(1982)] and their absence may reflect chance. The planktonic larvae of sponges are short-lived and may survive only short voyages.
11. True species diversity in these samples is underestimated because larval and postlarval forms of many species are morphologically indistinguishable. Also, animal, plant, and protist taxa smaller than 80 μm (the size of the plankton net mesh) were not efficiently retained.
12. We estimate (assuming 20 to 30 taxa per vessel, and several thousand ships out of a world fleet of 35,000 with ballast water at sea at any given time) that, on any one day, several thousand species may be in motion in ballast water "conveyor belts" around the world. Therefore, comparing known invasions (Table 2) with any particular ballast sample may rarely reveal the same taxa, underscoring the importance of recognizing this phenomenon at a supraspecific level.
13. Densities of plankton were estimated by mixing and splitting each replicate, counting the organisms, and multiplying by the volume of water sampled per replicate (*9*).
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16. Organisms with a short larval phase can be entrained in ballast water and then settle. We found metamorphosed ascidians (1 to 2 mm) settled on floating wood chips, and, in five ships that had completed voyages of 13 to 16 days, we found unattached ascidian tadpoles but no adults. Although we found few fish in our samples, more than 20 ship's captains in the Great Lakes, and on the U.S. Atlantic, Gulf, and Pacific coasts, have reported to us live fish in ballast water tanks.
17. Freshwater ballast transferred to other freshwater endpoints (such as from Europe to the Laurentian Great Lakes, or vice versa) may transfer encysted stages of many taxa (such as sponge gemmules and bryozoan statoblasts). Such stages remaining in ship's ballast sediments after open ocean exchange may resist saltwater immersion.
18. The recent increase in invasions caused by ballast water may be due to a variety of factors, including increases in the size, number, and speed of ships (*4, 5*).
19. We expect that several of the six species of Asian copepods now recognized on the Pacific coast of North America will also be found in eastern Australia, a region that receives large volumes of ballast water from the same sources in Japan and China as does the North American Pacific coast.
20. Over 50 examples are now known where introduced species were mistakenly described as "new" species, some several times from different places around the world (J. T. Carlton, in *San Francisco Bay: The Urbanized Estuary*, T. J. Conomos, Ed. (American Association for the Advancement of Science, Washington, DC, 1979), pp. 427–444; J. W. Chapman and J. T. Carlton, *J. Crustacean Biol.* **11**, 386 (1991); J. T. Carlton, unpublished results.
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27. Recent changes in policy have reflected a recognition of the damage caused by introductions mediated through ballast water. Federal regulations to control the discharge of ballast water into the Great Lakes, Australia, and New Zealand are in effect and national studies are under way in Australia, Canada, and the United States on control mechanisms to reduce the number of living specimens arriving in port-of-origin ballast water and sediments. The United Nations International Maritime Organization has ratified international protocols for ballast control.
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Regulation of Lymphoid-Specific Immunoglobulin μ Heavy Chain Gene Enhancer by ETS-Domain Proteins

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The enhancer for the immunoglobulin μ heavy chain gene (*IgH*) activates a heterologous gene at the pre-B cell stage of B lymphocyte differentiation. A lymphoid-specific element, μB, is necessary for enhancer function in pre-B cells. A μB binding protein is encoded by the *PU.1/Spi-1* proto-oncogene. Another sequence element, μA, was identified in the μ enhancer that binds the product of the *ets*-1 proto-oncogene. The μA motif was required for μB-dependent enhancer activity, which suggests that a minimal B cell–specific enhancer is composed of both the PU.1 and Ets-1 binding sites. Co-expression of both PU.1 and Ets-1 in nonlymphoid cells trans-activated reporter plasmids that contained the minimal μ enhancer. These results implicate two members of the Ets family in the activation of *IgH* gene expression.

