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Genetics

Analysis of lipopolysaccharide-response genes in B-lineage cells demonstrates that they can have differentiation stage-restricted expression and contain SH2 domains

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ABSTRACT **Bacterial lipopolysaccharide (LPS) is a potent stimulator of B-cell activation, proliferation, and differentiation. We examined the genetic response of B-lineage cells to LPS via trapping of expressed genes with a gene-trap retrovirus. This analysis showed that expression of only a small fraction of genes is altered during LPS stimulation of B-lineage cells. Isolation of the cellular portion of the trapped LPS-response genes via 5′ RACE (rapid amplification of cDNA ends) cloning identified novel genes for all the cloned loci. These novel LPS-response genes were also found to have differentiation stage-restricted expression within the B-lymphoid lineage. That LPS-response genes in B cells also have differentiation stage-restricted expression suggests that these genes may be involved in the control of B-cell function and differentiation, since the known members of this class of genes have frequently been found to play a role in the function and differentiation of B-lineage cells. The isolation of novel members of this class of genes, including a gene that contains a putative SH2 domain, will further increase our understanding of the molecular events involved in the control of B-cell differentiation and function.**

In the B-lymphoid lineage, the differentiation pathway is defined by several discrete stages of maturation beginning with a lineage-committed progenitor cell and ending with the immunoglobulin-secreting plasma cell (1–3). For a B-lineage committed progenitor cell to successfully traverse this differentiation pathway, it must achieve several different milestones, each of which is specific to a given stage of differentiation (e.g., immunoglobulin heavy-chain gene rearrangement) (4). In many instances, these milestones could not be reached without the function of gene products that have a stage-specific or -restricted pattern of expression (5, 6). Thus, there is a requirement for this class of genes and their products to carry out critical steps in B-cell differentiation (e.g., EBF, RAG-1) (6, 7) or enable a B-lineage cell to perform its function (e.g., J chain) (8).

It has been well documented that lipopolysaccharide (LPS) can stimulate the differentiation of B-lineage cells without the help of other cells (e.g., T cells) or cytokines (e.g., interleukin 4) (9). Since LPS can stimulate the differentiation of purified B-lineage cells, we proposed that LPS may act directly on B cells to alter the expression patterns of genes that control the differentiation process at the molecular level (10). Because genes with differentiation-restricted expression frequently play a role in regulation of B-cell differentiation, we also proposed that the expression of this class of genes would be responsive to LPS stimulation (10). Thus, we sought to identify and quantitate the fraction of genes whose expression is altered in a B cell when stimulated by LPS. Such a study could elucidate how LPS affects the genetic program of B-lineage cells as well as lead to the identification of specific genes that are responding to LPS which may be involved in the differentiation process.

We and others had previously developed enhancer-trap (11, 12) and gene-trap vectors (11–15). We had shown that enhancer- and gene-trap retroviruses are capable of detecting transcriptionally active chromatin domains and genes that are responsive to LPS and cytokine stimulation (11, 13). The enhancer-trap retrovirus was found to be effective at identifying regions of chromatin that are regulated in a differentiation stage-specific fashion in a transformed B-lineage cell line, 70Z/3, that will differentiate *in vitro* in response to LPS (11). However, isolation and cloning of the gene(s) contained within these transcriptionally active chromatin domains might pose difficulties given the distance over which enhancers can act. We developed the gene-trap virus (Gensr1), which requires the fusion of *Escherichia coli lacZ* and a cellular gene at both the transcriptional and translational level in order for the cell to express β-galactosidase (β-gal) (11, 13), as a more feasible alternative than the enhancer-trap virus for the cloning of the trapped genes. We specifically designed our gene-trap vector so that it requires both a transcriptional fusion and a translational fusion with the cellular gene. The additional requirement for translational fusion eliminates the possibility of *lacZ* expression from unspliced transcripts. This additional level of stringency ensures that β-gal$^+$ cells are not the result of readthrough transcripts from promoter sequences present in either the flanking cellular chromatin or the vector.

Here we document that our gene-trap retrovirus can form both transcriptional and translational fusions with cellular genes in their native chromosomal locations. We show that one of the gene-trap integrations created a transcriptional and translational fusion with a previously identified cellular gene, eIF-4AI (16). We identified a novel LPS-response gene (7a291), which also has differentiation stage-specific expression. Another novel gene (7a33) that we identified by trapping a LPS-reponse gene also showed expression that is differentiation-stage restricted. It is expressed at high levels in early and mature B-lineage cells but at only trace levels in plasmacytomas. Interestingly, this gene contains a region that has strong homology to phosphotyrosine recognition domains (i.e., SH2 domains), which would suggest that it has a role in receptor-mediated signal transduction events.

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Abbreviations: LPS, lipopolysaccharide; β-gal, β-galactosidase; FACS, fluorescence-activated cell sorting; RACE, rapid amplification of cDNA ends.

