DNA Binding by Sgf11 Protein Affects Histone H2B Deubiquitination by Spt-Ada-Gcn5-Acetyltransferase (SAGA)*

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**Background:** Histone deubiquitination by SAGA is a core mechanism in gene expression regulation.

**Results:** The zinc finger domain of SAGA subunit Sgf11 interacts with nucleosomal DNA via a positively charged surface patch.

**Conclusion:** This interaction is required for optimal deubiquitination activity.

**Significance:** This work highlights the unexpected role of C2H2-like zinc fingers in SAGA complex.

The yeast Spt-Ada-Gcn5-acetyltransferase (SAGA) complex is a transcription coactivator that contains a histone H2B deubiquitination activity mediated by its Ubp8 subunit. Full enzymatic activity requires the formation of a quaternary complex, the deubiquitination module (DUBm) of SAGA, which is composed of Ubp8, Sus1, Sgf11, and Sgf73. The crystal structures of the DUBm have shed light on the structure/function relationship of this complex. Specifically, both Sgf11 and Sgf73 contain zinc finger domains (ZnF) that appear essential for the DUBm activity. Whereas Sgf73 N-terminal ZnF is important for DUBm stability, Sgf11 C-terminal ZnF appears to be involved in DUBm function. To further characterize the role of these two zinc fingers, we have solved their structure by NMR. We show that, contrary to the previously reported structures, Sgf73 ZnF adopts a C2H2 coordination with unusual tautomeric forms for the coordinating histidines. We further report that the Sgf11 ZnF, but not the Sgf73 ZnF, binds to nucleosomal DNA with a binding interface composed of arginine residues located within the ZnF α-helix. Mutational analyses both in vitro and in vivo provide evidence for the functional relevance of our structural observations. The combined interpretation of our results leads to an uncommon ZnF-DNA interaction between the SAGA DUBm and nucleosomes, thus providing further functional insights into SAGA’s epigenetic modulation of the chromatin structure.
The C2H2 Sgf11 ZnF shares common structural features with another zinc finger located within the N-terminal part of Sgf73 (residues 1–100), a region involved in stabilizing the two lobes of the DUBm with respect to each other. The remaining part of Sgf73 (residues 100–657) anchors the DUBm into SAGA and contains an atypical SCA7 zinc finger involved in the binding to nucleosomes (7). The two C2H2-like ZnF domains within the N-terminal parts of Sgf73 and Sgf11 are conserved among vertebrates (Fig. 1A) (8). As for its yeast counterpart, the N-terminal ZnF of ATXN7, the human ortholog of Sgf73, is crucial for the assembly of the human DUBm, whereas the ZnF of ATXN7L3, the human ortholog of Sgf11, is dispensable for the formation of a stable DUBm but is involved in the regulation of its enzymatic activity (8).

Contrasting with the functional importance of these ZnF domains, current structural data do not provide a consistent description of their zinc coordination. In particular, the last residue involved in the zinc coordination of Sgf73 was identified as either Cys-98 (5), Glu-95 (6), or His-97 (9), an ambiguity that possibly occurred due to the different truncation of Sgf73 ZnF in these various studies.

