cells were grown as above in RPMI 1040. Transfectants expressing MHC class II molecules were used as antigen-presenting cells (APCs) to stimulate T cell hybridomas in the presence of SEB or TSST-1. Briefly, 2×10^4 APCs were incubated in the presence of 6×10^4 T cells at various concentrations of toxins (0 to 1 μg/ml). Co-cultures were performed in a final volume of 200 μl in 96-well flat-bottom plates for 18 hours at 37°C in 5% CO_2. Stimulation was evaluated by the amount of interleukin-2 (IL-2) released by the T cells in the co-culture supernatants. Amounts of IL-2 were determined by the ability of the co-culture supernatant to support the proliferation of the IL-2–dependent cell line CTLL-2 and were measured with the hexosaminidase colorimetric assay (*31*).

34. Stainings were done on 5×10^5 cells for 1 hour on ice, washed twice with cold phosphate-buffered saline (PBS), and incubated for another hour on ice with fluorescein isothiocyanate–conjugated goat antibody to mouse (Caltag Laboratories, San Francisco). Flow cytometric analysis was performed on a FACScan (Becton Dickinson) with a four-decade logarithmic scale. Live cells were gated by light scatter.
35. Twenty micrograms each of SEA, TSST-1 (Toxin Technology, Sarasota, FL), and recombinant SEB were labeled for 10 min in PBS with 0.5 μg of Iodogen (Pierce, Rockford, IL) and 250 μCi of ^{125}I-Na (Amersham, Toronto) in a final volume of 60 μl. Radiolabeled products were separated from free iodine by Sephadex G-25 (Pharmacia, Sweden) exclusion chromatography in PBS containing 0.01% sodium azide and 0.1% bovine serum albumin. Specific activities varied between 7.5×10^6 and 18×10^6 cpm/μg. Adherent cells (DAP and HeLa) were trypsinized and counted. Cells (10^6) were incubated in Eppendorf tubes with 100 ng (unless otherwise specified) of radiolabeled toxins in a final volume of 200 μl of binding buffer (DMEM supplemented with 2% fetal calf serum and 0.1% NaN_3). Each point was done in duplicates, and each mutant was tested a minimum of two times with different preparations of iodinated toxins. For competition experiments, cold competitors (0.05 to 50 μg/ml) were added before the addition of labeled toxins. After 4 hours of incubation at 37°C, cells were transferred to a fresh tube over a 200-μl cushion of oil (84:16, silicone oil:mineral oil) and spun down for 1 min in an Eppendorf centrifuge. The tubes were immediately frozen at −80°C, and the tips of the tubes containing the cell-bound ^{125}I-labeled toxins were cut off and counted on a gamma counter.
36. We thank J. W. Kappler and P. Marrack for discussions and for the generous gift of recombinant SEB and the T cell hybridomas. The LG-2 cells were from L. Stern, Harvard University. We also thank J. Fraser for recombinant SEB and SEA. We are grateful to D. C. Wiley, F. Denis, and S. Gratton for discussions and critical reading of the manuscript; C. Lemire for expert secretarial assistance; C. Cantin for cell sorting; and H. McGrath, J.-P. Fortin, and H. Soudeyns for technical support. J.T. is supported by a postdoctoral fellowship from National Health Research and Development Program. N.L. is a student fellow of the Medical Research Council (MRC) and of the Université de Montréal. P.M.L. is supported by studentships from Fonds de la Recherche en Santé de Québec and MRC. R.-P.S. holds an MRC Scientist Award. This work was supported by grants to R.-P.S. from National Cancer Institute of Canada and MRC.

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Specification of C/EBP Function During *Drosophila* Development by the bZIP Basic Region

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The biologically relevant interactions of a transcription factor are those that are important for function in the organism. Here, a transgenic rescue assay was used to determine which molecular functions of *Drosophila* CCAAT/enhancer binding protein (C/EBP), a basic region–leucine zipper transcription factor, are required for it to fulfill its essential role during development. Chimeric proteins that contain the *Drosophila* C/EBP (DmC/EBP) basic region, a heterologous zipper, and a heterologous activation domain could functionally substitute for DmC/EBP. Mammalian C/EBPs were also functional in *Drosophila*. In contrast, 9 of 25 single amino acid substitutions in the basic region disrupted biological function. Thus, the conserved basic region specifies DmC/EBP activity in the organism.

