Dolichol-phosphate mannose (DPM) synthase is required for synthesis of the glycosylphosphatidylinositol (GPI) anchor, N-glycan precursor, protein O-mannose, and C-mannose. We previously identified DPM3, the third component of this enzyme, which was co-purified with DPM1 and DPM2. Here, we have established mutant Chinese hamster ovary (CHO) 2.38 cells that were defective in DPM3. CHO2.38 cells were negative for GPI-anchored proteins and microsomes from these cells showed no detectable DPM synthase activity, indicating that DPM3 is an essential component of this enzyme. A coiled-coil domain near the C terminus of DPM3 was important for tethering DPM1, the catalytic subunit of the enzyme, to the endoplasmic reticulum membrane and, therefore, was critical for enzyme activity. On the other hand, two transmembrane regions in the N-terminal portion of DPM3 showed no specific functions. DPM1 was rapidly degraded by the proteasome in the absence of DPM3. Free DPM1 was strongly associated with the C terminus of Hsc70-interacting protein (CHIP), a chaperone-dependent E3 ubiquitin ligase, suggesting that DPM1 is ubiquitinated, at least in part, by CHIP.

Dolichol phosphate mannose (DPM) is the sole donor substrate for mannosyltransferases working on the luminal side of the endoplasmic reticulum (ER) that transfer a mannose (Man) residue onto lipid-linked can precursor, protein synthesis of the glycosylphosphatidylinositol (GPI) anchor, PIG, phosphatidylinositol glycan; uPAR, urokinase plasminogen activator receptor.

O-mannosylation and C-mannosylation that occur in the ER lumen have also been shown to be DPM-dependent (9, 10).

DPM is synthesized from dolichol phosphate and GDP-Man on the cytosolic surface of the ER membrane by DPM synthase (GDP-α-Man: dolichol-phosphate β-mannosyltransferase; EC 2.4.1.83) and then flipped onto the luminal side and used as a donor substrate. In lower eukaryotes, such as Saccharomyces cerevisiae and Trypanosoma brucei, DPM synthase consists of a single component (Dpm1p and Tbp1p, respectively) that possesses one predicted transmembrane region near the C terminus for anchoring to the ER membrane (11, 12). In contrast, the Dpm1 homologues of higher eukaryotes, namely fission yeast, fungi, and animals, have no transmembrane region (13), suggesting the existence of adapter molecules for membrane anchoring. In fact, we previously demonstrated that S. cerevisiae Dpm1p was able to complement two different DPM synthase-defective mutants, namely a Thy-1-negative class E mutant of mouse T cell lymphoma and Chinese hamster ovary (CHO) Lec15 cells, whereas human DPM1 was only able to complement the class E mutant (14). We cloned a second component of DPM synthase, DPM2, which is the gene responsible for CHO Lec15 cells (15), and then further identified a third component, DPM3, in the biochemically purified active enzyme complex (16). Mammalian DPM1 was predicted to be a soluble protein and found to be associated with two small membrane proteins, DPM2 and DPM3. Our previous analyses using mammalian cells revealed that: (i) DPM2 stabilizes the expression of DPM3; (ii) DPM3 binds both DPM2 and DPM1; (iii) the N-terminal transmembrane regions of DPM3 are important for binding to DPM2; and (iv) the C-terminal hydrophilic region of DPM3 is important for binding to DPM1 (15, 16). Thus, DPM3 is most likely to be the anchoring molecule that tethers DPM1 to the ER membrane, although direct evidence was missing due to the lack of DPM3 mutant or knock- out cells. In the present study, we identified a DPM3 mutant CHO cell line for the first time and addressed the biological functions of DPM3 using this mutant. Here we describe the essential role of the C-terminal coiled-coil domain of DPM3 and the fate of DPM1 in DPM3-defective mutant cells.

