14-3-3 proteins mediate interactions between proteins involved in signal transduction and cell cycle regulation. Phosphorylation of target proteins as well as 14-3-3 are important for protein-protein interactions. Here, we describe the purification of a protein kinase from porcine brain that phosphorylates 14-3-3 \( \alpha \) on Thr-233. This protein kinase has been identified as casein kinase \( \alpha \) (CK\( \alpha \)) by peptide mapping analysis and sequencing. Among mammalian 14-3-3, only 14-3-3 \( \tau \) possesses a phosphorylatable residue at the same position (Ser-233), and we show that this residue is also phosphorylated by CK\( \alpha \).

In addition, we show that 14-3-3 \( \zeta \) is exclusively phosphorylated on Thr-233 in human embryonic kidney 293 cells. The residue 233 is located within a region shown to be important for the association of 14-3-3 to target proteins. We showed previously that, in 293 cells, only the unphosphorylated form of 14-3-3 \( \zeta \) associates with the regulatory domain of c-Raf. We have now shown that in vivo phosphorylation of 14-3-3 \( \zeta \) at the CK\( \alpha \) site (Thr-233) negatively regulates its binding to c-Raf, and may be important in Raf-mediated signal transduction.

The name 14-3-3 was given to an abundant mammalian brain protein family due to its particular migration pattern on two-dimensional DEAE-cellulose chromatography and starch gel electrophoresis (1). The proteins were subsequently named by Greek letters according to their respective elution positions on HPLC.\(^1\) Seven mammalian forms of 14-3-3 (\( \beta, \gamma, \varepsilon, \tau, \eta, \xi, \) and \( \sigma \)) have been found, and two are specifically expressed in T cells (\( \tau \)) and epithelial cells (\( \sigma \)). The 14-3-3 family is highly conserved, and individual proteins are either identical or contain a few conservative substitutions over a wide range of mammalian species. All are dimeric proteins with a pl around 4.5 and a subunit mass of 30–33 kDa. Homologues of 14-3-3 proteins have also been found in a broad range of eukaryotic organisms.

Although the exact function of 14-3-3 is not known, various biological activities have been ascribed for 14-3-3: activation of tyrosine and tryptophan hydroxylases (2), regulation of protein kinase C (3), stimulation of calcium-dependent exocytosis (4), cofactor activity for ADP-ribosylation by Pseudomonas aerugi-nosa exoenzyme S (5), and a role in cell cycle control (6).

New findings have suggested many additional roles for the 14-3-3 family, in particular mediating interactions between components involved in intracellular signal transduction (7). The discovery of the interaction of specific 14-3-3 proteins with Raf (8, 9) generated much interest in the 14-3-3 family. Whether 14-3-3 directly activates Raf is still controversial, and activation of Raf by 14-3-3 may in fact be due to stabilization rather than stimulation of Raf activity (10). However, it has been shown that dimerization may provide a mechanism for Raf activation (11, 12), and 14-3-3 may be involved in this process (13). 14-3-3 have also been shown to interact with other important signaling proteins including polyoma middle T antigen (14), Cdc25 phosphatases (15), protein kinase C \( \theta \) (16), Cbl (17), PI 3-kinase (18), Bcr and Bcr-Abl (19), KSR (20), and insulin-like growth factor I and insulin receptor substrate I (21). 14-3-3 proteins form homo- and heterodimers in cells (22). Since different signaling proteins have been shown to associate with distinct 14-3-3 isoforms, heterodimeric 14-3-3 could act as a scaffold protein to mediate the formation of protein complexes. Indeed, it has been shown that Raf can form a complex with Ber (23) or A20 (24), which is mediated in both cases by 14-3-3.

The crystal structures of 14-3-3 \( \tau \) (25) and \( \zeta \) (26) showed they are highly helical proteins, and the dimer forms a large negatively charged channel, the interior of which contains residues that are almost invariant throughout the family. The specificity of interaction of each 14-3-3 protein with diverse target proteins may involve the outer surface of the protein. 14-3-3 dimerization has been shown to be essential for target binding (17, 27).

