Characterization of the DOC1/APC10 Subunit of the Yeast and the Human Anaphase-promoting Complex*

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The anaphase-promoting complex/cyclosome (APC) is a ubiquitin-protein ligase whose activity is essential for progression through mitosis. The vertebrate APC is thought to be composed of 8 subunits, whereas in budding yeast several additional APC-associated proteins have been identified, including a 33-kDa protein called Doc1 or Apc10. Here, we show that Doc1/Apc10 is a subunit of the yeast APC throughout the cell cycle. Mutation of Doc1/Apc10 inactivates the APC without destabilizing the complex. An ortholog of Doc1/Apc10, which we call APC10, is associated with the APC in different vertebrates, including humans and frogs. Biochemical fractionation experiments and mass spectrometric analysis of a component of the purified human APC show that APC10 is a genuine APC subunit whose cellular levels or association with the APC are not cell cycle-regulated. We have further identified an APC10 homology region, which we propose to call the DOC domain, in several protein sequences that also contain either cullin or HECT domains. Cullins are present in several ubiquitination complexes including the APC, whereas HECT domains represent the catalytic core of a different type of ubiquitin-protein ligase. DOC domains may therefore be important for reactions catalyzed by several types of ubiquitin-protein ligases.

The initiation of anaphase and exit from mitosis depend on a multi-subunit ubiquitination complex called the anaphase-promoting complex (APC) or cyclosome (1–3) (for a recent review see Ref. 4). The APC ubiquitinates proteins such as Pds1 in budding yeast and Cut2 in fission yeast whose subsequent degradation by the 26 S proteasome is essential for the initiation of sister chromatid separation at the metaphase-anaphase transition (5, 6). Later in anaphase and telophase the APC promotes the inactivation of the mitotic cyclin-dependent protein kinase 1 by ubiquitinating its activating subunit cyclin B (1–3, 7). The APC also mediates the ubiquitin-dependent proteolysis of several other mitotic regulators, including Polo-like kinases (8, 9), spindle-associated proteins (10), the APC-activating protein Cdc20 (9, 11), and inhibitors of DNA replication (12).

Like all known ubiquitination reactions, the assembly of polyubiquitin chains on APC substrates depends on ubiquitin-activating and ubiquitin-conjugating enzymes, also called E1 and E2 enzymes, respectively (13). E1 first activates ubiquitin by forming a ubiquitin thioester and then transfers ubiquitin to an E2 enzyme, again forming a thioester. Subsequently, ubiquitin is transferred from the E2 to the substrate where polyubiquitin chains are assembled through isopeptide bonds on the e-amino side chains of lysine residues. The latter reaction usually depends on a third component, called ubiquitin-protein ligase or E3. The first E3s that were molecularly characterized were a yeast protein called Ubr1 (14) and a human protein called E6-AP (15). Ubr1 functions in the N-end rule pathway in which substrates are recognized based on the identity of their N-terminal amino acid residue (16), whereas E6-AP was identified as a protein required for the ubiquitination of the tumor suppressor protein p53 in human cells infected with oncogenic papilloma viruses (17). E6-AP and possibly also Ubr1 function as ubiquitin carriers that transfer the ubiquitin residue from the E2 to the substrate by forming a ubiquitin thioester intermediate (16, 18). The C-terminal domain of E6-AP where thioester formation takes place is also found in a number of other proteins (19, 20). Several of these proteins with homology to the E6-AP C-terminus (HECT domain proteins) have been shown to form ubiquitin thioesters and are therefore likely to function as ubiquitin-protein ligases (19, 21).

