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Biochemistry

Identification of the protein encoded by the human diffuse B-cell lymphoma (*dbl*) oncogene

(hybrid-selected translation/phosphoprotein/subcellular distribution/phosphoserine)

SHIV K. SRIVASTAVA, ROBERT H. P. WHEELOCK, STUART A. AARONSON*, AND ALESSANDRA EVA

Laboratory of Cellular and Molecular Biology, National Cancer Institute, Building 37, Room 1E24, Bethesda, MD 20892

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ABSTRACT **The *dbl* oncogene was initially isolated from a human diffuse B-cell lymphoma. Antisera from mice bearing tumors induced by this oncogene specifically detected a protein of about 66 kDa (p66) in *dbl* transformants. *dbl* cDNA-selected poly(A)$^+$ RNA isolated from a transfectant clone expressing p66 directed the *in vitro* synthesis of this protein, establishing that it is encoded by *dbl*. Subcellular localization studies revealed that p66 is a cytoplasmic protein distributed between cytosol and crude membrane fractions. Moreover, p66 was shown to be a phosphoprotein, with phosphorylation specific to serine residues. Our characterization of the *dbl*-encoded protein appears to distinguish this transforming gene product from those of other known oncogenes.**

DNA-mediated gene-transfer techniques have led to the detection of cellular sequences of human origin capable of inducing malignant transformation of NIH 3T3 cells. As many as 10–50% of human tumors and tumor cell lines score as positive in this assay. The great majority of transforming genes detected by this approach have been members of the *ras* family (for review, see refs. 1 and 2). However, a variety of other genes—including *B-lym* (3), *met* (4), NK 14 (5), *mcf-2* and *mcf-3* (6), *neu* (7), *dbl* (8), *ret* (9), *raf* (10), and *trk* (11)—have also been identified. Products of several of these transforming genes appear to be structurally related to those of the tyrosine kinase family of oncogenes (11–14). *B-lym* has been reported to share homology with the transferrin gene, although recent studies indicate a much greater homology to a mouse long interspersed repetitive sequence family (15, 16).

The *dbl* oncogene was initially detected as a transforming gene of a human diffuse B-cell lymphoma and was isolated as a 45-kilobase-pair transforming human DNA sequence by cosmid cloning (8). By molecular hybridization, *dbl* lacks detectable homology with a large number of cellular or retroviral oncogenes (8), including members of the tyrosine kinase family. Our present studies were undertaken in an effort to identify the *dbl* oncogene product and to characterize some of its structural and functional properties.

MATERIALS AND METHODS

Cell Culture. NIH 3T3 cells as well as *dbl* transfectants of NIH 3T3 cells have been described (8). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum.

Metabolic Labeling of Cells. Subconfluent cultures containing $\approx 5 \times 10^6$ cells were labeled with [^{35}S]methionine or [^{32}P]orthophosphoric acid as described (17). Labeling of cells with [*methyl*-^{3}H]thymidine (80 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) at 20 μCi/ml was carried out for 4 hr.

Antisera. Two- to six-week-old NFS mice were inoculated with 10^7 cells of *dbl* transfectant clone 109-6-3-4 by intramuscular, intraperitoneal, or subcutaneous routes. Sera from tumor-bearing mice were collected 3–4 weeks later. Other sera were obtained from mice injected with similar numbers of NIH 3T3 cells or NIH 3T3 cells transformed by the Hs242 (Ha-*ras*) oncogene (18).

Immunoprecipitation, Immunoblotting, and NaDodSO$_4$/PAGE. Procedures for immunoprecipitation and gel electrophoresis have been described (17). In brief, $\approx 5 \times 10^6$ cells were lysed in 1 ml of lysis buffer [10 mM sodium phosphate, pH 7.4/1% Triton X-100/0.1% NaDodSO$_4$/0.5% sodium deoxycholate/0.1 M NaCl/1 mM phenylmethylsulfonyl fluoride/aprotinin (100 kallikrein-inactivator units per ml)]. Clarified extracts were immunoprecipitated using *dbl* tumor-bearing mouse (*dbl* TBM) sera or other antisera as described for individual experiments. Immunoprecipitates were recovered on protein A-Sepharose (Pharmacia) beads. Immunocomplexes were washed with 1 ml of lysis buffer and treated with 1 ml of 50 mM Tris, pH 7.5/1 M $MgCl_2$ for 15 min with continuous shaking at 4°C. Finally, immunoprecipitates were washed twice with 1 ml of lysis buffer, solubilized in electrophoresis sample buffer, and resolved by NaDodSO$_4$/8% PAGE. After electrophoresis, gels were processed as described (17). For immunoblot analysis, unlabeled cell lysates were processed for immunoprecipitation and gel electrophoresis as described above, and proteins were transferred (19) onto nitrocellulose membranes (0.1-μm pore size, Schleicher & Schuell) for probing with antisera. ^{125}I-labeled protein A was used to localize immunocomplexes.