Eukaryotic transcription factors influence all aspects of development by regulating gene expression. For many transcription factors, the functional interactions with cognate DNA, the transcription initiation complex, and other regulatory proteins have been studied in detail. Such analyses are usually done by transfection of tissue culture cells and in vitro assays. The ultimate goal is to determine how transcription factors function in the organism and thus which interactions are biologically relevant. Here a transgenic approach was used to directly analyze the functional requirements of the DmC/EBP transcription factor during development.

C/EBP transcription factors are characterized by their amino acid sequence similarity in the COOH-terminal DNA-binding domains (*1*–*5*). Several distinct C/EBP family members have been identified in mammals (*1*–*4*). DmC/EBP was first identified as the protein encoded by *slow border cells*, a locus required during *Drosophila* oogenesis (*5*). Subsequently, DmC/EBP was found to be an essential gene and likely to be the only C/EBP in *Drosophila* (*6*). DmC/EBP is required during late embryogenesis and may perform functions similar to those of mammalian C/EBP (*6*).

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The introduction of an ectopic copy of the gene encoding DmC/EBP (the Pw15 transgene in Fig. 1) by germline transformation was found to overcome the lethality associated with loss of chromosomal DmC/EBP and to rescue flies to adulthood. Antibody staining showed that the Pw15 transgene directed expression of DmC/EBP (or of a heterologous gene) in the normal DmC/EBP pattern in the embryo, although at a reduced level (*7*). These observations provided a sensitive functional assay for authentic DmC/EBP activity in the organism. Transgenes were manipulated in vitro and introduced into flies by germline transformation (*8*). Rescue of the lethal phenotype indicated that the protein encoded by the transgene could fulfill the essential function or functions of DmC/EBP.

To determine which domains of the DmC/EBP protein were important for function in vivo, mutant variants were generated and tested in the transgenic rescue assay. DmC/EBP consists of a COOH-terminal basic region–leucine zipper (bZIP) domain and an uncharacterized NH_2-terminal region (occupying three-quarters of the protein).

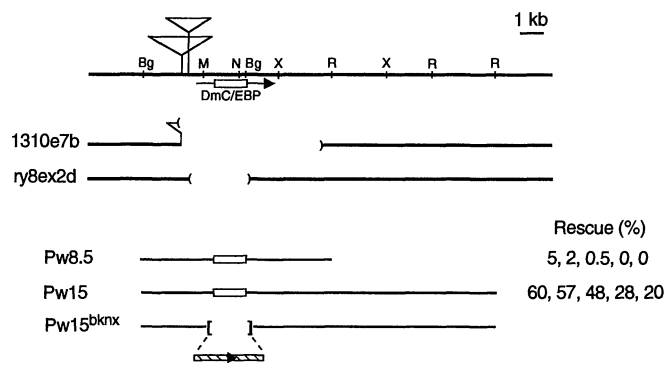


Fig. 1. Transgenic rescue assay (*8*). Top: Map of DmC/EBP genomic region. Bg, Bgl II; M, Mlu I; N, Not I; X, Xho I; R, Eco RI (only some sites are shown). P-elements associated with *slbo* alleles (*5*) are indicated as triangles, the DmC/EBP transcript is indicated by an arrow, and the open reading frame (ORF) is indicated by a white box. Represented below are DmC/EBP-null chromosomes used (1310e7b and ry8ex2d) and the fragments that were cloned into pCasPer to generate Pw8.5 and Pw15 transgenes. In Pw15^{bknx}, the DmC/EBP ORF was replaced by a linker with unique cloning sites (*23*).

The deletion of the NH_2-terminal 318 amino acids rendered DmC/EBP biologically inactive (N-bZIP in Fig. 2), which indicates that this region provides one or more essential functions. One likely function was transcriptional activation, as transfection of *Drosophila* Schneider cells showed that DmC/EBP, but not N-bZIP, was a transcriptional activator (*9*). Deletion of the 23–amino acid glutamine repeat, a potential activation domain (*10*), had little effect on transactivation and no detectable effect on in vivo activity (ΔOpa in Fig. 2).