To solve this discrepancy and further investigate the function of these ZnF domains within the DUBm, we used NMR spectroscopy to determine the solution structures of both Sgf73 N-terminal and Sgf11 C-terminal ZnF domains using longer constructs. This work unambiguously identified the native metal coordination site for both zinc fingers. Furthermore, we showed that Sgf11 ZnF, but not Sgf73 ZnF, domain binds to the short double-stranded DNA fragments as well as to nucleosomal DNA with a binding interface composed of arginine residues located within the ZnFα-helix. Mutational analyses both in vitro and in vivo confirmed the functional relevance of the identified contact site and enabled us to propose a model for the interaction between SAGA DUBm and nucleosomes.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification for NMR**—Sgf11_K63-R99 and Sgf73_N59-S102 were PCR-amplified from plasmids containing the full-length proteins and cloned into the pGex4T1 plasmid (GE Healthcare). In the case of Sgf11_K63-R99, an N-terminal tobacco etch virus cleavage site was added. After DNA sequencing of the expression vectors, the recombinant ZnF domains were expressed in BL21 (DE3). Expression cells were grown to an A600 of 0.5–0.6 at 37 °C in a bacterial culture supplemented by 100 μg/ml ampicillin. Protein expression was then induced by addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside, and the culture was further incubated overnight at 15 °C. The cells were harvested by centrifugation (6000 × g for 10 min), resuspended in Lysis Buffer (20 mM Tris/HCl, pH 7.8, 200 mM NaCl, 2 mM DTT) supplemented by protease inhibitor (Complete, Roche Applied Science), lysozyme, DNase, and RNase A, and lysed by ultrasonication. The soluble fraction was cleared from the cell debris by centrifugation at 40,000 × g for 1 h and loaded onto a glutathione affinity chromatography column (GE Healthcare). After elution with Lysis Buffer containing 20 mM glutathione, the GST fusion protein was cleaved with tobacco etch virus protease in the case of Sgf11_K63-R99 or thrombin in the case of Sgf73_N59-S102 overnight at 15 °C. A second step of purification was performed by size exclusion chromatography (GE Healthcare) using a low salt NMR buffer (20 mM sodium phosphate, pH 7.0, 100 mM NaCl, 1 mM DTT). Fractions containing the target protein were pooled and concentrated by ultrafiltration using a 3-kDa cutoff membrane (Millipore). Expression of uniformly labeled 15N and 15N/13C proteins was carried out by growing BL21 strains in M9 minimal media supplemented by 15NH4Cl and/or uniformly 13C-labeled glucose as nitrogen and carbon source, respectively.

**NMR Measurements/Structure Calculation**—NMR spectra were acquired at 258 K in the case of Sgf11_K63-R99 or 298 K in case of Sgf73_N59-S102 using Bruker Avance 600 and 700 spectrometers equipped with cryoprobes. All experiments were performed with solutions of 1 mM protein in NMR buffer supplemented by 0.01% NaN3 in either 90% H2O, 10% D2O or 100% D2O. All spectra were processed using Topspin 2.1 (Bruker Biospin GmbH). Backbone resonance assignment was carried out based on the triple-resonance experiment sets HNCA/HN(CO)CA, CBCA(CO)NH/CBCANH, and HN(CA)CO/HNCO (10). Side-chain resonances were identified using HBHA(CO)NH (11) and HCCH-COSY/HCCH-TOCSY (12) experiments recorded in 90% H2O, 10% D2O or 100% D2O, respectively, supplemented by the analysis of NOE spectra. Inter-proton distance information for structural calculations was derived from 15N-NOESY-HSQC as well as 13C-NOESY-HMQC (13) spectra recorded in 90% H2O, 10% D2O or 100% D2O, respectively. All NOESY spectra were acquired using a mixing time of 200 ms. The Sparky software package (14) was used for spectral interpretation. Dihedral angle restraints were predicted using the chemical shift-based dihedral angle prediction software TALOS+ (15). Automated assignment of NOEY spectra was performed with ATNOS/CANDID protocol implemented in Unio10 (16, 17). The resulting assigned distance restraints were imported back to Sparky and manually refined by iterative assignment corrections and structural calculations using the RECOORD scripts (18) for CNS1.2 (19). For these calculations, the assigned distance restraints were grouped into three classes (I < 4 Å, II < 5 Å, and III < 7 Å) according to the volume of the NOE peak. A zinc coordination site with ideal tetrahedral geometry was built into the CNS starting conformation by patching the topology file with additional bond and angle constraints. Furthermore, hydrogen bond restraints were defined within the secondary structural elements as suggested by characteristic short range NOE contacts. The 20 lowest energy structures were subjected to a refinement in water using the RECOORD scripts. The quality of the final ensemble was analyzed with PSVS 1.3 (20). Long range 1H,15N HSQC spectra for the characterization of the tauromeric forms of histidine residues were recorded using an NH coupling constant of 30 Hz and centered at a 15N chemical shift of 200 ppm.

