Biochemical and Biophysical Research Communications **256,** 104–109 (1999)
Article ID bbrc.1999.0292, available online at http://www.idealibrary.com on IDEAL®

The Identification and Characterization of Two Promoters and the Complete Genomic Sequence for the Wiskott–Aldrich Syndrome Gene

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Received January 26, 1999

The Wiskott–Aldrich syndrome (WAS) is an X-linked disorder characterized by immunodeficiency, eczema and thrombocytopenia. The gene responsible for WAS was identified through positional cloning, and the function of the encoded protein (WASP) is still the subject of much speculation. WASP is currently thought to be involved in the regulation of actin polymerization in hematopoietic cells. To study the elements that regulate the WASP gene, we have identified the sites for transcription initiation. We found that two promoters were responsible for controlling WASP expression. Multiple transcription initiation sites were found immediately adjacent to the translation start site, however an alternate exon with a second promoter region was identified 6 kb upstream. Examination of the 5′ sequence adjacent to the initiation sites in both promoters failed to reveal a TATA or CCAAT box, but numerous putative transcription factor binding sites including Sp1, Ets, c-Myb and PU.1 were apparent. Reporter constructs generated from each promoter showed functional activity in the Jurkat T-cell and HEL erythro-megakaryocytic cell lines. Although the alternate exon sequence was extremely GC rich and contained several potential binding elements, the primary promoter was stronger than the upstream promoter in the cell lines assayed. The transcription factor binding site profiles within each promoter suggested that they may play different roles in regulating WASP expression depending on the stage of differentiation and development, and the cell lineage. In this study we have also reported the complete nucleotide sequence of the coding and intervening sequences for the WASP gene. A comprehensive knowledge of the genomic structure and the further characterization of WASP gene expression will facilitate the continued investigation of mutations in WAS patients, and the eventual prospect of gene therapy.

The Wiskott-Aldrich syndrome is a X-linked recessive immunodeficiency that generally manifests during the first few years of life in males affected with the disorder. The disease usually presents with the hallmark combination of thrombocytopenia, eczema, and a deficient immune response. The gene encoding the protein responsible for WAS was identified through positional cloning (1,2). The 1.8 kb cDNA translates into a protein of 502 amino acids in length that is particularly rich in proline residues. The region that is most dense with proline has been shown to bind SH3 domains from several src-related protein tyrosine kinases and other adaptor proteins (3–9). The N-terminus contains a PH domain (10) that overlaps considerably with a second putative domain designated Whether (WASP homology), which is speculated to have a receptor binding function (11,12). A GTPase binding domain (GBD) has been identified between the WH1 domain and SH3 binding, poly-proline region. This domain has been shown to interact with the Rho family GTPase CDC42 (11,13,14), and has also been referred to as the CRIB (CDC42/Rac interactive binding) domain (15). At the C-terminus a verprolin homology domain has been identified (10). The verprolin-like region serves to bind actin, suggesting that the WAS protein (WASP) may provide a link between signal transduction at the cell surface and actin polymerization. This proposed WASP function would correlate with the aberrant cytoskeletal morphology observed in the T cells and platelets of WAS patients (16), and the defects exhibited in chemotaxis and migratory response in the monocytic lineages (17–19).

Mutations resulting in WAS have been found throughout the coding sequence, but primarily in the exons from the N-terminal region (20). WASP gene

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GenBank accession numbers included in this report: AF115548, AF115549.

0006-291X/99 $30.00

mutations are also responsible for the less severe disorder X-linked thrombocytopenia (XLT) in which patients do not exhibit immune dysfunction (21). In some WAS patients, analysis of the WASP gene has failed to identify alterations in the coding regions or the splice sites (Kwan, unpublished results). To enable further analysis of the WASP, gene we have completed the genomic sequence including the 5′ untranslated region. In this report, we have discovered two WASP promoter regions. The primary WASP promoter lies immediately adjacent to the translation initiation site (22). Transcripts derived from a second promoter lying within an alternate exon further upstream were also identified and characterized.