It has been reported that target protein phosphorylation is important for 14-3-3 binding to tryptophan hydroxylase (28), nitrate reductase (29), keratin (30), BAD protein (31), Cbl (32), and insulin-like growth factor I receptor and insulin receptor substrate I (21). In addition, phosphatase treatment of Raf-1 and Bcr inhibits their associations with 14-3-3 in vitro (33). Analysis of the major phosphorylation site of Raf has led to the identification of a novel sequence motif RSXS\( ^P \)XP (where \( \text{SP} \) is phosphoserine) that may represent a conserved interaction sequence within 14-3-3-binding proteins (34).

14-3-3 \( \zeta \) was shown to be phosphorylated in human embryonic kidney cells, and only the unphosphorylated form bound to the N-terminal regulatory domain of Raf (35). Therefore, the phosphorylation of 14-3-3 may also play an important role in the regulation of protein complex formation, and therefore in
signal transduction. Other 14-3-3 isoforms have been shown to be phosphorylated. 14-3-3 ρ is phosphorylated on Ser residues and on Ser/Tyr residues in vivo by the kinase activities of Bcr and Bcr-Abl, respectively (19). 14-3-3 ρ also binds to Bcr, but is not phosphorylated (19). 14-3-3 β, γ, and τ are phosphorylated in vitro by a sphiinosine-dependent kinase (36). In all cases described above, the phosphorylation sites in 14-3-3 were not identified. In addition, some 14-3-3 forms are phosphorylated on Ser-64 by protein kinase C at a low stoichiometry (37).

In conclusion, the regulation of 14-3-3-mediated protein complex formation may be regulated by the ratio of homo- and heterodimers in cells, and by the phosphorylation of 14-3-3 targets as well as the phosphorylation of 14-3-3 itself. Therefore, the identification of the protein kinases that phosphorylate 14-3-3 proteins is important in the study of the role of 14-3-3 in signal transduction.

**EXPERIMENTAL PROCEDURES**

**Materials**—γ-<sup>32</sup>P[ATP] was from Amersham. Casein, histone H1, phosphocellulose P-40 were purchased from Sigma. Antibodies against PSTAIRE motif, PCTAIRE-1, PCTAIRE-2, and cdk5 were from Santa Cruz Biotechnology. Recombinant cassein kinase I (CKI) of the Schizosaccharomyces pombe gene skil was produced in E. coli. Recombinant human 14-3-3 was kindly provided by Dr. L. A. Pinna (Dipartimento di Chimica Biologica, Università di Padova, Padova, Italy).

**In Vitro Recombinant Proteins—**14-3-3 ε was purified as a maltose-binding protein as described (39).

The cDNA corresponding to human 14-3-3 ε was originally cloned in pKK233-2 (39). 14-3-3 cDNA from this clone was amplified by polymerase chain reaction (PCR) using two oligonucleotides (5'-GGGATCCT-GGATCCATGATTTGAGAACTGAGACGTC-3' and 5'-GGGATCCT-GGATCCATGATTTGAGAACTGAGACGTC-3') and (5'-GGGAATTCTTAGTTTTCAGCCCCTGCTCG-3') to create a 5' BamHI site and a 3' EcoRI site. Amplified cDNA was inserted in a pGEX-2T vector (Pharmacia Biotech Inc.) at BamHI/EcoRI restriction sites to express it as a GST fusion protein. To substitute Thr-233 (ACC) for Ser-233 (AGC), Antibodies against PSTAIRE motif, PCTAIRE-1, PCTAIRE-2, and cdk5. The immunoprecipitation was carried out as described previously (40). For in vitro kinase assays, immunoprecipitates were washed several times with lysis buffer and once with kinase buffer (50 mM Hepes (pH 7.5), 10 mM MgCl₂, 1 mM DTT, and 20 μM cold ATP). The washed beads were incubated with kinase buffer containing 2 μg of histone H1 (as control) or 14-3-3 ε, and 5 μCi of γ-<sup>32</sup>P[ATP] in a final volume of 50 μL. Reactions were stopped by adding sample buffer, and the samples were analyzed by SDS-PAGE.

