TECHNIQUES

Analyzing Protein Complexes in *Drosophila* With Tandem Affinity Purification–Mass Spectrometry

Alexey Veraksa,[1]* Andreas Bauer,[2] and Spyros Artavanis-Tsakonas[1,3]

We describe the application of tandem affinity purification–mass spectrometry (TAP-MS) to the study of protein complexes in *Drosophila*. We have constructed vectors for inducible expression of TAP-tagged fusion proteins in *Drosophila* cultured cells and in vivo. Using these vectors, we tagged, as a paradigm, several components of the Notch signaling pathway, isolated protein complexes containing the baits and associated proteins from cells and embryos, and identified the subunits by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Several known interactions involving Notch pathway elements were confirmed, and many novel potential interactions were uncovered. For some of the novel associations, we validated the interaction genetically and biochemically. We conclude that TAP, in combination with MS, can be used as an effective method for the studies of the *Drosophila* proteome. *Developmental Dynamics 232: 827–834, 2005*. © **2005 Wiley-Liss, Inc.**

Key words: Notch; *Drosophila*; mass spectrometry; protein complexes

Received 9 September 2004; Revised 7 October 2004; Accepted 13 October 2004

INTRODUCTION

Supplementing traditional genetic approaches, the study of protein interactions has emerged as a valuable method for discovering novel components of signaling pathways. Recent advances in mass spectrometry (MS) made it possible to identify the components of multisubunit protein complexes isolated from cell lysates with high sensitivity and accuracy, thereby facilitating analysis of protein interactions (Bauer and Kuster, 2003). A suitable method of isolating relatively pure native complexes from cells is a key requirement for this type of proteomic analysis.

Comparative studies of different isolation procedures based on affinity chromatography have led to a conclusion that purification of a complex by means of a protein bait tagged with the tandem affinity purification (TAP) tag is optimal for subsequent analysis by MS (Rigaut et al., 1999; Bauer and Kuster, 2003). The TAP tag is a double-affinity module composed of a calmodulin binding peptide and two IgG binding domains of *Staphylococcus aureus* protein A, separated by a TEV protease cleavage site (Rigaut et al., 1999). The use of the TAP tag allows rapid and efficient purification of complexes from cell extracts under native conditions. TAP in combination with MS now has been validated by many studies in yeast and higher eukaryotes and has led to the identification of multiple protein complexes and large-scale protein interaction networks (Puig et al., 2001;

[1]Massachusetts General Hospital Cancer Center, Department of Cell Biology, Harvard Medical School, Charlestown, Massachusetts
[2]Cellzome AG, Heidelberg, Germany
[3]Collège de France, Paris, France
Grant sponsor: National Institutes of Health; Grant numbers: NS26084; GM62931; CA098402.
***Correspondence to: Alexey Veraksa, Massachusetts General Hospital, Building 149, 13th Street, 7th Floor, Cancer Center, Charlestown, MA 02129. E-mail: veraksa@helix.mgh.harvard.edu**

DOI 10.1002/dvdy.20272
Published online 9 February 2005 in Wiley InterScience (www.interscience.wiley.com).