Transcription of the immunoglobulin (Ig) heavy chain gene (*IgH*) is activated at the pre-B cell stage of B cell differentiation. The μ enhancer (μE), residing in the *IgH* gene, is a lymphocyte-specific regulatory element (*1, 2*) that enhances transcription in transfection assays but is also sufficient to activate a heterologous gene in the pre-B cells of transgenic mice (*3–7*). Thus, the μ enhancer may regulate *IgH* locus activation during B cell ontogeny, perhaps by inducing sterile μ transcripts that may be required for the initiation of gene rearrangements (*8–10*).

The μ enhancer is a composite of multiple positive- and negative-acting sequence

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elements that function by binding transacting nuclear factors (*1, 2*). Proteins that bind to the enhancer can be ubiquitous factors, such as the E motif–binding proteins, or lymphoid-specific ones, such as the octamer and μB binding proteins. Mutational and transfection analysis has indicated that most of these protein binding sites contribute to enhancer function. However, the extent of the contribution of the ubiquitous and lymphoid-specific factors to the tissue- and developmental stage–specific activation of this enhancer is unclear. In contrast to other reports (*11*), the basic helix-loop-helix (bHLH) proteins that bind the E sites have been implicated in tissue-specific control (*12, 13*). We identify and characterize here lymphoid-specific factors that may affect enhancer activation during B cell ontogeny.

The nine–base pair (bp) μB element is a lymphoid-specific component of the μ enhancer (*14, 15*). Although either μB or octamer elements are sufficient in transfection assays for enhancer activity in plasma cell lines, both elements are necessary for optimal enhancer function in pre-B cell lines (*14*). Furthermore, μB multimers are insufficient to activate a minimal promoter, which suggests that other proximal elements are necessary for μB-dependent enhancer activity (*14*). To further characterize the role of the μB site in enhancer function, we found that PU.1/Spi-1, a proto-oncogene (*16*) product, is a μB-binding protein and determined that, in addition to μB, a sequence element that we term μA is necessary to generate a minimal B cell–specific enhancer.

Two criteria were established to identify proteins that might mediate B cell–specific transcription through the μB site. (i) A good correlation was expected between enhancer activity and the ability of a factor to bind (or not bind) to a wild-type (WT) μB element and to a panel of mutations that functionally define the μB element. (ii) The functional μB binding protein would be expressed in pre-B cells, where μB is necessary for enhancer function. The μB element, identified by mutational analysis, contains nine nucleotides with the sequence TTTGGGGAA (*14*). The purine-rich character of this sequence was suggestive of the ETS-domain proteins that have been proposed to require a minimal GGAA recognition sequence (*17*) for DNA binding. Whereas Ets-1 did not protect the μB site in a deoxyribonuclease I footprint assay, the ETS-domain protein PU.1 (*16*) generated a specific nucleoprotein complex in an electrophoretic mobility-shift assay (Fig. 1A). The complex formed was specific to the μB site because excess nonradioactive μB sequences successfully competed with the radiolabeled μB, whereas a fragment containing the μE3 motif did not (Fig. 1A). The PU.1 binding site from the simian virus 40 (SV40) enhancer was also an efficient competitor (Fig. 1A). When a 9-bp oligonucleotide that encompassed the μB element cloned into the polylinker of pSP72 was used as a probe, identical binding and competition patterns were seen. The amount of nonradioactive DNA needed to compete PU.1 binding to μB indicated that the μB site had a lower affinity for PU.1 protein than did the SV40 PU.1 element. Thus, PU.1 protein interacts specifically with the μB element of the murine μ enhancer.

We investigated if PU.1 protein binding to μB mutants in vitro correlated with transcriptional activity of previously characterized μB mutations in vivo. Clustered mutations throughout the μB element that change either the first three or the last three nucleotides (mutations M102 and M104, respectively) resulted in little or no enhancer activity, whereas the internal 3-bp alteration (M103) had no discernible effect (*14*). A functional μB binding protein was predicted to bind to M103 but not to M102 and M104. To study PU.1 protein binding to these altered sequences, we radiolabeled DNA fragments that contained mutants M102 to 104 and used them in binding assays in vitro (Fig. 1B). Whereas the WT and the M103 mutant fragments bound PU.1, no complex formation was seen with either mutant M102 or M104. The correlation between the pattern of PU.1 binding in vitro and the in vivo activity of these clustered μB mutations suggests that the factor encoded by the *PU.1/Spi-1* gene may be the protein necessary for μB element activity in vivo.

