Tra1p Is a Component of the Yeast Ada-Spt Transcriptional Regulatory Complexes*

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The yeast Ada and TBP class of Spt proteins interact in multiple complexes that are required for transcriptional regulation. We have identified Tra1p as a component of these complexes through tandem mass spectrometry analysis of proteins that associate with Ngg1p/Ada3p. TRA1 is an essential gene and encodes a 3744-amino acid protein that is a member of a group of proteins including the catalytic subunit of DNA-dependent protein kinase, ATM and TRRAP, with carboxy-terminal regions related to phosphatidylinositol 3-kinases. The interaction between Tra1p and Ada/Spt components was verified by the reciprocal coimmunoprecipitation of Ada2p and Tra1p from whole cell extracts in one or more complexes containing Spt7p. Tra1p cofractionated with Ngg1p and Spt7p through consecutive chromatography on Mono Q, DNA-cellulose, and Superose 6 columns. Binding of Tra1p to DNA-cellulose required Ada components. The association of Tra1p with two Ada-Spt complexes was suggested by its cofractionation with Ngg1p and Spt7p in two peaks on the Mono Q column. In the absence of Ada2p, the elution profile of Tra1p shifted to a distinct peak. Despite the similarity of Tra1p to a group of putative protein kinases, we have not detected protein kinase activity within immunoprecipitates of Tra1p or the Ada-Spt complexes.

The ADA genes were identified in Saccharomyces cerevisiae based on their requirement for the regulated activation and repression of transcription (1–3). In initial studies, Ada2p, Ngg1p/Ada3p, and Gen5p/Ada4p were shown to function in a complex (4–6). The identity of Gen5p and its human homolog hGCN5 as histone acetyltransferases suggested that one role of these complexes is to modulate nucleosome structure (reviewed in Refs. 7–9). The identity of Gcn5p and its human homolog hGCN5 as histone acetyltransferases suggested that one role of these complexes is to modulate nucleosome structure (reviewed in Refs. 7–9). The association between the Spt and Ada proteins agrees with the functional link between the Ada proteins and TBP which was suggested by immunoprecipitation and affinity chromatography (8, 14–16).

A detailed understanding of the mechanisms and regulation of the Ada and Spt protein-containing complexes (Ada-Spt complexes for simplicity) requires the identification of component proteins. We now identify Tra1p as a component of the Ada-Spt complexes. Tra1p is a member of a group of putative protein kinases, including the catalytic subunit of human DNA-dependent protein kinase (DNA-PKcs) and ATM, that contain a carboxy-terminal region related to phosphatidylinositol 3-kinases (PI3K)1; reviewed in Refs. 17–20. In addition, Tra1p shows extensive sequence similarity throughout its entire length to the human protein TRRAP that associates with both c-Myc and E2F-1 (21).

MATERIALS AND METHODS

DNA Constructs, Yeast Strains, and Genetic Analysis

Epitope-tagging of TRA1. A BsrI107I-BglII fragment of Ycp88-NGG1(3), containing the DED1 promoter, Myc tag, and NosI restriction site, was cloned into HindIII-BamHI sites of YCplac111. A 11,832-base pair FspI fragment from ATCC cosmid 70,897 was cloned into the Smal site of this construct placing TRA1 downstream of the promoter. The 5′ segment of TRA1 was synthesized by PCR, with a NosI site at the position of the translational start. Alleles expressing myc-NGG1 and HA-ADA2 have been described (5, 8). Six histidine-tagged myc-NGG1 (HM-NGG1) was constructed by inserting a NosI fragment expressing 6-His into pDMYC-NGG1 (5).

All CY designated yeast strains are derivatives of KY320 (MATa ura3–52 ade2–101 trp–1 lethal2:PET56). CY927 and CY947 contain Tr10 LUK disruptions of the ADA2 coding region (5). CY947 and CY1077 contain Myc-tagged NGG1 integrated at his3 (5). CY979 contains disruptions of ngl1 and gal80 and has HM-NGG1 integrated at his3. CY1013, carrying a Tr10LUK disruption of TRA1 encompassing the coding region from amino acids 168 to 3618, was constructed in KY320 containing myc-TRA1-YCplac111. After selecting for the loss of UR3A (CY1020), the TRP1 marker on myc-TRA1-YCplac111 was changed to URA3 by single step recombination to give CY1021 (22). FY1093 is a derivative of FY630 (MATa ura3–52 ade8 trp–363 his3 his107 lys2–801173R2 leu2::PET56) that contains spfΔ402::LEU2 strain see Ref. 23; generously provided by Fred Winston).