If transactivation is the only function required of the NH_2-terminal region, then heterologous activation domains should be able to substitute for this function. To test this possibility, polypeptides from different transcription factors were fused to the DmC/EBP bZIP region at amino acid 318 (Fig. 2). Chimeras with potent activation domains from VP16 or GAL4 (GAL4L) rescued flies to adulthood almost as efficiently as DmC/EBP itself. Transcriptionally active chimeras containing NH_2-terminal regions from C/EBP-α or C/EBP-δ were also functional in vivo. Furthermore, there was a positive correlation between the rescue ability of various chimeras and their relative transactivation strength. Thus, the NH_2-terminal region of DmC/EBP could be substituted for by unrelated sequences without disruption of its biological function; providing an activation domain appeared to be the only requirement.

The basic region of the bZIP domain mediates DNA binding but may also have other functions. The crystal structures of the GCN4 bZIP domain bound to DNA show that some, but not all, basic region amino acids directly contact DNA (*11*). By analogy, the amino acid conservation between mammalian and *Drosophila* C/EBP (Fig. 3) includes both DNA-contacting and -noncontacting residues. The biological function of the basic region was analyzed by the introduction of 27 individual amino acid substitutions at positions conserved among all C/EBP proteins. Each mutant was tested for DNA binding in quantitative electrophoretic mobility shift assays (EMSAs) and for biological function in the rescue assay (Fig. 3). The presence of dysfunctional mutants with normal DNA binding would indicate that interactions in addition to DNA binding might be important in vivo.

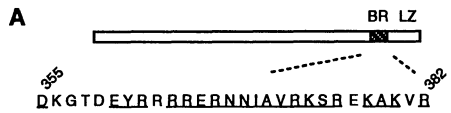
Correct DNA binding was critical for DmC/EBP biological function. Mutations that affected DNA binding (residues 365, 366, 368, 369, 375, and 376), even those with subtle effects (residues 361, 373, and 380), severely decreased or eliminated rescue. Direct involvement of the latter residues in DNA binding was indicated by slightly elevated dissociation constants (K_d) of the mutant proteins and was substantiated by the following observations. In GCN4 crystal structures (*11*), all basic residues contact phosphates of the DNA backbone. If each residue makes a contribution to the bZIP-DNA interaction, a more severe DNA-binding defect would be expected in a double mutant, as was found in 373RS/380KE (Fig. 3). Also, mutagenesis of corresponding residues in GCN4 and C/EBP-α affected DNA binding (*12*). Finally, an alpha-helical representation of the basic region (Fig. 3B) shows that all residues discussed above are located on the same face (contacting DNA) of the helix. The sensitivity of the rescue assay to minor perturbations of DNA binding may reflect that, in vivo, DmC/EBP competes with other proteins for DNA binding and must bind with high selectivity to the correct target sites in the genome.

All mutations disrupting DmC/EBP biological function directly affected DNA binding. Correspondingly, residues not required for DNA binding were not essential for in vivo function. Although additional functions may have gone undetected (*13*), a tentative conclusion is that correct DNA binding is the only function required of the basic region in vivo.

The bZIP domain contains a dimerization motif, the leucine zipper. Dimerization is required for DNA binding, but

	Rescue (%)	Transactivation
DmC/EBP	60, 57, 48, 28, 20	4.4x
N-bZip	0, 0	1.1x
ΔOpa	40, 34, 30	3.8x
(428–490) VP16/Dm	33, 24	7.0x
(768–881) GAL4L/Dm	42, 28, 15	5.8x
(851–881) GAL4S/Dm	3, 2,<1, <1, 0	1.9x
(1–257) α/Dm	2, <1, 0, 0	2.0x
(1–153) α/Dm	16, 7, 7, 6, 2	3.3x
(1–164) δ/Dm	23, 21, 6, 3, 2	4.8x
(1–144) δ/Dm	5, 5, 4, 2, 1, <1	3.2x

Fig. 2. Biological activity of DmC/EBP chimeras. N-bZIP contains only amino acids 318 to 444. In ΔOpa, amino acids 45 to 67 are deleted (*6*). The remaining chimeras contain the indicated amino acids from VP16, GAL4, C/EBP-α (α), or C/EBP-δ (δ) and the bZIP region (amino acids 318 to 444) from DmC/EBP (Dm) (*24*). For rescue determination, the derivatives were cloned into $Pw15^{bknx}$. Each listed rescue percentage represents a separate transformant line (see *8*). Transactivation was determined by transient transfection (*9*).



