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Identification of a large Myc-binding protein that contains RCC1-like repeats

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ABSTRACT **The protooncogene *MYC* plays an important role in the regulation of cellular proliferation, differentiation, and apoptosis and has been implicated in a variety of human tumors. *MYC* and the closely related *MYCN* encode highly conserved nuclear phosphoproteins (Myc and NMyc) that apparently function as transcription factors in the cell. We have identified a large and highly conserved nuclear protein that interacts directly with the transcriptional activating domain of Myc (designated "protein associated with Myc" or Pam). Pam contains an extended amino acid sequence with similarities to a protein known as regulator of chromosome condensation (RCC1), which may play a role in the function of chromatin. The gene encoding Pam (*PAM*) is expressed in all of the human tissue examined, but expression is exceptionally abundant in brain and thymus. Pam binds specifically to Myc, but not NMyc. The region in Myc required for binding to Pam includes a domain that is essential for the function of Myc and that is frequently mutated in Burkitt's lymphomas. *PAM* is located within a 300-kb region on chromosome 13q22.**

The *MYC* protooncogene is the archetype for a small gene family that also includes *MYCN*, *MYCL*, *MYCB,* and *MYCS*. The members of this family share four conserved domains: the Myc homology boxes I and II, a highly acidic region, and a composite known as the basic region/helix–loop–helix/leucine zipper domain (1–3). *MYC* has been implicated in the control of normal cellular proliferation, differentiation, and apoptosis (1–3). In addition, diverse forms of evidence indicate that *MYC* can contribute to tumorigenesis. Aberrant expression of the gene has been found in many human tumors, overexpression of the gene can act either alone or with the activated *H-RAS* oncogene to transform cells, and certain transgenes of *MYC* are tumorigenic (1–3).

Previous studies have suggested that the product of *MYC* (Myc) may function as a transcription factor (1–3). The N-terminal domain containing the Myc homology boxes is essential to transcriptional activation by Myc (1–3). The C-terminal basic region/helix–loop–helix/leucine zipper domain mediates dimerization of Myc with other proteins and binding to a specific site in DNA (1–3). Myc also interacts with a wide variety of other proteins. Some of these interactions have well-established functional consequences, others do not. For example, dimerization with a protein known as Max is essential for the binding of Myc to DNA (4). In addition, the C-terminal domain of Myc binds the transcription factors TFII-I, YY1, and AP-2 (5–7).

The N-terminal domain of Myc also interacts with several proteins. These include the tumor suppressor protein p107, which represses transcriptional activation by Myc (8); Bin1, another tumor suppressor protein, which binds to Myc box I and inhibits transformation by *MYC* (9), the TATA-box binding protein TBP (10); mitogen-activated protein kinase (11); and α-tubulin (12).

We now report the identification and characterization of a large nuclear protein that interacts with the transcriptional-activating domain of Myc ("protein associated with Myc" or Pam). The gene encoding Pam (*PAM*) is contained within a 300-kb domain at chromosome 13q22, which also carries a recently identified gene *CLN5* responsible for a variant form of late infantile neuronal ceroid lipofuscinosis (13).

MATERIALS AND METHODS

Cell Cultures. Normal human aortic endothelial cells (HAEC) were from Clonetics (San Diego) and cultured in endothelial cell growth medium (EGM) (Clonetics) with 2% fetal bovine serum. CB33-Myc cell is an Epstein–Barr virus-transformed lymphoblastoid cell line overexpressing Myc from a transfected *MYC* gene (8). Tissue culture cell lines Hela S3, JAR, and JEG-3 were from the American Type Culture Collection.

Construction of Plasmids. The *Eco*RI–*Ava*I insert of the plasmid pSP65-cMyc (14) was cloned into the *Ava*I site of pGEX-2TK vector (Pharmacia) to make the plasmid pGST-Myc1. An *Eco*RI linker was inserted into the *Xho*I site at the 5′ end of *MYCN* in the plasmid pcN64RX (15), and the insert then was cloned into the *Eco*RI site of pGEX-3X vector (Pharmacia) to make the plasmid p3X-NMyc. The insert of p3X-NMyc was cut out with *Bam*HI and cloned into the *Sma*I site of pGEX-2TK vector (Pharmacia) to make the plasmid pGST-NMyc1.

To create a series of C-terminal truncation mutants of Myc protein, the plasmid pGST-Myc1 was linearized with *Sac*II and treated with *Bal*31Slow (IBI/Kodak). The *Bal*31Slow-treated plasmids were ligated, transformed into bacteria, and examined by restriction digests and sequencing. Plasmids pGST-Myc5–10 constructed were selected for protein expression in bacteria. Plasmid pGST-Myc3 was made by cloning the *Eco*RI–*Tth*3I fragment of pSP65-cMyc plasmid into the *Ava*I site of pGEX-2TK. Plasmids pGST-Myc2, 4, and 11–13 were cloned in pGEX-2TK by PCR with Vent DNA Polymerase (NEB, Beverly, MA).