MATERIALS AND METHODS

Sequencing of the WASP gene. The WASP positive cosmid ICRFc104A02167 (23) was utilized in the restriction mapping and sequencing of the WASP gene. *Eco*R1 fragments of the cosmid insert were subcloned into pBluescript SK (Stratagene, La Jolla, CA). These clones were mapped and subcloned further with various restriction enzymes including *Bam*HI, *Hin*dIII and *Pst*I. An ABI 377 DNA Sequencer was used to generate the sequence data from the plasmid templates.

Computer database searches. The following search algorithms were used to analyze the sequence data: BLAST (ref. 24; URL: http://www.ncbi.nlm.nih.gov/BLAST/), RepeatMasker (Smit, A.F.A. and Green, P. URL: http://ftp.genome.washington.edu/RM/RepeatMasker.html/), and TESS (ref. 25; URL:http://agave.humgen.upenn.edu/tess/index.html/).

Cell lines. The cell lines used for this report included the Jurkat T-cell leukemia line (clone E6-1; TIB-152, American Type Culture Collection, Rockville, MD; ref. 26), the HEL erythroleukemia line (clone 92.1.7; TIB-180, American Type Culture Collection, Rockville, MD; ref. 27), and two EBV transformed B-cell lines derived from normal individuals.

Identification of transcription initiation sites. The 5′RACE system was used to identify the initiation sites for WASP mRNA transcripts (Gibco-BRL, Life Technologies, Gaithersburg, MD). A set of nested primers was generated to amplify the cDNA ends. Primer 198 (5′-ACA ATG CTC CTT GGT CCA GT-3′) from the complimentary sequence of exon 2 was used to synthesize the first strand cDNA. For the second round of amplification and subsequent cloning, primer 253 (5′-CAU CAU CAU CAU TCC AAG CAT CTC AAA GAG TC-3′) from the complementary strand of exon 1 was used in conjunction with the Gibco-BRL anchor primer as described in the manufacturer's protocol. Amplified cDNAs were cloned into pAMP1 and sequenced with a third nested primer 255 (5′-CTG GTT CTC GTG GTC CTG GA-3′) complementary to exon 1.

Generation of promoter reporter plasmids. For the primary promoter, a *Bbs*I site 14 bp 5′ of the translation start site was used in conjunction with an upstream *Pst*I site to cleave out the promoter region and clone the 1.6 kb fragment into the firefly luciferase reporter vector pGL3 (Promega, Madison, WI). The resulting clone pGL3-479 was sequenced to confirm that the sequence remained intact and in the correct orientation. After using BLAST (24) to screen the sequence against the GenBank database, an Alu element was apparent in a region spanning 460-710 nt. upstream of the *Bbs*I site. To eliminate the Alu element from the construct, a smaller promoter clone was generated by designing a primer 487 nt. upstream of the *Bbs*I site. A *Kpn*I site was incorporated into the 5′ end of the primer (primer 265: 5′-ATG AGC TCG GTA CCT GGG ATT ACA GGT GTG AG-3′). Primer 265 was paired with the pGL3 vector primer GL2 to amplify the 3′ end piece from the pGL3-479 insert. The resulting PCR product was cleaved with *Kpn*I and *Xho*I, and cloned into the corresponding sites from the pGL3 linker. The clone was analyzed for the correct sequence and orientation, and was subsequently designated as pGL3-481. An additional subclone of the WASP promoter region pGL3-482R was generated from a 1kb *Sma*I/*Bbs*I fragment in the reverse orientation as a negative control.

To clone the alternate promoter, flanking primers were generated 284 bp upstream and 150 bp downstream of the start site for PCR amplification of the putative promoter region (primer 321: 5′-AAT TGC CAG CTC GTG TGC-3′; primer 322: 5′-AGT CAA GCT TCC TGC GCC TCA GTC T-3′). An *Alu*I restriction site within the primer 321 sequence (restriction sites underlined in primer sequence above) and a *Hin*dIII tail incorporated into primer 322 were used to clone the PCR product directly into the corresponding *Sma*I and *Hin*dIII sites within pGL3. The cloned alternate exon was sequenced to confirm there were no PCR artifacts and the resulting construct was designated pGL3-535.