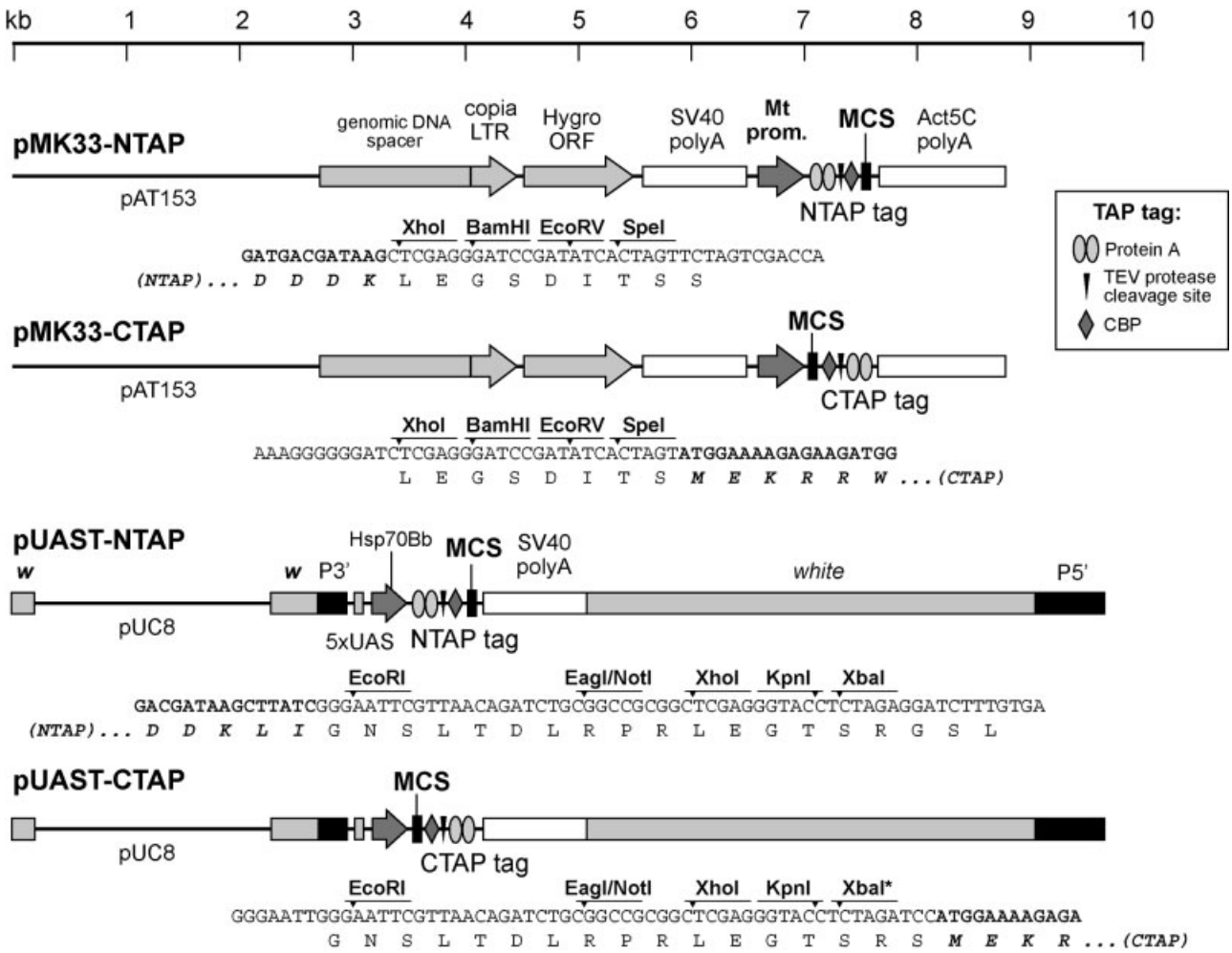

Fig. 1. Vectors for expression of tandem affinity purification (TAP) -tagged proteins in *Drosophila* cultured cells and transgenic flies. Polylinker region (MCS, multicloning site) is shown under a diagram of each vector, with unique cloning sites indicated above the sequence. Note that the elements of the amino terminal TAP tagging (NTAP) and carboxy terminal TAP tagging (CTAP) tags (two tandem IgG-binding Protein A modules, TEV protease cleavage site, and calmodulin binding peptide, CBP) are arranged in mirror-image orientation, so that Protein A modules are the most distal element in both tags. pMK33-based vectors carry metallothionein (Mt) promoter for copper-inducible expression and a constitutive hygromycin phosphotransferase gene (Hygro ORF) for selection of stably transfected cell lines (Koelle et al., 1991). pAT153 vector backbone is a low copy number plasmid. pUAST-based vectors for making transgenic flies express fusion constructs under the control of 5xUAS sequences and a minimal Hsp70Bb promoter that can be activated by using a variety of GAL4 drivers (Brand and Perrimon, 1993). *Note that the *Xba*I site in pUAST-CTAP is *dam*-methylation sensitive. GenBank accession numbers for complete vector sequences are AY727853-AY727856. LTR, long terminal repeat.

Gavin et al., 2002; Westermarck et al., 2002; Forler et al., 2003; Bouwmeester et al., 2004; Dziembowski and Seraphin, 2004).

We have constructed vectors for inducible expression of TAP-tagged proteins in *Drosophila melanogaster* cultured cells and in vivo. Using these vectors, we tagged several components of the Notch pathway and expressed these baits in two widely used *Drosophila* cell lines and embryos. Functionality of TAP-tagged constructs in the context of an organism was validated, among other criteria, by the rescue of corresponding mutant phenotypes. Protein complexes containing the baits and associated proteins were isolated by TAP, and the composition of these complexes was analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Our results confirmed several known interactions involving Notch pathway elements and uncovered novel interactions, some of which we validated biologically. Thus, TAP–LC-MS/MS should prove to be a useful tool for proteomic analysis in *Drosophila*.

METHODS AND RESULTS

Vectors for Inducible Expression of TAP-Tagged Proteins in *Drosophila*

pMK33-NTAP and pMK33-CTAP vectors for use in cell culture were constructed on the basis of the pMK33 (pMt/Hy) vector (Koelle et al., 1991; Fig. 1). To generate these vectors, TAP cassettes were amplified by polymerase chain reaction (PCR) from plasmids pBS1761 (for amino terminal TAP tagging, NTAP) and pBS1479

(for carboxy terminal TAP tagging, CTAP) and cloned into pMK33, preserving the unique sites in the polylinker and allowing in-frame fusions of the TAP tag with a protein of interest. The sequences of the source plasmids pBS1761 and pBS1479 (Puig et al., 2001) are available at http://www.uni-frankfurt.de/fb15/mikro/euroscarf/cz_plas.html. pMK33-NTAP and pMK33-CTAP provide copper-inducible expression of the fusion proteins driven by metallothionein (Mt) promoter, reducing potential toxicity problems associated with expression from constitutive promoters. In addition, the "on-board" hygromycin phosphotransferase gene provides Hygromycin B resistance and facilitates selection of stable cell lines by eliminating a need to cotransfect protein expression constructs with a helper plasmid (Fig. 1).

