A20, a novel zinc finger protein, is an inhibitor of tumor necrosis factor-induced apoptosis. The mechanism by which A20 exerts its protective effect is currently unknown. Several isoforms of the 14-3-3 proteins were found to interact with A20 in a yeast two-hybrid screen. A20 bound several 14-3-3 isoforms in vitro. Moreover, transfected A20 was found to preferentially bind the endogenous 14-3-3 isoform, whereas the β+ isoforms co-immunoprecipitated much less efficiently, and ε14-3-3 had an intermediate affinity. Importantly, c-Raf, a previously described 14-3-3-interacting protein, also preferentially bound the ε isoform. The cellular localization and subcellular fractionation of A20 was dramatically altered by co-transfected 14-3-3, providing the first experimental evidence for the notion that 14-3-3 can function as a chaperone. Furthermore, c-Raf and A20 co-immunoprecipitated in a 14-3-3-dependent manner, suggesting that 14-3-3 can function as a bridging or adapter molecule.

Tumor necrosis factor-α (TNF) is a catabolic pro-inflammatory cytokine that is capable of inducing apoptotic death in a number of tumor cell lines. The systemic toxicity of TNF and the generation of TNF resistant clones have in large part contributed to the therapeutic failure of TNF as an anti-cancer agent. To understand the molecular mechanism of TNF and the basis of sensitivity and resistance to TNF killing, we cloned TNF-inducible primary response genes by differential hybridization (1). One of the genes, designated A20, conferred resistance to TNF killing when transfected into sensitive NIH3T3, WEHI 164, and MCF7 cells (2). Further, breast carcinoma cell lines that were resistant to TNF cytotoxicity expressed higher levels of A20 than corresponding sensitive lines (2). Interestingly, the expression of A20 is subverted by a number of genes including the LMP-1 gene product of the Epstein-Barr virus, a known inhibitor of apoptosis (3). It is plausible that virally mediated induction of A20 and subsequent resistance to apoptosis and cytokine killing contributes to host immune response attenuation and viral persistence.

Analysis of the full-length A20 cDNA revealed an open reading frame coding for a protein of 790 amino acid residues, which showed no significant homology to previously described proteins (4). However, within A20 a distinct repeated element was found that included seven novel zinc finger motifs of the form CXXCXXCXXCXXC. The repression of its own promoter activity, coupled with the presence of zinc fingers, initially suggested that A20 might be a transcriptional factor (5). However, immunolocalization studies revealed A20 to be a cytoplasmic protein, consistent with the finding that other zinc finger proteins, including protein kinase C and c-Raf, are non-nuclear.

To derive a biochemical understanding of how A20 functions, a yeast two-hybrid screen was utilized to identify A20-interacting proteins. The 14-3-3 family of proteins was found to specifically bind A20 and could function as adapter molecules mediating the interaction of A20 with c-Raf, a Ser/Thr kinase of pivotal importance to a number of signaling pathways. 14-3-3 proteins are highly conserved and are ubiquitous in the animal and plant kingdoms. At least seven mammalian isoforms have been identified. Previously, 14-3-3 proteins had been shown to function as regulators of protein kinase C, trypthophan, and tyrosine hydroxylases and to be essential for the stimulation of exocytosis from chromaffin cells (see Ref. 6 and references therein). However, interest in 14-3-3 has recently been stimulated by the discovery that oncogene products, including Raf-1(7–13), Bcr-Abl, Bcr (14, 15), polyoma middle T antigen (16), and cell cycle control proteins such as Cdc25 phosphatases (17) associate with 14-3-3. Moreover, the Schizosaccharomyces pombe 14-3-3 homologs, rad24 and rad25, are required for DNA damage checkpoint control and, therefore, the timing of mitosis (18). The importance of 14-3-3 in signal transduction is further corroborated by reports that 14-3-3 interacts with phosphatidylinositol 3 kinase (19) and glycoprotein Iib-IX (20).