15N relaxation rate measurements were performed using series of 1H,15N HSQC-type spectra (21) recorded at a 600 MHz-proton frequency. Relaxation rates of the amide nitrogens were extracted from 11 spectra with delays of 10, 20, 40, 60, 80, 100, 150, 200, 500, 1000, and 2000 ms for T1 and 5, 10, 20, 30, 40, 50, 60, 80, 100, 150, and 200 ms for T2 by fitting the signal.
intensities to a mono-exponential decay as implemented in the Sparky software.

**NMR Measurements/CSP Experiments**—CSP were measured between a reference NMR sample containing 100 μM protein in the previously described NMR buffer and test samples additionally containing either mononucleosomes extracted from human HeLa cells or purified dsDNA oligomers of the palindromic sequences (5’ to 3’) GCTGTACAGC, CCTCTATAGAGG, and CCTCTGCAGAGG at 11 DNA/protein stoichiometric ratios ranging from 0.15:1 to 1:3.1. The fast exchange between free and bound forms of the protein allowed a quantitative interpretation of the 15N and 1H frequencies measured at 285 K as the DNA-bound fraction using the formulas described in Ref. 22. The presence of DNA oligonucleotide double strand base pairing was assessed by the observation of characteristic resonances from hydrogen-bonded guanine and thymine imino protons around 13 ppm.

The nucleosomal extract was prepared from human HeLa cells as described previously (23). The presence of intact histone and DNA moieties within this preparation was checked by SDS-PAGE and agarose gel electrophoresis, respectively. No tone and DNA moieties within this preparation was checked by cells as described previously (23). The presence of intact histone and thymine imino protons around 13 ppm.

TABLE 1
Ambiguous and unambiguous distance restraints used for HADDOCK calculations

| Atom 1                  | Atom 2                  | Distance (Å) |
|------------------------|------------------------|--------------|
| Cz of Arg-78, Arg-84, Arg-91, and Arg-95 in Sgf11 ZnF | Any DNA backbone phosphate | 1.8–4.0     |
| NH1 and NH2 of Arg-78, Arg-84, Arg-91, and Arg-95 in Sgf11 ZnF | Any DNA backbone phosphate linked oxygen | 1.8–3.0     |
| Nζ of Lys-123 in histone H2B | Any atom of the DUB catalytic triad (Cys-146, His-427, and Asn-443) | 1.8–4.0     |
| Nζ of Lys-123 in histone H2B | C-terminal C of ubiquitin | 1.8–7.0     |

**Generation of Yeast Strains**—Y2524 sfg11Δ yeast (5) were transformed with either an empty pRS314 vector or pRS314 vectors carrying wild type or mutant Sgf11 alleles. Selection and growth were performed on complete synthetic medium minus leucine, histidine, and tryptophan. All plasmids that were used are listed in Table 2.

**Analysis of Ubiquitinated H2B Levels in Vivo**—Each tested strain was grown to the midlog phase. A volume of 1 ml of each culture was resuspended in 100 μl of 200 mM NaOH for 5 min at room temperature (20 °C). Pellets of cells were then boiled for 5 min in 100 μl of Laemmli buffer. Extracts were diluted in IP100 buffer (25 mM Tris/HCl, pH 7.5, 5% glycerol, 5 mM MgCl2, 0.1% Nonidet P-40, 100 mM KCl) to decrease the concentration of SDS to 0.1% and were subjected to anti-FLAG immunoprecipitation. The purified material was recovered by peptide elution, and 50% of the eluates were analyzed by Western blot revealed with the FLAG antibody (M2 from Sigma).

**Immunoprecipitation of the SAGA Complex**—Yeast cells were harvested at the midlog phase, resuspended in lysis buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 8.7% glycerol, 0.1% Tween 20, and protease inhibitors (from Roche Applied Science)), and lysed with glass beads. 500 μg of protein extract were incubated on 50 μl of anti-HA-agarose beads (Sigma) overnight at 4 °C. Beads were washed five times with PBS containing 0.05% Tween 20. To recover the immunoprecipitated material, Laemmli buffer was added to the beads that were then incubated for 5 min at 95 °C. Western blots were revealed with anti-Taf12 and anti-Gcn5 antibodies (gift from T. Weil) as well as with an anti-HA antibody (3F10 from Roche Applied Science) to visualize HA-Sgf11.