In the APC pathway, the APC fulfills the role of an E3, because it is required for the transfer of ubiquitin from E2s to substrates. However, it is not known whether the APC also functions as a covalent ubiquitin carrier. A direct physical association of ubiquitin with APC has not so far been detected (2), raising the possibility that the complex facilitates ubiquitination reactions indirectly by bringing substrates and E2s into close proximity and perhaps by altering their conformation. The cloning of APC subunits has not revealed any similarity with Ubr1 or HECT domain proteins (22–27). The APC2 subunit, however, is a distant member of another protein family involved in ubiquitination, called cullins (26–28). The best studied cullin is the budding yeast protein Cdc53, which is part of a multi-subunit ubiquitin-protein ligase called Skp1-cullin-F-box protein complex (SCF) (for review see Refs. 4 and 29). SCF-dependent proteolysis is required for the initiation of DNA replication at the G1-S transition but also has multiple other physiologic roles. The E2 enzyme Cdc34 has been reported to interact with a C-terminal domain in Cdc53 (30), which is conserved in all cullins (28), including APC2. The conservation of this domain raises the possibility that also other cullins are part of ubiquitin-protein ligases and interact with E2 enzymes.

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¶ The abbreviations used are: APC, anaphase-promoting complex; HECT, homology to E6-AP C-terminus; PAM, protein-associated with Myc; RJS, runty-jerky-sterile protein; SCF, Skp1-cullin-F-box protein complex; PAGE, polyacrylamide gel electrophoresis; EST, expressed sequence tag.
SCF and APC differ from Ubr1 and HECT domain ubiquitin-protein ligases with respect to their subunit complexity: SCF and APC are both multi-subunit complexes, whereas Ubr1 and HECT proteins appear to be functional as single polypeptide chain enzymes. The vertebrate APC was believed to be composed of 8 constitutive subunits, 7 of which are related to subunits of the budding yeast APC (22, 26). APC immunoprecipitates from budding yeast contain five additional proteins, which have so far not been identified in APC preparations from animal cells (23, 27). Recently, a 33-kDa protein called Doc1 has been identified in a genetic screen for mutants defective in the degradation of mitotic cyclins (24). DOC1 deletion strains are barely viable and temperature-sensitive mutations causing a defect in the ubiquitination of mitotic cyclins in vitro could be assigned to the DOC1 gene (31). The Doc1 protein was found to cofractionate with other APC subunits suggesting that it is an APC subunit (24). Indeed, DOC1 was found to encode the 33-kDa protein Apc10 detected in the purified APC (27). We therefore refer to the yeast protein as Doc1/Apc10. Recently, Kominami et al. (32) reported the characterization of a fission yeast ortholog of Doc1/Apc10. The fission yeast apc10+ gene is essential and is required for the turnover of the mitotic cyclin Cdc13, a known APC substrate. Based on the observation that an association between fission yeast Apc10 and known APC subunits could only be detected by communoprecipitation but not in density gradient centrifugation experiments, it has been suggested that this protein may be a regulatory protein that only transiently associates with the APC.

Here, we have further analyzed budding yeast Doc1/Apc10 and have for the first time characterized its human ortholog APC10. Our results demonstrate that these proteins are constitutively associated with the APC in yeast and vertebrates, respectively, and suggest that Doc1/Apc10 has an important role in APC-dependent ubiquitination reactions. We have further identified a conserved protein domain homologous to the amino acid sequence of APC10 in several high molecular weight protein sequences. This homology region, which we call the DOCC domain (from Doc1), is found in several putative cullins and HECT domain proteins. This finding implies a conserved role for the APC10 sequence in diverse types of ubiquitin-protein ligases that were so far not known to have structural features in common.

