RL72 (IgM antibodies to mouse CD4), 31M (IgM antibodies to mouse CD8), and BP-107 (antibodies to mouse MHC class II $A^b$) [R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *Nature* **314**, 98 (1985); F. W. Symington and J. Sprent, *Immunogenetics* **14**, 53 (1981); M. Sarmiento, A. L. Glasebrook, F. W. Fitch, *J. Immunol.* **125**, 2665 (1980)]. Cedarlane Lo-Tox complement was added to 10% concentration, and the cells were incubated for a further 1 hour at 37°C. After two washes in Dulbecco's minimum essential medium containing 5% fetal bovine serum, live cells were counted and resuspended ($1 \times 10^7$ cells per milliliter) for use in cytotoxicity assays.


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28. Recipient mice were exposed to γ radiation (9.4 Gy) and then injected intravenously with $5 \times 10^6$ bone marrow cells from $\beta_2M^{-/+}$ or $\beta_2M^{-/-}$ mice (*12*). After 5 days, the mice were injected intraperitoneally with 3 μCi of 5-[$^{125}$I]iodo-2′-deoxyuridine. Isotope incorporated by the spleens of the recipients was determined the next day after extensive rinsing of the spleens with phosphate-buffered saline.


29. We thank E. Long and D. Raulet for helpful comments on the manuscript; D. Littman for the CD4 mutant mice; M. Digelmann, J.-M. Kuhry, P. André, and M. Duval for help in creating the knockout mice; C. Schricke and F. Fischer for help with care of the mice; P. Gerber and C. Ebel for assistance; P. Eberling and F. Ruffenach for peptides and oligonucleotides; and C. Waltzinger for help with flow cytometry. Supported by funds from INSERM, CNRS, and the Centre Hospitalier Universitaire Régional, and by grants to D.M. and C.B. from the Association pour la Recherche sur le Cancer and the Human Frontier Science Program. T.M. was supported by a fellowship from the Human Frontier Science Program Organization.

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# A Mammalian Histone Deacetylase Related to the Yeast Transcriptional Regulator Rpd3p


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**Trapoxin is a microbially derived cyclotetrapeptide that inhibits histone deacetylation in vivo and causes mammalian cells to arrest in the cell cycle. A trapoxin affinity matrix was used to isolate two nuclear proteins that copurified with histone deacetylase activity. Both proteins were identified by peptide microsequencing, and a complementary DNA encoding the histone deacetylase catalytic subunit (HD1) was cloned from a human Jurkat T cell library. As the predicted protein is very similar to the yeast transcriptional regulator Rpd3p, these results support a role for histone deacetylase as a key regulator of eukaryotic transcription.**


Reversible acetylation of lysine residues clustered near the $NH_2$-terminus of nucleosomal histones is thought to modulate the accessibility of transcription factors to their respective enhancer and promoter elements. Transcriptionally silent regions of the genome are enriched in underacetylated histone H4 (*1*), and histone hyperacetylation facilitates the ability of transcription factor TFIIIA to bind to chromatin templates (*2*). Although a histone acetyltransferase gene (*HAT1*) has been identified in yeast (*3*), the molecular entities responsible for histone deacetylation are unknown.

A requirement for a functional histone deacetylase in cell cycle progression has been implicated by the discovery that two cytostatic agents, trapoxin and trichostatin (Fig. 1A), inhibit histone deacetylation in cultured mammalian cells and in fractionated cell extracts. In addition to causing $G_1$ and $G_2$ phase cell cycle arrest, these natural products alter gene expression and induce certain mammalian cell lines to differentiate (*4*). Whereas sodium butyrate also has these properties, both trapoxin and trichostatin are five orders of magnitude more potent.

