Human UBC9 is a member of the E2 (ubiquitin conjugation enzyme) family of proteins. Instead of conjugating to ubiquitin, it conjugates with a ubiquitin homologue UBL1 (also known as SUMO-1, GMP1, SMTP3, PIC1, and sentrin). UBC9 has been shown to be involved in cell cycle regulation, DNA repair, and p53-dependent processes. The binding interfaces of the UBC9 and UBL1 complex have been determined by chemical shift perturbation using nuclear magnetic resonance spectroscopy. The binding site of UBL1 resides on the ubiquitin domain, and the binding site of UBC9 is located on a structurally conserved region of E2. Because the UBC9-UBL1 system shares many similarities with the ubiquitin system in structures and in conjugation with each other and with target proteins, the observed binding interfaces may be conserved in E2-ubiquitin interactions in general.

The ubiquitin pathway is crucial in cellular regulation (1–3). It is now clear that this pathway regulates the life span of many important proteins, including some cyclins, cyclin-dependent kinase inhibitors, histones, oncoproteins, and tumor suppressors. Therefore, this pathway regulates many cellular processes, including cell cycle progression and apoptosis, transcription regulation, and antigen presentation (4–6). Ubiquitin is a protein with 76 amino acids. Its C-terminal Gly residue is involved in covalent conjugation to a Lys residue of other proteins. The C-terminal Gly residue of one ubiquitin molecule can also conjugate to Lys48 of another ubiquitin molecule in multiubiquitination. In the ubiquitination pathway, the ubiquitin activation enzyme E11 activates ubiquitin by hydrolyzing ATP to form a high energy bond with ubiquitin. Ubiquitin is then transferred to a ubiquitin conjugation enzyme (UBC) also known as E2. In this step, the C-terminal Gly is conjugated to the SH group of the active-site Cys residue of E2. Then E2 interacts with substrate proteins to transfer ubiquitin to the substrate proteins. In some cases, this process requires the participation of ubiquitin-protein ligase (E3). Proteins with high sequence homology to ubiquitin and to the E1 and E2 enzymes have been found.

Human UBC9, composed of 158 amino acid residues, is a member of the E2 protein family. Among the conserved residues in the E2 family of proteins, the fragment containing the ubiquitin-accepting Cys residue is highly conserved. UBC9 conjugates with a ubiquitin homologue, UBL1, instead of ubiquitin. The interaction between UBC9 and UBL1 is specific, because UBC9 does not interact well with ubiquitin, and UBL1 does not interact with UBC2 in a yeast two-hybrid system (7–9). UBC9 in yeast, mouse, and human have been identified (10, 11, 7). Many proteins interact with UBC9. The human UBC9 has been shown to interact with several important proteins, such as DNA repair proteins RAD51 and RAD52, p53, c-JUN, glucocorticoid receptor, the negative regulatory domain of the Wilms’ tumor gene product, and human papillomavirus type 16 E1 replication protein (12–14). Seufert et al. (10) show that yeast UBC9 may interact with CLB2, an M-phase cyclin, and CLB5, an S-phase cyclin (10). Hateboer et al. (15) show that murine UBC9, which has an identical amino acid sequence to the human UBC9, binds to the adenovirus E1A protein. UBC9 plays critical roles in DNA repair, cell cycle regulation, and p53-dependent processes.

The three-dimensional structures of human UBC9 and several other E2 proteins have been determined (8, 16–19). In addition, conformational flexibility of UBC9 has been characterized by NMR methods (20). The E2 proteins have highly conserved tertiary structures with root mean square deviation of Cα atoms less than 2 Å, excluding two surface loops. The two surface loops occur around amino acid residues 30 and 100 and vary significantly in the lengths of the sequences and three-dimensional structures among different UBC proteins. Several regions on UBC9 have higher conformational flexibility than average. The N-terminal site has the highest mobility over a wide range of time scales. A region between the C terminus and the active site also displays higher mobility on the ps–ns and ms time scales.