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MATERIALS AND METHODS

Transduction, Detection, and Isolation of *lacZ*$^+$ Gene-Trap Integrants. We have previously described in detail the procedure for transduction of cell lines with Gensr1 packaging cell lines, fluorescence-activated cell sorting (FACS) of β-gal$^+$ cells from these transductions, and a 96-well format assay for large scale screening of β-gal$^+$ gene-trap cell clones that have integrations in LPS-responsive genes (11, 13). For the experiments described here, 70Z/3 cells were transduced by coculture on a packaging cell line producing either the ecotropic version of Gensr1 (ψ-2/A8) or the amphotropic version (PA/A36). 70Z/3 cells were harvested from the producer cell monolayer, loaded with FDG, the fluorogenic substrate for β-gal, and incubated on ice for 2–3 hr before FACS analysis and sorting (17). In order for a sorted cell clone to be considered a gene-trap clone, the presence of β-gal activity in the clone was confirmed by either FACS-Gal analysis or a cell lysate assay for β-gal activity. In total, 334 β-gal$^+$ gene-trap cell clones from a minimum of 16 independent Gensr1 transductions of 70Z/3 were isolated during the course of this study. The gene-trap clones in this study comprised 112 β-gal$^+$ gene-trap cell clones isolated from transductions of LPS-stimulated 70Z/3 cells and 222 β-gal$^+$ gene-trap cell clones isolated from transductions of 70Z/3 cells transduced in the absence of LPS.

Identification of Gensr1 Integrants with Differential *lacZ* Expression in Response to LPS. Single β-gal$^+$ cells were cloned directly into individual wells of a 96-well plate containing sterile growth medium and propagated for 10–14 days until the clones were sufficiently expanded to allow plating of a portion of each clone into replica 96-well plates containing either normal growth medium or the same medium supplemented with LPS (10 μg/ml). The LPS-responsiveness of *lacZ* expression in each gene-trap cell clone was determined by a fluorogenic assay utilizing methylumbelliferyl galactoside, which was carried out on cell lysates prepared directly in the 96-well trays (13, 18). The LPS responsiveness of each gene-trap cell clone as determined by the lysate assay for β-gal activity was subsequently confirmed by FACS-Gal analysis.

Cloning of Cellular Exons Fused to the *lacZ* Gene-Trap Exon. Cellular exons fused by splicing to the splice-acceptor *lacZ* exon of Gensr1 were cloned by 5′ rapid amplification of cDNA ends (RACE) (19) with some modifications (unpublished data). First-strand cDNA synthesis was primed with random deoxyhexanucleotide (Pharmacia) using either poly(A)$^+$ RNA (gene-trap clones 7a291, 7a305, and 7a309) or total cellular RNA (gene traps 7a24, 7a33, and 7a65). Reverse transcription was performed using Moloney MuLV Superscript reverse transcriptase (GIBCO/BRL) per the recommended reaction conditions. First-strand cDNA was tailed with dATP using terminal transferase (GIBCO/BRL). One-fortieth of each dA-tailed first-strand cDNA was amplified by RACE. Second-strand cDNA synthesis was primed with RACE oligonucleotide (5′-AAGGATCCGATCGTCGACATCGATAATACGACTCACTATAGGGATTTTTTTTTTTTTTTT-3′) in PCR mixture (Promega) with *Taq* polymerase (Promega). Second-strand synthesis was carried out for five cycles (94°C, 1 min; 37°C, 1.5 min; 40°C, 2 min; 45°C, 1 min; 72°C, 5 min). Amplification of double-stranded cDNA was initiated in the same tubes following a hot start by addition of a primer mix containing 10 pmol each of an antisense gene-trap oligonucleotide (5′-CCTCTTCGCTATTACGCCAGCTG-3′) and R_O (5′-AAGGATCCGATCGTCGACATC-3′). Reactions were amplified by 35 PCR cycles. One-fiftieth of the primary RACE amplification was subjected to a secondary, nested amplification with 20 pmol each of antisense gene trap (5′-GTCACGACGTTGTAAAACGACG-3′) and R_I (5′-GACATCGATAATACGACTCACTATAGG-3′) for 25 PCR cycles. Agarose gel-purified 5′-RACE products were TA cloned in pBluescriptII(SK−) (Stratagene), which was linearized at the *Sma* I site and T-tailed with dTTP and *Taq* polymerase (20). Gene-trap 5′-RACE clones were sequenced by a manual Sanger dideoxynucleotide method using [^{35}S]dATP (Amersham) and Sequenase 2.0 T7 DNA polymerase (United States Biochemical).