Cloning of Human *PAM* cDNA. The glutathione *S*-transferase (GST)-Myc1 and GST-NMyc1 fusion proteins were ^{32}P-labeled and used to screen a human λgt11 cDNA expression library made from the Akata cell line as described

Abbreviations: GST, glutathione *S*-transferase; PAC, P1-derived artificial chromosome; PAM, protein associated with Myc; RCC1, regulator of chromosome condensation; YAC, yeast artificial chromosome.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF075587).

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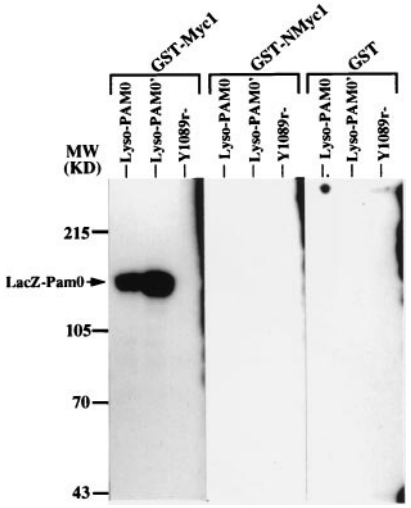

FIG. 1. Pam binds specifically to Myc but not NMyc. Lyso-PAM0 and Lyso-PAM0′ represent two lysogens isolated from the positive phage λgt11- PAM0 by using the host bacterial strain Y1089r-. Equal amounts of extracts from Lyso-PAM0, Lyso-PAM0′ or host bacteria Y1089r- were fractionated by electrophoresis through a PAGE gel. The proteins then were transferred to a nitrocellulose filter, and the filter was incubated with various probes.

(16). The lysogens were isolated by using λgt11-PAM0 phage and *Escherichia coli* starin Y1089r-, and the cell lysate was prepared as described (17). The conditions for the Far-Western hybridization were the same as those used for the original screening.

Human cDNA libraries made from Akata cells, T cells, testis, Raji cells, placenta, or brain were screened sequentially with the 5′-most or 3′-most cDNA fragments of the identified portion of *PAM* gene. After 13 rounds of screening, 15 kb of cDNA sequence was obtained and sequenced in both orientations.

The database searches and motif identifications were carried out by using the BLAST program. The sequence comparison and multiple-sequence alignment were carried out by using the programs of the Wisconsin Package Version 9.0, Genetics Computer Group (Madison, WI).

Immunofluorescence Staining and Immunoprecipitations. The protein GST-Ab1 containing residues 4312–4641 of Pam was expressed in bacteria and used to raise antibodies in rabbits. The antisera were depleted with GST and then affinity-purified by using GST-Ab1 on glutathione agrose beads. Cells were fixed in cold methanol and permeablized in 0.1% Triton X-100. The secondary antibody was the Cy3-conjugated goat antibody against rabbit IgG.

For immunoprecipatation, 1 ml of Hela nuclear extract (6 mg/ml, from Upstate Biotechnology) was incubated with 50 μg of random rabbit IgG and 100 μl of Protein A/G PLUS-Agrose beads (Santa Cruz Biotechnology) at 4°C for 2 hr. After the IgG bound to the beads was removed, 200 μl of the depleted Hela nuclear extract aliquots was incubated with the primary antibodies Ab1 (anti-Pam), anti-c-Myc (Upstate Biotechnology), cv3 (anti-c-Myc), or random IgG. After 20 μl of Protein A/G agrose was added to the nuclear extract and incubated for another hour at 4°C, the agarose beads were washed with cold PBS four times. The immunoprecipitated proteins were fractionated on a PAGE gel and analyzed by Western blot.

P1-Derived Artificial Chromosome (PAC) and Yeast Artificial Chromosome (YAC) Clones. YAC clones containing the genomic *PAM* gene were selected by screening the whole genome YAC library (18) by using oligos in *PAM* gene CCTACTGTGGGAATCTGACCTC and CTGCCCGT-GAAGAGGCGGGCATG. The chromosomal locations for the YACs identified were obtained via the human genome web site (http://www-genome.wi.mit.edu). PAC clones were ob-