Trapoxin is an "irreversible" inhibitor of histone deacetylase activity, and its molecular structure offers clues as to how it could form a covalent bond with a nucleophilic active site residue. First, trapoxin contains an electrophilic epoxyketone that is essential for biological activity (*5*). Second, the aliphatic epoxyketone side chain is approximately isosteric with *N*-acetyl lysine (Fig. 1A). Trapoxin likely acts as a substrate mimic, with its expoxyketone poised to alkylate an active site nucleophile. We therefore regarded trapoxin as a tool that could reveal the molecular identity of histone deacetylase, so that its role in transcriptional regulation and cell cycle progression could be elucidated.

Tritium-labeled trapoxin was prepared by total synthesis and used to identify trapoxin binding proteins in crude extracts from bovine thymus. We used a charcoal precipitation assay to detect a specific trapoxin binding activity primarily in the nuclear fraction of the extracts (*6*). The binding activity was saturable with nanomolar concentrations of [$^3$H]trapoxin and was not detected in the presence of unlabeled trapoxin. Trichostatin also competed for binding with [$^3$H]trapoxin, which suggests that both of these compounds exert their cellular effects by targeting the same molecule.

If trapoxin and trichostatin induce cell cycle arrest by directly inhibiting histone deacetylase, then their binding and enzymatic activities should copurify. To investigate this possibility, we fractionated nuclear thymus proteins by ammonium sulfate precipitation and MonoQ anion-exchange chromatography (*7*). Two peaks of histone deacetylase activity eluted from the MonoQ column between 250 and 350 mM $NH_4Cl$ (Fig. 1B). Trapoxin binding activity, as revealed by the charcoal precipitation assay (40 nM [$^3$H]trapoxin), precisely coeluted

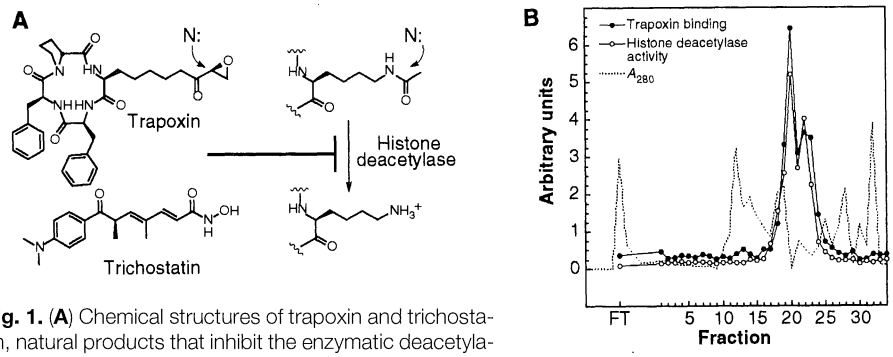


**Fig. 1.** (**A**) Chemical structures of trapoxin and trichostatin, natural products that inhibit the enzymatic deacetylation of lysine residues near the $NH_2$-terminus of histones. The epoxyketone side chain of trapoxin is approximately isosteric with *N*-acetyl lysine and likely alkylates an active site nucleophile. (**B**) Copurification of trapoxin binding and histone deacetylase activities. Nuclear proteins from bovine thymus were precipitated with ammonium sulfate and fractionated on a MonoQ column (*7*). Trapoxin binding was assayed by charcoal precipitation with [$^3$H]trapoxin (*6*). For the histone deacetylase assay, a peptide corresponding to bovine histone H4 (residues 1 to 24) was synthesized. The peptide was chemically acetylated with sodium [$^3$H]acetate (5.3 Ci/mmol, New England Nuclear)–BOP reagent (Aldrich) and purified by reversed-phase HPLC. Two microliters of [$^3$H]peptide (~40,000 dpm) was used per 200-μl assay. After incubation at 37°C for 1 hour, the reaction was quenched with 1 M HCl and 0.16 M acetic acid (50 μl). Released [$^3$H]acetic acid was extracted with 600 μl of ethyl acetate and quantified by scintillation counting. Pretreatment of crude or partially purified enzyme with trapoxin or trichostatin (20 nM) for 30 min at 4°C abolished deacetylase activity. $A_{280}$, absorbance at 280 nm; FT, flow-through.