To construct a disruption allele of TRA1 for genetic analysis, tagged PCR was performed using a 5′-oligonucleotide containing 60 base pairs corresponding to the sequence immediately upstream of TRA1 followed

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1 The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; PCR, polymerase chain reaction; PFLC, fast protein liquid chromatography; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin; kb, kilobase pair; NTA, nitrosoacetic acid.
by sequence from the 5’ end of HIS3. The 3’ primer included 3’ HIS3 sequence flanked by 60 base pairs corresponding to sequence immediately downstream of the TRA1 stop codon. HIS3-containing plasmid was used as template, and the resulting PCR fragment was used to transform the diploid strain YPH501 using lithium acetate (24, 25). Transformants were plated onto histidine-deficient media, and colonies were obtained. DNA was isolated from transformants and subjected either to NheI digestion and Southern analysis with a HIS3 probe or used as a template in PCR reactions designed to amplify the unique junctional region formed between sequences upstream of TRA1 and the recombined HIS3 gene. Sporulation and tetrad dissection were performed using S. cerevisiae 6H8B30 column (Amersham Pharmacia Biotech). Yeast were grown at 30°C in YPD broth (1% yeast extract, 2% peptone, 2% glucose) or in minimal media (0.67% yeast nitrogen base without amino acids, 2% glucose) and supplemented as required.

**Purification of HM-Ngg1p**

Whole cell extract was prepared from 8-liter cultures of strains expressing HM-Ngg1p (CY979) or Myc-Ngg1p (CY1077) in Buffer E (50 mM HEPES (pH 7.8), 50 mM NaCl, 20 mM imidazole, 10% glycerol and 0.1% Nonidet P-40; see Ref. 8). Protease inhibitors (8) and 1.0 M dithiothreitol were included in all solutions. 150 mg of protein (~4 ml) was rotated with 1.5 ml of Sepharose CL-4B for 30 min at 4°C. Unbound protein was rotated with 2.5 ml of Ni²⁺-nitrilotriacetic acid agarose (Ni²⁺-NTA; Qiagen) for 1 h. The mix was applied to a column, washed with 50 ml of Buffer E, then consecutively with 5 ml of Buffer E containing 50 mM NaCl, 200 ml imidazole fractions were collected (Centricron-30; Amicon) and separated on a 6% SDS gel. From the Coomassie-stained gel of the 350 mM imidazole fraction, gel slices corresponding to ~400-kDa protein from the HM-NGG1 strain and its parallel position from the myc-NGG1 control were excised and washed with water for 20 min.

**Protein Identification**

Protein was eluted and in-gel trypsin digested by the method of Shevchenko et al. (27). Protein was identified by micro-column high performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and data base searching. A 100- to 200-μm fused silica capillary (Ref. 28; Polymetrics, Inc.) was packed to a length of ~15 cm with 10 mm POROS 10 R2 reverse phase material (Percy Biosystems). The fritted end of the column was inserted into the needle of the electrospray ion source and sample loaded by helium pressurization in a stainless steel bomb (29). Chromatography was performed with a dual syringe pump (Applied Biosystems). The mobile phase consisted of 0.5% acetic acid (solvent A) and 80:20 acetonitrile/water containing 0.5% acetic acid (solvent B). A 100:1 precolumn split was used to deliver a flow rate of 1 to 1.5 ml/min. The high performance liquid chromatography program was programmed to ramp solvent B from 0 to 80% over 15 min. Electrospray ionization was carried out at voltage of 4.6 kV. Tandem mass spectra were acquired automatically during the entire gradient run (30).

Tandem mass spectra were searched against an S. cerevisiae protein data base (Saccharomyces Genome Data Base) using the SEQUEST program (31). Parameters for the SEQUEST program were set to locate potential sites of phosphorylation at serine, threonine, and tyrosine residues (32). Every sequence with high scores that matched a tandem mass spectrum was manually verified.