**EXPERIMENTAL PROCEDURES**

**Yeast Methods**—Strains were derivatives of W303. CDC16-myc6 strains have been described (23). For epitope-tagging of DOC1, a cassette containing an HA3 or myc9 epitope tag and the *Kluyveromyces lactis* TRP1 gene was amplified by polymerase chain reactions with DOC1-specific primers and integrated in front of the stop codon at the genomic locus. The doc1 mutants used in this study have been isolated in screens for mutants with a defect in the degradation of Clb2 (1, 7). The *doc1*-22 mutation is identical to the previously characterized mutation *cse1*-22 (1, 7) and was renamed after finding that the mutation is in the *DOC1* and not in the *CSE1* gene (31). For metabolic labeling, strains were grown in synthetic complete medium lacking methionine. In a typical experiment, 5 × 10⁶ cells were resuspended in 0.5 ml of medium containing 1 mCi of [35S]methionine and [35S]cysteine and incubated at 25 °C. Fresh medium was added after 60 min (0.5 ml) and 120 min (2 ml), and labeled cells were harvested after 180 min. We were not able to arrest cells with nocodazole in synthetic medium. For Fig. 1B, strains (MATa bar1) were grown to exponential phase or arrested for 90 min with α-factor (0.5 μg/ml) or nocodazole (15 μg/ml) in rich medium at 30 °C. As judged by microscopic examination, approximately 95% of cells were arrested in G1 (unbudded cells) or mitosis (large-budded cells), respectively. Cells were labeled (3 μCi/ml) for 150 min in 1 ml of YEPD medium. α-Factor or nocodazole was added to maintain cell cycle arrest. Preparation of extracts and immunoprecipitations were performed as described (27). Cells were broken in 0.25 ml of buffer B6 containing bovine serum albumin (5 mg/ml) and extract from an unlabelled Δαpep1 strain (5 mg/ml) grown under similar conditions. In Fig. 3C, the yeast extract was omitted from the breakage buffer. Myc-tagged proteins were immunoprecipitated with the monoclonal antibody 9E10. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by fluorography.

cDNAs, Reombinant Protein Expression, and Generation of Antibodies—Sequence analysis revealed that the human expressed sequence tag (EST) clone AA234528 (GenBank™ nomenclature of the National Center for Biotechnology Information) is composed of 843 base pairs and contains an open reading frame encoding APC10 (see “Results”). The complete APC10 nucleotide and amino acid sequences have been deposited at the National Center for Biotechnology Information (GenBank™ accession number AF 132794). [35S]Methionine- and [35S]cysteine-labeled APC10 protein was prepared from clone AA234528 by coupled transcription-translation reactions in rabbit reticulocyte lysate (Promega). For the generation of antibodies, CDNA fragments corresponding to amino acid residues 1–140 of APC10, to amino acid residues 332–619 of human CDC16/APC6 (33), and to amino acid residues 257–591 of human CDC23/APC8 (26) were cloned into a PET28 expression vector (Novagen). Reombinant protein containing N-terminal hexa-histidine tags were expressed in *Escherichia coli* BL21(DE) cells (Novagene), purified on nickel-nitrilotriacetic acid agarose (Qiagen) and used for the immunization of rabbits.

**Peptide Sequencing by Mass Spectrometry**—The 24-kDa protein present in immunoprecipitates of human APC was analyzed by nanoelectrospray tandem mass spectrometry (34). Briefly, the silver-stained protein band was excised from the gel, reduced and S-alkylated and subjected to in-gel digestion with trypsin (35, 36). After overnight digestion the supernatant was loaded on 50 nl of reversed phase resin (POROS R2) in a capillary, washed with acidified water and eluted with organic phase directly into a nanoelectrospray needle (Protana, Odense, Denmark). Peptide sequencing by tandem mass spectrometry was performed on a prototype quadrupole-time-of-flight instrument (PE-Sciex, Toronto, Canada). After fragmentation, peptide sequence tags (37) were assigned in the resulting spectra. The program PepsiSearch written by Mortensen and Mann was used to retrieve protein sequences from both a nonredundant protein data base and an EST data base. The retrieved data base hits were compared with the complete fragmentation spectrum to verify the complete peptide sequence.

**Other Techniques**—Extracts from logarithmically growing HeLa cells and from *Xenopus* eggs were prepared and fractionated as described (38, 39). Resource Q column fractions obtained from HeLa 100,000 × g supernatant (S100) fractions were the kind gift of I. Waizenegger, and extracts prepared from HeLa cells synchronized by cell cycle inhibitors were the kind gift of E. R. Kramer (both of Research Institute of Molecular Pathology). Immunoprecipitation, immunoblotting, and ubiquitination experiments were done as described (38, 39).