	DNA[a] binding	K_d (nM)[b]	Rescue[c]	Rescue (%)[d]
Wild-type	+	0.19	+	60, 57, 48, 28, 20
355DN	+		+	
355DH	+		+	
360EK	+		+	
360EQ	+		+	
361YN	(+)	0.42	-	1, <1, 0
362RS	+		+	
364RK	+		+	
364RQ	+		+	
365RW	-		-	
365RQ	-	3.1	n	<1, 0, 0, 0
366EK	-	3.4	-	<1, 0, 0
366EQ	+	0.20	+	50, 32, 24, 15
367RQ	+		n	
368NS	-		-	0, 0, 0, 0
369ND	-	2.2	-	0, 0, 0, 0
370IV	+		+	
371AS	+		+	56, 47
373RS	(+)	0.28	1	4, 2, 1, 0
374KQ	+		+	45, 20, 6
375SC	+		+	49, 29
375SR	-		-	
376RQ	-		-	
378KQ	+		+	
378KR	+		+	
379AR	+		+	
380KE	(+)	0.29	-	1, <1, 0, 0
382RS	+		+	
373RS/380KE	-	2.1	n	

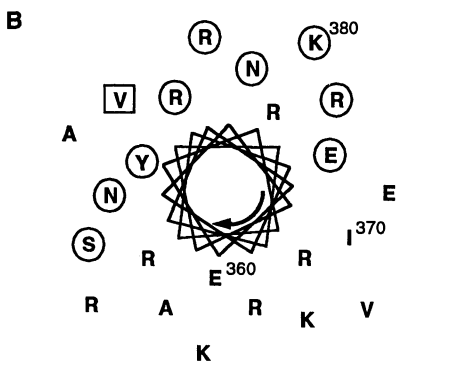


Fig. 3. Effect of single amino acid substitutions in the basic region (BR). LZ, leucine zipper (**A**) Basic region of DmC/EBP, with residues conserved among all C/EBP proteins underlined (*22*). Substitutions resulted from site-directed mutagenesis. a, DNA-binding activity of purified full-length proteins measured by EMSA (*6*). Dilution series of two independent preparations were tested for each mutant. Plus sign, as wild type; plus sign in parentheses, similar to wild type; minus sign, visibly decreased DNA binding or no DNA binding. b, Average of two K_d measurements by Scatchard plot analysis of saturation binding data (*3*). c, Qualitative determination of rescue in the Pw8.5 background (see Fig. 1). Minus sign, no rescue; 1, one rescued fly; plus sign, up to 5% rescue (as wild type); n, not tested. d, Rescue in the Pw15 background (*8*). Mutant proteins were expressed from Pw15 at the same low level as was wild-type DmC/EBP (detected by immunostaining of embryos). Mutants defective in DNA binding did not activate transcription. Complete analysis is found in (*25*). (**B**) Schematic representation of the basic region as an alpha helix; residues critical for biological function are circled (*22*). The boxed valine residue is also implicated directly in DNA binding (*12*).

several studies suggest that C/EBP leucine zippers also mediate heteromeric interactions with negative regulators (*14*), with other bZIP proteins (*15*), and with non-bZIP transcription factors (*16*) and directly regulate transcriptional activation (*17*). To investigate whether analogous interactions were important for DmC/EBP function, the leucine zipper was exchanged precisely for another zipper with nonoverlapping dimerization specificity (the GCN4 zipper in Fig. 4A). The resulting chimera could homodimerize and bind C/EBP sites but should be unable to participate in other zipper-specific interactions. This chimera rescued efficiently (Fig. 4B), which indicates that homodimerization was the only function required of the DmC/EBP zipper in vivo.

These results implicate the basic region as the unique element specifying DmC/EBP function in vivo. To test this possibility more directly, a chimera was constructed that contained only the basic region and flanking amino acids from DmC/EBP (VP16/Dm/GCN4 in Fig. 5A). This chimera substituted efficiently for DmC/EBP. Thus, the DmC/EBP basic region, coupled to heterologous activation and dimerization domains, is sufficient to mediate the biological function of the native protein. In conjunction with the mutational analysis of the basic region, this observation indicates that the functional requirements of DmC/EBP may simply be transactivation and sequence-specific DNA binding as a homodimer.