To see if PU.1 satisfied our second criterion for a functional μB binding protein, we analyzed PU.1 mRNA expression. Initial Northern (RNA) analysis indicated that PU.1 mRNA expression was restricted to lymphoid and macrophage lineages (*16*), consistent with the lymphoid specificity of the μB element. To assess whether PU.1 mRNA was expressed at the pre-B cell stage, we examined PU.1 mRNA expression in a panel of cells that represented

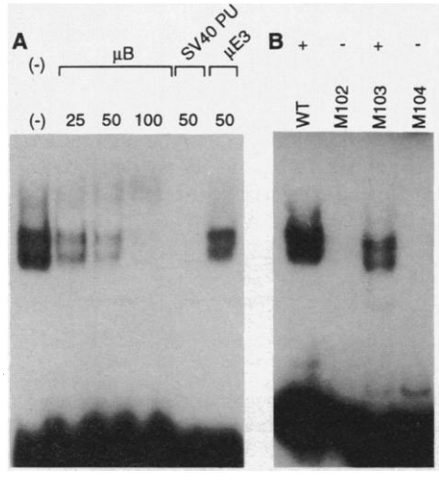

Fig. 1. In vitro binding analysis of the PU.1 protein to the μB element of the murine μ enhancer. (**A**) In vitro competition assays. Electrophoretic mobility-shift analysis was performed as described (*28*) with a 53-bp radiolabeled enhancer fragment [nucleotides 380 to 433, according to Ephrussi and co-workers (*29*)], bacterial extracts (Ly 25.1) (*16*) containing the PU.1 protein, and competitor DNAs as indicated. The amounts of competitor DNA are indicated in nanograms above the lanes. We obtained the competitor DNA by annealing coding and noncoding strands of synthetic oligonucleotides to create the double-stranded sequence element. The coding-strand sequences of the oligonucleotides we used to create each competitor element were as follows: μB, 5′-TCGACAAGGCTATTTGGGGAAG-3′; SV40 PU.1, 5′-GATCCCTCTGAAAGAGGAACTTGG-3′; and μE3, 5′-TCGACAGGTCATGTGGCAAGGC-3′. (**B**) PU.1 protein binding to WT and mutant μB elements. Radioactive DNA that contained mutations M102 to M104 was generated from plasmids M102, M103, and M104 as described (*14*). In vitro binding was carried out with 1 μl of Ly 25.1 (*16*) extract. Effects of the mutations on the activity of a μB-dependent μ enhancer are indicated above each lane; plus signifies WT enhancer activity, and minus signifies loss of enhancer activity.

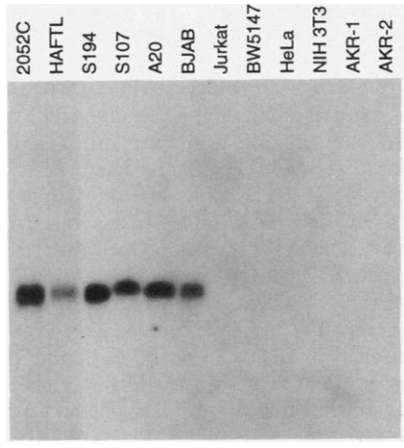

Fig. 2. The expression of PU.1 mRNA in lymphoid and nonlymphoid cells. Northern analysis was performed with 20 μg of total RNA from 2052C and HAFTL cells and 30 μg of total RNA from all other cell lines. RNA was fractionated by electrophoresis through formaldehyde-containing agarose gels (1%), transferred to Nytran, and probed with a 400-bp Eco RI–Nco I fragment of the *PU.1* gene (*16*). Characteristics of the cell lines are as follows: 2052C, pro-T; HAFTL, pre-B; S194 and S107, murine plasma; A20, murine B; BJAB, human B; Jurkat, human T; BW5147, murine T; HeLa and NIH 3T3, human and murine nonlymphoid; and AKR-1 and AKR-2, murine T.