The ubiquitin homologue UBL1 is also known as SUMO-1, GMP1, SMTP3, PIC1, or sentrin (for a review, see Ref. 21). It contains 101 amino acid residues. Residues 22–97 of UBL1 share approximately 48% sequence homology with ubiquitin. Residues 98–101 of UBL1 are cleaved before conjugation with E1. A unique feature of ubiquitin and ubiquitin-like proteins is that they all contain a diglycine sequence at the C terminus that is capable of conjugating to other proteins. An E1 homologue specific for the yeast UBL1 and UBC9 has also been found (22).

The three-dimensional structures of ubiquitin and UBL1 have been determined (23, 24). In addition, information on their conformational flexibilities has been obtained from NMR studies (25, 24). As expected from sequence conservation, res-
idues 22–97 of UBL1 have a similar three-dimensional structure as ubiquitin. In addition, the C-terminal residues of UBL1 are as flexible in solution as those of ubiquitin. The 21 extra amino acid residues at the N terminus of UBL1 have mainly a random conformation. This segment contains mostly hydrophilic amino acid residues and is overall negatively charged. There are only three hydrophobic residues, one Met, Ala, and Leu in this segment.

Despite the intensive studies on ubiquitination, the mechanism of protein-protein interactions in ubiquitination is not well understood. Details of the interaction and conjugation between an E2 and a ubiquitin are still not clear. One of the major difficulties in investigating these interactions is that they are not strong and cannot be easily detected by conventional biochemical approaches. NMR methods have contributed greatly to the characterization of molecular interactions. Chemical shift perturbation is one of the most sensitive methods to monitor specific protein complex formation (26). Chemical shift changes are capable of mapping the binding interfaces of protein complexes with dissociation constants in the mM to nM ranges. This paper describes the identification of the binding interfaces between UBC9 and UBL1 using NMR chemical shift perturbation. Because the binding site of UBL1 resides on the ubiquitin domain and the binding site of UBC9 resides on a structurally conserved region of E2, the observed interaction may represent a general feature of E2-ubiquitin interactions.

**MATERIALS AND METHODS**

**NMR Studies**—Both human UBC9 and UBL1 were subcloned into vector PET28 (from Novagen, Inc.). The modified plasmids have an open reading frame that encodes the His6 tag at the N terminus followed by the sequence of UBC9 or UBL1. *Escherichia coli* containing the expression plasmid were grown at 37 °C in M-9 minimal media supplemented with trace minerals and basal medium Eagle vitamins (Life Technologies, Inc.). (15NH₄)₂SO₄ (1.5 g/liter) was used as the nitrogen source. Both proteins were purified using nickel nitrilotriacetate columns. Approximately 20–30 mg of UBC9 and 10 mg of UBL1 were purified from 1 liter of the M-9 culture. Both samples were of high purity, because no impurities were observed on SDS-polyacrylamide gel electrophoresis. All NMR samples contained 100 mM phosphate buffer (pH 6.0) in 90% H₂O, 10% D₂O. The concentrations of protein samples were estimated with the Bio-Rad protein assay.

**1H-15N HSQC experiments** (27) were performed on a Varian Unity plus 500 spectrometer equipped with four channels, pulse shaping, and pulsed field gradient capabilities. The digital resolutions of the spectra were 0.02 ppm in the proton dimension and 0.05 ppm in the amide 15N dimension. For observing the UBL1 bind site on UBC9, 0.3 mM 15N-labeled UBC9 was used. Unlabeled UBL1 was titrated into the sample containing UBC9. The final concentrations of both proteins at the end of the titration were approximately 0.2 mM. The spectra were acquired at 30 °C, a condition used for obtaining the resonance assignments of UBC9. For detecting the UBC9 binding site on UBL1, 0.5 mM 15N-labeled UBL1 was used and titrated with unlabeled UBC9. The final concentrations of both proteins at the end of the titration were approximately 0.3 mM. The HSQC spectra for obtaining the binding interface on UBL1 were performed at 17 °C, which was used for the resonance

**FIG. 1.** Superposition of 1H-15N HSQC spectra of 15N-labeled human UBC9, free and in complex with unlabeled human UBL1. The UBC9/UBL1 ratio in the complex is approximately 1:1. The cross-peaks of free UBC9 are shown in green, and those in the complex are shown in red. Only peaks that were affected by the complex formation are labeled.
The NMR resonance assignments for UBC9 have been described previously (28). The NMR resonance assignments for UBL1 will be described elsewhere.2

Calculations of Electrostatic Potentials—we calculated the surface electrostatic potentials for UBL1, ubiquitin, UBC9, UBC4, and UBC7 (8, 17–19, 23, 24) using the DelPhi module of INSIGHTII (MSI, Inc.) with the NMR and crystal structures to better understand our results. The solvent dielectric constant was set to 80. The radius of the probe water molecule was 1.4 Å. The grids in the calculation of the electrostatic potentials were with a spacing of 1.5 Å.