MATERIALS AND METHODS

Cell Culture—CHO K1 and CHO Lec35 cells were cultured in Ham’s F-12 medium containing 10% fetal bovine serum. CHO F21 and CHO2.38 cells were cultured in the same medium supplemented with 600 μg/ml G418 (Nacalai Tesque), 400 μg/ml hygromycin B (Wako), and 50 μg/ml blasticidin S (InvivoGen). CHO2.38/DPM3, CHO K1/3HSV-DPM1, and CHO2.38/3HSV-DPM1 cells were cultured in medium containing 20 μg/ml puromycin (Sigma).

Flow Cytometric Analysis—Cells were stained with anti-CD59 (clone 5H8), anti-decay-accelerating factor (DAF, clone IA10) or anti-Chinese hamster urokinase plasminogen activator receptor (uPAR; clone 5D6) primary antibodies and then with a fluorescein isothiocyanate-conju-
gated anti-mouse IgG secondary antibody (ICN/Cappel). When cells were stained with a biotinylated antibody, phycoerythrin-streptavidin (Biomedex) was used as the secondary conjugate. Stained cells were analyzed using a FACScan (BD Biosciences).

Establishment of GPI-anchored Protein-defective CHO Mutant Cells—
The parental CHO cell line F21 was established by stable transfection of the wild-type CHO K1 cells with cDNAs of GPI-anchored protein markers (human CD59 and DAF) and previously known GPI biosynthetic enzymes (DPM1, DPM2, SL15, PIG-A, PIG-L, PIG-V, PIG-B, PIG-N, PIG-F, GAA1, and PIG-U) as described previously (3). CHO F21 cells (3 × 10^6) were mutagenized with 1.2 μg/ml N-methyl-N'-nitro-N-nitrosoguanidine (Nacalai Tesque) for 2 days, cultured in fresh medium for 1 day, transferred to 12-well plates, and cultured for 6 days. Next, the cells were treated with 2.0 nM proaerolysin from *Aeromonas hydrophila* (Protop Biotech) for 3 days and 2.0 nM α-toxin from *Clodrdium septicum* (a gift from Dr. N. Sugimoto, Osaka University) for 3 days. Surviving cells were isolated by limiting dilution.

Transfection of Cells—Cells were transfected by either electroporation or lipofection. For electroporation, CHO cells (4 × 10^6) suspended in 0.4 ml of culture medium were electroporated at 260 V and 1,000 microfarads with 10 μg of DNA using a Gene Pulser (Bio-Rad). For lipofection, CHO cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

Labeling of GPI Precursors and Dolichol-linked Oligosaccharides—For labeling with Man, CHO cells (1 × 10^6) were preincubated in medium containing 100 μg/ml glucose and 10 μg/ml tunicamycin (Sigma) for 1 h and then incubated in the same medium containing 40 μCi/ml [3H]-Man (American Radiolabeled Chemicals) for 45 min. Lipids were extracted from the cells with chloroform:methanol (2:1, v/v) and then partitioned into water-saturated 1-butanol. The extracts were applied to a silica gel TLC plate (Merck), and developed with chloroform:methanol:water (10:10:3, v/v/v) to analyze the GPI anchor precursors. The radiolabeled lipids were detected using a Fuji BAS1500 image analyzer (Fuji Film).

For analysis of dolichol-linked N-glycan precursors, cells were metabolically labeled with [3H]-Man in the absence of tunicamycin. Dolichol-linked oligosaccharides were extracted and hydrolyzed with 0.1 M HCl, 80% tetrahydrofuran at 65 °C for 30 min to release the oligosaccharides. After neutralization, the oligosaccharides were analyzed by TLC using 1-propanolacetic acid:water (3:3:2, v/v/v) (3).