**Data Base Searches and Sequence Alignments**—Protein data bases were searched by using the Gapped BLAST and the Psi-BLAST program (40). Sequence alignments were performed by using Clustal X (41) using the Blossum 62 substitution matrix and were manually modified in the GeneDoc program (42). The degree of identity between different amino acid sequences was determined by the gap program of the Genetic Computer Group.

**RESULTS**

**Characterization of Budding Yeast Doc1/Apc10**—The DOC1 gene was first identified in a genetic screen for budding yeast mutants defective in the proteolysis of mitotic cyclins (24). DOC1 encodes a protein of 33 kDa that was shown to be associated with known APC subunits (24). To determine whether Doc1 represents one of the 12 proteins detected after purification of the yeast APC (27), we constructed strains in which either Cdc16 or Doc1 or both proteins carry epitope tags at their C termini. Strains were metabolically labeled with [35S]methionine and [35S]cysteine, and the APC was isolated by immunoprecipitation of the Cdc16-myc6 protein with antibodies to the Myc epitope (Fig. 1A). The APC from CDC16-myc6 cells contains a 33-kDa protein called Apc1 (27). The identity of this protein was significantly revealed when the APC was reombinant from CDC16-myc6 DOC1-HA3 cells, demonstrating that the 33-kDa protein is encoded by the *DOC1* gene. We therefore refer to this subunit as Doc1/Apc10. The same set of proteins was coprecipitated with Doc1-myc9 and with Cdc16-myc6, confirming that the association of Doc1/Apc10 with other APC subunits is specific. Doc1/Apc10 was labeled less intensely than other subunits of higher molecular mass. This difference
could be due to the lower number of methionine and cysteine residues in Doc1/Apc10 than in larger APC subunits. Alternatively, Doc1/Apc10 could bind to the APC in substoichiometric amounts.

Most known APC subunits are part of the complex during all phases of the cell cycle (22). In contrast, two proteins that regulate APC activity, Cdc20 and Cdh1, are predominantly associated with the APC in mitosis and G1 phase, respectively (38, 43–47). To analyze whether Doc1/Apc10 is a constitutive or a cell cycle-regulated subunit, we compared the subunit composition of the APC isolated from different cell cycle phases (Fig. 1B). APC was immunopurified from metabolically labeled CDC16-myc6 cells that were either growing logarithmically or arrested in G1 with α-factor (α-fact) or in mitosis with nocodazole (noc). Growing (cyc) and arrested cells were labeled for 150 min. C. subunit composition of the APC in extracts from different doc1 mutant strains. Cells were grown in synthetic medium and labeled for 80 min at 25 °C and then for 100 min at the restrictive temperature (36 °C). Polypeptide bands that bound nonspecifically to the antibody beads are marked by stars.

Characterization of Human APC10 cDNAs—To analyze whether proteins related to budding yeast Doc1/Apc10 are associated with the APC in vertebrates, we characterized EST clones and cDNAs obtained by polymerase chain reactions that encode a related human protein. Clone AA234328 contained an open reading frame that codes for a protein of 185 amino acid residues with a predicted molecular mass of 21,300. This sequence is 29% identical to budding yeast Doc1/Apc10. Data base searches identified several other closely related sequences in fission yeast (SPBC1E8.06/Apc10) (32), Caenorhabditis elegans (Y48G1.0Contig49; St. Louis unfinished sequences from chromosome I) and Drosophila (AA141768), which were 38, 38, and 56% identical to the human sequence, respectively (Fig. 2). The high degree of sequence similarity and our biochemical data (see below) suggest that these proteins are orthologs of budding yeast Doc1/Apc10. We therefore refer to the human protein as APC10. To address whether the protein detected by APC10 antibodies is associated with the APC, we immunoprecipitated APC10 antibodies were able to immunoprecipitate the APC in extracts from different doc1 mutant strains. Cells were grown in synthetic medium and labeled for 80 min at 24 °C and then for 100 min at the restrictive temperature (36 °C). Polypeptide bands that bound nonspecifically to the antibody beads are marked by stars.