**RESULTS**

**Solution Structures and Dynamics of Sgf11 and Sgf73 Zinc Finger Domains**—To identify unambiguously the atoms of the histidine residues that are involved in the zinc coordination, chemical shifts of imidazole Nδ and Ne atoms were measured using long range 1H-15N HSQC spectra (Fig. 1B) (28). A nitrogen chemical shift around 170–180 ppm unambiguously indicates a protonated position, whereas higher shifts are observed for nonprotonated and zinc-coordinated nitrogens. The frequencies of His-72 imidazole nitrogens in Sgf11 indicate a fully protonated form, which excludes this residue from the zinc coordination sphere. In contrast, His-88 was shown to bind zinc via the Ne atom of the imidazole ring. In Sgf73 ZnF, the
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coordination of zinc was shown to be mediated by the Ne of His-93 and the Nδ of His-97, an atypical situation for C2H2 zinc fingers.

Besides the ambiguously described zinc coordination within the N-terminal Sgf73 ZnF, the two x-ray structures of DUUb differ remarkably in the backbone trace of the Sgf11 ZnF (5, 6). To address these uncertainties, we solved the solution structure of these two ZnF domains comprising residues Lys-63–Arg-99 of Sgf11 and Asn-59–Ser-102 of Sgf73. Furthermore, insights on their dynamic properties were obtained from 1H-15N heteronuclear relaxation experiments. Structural calculations based on inter-proton distances and backbone dihedral angle restraints predicted from backbone atom frequencies led to well defined structures with a backbone root mean square deviation of 0.3 and 0.5 Å for Sgf11 and Sgf73, respectively (Table 3). Both ZnF domains adopt a C2H2-like fold comprising a pair of antiparallel β-strands and one α-helix (Fig. 2, A and B). The backbone superposition of the ZnF structures obtained from both DUUb x-ray structures (in blue and yellow) with the NMR solution structure (red) are shown in Fig. 2, C and D. In the case of Sgf73, the root mean square deviation of backbone atoms to the NMR structure are 0.63 Å (3M99 (5), blue) and 0.41 Å (3MHH (6), yellow), reflecting a high backbone similarity between all three structures except for the different zinc coordination patterns in the C-terminal part. For Sgf11 ZnF, the backbone disparity between the three structures is higher (0.82 Å to 3M99 and 0.76 Å to 3MHH). A notable difference concerns the orientation of Cys-76 in 3M99. Because Cys-76 forms a disulfide bridge with Cys-92 in this structure, we interpret this discrepancy as an artifact of the x-ray structure.

In addition, insights into the rotational diffusion and internal backbone motions of the Sgf11 and Sgf73 ZnF were provided by the analysis of T1/T2 relaxation rates as well as the heteronuclear nuclear Overhauser effect (NOE) (Fig. 3). Both ZnF display similar global dynamics, with an anisotropic tumbling of 3.9 and 2.4 ns along the major and the minor diffusion axis for Sgf11, as compared with 3.7 and 1.8 ns for Sgf73. However, the two ZnF display some differences in their dynamic behavior. The 1H-15N NOE profile of Sgf73 shows that residues Ala-62 to Gln-74, which were crucial for the expression of a stable and soluble domain, are disordered in solution. Furthermore, as indicated by consistently lower 1H-15N NOE values, the Sgf73 ZnF seems to be characterized by higher internal motions. The similar root mean square deviation values observed for both ZnF sets of structures most likely result from zinc coordination, as well as from hydrogen bond restraints. Another difference is the presence of conformational exchange observed for residue