Cloning and Sequence Analysis of 7a33 cDNA. A random hexamer-primed cDNA library was constructed from 70Z/3 poly(A)$^+$ RNA in pBluescriptII(SK−) (Stratagene) (unpublished data). Specific cDNAs were recovered from the orientation-specific library by PCR amplification using a vector primer and different gene-specific primers obtained from 5′-RACE cDNA sequences. 7a33 cDNA was isolated from the 70Z/3 library for sequencing or for use as probes in Northern blot analysis (21).

Northern Blot Analysis of the Cellular mRNAs. Total cellular RNA was prepared from cell lines by a hot-phenol procedure and poly(A)$^+$ RNA prepared by oligo(dT) chromatography as described (22). RNA analyses (Northern blots) using formaldehyde-agarose gels were performed as described (22). To probe for 7a33 mRNA, we used a 7a33 cDNA clone. The cDNAs used to probe for β-actin and 7a291 mRNAs were generated by a reverse transcription PCR using primers specific for 7a291 (sense primer, 5′-GAGCAGTTTCAACTGAAGGATG-3′; anti-sense primer, 5′-CATCTTGTAAGGCGAATGTGAGG-3′) or primers specific for β-actin (sense primer, 5′-TGGGTCAGAAGGACTCCTATG-3′; anti-sense primer, 5′-ACCAGACAGCACTGTGTTGGC-3′).

RESULTS

The Gene-Trap Virus, Gensr1, Forms Fusions with Cellular Genes via Splicing of *lacZ* to Cellular Exons Creating Transcriptional and Translational Fusions Between *lacZ* and Cellular Genes. To directly assess whether cellular exons have been fused to *lacZ* in β-gal$^+$ gene-trap clones, we carried out cDNA cloning of the putative cellular–*lacZ* fusion mRNAs via a modified 5′ RACE procedure (19). The partial cDNA clones containing cellular exons fused to *lacZ* isolated via this procedure can be used to rapidly conduct Northern blot analyses to determine the size and expression pattern of the native cellular mRNAs, to identify regions of sequence homology as well as provide nucleotide sequence information for synthesis of oligonucleotides to facilitate the isolation of full-length clones from cDNA libraries.

For one gene-trap clone, 7a305, we verified that expression of *lacZ* from our gene-trap virus is the result of a transcriptional and translational fusion with a cellular gene. Sequence

FIG. 1. Expression of *lacZ* in gene-trap integrants is the result of transcriptional and translational fusions with cellular genes. The gene-trap clone 7a305 contains an integration in a previously sequenced gene, eIF-4AI, a protein synthesis initiation factor. Genomic sequence of the eIF-4AI promoter region is shown (TATA box is underlined) with the putative transcriptional start site (circle with horizontal arrow) and the junction between the first exon and intron of eIF-4AI (vertical line) indicated. Under the genomic sequence, cDNA sequence of the *lacZ* fusion mRNA in 7a305 derived from 7a305 5′ RACE clones is shown. Alignment of the 7a305 cDNA sequence with the genomic sequence of the eIF-4AI first exon and intron demonstrates that the splice-acceptor *lacZ* exon is fused to the 3′ end of the eIF-4AI first exon and that the putative ATG of the eIF-4A1 gene is in-frame with the coding frame (underlined triplets) of *lacZ*.

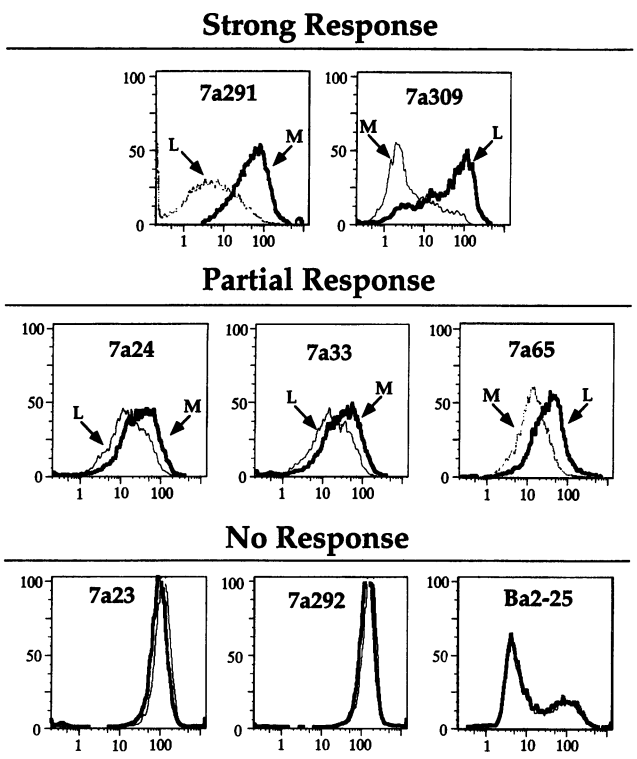

FIG. 2. LPS-responsive gene-trap clones show differential regulation of *lacZ* expression. Equal numbers of gene-trap cell clones were cultured in either normal medium (M) or medium containing LPS (10 μg/ml) (L) for 20 hr. *lacZ* expression of the individual gene-trap cell clones was then assessed by FACS-Gal analysis. For the "no response" clones, analysis of cells cultured in normal medium is indicated by a thin-lined histogram and analysis of the LPS-stimulated cells is indicated by a thick-lined histogram.