different stages of lymphoid development. No PU.1 mRNA expression was evident in T cell lines or nonlymphoid cell lines, but PU.1 mRNA was present in all B cell lines examined (Fig. 2). PU.1 mRNA was observed in the earliest pre-B cell line examined, HAFTL, as well as in 2052C, a pro-T cell line (Fig. 2). Thus, the expression pattern of PU.1 mRNA is consistent with its potential participation in μB-dependent enhancer activity in pre-B cells and in the early activation of the *IgH* gene during B cell development.

We had already shown that whereas μB multimers do not activate transcription from a minimal promoter, a 53-bp fragment that contains the μE2, μE3, and μB elements does. These results suggested that μB requires additional elements to generate enhancer activity (*14*). To identify the minimal components of a μB-dependent enhancer, we assayed a 70-bp fragment that contained only μE3 and μB (termed μ70) for transcriptional activity. Monomers and dimers of this fragment were cloned into the reporter plasmid Δ56fosCAT and tested for activity by transfection into S194 (murine plasma) cells. Because a monomer of the μ70 fragment activated transcription only 3.5-fold, whereas dimerization of this fragment activated transcription 14.4-fold over the enhancerless plasmid (Fig. 3), we used dimers in the experiments reported here in order to amplify small differences in activity. These experiments showed that the μE2 element was dispensable for μB-dependent activity, which suggests that the μE3 element functioned synergistically with μB. To test this possibility, we assayed μ70 dimers mutated at either the μB or the μE3 sites for enhancer function. The μB mutation ($\mu 70_2\mu B^-$) abolished activity (Fig. 3), which confirms that transcriptional activation was completely dependent on this element. Mutation of the μE3 ($\mu 70_2\mu E3^-$) site reduced activity 75% (Fig. 3). This residual enhancer activity was more than would have been expected if the activity was due solely to the remaining μB element and suggested that a third element in μ70, distinct from μE3, cooperates with the μB element. The possible location of this element came from DNase I footprint analysis of the μ enhancer performed with bacterially expressed Ets-1 (*18*). Although this factor did not bind to the μB element, a distinct region was protected 5′ to μE3, corresponding to the sequence GCAGGAAGCA (Fig. 4A). We refer to this site, which lies in the μ70 fragment, as μA. Electrophoretic mobility-shift assays confirmed Ets-1 binding to this fragment (Fig. 4B). Mutation of the GGA sequence in the μA site abolished Ets-1 binding, whereas mutations in the μE3 or μB elements had no effect (Fig. 4B). Because the μB element is unchanged in the μA mutant fragment, these results further suggest that Ets-1 does not bind efficiently to the μB site.

To check if the Ets-1 binding site was essential for μB-dependent enhancer activity, we analyzed a μ70 fragment mutated at this site by transfection assays in S194 cells. In contrast to the μE3 mutation that retained 25% of the activity relative to a WT μ70 dimer, mutation of the GGA sequences in μA ($\mu 70_2\mu A^-$) decreased the activity of μ70 dimers to background amounts (Fig. 5). Thus, both μB and μA sites were essential for μ70 enhancer activity, whereas the μE3 element contributed in a quantitative manner to raise the amount of transcription.

To further confirm the involvement of the μA motif, we analyzed a larger enhancer fragment (μ170) (*14*) that contained the μE1, μE2, μE3, μE5, μB, and μA elements, whose activity is dependent on the μB motif (*14*). Mutation of the μE3 motif in μ170 ($\mu 170\mu E3^-$) decreased, but did not abolish, enhancer activity (Fig. 5). The decrease in activity was only about 50%, presumably because loss of the μE3 site was functionally compensated for by the other E motifs present in this fragment. Mutation of the μA site ($\mu 170\mu A^-$), however, like mutation of the μB site ($\mu 170\mu B^-$), reduced activity to background levels, which suggests that this site was critical for enhancer function. Thus, the μA and μB elements together define an enhancer domain whose activity is increased in proportion to the number of proximal E elements.