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with the histone deacetylase peaks. Furthermore, all detectable histone deacetylase activity was abolished by treatment with either trapoxin or trichostatin (20 nM). Similar results were obtained with MonoQ-fractionated nuclear extracts prepared from human Jurkat T cells.

To purify the histone deacetylase further, we synthesized an affinity matrix based on the trapoxin structure. Because trapoxin itself is not amenable to direct modification and the epoxyketone side chain is indispensable for activity, we chose to replace one of the phenylalanine residues of trapoxin's cyclic core with a lysine that could then be covalently linked to a solid support. This molecule, which we call K-trap, was prepared by a 20-step synthesis starting with commercially available (*R*)-proline and (*S*,*S*)-threitol acetonide (Fig. 2A). Synthetic K-trap inhibited [$^3$H]thymidine incorporation in MG-63 human osteosarcoma cells with a potency approximately one tenth that of trapoxin. In vitro histone deacetylase activity was also inhibited potently by this compound (with complete inactivation at 20 nM) (*8*).

K-trap was deprotected with $Pd(Ph_3P)_4$ and coupled to an activated agarose matrix (Fig. 2A). MonoQ fractions containing nuclear proteins from bovine thymus were incubated with the K-trap affinity matrix and then tested for both trapoxin binding and histone deacetylase activity. Both activities were depleted (>90%) by treatment with the K-trap matrix, although a control matrix capped with ethanolamine had no effect on either activity (*8*). Bound polypeptides were eluted by boiling the matrix in 1% SDS buffer and separated by polyacrylamide gel electrophoresis (PAGE) (*9*).

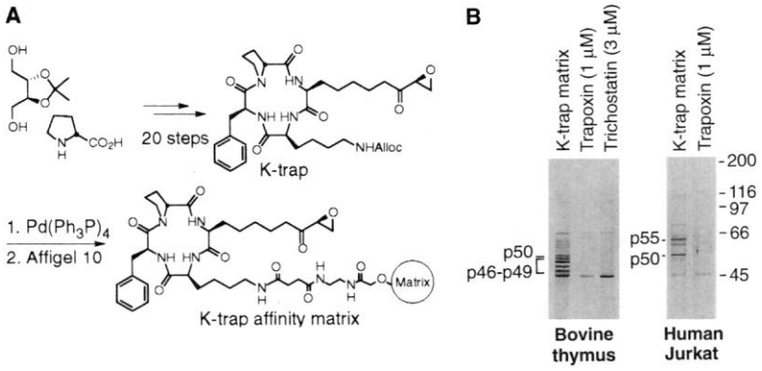


**Fig. 2.** Purification of trapoxin binding proteins by affinity chromatography. (**A**) Synthesis of K-trap and the K-trap affinity matrix. K-trap contains a protected lysine residue in place of the phenylalanine at position 2 in trapoxin. Alloc, allyloxycarbonyl; $Pd(Ph_3P)_4$, tetrakis(triphenylphosphine)palladium. (**B**) Silver-stained gel showing bovine and human trapoxin binding proteins. Proteins bound to the K-trap affinity matrix in the presence or absence of trapoxin or trichostatin were eluted by boiling in SDS loading buffer and analyzed by SDS-PAGE (9% gel). Nuclear proteins from human Jurkat T cells were prepared identically to those from bovine thymus (*7*). Molecular size standards (in kilodaltons) are indicated to the right.

The silver-stained gel of the affinity matrix eluates revealed six major polypeptides with apparent molecular sizes between 45 and 50 kD (Fig. 2B). The interaction between bovine p46-p50 and the K-trap matrix appeared to be specific, because these proteins were not retained when the incubation was done in the presence of either trapoxin or trichostatin (Fig. 2B) nor were they retained by an ethanolamine-capped control matrix (*8*). When the affinity purification was repeated with Jurkat nuclear extracts, only two major bands, p50 and p55, were observed by silver staining (Fig. 2B). Recovery of human p50 and p55 was similarly abolished by trapoxin (Fig. 2B) and trichostatin (*8*). Because the relative intensities of bovine p46 to p49 vary with each protein preparation, we suspect that they are proteolytic fragments derived from the bovine equivalent of human p55. One of the bands (p50) is common to both human and bovine sources.