The function of 14-3-3 in these cases has not been elucidated in detail. The interaction of Raf with 14-3-3 leads to Raf activation in several in vivo systems (7, 9, 10), while no effect is observed in vitro (13). Overexpression of 14-3-3 in J urkat cells leads to decreased phosphatidylinositol 3-kinase stimulation by anti-CD3 (19). One potential mechanism by which 14-3-3 could exert a variety of functions is by its ability to form dimers. It could act as an adapter between two molecules and thereby modulate their activity (21).

In this report we demonstrate that 14-3-3 can mediate the interaction of A20 and Raf by functioning as a bridging adapter molecule. Additionally, 14-3-3 proteins can serve as chaperones, promoting the transition of A20 from insoluble punctate cytoplasmic structures to the soluble cytoplasmic compartment.

MATERIALS AND METHODS

Yeast Two-hybrid Screen—A GAL4 DNA binding domain-A20 fusion construct in the pAS1CYH2 vector was used as bait (22) to screen a human B-cell cDNA library fused to the GAL4 activation domain in the pACT prey plasmid as described previously (23). Transformants were

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2 The abbreviations used are: TNF, tumor necrosis factor; PAG, polyacrylamide gel electrophoresis.
selected on Trp, Leu, His + 30 mM Triade and positive potentials identified by assaying for β-galactosidase activity using 5-bromo-4-chloro-3-indolyl-β-D-galactoside as a chromogenic substrate. Positive clones were cured of the bait plasmid by growth in Leu medium and the library plasmid rescued by transformation of XL1-Blue competent Escherichia coli cells. Recovered plasmids were initially characterized by partial DNA sequencing. To demonstrate specificity, nonidentical clones were co-transformed into yeast with full-length A20 bait, A20 NH2-terminal domain (amino acid residues 45-366), A20 COOH-terminal zinc finger domain (379-749), or a battery of heterologous proteins expressed as GAL4-DNA binding domain fusions in the pAS1CYH2 vector.

Plasmids—Standard recombinant DNA techniques were used to clone β14-3-3 in yeast bait and prey vectors, as well as that of epitope-tagged β14-3-3 (HA) and γ14-3-3 (AU1) in the eukaryotic expression vector pDNA3 (24). Full-length c-Raf in the yeast bait vector, pGBT8 c-Raf (19), was 30% from M. Ruggieri (Onyx Pharmaceuticals, Richmond, CA). Full-length c-Raf epitope-tagged (KT3) at the carboxyl terminus and pMv-Src were obtained from Rich J ove (Mossit Cancer Center, Tampa, FL).

293T Transfections—Transfection experiments with the CaPPO4 method were performed as previously published (25).

1 A Viro Binding—A20 containing an NH2-terminal FLAG epitope tag was expressed by transient transfaction in 293T cells. Following lysis in RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.4, 10% glycerol, 5 mM EDTA, 1% Triton X-100, 1% deoxycholate, and protease inhibitors; 5 μg/ml leupeptin, 5 μg/ml aprotinin, 5 μg/ml pepstatin, 50 μg/ml soybean trypsin inhibitor, and 0.5 mM phenylmethylsulfonyl fluoride), the lysates were incubated overnight at 4°C with anti-FLAG antibody-coupled agarose (Eastman Kodak Co.) and the beads subsequently washed in 50 mM NaCl, 10 mM Tris, pH 8.0, 1% Triton X-100. As a negative control, an unrelated protein, B94 (26) similarly FLAG tagged, was also expressed and bound to anti-FLAG beads. Library-encoded cDNAs to be tested for A20 binding were amplified by polymerase chain reaction and in vitro transcribed/translated in the presence of [35S]methionine according to the manufacturer’s instructions (Promega, Madison, WI). Five μl of the radiolabeled translation product was mixed with anti-FLAG beads containing either FLAG-A20 or FLAG-B94 and 30 μl of a 50% slurry of bovine serum albumin agarose beads (Sigma) as carrier. Binding reactions were incubated for 4 h at 4°C in 200 μl of binding buffer (50 mM NaCl, 10 mM Tris pH, 7.6, 1% Triton X-100, 100 μM CaCl2, 13.5 mM MgCl2, 1 mM diethiothreitol, and protease inhibitors), followed by three 500-μl washes with binding buffer, boiling in sample buffer, and analysis of the eluted proteins by SDS-PAGE and fluorography.