RESULTS

We have used 15N-1H HSQC spectra to map the binding interfaces of the ubiquitin homologue UBL1 and human UBC9. 15N-1H HSQC has been frequently used to map the binding surfaces of protein-protein, protein-nucleic acid, and protein-ligand interactions. In this spectrum, resonances are usually well resolved because of large 15N chemical shift dispersion.

2 C. Jin, Z. Shen, X. Liao, manuscript in preparation.
Because each amino acid residue gives one peak in this spectrum, it is easy to monitor the chemical shift perturbation of each amino acid. The binding interface on UBC9 was obtained using 15N-labeled UBC9 in complex with unlabeled UBL1. 15N-1H HSQC spectra of UBC9, free and in complex with UBL1, were compared. Similarly, the binding interface on UBL1 was mapped using 15N-labeled UBL1 and unlabeled UBC9. 15N-1H HSQC spectra of UBL1, free and in complex with UBC9, were compared.

The Binding Interface on UBC9—Specific chemical shift perturbation and changes in the line widths were observed in the 1H-15N HSQC spectrum of UBC9 upon forming complex with UBL1. These changes were observed from the beginning of the titration, when the concentration of UBC9 was approximately 0.3 mM and that of UBL1 was 0.03 mM until the final concentrations of UBL1 and UBC9 reached approximately 0.2 mM. Superposition of the HSQC spectra of free UBC9 and that in complex with UBL1 is shown in Fig. 1. The assignments of the resonances in the complex that undergo fast exchange were made by following resonance shifts during titration. Details of the chemical shift changes of UBC9 at the end of the titration are given in Table I. Variations in line widths between the resonances of free UBC9 and UBC9 at the end of the titration are indicated by relative changes in peak heights, also given in Table I. Most peaks of UBC9 were not affected, indicating that complex formation between the two proteins does not cause large conformational changes in UBC9. Some residues were significantly affected by the complex formation. The line widths of residues Ala10, Ala15, Arg17, Lys18, Phe22, Gly23, and Phe24 became so broad in the complex that their cross-peaks cannot be observed from the beginning of the titration when the ratio of UBC9:UBL1 was 10. These residues are colored in dark blue in the ribbon diagram of the UBC9 structure (Fig. 2A). Residues Ser7, Lys14, Trp16, Glu19, His20, Ala26, Val27, Met36, Gln37, Lys59, Leu63, and Ser158 moved more than 0.2 ppm in the 15N dimension or more than 0.18 ppm in the 1H dimension at the end of the titration. These residues are colored in cyan in Fig. 2A. In addition, residues Ile4, Thr29, and Gly115 moved more than 0.14 ppm in the 15N dimension at the end of the titration. The proton resonances of these residues did not change more than 0.04 ppm. These residues are shown in green in Fig. 2A. The chemical shift changes of these residues are linear with the addition of UBC1. Some residues had larger increases in line widths than average but had small chemical shift changes. These residues are labeled in the spectra in Fig. 1. Some of these residues are close to the region that showed the largest perturbation upon the formation of the complex, and some are located on the main b-sheet. Most of the chemical shift changes on UBC9 occur in the nitrogen dimension. Residues that are most affected by the complex formation are clustered together in the three-dimensional structure, suggesting that this region is the binding interface. A few residues on these surfaces, including Leu9, Asn11, Glu12, Arg13, Lys20, and Gln31, have resonances that overlap with other resonances and, therefore, could not be monitored for their chemical shift perturbations. These residues are colored in gray in Fig. 2A. Thus, the region of UBC9 interacting the UBL1, as suggested by the chemical shift perturbations, consists of the first a-helix, the first b-strand, the loop between them, and the second b-strand.