**Purification of 14-3-3 Protein Kinase from Mammalian Brain—**Pig brains were obtained from Dalehead Foods (Cambridgeshire, UK) and homogenized at 4°C in buffer A (20 mM MES (pH 6.5), 20 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 2 mM DTT, 5% glycerol, 0.1% Nonidet P-40) containing 50 mM β-glycerophosphate, 20 mM sodium fluoride, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL each of leupeptin, pepstatin, and aprotinin, and centrifuged at 15,000 × g for 1 h at 4°C. The supernatant was then centrifuged at 150,000 × g for 1 h at 4°C.

**Kinase Assay—**5 μL aliquots of column fractions or purified CKI were added to a solution of 20 μL HEPS (pH 7.2), 1 mM DTT, 10 mM MgCl₂, 20 μM γ-<sup>32</sup>P[ATP] (8–10 Ci/mmol) containing 1.5 μg of purified 14-3-3, to a final volume of 30 μL. After incubation at 30°C for 20 min, the reaction was stopped after the addition of electrophoresis sample buffer and analyzed on 12.5% SDS-PAGE. Gels were stained with Coomassie Blue and autoradiographed.

**In Gel Digestion of Phosphorylated Proteins—**Pig brains were obtained from Dalehead Foods (Cambridgeshire, UK) and homogenized at 4°C in buffer A (20 mM MES (pH 6.5), 20 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 2 mM DTT, 5% glycerol, 0.1% Nonidet P-40) containing 50 mM β-glycerophosphate, 20 mM sodium fluoride, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL each of leupeptin, pepstatin, and aprotinin, and centrifuged at 15,000 × g for 1 h at 4°C. The supernatant was then centrifuged at 150,000 × g for 1 h at 4°C.

**RESULTS**

The brain-specific phosphorylation of 14-3-3 β and γ at the SPOE motif (38) could be due to a proline-directed kinase similar to cyclin-dependent kinase or mitogen-activated kinase. Therefore, initial experiments were designed to phosphorylate 14-3-3 by different proline-directed kinases as well as other kinases.

In vitro kinase assays were performed using purified protein kinases or by immunoprecipitation with specific antibodies. None of them was able to significantly phosphorylate 14-3-3 by itself. The extracts were then dried in a SpeedVac vacuum centrifuge, and made up to the injection volume with water for on-line liquid chromatography MS.

**Mass Spectrometry (MS) of Phosphorylated Peptides—**Electrospray MS of in gel digested phosphoprotein and solid phase sequencing on arginine membrane were carried out as described (42).

**In Vivo γ<sup>32</sup>P Labeling of 14-3-3 —**adenovirus 5E1A/B transformed human embryonic kidney 293 cells were grown and transiently transfected using LipofectAMINE (35). Cells at 80% confluence were transfected with a total amount of 8 μg of DNA from 14-3-3 ε alone or with Ras-V12 (35). Cells were metabolically labeled with [γ-<sup>32</sup>P]orthophosphate (1.5 mCi/ml) for 3 h at 37°C. Cell lysate preparation and immunoprecipitation of Myc-tagged 14-3-3 ε with 9E10 antibodies have been described (35).

**Purification of the T233 Kinase—**The labeling of the 14-3-3 kinase with 8-azido-γ-<sup>32</sup>P[ATP] was performed as described (41) with some modifications. An aliquot containing the 14-3-3 kinase was incubated in 50 μL of buffer A (pH 7.5) containing 1 mM DTT, 5 μCi of 8-azido-γ-<sup>32</sup>P[ATP], and 0.3 μg of 14-3-3 ε (Table 1). The extracts were then dried in a SpeedVac vacuum centrifuge, and made up to the injection volume with water for on-line liquid chromatography MS.