Transfection and reporter gene expression assay. Cells were cultured at 10^5cells/ml and split 20 hrs before electroporation. Cultures were pelleted and resuspended at $2–3\times10^7$/ml in RPMI1640 medium without serum. For each construct, 20μg of CsC1 gradient purified DNA and 2μg of pRL-TK (an internal control plasmid; Promega, Madison, WI) were added to 500μl of cells and electroporated at 960μF, 250V (Jurkat) or 280V (HEL) in a 0.4cm cuvette (BioRad Gene Pulser apparatus, Hercules, CA). The cells were transferred to 12 ml 10%FCS/RPMI1640 medium. After incubating for 18 hours, the cultures were processed with the Promega Dual Luciferase Kit to detect expression from the reporter constructs. Luminescence was quantitated for the firefly luciferase expressed from the pGL3 vector and subsequently normalized against the luminescence of the *Renilla* luciferase from the internal control vector pRL-TK. A Zylux FB-12 Luminometer was used to measure the relative luminescence.

RESULTS

We had previously isolated the genomic cosmid clone A02167 from the Xp11.23 region of the WAS locus (28,29). This cosmid contains the entire length of the WASP gene and was in turn used to isolate the WASP positive cDNA p427 (2). To generate a PCR based assay for the identification of mutations in WAS patient DNA, the exon/intron boundaries were identified and sequenced from restriction fragment subclones of the A02167 cosmid. From these cosmid subclones we have now sequenced the WASP gene in its entirety. The data have been entered into GenBank under the accession # AF115549. Primers were designed sequentially to continue analysis into the introns. The coding sequence from cosmid A02167 was the same as that described for the cDNA (2) with the exception of a single nucleotide change from T to C at position 1029 (cDNA numbering; ref. 2) within exon 10. This exchange would result in a conserved amino acid substitution of Val332 to Ala within the proline rich region of the protein, and may not have any deleterious effects on protein function. We have not observed this alteration in genomic DNA derived from WAS patients or normal individuals (2,30), and this substitution does not appear to be a polymorphism in the general population. The introns ranged in size from 93 to 1672 bp (Table 1), and the

TABLE 1

Exon/Intron Positions and Sizes

Feature	Position[a]	Size (bp)
Exon 1	(1537–1608)[b]–1759	152–223[b]
Intron 1	1760–2056	297
Exon 2	2057–2197	141
Intron 2	2198–3320	1123
Exon 3	3321–3407	87
Intron 3	3408–3507	100
Exon 4	3508–3610	103
Intron 4	3611–3719	109
Exon 5	3720–3761	42
Intron 5	3762–3854	93
Exon 6	3855–3908	54
Intron 6	3909–4554	646
Exon 7	4555–4729	175
Intron 7	4730–5827	1098
Exon 8	5828–5870	43
Intron 8	5871–6073	203
Exon 9	6074–6227	154
Intron 9	6228–6433	206
Exon 10	6434–6840	407
Intron 10	6841–7093	253
Exon 11	7094–7208	115
Intron 11	7209–8880	1672
Exon 12	8881–9201	321

[a] Nucleotide positions are based on the *Pst*I cloning site as position 1 as listed under Genbank accession # AF115549.

[b] The size of exon 1 is listed as variable due to the multiple transcription start sites in the primary promoter. The alternate promoter is not listed in this table.

consensus sequences for donor and acceptor splice junctions were apparent at each exon/intron boundary (31). A search for repetitive elements within the introns with the program RepeatMasker revealed a variety of repeats at different locations within the WASP gene. Introns 1, 6, and 7 each consisted largely of simple repeats. Intron 9 carried a short repeat of the MIR family, and intron 11 contained three regions of Alu element homology.