analysis of the 5′ RACE cDNAs isolated from 7a305 cells revealed that the Gensr1 had integrated in a known gene whose exon and intron structure had previously been determined, eIF-4AI (16). In Fig. 1, we show the sequence of the cDNA derived from 7a305 cells. This sequence represents splicing of the first exon of eIF-4AI to the splice acceptor–*lacZ* exon. In addition, the ATG translational initiatior for the eIF-4AI gene is in-frame with the translational frame of *lacZ* (Fig. 1). The cDNA of the 7a305 fusion transcript confirms our model for how Gensr1 functions as a gene trap.

The Expression of a Very Small Fraction of Genes Is Altered During LPS Stimulation of B-Lymphoid Cells. We next looked for the fraction of genes that are altered in B-lineage cells upon stimulation with LPS, including genes that are both stimulated and repressed. To accomplish this, we performed several independent transductions of a LPS-responsive murine B-cell line with Gensr1. Following each of these transductions, individual β-gal$^+$ cells were cloned into individual wells of sterile 96-well plates for subsequent propagation and screening of clones for gene-trap integrations in genetic loci whose expression is altered during LPS stimulation of B-lineage cells. In Fig. 2, we show the flow cytometric histograms representing the relative β-gal activity (FACS-Gal) of five representative gene-trap cell clones harboring integrations in genes where expression of the *lacZ* reporter gene is altered when the cells are stimulated with LPS. For comparison, we show FACS-Gal histograms of gene-trap cell clones where *lacZ* expression showed no response to LPS stimulation such as 7a292, 7a23, and Ba2–25 (Fig. 2). The overwhelming majority of gene-trap integrations showed no alteration in *lacZ* expression in response to LPS stimulation. In total, we found that in 12 of 334 β-gal$^+$ gene-trap cell clones *lacZ* expression was altered in a significant and reproducible manner following LPS stimulation. Thus, we conclude that LPS alters the expression of a small minority of genes in B-lymphoid cells (≈3%; 12/334 trapped genes).

Two of the 12 LPS-responsive cell clones showed a strong response of *lacZ* expression to LPS stimulation (7a291, 7a309). In the other LPS-responsive gene-trap clones the effects on *lacZ* expression following LPS stimulation are less pronounced but reproducible (Fig. 2; data not shown).

Gensr1 Integrations in Novel Genes Whose Expression Is Altered by Stimulation with LPS. We performed the 5′ RACE procedure on RNA derived from the gene-trap clones that showed changes in *lacZ* expression in response to LPS. Sequence analysis of the 5′ RACE cDNAs derived from the LPS-responsive gene traps also revealed the presence of cellular sequences fused to the splice acceptor–*lacZ* exon for all gene-trap clones analyzed. Because translation of β-gal from the cellular–*lacZ* gene fusion requires that translation initiate in-frame with *lacZ*, we can predict the translated open reading frame for the cellular exons fused to *lacZ*. In all LPS-responsive gene fusions examined by 5′ RACE we found an open reading frame preceded by an AUG translation initiatior which is in-frame with *lacZ*. Thus, sequence analysis of the 5′ RACE clones provides both nucleotide and predicted amino acid sequence information for comparison with sequences in GenBank. In Fig. 3, we show the predicted amino acid sequence that is in-frame with the coding sequence of *lacZ* for each of the five LPS-responsive clones examined by 5′ RACE. In general, the number of amino acids predicted to be fused to β-gal from the cellular gene product is relatively small, ranging from 7 (7a309) to 47 (7a33) amino acids. Comparisons of the cellular exon sequences to the nucleotide sequences currently in GenBank did not retrieve any identical sequence matches. Thus, all five of the cDNAs represent heretofore unidentified genes whose expression is altered during LPS stimulation of B-lineage cells.