Subcellular Fractionation. Cell fractionation was performed by a modification of the method described by Robbins *et al.* (20). Cultures of NIH 3T3 *dbl* transfectants representing $\approx 5 \times 10^7$ cells were washed with phosphate-buffered saline and suspended in 1.25 ml of 1 mM Tris (pH 7.5) containing 0.01% aprotinin. Cells were disrupted with 25–30 strokes in a Dounce homogenizer as described (20). Nuclei and cell debris were removed by centrifugation at 600 $\times$ *g* for 15 min, and the pellet was washed twice by centrifugation in the above buffer (crude nuclear fraction). The supernatant was fractionated into soluble (S-100) and particulate (P-100) fractions as described (20). All fractions were made up to a volume of 1 ml with the above buffer and treated with an equal volume of 2$\times$ lysis buffer. The lysates from different fractions were processed for immunoprecipitations and immunoblotting as above. Aliquots from various fractions (labeled with [^{3}H]thymidine) were analyzed for trichloroacetic acid-precipitable ^{3}H by liquid scintillation counting.

Phospho Amino Acid Analysis. The ^{32}P-labeled pp66 band was cut from the gel and electroeluted. Electroeluted samples were acid-hydrolyzed and analyzed for phospho amino acids by thin-layer electrophoresis and chromatography in the first and second dimensions, respectively (21).

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Abbreviations: TBM, tumor-bearing mouse; kb, kilobase(s).

*To whom reprint requests should be addressed.

RNA Purification and Blot Hybridization. Total RNA and poly(A)$^+$ RNA were isolated as described (22–24). Poly(A)$^+$ RNAs (2 μg per lane) were denatured in 50% formamide/2.2 M formaldehyde at 60°C for 5 min, size-fractionated in 1% agarose gel containing 2.2 M formaldehyde, and transferred to nitrocellulose filters as described (25). *dbl*-specific RNA sequences were identified by hybridization with nick-translated *dbl* DNA fragment (8) at 2.5 × 10^6 cpm/ml. Hybridization conditions were as described (25).

Hybrid-Selected Translation. Hybrid-selected translation was performed as described by Miller *et al.* (26). Five micrograms of ≈1.8-kilobase pair *dbl* cDNA insert was bound to diazobenzyloxymethyl (DBM) circles and prehybridized in 10 mM Pipes, pH 6.4/0.4 M NaCl/65% formamide for 1 hr at 50°C. Ten micrograms of poly(A)$^+$ RNA isolated from individual NIH 3T3 *dbl* transfectants was then hybridized to *dbl* cDNA prebound to DBM circles for 12 hr at 50°C. Following a wash with 75 mM NaCl/7.5 mM sodium citrate, pH 7/0.5% $NaDodSO_4$ at 65°C, the poly(A)$^+$ RNA bound to *dbl* cDNA was eluted in 1 mM EDTA at 100°C. The eluted mRNA was translated *in vitro*, using a rabbit reticulocyte lysate system (New England Nuclear), and proteins were analyzed by immunoprecipitation and $NaDodSO_4$/PAGE.

RESULTS

Detection of a 66-kDa Protein in NIH 3T3 Cells Transformed by the *dbl* Oncogene. The ability of sera from animals bearing tumors induced by tumor viruses or oncogenes to recognize products of such genes (refs. 27 and 28; for review, see ref. 29) led us to inquire whether antisera raised in mice to *dbl* transfectants might specifically detect the *dbl* gene product. Sera obtained at 3–4 weeks following the appearance of tumors induced by *dbl* transfectants were screened for reactivity against [^{35}S]methionine-labeled cell extracts of control NIH 3T3 cells and a *dbl*-transfected NIH 3T3 clone, 109-6-3-4. *dbl* TBM serum from each of three individual animals readily detected a 66-kDa species (p66) specific to the *dbl* transfectant (Fig. 1*A*, lanes 4, 6, and 8). As controls, neither normal serum (lanes 1 and 2) nor serum from a mouse immunized with NIH 3T3 cells transformed by the Hs242 oncogene, a human Ha-*ras* oncogene (18), showed reactivity against p66 (lanes 9 and 10). Moreover, none of the TBM sera detected p66 in a Hs242 transfectant. p66 was also undetectable in NIH 3T3 cells transformed by *mos, raf, fgr, fms, src*, or *sis* oncogenes (data not shown).