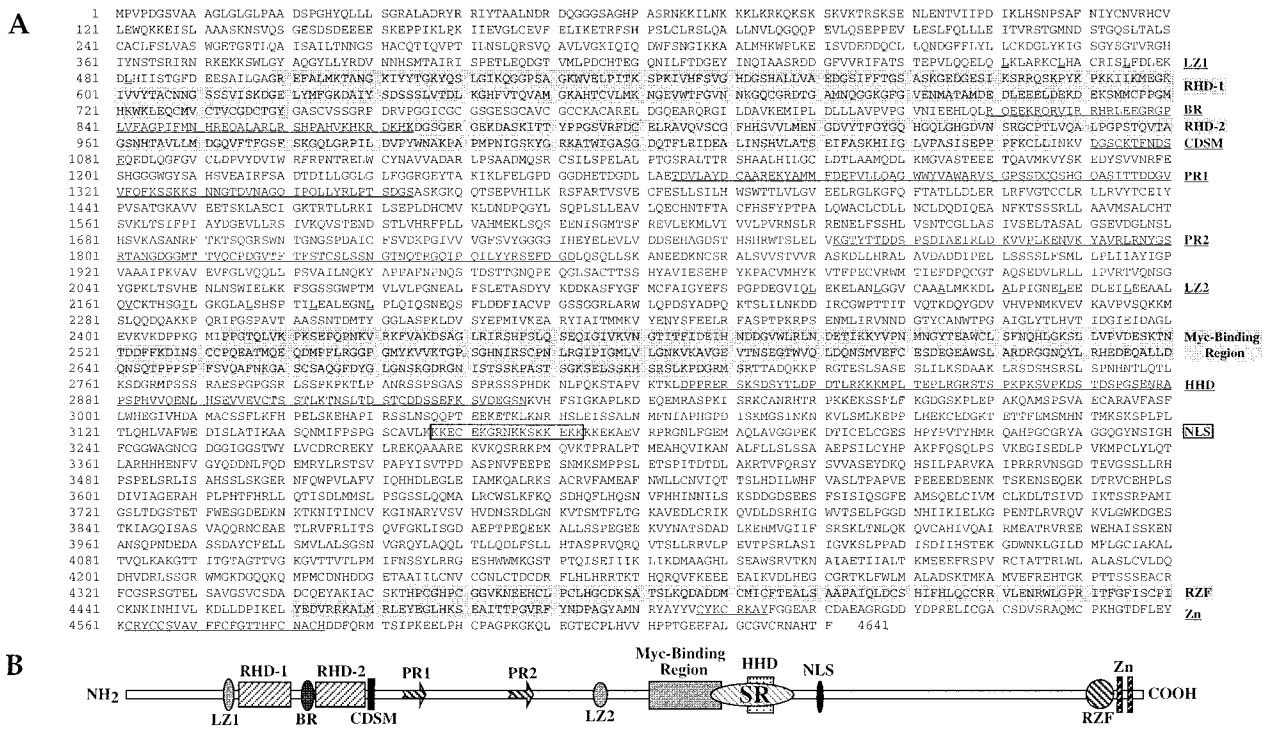

FIG. 2. The structure and conservation of Pam. (*A*) Amino acid sequence of human Pam predicted from overlapping cDNA clones. The sequence includes the following motifs: LZ1 and LZ2, potential leucine zippers; RHD-1 and RHD-2, RCC1 homology domains; PR1 and PR2, Pam repeats; CDSM, cell division sequence motif; Myc-binding Region, insert in the original phage λgt11-PAM0; HHD, histone-binding protein homology domain; NLS, putative nuclear localization signal; RZF, potential ring zinc finger domain; and Zn, two putative C2H2-type zinc finger motifs. (*B*) Topography of human Pam. All abbreviations are as in *A,* except for SR, a serine-rich region occupying residues 2643–3057 (20% serine).

tained from the BacPac Resource Center at Roswell Park Cancer Institute, Buffalo, NY.

RESULTS

Identification of Pam by Protein Interaction Screening. To search for proteins that bind to the transcriptional-activating domains of Myc and NMyc, we fused N-terminal domains of the two proteins to the C terminus of GST, creating the plasmids pGST-Myc1 and pGST-NMyc1. The chimeric proteins were expressed in bacteria and ^{32}P-labeled as described in *Materials and Methods*. A mixture of the labeled proteins then was used to screen a λgt11 cDNA expression library constructed from the RNA of the Akata Burkitt's lymphoma cell line. Two positive phages were identified. The positive phages were not binding to the GST portion of the probes (data not shown). Nucleotide sequencing revealed that these phages contain identical inserts of 902 bp, which encode an ORF of 300 aa. This ORF is fused to and in-frame with the LacZ protein encoded by the λgt11 vector and contains neither a suitable start nor stop codon. Database searches failed to uncover any previous sightings of the ORF. We designated the encoded protein as Pam for "protein associated with Myc" and the phage containing the 902-bp insert as λgt11-PAM0.