The sequence homology between fly and mammalian C/EBP is limited to the bZIP region. In support of the above conclusion, all tested mammalian C/EBPs were functional in the rescue assay (Fig. 5B). However, they rescued inefficiently. Only C/EBP-δ rescued as a single copy transgene; for C/EBP-α and C/EBP-β, multiple copies were required. C/EBP-α and C/EBP-δ were substantially less active than were corresponding chimeras with the bZIP region from DmC/EBP (δ/Dm and α/Dm in Fig. 2). This indicates that nonconserved sequences retained in the chimeras, perhaps the 35 amino acids upstream of the conserved basic region core, also contribute quantitatively to DmC/EBP function. Mammalian C/EBP proteins show distinct properties in some tissue culture assays. Nevertheless, their ability to partially substitute for DmC/EBP suggests that they might substitute for each other if expressed appropriately during mammalian development. Having overlapping expression patterns, they may at least in part be genetically redundant.

This analysis may also be relevant for other transcription factors. It has been shown that Bicoid chimeras with heterologous activation domains can rescue *bicoid* mutants (*18*). Also, overexpression of *Drosophila* and mammalian proteins with very similar homeodomains results in identical dominant phenotypes (*19*). Finally, three Pax transcription factors with limited sequence homology can, if expressed appropriately, perform the same functions in *Drosophila* embryos (*20*). These experimental observations are consistent with the idea that the DNA-binding domain specifies the biological function of the transcription factor, as determined here for DmC/EBP. From an evolutionary perspective, the reason many transcription factor homologs or isoforms are similar exclusively within their DNA-binding domains may in some cases be quite simple. Sequences outside this domain may have diverged because many polypeptides can perform the only required function: namely, transactivation. In contrast, for transcription factors such as Dorsal and Extradenticle (*21*) that show more extensive sequence conservation, additional specific (and conserved) interactions are predicted to be required in vivo.

In conclusion, a transgenic assay for biological function led to a simple description of a bZIP transcription factor in action. Other developmentally important transcription factors may function similarly.

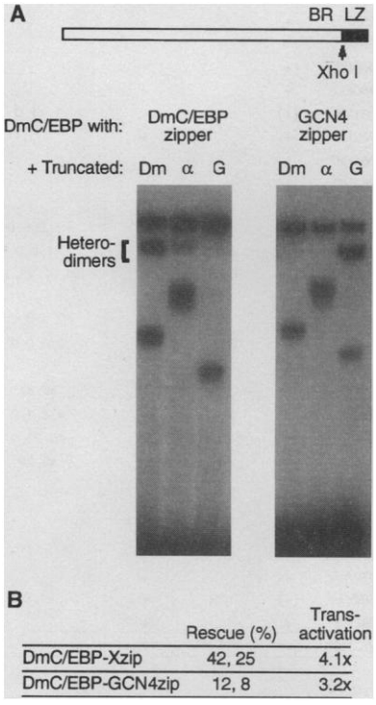


Fig. 4. Biological activity of DmC/EBP with a heterologous leucine zipper (LZ). BR, basic region. The zipper was exchanged at amino acid 384; making an Xho I site here introduced only a functionally neutral substitution (DmC/EBP-Xzip (*26*). (**A**) Full-length DmC/EBP derivatives (top bands) were mixed with truncated bZIP proteins (lower bands) (Dm, DmC/EBP$^{354-444}$; α, C/EBP-α$^{272-346}$; G, GCN4$^{228-281}$), followed by EMSA (*6*). A complex with intermediate mobility indicates heterodimerization. Purified proteins were premixed in 2 M guanidinium to promote subunit exchange and were serially diluted before addition of probe. (**B**) Determination of rescue ability and transactivation as in Fig. 2.

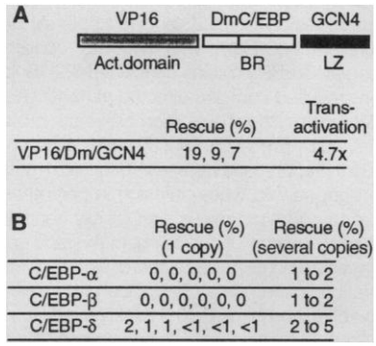


Fig. 5. (**A**) Rescue by VP16/Dm/GCN4. This chimera contains the VP16 activation domain (see Fig. 2), amino acids 318 to 384 from DmC/EBP, and the GCN4 leucine zipper (LZ) (see Fig. 4). BR, basic region. (**B**) Rescue by mammalian C/EBPs. The ORF for the indicated mammalian C/EBP (*1*, *3*) was cloned into Pw15^{bknx}. Single-copy values were obtained by standard rescue assay (*8*). For several copies, the rescue percentage fell within the given interval when flies each carrying two or three copies of the transgene were mated.