Using cloned *dbl* oncogene-derived subgenomic fragments as probes, we have observed an ≈2.9-kilobase (kb) mRNA species specific to *dbl*-induced transfectants. Variations in the level of this 2.9-kb transcript were found to correlate with the estimated number of *dbl* gene copies detected by Southern blot analysis (A.E., unpublished observations). As shown in Fig. 1*B*, the level of p66 in these transformants correlated well with the levels of *dbl*-specific mRNA and the estimated copy number of the *dbl* gene. These findings further substantiated p66 as being *dbl*-transformation specific but did not resolve whether p66 was the product of the *dbl* oncogene or a *dbl*-induced cellular antigen.

p66 Is Encoded by the *dbl* Oncogene. Evidence that p66 was encoded by *dbl* was obtained by immunoblot analysis of a *dbl*-induced transfectant clone, 109-5-1-1-2. In these cells, *dbl* TBM serum detected a faster-migrating protein of 60 kDa designated p60 (Fig. 2*A*, lane 6). Using the same antiserum, p60 was not observed in control NIH 3T3 cells (lane 2). Moreover, normal mouse serum failed to recognize this protein in the 109-5-1-1-2 transfectant (lanes 3 and 5). The detection of p60 in this transfectant by each of the *dbl* TBM sera capable of recognizing p66 implied an immunological relationship between the two proteins. When poly(A)$^+$ RNA extracted from *dbl* clone 109-5-1-1-2 was subjected to blot hybridization analysis, we observed a slightly smaller transcript of about 2.7 kb (Fig. 2*B*, lane 2). The smaller size of *dbl* mRNA in this transfectant was consistent with the ≈6-kDa difference in the sizes of p66 and p60. These findings strongly supported the concept that p66 and p60 specifically detected by *dbl* TBM sera were encoded by the *dbl* oncogene.

As an independent approach toward establishing the *dbl*-encoded nature of p66, we utilized hybrid-selected transla-