Large-scale purification of the bovine proteins led to the resolution of two major bands of ~46 and ~50 kD in the final preparative electrophoresis step, both of which were submitted for microsequencing (*10*). The ~50-kD bovine protein corresponds to a known protein, RbAp48 (*11*), that consists of seven WD repeat domains (where W is Trp and D is Asp) (*12*). Originally identified as a protein that binds to the retinoblastoma gene product (pRb), RbAp48 may constitute an adaptor subunit that targets the histone deacetylase to specific chromatin domains.

The ~46-kD bovine protein is related to the protein encoded by the yeast *RPD3* gene, which has been implicated by several genetic screens as a transcriptional regulator but whose biochemical function is unknown (*13*). Partial complementary DNA (cDNA) sequences for the human gene were identified in the expressed sequence tag database (dbEST) and were used to design polymerase chain reaction (PCR) primers (*14*). Screening a Jurkat T cell cDNA library with the resultant 1-kb PCR product allowed for the isolation of several positive clones, one of which was fully sequenced and found to contain a putative full-length open reading frame (Fig. 3A)

**A**

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  1  MAQTQGTRRK VCYYYDGDVG NYYYGQGHPM KPHRIRMTHN LLLNYGLYRK MEIYRPHKAN AEEMTKYHSD
 71  DYIKFLRSIR PDNMSEYSKQ MQRFNVGEDC PVFDGLFEFC QLSTGGSVAS AVKLNKQQTD IAVNWAGGLH
141  HAKKSEASGF CYVNDIVLAI LELLKYHQRV LYIDIDIHHG DGVEEAFYTT DRVMTVSFHK YGEYFPGTGD
211  LRDIGAGKGK YYAVNYPLRD GIDDESYEAI FKPVMSKVME MFQPSAVVLQ CGSDSLSGDR LGCFNLTIKG
281  HAKCVEFVKS FNLPMLMLGG GGYTIRNVAR CWTYETAVAL DTEIPNELPY NDYFEYFGPD FKLHISPSNM
351  TNQNTNEYLE KIKQRLFENL RMLPHAPGVQ MQAIPEDAIP EESGDEDEDD PDKRISICSS DKRIACEEEF
421  SDSEEEGEGG RKNSSNFKKA KRVKTEDEKE KDPEEKKEVT EEEKTKEEKP EAKGVKEEVK LA
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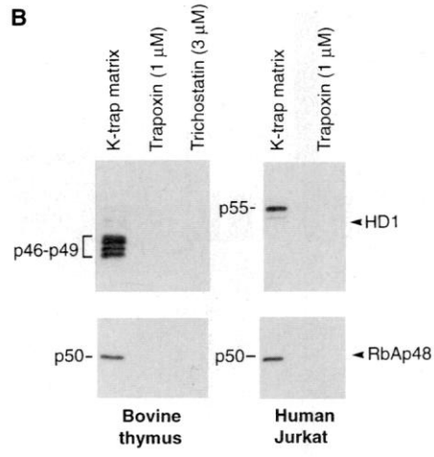


**Fig. 3.** Molecular characterization of the trapoxin receptor HD1, a nuclear histone deacetylase. (**A**) Predicted amino acid sequence of human HD1. An in-frame stop codon was found upstream of the starting methionine. Regions equivalent to microsequenced tryptic peptides from the purified bovine protein are boxed. Underlined amino acids 319 to 334 and 467 to 482 denote the sequences of synthetic peptides that were conjugated to keyhole limpet hemocyanin and used to generate polyclonal antisera. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**B**) Protein immunoblot analogous to the silver-stained gel in Fig. 2B, showing the relation between bovine p46 to p49 and human p55 (top) and confirming the identity of p50 (bovine and human) as RbAp48 (bottom). Proteins eluted from the K-trap affinity matrix (Fig. 2) were separated by SDS-PAGE and transferred to Immobilon-P (Millipore). Blots were probed with polyclonal antibody to HD1 (residues 319 to 336; top panels) or monoclonal antibody to RbAp48 (bottom panels), and bound antibodies were detected with enhanced chemiluminescence (Amersham).