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8. P-element germline transformation was done by injection into *w*^{1118}*;;Sb,P[ry*$^{+}$*, Δ2-3]*/TM6,*Ubx* embryos; additional insertion sites were obtained by exposing P-elements to a *P[Δ2-3]99B* transposase source. Transgenic flies were checked by Southern (DNA) blot and polymerase chain reaction (PCR) analysis; for point mutants, the appropriate PCR product from genomic DNA was sequenced. Rescue crosses were done at 25°C under uncrowded conditions with single-copy transgenes. Rescue ability is given as a percentage: the number of surviving adults having a transgene and no chromosomal DmC/EBP, relative to complete complementation. Each value is from an independent insertion site, with 1000 to 2000 progeny counted. DmC/EBP was expressed at a much lower level from Pw15 than from the chromosomal locus. Rescue percentage correlated with amounts of expression at different insertion sites, and multiple copies of the same transgene gave more efficient rescue than did one copy. Thus, DmC/EBP was expressed in subsaturating amounts, which was an advantage for the rescue assay. At larger amounts of expression, minor defects in DmC/EBP functionality might be tolerated and thus not be detected.
9. Schneider cells, which do not express endogenous DmC/EBP, were transfected as described [P. P. Di Nocera and I. B. Dawid, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7095 (1983)]. A vector (20 to 40 ng) with Actin5C promoter driving expression of DmC/EBP

or derivatives was cotransfected with 0.25 μg of a copia-lacZ internal control and 1 μg of a reporter construct containing two C/EBP binding sites upstream of a minimal thymidine kinase promoter and the gene encoding luciferase. After 48 hours, luciferase and β-galactosidase activities were measured. Fold transactivation relative to no transactivator is listed as the average of three experiments (Figs. 2 through 5). All DmC/EBP derivatives, C/EBP-α, and C/EBP-δ were of the expected size and expressed in amounts similar to that of DmC/EBP, as judged by protein immunoblot with antibodies to DmC/EBP (*6*), C/EBP-α [L. M. Scott, C. I. Civin, P. Rørth, A. D. Friedman, *Blood* **80**, 1725 (1992)], and C/EBP-δ.

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22. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; G, Gly; H, His; I, Ile; K, Lys; L, Leu; N, Asn; P, Pro; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.
23. Pw15 was digested with Bgl II and partially digested with Mlu I, and the oligonucleotide 5′-(CGCG)AAAATGAGATCTTAGGTACCGCGGCCGCTCTAGAG(GATC)-3′ was inserted.
24. Activation domains were cloned by PCR (confirmed by sequencing), with the following primers and complementary DNA or genomic DNA as template. N-bZIP: 5′-CCGGATCCATG**GCGGCCGC**AGGAGC-3′; VP-16: 5′-CGGGATCCATGGGCGAGGACGTG-GCGAT-3′ and 5′-TTGAA**GCGGCCGC**CAGGC-CCACCGTACTCGTC-3′; GAL4: 5′-TTGAA**GCG-GCCGC**CAGGCTCTTTTTTTGGGTTTGG-3′ and $GAL4_{short}$: 5′-CGGGATCCATGATCACTACAGG-GATGTTT-3′, $GAL4_{long}$: 5′-CGGGATCCATGGC-CAATTTTAATCAAAGTGG-3′; C/EBP-α_{1-257}: 5′-CGGGATCCATGGAGTCGGCCGAC-3′ and 5′-TT-GAA**GCG GCC GC**GGGGTGCGGACCAGC-3′; C/EBP-δ_{1-164}: 5′-CGGGATCCATGAGCGCCGC-GCTT-3′ and 5′-TTGAA**GCGGCCGC**AGGAGGCT-CCGGCGA-3′. Deletion of internal Not I fragments created C/EBPδ_{1-144} and C/EBP-α_{1-153}; for C/EBP-α_{1-153}, the Not I sites were filled in. Bam HI–Not I fragments were cloned into a vector with Bam HI 5′ of DmC/EBPs ATG (*6*), with the use of the internal Not I site. The sequence surrounding this site has been corrected to LPHLAAAAGAHNLLK (*22*) on the basis of new DNA sequencing. Mutagenesis with 5′-CCTG**GCGGCCGC**·GCAGGAGCA-3′ recreated the corrected reading frame in affected fusions.
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27. I thank P. Beachy for Schneider cells, copia-lacZ, and an Actin5C plasmid and S. L. McKnight for the C/EBP-δ antibody. I am grateful to J. Shuman, C. Thompson, A. Fire, A. Spradling, and S. L. McKnight for discussions and helpful comments on the manuscript. Supported by fellowships from the Danish Medical Research Council and the Danish Cancer Society and by the Carnegie Institution of Washington.