To construct a vector for inducible expression of TAP-tagged proteins in vivo, we used the pUAST plasmid, which allows flexibility in terms of both the amounts and patterns of expression, using a multitude of available GAL4 drivers (Brand and Perrimon, 1993). NTAP and CTAP modules were amplified by PCR from pBS1761 and pBS1479 plasmids described above, and inserted into pUAST (Fig. 1). Complete sequences of *Drosophila* TAP vectors were submitted to GenBank (accession nos. AY727853-AY727856).

Generation of Stable S2 and Kc Cell Lines and Transgenic Flies

Using these vectors, we TAP-tagged selected molecules known to be involved in Notch signaling, representing membrane-bound, cytoplasmic and nuclear subcellular compartments. We made two versions of each bait: pMK33-based (for expression in cultured cells) and pUAST-based (for expression in flies). Full-length Notch (NFL), known to be localized at the membrane and in the cytoplasm (Fehon et al., 1990), was tagged at the carboxy terminus, to make NFL-CTAP. Notch intracellular domain (NICD) and full-length Mastermind, both nuclear proteins (Fortini et al., 1993; Bettler et al., 1996), were tagged at the amino terminus to generate NTAP-NICD and NTAP-Mam, respectively. Full-length Deltex, a cytoplasmic regulator of Notch (Busseau et al., 1994), was tagged at either end to make NTAP-Dx and Dx-CTAP fusion proteins.

Drosophila S2 and Kc cells were grown in Schneider's medium (JRH Biosciences) in the presence of 10% fetal calf serum. Cells were transfected with 2 μg of pMK33-based constructs in six-well plates by using Effectene transfection reagent (Qiagen), according to manufacturer's instructions. After 2 days of incubation with transfection mixes, cells were subjected to selection in the presence of Hygromycin B (Sigma). For S2 cells, the optimal concentration of Hygromycin B was found to be 300 μg/ml, whereas Kc cells tolerated up to 600 μg/ml. Stable lines were established after approximately 1 month. Transgenic fly lines were generated using standard methods (Spradling and Rubin, 1982), with at least three homozygous viable lines established for each construct. pUAST-NTAP and pUAST-CTAP vectors were also used to create control fly lines.

Verification of Construct Functionality

We probed the functionality of the TAP-tagged constructs according to the following criteria: (1) protein size on Western blots; (2) subcellular localization; (3) rescue of loss of function mutant phenotypes by the transgenes; and (4) examination of phenotypes resulting from overexpression of the transgenes in flies.

To verify expression levels and the size of TAP-tagged proteins, S2 and Kc cell lines stably transfected with pMK33-based constructs were induced overnight with 0.35 mM $CuSO_4$, after which cells were harvested and resuspended in sodium dodecyl sulfate (SDS) sample buffer. pUAST-based constructs were induced by crossing the transformants with an *arm-GAL4* driver, which is expressed ubiquitously at medium levels (Sanson et al., 1996). The 0–16 hr embryos were collected, dechorionated with bleach, and lysed in SDS sample buffer. After electrophoresis and blotting on Immun-Blot PVDF membrane (Bio-Rad), proteins were visualized in a one-step Western detection procedure using anti–Protein A antibody conjugated to horseradish peroxidase (HRP; Rockland Immunochemicals). All of the stably transfected S2 and Kc lines, and all of the fly lines expressed proteins of expected sizes, taking into account the additional 20 kDa contributed by the presence of the TAP tag (Fig. 3 and data not shown).

Immunocytochemical analysis demonstrated that the TAP tagged proteins were localized in the expected subcellular compartments. NTAP-NICD and NTAP-Mam were exclusively nuclear (Fortini et al., 1993; Bettler et al., 1996), NFL-CTAP was localized on the cell surface and in the cytoplasm (Fehon et al., 1990), and NTAP-Dx and Dx-CTAP were exclusively cytoplasmic (Busseau et al., 1994; Fig. 2).

Finally, the functionality of the TAP-tagged constructs was tested in vivo for their ability to rescue relevant loss of function mutants, as well as to elicit overexpression phenotypes, using pUAST-based transgenes. Thus, NFL-CTAP, NTAP-Mam, and Dx-CTAP provided complete rescue of the corresponding mutant wing phenotypes and gave expected overexpression phenotypes (Fig. 2 and data not shown). NTAP-Dx gave a partial rescue and was somewhat toxic upon overexpression. NTAP-NICD could not be tested in a rescue experiment due to lethality with most drivers, but produced phenotypes indistinguishable from those obtained with untagged NICD when ectopically expressed with *GMR-GAL4* (data not shown). Overexpression of NTAP or CTAP tag alone resulted in no observable phenotype, using a variety of drivers, suggesting that the TAP tag does not lead to general toxicity in living animals.