LPS-Responsive Genes Have Stage-Specific or -Restricted Expression Within the B-Lineage Pathway. To determine whether cellular genes identified as LPS-responsive were also expressed at different stages in B-cell differentiation, we performed Northern blot analyses with their respective cDNA probes. In Fig. 4 we probed total poly(A)$^+$ RNAs isolated from a panel of murine B-cell lines representative of the major stages of B-cell differentiation with 7a291 or 7a33 cDNAs and

Gene-trap	LPS Response	Amino Acid Translation of Exon(s) Fused to AcLac
7a24	repressed	MDRGSSGPRAAAAARWGQASPRRDCWTGCTRSPTAPCSKWRRSL
7a33	repressed	MPAMVPGWNHGNITRSKAEELLSRAGKDGSFLVRASESIPRAYALCVSL
7a65	induced	MCDHLIRAAKYRDHVTATQLIQKIINLLTDKHGAWGSSAVSL
7a291	repressed	MCGQLFEMEFANSSL
7a309	induced	MNSSMEVSL

FIG. 3. LPS-responsive gene traps are fusions with novel cellular genes. Amino acid sequence predicted from nucleotide sequence of five LPS-responsive gene traps analyzed by 5′ RACE. We show the amino acid sequence for the translational frame which is in-frame with *lacZ*. In boldface, we indicate the methionine residue (M) where translation could initiate. In addition, we show the first 2 amino acids derived from the splice-acceptor *lacZ* exon at the right end of each sequence in boldface (SL).

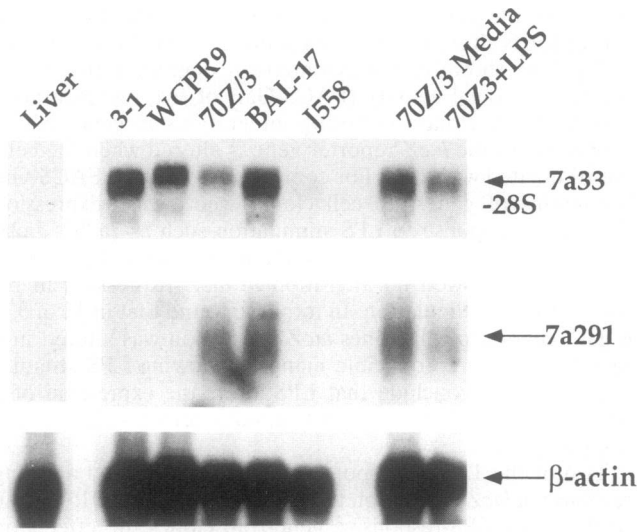

FIG. 4. Cellular genes trapped in the LPS-responsive gene traps 7a291 and 7a309 have differentiation stage-restricted expression. Northern blot analysis of total poly(A)$^+$ (5 μg per lane) with 5′ RACE cDNA clones from gene-traps 7a33 (*Top*) and 7a291 (*Middle*). To control for the amount of RNA loaded, we show hybridization of the filter with a β-actin cDNA probe. The filter was initially probed with a 7a33 cDNA clone (87–7-1), stripped, and probed with β-actin cDNA, stripped again, and probed with 7a291 cDNA. The 7a33 mRNA is ≈5.0 kb, while the 7a291 mRNA(s) are 1.0–1.1 kb.

found that neither gene is expressed throughout all stages of B-cell differentiation (Fig. 4). 7a291 has the most restricted expression pattern, being found only in surface IgM$^+$ cell lines (e.g., BAL17, 70Z/3). Its mRNA is not detected in pre-B-cell lines (3–1, WCPR9) or in a plasmacytoma (J558). Analysis of 7a33 mRNA expression showed that it is expressed at both the early (3–1, WCPR9) and mature (70Z/3, BAL-17) B-cell stages but at only trace levels in the plasmacytoma (J558). Normal adult liver did not express 7a291 or 7a33 mRNA, suggesting that these genes do not have widespread, multiorgan expression.

Also shown in Fig. 4 is that expression of 7a291 mRNA is not completely shut off during LPS stimulation in 70Z/3 cells, which is in agreement with FACS-Gal analysis of *lacZ* expression in the 7a291 gene-trap cells (see Fig. 2). A similar comparison for 7a33 showed that expression of 7a33 mRNA is partially repressed by LPS stimulation (Fig. 4), in agreement with FACS-Gal expression (Fig. 2). That the response of *lacZ* expression to LPS in these gene traps mirrors the response of their native mRNAs to LPS confirms the utility of the gene-trapping procedure to identify and quantitate the frequency of LPS-responsive genes.

The 7a33 Gene Contains a Putative SH2 Domain. Computer homology analysis of the sequences for 7a33 gene-trap 5′ RACE cDNAs and additional 7a33 cDNA clones derived from a 70Z/3 cDNA library indicated a strong homology to the SH2 motif originally described as a noncatalytic domain in protein tyrosine kinases. The SH2 domain is a protein–protein interaction motif that enables recognition and binding to target protein sequences that contain a phosphotyrosine (23–28). Different SH2 regions have specificity for high-affinity binding to particular tyrosine-phosphorylated sites on other proteins and suggests the involvement of SH2-containing proteins in signal transduction. A 100-amino acid region of the 7a33 open reading frame has the conserved features of an SH2 domain (Fig. 5).