**Fractionation of Tra1p, Ngg1p, Ada2p, and Spt7p**

**Ion Exchange Chromatography**—32 mg (2 ml) of a mixture of whole cell extract containing Myc-Tra1p, Spt7p, and Myc-Ngg1p was prepared in 40 mM Tris-HCl (pH 7.7) buffer containing 20 mM NaCl, 10% glycerol, 0.08% Nonidet P-40, 0.2 mM EDTA, and 0.1 mM dithiothreitol. The extract was applied to a column (1 ml) of Q Sepharose FF (Amersham Pharmacia Biotech) at a flow rate of 0.1 ml/min. After washing the column with 4 ml of the extraction buffer, protein was eluted with a 21-ml gradient of 20–700 mM NaCl at a flow rate of 0.25 ml/ml. Protein from 45–μl aliquots of 250-μl fractions was separated by SDS-PAGE and analyzed by Western blotting with anti-Myc and anti-SSN6 antibodies. Similarly, extracts prepared from wild-type strain (TRA1 ade2Δ) or from wild-type strain (TRA1 ade2Δ) were co-fractionated on theMono Q column and the peak fraction containing Myc-Tra1p and/or Spt7p used for subsequent analysis.

**Chromatography on DNA-Cellulose**—The peak fraction containing Myc-Tra1p, Spt7p, and Myc-Ngg1p from the ~350 mM NaCl Mono Q fraction (Complex I) was dialyzed against DB buffer (5 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM MgCl₂, 0.02% Nonidet P-40, 5% glycerol) and loaded onto a 0.5-ml DNA-cellulose column (Amersham Pharmacia Biotech) at a flow rate of 1.5 ml/h. After washing to remove unbound protein, bound protein was eluted with 0.6 ml of DNA-binding buffer containing 100, 300, and 500 mM NaCl and analyzed by Western blotting. The ~250 mM NaCl Mono Q fraction from the ade2Δ strain CY927 was handled similarly.

**Gel Filtration Chromatography**—The DNA-cellulose fraction containing Myc-Tra1p, Spt7p, and Myc-Ngg1p (300 mM NaCl fraction) was dialyzed against 40 mM Tris-HCl buffer containing 300 mM NaCl, 4 μg/ml ethidium bromide, 10% glycerol, 0.08% Nonidet P-40, 0.2 mM EDTA, 0.1 mM dithiothreitol and loaded at a flow rate of 0.2 ml/min onto an FPLC Superose 6 column (Amersham Pharmacia Biotech). Protein from 150-μl aliquots of 500-μl fractions was precipitated with 10% trichloroacetic acid, solubilized in SDS sample buffer, and separated on a 5.5% SDS-polyacrylamide gel. Similarly, 16 mg of whole cell extract was prepared from strains expressing Myc-Tra1p, Spt7p, and Myc-Ngg1p, filtered through 0.22-mm membrane and applied to the Superose 6 column in the presence of ethidium bromide. Alternate fractions were analyzed by Western blotting with anti-Myc and anti-Spt7p antibody.

**Immunoprecipitation and Immunoblotting**

Immunoprecipitation of HA-Ada2p and Myc-Tra1p from whole cell extracts was performed as described (8). Immunoprecipitation from the Mono Q fraction containing AdaSpt Complex I was done after adjusting the buffer to 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.08% Nonidet P-40, and 5% glycerol (IP buffer) in a volume of 1.5 ml. 150 μg of protein was rotated for 20 min with 50 μl of protein A-Sepharose beads for 3 h. Beads were washed four times with 1.5 ml of IP buffer and twice with 1.5 ml of buffer containing 25 mM HEPES (pH 7.5), 50 mM KCl, and 5% glycerol and then eluted in SDS loading buffer at 60 °C for 5 min and separated by SDS-PAGE. To analyze for the association of Spt7p with Tra1p in the presence and absence of Ada2p, protein from the ~350 mM (50 μg) and ~250 mM NaCl (70 μg) Mono Q eluting fractions from strains expressing Myc-Tra1p (KY320, myc-TRA1 ADA2, CY927, Myc-TRA1 ada2Δ) and untagged Tra1p (TRA1 ADa2) were incubated with 4 μg of anti-Myc antibody and 15 μl of protein A-Sepharose after dialysis in IP buffer. Bound protein was eluted from the beads and analyzed by Western blotting for Spt7p. Western blotting with polyclonal anti-Spt7p antibody and monoclonal anti-Myc and HA antibodies has been described (5, 8, 16).