(GenBank accession number U50079). The peptide sequences obtained from the purified bovine protein align with 100% identity to sequences deduced from this coding region (Fig. 3A). We call this human protein HD1 (for histone deacetylase), and its predicted size of 55 kD agrees well with the estimated size of p55 isolated from Jurkat nuclear extracts with the use of the K-trap affinity matrix (Fig. 2B). A dbEST search indicated the existence of at least two other related human genes.

To determine the relation between the proteins from bovine thymus (p46 to p50) and the proteins isolated from human Jurkat T cells (p50 and p55), we generated an antiserum to a peptide specified by the HD1 open reading frame (Fig. 3A, amino acids 319 to 334). Immunoblot analysis of the bovine proteins p46 to p49 and the human protein p55 showed that they all react with the antiserum and provides additional evidence that these bands correspond to bovine and human HD1 (Fig. 3B). A monoclonal antibody that specifically recognizes RbAp48 was used to confirm the identity of bovine and human p50. Importantly, neither HD1 nor RbAp48 was detected when the affinity purification was done in the presence of trapoxin or trichostatin (Fig. 3B).

We used affinity-purified antibodies directed against a COOH-terminal peptide (amino acids 467 to 482) to immunoprecipitate HD1 from crude nuclear extracts. The immunoprecipitates contained histone deacetylase activity that was inhibited by both trapoxin and trichostatin (Fig. 4A). Consistent with the idea that HD1 and RbAp48 form a complex in vivo, the two proteins coprecipitated with the HD1 antibodies (Fig. 4B). Neither HD1, RbAp48, nor the associated histone deacetylase activity was immunoprecipitated in the presence of the HD1 COOH-terminal peptide (Fig. 4, A and B) (*15*). HD1, like RbAp48 (*11*), is detected predominantly in the nucleus by immunostaining with the aforementioned antibodies (*8*). Given that HD1 and RbAp48 are the major proteins eluted from the K-trap matrix (Figs. 2B and 3B), it is likely that they interact directly with one another.

We extended the results obtained with the endogenous protein by expressing recombinant FLAG epitope–tagged HD1 (HD1-F) in Jurkat T cells. FLAG antibody immunoprecipitates from cells transfected with pBJ5–HD1-F contained histone deacetylase activity that was sensitive to both trapoxin and trichostatin (Fig. 4C). Histone deacetylase activity was not precipitated when the antibody was blocked with excess FLAG peptide (*15*). Interestingly, endogenous RbAp48 did not coprecipitate with overexpressed HD1-F (*8*), which demonstrates that RbAp48 is not required for either histone deacetylase or trapoxin binding activity. This result is consistent with the idea that RbAp48 serves a targeting rather than an enzymatic function. Finally, lysates from cells transfected with pBJ5–HD1-F were incubated with the K-trap affinity matrix in the presence or absence of trapoxin and trichostatin. Protein immunoblot analysis demonstrated an interaction between recombinant HD1-F and the K-trap affinity matrix that was prevented by nanomolar concentrations of trapoxin or trichostatin (Fig. 4D).