Therefore, using the criteria outlined above, we found that NFL-CTAP, NTAP-Mam, Dx-CTAP, and NTAP-NICD are functional and, thus, could be incorporated into relevant protein complexes in vivo, while in the

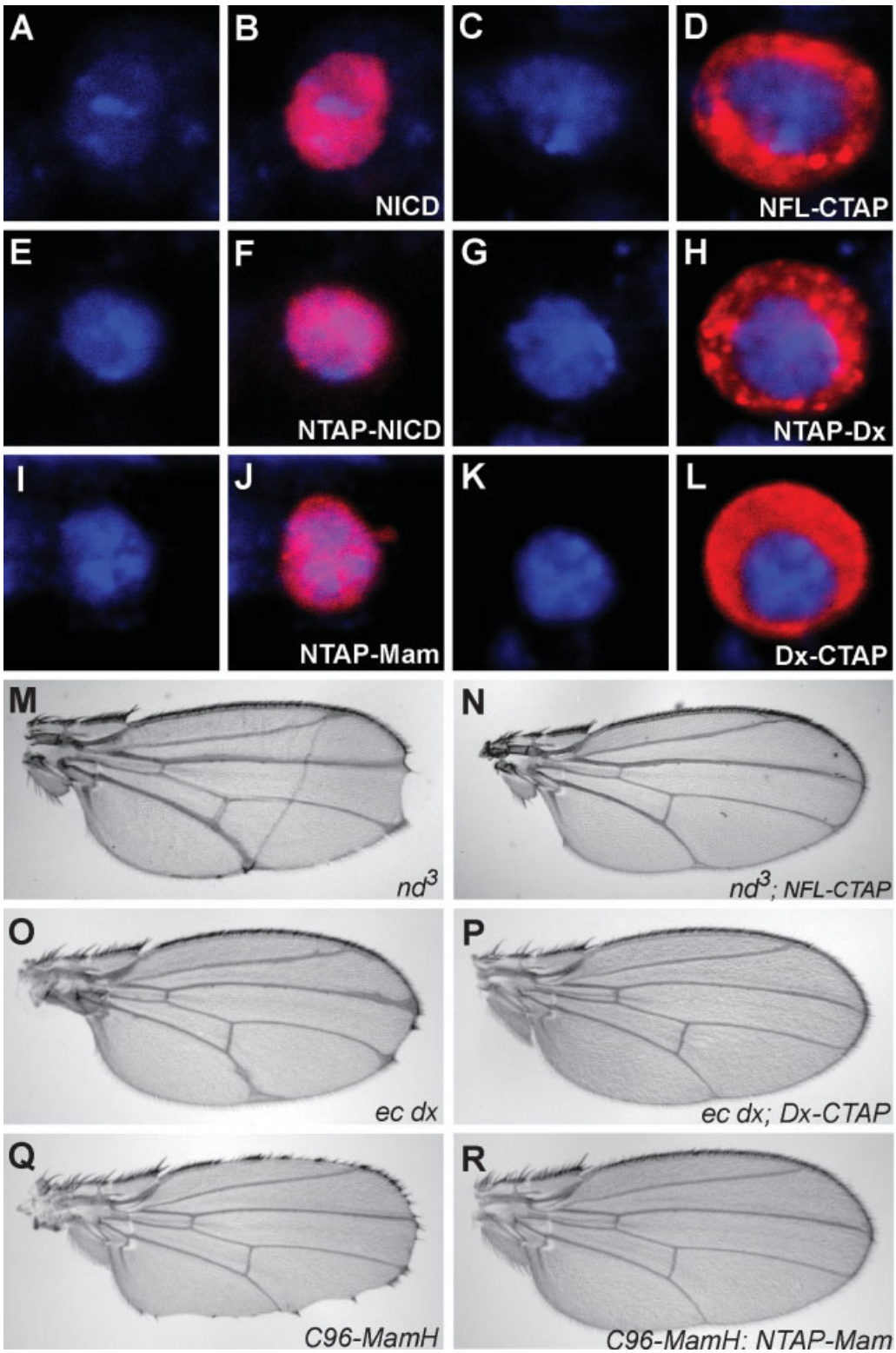

Fig. 2. Verification of tandem affinity purification (TAP) construct functionality. A–L: Subcellular localization of TAP protein fusions used in this study. S2 cells were transfected with pMK33-based constructs, induced overnight with 0.35 mM $CuSO_4$, fixed, permeabilized with Triton X-100, and incubated with mouse IgG (Sigma), followed by incubation with Cy3-conjugated goat anti-mouse antibodies (Jackson). **A,C,E,G,I,K:** Nuclear staining (TO-PRO-3, Molecular Probes). **B,D,F,H,J,L:** Merged images. Amino-terminally TAP-tagged (NTAP)/Notch intracellular domain (NICD) was localized in the nucleus (E,F), similar to the control untagged NICD (A,B; Fehon et al., 1990). NTAP-Mam was also exclusively nuclear (I,J; Bettler et al., 1996). C,D,G,H,K,L: In contrast, full-length Notch-carboxy terminal TAP tag (NFL-CTAP) showed membrane and cytoplasmic staining (C,D; Fehon et al., 1990), whereas both NTAP-Deltex (NTAP-Dx; G,H) and Dx-carboxy terminal TAP tag (Dx-CTAP; K,L) were cytoplasmic (Busseau et al., 1994). M-R: Rescue of mutant phenotypes with TAP-tagged constructs. All crosses were set up at 25°C. **M:** *nd*^{3}*/Y; UAS-NFL-CTAP/CyO* males showed characteristic notching at the wing tip. *nd*^{3} is a hypomorphic allele of *N* (Hing et al., 1994). **N:** In *nd*^{3}*/Y; UAS-NFL-CTAP/T113-GAL4* males, the wing notching was undetectable in 56–80% of all wings scored, depending on the particular transgenic line. This degree of rescue was similar to the rescue with an untagged full-length Notch (data not shown). **O:** A wing from an *ec dx/Y; arm-GAL4/+* male, showing wing notching and widening of wing veins associated with the *dx* loss-of-function phenotype. **P:** This phenotype was rescued completely by expressing Dx-CTAP with the same driver (genotype *ec dx/Y; arm-GAL4 UAS-Dx-CTAP/+)*. **Q:** Overexpression of a dominant-negative Mam isoform using the *C96-GAL4* driver (Helms et al., 1999) resulted in severe wing notching around the wing margin (genotype *C96-GAL4 UAS-MamH/+*). **R:** Introduction of an NTAP-Mam construct suppressed this phenotype (*UAS-NTAP-Mam/+; C96-GAL4 UAS-MamH/+*).