The Binding Interface on UBL1—Specific chemical shift changes were also observed on UBL1. Unlabeled UBC9 was used to titrate 15N-labeled UBL1. The concentrations of UBL1 and hUBC9 at the end of the titration are about 0.45 and 0.39 mM, respectively. The superposition of the HSQC spectra of UBL1, free and in complex with UBC9, is shown in Fig. 3. The assignments of the resonances in the complex that undergo fast exchange were made by following resonance shifts during titration. Details of the chemical shift changes of UBL1 at the end of the titration are given in Table II. Variations in line widths between the resonances of free UBL1 and UBL1 at the...
end of the titration are indicated by relative changes in peak heights, given in Table II. The resonances of residues Ile27, Ser31, Leu65, Glu67, Asp86, Ile88, and Val90 were significantly broadened, because their peaks disappeared in the HSQC spectrum. These residues are shown in dark blue in the ribbon diagram of the structure of UBL1 (Fig. 4A). Residues Val26, Phe64, Gly68, Ile71, Gly81, Met82, Glu83, Glu85, and Val87 showed chemical shift changes of more than 0.25 ppm in the 15N dimension and/or more than approximately 0.1 ppm in the 1H dimension. Many of these residues have large chemical shift changes in both the proton and nitrogen dimensions. The chemical shift changes of these residues are linear with the addition of UBC9. These residues are colored in cyan in Fig. 4A. These residues are also clustered together in the three-dimensional structure of UBL1, suggesting that this region is the binding interface with UBC9. This surface is located on the main \(\beta\)-sheet in the ubiquitin domain of UBL1. No chemical shift changes were observed for the N-terminal 21-amino acid residues, indicating that this region is not involved in interaction with UBC9.

**DISCUSSION**

Chemical shift perturbation is extremely effective in mapping the binding surfaces. The chemical shift of a nuclei is sensitive to the changes of its local environment including aromatic ring current effects, peptide bond anisotropy, electrostatic interactions, and hydrogen bonding. When two proteins form a complex, the interactions between them cause changes in the environment of the amino acids at the interfaces, resulting in chemical shift changes. Any small additional conformation changes near the direct contacting surfaces will cause additional chemical shift perturbation. Thus the surface mapped by chemical shift perturbation contains but extends beyond the direct binding surface. However, residues that have the largest chemical shift changes are usually located within the binding interface.

**Sequence Conservation**—The amino acid residues involved in the recognition between UBC9 and the ubiquitin homologue UBL1 are highly conserved throughout different species. Residues 10–27 of UBC9 had the largest changes in resonance line
The Binding Interfaces between UBC9 and UBL1