Pam Binds to Myc but Not NMyc. We explored the specificity of Pam binding in three ways. First, we demonstrated that plaques of λgt11-PAM0 hybridized to GST-Myc1, but not to GST-NMyc1 (data not shown). Second, we removed the GST portion from the Myc and NMyc probes by cleavage with thrombin; the pattern of binding to λgt11-PAM0 remained the same (data not shown). Third, we used GST-Myc1, GST-NMyc1, and the GST alone as probes in Far-Western blots (Fig. 1). Only the Myc probe bound to the LacZ fusion protein made from λgt11-PAM0. We conclude that the interaction between Myc and Pam is sufficiently specific to exclude detectable binding to NMyc.

PAM Encodes a 510-kDa Protein with a Domain Related to Regulator of Chromosome Condensation, RCC1. To obtain a full-length cDNA representing the gene encoding Pam, we screened several cDNA libraries and isolated overlapping clones covering 15 kb of *PAM* cDNA. Sequencing of the overlapping cDNAs revealed an ORF of 4,641 aa with a predicted mass of 510 kDa (Fig. 2*A*). The encoded protein has several notable features (Fig. 2*B*): a ring zinc-finger motif (RZF) and two zinc-finger motifs (Zn); a bipartite nuclear localization signal (NLS); two putative leucine zipper motifs (LZ1 and LZ2); a cell division sequence motif (CDSM), found in a variety of viral and cellular proteins that have been implicated in control of the cell cycle (19); and a large region (residues 498-1065) exhibiting significant homology to a protein known as RCC1 (20). The original fragment of *PAM* accounting for 300 aa isolated by interaction screening encodes the middle of Pam; this fragment must contain a domain responsible for binding to Myc, but we have yet to localize that domain with any greater resolution.

The resemblance to RCC1 deserves further comment. RCC1 contains a motif of 50–60 aa that is repeated seven times in tandem. These repeats form a seven-bladed propeller structure as determined recently by x-ray crystallography (21). A similar 7-fold repeat is present in Pam, but is divided into two elements (RHD-1 and RHD-2) by an insertion of 134 aa after the fourth repeat (Fig. 2 and Fig. 3 *A* and *B*). The insertion between RHD-1 and RHD-2 contains a C-terminal region of 55 aa that is rich in basic amino acids (BR in Fig. 2*A*). Such a short basic region (40–50 aa) also is present in RCC1 proteins at their N termini, which are important for chromatin binding (20). Although most RCC1 proteins end with a repeated element, the *Drosophila* RCC1 protein BJ1 has a substantial C-terminal extension, which has limited homology to chromatin proteins such as *Xenopus* histone-binding protein

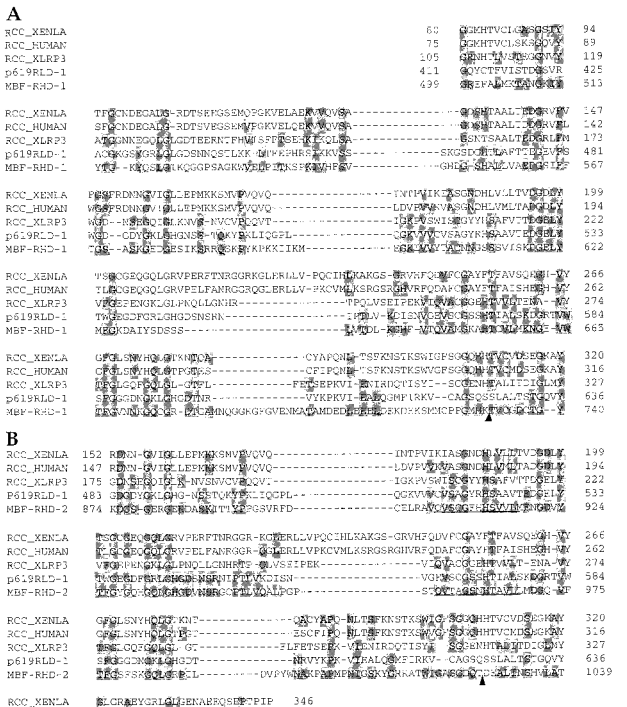

FIG. 3. Sequence alignments of the RCC1 homology domains (RHD-1 and RHD-2) and two additional direct repeats (PR1 and PR2). (*A*) Alignment of RHD-1 of Pam with RCC1 proteins. RCC_XENLA and RCC_HUMAN are the RCC1 proteins from *Xenopus* and human (P25183 and P18754). RCC_XLRP3 is the X-linked retinitis pigmentosa 3 protein (O92834), which belongs to the RCC1 family. p619RLD-1 is the first RCC1-like domain in p619 protein (U50078). The underlining indicates the signature sequences of RCC1 family (PS00625 and PS00626). The arrow indicates the amino acid insertion of WKLEQCMVC. (*B*) Alignment of RHD-2 of Pam with RCC1 proteins. The arrow indicates the amino acid insertion of FLRI. All details are as in *A*. (*C*) Alignment of the two direct repeats of human Pam (PR1 and PR2).