The predicted 7a33 SH2 domain has 41% and 33% amino acid identity to the c-*abl* and v-*src* SH2 domains, respectively. In comparison, c-*abl* and v-*src* SH2 regions share 37% amino acid identity when their sequences are optimally aligned (Fig. 5) based on crystal structures and NMR solution structure (24–28). Structural analyses of the SH2 motif and its binding recognition of phosphotyrosine ligand have revealed conserved amino acids that contribute to the core element structure of an antiparallel β-sheet sandwiched between two α-helices and amino acids that contribute to phosphotyrosine peptide ligand binding (24–28). 7a33 chemically conserves all 15 of the buried aromatic/aliphatic residues that determine the hydrophobic core interactions of the SH2 domain; there is 9 of 15 and 13 of 15 amino acid identity of 7a33 to c-*abl* and v-*src*, respectively.

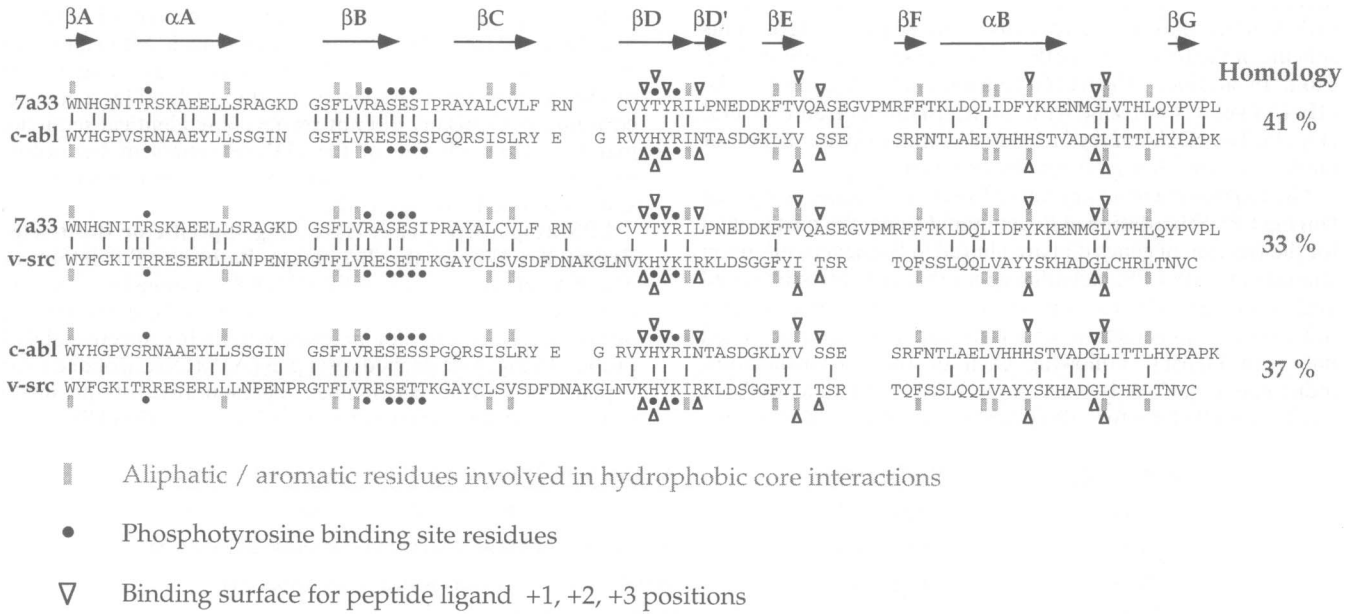

FIG. 5. The 7a33 open reading frame contains a region that is highly homologous to known SH2 domains. The major open reading frame of 7a33, which is also found to be in-frame with *lacZ* in the 5′ RACE clones, was searched against all protein sequences entered in GenBank using the FASTDB program in the Intelligenetics package. Top matches were found to be with regions of proteins corresponding to SH2 domains. Structural and functional significance of various residues is as indicated. Secondary structure elements are shown above the aligned sequences such as α-helices (αA, αB), β-helices (βA-βG), or loops connecting helices (loops are named with letters of the adjacent helices, thus BC is the loop between βB and βC) according to Eck *et al.* (26). Amino acid residues described in *Results* refer to positions within these structural elements.