Purified tagged 14-3-3 isoforms were obtained by immunoprecipitation of the in vitro transcription/translation mixture with tag-specific antibodies. Phosphotagged and unphosphorylated salmon washes were followed by elution in 0.1 M glycine, pH 3.0. The pH of the eluted fractions was adjusted to 8.0 by 1 M Tris base.

In Vivo Binding—FLAG-A20 was expressed in 293T cells by transient transfection. Forty-eight hours following transfection, cells were washed in phosphate-buffered saline and lysed in 3 ml of lysis buffer (10 mM NaCl, 10 mM Tris pH 7.6, 1% Triton X-100, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 mM NaF, 1 mM Na3VO4, 5 mM dithiothreitol, protease inhibitors). Lysates were cleared by microcentrifugation, diluted 3-fold in lysis buffer, anti-FLAG beads added (25 μl of a 50% slurry), and incubated at 4°C for the indicated times. Beads were washed three times in lysis buffer, boiled in sample buffer, and the eluted proteins resolved by SDSPAGE, transferred to Immobilon P membrane (Millipore, Bedford, MA), and probed with isoform-specific 14-3-3 antibodies. 14-3-3 binding was demonstrated by chemiluminescent imaging using the ECL detection system (Amersham Corp.) To strip bound antibody, membranes were incubated at 50°C for 30 min in 1% SDS, 2% 50 mM Tris-HCl, pH 8.8, followed by two 10-min washes in 150 mM NaCl, 20 mM Tris, pH 7.6, 0.05% Tween 20.

Immunostaining—Immunostaining was performed on transiently transfected 293 cells grown on gelatin-coated glass coverslips as described (28).

RESULTS AND DISCUSSION

Two-hybrid Screen—A total of 1.3 × 109 primary transformants were screened following transformation of a Y190 yeast strain expressing A20 bait with a B-cell library. Sixty-two colonies were β-galactosidase-positive. Forty-three of the positive colonies were sequenced and 18 clones identified as fusions between 14-3-3 and the library vector encoded Gal4 activation domain (Table I). Specificity of the interaction was corroborated by co-precipitations with heterologous baits.

Intriguingly, all 14-3-3 sequences contained an untranslatable segment fused in-frame to the Gal4 activation domain. To assess the significance of the 5'-untranslated sequence, β14-3-3 constructs with only the coding region fused to the Gal4 DNA binding domain were tested for two-hybrid interaction with A20 (Table II). The β14-3-3 without the untranslated segment interacted with A20 in the prey vector; therefore, the non-coding region was not necessary for binding A20. Similar untranslated 14-3-3 sequences were also obtained during a two-hybrid screen with Raf as bait (9). Moreover, both the amino- and carboxyl-terminal halves of A20 were found to interact with 14-3-3, indicating that binding was not restricted to a distinctly defined single domain. Full-length A20 also interacted with the amino- and carboxyl-terminal halves of A20 (Table II) signifying that A20 also bound itself through multiple domains.