The sequence data upstream from the translation start site did not demonstrate a TATA or CCAAT box nor homology to other mammalian promoters. To determine the boundary of the 5′ promoter region and to eliminate the possibility of additional upstream exons, we performed 5′ RACE experiments from the Jurkat T-cell line and two EBV transformed B-cell lines. After sequencing 80 individual cDNA ends (43 from the B-cell lines and 37 from the Jurkat T-cell line), several transcription initiation sites were apparent within a 71 bp region between 20–91 bp upstream of the translation start site (Fig. 1A). Multiple start sites are common in promoters without TATA or CCAAT boxes, and 4 of the sites in this region roughly match the initiator consensus sequence for TATA-less promoters (32). However, of the cDNAs derived from the Jurkat T-cell mRNA, 5 clones demonstrated a start site that was apparently expressed from an alternate promoter. These clones (Fig. 1B) showed sequence identity to the primary promoter (Fig. 1A) in the region adjacent to the translation start site, but diverged into separate sequences 34 bp upstream. At this junction, a putative intron acceptor site was apparent in the corresponding genomic sequence with the consensus CAG sequence preceded by a pyrimidine rich tract (31). All of the cDNA clones derived from the alternate promoter demonstrated a single transcription initiation site (Fig. 1B). B-cell derived cDNA end clones did not exhibit the alternate exon sequence. Primers generated from this cDNA data were used to sequence the A02167 cosmid directly and determine the genomic sequence containing the alternate exon (accession # AF115548). The region upstream of the alternate transcription initiation site was very GC rich. The downstream sequence showed a potential donor splice site where the intron junction had been anticipated from the cDNA data (Fig. 1B). The alternate promoter was placed through restriction mapping to a position approximately 6kb upstream from the translation initiation codon.

To search for potential transcription factor binding sites, the TESS (25) combined and string search algorithms were employed to screen the promoter sequences against the TRANSFAC and TFDsite databases (33,34). Several putative transcription factor binding sites were apparent (35). Both the primary and alternate promoters were predicted to have sites for PU.1 (Fig. 1). With the exception of these sites, the two promoters were considerably different. In the primary promoter two Ets-1 sites were identified, which have also been shown to be functionally significant (22). In the alternate promoter several potential Sp1 and AP-2 sites were evident. Two c-Myb sites and a EGR2 site were identified, and in contrast to the primary promoter a putative Ets-2 site was present. Ets-1 and Ets-2 are expressed in a recipricol fashion in T cells depending on the state of activation (36). Two sites for the transcription repressor GCF were also apparent.

To confirm promoter function from the two identified regions, luciferase reporter constructs were generated by cloning the genomic DNA flanking the transcription initiation sites into the reporter vector pGL3 (see Materials and Methods). Since T-cells and platelets are the most obviously affected of the hematopoietic cells in patients with the WAS disorder, the promoter constructs were assayed for reporter activity in the HEL megakaryocyte-like erythroleukemia and Jurkat T-cell lines. The results showed that the 487 bp primary promoter fragment in pGL3-481 could promote expression at a high level compared to both the negative promoterless pGL3 vector (with no insert) and a positive SV40 control (Fig. 2). The pGL3-479 plasmid (primary promoter, 1.6kb insert) showed expression levels similar to pGL3-481, whereas the same promoter in reverse orientation (pGL3-482R) showed no activity (data not shown). As expected from the proportion of