**Fig. 1. Characterization of Doc1/Apc10 as a subunit of the budding yeast APC.** Wild type cells (WT) and cells expressing epitope-tagged versions of the APC subunit Cdc16 and of the Doc1/Apc10 protein were metabolically labeled with [35S]methionine and [35S]cysteine. Protein extracts were prepared and immunoprecipitated with an antibody to the Myc epitope. Bound proteins were detected by fluorography. A, identification of the 33-kDa APC subunit as the DOC1 gene product. Cells were labeled for 180 min at 25 °C in synthetic medium. B, association of Doc1/Apc10 with the APC at different cell cycle stages. Wild type or CDC16-myc6 cells were grown in rich medium at 30 °C and arrested in G1 with α-factor (α-fact) or in mitosis with nocodazole (noc). Growing (cyc) and arrested cells were labeled for 150 min. C, subunit composition of the APC in extracts from different doc1 mutant strains. Cells were grown in synthetic medium and labeled for 80 min at 24 °C and then for 100 min at the restrictive temperature (36 °C). Polypeptide bands that bound nonspecifically to the antibody beads are marked by stars.

2 W. Zachariae, unpublished observations.
Identification of an APC10 Homology Domain—Data base searches with the APC10 sequence not only identified putative APC10 orthologs in different species, but also several high molecular weight proteins that contain regions of significant homology with other APC10 relatives. Note that only for Hs APC10, Dm Apc10, and Sp Apc10, the full-length sequences are deduced from genomic DNA sequences (because the DOC domains of KIAA0076 and KIAA0708 are 90% identical, a weight of only 0.5 instead of 1.0 was assigned to these two sequences).

When we immunoprecipitated APC from human HeLa and mouse myeloma cells and analyzed its subunit composition by SDS-PAGE and silver staining, we observed a 24-kDa polypeptide band (data not shown), suggesting that the epitope recognized by these antibodies is inaccessible in the native complex. A protein of similar electrophoretic mobility to APC10 was recognized by APC10 antibodies in APC immunoprecipitates isolated from mouse and bovine cells, and two polypeptide bands were recognized in APC preparations obtained from Xenopus egg extracts (Fig. 3B). We conclude that APC10 is specifically associated with the APC in different vertebrates.

To analyze whether the association of APC10 with the APC is cell cycle-regulated, we synchronized logarithmically growing HeLa cells by drug treatment and determined the presence of APC10 in cell extracts and in APC immunoprecipitates by immunoblotting. Similar amounts of APC10 were present in cell extracts and in APC immunoprecipitates in all cell cycle phases (Fig. 3D and data not shown), indicating that APC10 is a constitutive APC subunit.

When we immunoprecipitated APC from human HeLa and mouse myeloma cells and analyzed its subunit composition by SDS-PAGE and silver staining, we observed a 24-kDa polypeptide band that could not be detected in immunoprecipitates obtained with preimmune sera (indicated by an arrow in Fig. 4A). To analyze whether this protein represents APC10, we isolated tryptic peptides from this band and sequenced them by nanoelectrospray tandem mass spectrometry (50) using a novel hybrid quadrupole-time-of-flight mass spectrometer instrument (51). The unseparated peptide mixture resulted in the mass spectra shown in Fig. 4B. The main detected peaks corresponded to trypsin autolysis products (T) due to low amounts of protein in the gel. Several smaller peaks in the spectrum at a mass to charge ratio of 840, 92, 574, 97, 661, 84, 671, 34, 845, 67, and 921, 44 were fragmented and found not to belong to any entry in the protein data base. As an example, Fig. 4C shows that the peptide with m/z 661.84 was sequenced completely, revealing the sequence YTPVVEESSIGK. In total, 6 peptides were sequenced (Table I). Data base searches with these peptide sequences identified a total of 11 ESTs, all of which encode parts of APC10. The 24-kDa band present in APC preparations does therefore represent APC10.