To directly test if Ets-1 activates the μ enhancer, we co-transfected μ enhancer–dependent reporter plasmids with an Ets-1 expression plasmid into S194 murine plasma cells. A fivefold increase in the activity of μ70 dimers was observed when the Ets-1 expression plasmid was included in the assay instead of a control plasmid (Fig. 6A). Furthermore, trans-activation required an intact μA site, and no activity was seen when the corresponding μA site mutant enhancer was used (*19*). These results provide evidence that the product of the *ets*-1 proto-oncogene can bind and activate the Ig μ enhancer through the μA element.

Characterization of μ70 dimer function in S194 (B) cells showed that both μA and μB sites are necessary to activate transcription (Fig. 5). Trans-activation of this fragment by Ets-1 in S194 cells (Fig. 6A)

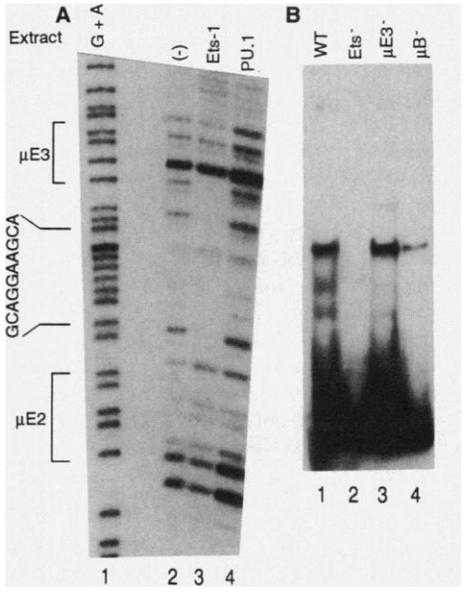

Fig. 4. Identification of an Ets-1 binding site in the μ enhancer. (**A**) Deoxyribonuclease I footprinting analysis of the μ enhancer with Ets-1 protein. Radioactively labeled (30,000 cpm) μ enhancer DNA (coding strand) was incubated with the extract indicated and subjected to DNase I treatment as described (*14*), except that no polydIdC was included. Lane 1, G + A ladder; lane 2, no protein; lane 3, bacterially expressed Ets-1 (*18*), and lane 4, PU.1 (*16*). Brackets show the location of the μE2 and μE3 elements. The sequence shown identifies the region protected by Ets-1. (**B**) Binding analysis in vitro of Ets-1 to enhancer DNA. Radioactively labeled probes (nucleotides 380 to 433) were incubated with bacterially expressed Ets-1 (*18*) and then subjected to electrophoretic mobility-shift assays. The binding analyses and electrophoresis were performed at 4°C. The probes used were either WT or contained a mutation in the Ets-1 binding site μA (lane 2), the μE3 site (lane 3), or the μB site (lane 4), as indicated above the lanes.

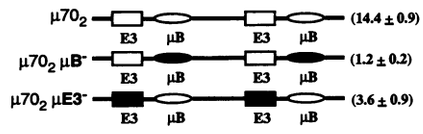

Fig. 3. Contribution of μB and μE3 to μ70 enhancer activity. We performed transfection analysis of WT ($\mu 70_2$), μB mutant ($\mu 70_2\mu B^-$), and μE3 mutant ($\mu 70_2\mu E3^-$) CAT reporter plasmids by transfecting 1×10^7 S194 cells with 5 μg of DNA with the DEAE-dextran protocol and then performed CAT assays as described (*4*). Relative CAT activity is expressed as the increase relative to the enhancerless plasmid Δ56fosCAT, whose background activity equals 1. The results represent at least two sets of transfections performed in duplicate ± SD. Boxes and ovals represent sequence elements in the μ enhancer; darkened elements indicate those mutated. We used site-directed mutagenesis (*14*) to create mutations in the μB element (TTTGGGGAA → CCCGGGGAA) or in the μE3 element (CATGTGG → CATGCAT) in the μ enhancer. From these mutations, the 70-bp Alu I–Alu I enhancer fragments (nucleotides 385 to 456) were obtained, and we inserted them as dimers into the reporter plasmid Δ56fosCAT (*30*) at the Sal I site to create WT ($\mu 70_2$), μB mutant ($\mu 70_2\mu B^-$), and μE3 mutant ($\mu 70_2\mu E3^-$) CAT reporter plasmids.