**RESULTS**

**Association of Tra1p with Ngg1p**—To search for components of the Ada-Spt complexes, we analyzed proteins associated with Ngg1p after affinity purification of Ngg1p from whole cell extracts. A derivative of NGG1 was constructed that encodes a protein with both six histidine and Myc-epitope tags (HM-Ngg1p). Extracts from a strain containing HMG-NGG1 and as a control from a strain containing myc-NGG1 were loaded onto parallel Ni²⁺-NTA columns. HM-Ngg1p eluted from the Ni²⁺-NTA column with buffer containing 350 mM imidazole as compared with Myc-Ngg1p which eluted in 50 mM imidazole. 350 mM imidazole eluants from both strains were compared by SDS-PAGE, revealing potential HM-Ngg1p-associated proteins (not shown). One of these proteins had an apparent molecular mass of ~400 kDa and thus did not match previously described Ada components. With the purification of the Ada components obtained by chromatography on Ni²⁺-NTA and the resolution afforded by its migration on SDS-PAGE, the ~400-kDa protein was isolated and its identity determined by micro-column high performance liquid chromatography coupled to tandem mass spectrometry and database searching.

The gene product Tra1p (YHR099W) was identified from 36 unannotated peptides that correspond to 15.2% of the mass of the previously identified Tra1p (yhr099w) gene encoding a protein of 3744 amino acids (433 kDa) with 20–50% sequence similarity throughout its length to TRRAP, a human protein that associates with c-Myc and E2F-1 (21). The absence of Tra1p in the parallel gel slice from the control strain suggested that its association with HM-Ngg1p was specific. In addition, mass spectrometry revealed that the peptide AEQGDLDSPKEQADLEGFSK between residues...
of 165 and 187 of Tra1p contained one phosphoserine.

**TRA1 Encodes an Essential Gene**—The approaches used to study further Tra1p in the Ada:Spt complexes depended on whether TRA1 was essential for cellular viability. The entire 11.2-kb coding region of one TRA1 allele was replaced with HIS3 in the diploid strain YPH501. To ensure that a TRA1 allele was disrupted several isolated His’ colonies were selected and their DNA analyzed by Southern blotting after digestion with NheI. Membranes were hybridized with a HIS3 probe. The common band at 7 kb represents the endogenous HIS3 genes in this strain (his3Δ200). The new band at 6.5 kb in lanes 2, 4, 5, and 6 represents a correctly targeted replacement of one TRA1 allele with tra1::HIS3. The smaller band in lane 7 represents HIS3 insertion insertion at another site in the genome. B, sporulation of diploid strains and tetrads dissection. The parental strain TRA1/TRA1 gave four viable spores from each tetrad (left panel). A representative TRA1 knock-out strain (TRA1/tra1::HIS3) yielded a 2:2 distribution of viable spores (right panel), indicating that TRA1 is essential for normal growth. wt, wild type.

**Fig. 1. TRA1 is essential for cell growth.** A, homologous recombination was used to replace the complete coding region of TRA1 with HIS3 in diploid strain YPH501. Individual His’ colonies were selected and their DNA analyzed by Southern blotting after digestion with NheI. Membranes were hybridized with a HIS3 probe. The common band at 7 kb represents the endogenous HIS3 genes in this strain (his3Δ200). The new band at 6.5 kb in lanes 2, 4, 5, and 6 represents a correctly targeted replacement of one TRA1 allele with tra1::HIS3. The smaller band in lane 7 represents HIS3 insertion insertion at another site in the genome. B, sporulation of diploid strains and tetrads dissection. The parental strain TRA1/TRA1 gave four viable spores from each tetrad (left panel). A representative TRA1 knock-out strain (TRA1/tra1::HIS3) yielded a 2:2 distribution of viable spores (right panel), indicating that TRA1 is essential for normal growth. wt, wild type.

**Fig. 2. Myc-Tra1p is sufficient for cell viability.** Yeast strains KY320 (TRA1) and CY1021 (tra1, myc-TRA1-YCplac111/URA3) both containing myc-TRA1-YCplac111/URA3 were transformed with myc-TRA1-YCplac111. These strains along with CY1021 lacking myc-TRA1-YCplac111/URA3 were plated on YPD after growth on rich media or directly on plates containing 5-fluoro-orotic acid (5-FOA). Acid (Fig. 2). Cells containing myc-TRA1-YCplac111 were able to lose the URA3 plasmid and thus grow on 5-fluoro-orotic acid, in contrast to CY1021 without myc-TRA1-YCplac111.