The phosphotyrosine peptide ligand binding domain is a solvent-accessible binding pocket with two components. One site interacts with the phosphotyrosine via ion-pairing, hydrogen-bonding, amino–aromatic, and hydrophobic interactions (residues αA2, βB5, βB7, BC1, BC2, BC3, βD4, βD5, and βD6) (24–28). [In this description, residue positions are as defined by Eck et al (26) and designate a position in a secondary structure element (Fig. 5) such as α-helices (αA, αB), β-helices (βA-βG), or loops connecting helices (loops are named with letters of the adjacent helices; thus, BC is the loop between βB and βC).] These residues are largely identical or chemically conserved among 7a33, *abl*, and *src*, except for position BC3 (Ile in 7a33), which lacks the hydroxyl group (Ser or Thr in *abl* and *src*, respectively), which stabilizes an amino group at position βD6. The other site has both polar and hydrophobic residues (residues βD3, βD4, βD5, βD′1, βE4, EF2, αB9, BG3, and BG4) and contributes a binding surface for the ligand residues at positions +1, +2, and +3 relative to tyrosine (residues at +3 binding surface are underlined) in the peptide ligand (YEEI preferred for *src*; YENP for *abl*) (24–28). The strongly hydrophobic character of the +3 position interaction residues in 7a33 SH2 suggests its putative phosphotyrosine peptide ligand has *src*-like hydrophobic specificity at this position. Finally, residue βD′1, which interacts with the +2 ligand position, is hydrophobic in 7a33 (Leu) vs. polar (Asn in *abl*, Arg in *src*), suggesting a less polar +2 residue in the putative 7a33 ligand.

DISCUSSION

We have shown that transduction of mammalian cells with a retrovirus containing a mammalian splice acceptor fused to a *lacZ* reporter gene results in transcriptional and translational fusions with cellular genes. All six β-gal$^+$ gene trap integrations we examined contained cellular sequences spliced to the splice acceptor–*lacZ* exon. One of these fusions, 7a305, unambiguously shows expression of *lacZ* from the gene-trap virus is due to transcriptional and translational fusion with a cellular gene. We demonstrate that gene fusions to loci that are responsive to LPS resulted in identification of novel genes (5 of 5 examined); these loci have differentiation stage-restricted (7a33) or stage-specific (7a291) expression within the B-lymphoid lineage. In addition, the molecular characterization of these gene fusions has allowed us to predict that the 7a33 gene product plays a role in signal transduction due to the presence of a phosphotyrosine recognition domain.

Our work demonstrates that gene trapping is an efficient methodology for isolation of a small subset of genes whose expression is differentially regulated with respect to a defined stimulus such as LPS. Thus, gene trapping is a reasonable alternative to molecular cloning approaches where the isolation of differentially regulated genes depends on adequate differences in steady-state levels of mRNA in one state vs. another (e.g., differential screening, subtractive hybridization, differential display PCR) (29–31). Of a total of 334 gene-trap cell clones that were sorted solely on the basis of having *lacZ* expression in either the presence or absence of LPS, only 12 showed altered β-gal expression after LPS stimulation. Sequence analysis of molecular clones for 5 of these LPS-responsive gene fusions revealed that all of them are novel cellular sequences. Trapping of genes whose expression is altered in response to a defined stimulus rather than utilizing differential cDNA cloning procedures has an inherent advantage in that gene trapping allows genes that have only a partial response to a stimulus to be tagged, screened, and subsequently cloned. Expression of the cellular genes 7a291 and 7a33 is partially, but not completely, repressed by LPS. It is unlikely that these genes would have been isolated by differential cloning procedures, since their mRNAs have low abundance in LPS-stimulated 70Z/3 cells. Despite the partial response of these genes to LPS *in vitro* in a transformed cell line, they are still found to have differentiation stage-specific (7a291) or stage-restricted (7a33) expression in the various stages of B-cell differentiation, thus demonstrating that isolation and characterization of partially responsive gene-trap cell clones can result in identification of genes with a stage-specific pattern of expression within the developmental pathway of a mammalian cell lineage.

The gene-trapping approach exploited here represents a tool to study not only genetic programs active in mammalian development and differentiation but also genetic programs in mammalian cells that control their response to a defined stimulus. Application of this procedure to lymphoid differentiation, a very well-worked area of study, resulted in the discovery of novel genes with different expression patterns in various stages of B-cell differentiation. Our success with the gene-trap approach indicates its broad utility in characterization of other genetic programs.

LPS is a potent stimulus for cells of the immune system, especially B cells and macrophages. It brings about dramatic changes in the morphology, function, and differentiation state of B-lineage and myeloid-lineage cells. After quantifying the fraction of genes whose expression is LPS responsive in a murine B-cell line, we found that a small fraction (≈3%) of the expressed genes are responsive. However, when the expression pattern of these genes was examined, they were found to belong to another class of rare genes, those that have differentiation stage-specific or stage-restricted expression within the B-lymphoid lineage, including a gene that encodes a putative signal transduction protein due to the presence of an SH2 domain. Many of the known and well-characterized members of this differentiation-stage-restricted gene family have been found to play a crucial role in regulation of B-cell differentiation. Given the ability of LPS to drive the differentiation of B-lineage cells at all stages, it would seem apparent that LPS would modulate the expression of this class of genes in order to achieve its effect on differentiation. Thus, mammals may have evolved a signal transduction mechanism(s) that allows B-lineage cells to directly and therefore rapidly respond to bacterial cell wall components such as LPS to alter the expression of differentiation stage-restricted genes (e.g., J-chain, 7a291, 7a33). This LPS-responsive signal transduction pathway would effect the expression of those genes involved in control of B-cell differentiation and thus would allow differentiation to be "jump started" during a period of bacterial stress in an antigen-independent fashion without the usual support of antigen-presenting cells and T cells.