To determine what fraction of APC10 is associated with the APC, we separated extracts from logarithmically growing HeLa cells by Q column anion exchange chromatography and by linear sucrose density centrifugation (Fig. 5). The column and gradient fractions were analyzed by immunoblotting for the presence of APC10 and the APC subunits CDC16 and CDC27 (Fig. 5, B and C). In addition, individual Q column fractions were analyzed for their ability to ubiquitinate a radiolabeled N-terminal fragment of cyclin B in a reconstituted reaction containing purified E1 and E2 enzymes (Fig. 5A). In both experiments, APC10 cochromatographed with the peak of CDC16 and CDC27 proteins, whereas there was no APC10 detectable in other fractions. The presence of APC10 correlated closely with the ability of the Q column fractions to ubiquitinate cyclin B. However, the elution and sedimentation behavior of APC10 was not identical to that of CDC16 and CDC27; the latter two proteins could be detected in multiple fractions in both separation experiments, but APC10 could only be detected in the APC peak fractions (as defined by the peak of cyclin B ubiquitination activity and CDC16 and CDC27 concentration), even after prolonged exposure times during the immunoblotting procedure. These experiments suggest that the large majority if not all APC10 molecules are tightly associated with the APC, whereas CDC16 and CDC27 may either be more loosely associated or may be present in multiple complexes or aggregates.
and analyzed by immunoblotting. Corresponding preimmune antibodies (IP pre-CDC16) were added in growing HeLa cells (38) and from mouse myeloma Ag8.653 cells (39), from mouse myeloma Ag8.653 cells (39), and from Xenopus interphase egg extracts (40) were analyzed by immunoprecipitation with antibodies against CDC16, CDC23, CDC27, CDC16, and APC7 or with preimmune antibodies corresponding to the CDC23 cDNA, respectively. Whole cell extracts from logarithmically growing HeLa cells (HeLa xT) and immunoprecipitates obtained from these extracts either with CDC16 antibodies (IP a-CDC16) or with the corresponding preimmune antibodies (IP pre-CDC16) were also analyzed by immunoblotting. B, whole cell extracts from logarithmically growing HeLa cells (HeLa), from mouse myeloma Ag8.653 cells (Mm), and from Xenopus interphase egg extracts (Xl) were analyzed by immunoprecipitation with antibodies against CDC23, CDC27, CDC16, and APC7 or with preimmune antibodies corresponding to the CDC23 antibodies (pre-CDC23). All immunoprecipitates (IP) were analyzed by SDS-PAGE and Western blot (WB) with CDC16 and APC10 antibodies. C, CDC20 and APC7 immunoprecipitates obtained from whole cell extracts of HeLa cells arrested in mitosis with nocodazole were analyzed by immunoblotting with CDC20 and APC10 antibodies. D, whole cell extracts of logarithmically growing HeLa cells (HeLa), from cells enriched in different cell cycle phases were analyzed by immunoblotting with CDC16 and APC10 antibodies. Cells were either enriched in G1 by mimosine, in S by hydroxyurea (HU), in G2 by nitrogen mustard (NM), or in mitosis by colcemid (COL). Cell cycle synchronization was monitored by flow cytometry (see Ref. 38, and data not shown). The APC10 polypeptide band detected by phosphorimaging and by immunoblotting is marked by an arrow in A and D.

is not known because the corresponding cDNA may not be complete at the 5' end). The two amino acid sequences are 57% identical to each other. Both contain a region homologous to APC10, which is 90% identical between KIAA0076 and KIAA0708 (Fig. 2), and a more C-terminal region homologous to cullins (28). The cullin domain of KIAA0076 (amino acid residue 1187–1576) is most closely related to human CUL1, whereas the cullin domain of KIAA0708 (amino acid residue 782–1065) is similar to both human CUL4B and CUL1 (as determined by the BLAST 2.0 program). In addition, KIAA0708 contains a zinc-binding RING finger motif (54) at amino acid positions 1472–1501.