Ngg1p and Ada2p are intimately associated in multiple complexes, some of which contain the TBP class of Spt proteins (8–11). To verify the interaction of Tra1p with the ADA components, we determined if Tra1p coimmunoprecipitates with Ada2p. Whole cell extracts were prepared from strains expressing myc-TRA1 and HA-ADA2 independently and from a strain expressing both myc-TRA1 and HA-ADA2. HA-Ada2p and associated proteins were immunoprecipitated with anti-HA antibody, separated by SDS-PAGE, and Western blotted with anti-Myc antibody (Fig. 3A). The presence of Myc-Tra1p in immunoprecipitates with HA-Ada2p (lane 3), but not in immunoprecipitates lacking either HA-Ada2p (lane 2) or Myc-Tra1p (lane 1), indicated that Tra1p associates with Ada2p. Reprobing the blot with anti-HA antibody demonstrated that equivalent amounts of HA-Ada2p were immunoprecipitated from the HA-ADA2 containing strains (not shown).

The reciprocal experiment was performed in which Myc-Tra1p was immunoprecipitated and the presence of HA-Ada2p assayed by Western blotting. As a positive control an extract containing Myc-Ngg1p and HA-Ada2p was included. As shown in Fig. 3B, HA-Ada2p was found in Myc immunoprecipitates from strains expressing myc-TRA1 (lane 1) or myc-NGG1 (lane 4) in combination with HA-ADA2 but not from the strain lacking myc-TRA1 (lane 3). No band was detected in the strain lacking HA-tagged Ada2p (lane 2). To determine if other components of the SAGA complex associate with Myc-Tra1p, the blot was reprobed with anti-Spt7p antibody. As with HA-Ada2p, Spt7p specifically coimmunoprecipitated with Myc-Tra1p and Myc-Ngg1p.

In certain whole cell extracts Spt7p appeared as multiple bands (Fig. 3B, lanes 1 and 4). These may arise as a result of the modification of Spt7p by ubiquitination (16), although the reason for variability in the appearance of the bands is unclear.

**Tra1p Cofractionates with ADA and SPT Proteins**—To examine if Tra1p interacts with one or more of the Ada complexes, we compared the elution of Myc-Ngg1p, Spt7p, and Myc-Tra1p after fractionation by gel filtration and ion exchange chromatography. First, whole cell extract was chromatographed on a Superose 6 column, and equal volumes of alternate fractions were examined for Myc-Ngg1p, Spt7p, and Myc-Tra1p by Western blotting. As shown in the densitometric scanning profile in Fig. 4 and as described previously (8), Myc-Ngg1p elutes from this column in three peaks corresponding to complexes with sizes of approximately 2 MDa, 900 kDa, and 200 kDa. Spt7p elutes with the 2-MDa complex(es). A peak of Spt7p eluting at fraction 20 (approximately 1.5 MDa) suggests the possibility of an Ada-independent Spt7p complex (see be-
Myc-Tra1p and Ada2p with Tra1p. 25 mg of cell extract from KY320 containing TRA1 and HC body. Precipitated protein was Western blotted with anti-Myc antibody. Strain KY320 containing HA-Ada2p (lane 1), Myc-Ngg1p, and Spt7p was fractionated on Superose 6. Equal volumes of alternate fractions (150 μl) were precipitated with 10% trichloroacetic acid, separated by SDS-PAGE, and assayed for the presence of Myc-Tra1p (solid line), Myc-Ngg1p (dashed-dotted line), and Spt7p (dashed line) by immunoblotting. The void volume was determined with high molecular mass DNA and occurs at fraction 10. Arrowheads indicate the peak fractions containing the following calibration proteins: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), and bovine serum albumin (66 kDa).