supports the idea that Ets-1 is a functional μA binding protein. To provide further evidence that PU.1 is a functional μB binding protein, we co-transfected μ70-dependent reporter plasmids with Ets-1 and PU.1 expression vectors into COS (nonlymphoid) cells. In these experiments, the amounts of reporter plasmid and total DNA were held constant, and the amounts of Ets-1 and PU.1 expression plasmids were varied (Fig. 6B). Neither PU.1 nor Ets-1 individually were able to trans-activate efficiently (Fig. 6B). However, co-expression of both Ets-1 and PU.1 led to dose-dependent, synergistic transcriptional activation. In the presence of 0.5 μg or 2 μg of each expression plasmid, we observed a 6- or 21-fold increase in transcription from the μ70 dimer chloramphenicol acetyltransferase (CAT) construct (Fig. 6B). To confirm that transactivation was dependent on the μA and μB elements in μ70, we used plasmids mutated at these sites in co-transfection assays. These mutations reduced transcriptional activity observed in the presence of Ets-1 and PU.1 (Fig. 6B, last two columns), thereby mimicking closely the pattern observed in S194 (B) cells. We conclude that in COS cells, Ets-1 and PU.1 together can complement the absence of lymphocyte-specific factors that are necessary for activity of the B cell–specific core μ enhancer.

Analysis of Ets-1 mRNA in lymphoid cell lines demonstrated that the expression of the *ets*-1 gene is consistent with a role for Ets-1 in the developmental activation of the μ enhancer (Table 1). Other work has shown that Ets-1 transcripts are present in spleen and thymus cells, which suggests that the gene is expressed in both B and T lymphocytes (*20*). We found varying amounts of Ets-1 RNA in both B and T cell lines, including the pre-B cell line HAFTL, but not in HeLa cells or the pro-T line 2052C (Table 1). The *PU.1* and *ets*-1 genes have overlapping, yet distinct, expression patterns in the lymphoid lineages (Table 1), and *IgH* gene expression coincides largely with that subset of lymphocytes where both genes are expressed.

The Ig μ enhancer is a complex regulatory element that contains binding sites for many different DNA binding proteins. However, mutations at most of these sites do not significantly affect enhancer activity, which suggests functional redundancy. On the basis of several observations, we propose that a critical enhancer domain is composed of the μA and μB sites. First, a μE3-mutated μ70 fragment (containing only the μA and μB elements) still retains enhancer activity. Second, although the number of E elements increases the overall enhancer activity, mutation of either μB or μA sites abolishes activity. Thus, the E motifs may contribute in an additive manner. Third, μB and μA motifs are conserved in the human and murine enhancers, whereas the intervening μE3 element is unrecognizable at the corresponding loca-