case of NTAP-Dx, addition of the TAP tag compromised some, but not all of the functional activity of Dx.

Tandem Affinity Purification

The general procedure for TAP has been discussed elsewhere (Puig et al., 2001; Cox et al., 2002; Bouwmeester et al., 2004; Gould et al., 2004). We have followed the established protocols with only minor modifications. For protein extraction, we used, in most cases, a detergent-based lysis buffer that was a modified version of the buffer described in Bouwmeester et al. (2004; 50 mM Tris pH 7.5, 125 mM NaCl, 5% glycerol, 0.2–0.4% NP-40, 1.5 mM $MgCl_2$, 1 mM dithiothreitol, 25 mM NaF, 1 mM Na_3VO_4, 1 mM ethylenediaminetetraacetic acid, Complete protease inhibitor from Roche). For NTAP-NICD, which is a nuclear protein, we also used hypotonic lysis followed by high-salt extraction (Kamakaka and Kadonaga, 1994), and obtained qualitatively similar results (data not shown). However, for other protein complexes, an extraction procedure that specifically targets the compartment where the complex is localized may increase the recovery of the bait and associated components.

Using extracts from S2 and Kc cells and embryos, we found that $1–2 \times 10^9$ S2 or Kc cells (0.5–1 L of culture volume), or 3–6 g of embryos provide sufficient starting material for the TAP to obtain a visible band after colloidal Coomassie staining (Fig. 3). Kc and S2 cells stably transfected with pMK33-based TAP constructs were induced overnight by addition of $CuSO_4$ to a final concentration of 0.07 mM, which provides a medium-level induction of the proteins. Cells were pelleted at $500 \times g$, washed three times with cold phosphate buffered saline, and lysed by homogenization with 5–10 volumes of lysis buffer. pUAST-based fly transgenes were expressed in *Drosophila* embryos by crossing or recombining the lines with the *arm-GAL4* driver (Sanson et al., 1996). The 0–24 hr embryos were collected in population cages, dechorionated with bleach, washed, and immediately homogenized on ice in Lysis Buffer, by using a Wheaton dounce homogenizer with tight pestle. After a 20-min incubation on ice, both cell and embryo extracts were cleared by centrifugation at $20,000 \times g$ for 15 min, followed by ultracentrifugation at $100,000 \times g$ for 45 min, to obtain S100 supernatants that were snap-frozen in liquid nitrogen and stored at −80°C. We found that ultracentrifugation reduced clogging of the columns during subsequent binding and washing steps.

Using S100 lysates, we performed TAP essentially as described in Puig et al. (2001). At each step during TAP, we collected analytical samples, and probed these fractions in a Western blot with bait-specific antibodies. Two examples of purification profiles from Kc cells are shown in Figure 3A,B, for NTAP-NICD and Dx-CTAP. Densitometry measurements of band intensity showed that the yield of the final bait protein was approximately 6% for NTAP-NICD and 2% for Dx-CTAP, relative to the amount of the bait in the S100 extract. Although recovery rates were somewhat different for different bait proteins, in all cases presented here, they were sufficient for an unambiguous identification of the baits and interacting partners.

Purified protein complexes were treated with iodoacetamide at a concentration of 20 mg/ml for 30 min at room temperature and resolved on 4–12% Novex NuPage denaturing protein gels (Invitrogen). Gels were fixed and stained with colloidal Coomassie reagent (Sigma). Estimation of protein amounts in Coomassie-stained gels relative to standards showed that the amount of purified bait protein was between 0.1 and more than 5 μg (Fig. 3 and data not shown). In addition to the bait proteins, in all cases, we also detected other bands that corresponded to putative associated components (detection limit of colloidal Coomassie stain is approximately 10 ng per band). These amounts are sufficient for unambiguous protein identification by LC-MS/MS, which is effective down to subpicomolar range (Bauer and Kuster, 2003). Band patterns were very reproducible in repeated purifications using the same bait/cell type combination (Fig. 3 and data not shown). In our experience, purifications from cultured cells generally resulted in higher yields and less overall background, compared with embryos.