Cloning of Chinese Hamster DPM3 cDNA—The cDNA of Chinese hamster (*Cricetus griseus*) DPM3 was cloned by degenerate reverse transcription-PCR using mRNA prepared from CHO K1 cells as a template and the following primers: forward, 5′-GACGAAARTTACRCA-GTGGCCCTTTGAGGACT; reverse, 5′-TCGGGCCTCCWSKATC-CTGCTCTGACCT. The 5′ and 3′ sequences were obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) PCR. The GenBank™ accession number of Chinese hamster DPM3 cDNA is AB219149.

Plasmids—Site-directed mutagenesis of pME/FLAG-DPM3 was carried out using a PCR-based method. To construct pME/3HSV-DPM3tail and pME/GST-DPM3tail, a DNA fragment encoding the C-terminal 35 amino acids (Gly89–Phe92) of DPM3 was amplified with a specific forward primer containing a SalI site (5′-AAGTCGACACTCTTCATTCGTTCTGAGGAG; the SalI site is underlined) and a reverse vector primer (5′-GACGAAARTTACRCA-GTGGCCCTTTGAGGACT) using pME/FLAG-DPM3 as a template and then ligated into the pME/3HSV and pME/GST vectors, respectively. To prepare the chimeric construct pME/PIG-Lm-DPM3tail, a DNA fragment encoding the N-terminal 30 amino acids (Met1–Ser30) of PIG-L was amplified with a forward SRα primer (5′-TGAACCTGCTTGTGCT-CAACTCTACG) and a specific reverse primer containing a Sall site (5′-AAGTCGACACTCTTCATTCGTTCTGAGGAG; the Sall site is underlined) using pME/py/PIG-L as a template, and the PCR product was used to replace the 3HSV sequence of pME/3HSV-DPM3tail. Human CHIP (C terminus of Hsc70-interacting protein) cDNA was amplified by PCR using a cDNA library prepared from Hep3B cells as a template.

Affinity Precipitation and Western Blotting—Cells were lysed in TEN buffer (1.0% Nonidet P-40, 50 mM Tris-HCl, pH 7.7, 5.0 mM EDTA, 150 mM NaCl, Complete protease inhibitor mixture (Roche Applied Science)), and cell debris was removed by ultracentrifugation (100,000 × g for 1 h). For immunoprecipitation of FLAG-tagged proteins, anti-FLAG M2-agarose (Sigma) was added to the supernatant and rotated at 4 °C for 2 h. For immunoprecipitation of HSV-tagged or GST-tagged proteins, anti-HSV (Novagen) or anti-GST (Amershams Biosciences) antibodies plus protein G-Sepharose (Amershams Biosciences) were added. The beads were collected and washed twice with TEN buffer. Next, the absorbed proteins were eluted with reducing SDS sample buffer. Aliquots of the samples were separated in a 10–20% gradient SDS-polyacrylamide gel and electroblotted onto a polyvinylidene difluoride membrane. The membrane was treated with a primary antibody and then with horseradish peroxidase-conjugated protein G (Bio-Rad). Detection was carried out using Western Lightning chemiluminescence reagents (PerkinElmer Life Sciences).

RESULTS

**DPM3 Is Essential for DPM Synthase Activity**—We previously identified DPM3 in the DPM synthase complex that was affinity-purified using DPM1 double-tagged with GST and FLAG (16). Because neither mutant nor knock-out cells of DPM3 have been established to date, it is unknown whether DPM3 is essential for the function of DPM synthase. To obtain DPM3 mutant cells, we screened GPI-anchored protein-defective mutant cells, which were generated from the parental CHO F21 cells by chemical mutagenesis, followed by selection with *A. hydrophila* proaerolysin and/or *C. septicum* α-toxin, both of which are GPI anchor-recognizing pore-forming cytolytic toxins (3). We found one mutant cell line, CHO2.38, in which surface expressions of the GPI-anchored proteins CDS9, DAF, and uPAR were markedly decreased compared with the parental cells (Fig. 1A). The surface expressions of these GPI-anchored proteins were restored by transient transfection of DPM3 cDNA but not by transfection of DPM1 or DPM2 cDNA (Fig. 1B).