In Vitro Interactions—To confirm the interaction between 14-3-3 and A20, in vitro binding experiments were performed using recombinant A20 expressed in 293T cells and in vitro transcription/translation 14-3-3 (Fig. 1). All isoforms specifically bound A20 but not an unrelated protein B94. Quantitative comparison of the affinities of 14-3-3 isoforms for A20 was difficult due to the presence of endogenous 14-3-3 proteins in the reticulocyte lysates. Endogenous proteins also lowered the binding capacity of the FLAG-A20 beads for 14-3-3 so that significant depletion of the supernatant was only achieved with an excess of A20 beads (data not shown). Therefore, in vitro binding experiments were repeated by using tagged and purified in vitro transcription/translation β and γ14-3-3. Using the phosphomannase, it was determined that 32% (± 13) of the
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Table II
A20 interactions in yeast

| Bait | Prey       | β-Galactosidase |
|------|------------|-----------------|
| A20  | S' Un, β14-3-3 | +               |
| N-A20| S' Un, β14-3-3 | +               |
| C-A20| S' Un, β14-3-3 | +               |
| β 14-3-3 | A20 | +               |
| A20  | A20        | +               |
| N-A20| A20        | +               |
| C-A20| A20        | +               |
| Raf  | A20        | –               |
| S' Un, β14-3-3 | –          |

**Fig. 1.** In vitro interaction of A20 with 14-3-3. FLAG-A20 and FLAG-B94 were expressed in 293T cells and immobilized onto anti-FLAG-beads. [35S]Methionine-labeled 14-3-3 isoforms obtained by coupled in vitro transcription/translation were added to the beads, incubated, and washed, and bound proteins were analyzed by SDS-PAGE and autoradiography. One fifth of the amount used in the binding reaction (input) was run in parallel for comparison to what was left in the supernatant after binding (Sup) and what bound to the beads (bound). The 14-3-3 isoforms analyzed are indicated at the bottom of the panels.

η14-3-3 bound to A20, while only 11% (± 4) of the β14-3-3 isoform bound to the same amount of A20 beads under identical conditions. The κ isoform has therefore an affinity for A20 that is 2.8-fold higher than the β isoform. The same experiments were performed with c-Raf expressed in 293 cells, and the ratio of η/β affinity for Raf binding was 1.6-fold.

These experiments demonstrate that A20 and 14-3-3 proteins interact directly and in an isoform-specific manner.

**Isoform Specificity of in Vivo Interactions**—The overall amino acid sequence of the 14-3-3 proteins is highly conserved (6). The different isoforms are even more conserved between species, which suggest distinct conserved functions within each isoform. To assess the affinity of each isoform for A20, we analyzed the in vivo interaction between FLAG-A20 and endogenous 14-3-3 isoforms in 293T cells. The endogenous γ, ε, η, and βζ 14-3-3 isoforms can be detected with the available antibodies by immunoblotting 293T cell lysates (Fig. 2A, total lysate). No signal was obtained in the lysate nor immunoprecipitate with antibodies against τ and α isoforms. The relative affinity of A20 for the different isoforms was estimated by comparison of the ECL signal of the total lysate to that obtained with the same antibody in the immunoprecipitate. Reproducibly, the largest relative signal in the anti-FLAG-A20 immune complex compared to total cell lysate was obtained with anti-γ antibodies. The immunoprecipitate to lysate ratio for the ε isoform was also significantly higher, while much lower ratios were obtained with anti βζ/γ and γ antibodies. A20 therefore preferentially bound the γ isoform, followed by the ε isoform. The βζ and γ isoforms bound least well. Probing the blot with PAN 14-3-3 antibodies revealed an additional band between the slow migrating ε band and the 30-kDa band that represents the bulk of the isoforms (Fig. 2A, arrow). The same pattern was obtained using a different PAN-14-3-3 antibody raised against the peptide KSELVQKAKLAEQAERYDD (S.C. PAN 14-3-3). As this band was highly enriched in the A20 immunoprecipitate, A20 binds strongly a 14-3-3 protein that is not recognized by the available isoform-specific antibodies. This protein could be a new 14-3-3 isoform or a post-translational modification that modifies the epitopes recognized by the polyclonal isoform-specific antibodies. Posttranslational modifications of the β and ζ isoforms have been identified as the α and δ isoform, respectively (29). These post-translational modifications are still recognized by the antibodies used in our study (27), making it more likely that the A20-associated 14-3-3 protein is a new isoform.