Mass Spectrometry, Protein Identification, and Functional Validation of Identified Interactors

Mass-spectrometric identification of proteins was carried out as described in Bouwmeester et al. (2004). Complete lanes from protein gels were cut into slices (narrow for specific bands and wider for interband areas) and analyzed by LC-MS/MS. We have carried out a total of 105 TAPs using TAP-tagged Notch pathway components as well as several other TAP-tagged proteins and control samples. Protein complexes from 41 purifications were analyzed by LC-MS/MS, and a total of approximately 400 proteins were identified. We would like to emphasize that it is essential to perform several purifications for a given bait protein to obtain a reliable view of its interaction network, as significant interactors are expected to be reproducibly identified in more than one experiment.

Using TAP-MS, we were able to confirm the existence of a molecular complex composed of the Notch intracellular domain, Su(H) and Mam proteins, previously characterized by other approaches (Wu et al., 2000; Fryer et al., 2002; Jeffries et al., 2002). We observed this association in Kc cells using three independent TAP-tagged entry proteins (Notch ICD, Mam and full-length Notch; Fig. 3C, lanes 1, 2, and 4). For example, endogenous Su(H) and Mam proteins were copurified with NTAP-NICD, used as a bait, in three independent experiments. Significantly, we were able to obtain very similar results from *Drosophila* embryos using *UAS-NTAP-NICD* transgene crossed to the *arm-GAL4* driver (Fig. 3C, lane 3). To our knowledge, this is the first demonstration of a protein complex identified by TAP-MS from a whole organism.

It has been shown previously that *Drosophila* S2 cells expressing Notch protein aggregate with S2 cells expressing its ligand Delta and that the two proteins can be coimmunoprecipitated (Rebay et al., 1991). We have