To confirm the presence of a mutation in the *DPM3* gene, we sequenced and compared the *DPM3* mRNAs from CHO K1 and CHO2.38 cells. Chinese hamster *DPM3* was found to encode 92 amino acid residues, as is the case for humans and mice, and showed 92% (85/92) and 97% (89/92) identities with these species, respectively. By comparison of the nucleotide sequences, we found an 8-base deletion at nucleotides 108–115 in the *DPM3* cDNA from CHO2.38 cells (Fig. 1C). This mutation, which causes a frameshift, may completely abolish the function of DPM3. CHO2.38 cells stably transfected with pME/puro/DPM3 (CHO2.38/DPM3 cells) showed the same expression levels of surface GPI-anchored proteins as the parental CHO F21 cells (Fig. 1D). In *in vitro* enzyme assays using microsomes revealed that CHO2.38 cells did not synthesize either DPM or Man-containing GPI anchor precursors (Fig. 1E, lane 3), whereas CHO2.38/DPM3 cells produced more DPM than CHO F21 cells (Fig. 1E, lane 2 versus lane 1). Furthermore, CHO2.38 cells did not synthesize the mature N-glycan precursor Dol-P-P-GlcNAc2Man2Glc, and, instead, accumulated two immature precursors (Fig. 1F, lane 2). The upper large spot (Fig. 1F, lane 2) was GlcNAc2Man2, which was identical to the spot accumulated in CHO.
FIGURE 1. CHO2.38 cells are DPM3-defective mutant cells. A, surface expressions of the GPI-anchored proteins CD59, DAF, and uPAR on the parental CHO F21 (upper panels) and mutant CHO2.38 (lower panels) cells. The thin lines indicate control staining using isotype control antibodies. B, restoration of CD59 expression on CHO2.38 cells by transient transfection of DPM1 (top), DPM2 (middle), and DPM3 (bottom) cDNAs. The thin lines indicate control staining. C, cDNA and deduced amino acid sequences of DPM3 from Chinese hamster C. griseus (GenBank™ accession number AB219149). The 8 underlined bases are deleted in CHO2.38 cells. D, surface expressions of CD59 on CHO F21 cells (line 1), CHO2.38 cells stably transfected with pME/puro/DPM3 (line 2), and CHO2.38 cells (line 3). E, in vitro enzyme assays using microsomes from CHO F21 cells (lane 1), CHO2.38 cells stably transfected with pME/puro/DPM3 (lane 2), and CHO2.38 cells (lane 3).
Stabilization and Degradation of the DPM Synthase Complex

A Cytosolic Coiled-coil Domain Near the C Terminus of DPM3 Is Critical for DPM Synthase Activity—DPM3 is a small protein that consists of 92 amino acid residues. In silico analyses of human DPM3 predicted the presence of two transmembrane regions (Trp$^9$–Pro$^{31}$ and Glu$^{66}$–Gly$^{86}$) in the N-terminal portion and a coiled-coil domain (Glu$^{68}$–Arg$^{87}$) in the C-terminal hydrophilic portion (Fig. 2A). Because a FLAG tag attached to the N terminus of DPM3 faced the cytosolic surface of the ER membrane, as revealed by immunomicroscopic analysis (data not shown), the C-terminal coiled-coil domain must also be oriented toward the cytosol. To form such a coiled-coil structure, regularly aligned hydrophobic amino acid residues need to be on the same side of an α-helix. A comparison of the amino acid sequences of DPM3 with pME/puro/DPM3 (lane 2), and CHO2.38 cells (lane 3). The microsomes were incubated with GDP-[3H]Man, and the Man-containing glycolipids were analyzed by TLC. The labels are indicated on the right (3, 17).