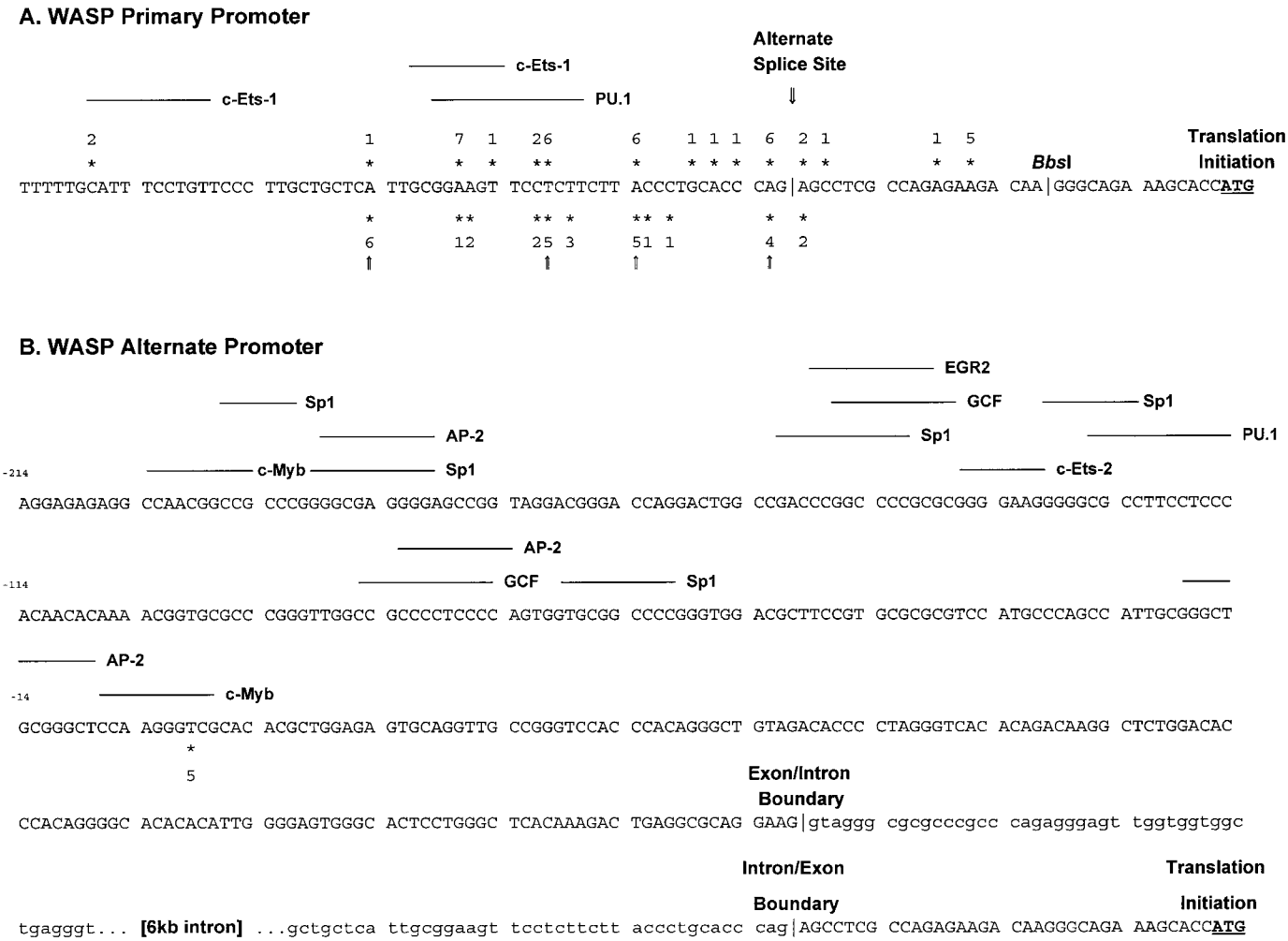

FIG. 1. Primary and alternate WASP promoter sequences, transcription initiation sites, and putative transcription factor binding sites. Transcription start sites as identified by sequence analysis of 5′RACE clones, are positioned with an asterisk at the determined initiation site. The asterisks above the sequence represent cDNA end clones isolated from EBV transformed B-cell lines, while those below the sequence were identified from the Jurkat T-cell line. The numbers at the asterisk positions indicate the number of end clones sequenced with that initiation site. The putative transcription factor binding sites as identified by the TESS search algorithm are indicated with horizontal lines above the sequence with the name of the factor to the right. In the primary promoter (A) the *Bbs*I restriction site utilized in cloning the pGL3-481 reporter construct is indicated upstream of the translation initiation site. The position of the alternate splice site that is used in transcripts generated from the alternate promoter is also indicated. Transcription initiation sites that most closely match the initiator consensus sequence are indicated with an arrow. In the alternate promoter (B), only one transcription initiation site is apparent and the downstream exon boundary/intron donor is shown. The 6kb intron resides between the alternate promoter and translation initiation site, and the intron/exon sequences are also shown for the acceptor site. The intron sequences are shown in lower case.

cDNA clones carrying the alternate exon sequence, expression from the alternate promoter construct pGL3-535 was present but considerably lower than that of the primary WASP promoter in both Jurkat and HEL cell lines. Both promoters showed increased activity in the HEL line, and the alternate promoter showed a less disparate level of expression.