Co-precipitations with c-Raf were performed to determine if Raf also interacted preferentially with 14-3-3 isoforms. c-Raf was found to bind 14-3-3 (Fig. 2B) with an isoform preference similar to that of A20 (i.e. η bound more strongly than βζ/ε showing intermediate binding). The affinity of c-Raf for the 14-3-3 isoforms was not identical to A20, as most clearly demonstrated by the absence of the novel isoform in the Raf immunoprecipitates.

Surprisingly, the sequence of η14-3-3 is more closely related to the weak binding βζ isoforms than to the intermediate binding ε isoform (6). The fraction of 14-3-3 proteins that co-immunoprecipitated with FLAG-A20 as well as c-Raf was low, even for the strongly interacting η isoform (1–5%). It is likely that only a small fraction of FLAG-A20 in 293T cells localizes to the subcellular compartment where it can interact with endogenous 14-3-3 (Fig. 3).

**Fig. 2.** A20 displays binding specificity for endogenous 14-3-3 isoforms. A, 293T cells were transfected with FLAG epitope-tagged A20. Cleared lysates (lane 1, 1/500 of total) were incubated either for 8 h (lane 3a) or 2 h (lane 3b) with anti-FLAG beads. 293T cells transfected with FLAG-B94 and immunoprecipitated for 8 h served as a negative control (lane 2). Precipitated 14-3-3 isoforms were resolved on a 15% SDS-PAGE gel, transferred to a membrane, and probed with isoform-specific antibodies. Membranes were stripped and reprobed. The X indicates a reference point and the arrow an unknown 14-3-3 isoform that preferentially binds A20. The panel is representative of three independent experiments. S.C. PAN 14-3-3, gel probed with PAN 14-3-3 antibodies from Santa Cruz Biotechnology. B, 293T cells were transfected with c-Raf. Lanes 1 and 3 were cotransfected with v-Src. Anti-c-Raf immunoprecipitates (lanes 3 and 4) were compared to total lysates (lanes 1 and 2; 1/100 of total) by immunoblotting for 14-3-3 isoforms as in A.
Also of note is the fact that the difference in affinities of the β and η isoforms for A20, albeit with the same preferences, are much larger in vivo than in vitro. This suggests that post-translational modifications of 14-3-3 also contribute to the binding.

Interestingly, a recent peptide binding study defined the 14-3-3 binding sequence as RS\textsubscript{X}phosphoS\textsubscript{X}P (30). No isoform preference was found in these studies, indicating that additional motifs are likely responsible for the isoform specificity observed with native proteins.

Raf immunoprecipitates from 293T cells transfected with c-Raf alone or c-Raf and v-Src (a potent activator of c-Raf activity) display identical 14-3-3 isoform binding (Fig. 2B, lanes 3 and 4). Therefore, the 14-3-3/c-Raf interaction and isoform preference was not influenced by the state of activation of Raf.

Whereas this is the first report on Raf isoform specificity, the association of Raf with 14-3-3 has been reported previously to be independent of Raf activation in baculovirus overexpression systems (31, 32). In contrast to our work is the finding that activated c-Raf from serum-activated NIH3T3 does not associate with 14-3-3 (12).

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**Fig. 3.** **14-3-3 functions as a chaperone mobilizing A20 in a soluble form.** A, 293 cells were transfected with FLAG-A20 (lanes 1-4) and AU1 epitope-tagged η14-3-3. The cells were continuously cultured in 10% fetal calf serum (lanes 1 and 3) or serum-starved prior to lysis. The fraction containing soluble A20 was obtained following a 10-min centrifugation (12,000 × g). The remaining insoluble pellet was solubilized in SDS-sample buffer (Insoluble A20). Identical cell equivalents were resolved by SDS-PAGE and probed with anti-FLAG antibodies to detect epitope tagged A20. B, 293 cells were co-transfected with FLAG-A20 and pcDNA3 vector (1), AU1-η14-3-3 and vector (3), or with FLAG-A20 and AU1-η 14-3-3 (2 and 4). Cells were stained 48 h following transfection with anti-FLAG antibodies (1 and 2) or anti-AU1 antibodies (3 and 4).
14-3-3 Associates with A20