N1/N2 (20). Pam contains a similar region (designated HHD, see Fig. 2*A*), situated in the midst of a serine-rich domain (SR) and in the vicinity of the Myc-binding domain, but relatively distant from RHD-1/2 (see Fig. 2*B*).

In addition to the RCC1 repeats, Pam contains two additional direct repeats of 91 aa (PR1 and PR2, Fig. 3*C*). These two repeats are 407 aa apart.

By searching several databases, we uncovered expressed sequence tags (ESTs) from the mouse and rat genomes that are represented within *PAM*, as well as a complete homologue of *PAM* in *Caenorhabditis elegans* (GenBank accession no. U53147). The sequences of human and *C. elegans* Pams display 32% identity and 55% similarity (data not shown). There are focal regions of exceptional conservation, but similarity is distributed throughout the whole ORF. The most conserved C-terminal region of 324 aa shows 55% identity and 73% similarity. Because the homologue in *C. elegans* was uncovered by genomic sequencing rather than genetic analysis, nothing is yet known of its function.

The rodent expressed sequence tags represent only portions of Pam, but are extremely conserved when compared with each other and to human Pam. The mouse ESTs cover the following residues in human Pam: 2575–2810 (90% identity, 93% similarity), 2912–3152 (94% identity, 97% similarity), 4061–4268 (99% identity, 100% similarity), and 4458–4641 (98% identity,

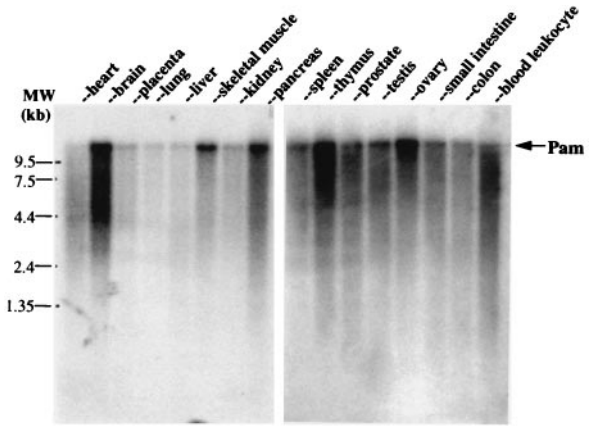

FIG. 4. Pam RNA in human tissues. Northern blots prepared with RNAs from multiple human tissues were obtained from CLONTECH (MTN I and II). The filters were analyzed with radioactive probe prepared from *PAM* cDNA following the manufacturer's instructions.

99% similarity). The accession numbers for the mouse *PAM* ESTs are W44173, R75243, AA727659, AA689905, AA545634, AA547620, AA414366, AA734728, AA119999, AA647417, and AA174369.

***PAM* Is Expressed in Many Tissues but Most Abundantly in Brain and Thymus.** We examined the expression of *PAM* by performing Northern blots with polyadenylated RNA from various human tissues (Fig. 4). A single RNA with a size of approximately 15 kb was detected in all tissues examined. The level of expression was relatively low in heart, placenta, lung, liver, kidney, spleen, prostate, testis, small intestine, colon, and peripheral blood leukocytes; somewhat higher in skeletal muscle, pancreas, and ovary; and highest in brain and thymus.

Pam Is Located in the Nucleus and Binds to Myc in Cellular Extracts. We prepared a polyclonal rabbit antiserum against the C terminus of Pam and purified the antiserum by affinity chromatography on the antigen. Immunostaining with the antiserum revealed strong nuclear fluorescence in interphase nuclei of normal human aortic endothelial cells (Fig. 5 *A–C*) and cultured cells of human choriocarcinomas (lines JAR and JEG-3; data not shown). In contrast, the fluorescence dispersed throughout the whole cell during mitosis, when the nuclear envelope is disintegrated (Fig. 5 *D–F* for a telophase cell).