**In Vivo γ<sup>32</sup>P Labeling of 14-3-3 —**adenovirus 5E1A/B transformed human embryonic kidney 293 cells were grown and transiently transfected using LipofectAMINE (35). Cells at 80% confluence were transfected with a total amount of 8 μg of DNA from 14-3-3 ε alone or with Ras-V12 (35). Cells were metabolically labeled with [γ-<sup>32</sup>P]orthophosphate (1.5 mCi/ml) for 3 h at 37°C. Cell lysate preparation and immunoprecipitation of Myc-tagged 14-3-3 ε with 9E10 antibodies have been described (35).

**RESULTS**

The brain-specific phosphorylation of 14-3-3 β and γ at the SPEK motif (38) could be due to a proline-directed kinase such as cyclin-dependent kinase or mitogen-activated kinase. Therefore, initial experiments were designed to phosphorylate 14-3-3 by different proline-directed kinases as well as other kinases. Experiments were performed using purified protein kinases or by immunoprecipitation with specific antibodies. None of them was able to significantly phosphorylate 14-3-3 in vivo (Table 1). We then attempted to purify the protein kinase from mammalian brain and found that 14-3-3 ε is phosphorylated in vitro on Thr233 by a protein kinase (termed “T233 kinase”) present in mammalian brain (42). In the present study, we identify this kinase as casein kinase Ia.

**Purification of the T233 Kinase from Pig Brain—**No kinase activity toward recombinant 14-3-3 ε was detectable in crude extract of pig brain (Fig. 1A, lane 7), possibly due to the presence of inhibitors as already shown for other kinases. The extract (lane 7) was loaded on a SP-Sepharose column, and bound proteins were eluted using a salt gradient. Under these conditions, we recovered a kinase activity eluting...
in fractions 16–18 at 0.35 M NaCl, which phosphorylated 14-3-3 ζ (Fig. 1A). The kinase activity from the SP-Sepharose was eluted from an Affi-Gel Blue column in fractions 32–42 between 0.6 and 0.8 M NaCl (Fig. 1B). Fractions containing the kinase activity were pooled and chromatographed on a Mono S column. The kinase eluted in fractions 12–14 at 0.55 M NaCl (Fig. 1C) and was subjected to gel filtration chromatography (Fig. 1D). The absolute levels of kinase activity at each step of chromatography are not known, and no specific activity can be measured because of the high sensitivity of the kinase to salt concentration as shown in Fig. 1, B and C, before (lane b) and after (lane a) dialysis to remove salt.

The T233 Kinase Is a Protein of 38–40 kDa—The chromatographic procedures did not lead to a complete purification of the T233 kinase, and a few protein bands remained. Nevertheless, one major protein of 38 kDa was visualized with silver staining (Fig. 2A). Photoaffinity labeling of the kinase using 8-azido-[γ-32P]ATP was performed to establish its molecular weight. This revealed the presence of one radiolabeled band around 40 kDa (Fig. 2B). The Mₐ of the kinase after gel filtration was 30–35 kDa (Fig. 1D). When gel filtration was performed at low (50 mM) NaCl, the kinase eluted from the column with a lower apparent Mₐ, suggesting that some nonspecific interactions with the column occurred (data not shown). SDS-PAGE analysis of the kinase activity eluting at an apparent Mₐ of 30–35 kDa from the gel filtration column revealed a phosphorylated band of 40 kDa, corresponding possibly to the autoposphorylated form of the kinase (Fig. 1, B and D). We therefore concluded that the kinase has an Mₐ of 38–40 kDa.