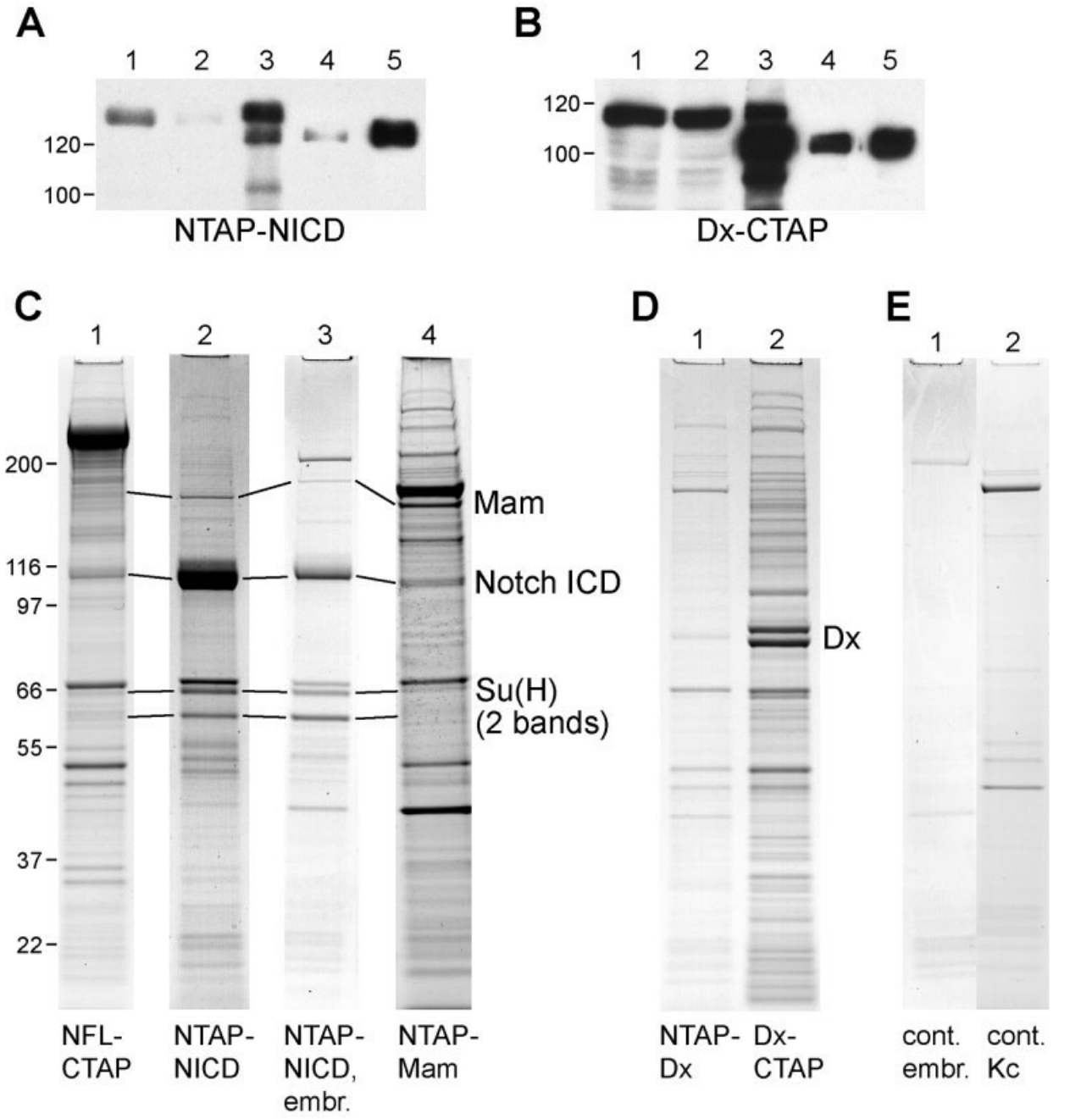

Fig. 3. Protein complexes containing Notch pathway components isolated by tandem affinity purification (TAP). **A,B:** Protein fractions collected during purification of amino-terminally TAP-tagged–Notch intracellular domain (NTAP-NICD; A) and Deltex–carboxy terminal TAP tag (Dx-CTAP; B) from stably transfected Kc cell lines. The following fractions indicate the portion of the total sample loaded on the gel. Lane 1, S100 lysates, 1/500; lane 2, flow-through after binding to IgG beads, 1/500; lane 3, material left on IgG beads after TEV cleavage, 1/10; lane 4, eluate after TEV cleavage, 1/50; lane 5, final complexes eluted from calmodulin beads, 1/10. Note an approximately 16-kDa shift in the size of the tagged proteins due to the removal of the Protein A module by TEV protease (compare lanes 1 and 4). Proteins were immunoblotted with mouse monoclonal anti-N antibody (A; Fehon et al., 1990) or rat monoclonal anti-Dx antibody (B; Busseau et al., 1994). **C:** A trimeric complex between NICD, Mam and Su(H) proteins identified in multiple TAP-mass spectrometry experiments in Kc cells and *Drosophila* embryos. Colloidal Coomassie-stained gradient protein gels are shown, with representative examples of the final TAP fractions. Lane 1, protein complexes isolated from Kc-NFL-CTAP cells; lane 2, Kc-NTAP-NICD cells; lane 3, *UAS-NTAP-NICD/arm-GAL4* embryos; lane 4, Kc-NTAP-Mam cells. Note similar band patterns in lanes 2 and 3. Lines connect protein bands of the same identity, whose positions varied slightly due to differences in migration conditions in different batches of gradient gels. Indicated proteins were identified unambiguously, based on the number of peptides and Mascot scores obtained in liquid chromatography–tandem mass spectrometry (data not shown). Positions of molecular weight markers (in kilodaltons) are given for lane 1. **D:** Representative final TAP fractions from Kc-NTAP-Dx cells (left panel) and Kc-Dx-CTAP cells (right panel). **E:** Representative final TAP fractions from control samples. Lane 1, *arm-GAL4* embryos; lane 2, Kc cells. NFL, full-length Notch.

identified Delta among the proteins associated with TAP-tagged full-length Notch, after mixing Kc cells stably transfected with pMK33-NFL-CTAP with S2 cells expressing Delta (data not shown). Thus, TAP-MS can be used to study interactions of a receptor with its ligands expressed on adjacent cells.

Comparing the interacting partners of NTAP-Dx with proteins that copurified with Dx-CTAP, we found that the overlap between the two sets was not extensive, which was also reflected in different band patterns observed in Coomassie-stained gels (Fig. 3D). Differences in the sets of interacting partners of these two constructs may explain their different abilities to rescue the *dx* mutant phenotype.

The specificity of the protein bands in the final TAP fractions can be assessed by comparing experimental band patterns with those of controls. Purifications using extracts from untransfected Kc cells and *arm-GAL4* embryos, performed in parallel with experimental samples, gave a reproducible pattern of low-level contaminating bands that were also observed in experimental lanes (Fig. 3E, lane 2 is a typical example). Common contaminants were represented by abundant cytoplasmic proteins, such as myosins, heat-shock proteins, tubulin, actin, and ribosomal proteins.

Our TAP-MS approach has identified several potential novel interactors involved in the regulation of Notch signaling. We currently are validating the functional significance of these interactions by using conventional genetic and biochemical approaches. One of such validated interactors, identified as a binding partner of NTAP-Dx, is a *Drosophila* homolog of mammalian nonvisual β-arrestins, Kurtz. We have confirmed a direct interaction between Kurtz and Dx proteins, demonstrated their genetic and molecular interactions in vivo, and have shown that the formation of a trimeric complex between Notch, Kurtz, and Deltex is involved in down-regulation of Notch signaling (Mukherjee, Veraksa et al., manuscript submitted for publication). In another study, we used the TAP-MS approach to uncover an interaction between *Drosophila* Myc and an E3 ubiquitin ligase Archipelago and showed that this interaction results in down-regulation of Myc in vivo (Moberg et al., 2004).

It is worth noting that our analysis does not directly provide an estimate of the minimum abundance of the TAP-tagged protein bait that is required to give results with this approach. In all cases presented here, TAP-tagged proteins were overexpressed in cultured cells and in embryos at a level sufficient for the identification of the baits and interacting partners. We do note that, based on our results and other TAP-MS studies, controlled overexpression of the baits does not interfere with reliable identification of complexes whose functional significance has been validated extensively by other approaches. We conclude that the TAP-MS approach can be successfully applied to the study of protein interaction networks in *Drosophila*, both in cultured cells and in vivo.