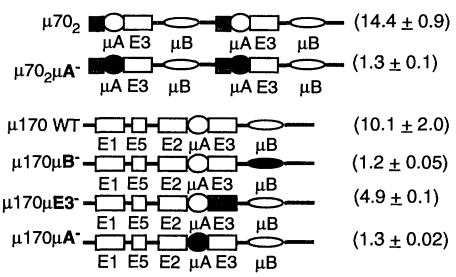

Fig. 5. Functional analysis of the murine μ enhancer μA element (Ets-1 binding site) in S194 plasma cells. The contribution of μA to the activity of μ70 dimers and a μB-dependent enhancer (μ170) was analyzed by transient transfections into S194 cells as described (Fig. 3). The activity of μ70$_2$ with a mutant Ets-1 binding site (μ70$_2$μA$^-$), as compared with WT μ70$_2$, is indicated in the top two lines. The μ70$_2$μA$^-$ plasmid was generated as described (Fig. 3) with △56fosCAT. The μ170 reporter plasmids were generated by the insertion of the 170-bp Hinf I–Dde I fragment (nucleotides 345 to 518) from WT and mutant enhancers into the pSPCAT vector as described (*14*). Various mutations in the larger enhancer fragment are indicated as μ170μB$^-$, μ170μE3$^-$, and μ170μA$^-$ and are compared with the activity of the μ170 WT enhancer. The μ170μA$^-$ mutation contains the clustered mutation GCAGGAAGCA → GCATCGAGCA in the μA element. Transfection analysis was performed as described (Fig. 3), and activity is expressed relative to that of the enhancerless vector. Results shown are the average ± SD of three independent experiments conducted in duplicate.

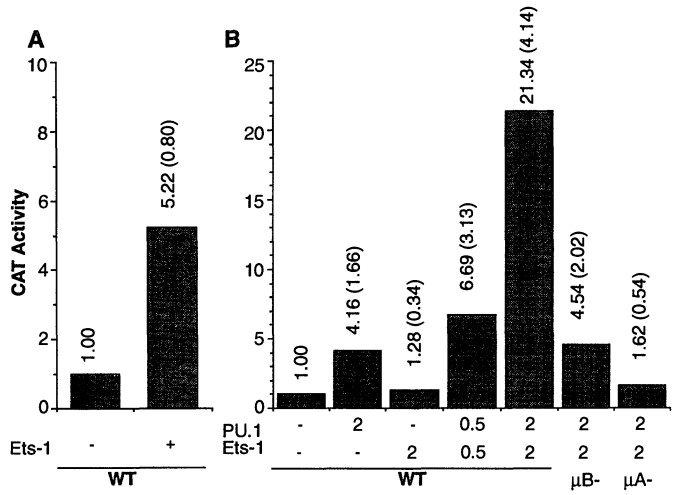

Fig. 6. Trans-activation of the μ enhancer core by Ets-1 and PU.1. (**A**) Effect of Ets-1 expression on the activity of μ70. Cotransfection experiments were performed in S194 cells with 2 μg of reporter plasmids indicated (WT refers to μ70$_2$, μA– refers to μ70$_2$μA$^-$, and μB– refers to μ70$_2$μB$^-$; Figs. 3 and 5) and 2 μg of an Ets-1 mammalian expression plasmid that contained the cytomegalovirus (CMV) promoter and enhancer (indicated by a plus) or a plasmid that expresses no protein [either pEVRFO plasmid (*31*) or a CMV antisense Ets-1 vector indicated by a minus]. CAT activity is expressed relative to the WT reporter control in each experiment. The CMV–Ets-1 mammalian expression plasmid contains the complete coding sequence of the 54-kD murine Ets-1 protein and 57-bp 3′ untranslated region cloned into the Bam H1 site of pEVRFO plasmid. CMV antisense Ets-1 contains this insert in the opposite orientation. Results shown are the mean ± SD of five independent experiments conducted in duplicate. (**B**) Synergistic transactivation of the μ70 enhancer by Ets-1 and PU.1 in COS cells. Cells (10^6) were transfected with the calcium phosphate precipitation procedure (*32*). Reporter plasmids (2 μg), together with either 0, 0.5, or 2 μg of Ets-1 and PU.1 (*16*) expression plasmids as indicated, were used. The plasmid pEVRF was included as a carrier to maintain a total of 6 μg of DNA per transfection. Cells were harvested 2 days after being transfected, and CAT analysis was performed with 20 to 100 μg of cellular proteins in a 1-hour assay. CAT activity is relative to that obtained in the absence of cotransfected Ets-1 or PU.1 expression plasmids (assigned a value of 1). Data shown are the average ± SD of five independent experiments conducted in duplicate, except for that with the WT reporter with 0.5 μg of the transactivators, which was done twice in duplicate.