HD1 is 60% identical to the protein encoded by the yeast *RPD3* gene, which was isolated in four independent mutant suppressor screens designed to identify transcriptional repressors (*13*, *16*–*19*). No biochemical function for the yeast protein has yet been postulated. A negative regulator of the *TRK2* gene, *RPD3* is necessary for the transcriptional repression of several genes whose expression is regulated according to specific environmental conditions. Loss of *RPD3* also leads to decreased transcriptional activation of certain genes, but this effect may be indirect (*13*, *17*). Although *RPD3* has yet to be implicated in silencing at telomeres or at the mating loci, the fact that silencing is eliminated by point mutations in specific lysine residues near the $NH_2$-terminus of histones H3 and H4 suggests that lysine deacetylation may contribute to the maintenance of silenced chromatin (*20*–*23*). Indeed, silencing at telomeres and at the mating loci has been correlated with the presence of hypoacetylated histones, and *sir* mutants that are defective in silencing show a corresponding increase in the extent of histone acetylation at these loci (*24*). The Sir3p and Sir4p proteins have been shown to interact with a bacterially expressed histone H4 $NH_2$-terminal domain in vitro (*25*), and it is possible that deacetylation of one or more lysine residues is required for this interaction in vivo. Our results further support a role for histone deacetylase as a transcriptional regulator and establish a biochemical connection to the genetic studies that originally characterized *RPD3*.

How does inhibition of histone deacetylase in mammalian cells lead to $G_1$ and $G_2$ phase cell cycle arrest? One possibility is that specific cell cycle regulatory proteins such as the cyclin-dependent kinase inhibitors are transcriptionally up-regulated in response to histone deacetylase inactivation. Alternatively, cell cycle checkpoints

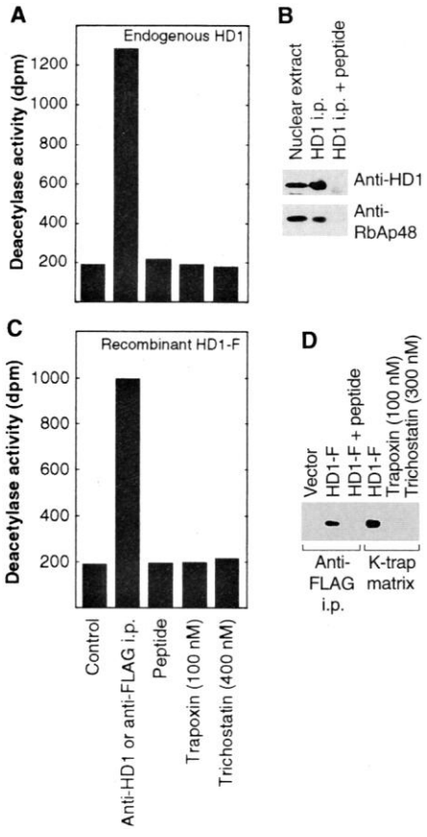


**Fig. 4**. Association of histone deacetylase activity with endogenous and recombinant HD1. (**A**) Immunoprecipitation (i.p.) of endogenous histone deacetylase activity with affinity-purified antibody to HD1 (anti-HD1) (residues 467 to 482). Anti-HD1(residues 467 to 482) immunoprecipitates from equivalent amounts of Jurkat nuclear extract (1 mg of nuclear protein supplemented with 0.5 M NaCl, 1% bovine serum albumin, and 0.1% NP-40) were isolated in the presence or absence of HD1 (residues 467 to 482) peptide competitor. After resuspending the immunoprecipitates in HD buffer [20 mM tris (pH 8), 150 mM NaCl, and 10% glycerol], we added inhibitors as indicated, and histone deacetylase activity was measured as described in Fig. 1. The inherent background of the assay, without added protein, is also shown (Control). (**B**) Coprecipitation of HD1 and RbAp48, as detected by protein immunoblot analysis. (**C**) Histone deacetylase activity of recombinant HD1-F. Tag Jurkat cells (*26*) were transfected with pBJ5 (Control) or pBJ5–HD1-F (encoding COOH-terminal FLAG epitope–tagged HD1) by electroporation, and detergent lysates were prepared [0.5% Triton X-100, 50 mM tris (pH 8), 100 mM NaCl, and 10% glycerol]. FLAG antibodies (anti-FLAG) conjugated to agarose beads (IBI) were added to lysates in the presence or absence of FLAG peptide competitor, and histone deacetylase activity was measured as described above. Deacetylase activity was undetectable in immunoprecipitates from cells transfected with vector alone (Control). (**D**) Interaction between recombinant HD1-F and the K-trap affinity matrix. Lysates from Jurkat cells transfected with pBJ5–HD1-F were incubated with the K-trap affinity matrix in the presence or absence of inhibitors. Proteins from anti-FLAG immunoprecipitates (Fig. 4C) and the K-trap affinity matrix were blotted and probed with the M2 monoclonal antibody (IBI).

may exist that monitor histone acetylation or higher order chromatin structure. It should now be possible to study the regulation of histone deacetylase during the cell cycle, its substrate specificity, and the mechanism by which it is targeted to specific regions of the genome.