ACKNOWLEDGMENTS

We thank the scientists at Cellzome for sharing their expertise in TAP technology. Constructs pBS1479 and pBS1761 were provided by B. Seraphin. The *C96-GAL4 UAS-MamH* line was a gift from B. Yedvobnick. This work was supported by National Institute of Health grants NS26084, GM62931, and CA098402 to S.A.-T. A.V. was supported by the MGH Fund for Medical Discovery postdoctoral fellowship.

REFERENCES

Bauer A, Kuster B. 2003. Affinity purification-mass spectrometry. Powerful tools for the characterization of protein complexes. Eur J Biochem 270:570–578.

Bettler D, Pearson S, Yedvobnick B. 1996. The nuclear protein encoded by the Drosophila neurogenic gene mastermind is widely expressed and associates with specific chromosomal regions. Genetics 143:859–875.

Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S, Hopf C, Huhse B, Mangano R, Michon AM, Schirle M, Schlegl J, Schwab M, Stein MA, Bauer A, Casari G, Drewes G, Gavin AC, Jackson DB, Joberty G, Neubauer G, Rick J, Kuster B, Superti-Furga G. 2004. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 6:97–105.

Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415.

Busseau I, Diederich RJ, Xu T, Artavanis-Tsakonas S. 1994. A member of the Notch group of interacting loci, deltex encodes a cytoplasmic basic protein. Genetics 136:585–596.

Cox DM, Du M, Guo X, Siu KW, McDermott JC. 2002. Tandem affinity purification of protein complexes from mammalian cells. Biotechniques 33:267–268, 270.

Dziembowski A, Seraphin B. 2004. Recent developments in the analysis of protein complexes. FEBS Lett 556:1–6.

Fehon RG, Kooh PJ, Rebay I, Regan CL, Xu T, Muskavitch MA, Artavanis-Tsakonas S. 1990. Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila. Cell 61: 523–534.

Forler D, Kocher T, Rode M, Gentzel M, Izaurralde E, Wilm M. 2003. An efficient protein complex purification method for functional proteomics in higher eukaryotes. Nat Biotechnol 21:89–92.

Fortini ME, Rebay I, Caron LA, Artavanis-Tsakonas S. 1993. An activated Notch receptor blocks cell-fate commitment in the developing Drosophila eye. Nature 365:555–557.

Fryer CJ, Lamar E, Turbachova I, Kintner C, Jones KA. 2002. Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex. Genes Dev 16:1397–1411.

Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415:141–147.

Gould KL, Ren L, Feoktistova AS, Jennings JL, Link AJ. 2004. Tandem affinity purification and identification of protein complex components. Methods 33:239–244.

Helms W, Lee H, Ammerman M, Parks AL, Muskavitch MA, Yedvobnick B. 1999. Engineered truncations in the Drosophila mastermind protein disrupt Notch pathway function. Dev Biol 215:358–374.

Hing HK, Sun X, Artavanis-Tsakonas S. 1994. Modulation of wingless signaling

by Notch in Drosophila. Mech Dev 47: 261–268.

Jeffries S, Robbins DJ, Capobianco AJ. 2002. Characterization of a high-molecular-weight Notch complex in the nucleus of Notch(ic)-transformed RKE cells and in a human T-cell leukemia cell line. Mol Cell Biol 22:3927–3941.

Kamakaka RT, Kadonaga JT. 1994. The soluble nuclear fraction, a highly efficient transcription extract from Drosophila embryos. Methods Cell Biol 44: 225–235.

Koelle MR, Talbot WS, Segraves WA, Bender MT, Cherbas P, Hogness DS. 1991. The Drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. Cell 67: 59–77.

Moberg KH, Mukherjee A, Veraksa A, Artavanis-Tsakonas S, Hariharan IK. 2004. The Drosophila F box protein archipelago regulates dMyc protein levels in vivo. Curr Biol 14:965–974.

Puig O, Caspary F, Rigaut G, Rutz B, Bouveret E, Bragado-Nilsson E, Wilm M, Seraphin B. 2001. The tandem affinity purification (TAP) method: a general procedure of protein complex purification. Methods 24:218–229.

Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S. 1991. Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell 67:687–699.

Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Seraphin B. 1999. A generic protein purification method for protein complex characterization and proteome exploration. Nat Biotechnol 17:1030–1032.

Sanson B, White P, Vincent J-P. 1996. Uncoupling cadherin-based adhesion from wingless signalling in Drosophila. Nature 383:627–630.

Spradling AC, Rubin GM. 1982. Transposition of cloned P elements into Drosophila germ line chromosomes. Science 218: 341–347.

Westermarck J, Weiss C, Saffrich R, Kast J, Musti AM, Wessely M, Ansorge W, Seraphin B, Wilm M, Valdez BC, Bohmann D. 2002. The DEXD/H-box RNA helicase RHII/Gu is a co-factor for c-Jun-activated transcription. EMBO J 21:451–460.

Wu L, Aster JC, Blacklow SC, Lake R, Artavanis-Tsakonas S, Griffin JD. 2000. MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. Nat Genet 26:484–489.