Knowing that Pam is located in the nucleus during interphase, we examined the interaction between Pam and Myc in nuclear extracts. We used two experimental tactics. First, Myc protein in extracts could be bound to GST-Pam0 and then

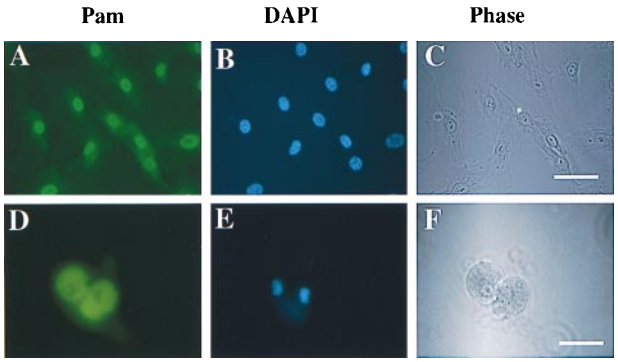

FIG. 5. Subcellular localization of Pam. Immunofluorescence microscopy was performed on normal human aortic endothelial cells with antibody directed against Pam. (*A*) and (*D*) Immunofluorescence with Pam-specific antiserum. (*B*) and (*E*) Staining with 4′,6-diamidino-2-phenylindole (DAPI). (*C*) and (*F*) Phase contrast microscopy. The bars represent 40 μm in *C* and 20 μm in *F*.

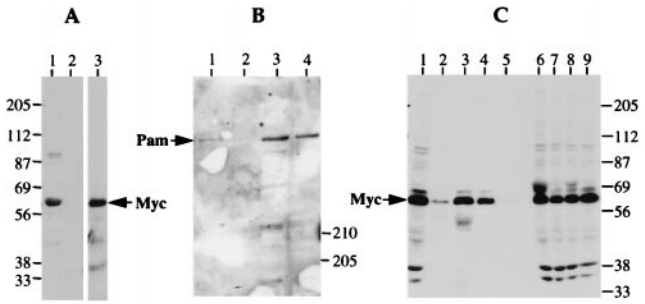

FIG. 6. Myc interacts with Pam *in vivo*. (*A*) Binding of Myc to GST-Pam0. GST and GST-Pam0 proteins were incubated with a nuclear extract of CB33-Myc cells, recovered on glutathione agarose beads, and analyzed by Western blotting by using antibody directed against Myc (9E10). Lane 1, binding with GST-Pam0 protein. Lane 2, binding with GST protein. Lane 3, total nuclear extract of CB33-Myc cells. (*B*) Binding of Pam to GST-Myc1. Purified GST or GST-Myc1 protein was incubated with a nuclear extract of Hela cells, recovered on glutathione agarose beads, and analyzed by Western blotting with an antiserum against Pam. Lane 1, binding with GST-Myc1 protein. Lane 2, binding with GST protein. Lane 3, the supernatant after adsorption of the nuclear extract with GST protein. Lane 4, the supernatant after adsorption of the nuclear extract with GST-Myc1 protein. (*C*) Coprecipitation of Pam and Myc from nuclear extracts of Hela cells. Extracts were subjected to immunoprecipitation with various antisera. The precipitates were analyzed by Western blotting with antiserum against Myc (9E10). Lane 1, total nuclear extract. Lanes 2–5, immunoprecipitates. Lanes 6–9, supernatants after immunoprecipitation. Lanes 2 and 6, immunoprecipitation with anti-Pam. Lanes 3 and 7, polyclonal anti-Myc from UBI. Lanes 4 and 8, polyclonal anti-Myc prepared by investigators (cv3). Lanes 5 and 9, nonspecific rabbit IgG.

recovered on beads coated with glutathione (Fig. 6*A*). Similarly, Pam could be recovered from nuclear extracts by incubation with GST-Myc1 (Fig. 6*B*). No binding could be detected between GST itself and either Myc or Pam (Fig. 6 *A* and *B*). Second, Myc coprecipitated with Pam when the latter was recovered by reaction with the specific antiserum described above (Fig. 6*C*). We were unable to perform the reciprocal experiment, possibly because of the low abundance of Pam.

Pam Binds to a Region of Myc that Is Important for Transactivation of Transcription and that Is Frequently Mutated in Burkitt's Lymphomas. To localize the regions in Myc important for binding to Pam, we constructed a series of truncations of Myc and then examined their binding to LacZ-Pam0 in Far-Western blots (Fig. 7). Representative data are illustrated in Fig. 7*A* and summarized in Fig. 7*B*. Short truncations from the N terminus, up to the border of the Myc box I domain, did not disturb the binding of Pam (Myc11, 12). In contrast, those truncations that extended into the Myc box I domain eliminated binding to Pam (Myc13). Truncations extending from the C terminus to residue 154 did not affect binding to Pam (Myc1–6). C-terminal truncations beyond amino acid 154 (Myc7–9) reduced but did not eliminate the binding, whereas truncations from the C terminus to amino acid 75 (Myc10) abolished all detectable binding.

In aggregate, the data indicate that the portion of Myc between amino acids 44 and 107 is essential for binding to Pam (Fig. 7*B*). This region contains the highly conserved Myc homology box I. Residues 140–154 also contribute to maximal binding, because the extent of binding drops substantially when this portion of Myc is removed. The Pam-binding region in Myc is important for the transcriptional-activating activity of Myc (1–3) and is frequently mutated in Burkitt's and AIDS-associated lymphomas (2).