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Potentiated Transmission and Prevention of Further LTP by Increased CaMKII Activity in Postsynaptic Hippocampal Slice Neurons

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Calcium-calmodulin–dependent protein kinase II (CaMKII) is a necessary component of the cellular machinery underlying learning and memory. Here, a constitutively active form of this enzyme, CaMKII(1–290), was introduced into neurons of hippocampal slices with a recombinant vaccinia virus to test the hypothesis that increased postsynaptic activity of this enzyme is sufficient to produce long-term synaptic potentiation (LTP), a prominent cellular model of learning and memory. Postsynaptic expression of CaMKII(1–290) increased CaMKII activity, enhanced synaptic transmission, and prevented more potentiation by an LTP-inducing protocol. These results, together with previous studies, suggest that postsynaptic CaMKII activity is necessary and sufficient to generate LTP.

CaMKII mediates numerous physiological processes triggered by a rise in intracellular Ca^{2+} ions (*1*). In the brain, its abundance at excitatory synaptic contacts (*2*) makes it strategically placed to respond to localized Ca^{2+} transients. Its molecular properties suggest that this enzyme could convert a transient signal to a long-lasting modification (*3*). Particular attention has been paid to the role of CaMKII in LTP, a form of activity-dependent synaptic plasticity that may underlie some forms of learning. LTP is triggered by a conditioning protocol that transiently activates postsynaptic *N*-methyl-D-aspartate (NMDA) receptors (*4*) and produces an increase in postsynaptic Ca^{2+} concentration (*5*). The biochemical steps occurring after this increase in Ca^{2+} have not been established, although several protein kinases have been implicated (*6–9*). In particular, inhibition of CaMKII activity in neurons, either with peptide inhibitors (*10*) or genetically (*11*), prevents LTP.

To test the hypothesis whether postsynaptic CaMKII activity is sufficient to generate LTP, we used vaccinia virus (VV) infection to introduce recombinant products into neurons of hippocampal slices. Slices were prepared from 9- to 13-day-old rats by standard methods (*12*). Under visual guidance, the extracellular space of the CA1 pyramidal cell body layer was injected with a solution containing purified VV (*13*). Slices were then incubated at 35°C for 4 to 16 hours to allow for infection and expression of the recombinant gene products. Initially, experiments with a VV encoding β-galactosidase (BGVV) were performed to test for neuronal expression of recombinant virus product. Infected slices were fixed, stained with X-gal, and cleared (Fig. 1, A, B, and C). Within 4 hours of infection, recombinant expression could be detected (*14*). Overlapping injections, carefully controlled to ensure maximal delivery of virus-rich solution to the tissue, produced continuous staining of the injected area (Fig. 1, A and B). Thin sections (40 μm) through slices indicate that such injections can infect 100% of cells in the injected area (Fig. 1A). Injections can be restricted to targeted regions, for example, the postsynaptic CA1 region (Fig. 1B). Recombinant products in such infections are not expressed in presynaptic structures because to express recombinant products VV requires translational machinery not found in presynaptic axons or terminals (*15*). The cell bodies of the presynaptic neurons show no expression of recombinant products in infections of the CA1 region (Fig. 1B). Electrophysiological properties of cells infected with BGVV were indistinguishable 6 hours after infections compared with those of uninfected cells (input resistance: 290 ± 3 megohms for cells from BGVV-infected slices, $n = 22$; 276 ± 1 megohms for uninfected cells, $n = 8$, $P > 0.5$, t test; resting membrane potential: 54 ±

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