Identification of the 38-kDa Protein as Casein Kinase Iα—The fractions containing the T233 kinase activity from the gel filtration column were then separated on SDS-PAGE (Fig. 2A). The 38-kDa protein band was digested in gel with trypsin, and the mass of each peptide was measured by electrospray mass spectrometry. Analysis of the peptide mass map using the “Peptidesearch” program identified the 38-kDa protein as casein kinase Iα (CKIα) (Fig. 3A).

Is CKI Identical to the T233 Kinase?—To be certain that the T233 kinase was CKI, and not a copurifying protein kinase, in vitro kinase assays were performed using purified mammalian CKI and recombinant yeast CKI (Fig. 3B). Both kinases phosphorylated 14-3-3 ζ as well as the positive control proteins, i.e., casein and phosvitin. Moreover, the T233 kinase phosphorylated both the CKI substrates. The residue in 14-3-3 ζ phosphorylated by mammalian and yeast CKI was in both cases identified as Thr-233, since the mutant T233A was not phosphorylated.

14-3-3 ζ Is Phosphorylated in Vivo Exclusively on Thr-233 in 293 Cells—In human embryonic kidney 293 cells, only one tryptic peptide corresponding to the C terminus of 14-3-3 ζ was phosphorylated (Fig. 4A). Sequencing of the phosphopeptide revealed that only Thr-233 is phosphorylated (Fig. 4B). We have previously shown that in these cells only the unphosphorylated form of 14-3-3 ζ bound to Raf (35). Therefore, together with the present results, we conclude that in vivo, when phosphorylated on Thr-233, 14-3-3 ζ does not bind to the N-terminal regulatory domain of c-Raf.

CKI Phosphorylates in Vitro Only 14-3-3 τ and ζ among Mammalian 14-3-3—Among the mammalian 14-3-3 family, only 14-3-3 τ contains a potential phosphorylatable residue at the same position as 14-3-3 ζ (Table II). When different 14-3-3 isoforms were tested for their ability to be a CKI substrate in vitro, only 14-3-3 τ in addition to 14-3-3 ζ was phosphorylated by recombinant yeast CKI (Fig. 5A). The same result was obtained with mammalian CKI (data not shown).

Mass spectrometry after trypsin digestion of phosphorylated 14-3-3 τ revealed the phosphorylation of only one tryptic peptide and its sequencing showed the phosphorylation of Ser-233 (data not shown). Indeed, the mutant 14-3-3 τ S233A was not phosphorylated by purified mammalian CKI (Fig. 5B). In conclusion, CKI phosphorylates in vitro 14-3-3 τ on Ser-233.

DISCUSSION

Proteins of the 14-3-3 family bind in a phosphorylation-dependent manner to several target proteins. This may involve a novel consensus sequence RSX²XP, where S²P is serine (34). Indeed, most 14-3-3-binding proteins contain a putative consensus motif (7, 34), and it has been shown that for association with 14-3-3, this sequence must be phosphorylated in nitrate reductase (29) and BAD (31). However, the requirement for phosphorylation at this motif is still controversial for Raf because recombinant Raf purified from bacteria, which has been shown to electrophoresis MS not to be phosphorylated, is still able to bind 14-3-3. However, the phosphorylation of the motif may increase the affinity between Raf and 14-3-3.

The domain in 14-3-3 which is involved in target binding is not yet well defined. However, using deletion mutants of 14-3-3, Luo et al. (27) showed that the C-terminal 65 residues (176–245) of 14-3-3 τ were sufficient to interact with Raf. With a similar approach, Ichimura et al. (44) found that the last 76 residues (170–246) in 14-3-3 ν were essential for the binding to phosphorylated tryptophan hydroxylase, in particular the amino acids 171–213. Using the yeast two-hybrid system, the ligand-activated glucocorticoid receptor has been shown to interact to the C terminus (residues 187–246) of 14-3-3 ε (45). Liu et al. (17) showed that the last 15 residues (230–245) of 14-3-3 τ were required for efficient binding to Cbl, Raf, and PI 3-kinase. Therefore, the C terminus of 14-3-3 is essential for interaction with target proteins. As shown in the crystal structure of 14-3-3 τ (25) and ν (26), this region is not highly ordered.