TABLE II

| Residue | $\Delta^{1}H$ (ppm) | $\Delta^{15}N$ (ppm) | Ratio | Secondary Structure |
|---------|---------------------|----------------------|-------|---------------------|
| Ser$^7$ | -0.04               | 0.87                 |       |                     |
| Ala$^8$ | 0.00                | 0.80                 |       |                     |
| Lys$^9$ | 0.00                | 0.82                 |       |                     |
| Ser$^{10}$ | 0.00            | 0.79                 |       |                     |
| Thr$^{11}$ | -0.36          | 0.71                 |       |                     |
| Asp$^{12}$ | -0.01          | 0.94                 |       |                     |
| Leu$^{13}$ | 0.00             | 0.70                 |       |                     |
| Gly$^{14}$ | 0.00            | 0.76                 |       |                     |
| Lys$^{17}$ | 0.00             | 0.20                 |       |                     |
| Gly$^{19}$ | 0.02             | 0.60                 |       |                     |
| Tyr$^{21}$ | 0.00             | 0.30                 |       |                     |
| Ile$^{22}$ | -0.14           | 0.15                 |       |                     |
| Leu$^{24}$ | 0.08             | 0.19                 |       |                     |
| Val$^{26}$ | -0.01           | 0.12                 |       |                     |
| Ile$^{29}$ | disappear       |                      |       |                     |
| Ser$^{31}$ | 0.13             | 0.22                 |       | β STRAND I          |
| Val$^{32}$ | -0.22            | 0.20                 |       |                     |
| Met$^{34}$ | -0.01            | 0.18                 |       |                     |
| Thr$^{37}$ | 0.05             | 0.16                 |       |                     |
| Thr$^{38}$ | 0.00             | 0.25                 |       |                     |
| Leu$^{40}$ | 0.05             | 0.13                 |       |                     |
| Lys$^{43}$ | 0.00             | 0.85                 |       |                     |
| Leu$^{45}$ | 0.00             | 0.14                 |       |                     |
| Lys$^{48}$ | 0.00             | 0.14                 |       |                     |
| Ser$^{50}$ | 0.00             | 0.16                 |       |                     |
| Tyr$^{51}$ | 0.04             | 0.45                 |       |                     |
| Cys$^{52}$ | -0.04            | 0.16                 |       |                     |
| Arg$^{54}$ | 0.00             | 0.35                 |       |                     |
| Gln$^{56}$ | 0.00             | 0.21                 |       |                     |
| Gly$^{58}$ | 0.00             | 0.20                 |       |                     |
| Val$^{61}$ | -0.03            | 0.28                 |       |                     |
| Met$^{64}$ | 0.00             | 0.17                 |       |                     |
| Ser$^{66}$ | 0.03             | 0.21                 |       |                     |
| Leu$^{68}$ | -0.10            | 0.35                 |       |                     |
| Phe$^{70}$ | 0.07             | 0.19                 |       |                     |
| Leu$^{72}$ | disappear       |                      |       |                     |
| Glu$^{74}$ | disappear       |                      |       |                     |
| Ile$^{76}$ | 0.34             | 0.15                 |       |                     |
| Ile$^{77}$ | 0.00             | 0.10                 |       |                     |
| Asn$^{78}$ | -0.02            | 0.19                 |       |                     |
| His$^{79}$ | 0.00             | 0.18                 |       |                     |
| Thr$^{80}$ | 0.00             | 0.14                 |       |                     |
| Lys$^{81}$ | 0.09             | 0.13                 |       |                     |
| Glu$^{83}$ | 0.01             | 0.20                 |       |                     |
| Leu$^{85}$ | 0.03             | 0.15                 |       |                     |
| Gly$^{87}$ | 0.34             | 0.14                 |       |                     |
| Met$^{88}$ | 0.08             | 0.12                 |       |                     |
| Glu$^{90}$ | -0.02            | 0.15                 |       |                     |
| Glu$^{92}$ | 0.52             | 0.10                 |       |                     |
| Asp$^{94}$ | disappear       |                      |       |                     |
| Val$^{95}$ | -0.45            | 0.13                 |       | β STRAND III        |
| Ile$^{96}$ | -0.45            | 0.13                 |       |                     |
| Val$^{98}$ | -0.01            | 0.13                 |       |                     |
| Thr$^{91}$ | 0.07             | 0.73                 |       |                     |
| Gly$^{93}$ | 0.00             | 0.85                 |       |                     |
| Gly$^{95}$ | -0.02            | 0.86                 |       |                     |
| His$^{97}$ | -0.97            | 0.54                 |       |                     |

Fig. 4. A, ribbon diagram of the three-dimensional structure of UBL1. Chemical shift perturbation upon UBC9 binding is indicated with the coloring scheme described in the text. B displays the surface electrostatic potential of human UBL1. The orientation of the molecule in B is the same as that in A. The charge topology was calculated and displayed using INSIGHTII (MSI, Inc.). The color spectrum from red to blue corresponds to changes from negative to positive potentials over a range of $-5$ to $+5 \text{K}_B$/electron.
widths and chemical shifts upon complex formation with UBL1. Twelve of these 17 residues are identical among UBC9 of human, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (30). Two more residues have conservative mutations from Arg to Lys and Val to Tyr. Among the residues that are affected by complexation, four positively charged residues are conserved between the yeast and human proteins, including Arg13, Lys 14, Arg 17, and Lys 18. In addition, two negatively charged residue, Glu 12 and Asp 19, are identical between the yeast and human proteins. Six hydrophobic residues in this region are highly conserved including Trp16, Pro 21, Phe 22, Phe23, Val24, and Ala25. Five of them are identical between the human and yeast proteins.