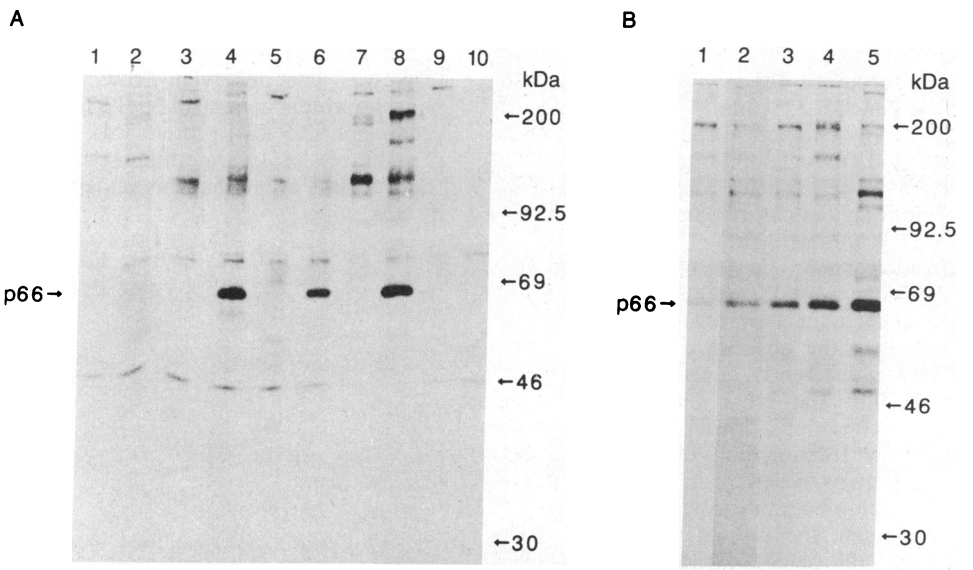

FIG. 1. Detection of p66 in NIH 3T3 cells transformed by the *dbl* oncogene. (*A*) Lysates from a [^{35}S]methionine-labeled NIH 3T3 *dbl* transfectant clone, 109-6-3-4, were immunoprecipitated with normal mouse serum (lane 2), three representative *dbl* TBM sera (lanes 4, 6, and 8), and a Ha-*ras* transfectant-derived TBM serum (lane 10). Control NIH 3T3 cells were processed under similar conditions for immunoprecipitation with normal mouse serum (lane 1), *dbl* TBM sera (lanes 3, 5, and 7), or Ha-*ras* TBM serum (lane 9). Immunoprecipitates were analyzed by $NaDodSO_4$/8% PAGE and fluorography. (*B*) NIH 3T3 *dbl* transfectant clones were labeled with [^{35}S]methionine, and lysates were immunoprecipitated using *dbl* TBM serum. Immunoprecipitates were analyzed by $NaDodSO_4$/8% PAGE and fluorography. The estimated copy number of the *dbl* gene in each transfectant is 1× (lanes 1 and 2), 2× (lanes 3 and 4), and 5× (lane 5), as determined by Southern blotting (see text).

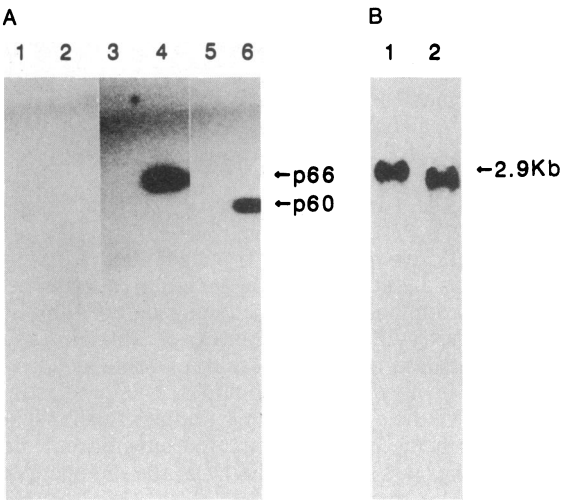

FIG. 2. Detection of a truncated *dbl* gene product and mRNA in one *dbl* transfectant clone. (*A*) Subconfluent cultures of control NIH 3T3 (lanes 1 and 2), and NIH 3T3 *dbl* transfectant clones 109-6-3-4 (lanes 3 and 4) and 109-5-1-1-2 (lanes 5 and 6) were lysed and immunoprecipitated with either normal mouse serum (lanes 1, 3, and 5) or *dbl* TBM serum (lanes 2, 4, and 6). After $NaDodSO_4$/8% PAGE, proteins were transferred to a 0.1-μm nitrocellulose membrane, and the blot was probed with *dbl* TBM serum and ^{125}I-labeled protein A. (*B*) Poly(A)$^+$ RNA (2 μg per lane) from NIH 3T3 *dbl* transfectant clones 109-6-3-4 (lane 1) and 109-5-1-1-2 (lane 2) was electrophoresed in 1% agarose/formamide gels and transferred to a nitrocellulose membrane. *dbl*-specific mRNA species were detected by use of a nick-translated *dbl* DNA fragment.