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1. Rajewsky, K. (1992) *Curr. Opin. Immunol.* **4,** 171–176.
2. Melchers, F., Rolink, A., Grawunder, U., Winkler, T. H., Karasuyama, H., Ghia, P. & Andersson, J. (1995) *Curr. Opin. Immunol.* **7,** 214–227.
3. Hardy, R. R., Carmack, C. E., Shinton, S. A., Kemp, J. D. & Hayakawa, K. (1991) *J. Exp. Med.* **173,** 1213–1225.
4. Kitamura, D. & Rajewsky, K. (1992) *Nature (London)* **356,** 154–156.
5. Kitamura, D., Kudo, A., Schaal, S., Muller, W., Melchers, F. & Rajewsky, K. (1992) *Cell* **69,** 823–831.
6. Lin, H. & Grosschedl, R. (1995) *Nature (London)* **376,** 263–267.
7. Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Tonegawa, S. & Papaioannou, V. E. (1992) *Cell* **68,** 869–877.
8. Mather, E. & Koshland, M. E. (1977) in *Regulatory Genetics of the Immune System*, ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. VI (N.Y. Acad. Press, New York), pp. 727–733.

9. Melchers, F. & Andersson, J. (1986) *Annu. Rev. Immunol.* **4,** 13–36.
10. Kerr, W. G., Nolan, G. P., Johnsen, J. B. & Herzenberg, L. A. (1991) *Adv. Exp. Med. Biol.* **292,** 187–200.
11. Kerr, W. G., Nolan, G. P., Serafini, A. T. & Herzenberg, L. A. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54,** 767–776.
12. Gossler, A., Joyner, A. L., Rossant, J. & Skarnes, W. C. (1989) *Science* **244,** 463–465.
13. Kerr, W. G. & Herzenberg, L. A. (1991) *Methods Compan. Methods Enzymol.* **2,** 261–271.
14. Friedrich, G. & Soriano, P. (1991) *Genes Devel.* **5,** 1513–1523.
15. Brenner, D. G., Lin-Chao, S. & Cohen, S. N. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5517–5521.
16. Nielsen, P. J. & Trachsel, H. (1988) *EMBO J.* **7,** 2097–2105.
17. Nolan, G. P., Fiering, S., Nicolas, J. F. & Herzenberg, L. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2603–2607.
18. Fiering, S., Roederer, M., Nolan, G. P., Micklem, D. R., Parks, D. R. & Herzenberg, L. A. (1991) *Cytometry* **12,** 291–301.
19. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
20. Marchuk, D., Drumm, M., Saulino, A. & Collins, F. S. (1991) *Nucleic Acids Res.* **9,** 1154.
21. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual*, ed. Nolan, C. (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed., pp. 8.46–8.52.
22. Kerr, W. G., Hendershot, L. M. & Burrows, P. D. (1991) *J. Immunol.* **146,** 3314–3321.
23. Pawson, T. (1995) *Nature (London)* **373,** 573–580.
24. Waksman, G., Kominos, D., Robertson, S. C., Pant, N., Baltimore, D. B., Birge, R. B., Cowburn, D., Hanafusa, H., Mayer, B. J., Overduin, M., Resh, M., Rios, C. B., Silverman, L. & Kuriyan, J. (1992) *Nature (London)* **358,** 646–653.
25. Overduin, M., Rios, C. B., Mayer, B. J., Baltimore, D. & Cowburn, D. (1992) *Cell* **70,** 697–704.
26. Eck, M. J., Shoelson, S. E. & Harrison, S. C. (1993) *Nature (London)* **362,** 87–91.
27. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B. & Cantley, L. C. (1993) *Cell* **72,** 767–778.
28. Waksman, G., Shoelson, S. E., Pant, N., Cowburn, D. & Kuriyan, J. (1993) *Cell* **72,** 779–790.
29. Sakaguchi, N. & Melchers, F. (1986) *Nature (London)* **324,** 579–582.
30. Yancopolous, G. D., Oltz, E. M., Rathbun, G., Berman, J. E., Smith, R. K., Lansford, R. D., Rothman, D., Okada, A., Lee, G., Morrow, M., Kaplan, K., Prockop, S. & Alt, F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5759–5763.
31. Liang, P. & Pardee, A. B. (1992) *Science* **257,** 967–971.