Residues 26, 64–71, and 81–91 of UBL1 had the most significant changes in line widths and chemical shifts upon complex formation with UBC9. Nine of these residues are identical between the human and yeast proteins (24). Four residues are highly conserved, including a Phe to Tyr mutation, two Glu to Asp mutations, and a Val to Ile mutation. Among the residues that are affected by complexation, four negatively charged residues are highly conserved including Glu13, Glu14, Asp17, and Glu19. In addition, a positively charged residue, Arg20, is identical between the yeast and human proteins. Lys15 on this surface is identical among UBL1s of different species. However, this residue has overlapping resonances in the 1H-15N HSQC, and it is not clear whether it had significant chemical shift changes. Six hydrophobic residues are conserved, including Leu66, Phe67, Met82, Val87, Ile88, and Val90. Most of these residues are identical between the yeast and human proteins, except Phe66 mutated to a Tyr and Val87 to a Ile in the *S. cerevisiae* protein.

Sequence conservation indicates important functions for these residues. For UBC9 and UBL1, similar numbers of hydrophobic residues are conserved at the binding interfaces. In addition, similar numbers of the opposite-charged residues at the binding interfaces are also conserved. This suggests the compatibility of the binding interfaces.

**Mechanism of Recognition and Specificity**—The binding interfaces of the UBC9-UBL1 complex are highly complementary in their electrostatic potentials and hydrophobicity. The surface electrostatic potentials of UBC9 and UBL1 are displayed in Fig. 2B and 4B, respectively. Surface hydrophobic side chains in the binding interfaces are displayed in yellow along with electrostatic potentials in Fig. 5. For UBC9, the surface of residues 10–30 and 35–37 are displayed in yellow. For UBL1, the surfaces of residues 25–27, 31, 65–71, and 81–90 are displayed in Fig. 5B. The sizes of the surface areas that showed significant changes in line widths and chemical shifts are similar and are approximately 1,500 Å². The surface of UBC9, which is involved in binding to UBL1, is mainly positively charged (Fig. 2B and 5A). The surface of UBL1, which is involved in binding to UBC9, is mainly negatively charged (24) (Fig. 4B and 5B). The opposite surface of UBL1 from the binding interface has an overall positive potential (24). The N-terminal 21-amino acid residues of UBL1 are mainly negatively charged and may contribute to the electrostatic energy when forming a complex with the positively charged surface of UBC9. However, no specific chemical shift changes have been observed for residues 1–16. Residue 17 showed some 15N chemical shift perturbation (0.19 ppm). Because this change is smaller compared with that in the ubiquitin domain, and this residue is close to the region that displays large changes in chemical shifts and line widths, this perturbation may not result from direct contacts. Electrostatic interactions can be long range and nonspecific and, therefore, may not result in specific chemical shift changes.

A detailed mechanism for UBL1 and UBC9 recognition is proposed. The conserved charged residues of UBC9, Arg13, Lys14, Arg17, and Lys18, are clustered together at the lower right corner of Fig. 5A. The conservative residues of UBL1,
Glu^{83}, Glu^{84}, Glu^{85}, and Asp^{86}, are clustered at the lower left corner in Fig. 5B. Both regions are adjacent to the surface hydrophobic side chains. These two regions may interact with each other in the complex. Conserved residues Glu^{12} of UBC9 and Lys^{26} of UBL1, located at the inner sides of Fig. 5, may form a salt bridge. Surface hydrophobic side chains are located near the outer upper sides on the binding surfaces shown in Fig. 5. These residues are in complementary positions and can readily form hydrophobic interactions. These hydrophobic interactions may contribute significantly to the specificity of the complex, because hydrophobic interactions are of short range. The outer lower regions, which have positive and negative potentials shown in Figs. 5, A and B, respectively, are composed of several polar residues that may form intermolecular hydrogen bonds. Because the highly conserved residues on the binding interfaces are charged and hydrophobic residues, the mechanism of recognition is likely through mainly hydrophobic and electrostatic interactions.