**Figure 4.** 14-3-3 functions as an adapter mediating A20c-Raf binding. 293T cells were transfected with c-Raf and FLAG-A20 (lanes 1, 3, and 4). Cleared cell lysates were prepared in 1% Nonidet P-40 buffer. For high stringency immunoprecipitation (H), deoxycholate and SDS were added to a final concentration of 0.5% and 0.1%, respectively. The relative amount of c-Raf (top panel) and 14-3-3 protein (bottom panel) present in the anti-FLAG-A20 immunoprecipitates (lanes 3–6) and cell lysate prior to immunoprecipitation (lanes 1 and 2; 1/75 of total) was determined by immunoblotting with α-Raf (upper panel) and α-PAN 14-3-3 (lower panel). Neither c-Raf or 14-3-3 associated nonspecifically with the FLAG antibody (lanes 5 and 6).

**Fig. 4.** 14-3-3 functions as an adapter mediating A20c-Raf binding. 293T cells were transfected with c-Raf and FLAG-A20 (lanes 1, 3, and 4). Cleared cell lysates were prepared in 1% Nonidet P-40 buffer. For high stringency immunoprecipitation (H), deoxycholate and SDS were added to a final concentration of 0.5% and 0.1%, respectively. The relative amount of c-Raf (top panel) and 14-3-3 protein (bottom panel) present in the anti-FLAG-A20 immunoprecipitates (lanes 3–6) and cell lysate prior to immunoprecipitation (lanes 1 and 2; 1/75 of total) was determined by immunoblotting with α-Raf (upper panel) and α-PAN 14-3-3 (lower panel). Neither c-Raf or 14-3-3 associated nonspecifically with the FLAG antibody (lanes 5 and 6).

**Table 1.**

| Flag Lysate | α-Flag I.P. |
|-------------|-------------|
| +/-         | +/-         |
| +/-         | +/-         |