An APC10 homology domain was also found in a 527-kDa mouse protein called RJS (runty-jerky-sterile) (55) and its human ortholog HERC2 (56). Both proteins contain a HECT domain in the C-terminal region (at position 4459–4796 of RJS). A fourth mammalian protein containing a region of less pronounced but still significant homology to APC10 is a 510-kDa protein associated with the transcription factor Myc (PAM) (57). The sequence of PAM does not contain cullin or HECT domains, but numerous other sequence motifs have been recognized previously (57), including a subtype of the RING finger, called RING-H2 finger (54), which is also present in the budding yeast APC subunit Apcl1 (27) and in Ub1 (58). In addition, PAM contains a putative protein binding domain originally identified in the RCC1 protein (regulator of chromosome condensation 1) where this domain has recently been shown to form a seven-bladed propeller structure (59). Three domains containing RCC1 repeats are also present in RJS/HERC2 (55, 56). The genome of *C. elegans* contains an open reading frame (C01B7.6) that codes for a protein whose sequence is 32% identical to PAM (57) and accordingly also contains a region related to APC10.

The sequence similarity between APC10 and the related regions in KIAA0076, KIAA0708, RJS/HERC2, PAM, and the PAM-related *C. elegans* sequence suggest that parts of these proteins are derived from a common ancestral protein domain. Based on the original name of APC10 in budding yeast, Doc1, we propose to call this homology region the DOC domain.

**DISCUSSION**

From our results, we conclude that budding yeast Doc1/Apcl1 and its vertebrate ortholog APC10 represent an evolutionary conserved subunit of the APC. Kominami *et al.* (32) have recently proposed that fission yeast Apcl1 may be loosely associated with the APC and may therefore be a regulator of the complex. However, our data suggest that this protein is tightly and constitutively associated with the APC, at least in budding yeast and human cells.

An association of Doc1/ Apcl1 with the budding yeast APC had already been reported previously (24), but APC10 had so far not been recognized as a subunit of the vertebrate APC (22, 26). We suspect that APC10 was not detected in previous analyses of the vertebrate APC because the 24-kDa APC10 polypeptide band is less intensely stained by silver than other APC subunits (Fig. 4A). We presently do not know whether these differences in staining intensity are due to substoichiometric amounts of APC10 or whether this protein is less aglyrophilic than other APC subunits. In either case, our characterization of human APC10 and the evolutionary conservation between most known APC subunits suggest that orthologs of other proteins, which so far were only identified as subunits of the budding yeast APC, may remain to be discovered in the vertebrate APC. This view is supported by the observation that a protein related to the budding yeast APC subunit Cdc26 is associated with the APC in fission yeast (49) and in human cells and that human cDNAs encoding proteins closely related to budding yeast Apcl1 exist (27).

Like the function of other APC subunits, the function of APC10 within the APC is unknown. Our result that mutation of budding yeast Doc1/ Apcl1 abolishes APC function without disrupting the complex (Fig. 1) may indicate that Doc1/ Apcl1 has a rather direct role in the ubiquitination function of APC. This notion is consistent with our observation that in biochemical fractionation experiments the abundance of APC10 correlates with the ability of human APC to ubiquitinate cyclin B in vitro (Fig. 5).

3 C. Gieffers, A. V. Podtelejnikov, M. Mann, and J.-M. Peters, unpublished results.
We noticed that the sequences of several high molecular weight proteins found in the data bases contain a region of significant homology to APC10, which we propose to call the DOC domain (from Doc1/Apc10). During preparation of this manuscript, Kominami et al. (32) also reported that fission yeast Apc10 is similar to a portion of one of these proteins, KIAA0076. The presence of either cullin or HECT domains in three of these proteins, KIAA0076, KIAA0708, and RJS/HERC2, implies that these proteins may be ubiquitin-protein ligases or subunits of such enzymes. KIAA0076, KIAA0708, and RJS/HERC2 may therefore participate in ubiquitination reactions or perhaps in related reactions in which ubiquitin-like proteins are conjugated to other proteins.