The amount of Myc-Tra1p in Complex II prohibited the same analyses done with Complex I. Therefore, to address whether coelution of Myc-Tra1p with the Ada/Spt components in this complex is indicative of their association, we analyzed whether removal of Ada2p altered the elution profile of Myc-Tra1p from gel filtration and ion exchange columns. As shown in Fig. 7, when extracts prepared from the ada2 deletion strain CY927 were chromatographed on a Mono Q column, Myc-Tra1p eluted in fractions centered at ~250 mM NaCl and was absent from both complexes (I and II) in which it was found when prepared from the wild-type ADA2 strain. This altered elution profile for Myc-Tra1p from the ada2 strain suggests that it is a component of both the Ada/Spt Complexes I and II.

Tra1p Associates with Spt7p in the Absence of Ada2p and with Ada2p in the Absence of Spt7p—To investigate the interdependence of the associations of Tra1p with Ada2p and Spt7p, Myc-Tra1p immunoprecipitates from yeast strain CY927 were analyzed for Spt7p by Western blotting (Fig. 6A). Immunoprecipitates from both the ~250 mM NaCl Mono Q fraction after chromatography of extract from CY927 (ada2; lane 5) and as a positive control from the ~350 mM fraction (Complex I) from KY320 (ADA2; lane 2) contained Spt7p. This association of Myc-Tra1p and Spt7p was specific since Spt7p was not found in any of the fractions which lacked Myc-Tra1p (lanes 1, 3, 4, and 6).

A similar experiment was performed to determine if Tra1p associates with Ada2p in the absence of Spt7p. The spt7 disruption strain FY1093 and its parent strain FY630 were transformed with plasmids expressing Myc-Tra1p and HA-Ada2p.
Immunoprecipitates were analyzed for the presence of Myc-Tra1p by Western blotting. As shown in Fig. 8B, Myc-Tra1p, identified by its absence in control extracts lacking tagged protein (lane 3), was found within immunoprecipitates from both SPT7 wild-type (lane 1) and spt7 deletion (lane 2) strains. Tra1p thus independently associates with Ada2p and Spt7p. Binding of Tra1p to DNA-Cellulose Requires ADA Proteins—The binding of AdaSpt Complex I to DNA-cellulose was of particular note since it may reflect a function of the complex. To determine if Ada proteins are required for the binding of Myc-Tra1p to DNA-cellulose the; NaCl, Myc-Tra1p-containing fraction after chromatography of a CY927 (ada2) extract on DNA-cellulose. In contrast to Myc-Tra1p from a wild-type ADA2 extract, for the CY927 extract Myc-Tra1p eluted in the flow-through fraction (Fig. 9; compare top two panels). To address whether factors in a wild-type extract could restore DNA binding of this Myc-Tra1p, Myc-Tra1p (~250 mM NaCl Mono Q fraction) from the ada2 deletion strain was mixed with the ~350 mM NaCl Mono Q fraction from a wild-type strain lacking tagged Ada2p (TRA1 ADA2). As shown in the second to last panel, binding of approximately 25% of the Myc-Tra1p to DNA-cellulose was restored upon mixing with the wild-type Ada/Spt-containing frac-
Tra1p Associates with Ada/Spt Complexes

**Discussion**

**Tra1p Is a Component of Two Ada/Spt Complexes**—Our identification of Tra1p within the Ada/Spt complexes was based on its association with ADA components after affinity purification on Ni²⁺-NTA. Several lines of evidence confirm that Tra1p associates with the Ada/Spt proteins. The association of Tra1p and Ada/Spt components was shown by their reciprocal coimmunoprecipitation from whole cell extracts. As well as their copurification on a Ni²⁺-NTA column, Tra1p, Ngg1p, and Spt7p remain associated through an approximately 1200-fold purification over consecutive chromatography on FPLC Mono Q, DNA-cellulose, and Superose 6 columns. Furthermore, chromatography on the FPLC Mono Q column revealed that all of the Tra1p coeluted with Ngg1p and Spt7p in two distinct peaks. The validity of this cofractionation as a measure of association was also verified by the finding that Tra1p coimmunoprecipitated with Ada2p in the ~350 mM NaCl Mono Q fraction (Complex I) and the demonstration that the elution profile of Tra1p on Mono Q and DNA-cellulose was altered when performed with an extract from a strain lacking Ada2p. Association of Tra1p with the Ada/Spt proteins was also supported by the ability of partially purified Ada/Spt proteins to reconstitute binding of Tra1p to DNA-cellulose.