***PAM* Is Located at Chromosome 13q22.** We mapped the chromosomal location of *PAM* by screening a human genomic YAC library. The four YAC clones identified through the screening all have been localized to chromosome 13q22–31

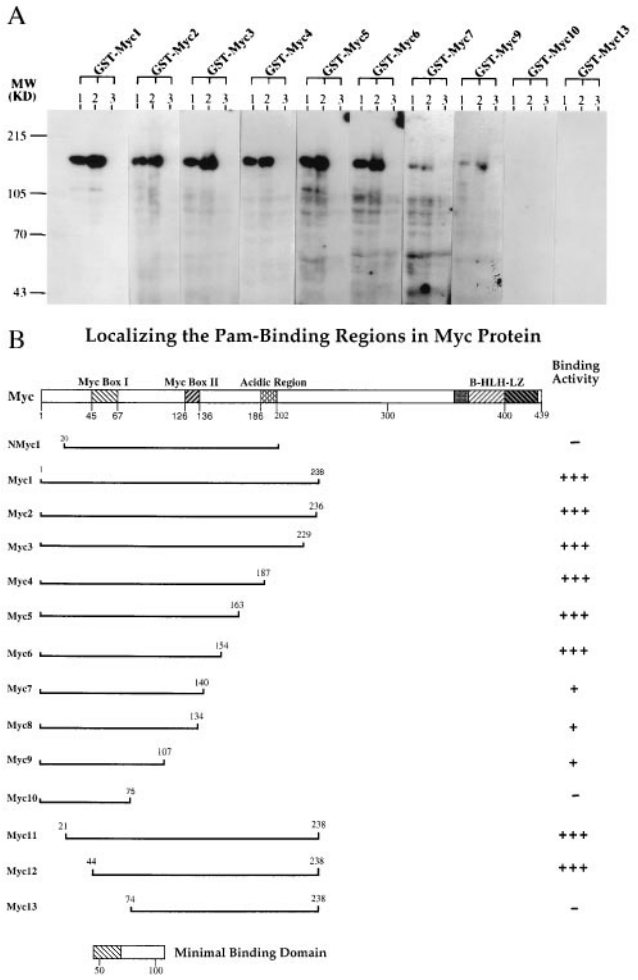

FIG. 7. Localization of the Pam-binding site within Myc. (*A*) Binding of ^{32}P-labeled truncations of GST-Myc to LacZ-Pam0. Binding was assayed by using the Far-Western assay. (*B*) Summary of results. The binding data for some of the truncation mutants listed here are not shown in *A*. The numbers indicate the amino acid residues in human Myc.

(854b5, 852g2, 885a10, and 946c1). This region contains the *CLN5* gene, which is defective in a variant form of the human disease late infantile neuronal ceroid lipofuscinoses (13). The YAC that harbors the *CLN5* locus is identical to YAC 852g2, which contains sequences of *PAM*. To further authenticate the location of *PAM*, we compared the restriction patterns of YAC 852g2 and human genomic DNA when they were probed with *PAM* cDNA. The patterns obtained with 852g2 YAC were the same as those obtained with human genomic DNA (data not shown).

The location of *CLN5* has been refined to a 300-kb region between markers AC224 and COLAC1, covered by several PAC clones (see Fig. 8; ref. 13). We explored whether *PAM* is localized to this 300-kb region by hybridizing *PAM* cDNA probes to the restriction fragments of these PACs. A 3′ cDNA probe of *PAM* (3HP3) hybridized with the restriction fragments from PAC76n15, whereas a cDNA probe near the 5′ end (T25) hybridized with the restriction fragments of PAC189o20 (data not shown). These results demonstrate that the transcription of *PAM* must proceed from right to left along the *CLN5* locus as displayed in Fig. 8.

Recently, PAC264j2 and PAC76n15 were sequenced by the Whitehead Institute Genome Center (Cambridge, MA) (GenBank accession nos. AC001226 and AC000403). These two PACs overlap by 17 kb and cover a total of 199 kb of genomic

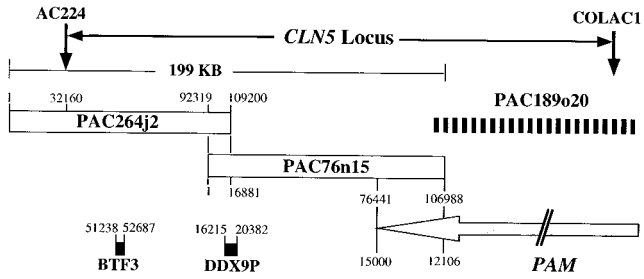

FIG. 8. Mapping the *PAM* gene to the chromosome 13q22 region between markers AC224 and COLAC1. BTF3 gene and a pseudogene for RNA helicase A (DDX9P) were identified by random sequencing of PAC224a14 (22).