Our results explain the specificity of UBC9 and UBL1 interactions. It was reported previously that UBC9 conjugated with UBL1 but not ubiquitin (9). Although the sequences and three-dimensional structures are highly conserved between UBL1 and ubiquitin, the surface electrostatic potentials between the two proteins are very different. The surface of UBL1 that binds UBC9 has an overall negative electrostatic potential. However, this surface of ubiquitin is positively charged (24). Electrostatic repulsion will prevent UBC9 from interacting with ubiquitin well. Titration of unlabeled ubiquitin to 15N-labeled UBC9 until the concentration ratio of ubiquitin to UBC9 reached 6–7 did not show any specific chemical shift changes (data not shown), indicating that the two proteins do not show specific binding. The surface electrostatic potentials were also calculated for UBC4 and UBC7 (17, 18), which interact with ubiquitin. The regions that superimpose with the UBL1 binding site of UBC9 on the two E2 proteins have negative or neutral potentials, which is compatible with the electrostatic potential of the ubiquitin surface that superimposes with the UBC9 binding site on UBL1.

**Binding of the E1-UBL1 Conjugate to UBC9—UBL1 and the E1 homolog for this system may bind to different locations on UBC9. It is likely that UBL1 contributes significantly to the affinity with UBC9 in the E1-UBL1 conjugate. It has been shown that deletion of the N terminus of UBL1 affected its interaction with UBC9 (31). This seems unrelated to E1, because the conjugation of UBL1 to E1 and other proteins is through its C-terminal Gly residue. Because the UCB9 binding site on UBL1 is not close to the C terminus of UBL1, and the C-terminal tail is flexible, the UBL1-E1 conjugate may be flexible relative to each other. From the above-described model of UBC9-UBL1 interaction, the C terminus of UBL1 should extend toward the main β-sheet. Significant chemical shift changes and line broadening have been observed for residues in the β-sheet (Fig. 2A). In addition, many residues in the β-sheet show larger than average line broadening. Residues 91–95 of UBL1 have overlapping resonances, and therefore, it is not clear whether chemical shift changes and line broadening occurred to these residues. Simple modeling shows that although the binding site on UBC9 is not close to the active-site Cys^{83}, the C-terminal Gly of UBL1 can span the β-sheet to reach Cys^{83} of UBC9 for conjugation. A region of high sequence conservation have been observed on E2 structures (19, 17). This region is between the conjugation active-site Cys residue and the second helix and is on the opposite surface from the UBL1 binding site. This surface has been predicted to bind the E1-ubiquitin conjugate. It is possible that this surface binds E1 in the E1-UBL1 conjugate to place the C terminus of UBL1 at the conjugation active site of UBC9. Because the C-terminal residues of ubiquitin and UBL1 are flexible in solution and the binding site of UBL1 on UBC9 does not involve the C terminus, UBL1 and E1 may bind to different surfaces on the E2. E1 should bind to a location close to the active site, because it catalyzes the conjugation between E2 and ubiquitin.

**A General Feature of E2-Ubiquitin Interaction—**The functional importance of the N terminus of E2 has also been implicated from previous studies; however, the exact function is still unclear. Mutating residues 6–8 of Arabidopsis thaliana UBC1 significantly reduces the conjugation of ubiquitin to the E2 (32). As suggested from the crystal structure of Rad6 (19), the tripeptide structure is likely to destabilize the protein structure, because the side chains of both Arg^{5} and Arg^{6} are involved in intramolecular hydrogen bonding. Therefore, the effect of these mutations may not be because of the disruption of direct binding interactions. Deletion of residues 1–22 of Rad6 resulted in failure to form a complex with Rad18, which is a single-stranded DNA-binding protein possibly functioning to target Rad6 to sites of DNA damages (29). Because residues 8–22 have extensive contacts with the main structure of Rad6, it is not clear whether deletion of these residues disrupted the structural integrity of the protein.

The UBL1-UBC9 system shares many similarities to other E2-ubiquitin systems in the mechanism of conjugation with each other and with other proteins. The binding interfaces between UBL1 and UBC9 are located on the regions that are highly conserved in the three-dimensional structures of E2 and ubiquitin. Therefore, the mechanism of recognition between UBC9 and UBL1 may represent a general feature of E2-ubiquitin interactions. Interestingly, the surface of the E2s that conjugate with ubiquitin does not have highly conserved sequences. This may result in differences in their affinities for the E1-ubiquitin conjugate.

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