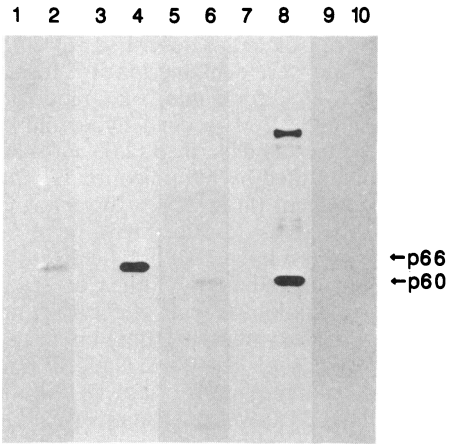

FIG. 3. Comparison of *in vitro*- and *in vivo*-synthesized *dbl* p66 and p60 proteins. Poly(A)$^+$ RNA from NIH 3T3 *dbl* transfectant clones 109-6-3-4 (lanes 1 and 2) and 109-5-1-1-2 (lanes 5 and 6) was hybrid-selected with *dbl* cDNA. pBR322-selected poly(A)$^+$ RNA from clone 109-5-1-1-2 (lanes 9 and 10) served as control. Hybrid-selected RNA species were translated in a rabbit reticulocyte system with [^{35}S]methionine as a tracer and immunoprecipitated with normal mouse serum (lanes 1, 5, and 9) or with *dbl* TBM serum (lanes 2, 6, and 10). For comparison, clones 109-6-3-4 (lanes 3 and 4) and 109-5-1-1-2 (lanes 7 and 8) were metabolically labeled with [^{35}S]methionine and immunoprecipitated either with normal mouse serum (lanes 3 and 7) or with *dbl* TBM serum (lanes 4 and 8). The labeled proteins were analyzed by $NaDodSO_4$/8% PAGE and fluorography.

tion (26). Hybrid-selected mRNAs from *dbl* transfectant clones 109-6-3-4 and 109-5-1-1-2 were obtained as described in *Materials and Methods*. When the mRNA molecules were translated *in vitro*, using rabbit reticulocyte lysate in the presence of [^{35}S]methionine, and were immunoprecipitated with *dbl* TBM sera, we observed major protein products (Fig. 3, lanes 2 and 6) whose sizes were indistinguishable from *in vivo*-synthesized p66 and p60, respectively (lanes 4 and 8). Smaller, minor species were also observed and may reflect degradation of the *in vitro*-translated proteins or synthesis of proteins on degraded RNA molecules. As controls, normal mouse serum did not recognize either *in vitro*- or *in vivo*-synthesized p66 or p60 (lanes 1, 3, 5, and 7). Moreover, when poly(A)$^+$ RNA from *dbl* transfectant clone 109-5-1-1-2 was selected with an unrelated DNA (*Eco*RI-linearized pBR322), a *dbl* TBM serum failed to detect p60 or any related protein (lane 10). All of these findings established that p66, as well as p60, was encoded by the *dbl* oncogene.

Subcellular Localization of p66. The subcellular localization of an oncogene product can provide insights into its possible functions. Thus, *dbl* transfectant clone 109-6-3-4 was disrupted by hypotonic shock in the absence of detergent, and lysates were subjected to differential centrifugation to yield nuclear, cytosolic (S-100), and crude membrane (P-100) fractions. Under these conditions, nuclei remained >90% intact as determined by fractionation of [^{3}H]thymidine-labeled cells (data not shown). When the individual fractions were solubilized in immunoprecipitation buffer and analyzed by immunoblotting for p66, the protein was found to be primarily distributed in P-100 and S-100 fractions (Fig. 4). p66 was undetectable in the nuclear fraction under these conditions.

p66 Is a Phosphoprotein Whose Major Site of Phosphorylation Is on Serine Residues. A number of known oncogene products are posttranslationally modified (for review, see ref. 29). To assess whether the *dbl* gene product is a phosphoprotein, representative third-cycle *dbl* transfectants as well as transfectants induced by the cloned *dbl* transforming gene were ^{32}P-labeled and analyzed for the presence of phosphorylated proteins by immunoprecipitation with *dbl* TBM sera. A phosphoprotein of about 66 kDa (pp66) was specifically immunoprecipitated from each *dbl* transfectant analyzed (Fig. 5*A*). The apparent molecular mass of pp66 was similar to that of [^{35}S]methionine-labeled p66 specifically immunoprecipitated by *dbl* TBM serum (lanes 4 and 6). Normal mouse serum (lanes 3 and 5) or Ha-*ras* TBM serum (data not shown) failed to immunoprecipitate pp66. Furthermore, pp66 was undetectable in control NIH 3T3 cells (lanes 1 and 2) or *ras*-transformed NIH 3T3 cells (data not shown). In addition to *dbl* p66, another abundant phosphoprotein, of about 92.5 kDa, was also observed. However, the presence of this phosphoprotein in control NIH 3T3 cells and the fact that no prominent 92.5 kDa protein was observed in the immunoprecipitates of [^{35}S]methionine-labeled lysates from *dbl* transfectants argue that this phosphoprotein is not encoded by *dbl*.