DISCUSSION

The difference in transcription factor binding sites within the two promoters suggests that their activity may be affected by cell lineage and the stage of development or differentiation. Both c-Myb and Ets have been shown to be important in the transactivation of myeloid specific genes (37–40). PU.1 is the only transcription factor binding site found in both promoters. PU.1 is expressed in cells of the myeloid lineage and in B cells, however it is not expressed in T cells (41). The presence of these sites suggests that both promoters, but particularly the alternate promoter, may be more active in cells of the myeloid lineage. This may explain the lower levels of expression observed in the Jurkat T-cell line compared to that in HEL cells.

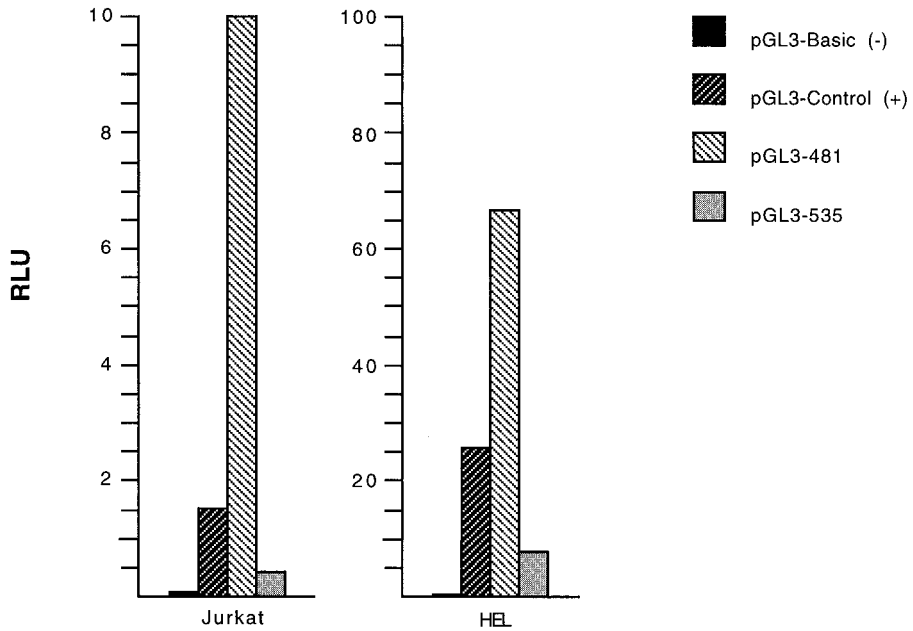

FIG. 2. Promoter activity measured from luciferase constructs in Jurkat T-cells and the HEL cell line. Promoter activity is reported in relative light units (RLU). Results are shown as a normalized value by dividing the readings from the pGL3 constructs (firefly luciferase reporter) by those produced by the pRL-TK internal control vector (*Renilla* luciferase). Expression was assayed at 18hrs. after transfection. Note the ten fold difference in the RLU scale for the level of expression in the Jurkat and HEL cell lines.

Both c-Myb and PU.1 are integral to hematopoietic development. Expression of c-Myb is highest in immature hematopoietic cells and decreases after differentiation (40). Targeted deletion of c-Myb in mice results in the failure of fetal liver hematopoiesis (42). PU.1 expression is restricted to specific stages of hematopoietic differentiation, depending on the cell lineage (41). Mutations in the murine PU.1 gene lead to defects in development of both the lymphoid and myeloid lineages, including progenitors for B and T cells, monocytes and granulocytes. (43). WASP expression is evident at very early stages of hematopoietic development (44). The requirement for WASP during early stages of hematopoiesis is demonstrated by the non-random X-inactivation pattern observed in early hematopoietic cell precursors (CD34+) of women who carry the Wiskott-Aldrich syndrome (45). Furthermore, WASP expression has been shown in the early embryonic stage of hematopoiesis within the CD34+ cell clusters of the aorta/gonad/mesonephros region (46). The presence of both c-Myb and PU.1 binding sites may indicate that the alternate promoter has a more important role in WASP expression in these earlier stages of hematopoiesis.