Lec35 cells (Fig. 1F, lane 3) (CHO Lec35 is a mutant cell line defective in the general usage of both DPM and dolichol-phosphate glucose (17)). The lower weak spot (Fig. 1F, lane 2) was probably GlcNAc$^2$Man$^5$Glc$^3$. Taken together, these results indicate that the CHO2.38 cell line is a DPM3-defective mutant and that DPM3 is an essential component for DPM synthase activity.

A Cytosolic Coiled-coil Domain Near the C Terminus of DPM3 Is Critical for DPM Synthase Activity—DPM3 is a small protein that consists of 92 amino acid residues. In silico analyses of human DPM3 predicted the presence of two transmembrane regions (Trp$^9$–Pro$^{31}$ and Glu$^{66}$–Gly$^{86}$) in the N-terminal portion and a coiled-coil domain (Glu$^{68}$–Arg$^{87}$) in the C-terminal hydrophilic portion (Fig. 2A). Because a FLAG tag attached to the N terminus of DPM3 faced the cytosolic surface of the ER membrane, as revealed by immunomicroscopic analysis (data not shown), the C-terminal coiled-coil domain must also be oriented toward the cytosol. To form such a coiled-coil structure, regularly aligned hydrophobic amino acid residues need to be on the same side of an α-helix. A comparison of the amino acid sequences of DPM3

with pME/puro/DPM3 (lane 2), and CHO2.38 cells (lane 3). The microsomes were incubated with GDP-[3H]Man, and the Man-containing glycolipids were analyzed by TLC. The labels are indicated on the left of the chromatogram (3). EtNP, ethanolamine phosphate; GlcN, glucosamine; *, unknown spot always observed with CHO2.38 cells. F, N-glycan precursors of CHO F21 cells (lane 1), CHO2.38 cells (lane 2), and CHO Lec35 cells, an SL15-defective mutant (lane 3). Following metabolic labeling of the cells with [3H]Man, the lipid-linked oligosaccharides were extracted and acid-hydrolyzed at phosphate bonds. The released oligosaccharides were analyzed by TLC. The labels are indicated on the right (3, 17).
produced point mutations into three of these aliphatic residues, namely DPM3(triple), or pME/FLAG-DPM3 and either pME/FLAG-DPM3(wt), pME/FLAG-DPM3. We analyzed the binding of DPM3 to DPM1 by co-immunoprecipitation of DPM3 function by interacting with the catalytic subunit DPM1, we were transfected CHO2.38 cells with the indicated antibodies.

Next, we expressed soluble forms of the DPM3 tail constructs. CHO2.38 cells were transiently transfected with the wild-type and triple-mutant forms of 3HSV-DPM3 tail and GST-DPM3 tail. All of the soluble constructs were strongly expressed (data not shown). Surprisingly, both the 3HSV-DPM3 tail(wt) and GST-DPM3 tail(wt) constructs showed some activity (Fig. 4B, panels 2 and 4), whereas the triple-mutant constructs did not (Fig. 4B, panels 3 and 5). These results indicate that DPM1 is partially active in the presence of the coiled-coil domain of DPM3, even in a soluble form, and that the transmembrane regions of DPM3 are not essential.

**DPM1 Is Degraded by the Proteasome in DPM3-defective Cells**—The expression of DPM1 was enhanced by co-transfection of the wild-type DPM3 into CHO K1 cells (Fig. 3), suggesting that DPM3 stabilizes DPM1 on the ER membrane. To examine whether DPM1 is expressed in the absence of DPM3, we transiently transfected CHO2.38 cells with pME/3HSV-DPM1 in the presence or absence of pME/3HSV-DPM3. Rat microsomal aldehyde dehydrogenase (ALDH) was used as a control for transfection efficiency. After 2 days of culture, the cells were lysed, and the protein expression was analyzed by Western blotting. Unexpectedly, 3HSV-DPM1 was hardly detected in the absence of DPM3 (Fig. 5A, lane 2), whereas it was strongly expressed in the presence of DPM3 (Fig. 5A, lane 1). Similar experiments were carried out for DPM2. Unlike DPM1, DPM2 was stably expressed in the presence or absence of DPM3 (Fig. 5A, lanes 3 and 4).