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7. All procedures were done at 4°C. Thymocytes (~12 g) prepared from fresh bovine thymus were homogenized in hypotonic lysis buffer [20 mM tris (pH 7.8), 20 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 10 μg/ml each of pepstatin, aprotinin, and leupeptin] by mechanical disruption, and the nuclei were isolated by centrifugation at 3000*g*. Nuclei were resuspended in lysis buffer, and the proteins were extracted with 0.4 M ammonium sulfate. The viscous lysate was sonicated and clarified by centrifugation at 100,000*g* for 1 hour. Proteins were then precipitated with 90% saturated ammonium sulfate and recovered by centrifugation (100,000*g* for 1 hour). After thorough dialysis against Q buffer (25 mM tris, pH 8, 10 mM $NH_4Cl$, 0.25 mM EDTA, and 10% glycerol), a portion of the nuclear proteins (~12 mg of total protein) was loaded onto an HR 10/10 MonoQ column (Pharmacia). The column was washed with 25 ml of Q buffer and eluted with a 50-ml linear gradient of 10 to 500 mM $NH_4Cl$. The column was further washed with 25 ml of 500 mM $NH_4Cl$ and 25 ml of 1 M $NH_4Cl$. Fractions (2.5 ml) were then analyzed for trapoxin binding and histone deacetylase activities or further purified with the K-trap affinity matrix.
8. J. Taunton, C. A. Hassig, S. L. Schreiber, unpublished results.
9. In vitro binding experiments with soluble [$^3$H]trapoxin indicated that the radiolabel is released into solution after protein denaturation with SDS or guanidinium hydrochloride. Thus, trapoxin binding proteins were expected to elute from the affinity matrix with SDS (J. Taunton, C. A. Hassig, S. L. Schreiber, unpublished observations).
10. To obtain enough trapoxin binding protein for microsequencing, we prepared nuclear ammonium sulfate pellets from 15 bovine thymuses as described (*7*). Sedimented proteins were resuspended in and dialyzed against buffer A [20 mM bis-tris (pH 7.2), 20 mM NaCl, and 10% glycerol] for 12 hours and brought to pH 5.8 by dialyzing against buffer A (pH 5.8) for 30 min. After centrifugation, the dialysate (~650 mg of protein) was loaded onto a Q Sepharose FF column (2.6 cm by 10 cm; Pharmacia), and the column was washed with 120 ml of buffer A (pH 5.8). Proteins were eluted with a 400-ml linear gradient of 20 to 600 mM NaCl in buffer A. Fractions (10 ml; each fraction contained 1 ml of 1 M tris, pH 8, to neutralize the acidic buffer A) were assayed for trapoxin binding activity. Tween-20 was added to active fractions at a final concentration of 0.05%, and these fractions were incubated with K-trap affinity matrix for 16 hours (25 μl per milliliter of Q fraction). After the matrix was washed three times with phosphate-buffered saline, bound proteins were eluted by boiling in 40 μl of SDS sample buffer per 25 μl of matrix. SDS eluates were combined and the proteins resolved by SDS-(PAGE) (12% gel) and transferred to polyvinylidene difluoride membrane (Bio-Rad). Staining with Ponceau S revealed two major bands (46 and 50 kD). The excised bands were proteolytically digested, and the peptide fragments purified by high-performance liquid chromatography were sequenced at the Harvard Microchemistry Facility.
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14. After noting sequence similarity between peptides derived from the purified bovine trapoxin binding protein and yeast Rpd3p, we checked the dbEST to see whether any partial sequences for the human homolog had been reported. Two ESTs (GenBank accession numbers D31480 and F07807) were identified whose predicted translation products aligned, with sequences similar to the $NH_2$- and COOH-terminal regions of Rpd3p, respectively. PCR primers were designed on the basis of these tags, and a 1-kb PCR product was obtained from a Jurkat cDNA library (Stratagene). A $^{32}$P-labeled probe prepared by random priming was used to screen the Jurkat library, and 10 positive clones were isolated.
15. Control experiments indicated that competitor peptides had no effect on histone deacetylase activity per se (J. Taunton, C. A. Hassig, S. L. Schreiber, unpublished observations).
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27. We thank J. L. Collins for preparing [$^3$H]trapoxin; W. S. Lane and colleagues for unparalleled expertise in peptide microsequencing; J. A. Simon for help with molecular biology; K. L. Morrison and L. F. Snow for help with polyclonal antibodies; and Y.-W. Qian and E. Y.-H. Lee for the RbAp48 monoclonal antibody. J.T. extends his gratitude to NSF and Eli Lilly and Co. for predoctoral fellowships. C.A.H. is supported by an NIH predoctoral training grant. S.L.S. is an investigator at the Howard Hughes Medical Institute.