The completion of the sequence for the WASP gene will be useful in the continued analysis of mutations in WAS patients. In cases where no mutation has been identified, the elimination of undetected defects in the intervening sequences or the transcription control elements of the WASP gene will help to determine whether a second protein defect could be responsible for the WAS phenotype. A full characterization of the elements necessary for controlling WASP expression will be useful in the further analysis of the WAS defect and the eventual design of vectors for gene-therapy.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health grant AI31587 to S-P.K., who is the recipient of the National Institutes of Health Research Career and Development Award KO4-01044.

REFERENCES

1. Derry, J. M. J., Ochs, H. D., and Francke, U. (1994) *Cell* **78,** 635–644. Derry, J. M. J., Ochs, H. D., and Francke, U. Erratum *Cell* **79,** 923.
2. Kwan, S-P., Hagemann, T. L., Radtke, B. E., Blaese, R. M., and Rosen, F. S. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4706–4710.
3. Rivero-Lezcano, O. M., Marcilla, A., Sameshima, J. H., and Robbins, K. C. (1995) *Mol. Cell. Biol.* **15,** 5725–5731.
4. Banin, S., Truong, O., Katz, D. R., Waterfield, M. D., Brickell, P. M., and Gout, I. (1996) *Curr. Biol.* **6,** 981–988.
5. Bunnell, S. C., Henry, P. A., Kolluri, R., Kirchhausen, T., Rickles, R. J., and Berg, L. (1996) *J. Biol. Chem.* **271,** 25646–25656.
6. Cory, G. O. C., MacCarthy-Morrogh, L., Banin, S., Gout, I., Brickell, P. M., Levinsky, R. J., Kinnon, C., Lovering, R. C. (1996) *J. Immunol.* **157,** 3791–3795.
7. Finan, P. M., Soames, C. J., Wilson, L., Nelson, D. L., Stewart, D. M., Truong, O., Hsuan, J. J., and Kellie, S. (1996) *J. Biol. Chem.* **271,** 26291–26295.
8. She, H., Rockow, S., Tan, J., Nishimura, R., Skolnik, E. Y., Chen, M., Margolis, B., and Li, Wei. (1997) *Mol. Biol. Cell.* **8,** 1709–1721.