In this study, we have shown that 14-3-3 τ is phosphorylated on Thr-233 in the C terminus. This site is accessible at the surface of the dimer, and we found that the dimer is phosphorylated in vitro (data not shown). The protein kinase responsi-

| Kinase          | Method   | Phosphorylation |
|-----------------|----------|-----------------|
| Proline-directed kinases |          |                 |
| GSK3 αβ = TPK-I | p        |                 |
| cdk5 = TPK-II   | p        |                 |
| Cdc2            | p        |                 |
| PSTAIRE related proteins | IP     |                 |
| PCTAIRE 1       | IP       |                 |
| PCTAIRE 2       | IP       |                 |
| ERK1            | p        |                 |
| RK (p38)        | p        |                 |
| Non-proline-directed kinases |        |                 |
| c-Raf           | p        | −               |
| B-Raf           | p        | −               |
| Mos             | p        | −               |
| MEKK            | p        | −               |
| MAPKAP          | p        | −               |
| CK-II           | p        | +/−            |
| p90S6 kinase (RSK) | p    | +/−            |
| p70S6 kinase    | p        | −               |
| PKC             | p        | −               |

Table I

In vitro phosphorylation of 14-3-3 ζ by different protein kinases

Purified protein kinases (p) were used to phosphorylate in vitro recombinant 14-3-3 ζ. In some cases, protein kinases were immunoprecipitated (IP) from rat brain before doing an in vitro kinase assay with recombinant 14-3-3 ζ as a substrate. No (+) or a weak (+/−) 14-3-3 phosphorylation was detected with all the kinases tested. All kinases were assayed with appropriate control substrates.

S. Howell and A. Aitken, unpublished results.
Casein Kinase I Phosphorylates 14-3-3

 FIG. 1. Purification of the T233 kinase from pig brain. A, the extract (lane T) from pig brains was loaded on a 100-ml FF SP-Sepharose column (Pharmacia) pre-equilibrated with buffer A. The bound proteins were eluted using a FPLC system (Pharmacia) with a gradient of 0–1 M NaCl, and 13 ml fractions were collected (top). The flow-through (lane FT) and the fractions were assayed for their phosphorylating activity toward recombinant 14-3-3 (bottom). B, the peak fractions (80 ml) corresponding to the 14-3-3 kinase activity (lane b) were dialyzed into buffer A (lane a) and loaded on a 35 ml Affi-Gel-Blue column (Bio-Rad) pre-equilibrated with buffer A. The bound proteins were eluted with a gradient of 0–1 M NaCl (top), and fractions of 5 ml were collected and assayed for their ability to phosphorylate 14-3-3 (bottom). A 40-kDa protein co-eluted with the T233 kinase activity and may represent the autophosphorylated form of the protein kinase (kinase?). C, active fractions (55 ml) were pooled (lane b), dialyzed into buffer A (lane a), and loaded onto a 1-ml Mono S column (Pharmacia) pre-equilibrated with buffer A. The bound proteins were eluted with a gradient of 0–1 M NaCl (top). Fractions of 0.5 ml were collected and assayed for their ability to phosphorylate 14-3-3 (bottom). The protein of 40 kDa may be the autophosphorylated form of the T233 kinase (kinase?).

ble has been identify as CKI by the following criteria: 1) mass spectrometric and sequencing analysis of the purified protein revealed that the kinase was CKIα, 2) this kinase phosphorylated specific substrates of CKI such as casein and phosvitin, and 3) CKI from mammalian and from S. pombe phosphorylated 14-3-3 τ and ζ at residue 233.