DNA. The AC224 marker is located within the PAC264j2 clone (nucleotides 32152–32173), whereas the COLAC1 marker lies outside the PAC264j2/PAC76n15 region (see Fig. 8). Sequence comparison between *PAM* cDNA and the sequenced genomic DNA in this region confirmed that *PAM* is located in this region as displayed in Fig. 8. The 3-kb cDNA at the 3′ end of the *PAM* gene lies in the 30-kb terminal region of PAC76n15. The rest of the *PAM* cDNA lies in the nonsequenced genomic region in PAC189o20 or beyond.

These analyses indicate that *PAM* is located on PAC76n15 and PAC189o20 between the markers AC224 and COLAC1 at the *CLN5* locus. To the best of our knowledge, however, *PAM* is not equivalent to *CLN5*.

DISCUSSION

We have identified a large nuclear protein, Pam, that interacts with a functionally important region of the protein encoded by the *MYC* protooncogene. *PAM* apparently is expressed in all of the tissues we examined, but expression is especially abundant in brain and thymus. *PAM* is located within a 300-kb domain at chromosome 13q22, in the vicinity of the recently identified *CLN5* gene (13).

Pam Binds to a Functionally Important Region of Myc. We have localized the site of Pam binding to a 64-aa region within Myc that includes the highly conserved Myc homology box I. The Myc homology box I is essential but not sufficient for Pam binding: the C-terminal truncation Myc10 leaves the box intact, yet abolishes the binding (see Fig. 7). For maximal binding, an additional region between amino acids 107 and 154 is required. This sequence might be essential for the correct folding of Myc or provide a second contact with Pam.

There is circumstantial evidence that the binding of Pam to Myc may be functionally significant. First, the binding occurs to a region of Myc that is essential for transcriptional activation by Myc. Second, point mutations cluster in this region of the translocated alleles of Myc found in Burkitt's and AIDS-associated lymphomas (2). These mutations are thought to augment the tumorigenicity of the translocated gene and bespeak a functional role for the affected regions of Myc (2). We hypothesize that the binding of Pam plays a role in transcriptional activation by Myc, either as facilitator or regulator.

Pam Binds to Myc but Not NMyc. Myc and NMyc are closely related proteins that share a number of biochemical functions, including binding to the same specific site in DNA (1–3, 23) and dimerization with the Max protein (4, 24). Both Myc and NMyc can induce S-phase DNA synthesis in established lines of rodent fibroblasts (25, 26), elicit extended proliferation of normal rodent fibroblasts (1, 27), transform Rat-1 cells (14, 28), and cooperate with *H-RAS* to transform embryonic rat fibroblasts (1, 29).

There are reasons to believe, however, that Myc and NMyc have different biological functions *in vivo*. First, the pattern of

expression differs for the two genes in both embryos and adults (30–33). Second, aberrant expression of *MYC* is associated with a large number and variety of human tumors, whereas *MYCN* has been implicated in a much more limited set of tumors (1–3). Third, homozygous deficiencies of either *MYC* or *MYCN* in mice are embryonic lethals (33–35). Thus, *MYC* and *MYCN* are not functionally redundant.

The biological differences between *MYC* and *MYCN* could be solely because of the different patterns of expression. But the specificity of binding between Myc and Pam suggests that an additional explanation for functional differences between *MYC* and *MYCN* may emerge once the function of the binding is understood.

The Myc homology box I is virtually identical between Myc and NMyc (20 of 23 amino acids in box I are the same). Because a truncation of Myc that leaves the Myc box I intact cannot bind to Pam (see above), it is likely that the specificity of Pam binding is determined by regions outside of the box.

RCC1 Motifs May Provide a Clue to the Function of Pam. Pam is located in the nucleus during interphase of the cell cycle, but disperses throughout the whole cell during mitosis, when the nuclear envelope is disintegrated and the condensed chromatin is neither transcribed nor replicated. Several motifs in Pam, such as leucine zippers, zinc fingers, putative histone-binding protein homologous domains, and the RCC1 motifs, suggest that the function of Pam may involve DNA binding and chromatin. Among these features, the RCC1 motifs occupy the largest region in Pam (≈500 aa). RCC1 may have multiple functions, but these remain poorly documented (20). One unifying hypothesis is that RCC1 may serve to either evoke or detect conformational changes of chromatin during the cell cycle (20). As a transcriptional activator, Myc must interact with chromatin. Pam may play a role in that interaction. The presence of a well-conserved Pam in *C. elegans* offers the prospect for a genetic analysis of the protein's function.

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