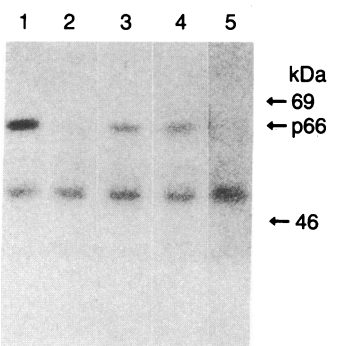

FIG. 4. Subcellular distribution of *dbl* p66. Unfractionated cells from NIH 3T3 *dbl* transfectant clone 109-6-3-4 (lane 1) or the same cells fractionated into nuclear (lane 2), S-100 (lane 3), and P-100 (lane 4) components were analyzed for p66. Following immunoprecipitation of each fraction with *dbl* TBM sera (lanes 1–4) and of the P-100 fraction with control serum (lane 5), immunoblotting was performed using *dbl* TBM serum.

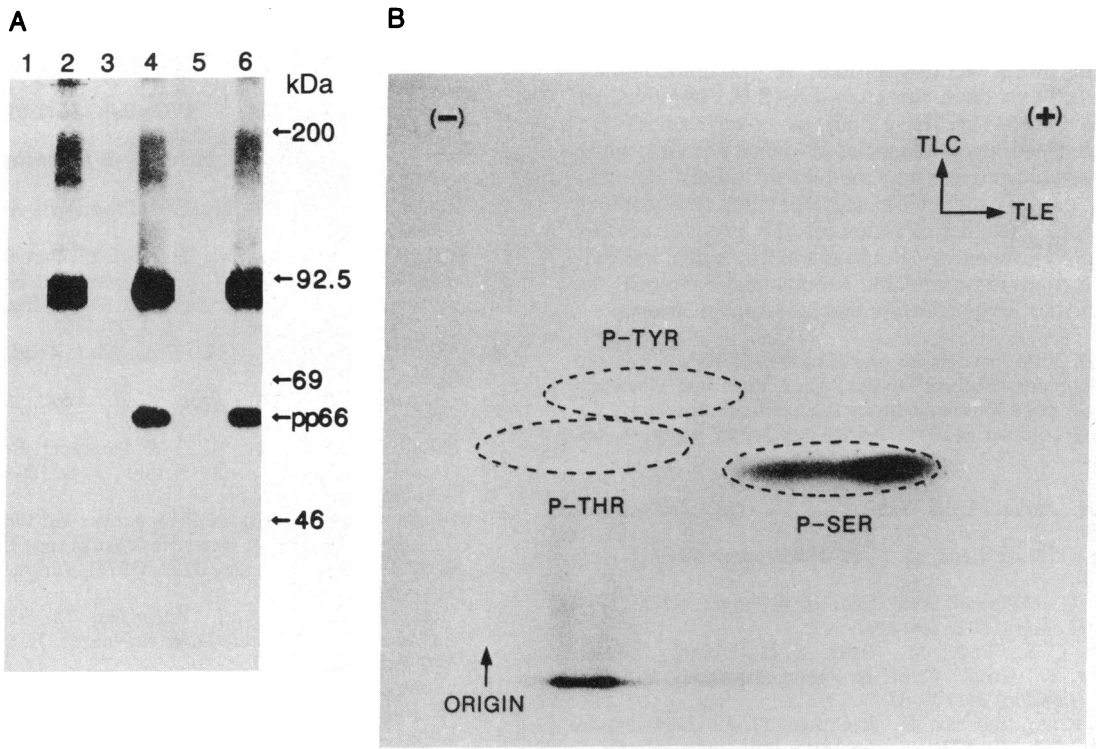

FIG. 5. *dbl* p66 is a phosphoprotein whose major site of phosphorylation is on serine residues. (*A*) Subconfluent cultures of control NIH 3T3 (lanes 1 and 2) and *dbl* transfectant clones 66-3-9-3 (lanes 3 and 4) and 109-6-3-4 (lanes 5 and 6) were labeled with [^{32}P]orthophosphoric acid. Cell lysates were immunoprecipitated either with normal mouse serum (lanes 1, 3, and 5) or with *dbl* TBM serum (lanes 2, 4, and 6). Immunoprecipitates were analyzed by NaDodSO$_4$/8% PAGE and fluorography. (*B*) ^{32}P-labeled *dbl* p66 band was excised from the gel and processed for phospho amino acid analysis. Phosphoserine, phosphothreonine, and phosphotyrosine were used as standards (positions detected by ninhydrin are enclosed by broken lines). Separation was achieved by thin-layer electrophoresis in the first dimension and thin-layer chromatography in the second dimension, as indicated.