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# Regulation of an Early Developmental Checkpoint in the B Cell Pathway by Igβ


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Many of the cell fate decisions in precursor B cells and more mature B cells are controlled by membrane immunoglobulin (Ig) M heavy chain (mμ) and the Igα-Igβ signal transducers. The role of Igβ in regulating early B cell development was examined in mice that lack Igβ ($Ig\beta^{-/-}$). These mice had a complete block in B cell development at the immature $CD43^+B220^+$ stage. Immunoglobulin heavy chain diversity ($D_H$) and joining ($J_H$) segments rearranged, but variable ($V_H$) to $DJ_H$ recombination and immunoglobulin messenger RNA expression were compromised. These experiments define an unexpected, early requirement for Igβ to produce B cells that can complete $VDJ_H$ recombination.


Membrane immunoglobulins are essential regulatory components in both developing and mature B cells (*1*). Specific events that are controlled by mμ in developing B cells include the precursor B cell (pre–B cell) transition (*2*), allelic exclusion (*3*), receptor editing (*4*), and deletion of lymphocytes that express self-reactive immunoglobulins (*5*). The earliest of these events, allelic exclusion and the pre–B cell transition, are induced by mμ through the mμ-associated Igα-Igβ signal-transducing proteins (*6*). In more mature B cells, the same signal transducers mediate B cell activation by triggering Src and Syk family tyrosine kinases (*7*). All of these mμ-induced cellular responses are thought to be mechanistically related because they share a requirement for phosphorylation of the tyrosine residues in the antigen receptor activation motifs (ARAMs) of the Igα-Igβ complex (*8*).


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Less is known about the regulation of portions of the B cell pathway that occurs before Ig gene rearrangement. A receptor complex composed of surrogate heavy and light chains, possibly associated with the Igα-Igβ signal transducers, has been proposed as a regulator of these earlier stages of the B cell pathway (*1*, *9*). Support for this hypothesis comes from the observation that Igα and Igβ are expressed when Ig genes are still in the germline configuration (*2*, *10*).

To determine whether the Igα-Igβ complex regulates the early stages of B cell development, we produced a targeted mutation in the mouse Igβ gene (*11*) (Fig. 1). Deletion of the promoter as well as of the first and part of the second coding exons of Igβ resulted in mice that did not express Igβ mRNA (Fig. 2). Northern (RNA) blots with an Igβ complementary DNA (cDNA) probe failed to detect Igβ RNA extracted from bone marrow of $Ig\beta^{-/-}$ mice, whereas a high-intensity signal was present in both wild-type and $RAG\text{-}1^{-/-}$ control RNA samples (Fig. 2). In contrast to Igβ, expres-