9. Wu, Y., Spencer, S. D., and Lasky, L. A. (1998) *J. Biol. Chem.* **273,** 5765–5770.
10. Miki, H., Miura, K., and Takenawa, T. (1996) *EMBO J.* **15,** 5326–5335.
11. Symons, M., Derry, J. M. J., Kariak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., and Abo, A. (1996) *Cell* **84,** 723–734.
12. Ponting, C. P., and Phillips, C. (1997) *J. Mol. Med.* **75,** 769–771.
13. Apenstrom, P., Lindberg, U., and Hall, A. (1996) *Curr. Biol.* **6,** 70–75.
14. Kolluri, R., Fuchs Tolias, K., Carpenter, C. L., Rosen, F. S., and Kirchhausen, T. (1996) *Proc. Natl. Acad. Science USA* **93,** 5615–5618.
15. Burbelo, P. D., Dreschel, D., and Hall, A. (1995) *J. Biol. Chem.* **270,** 29071–29074.
16. Remold-O'Donnell, E., Rosen, F. S., and Kenney, D. M. (1996) *Blood* **87,** 2621–2631.
17. Badolato, R., Sozzani, S., Malacarne, F., Bresciani, S., Fiorini, M., Borsatti, A., Albertini, A., Mantovani, A., Ugazio, A. G., and Notarangelo, L. D. (1998) *J. Immunol.* **161,** 1026–1033.
18. Binks, M., Jones, G. E., Brickell, P. M., Kinnon, C., Katz, D. R., and Thrasher, A. J. (1998) *Eur. J. Immunol.* **28,** 3259–3267.
19. Zicha, D., Allen, W. E., Brickell, P. M., Kinnon, C., Dunn, G. A., Jones, G. E., and Thrasher, A. J. (1998) *Br. J. Haematol.* **101,** 659–665.
20. Schwarz, K., *et al.* (1996) *Immunol. Today* **17,** 496–502.
21. Villa, A., Notarangelo, L., Macchi, P., Mantuano, E., Cavagni, G., Brugnoni, D., Strina, D., Patrosso, M. C., Ramenghi, U., Sacco, M. G., Ugazio, A., and Vezzoni, P. (1995) *Nat. Genet.* **9,** 414–417.
22. Petrella, A., Doti, I., Agosti, V., Carandente Giarrusso, P., Vitale, D., Bond, H. M., Cuomo, C., Tassone, P., Franco, B., Ballabio, A., Venuta, S., and Morrone, G. (1998) *Blood* **91,** 4554–4560.
23. Nizetic, D., Zehetner, G., Monaco, A. P., Gellen, L., Young, B. D., and Lehrachs, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3233–3237.
24. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucl. Acids Res.* **25,** 3389–3402.
25. Schug, J., and Overton, G. C. (1997) TESS: Transcription element search system on the WWW. Technical Report CBIL-TR-1997-1001-v0.0, of the Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania.
26. Weiss, A., Wiskocil, R. L., and Stobo, J. D. (1984) *J. Immunol.* **133,** 123–128.
27. Martin, P., and Papayannopoulou, T. (1982) *Science* **216,** 1233–1235.
28. Kwan, S-P., Lehner, T., Hagemann, T., Lu, B., Blaese, M., Ochs, H., Wedgwood, R., Ott, J., Craig, I. E., and Rosen, F. S. (1991) *Genomics* **10,** 29–33.
29. Kwan, S-P., Hagemann, T. L., Blaese, R. M., and Rosen, F. S. (1995) *Genomics* **29,** 247–252.
30. Kwan, S-P., Hagemann, T. L., Blaese, R. M., Knutsen, A., and Rosen, F. S. (1995) *Hum. Mol. Genet.* **4,** 1995–1998.
31. Mount, S. M. (1982) *Nucl. Acids Res.* **10,** 459–472.
32. Smale, S. T. (1997) *Biochim. Biophys. Acta* **1351,** 73–88.
33. Wingender, E. (1988) *Nucl. Acids Res.* **16,** 1879–1902.
34. Ghosh, D. (1991) *Trends Biochem. Sci.* **16,** 445–447.
35. Faisst, S., and Meyer, S. (1992) *Nucl. Acids Res.* **20,** 3–26.
36. Bhat, N. K., Thompson, C. B., Lindsten, T., June, C. H., Fujiwara, S., Koizumi, S., Fisher, R. J., and Papas, T. S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3723–3727.
37. Moscovici, C., Samarut, J., Gazzolo, L., and Moscovici, M. G. (1981) *Virology* **113,** 765–768.
38. Nunn, M. F., Seeburg, P. H., Moscovici, C., and Duesberg, P. H. (1983) *Nature* **306,** 391–395.
39. Ferrao, P., Macmillan, E. M., Ashman, L. K., and Gonda, T. J. (1995) *Oncogene* **11,** 1631–1638.
40. Gonda, T. J. (1998) *Int. J. Biochem. Cell Biol.* **30,** 547–551.
41. Hromos, R., Orazi, A., Neiman, R. S., Maki, R., Van Beveran, C. V., Moore, J., and Klemsz, M. (1993) *Blood* **82,** 2998–3004.
42. Mucenski, M. L., McLain, K., Kier, A. B., Swerdlow, S. H., Schreiner, C. M., Miller, T. A., Pietryga, D. W., Scott, W. J. Jr., and Potter, S. S. (1991) *Cell* **65,** 677–689.
43. Scott, E. W., Simon, M. C., Anastasi, J., and Singh, H. (1994) *Science* **265,** 1573–1577.
44. Parolini, O., Berardelli, S., Riedl, E., Bello-Fernandez, C., Strobl, H., Majdic, O., and Knapp, W. (1997) *Blood* **90,** 70–75.
45. Wengler, G., Gorlin, J. B., Williamson, J. M., Rosen, F. S., and Bing, D. H. (1995) *Blood* **85,** 2471–2477.
46. Thrasher, A. J., Jones, G. E., Kinnon, C., Brickell, P. M., and Katz, D. R. (1998) *Immunol. Today* **19,** 537–539.