To determine the amino acid residue(s) of the *dbl* gene product that had undergone phosphorylation, metabolically labeled pp66 was acid-hydrolyzed and subjected to thin-layer electrophoresis/chromatography. The only detectable ^{32}P-labeled phospho amino acid was phosphoserine, based on its mobility compared with those of standard phospho amino acids processed under similar conditions (Fig. 5*B*). Thus, p66 is a phosphoprotein whose major site of phosphorylation is on serine residue(s).

DISCUSSION

We have identified the p66 product of *dbl*, a human oncogene, by use of antisera raised against *dbl*-transformed cells. Evidence of the *dbl*-encoded nature of this protein included the specificity of its detection in *dbl* transfectants by such antisera. We also identified a rare *dbl* transfectant that expressed a smaller *dbl*-specific transcript and protein, p60. These findings argue strongly on a genetic basis that p60 represents a truncated form of p66. Finally, hybrid-selected translation of RNAs from *dbl* transfectants expressing either p66 or p60 led to the specific immunologic detection of *in vitro*-synthesized p66 and p60, respectively, further establishing their *dbl*-encoded nature. Since *in vitro* translational products are not posttranslationally modified by glycosylation or cleavage, our findings of similar sizes of the *dbl* products detected both *in vitro* and *in vivo* suggest no major size alterations of the primary translational product of the *dbl* oncogene *in vivo*.

We were able to establish that the *dbl* gene product is distributed equally within cytosol and crude membrane fractions. This subcellular localization of p66 argues against any likely functional relationship with nuclear proteins encoded by oncogenes such as *myc*, *fos*, *myb*, and *ets* (for review, see ref. 29); *ski* (30); and p53 (31, 32). The products of most other known oncogenes are localized to the cytoplasm, to the membrane, or to both subcellular components. Oncogenes encoding growth factors, such as *sis* (20, 33), or growth factor receptors, including *erbB* (34, 35) and *fms* (36, 37), are synthesized in association with the endoplasmic reticulum and become associated with peripheral cell membranes where they are exposed to the cell surface. Preliminary immunofluorescence evidence indicates that *dbl* antigenic determinants are not exposed on the cell surface (L. Varesio, personal communication), although analysis of a broader range of antisera will be required to exclude this possibility rigorously. The products of other known oncogenes, which comprise members of both the tyrosine kinase and *ras* gene families, are synthesized in the cytosol and either remain there or become posttranslationally modified and translocated to the inner face of the plasma membrane (for review, see ref. 29). Our detection of the *dbl* product in both cytosol and membrane subcellular components indicates that it is a member of this last category.

The products of oncogenes in the tyrosine kinase family exhibit autophosphorylation on tyrosine residues (38). The more distantly related *mos* and *raf* (14, 39) oncogenes encode proteins that have been reported to contain phosphoserine and phosphothreonine residues (40–42). Our findings that the *dbl* gene product is a phosphoprotein with site(s) of phosphorylation specific to serine residues seem to distinguish p66 from the products of most known oncogenes within the tyrosine kinase family. Independent evidence that *dbl* is unrelated to oncogenes of the tyrosine kinase or the *ras* gene families derives from the lack of detectable homology of the cloned *dbl* oncogene to known members of either group (8).

The functional significance of serine phosphorylation of the *dbl* product remains to be elucidated. Attempts to detect autophosphorylation of this protein in immunocomplex kinase assays have been unsuccessful (S.S., unpublished observations). However, these findings do not exclude the possibility that p66 has an associated kinase activity, since presently available antisera may inhibit this activity. It is also possible that the *dbl* product is a target for some other cellular protein kinase. Our identification of the product of this apparently novel oncogene should make it possible to compare its expression in normal and neoplastic human cells, as well as to further investigate its biochemical functions.