Table 1. Summary of relative mRNA expression patterns of the *ets*-1 and *PU.1* genes. B and T lymphoid cell lines, as well as nonlymphoid cell lines, were analyzed for the presence of Ets-1 and PU.1 transcripts. Northern analysis of Ets-1 mRNA was done as described (Fig. 2), with a 435-bp Pst I fragment (encoding amino acids 133 to 277) derived from the *ets*-1 gene used as the probe. The *ets*-1 gene diverges significantly from the highly related *ets*-2 gene in this region. Cell lines used are as in Fig. 2.

Cell type	Cell line	Ets-1	PU.1
Pro-T	2052C	–	++
Pre-B	HAFTL	+	++
B	A20	+	++
	BJAB	+	++
Plasma	S107	+/–	++
	S194	+	++
T	RLM11	++	–
	AKR-1 (++)	++	–
	AKR-2 (4$^+$8$^-$)	++	–
	Jurkat	+	–
	BW5147	+	–
Nonlymphoid	NIH 3T3	+/–	–
	HeLa	–	–

tion in the human enhancer. Fourth, alteration of the spacing between the μB and μA elements affects enhancer function (*21*), which suggests the need for appropriate spatial configuration of these elements.

On the basis of in vitro binding, mRNA expression, and trans-activation experiments, we propose that μB and μA binding proteins are encoded by the *PU.1/Spi-1* and *ets*-1 proto-oncogenes, which implicates them in the activation of the *IgH* locus (*22*). Other early B cell–specific genes such as λ5, *V–pre-B*, and *mb-1* also have μB homologous sequences in their promoters (*23*), which suggests that PU.1 may be a general regulator of early B cell gene expression. Although a role for Ets-1 has been suggested in T cell receptor α enhancer activity (*24*) and in the regulation of several T lymphotropic viruses (*18*, *25*), our results provide the first evidence of Ets-1 function in B lymphoid cells.

The expression of PU.1 and Ets-1 together in nonlymphoid cells is sufficient to activate the core μ enhancer, which suggests that no other lymphoid-specific components are required in COS cells to induce core enhancer activity. The requirement for both proteins for efficient trans-activation in COS cells may reflect a necessity for occupancy of both μA and μB sites for enhancer function (*26*). Such a complex may provide a joint activation domain for the basal transcription machinery or may recruit (*27*) a different activator protein for this purpose. Further, the coordinate requirement for two different ETS-domain proteins, with overlapping expression patterns, may be an example of regulatory specificity achieved by the combinatorial use of factors with differing properties. Identification of the sequence motifs and the gene products that govern the activity of a B cell–specific domain of the μ enhancer provides a starting point for the biochemical reconstruction of the multiple DNA-protein interactions that constitute a functional enhancer.

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Increased Activity of L-Type Ca^{2+} Channels Exposed to Serum from Patients with Type I Diabetes

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Type I diabetes [insulin-dependent diabetes mellitus (IDDM)] is an autoimmune disease associated with the destruction of pancreatic β cells. Serum from patients with IDDM increased L-type calcium channel activity of insulin-producing cells and of GH_3 cells derived from a pituitary tumor. The subsequent increase in the concentration of free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) was associated with DNA fragmentation typical of programmed cell death or apoptosis. These effects of the serum were prevented by adding a blocker of voltage-activated L-type Ca^{2+} channels. When the serum was depleted of immunoglobulin M (IgM), it no longer affected $[Ca^{2+}]_i$. An IgM-mediated increase in Ca^{2+} influx may thus be part of the autoimmune reaction associated with IDDM and contribute to the destruction of β cells in vivo.

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease associated with massive lymphocytic infiltration of the endocrine pancreas, reaction of circulating autoantibodies with autoantigens on the insulin-producing β cells, and destruction of the β cells (*1–4*). The latter effect is believed to precede the onset of IDDM. In serum from children with newly diagnosed IDDM, antibodies to the islet cell surface affect glucose-induced insulin release (*2*). Voltage-dependent Ca^{2+} channels participate in various cellular processes, including the stimulus-secretion coupling in the β cell. We were interested in clarifying a possible effect of IDDM serum on voltage-activated L-type Ca^{2+} channels, and the molecular consequences, in insulin-secreting cells.