C/EBP-independent *btl* expression could rescue border cell migration in *slbo* mutants indicates that *btl* is an important target for C/EBP in border cell migration. While this result at first seems to imply that *btl* is the only target for C/EBP regulation in the border cells, several observations indicate that additional *slbo* target genes are required for completely normal migration. First the rescue of border cell migration by *hs-btl* was partial. While 90% of stage 9 egg chambers displayed significant border cell migration, 10% did not. Furthermore, the migration was usually not as complete as that observed when migration was restored using the *hs-slbo* transgene (C. Andrews and D. Montell, unpublished results). Presumably additional C/EBP target genes contribute to efficient and complete border cell migration. In addition, mosaic analysis with a presumed null allele of *btl* indicated that loss of *btl* did not eliminate border cell migration, whereas loss or even reduction in C/EBP expression does. Therefore at least one other gene must be able to compensate for loss of *btl*. It is likely that this gene is also a target for regulation by C/EBP since border cell migration was detectably impeded by the loss of *btl* function when C/EBP levels were low, as in the weak *slbo* background, $slbo^{e14a}/slbo^{e2B}$. Consistent with these conclusions, we have identified at least two additional genes whose border cell expression depends upon C/EBP (B. Tinker, C. M. A. and D. J. M., unpublished results). Whether the redundant function(s) is an additional receptor tyrosine kinase or a completely different function remains to be clarified, however the observation that *btl* expression largely rescues *slbo* defects indicates that RTK action can promote border cell migration.

Functional redundancy has been observed for other proteins implicated in cellular motility, such as axon guidance. Fasciclins I and III and Connectin are cell adhesion proteins expressed on subsets of axons in the developing *Drosophila* nervous sytem (Hortsch and Goodman, 1991). Although, *fasI* and *fasIII* and *connectin* mutants develop normally, the biological activities of these proteins can be demonstrated in other ways. Embryos lacking both FasI and the Abl tyrosine kinase display severe disruption of nervous system development (Elkins et al., 1990); misexpression of FasIII causes predicted errors in axon guidance and synapse formation (Chiba et al., 1995), as does misexpression of Connectin (Nose et al., 1994).

The apparent redundancy of function of *btl* in the border cells contrasts with the strict requirement for *btl* function in tracheal cell migration; however this situation may be quite common. For example, the Kit receptor tyrosine kinase is essential for migration and survival of a variety of cell types in the mouse embryo. And yet, the receptor and its ligand are also expressed in complementary patterns in the mouse CNS, where no effect of Kit mutations has yet been found (Morrison-Graham and Weston, 1993). Thus as with Btl, it appears that Kit is absolutely required in some cells, but functionally redundant in other cell types. Another example can be found in the c-Ret RTK for which mouse knockouts have recently been reported (Schuchardt et al., 1994). Although this RTK is expressed in many neural crest cells (Pachnis et al., 1993), defects in the mutants appear to be limited to those crest cells that innervate the digestive tract.

RTKs, and FGF receptors in particular, are implicated in a growing number of developmentally regulated cell migrations (Montell, 1994), however, the mechanism by which RTKs affect cell movements in development is not yet clear. Whether RTK activity acts as a developmental switch, converting cells from an immotile to a motile state or whether RTKs act as chemotactic receptors, navigating cells toward increasing concentrations of ligand, remains to be elucidated. It is possible that different receptors perform different functions or that the same receptor may serve different purposes in different cells. It will be of interest then to compare the role of Btl signaling in tracheal cell migration in the embryo and in border cell migration in the ovary. For example, constitutively active Ras and Raf, when induced using a heat shock promoter can partially rescue tracheal cell migration in *btl* mutant embryos (Reichman-Fried et al., 1994). While we have observed dramatic effects of constitutively active and dominant negative Ras proteins on border cell migration, no effect of constitutively active Raf could be detected (T. Lee and D. Montell, manuscript in preparation). Further experiments will be required to elucidate the differences between these two migratory cell types and the role of RTK signaling in their migrations.

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