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1. Land, H., Parada, L. & Weinberg, R. A. (1983) *Science* **222,** 771–778.
2. Cooper, G. M. & Lane, M. A. (1984) *Biochim. Biophys. Acta* **738,** 9–20.
3. Goubin, G., Goldman, D. S., Luce, J. & Cooper, G. M. (1983) *Nature (London)* **302,** 114–119.
4. Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. M. & Vande Woude, G. F. (1984) *Nature (London)* **311,** 29–33.
5. Padua, R. A., Barrass, N. & Crouie, G. A. (1984) *Nature (London)* **311,** 671–673.
6. Fasano, O., Birnbaum, D., Edlund, L., Fogh, J. & Wigler, M. (1984) *Mol. Cell. Biol.* **4,** 1695–1705.
7. Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Greene, M. I. & Weinberg, R. A. (1984) *Nature (London)* **312,** 513–516.
8. Eva, A. & Aaronson, S. A. (1985) *Nature (London)* **316,** 273–275.
9. Takahashi, M., Ritz, J. & Cooper, G. M. (1985) *Cell* **42,** 581–588.
10. Shimizu, K., Nakatsu, Y., Sekiguchi, M., Hokamura, K., Tanaka, K., Terada, M. & Sigimura, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5641–5645.
11. Martin-Zanca, D., Hughes, S. H. & Barbacid, M. (1986) *Nature (London)* **319,** 743–748.
12. Stern, D. F., Heffernan, P. A. & Weinberg, R. A. (1986) *Mol. Cell. Biol.* **6,** 1729–1740.
13. Dean, M., Park, M., LeBeau, M. M., Robbins, T. S., Diaz, M. O., Rowley, J. D., Blair, D. G. & Vande Woude, G. F. (1985) *Nature (London)* **318,** 385–388.
14. Mark, G. E. & Rapp, U. R. (1984) *Science* **224,** 285–289.
15. Rogers, J. (1986) *Nature (London)* **320,** 579–580.
16. Cooper, G. M., Goubin, G., Diamond, A. & Neiman, P. (1986) *Nature (London)* **320,** 570–580.
17. Srivastava, S. K., Yuasa, Y., Reynolds, S. H. & Aaronson, S. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 38–42.
18. Yuasa, Y., Srivastava, S. K., Dunn, C. Y., Rhim, J. S., Reddy, E. P. & Aaronson, S. A. (1983) *Nature (London)* **303,** 775–779.
19. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
20. Robbins, K. C., Leal, F., Pierce, J. & Aaronson, S. A. (1985) *EMBO J.* **4,** 1783–1792.
21. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1311–1315.
22. Cox, R. A. (1968) *Methods Enzymol.* **12,** Part B, 120–129.
23. Adams, S. L., Sobel, M. E., Howard, B. H., Olden, K., Yamada, K. M., Crombrugghe, B. D. & Pastan, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3399–3403.
24. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
25. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
26. Miller, J. S., Roberts, B. E. & Paterson, B. M. (1982) in *Genetic Engineering*, eds. Setlow, J. K. & Hollander, A. (Plenum, New York), pp. 103–117.
27. Huebner, R. J. (1965) in *Perspectives in Virology IV*, ed. Pollard, M. (Harper & Row, New York), pp. 142–163.
28. Brugge, J. S. & Erikson, R. L. (1977) *Nature (London)* **269,** 346–348.
29. Hunter, T. (1984) *J. Natl. Cancer Inst.* **73,** 773–786.
30. Barkas, A. E., Brodeur, D. & Stavnezer, E. (1986) *Virology* **151,** 131–138.
31. Gurney, E. G., Harrison, R. O. & Fenno, J. (1980) *J. Virol.* **34,** 752–763.
32. Rotter, V., Abutbul, H. & Ben-Ze'ev, A. (1983) *EMBO J.* **2,** 1041–1043.
33. Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Robbins, K. C., Aaronson, S. A. & Antoniades, H. M. (1983) *Science* **221,** 275–277.
34. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, Y., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307,** 521–527.
35. Schatzman, R. L., Evan, G. I., Privalsky, M. N. & Bishop, J. M. (1986) *Mol. Cell. Biol.* **4,** 1329–1333.
36. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
37. Anderson, S. J., Gonda, M. A., Rettenmier, C. W. & Sherr, C. J. (1984) *J. Virol.* **51,** 730–741.
38. Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54,** 897–930.
39. Van Beveren, C., Galleshaw, J. A., Jonas, V., Berns, A. J. M., Doolittle, R. F., Donoghue, D. J. & Verma, I. M. (1981) *Nature (London)* **289,** 258–262.
40. Papkoff, J., Verma, I. M. & Hunter, T. (1982) *Cell* **29,** 417–426.
41. Kloetzer, W. S., Maxwell, S. A. & Arlinghaus, R. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 412–416.
42. Rapp, U. R., Reynold, F. H. & Stephenson, J. R. (1983) *J. Virol.* **45,** 914–924.