Because DPM1 was hardly expressed in DPM3-defective CHO2.38 cells, we speculated that it was rapidly degraded after translation. To elucidate this, we stably transfected CHO K1 and CHO2.38 cells with pME/puro/3HSV-DPM1 to generate CHO K1/3HSV-DPM1 and CHO2.38/3HSV-DPM1 cells, respectively, and analyzed their 3HSV-DPM1 expression levels. DPM1 was strongly expressed in CHO K1/3HSV-DPM1 cells (Fig. 5B, lane 1) but was hardly expressed in CHO2.38/3HSV-DPM1 cells (Fig. 5B, lane 2), as expected. When a DPM3 cDNA was transiently transfected into CHO2.38/3HSV-DPM1...
cells, the expression of DPM1 was recovered to some extent (Fig. 5B, lane 3), indicating that the cDNA of DPM1 was maintained in the cells but the protein was degraded.

Next, we speculated that DPM1 expressed in CHO2.38 cells may be dissociated from the ER membrane and degraded by the proteasome, because DPM1 is predicted to be located on the cytosolic surface of the ER membrane. To test this hypothesis, we cultured CHO K1/3HSV-DPM1 and CHO2.38/3HSV-DPM1 cells in the presence of protease inhibitors. In CHO K1/3HSV-DPM1 cells, the expression levels of 3HSV-DPM1 remained unchanged following the addition of lactacystin, an inhibitor of the proteasome, or leupeptin, an inhibitor of lysosomal proteases (Fig. 5C, lanes 1–3). In contrast, in CHO2.38/3HSV-DPM1 cells, the expression of 3HSV-DPM1 was restored after the addition of lactacystin (Fig. 5C, lane 5) but not leupeptin (Fig. 5C, lane 6). These results strongly suggest that DPM1 is highly unstable and degraded by the proteasome in the cytosol when DPM3 is absent. It is worth noting that CD59 expression was not restored at all in CHO2.38/3HSV-DPM1 cells transiently transfected with DPM3 cDNA (Fig. 5C, lane 5, versus Fig. 5B, lane 3). These results suggest that the coiled-coil domain of DPM3 is necessary not only for the stabilization of DPM1 but also for its enzyme activity.

**Free DPM1 Strongly Interacts with CHIP, a Chaperone-dependent E3 Ubiquitin Ligase**—There are several hundred known candidates for E3 ubiquitin ligases in mammalian genomes, and these define the specificity of the target protein degradation by the proteasome. To explore which ubiquitin ligase is responsible for the degradation of DPM1, we focused on CHIP, which was originally discovered as a co-chaperone containing a tetratricopeptide repeat domain (20) and later identified to be a chaperone-dependent U-box-type E3 ubiquitin ligase (21, 22). We transiently transfected CHO2.38 cells with GST-CHIP and either 3HSV-ALDH as a control or 3HSV-DPM1. GST-CHIP was strongly expressed in all transfectants (Fig. 6, bottom panel). 3HSV-ALDH was also strongly expressed in the presence or absence of lactacystin (Fig. 6, top panel, lanes 1–4; lanes 3 and 4 contain 10% of the loading of lanes 1 and 2, respectively). However, very little GST-CHIP co-immunoprecipitated with 3HSV-ALDH (Fig. 6, middle panel, lanes 1 and 2), and it was hardly detected in the lanes with the 10% loadings (Fig. 6, middle panel, lanes 3 and 4). In contrast to ALDH, the expression of 3HSV-DPM1 was very weak in the absence of lactacystin and increased after the addition of lactacystin (Fig. 6, top panel, lanes 5 and 6, respectively). Despite its weak expression, GST-CHIP was much more strongly co-immunoprecipitated with 3HSV-DPM1 than with ALDH (Fig. 6, middle panel, lanes 5 and 6 versus lanes 3 and 4). These results strongly suggest that free DPM1 is ubiquitinated by CHIP.