CKI are a family of ubiquitous monomeric Ser/Thr protein kinases ranging in size from 25 to 55 kDa (46). They were
Casein Kinase I Phosphorylates 14-3-3

Fig. 2. The $M_r$ of the T233 kinase is 38–40 kDa. A, an aliquot of the active fractions from the last step of purification was analyzed by SDS-PAGE followed by silver staining. B, the labeling of the T233 kinase with 8-azido-$\gamma$-32P-ATP was performed with $5 \mu$Ci of 8-azido-ATP, and without (lane 0) or with 300 $\mu$Ci of 8-azido-ATP (lane 300). Samples were then analyzed on 12.5% SDS-PAGE and autoradiographed.

Fig. 3. The T233 kinase is identical to CKI. A, the active fractions from the gel filtration column were pooled and loaded on SDS-polyacrylamide gel. The band corresponding to the 39-kDa protein (Fig 2A) was excised, subjected to trypsin digestion, and electrospray mass spectrometry. The 38-kDa protein was shown to be identical to CKIa by analyzing with the “Peptidesearch” program from the group of Matthias Mann at EMBL (Heidelberg) using the non-redundant data base of protein sequences (43). The boxes correspond to the peptides recovered after trypsin digestion of the 38-kDa protein, and match unequivocally with the sequence of CKIa. Some peptides were Edman sequenced and also matched (>) with CKIa, B, T233 kinase, purified mammalian CKI and $S. pombe$ CKI (Upstate Biotechnology Inc) were tested for their ability to phosphorylate wild-type (WT) and T233A 14-3-3 $\zeta$, as well as the positive control substrates casein (C) and phosphovitin (P). The first lane (0) represents the negative control without substrate.
highly ordered in the crystal structure (25, 26), but which has been shown to be required for efficient binding of 14-3-3 proteins to target proteins (17, 27, 44, 45). Therefore, the phosphorylation of 14-3-3 proteins has been proposed to be involved in this process (13). The phosphorylation of 14-3-3 on Thr-233 would therefore potentially affect Raf activity. The control of 14-3-3 phosphorylation at the CKI site will be investigated to provide further insights for its function in cells. In conclusion, phosphorylation of specific isoforms of 14-3-3 may play an important role in the regulation of protein complex formation in signal transduction.

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REFERENCES

1. Aitken, A., Collinge, D. B., van Heusden, G. P. H., Roseboom, P. H., Isobe, T., Rosenfeld, G., and Soll, J. (1992) Trends Biochem. Sci. 17, 498–501.
2. Ichimura, T., Isobe, T., Okuyama, T., Takahashi, N., Araki, K., Kuwano, R., and Takahashi, Y. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 7084–7088.
3. Aitken, A. (1995) Trends Biochem. Sci. 20, 95–97.
4. Morgan, A., and Burgoyne, R. D. (1992) Nature 355, 833–836.
5. Fu, H., Coburn, J., and Collier, R. J. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2230–2234.
6. Ford, J. C., Al-Khodairy, F., Foteu, E., Shieldrick, K. S., Griffiths, D. J. F., and Carr, A. M. (1994) Science 265, 533–535.
7. Aitken, A. (1996) Trends Cell Biol. 6, 341–347.
8. Morrison, D. (1994) Science 266, 56–58.
9. Burbelo, P. D., and Hall, A. (1995) Curr. Biol. 5, 95–96.
10. Morrison, D., and Ciechanover, A. (1997) Curr. Opin. Cell Biol. 9, 174–179.
11. Farrar, M. A., Alberola-Ila, J., and Perlmutter, R. M. (1996) Nature 383, 178–181.
12. Luo, Z., Trivion, G., Belshaw, P. J., Vavvas, D., Marshall, M., and Avruch, J. (1996) Nature 383, 181–185.
13. Marshall, C. J. (1996) Nature 383, 127–128.
14. Pallas, D. C., Fu, H., Haehtel, L. C., Weller, W., Collier, R. J., and Roberts, T. M. (1994) Science 265, 535–537.