These observations have several interesting implications. First, the presence of DOC domains in the APC and in high molecular weight cullin and HECT domain proteins suggests that the function of the DOC domain may be linked to ubiquitination reactions, although the function of more DOC domain proteins will need to be examined to test this hypothesis. The DOC domain appears to be able to perform its function

![Fig. 4. Sequencing of human APC10 by nanoelectrospray tandem mass spectrometry. A, immunoprecipitates obtained with CDC27 antibodies (a-CDC27) from whole cell extracts of logarithmically growing mouse Ag8.653 (Mm) or human HeLa cells were analyzed by SDS-PAGE and silver staining. As a negative control, immunoprecipitates obtained with the corresponding CDC27 preimmune antibodies (pre-CDC27) from HeLa extracts were analyzed side by side. The position of the subunits APC1, APC2, CDC27, APC4, APC5, CDC16, APC7, and APC8 was determined by immunoblotting (data not shown) and has been indicated by bars on the right side. The 24-kDa APC10 polypeptide band is marked by an arrow. Bars on the left indicate the position of marker proteins whose molecular masses are listed in kDa. B, mass spectrum of the unseparated peptide mixture of the 24-kDa band present in human APC immunoprecipitates separated by SDS-PAGE as in A. The peaks marked with T correspond to tryptic autolysis products. All peaks marked by their m/z value were sequenced by nanoelectrospray mass spectrometry. C, tandem mass spectrum of the peak at m/z 661.84. The high resolution and mass accuracy of the quadrupole-time-of-flight instrument allowed complete sequence assignment as IYTPVEESSIGK, which maps to a tryptic peptide of APC10.

| Mass to charge ratio | Charge | Molecular mass | Sequence | GenBank™ accession no. |
|----------------------|--------|----------------|----------|------------------------|
| m/z                  | Charge | Molecular mass | Sequence             | GenBank™ accession no. |
| 574.97               | 3      | 1721.91        | IYTPVEESSIGKFPR     | AA281117               |
|                      |        |                |                      | A1024091               |
|                      |        |                |                      | A1082438               |
| 661.84               | 2      | 1321.68        | IYTPVEESSIGK         | AA281117               |
|                      |        |                |                      | A1024091               |
|                      |        |                |                      | A1082438               |
| 671.34               | 2      | 1340.68        | GNNFHNQLEIR          | AA472445               |
|                      |        |                |                      | AA856395               |
| 845.67               | 3      | 2534.01        | EIGSQAVWSLSSCacrKPGFGVQDQLR<sup>a</sup> | AA234328               |
|                      |        |                |                      | AA367106               |
|                      |        |                |                      | AA472445               |
|                      |        |                |                      | AA590715               |
|                      |        |                |                      | AA856395               |
|                      |        |                |                      | AA947769               |
| 480.92               | 3      | 1439.76        | VGNNFHNQLEIR         | AA472445               |
|                      |        |                |                      | AA856395               |
| 921.44               | 3      | 2761.32        | DDNLEYWQSDGSPHLYNIQFR| H33761                 |
|                      |        |                |                      | AA234328               |
|                      |        |                |                      | AA472445               |
|                      |        |                |                      | AA590735               |
|                      |        |                |                      | AA856395               |
|                      |        |                |                      | AA947769               |
|                      |        |                |                      | A1052991               |

<sup>a</sup> Cacr, Cys acrylamide.
either as a single subunit in trans (in the case of APC10) or as a protein domain in cis (in the case of KIAA0076, KIAA0708, and RJS/HERC2). Second, the presence of the DOC domain in the human CDC16/APC6 cDNA. Because the WD40 repeats of the SCF subunit Cdc4 have been shown to form seven-bladed propeller-like structures (59–61). The occurrence of the DOC domain in conjunction with either cullin or HECT domains suggests a modular structure for ubiquitin-protein ligases. These observations and our finding that the DOC domain is found either in association with a cullin domain or in association with a HECT domain raises the interesting possibility that structural domains are shared between different types of ubiquitin-protein ligases such as Ubr1, HECT enzymes, and cullins that were so far not thought to have any features in common.

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