**DISCUSSION**

Four of nine Man residues in the mature precursor of N-glycan are transferred from DPM in the ER lumen. After the glycan is transferred to proteins, all four Man residues are sequentially removed by ER/ Golgi mannosidase I and II. These residues are not essential for the viability of cultured mammalian cells but are important for quality control of glycoprotein production in the ER. It has been
reported that the maturation, transport, and degradation of nascent glycoproteins in the ER are delayed in DPM synthase-defective cells (23). Recently, ER degradation-enhancing \( \alpha \)-mannosidase-like protein (EDEM) was found to play a key role in ER-associated degradation by recognition of the terminal \( \alpha1,2 \)-linked Man residue in the GlcNAc\(_2\)Man\(_8\) structure, which is derived from DPM (24). In humans, genetic diseases caused by mutations in DPM synthase are known and classified into congenital disorder of glycosylation type Ie (CDG-Ie). These patients show accumulation of the \( \text{N} \)-glycan precursor with GlcNAc\(_2\)Man\(_5\) (25, 26), similar to the case with CHO2.38 cells (Fig. 1F). To date, mutations that cause CDG-Ie have been found only in DPM1 and not in DPM2 or DPM3. Because DPM3 and DPM2 are essential for enzyme activity (Fig. 1E), CDG-Ie patients harboring mutations in these subcomponents may be identified in the future. Defects in DPM synthesis also affect GPI anchor biosynthesis, because the three core Man residues of GPI are derived from DPM (Fig. 1A). Knock-out mice for PIG-A, which encodes the catalytic subunit of GPI-GlcNAc-transferase, the first-step enzyme of GPI anchor biosynthesis, show embryonic...
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DPM3 is limited or depleted, excess DPM1 may be degraded by the proteasome, as demonstrated in CHO2.38 cells (Fig. 5). Although DPM1 does not have a transmembrane region, it does possess a hydrophobic peptide sequence near the C terminus that functions in its binding to DPM3 (15, 16). Thus, DPM1 dissociated from DPM3 must bear the hydrophobic peptide on the surface of the molecule, which could be a binding target for cytosolic chaperones. We demonstrated that free DPM1 formed a complex with an E3 ubiquitin ligase, CHIP (Fig. 6), which is known to bind an E2 ligase through its U-box domain and is also able to bind the cytosolic chaperones Hsc70/Hsp70 or Hsp90 through its tetratricopeptide repeat domain. It has been reported that CHIP accelerates the degradation of several cytosolic and membrane proteins and also that CHIP prefers misfolded proteins over correctly folded proteins (30, 31). The interaction between DPM3 and DPM1 must be relatively weak, because it was stable in digitonin but labile in Nonident P-40 (Fig. 3), suggesting that some intracellular stimuli may dissociate these two molecules under physiological conditions. It has been reported that cAMP-dependent protein kinase regulates mammalian DPM synthase activity by phosphorylating a serine residue in DPM1 (32, 33). The conformational changes of DPM1 caused by such post-translational modifications may trigger dissociation of the DPM synthase complex.

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FIGURE 6. Free DPM1 interacts with CHIP, a chaperone-dependent E3 ubiquitin ligase. CHO2.38 cells were co-transfected with pME/GST-CHIP and either pME/3HSV-ALDH or a control (lanes 1–4) or pME/3HSV-DPM1 (lanes 5 and 6). The expressed proteins were immunoprecipitated (IP) and analyzed by Western blotting (WB). Cells were harvested at 12 h after the addition of a control solvent (Me2SO; lanes 1, 3, and 5) or 10 μM lactacystin (lanes 2 and 4). Lanes 3 and 4 contain 10% of the sample amounts loaded in lanes 1 and 2, respectively.
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