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**REFERENCES**

1. Dixit, V. M., Green, S., Saras, V., Holzman, L. B., Wolf, F. W., O'Rourke, K., Ward, P. A., Prochownik, E. V., and Marks, R. M. (1990) J. Biol. Chem. 265, 2973–2978
2. Opipari, A. W., Jr., Hu, H. M., Yalkowitz, R., and Dixit, V. M. (1991) J. Biol. Chem. 267, 12424–12431
3. Laherty, C. D., Hu, H. M., Opipari, A. W., Wang, F., and Dixit, V. M. (1992) J. Biol. Chem. 267, 24157–24160
4. Opipari, A. W., Jr., Boguski, M. S., and Dixit, V. M. (1990) J. Biol. Chem. 265, 14705–14708
5. Krikos, A., Laherty, C. D., and Dixit, V. M. (1992) J. Biol. Chem. 267, 17971–17976
6. Alten, A., Cali, E. D., van Heusden, B. P., Isobe, T., Roseboom, P. H., Rosenfeld, G., and Soli, J. (1992) Trends Biochem. Sci. 17, 498–501
7. Fantl, W. J., Muslin, A. J., Kikuchi, A., Martin, J. A., MacNicol, A. M., Gross, A. W., Williams, L. T., and Fantl, W. J. (1994) Nature 371, 612–614
8. Fu, H., Xia, K., Pallas, D. C., Cui, C., Conroy, K., Narsimhan, R. P., Mamon, H., Collier, R. J., and Roberts, T. M. (1994) Science 266, 126–129
9. Freed, E., Synons, M., MacDonald, S. G., McCormick, F., and Ruggieri, R. (1994) Science 265, 1713–1716
10. Irie, K., Gotoh, Y., Yashar, B. M., Errede, B., Nishida, E., and Matsumoto, K. (1994) Science 267, 1716–1719
11. Yamamori, B., Kuroda, S., Shinizu, K., Fukui, K., Oshtuka, T., and Takai, Y. (1995) J. Biol. Chem. 270, 11723–11726
12. Li, S., Janschow, P., Tanji, M., Rosenfeld, G. C., Waymire, J. C., Mischak, H., Kolch, W., and Seliviv, J. M. (1995) EMBO J. 14, 685–690
13. Michaud, N. R., Fabian, J. R., Mathes, K. D., and Morrison, D. K. (1995) Mod. Cell. Biol. 15, 3390–3397
14. Reuther, G. W., Fu, H., Cripes, L. D., Collier, R. J., and Pendergast, A. M. (1994) Science 266, 129–133
15. Braselmann, S., and McCormick, F. (1995) EMBO J. 14, 4839–4848
16. Pallas, D. C., Fu, H., Haehnel, L. C., Weller, W., Collier, R. J., and Roberts, T. M. (1994) Science 265, 553–557
17. Conklin, D. S., Galaktionov, K., and Beach, D. (1995) Proc. Natl. Acad. Sci. USA 92, 7892–7896
18. Ford, J. C., al-Khodairy, F., Fotou, E., Sheldrick, K. S., Griffiths, D. J., and Carr, A. M. (1994) Science 265, 353–355
19. Bonnefoy-Berard, N., Liu, Y., von Willibrand, M., Sung, A., Elly, C., Mustelin, T., Yoshida, H., Ishizaka, K., and Altmann, A. (1995) Proc. Natl. Acad. Sci. USA 92, 10142–10146
20. Du, X., Harris, S. J., Tetaz, T. J., Ginsberg, M. H., and Berndt, M. C. (1994) EMBO J. 13, 1297–1303
21. Jones, D. H., Lee, S., and Alkon, D. (1995) FEBS Lett. 368, 55–58
22. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) Cell 75, 805–816
23. Durfee, T., Becherer, K., Chen, P. L., Yang, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Elledge, S. J. (1993) Genes Dev. 7, 555–569
24. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (eds) (1994) Current Protocols in Molecular Biology, Vol. 1, 10142–10146
25. O'Rourke, K. M., Laherty, C. D., and Dixit, V. M. (1992) J. Biol. Chem. 267, 24921–24924
26. Wolf, F. W., Saras, V., Seldin, M., Drake, S., Suchard, S. J., Shao, H., O'Shea, V. M. Dixit, unpublished results.
14-3-3 Associates with A20

27. Martin, H., Patel, Y., Jones, D., Howell, S., Robinson, K., and Aitken, A. (1993) FEBS Lett. 331, 296-303
28. Duan, H., Chinnaian, A. M., Hudson, P. L., Wing, J. P., He, W., and Dixit, V. M. (1996) J. Biol. Chem. 271, 1621-1625
29. Aitken, A., Howell, S., Jones, D., Madrazo, J., and Patel, Y. (1995) J. Biol. Chem. 270, 5706-5709
30. Muslin, A. J., Tanner, J. W., Allen, P. M., and Shaw, A. S. (1996) Cell 84, 889-897
31. Koyama, S., Williams, L. T., and Kikuchi, A. (1995) FEBS Lett. 368, 321-325
32. Aitken, A. (1995) Trends Biochem. Sci. 20, 95-97
33. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Annu. Rev. Cell Biol. 10, 251-337
34. Du, X., Fox, J. E., and Pei, S. (1996) J. Biol. Chem. 271, 7362-7367
35. Jaattelä, M., Mouritzen, H., Elling, F., and Bastholm, L. (1996) J. Immunol. 156, 1166-1173
36. Song, H. Y., Rothe, M., and Goeddel, D. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6721-6725