We have previously identified biochemically four complexes that contain the ADA proteins as follows: two with sizes of approximately 2 MDa and single complexes of approximately 900 and 200 kDa (8). The appearance of Tra1p with Ngg1p in the 2-MDa peak on Superose 6 and in two peaks on a Mono Q column suggests that it is found within both of the 2-MDa complexes. Since the Tra1p-containing complexes also contained Spt7p, one of these probably represents the previously described 1.8-MDa SAGA complex (9); the second thus identifies an additional Ada/Spt complex that has also been observed by Grant and Workman. Tra1p is likely a central molecule in the formation or stability of these complexes since the interaction of Ada2p with Tra1p was independent of Spt7p and similarly the interaction of Spt7p with Tra1p was independent of Ada2p.

The fractionation of the Ada and Spt proteins on Superose 6 and Mono Q also identified a possible Spt7p-containing complex that lacked the Ada proteins and Tra1p. This complex has an approximate size of 1.5 MDa based upon its elution from Superose 6. The existence of Ada-independent Spt7p agrees with the broader range of phenotypes seen in spt7 disruptions as compared with those seen for the adas (10, 15).

**Similarity of Tra1p to PI3K-related Molecules**—Tra1p is a member of a group of molecules with carboxy-terminal sequences similar to PI3Ks (33). Although related to the PI3Ks, these molecules can be distinguished from the PI3Ks by having a common region directly at their carboxyl terminus, their large size, and in some cases an ability to phosphorylate proteins (19, 34). Included in this group are the mammalian proteins DNA-PKζ, ATM, FRAP, TRRAP; the Drosophila protein MEI-41; the S. cerevisiae proteins Tor1p, Tor2p, Mec1p, and Tel1p; and from Schizosaccharomyces pombe, Rad3p (reviewed in Refs. 17–20 and see also Ref. 21) and the product of open reading frame C1F5.11C; the products of the Arabidopsis gene C47D12.1; and from Caenorhabditis elegans gene C47D12.1; and from Arabidopsis the product of 19K4.210. Tra1p shares approximately 25% (or greater) sequence similarity to all members of the group in its carboxy-terminal PI3K region and short stretches of similarity can also

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2 P. Grant and J. Workman, personal communication.
be identified with a BLAST search throughout the molecules. Of the PI3K-like molecules, two seem to be most closely related to Tra1p as follows: the product of the S. pombe gene C1F5.11C and the mammalian protein TRRAP (21). Both share 20–50% homology with Tra1p along the entire length of the molecules.

Although closely related to the PI3K family members, Tra1p lacks the DFG sequence found in many protein kinases (35). It does contain the DXXXXX kinase motif but in a flanking sequence context different from other family members. BLAST and FASTA searches identify other sequence relationships that could relate to function. The PI3K region shows similarity to the transcriptional repressor RGM1p (27% identity over a 136-amino acid overlap; see Ref. 36) and SIR4p (24% identity over a 244-amino acid overlap; see Refs. 37 and 38). Central regions of the protein are also similar to the transcriptional regulator TEC1p (39) and to MTR1p which is involved in nuclear protein import (40).

As a group the PI3K-related molecules are involved in many key cellular processes including DNA repair, meiotic recombination, V(D)J recombination, cell-cycle regulation, DNA damage recognition, and transcription (17, 18, 20). Interestingly, the human molecule most likely the homologue of Tra1p (TRA1) has a broader range of function(s) than the other components of the ADA complex because they retained histone acetyltransferase activity (not shown, Ref. 16). Furthermore, it is unlikely that Tra1p is inhibited by components of the ADA complex because immuno-precipitates of Tra1p isolated from the ada2 deletion strain were inhibited by components of the ADA complex because immunoprecipitates of Tra1p isolated from the ada2 deletion strain also lacked kinase activity. Clearly, we cannot at present exclude the possibility that Tra1p has unique substrates or assay conditions; however, the lack of kinase activity is consistent with the absence of the normally conserved DFG sequence in the kinase motif.

The sequence similarity of Tra1p to several key cellular regulators, its appearance in at least two of the Ada-Spt complexes, and even its large size suggests that Tra1p plays a key role in the structure, function, or regulation of the Ada-Spt complexes. Since all the detectable Tra1p cofractionated with Myc-Ngg1p, it appears to function principally through its association with the Ada-Spt proteins. The fact that unlike other Ada-Spt complex proteins, Tra1p is essential, does predict that it has a broader range of function(s) than the other components.

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