TABLE 3

| NMR and structural refinement statistics for the ensemble of 20 conformers obtained from PSVS (20) |
|-----------------|-----------------|-----------------|-----------------|
|                 | Sgf11 ZnF       | Sgf73 ZnF       |
| NMR distance and dihedral constraints | | |
| Total NOE | 662 | 329 |
| Intra-residue | 118 | 76 |
| Inter-residue | 584 | 253 |
| Dihedral angle constraints (>1°) | 1.25 | 2.55 |
| Maximum dihedral angle violation | 2.7° | 4.2° |
| Maximum distance constraint violation | 0.26 Å | 0.34 Å |
| Deviations from idealized geometry | | |
| Bond lengths | 0.016 Å | 0.016 Å |
| Bond angles | 1.3° | 1.3° |
| Average pairwise root mean square deviation between 20 refined structures (Å) | | |
| Heavy | Residues | Residues |
| Backbone | 1.0 | 1.1 |
| MolProbity Clashscore (Raw score/Z-score) | 14.74/-1.00 | 7.82/0.18 |
| Ramachandran Plot (Procheck Analysis) | Residues | Residues |
| Most favored region | 94.0% | 97.3% |
| Additionally allowed | 6.0% | 2.7% |

Structural statistics

Violations (average of 20 conformers)

Distance constraints (>0.2 Å) | 0.4 | 1.35 |
Dihedral angle constraints (>1°) | 1.25 | 2.55 |
Maximum dihedral angle violation | 2.7° | 4.2° |
Maximum distance constraint violation | 0.26 Å | 0.34 Å |

Deviations from idealized geometry

Bond lengths | 0.016 Å | 0.016 Å |
Bond angles | 1.3° | 1.3° |
Average pairwise root mean square deviation between 20 refined structures (Å) | | |
Heavy | Residues | Residues |
Backbone | 1.0 | 1.1 |
MolProbity Clashscore (Raw score/Z-score) | 14.74/-1.00 | 7.82/0.18 |
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Average pairwise root mean square deviation between 20 refined structures (Å) | 0.26 Å | 0.34 Å |

Dihedral angle constraints (1°) | 1.25 | 2.55 |
Average pairwise root mean square deviation between 20 refined structures (Å) | 0.26 Å | 0.34 Å |
Cys-98 of Sgf73, which may be due to an exchange with a minor alternative conformation of the zinc coordination sphere. In addition to this conformational exchange, the presence of an extended disordered N-terminal tail may also contribute to the peculiar relaxation parameters measured for Sgf73 ZnF.

Sgf11 but Not Sgf73 ZnF Interacts with Double-stranded DNA Oligomers as Well as Nucleosomal DNA—The DNA binding properties of Sgf11 and Sgf73 ZnF domains were investigated using NMR titration experiments with either double-stranded nonspecific DNA oligonucleotides (dsDNA) with palindromic sequences (5'-GCTGTACAGC-3', 5'-CCTCTATAGAGG-3', and 5'-CCTCTGCAGAGG-3') or mononucleosomes extracted from human HeLa cells. The resulting CSP data consistently identified a DNA binding interface encompassing the C-terminal residues of Sgf11 ZnF, although no binding was observed for Sgf73 ZnF (Fig. 4). The frequency shift of 1H-15N correlation peaks observed upon successive addition of dsDNA was interpreted as a fast exchange between free and DNA-bound forms that allowed us to get an estimate for the equilibrium dissociation constant ($K_D$) (Table 4). The three tested DNA sequences gave similar dissociation constants around 200 µM. This rather weak binding is consistent with an expected lack of sequence specificity, although a significant increase of affinity was observed by replacing the AT-rich se-
sequence in the middle of the oligonucleotide by a GC pair (Table 4). By contrast, the addition of mononucleosomes into 15N-labeled Sgf11 ZnF preparations led to the appearance of separate sets of peaks for the DNA-bound and free form of Sgf11 ZnF, a phenomenon that is indicative of a slow exchange between free and bound forms in the NMR time scale that results from submicromolar affinity. Remarkably, both the nucleosomal DNA and dsDNA interact with the same region of the protein encompassing the C-terminal part of the helix (Arg-91–Arg-95) together with the side chain of Asn-75. This residue is located at the tip of the loop between both β-strands and is facing the C-terminal part of the α-helix. Altogether, these observations suggest a common mode of binding between either dsDNA or nucleosomal DNA. The tight binding observed in the latter case may be either due to additional interactions within the nucleosome or to a more favorable DNA conformation.

Functional Implication of Sgf11 DNA Interaction for DUBm Activity—Although residues Arg-91 to Arg-95 from the C-terminal part of the Sgf11 α-helix had their backbone nitrogen frequencies affected by the presence of DNA, their direct involvement in DNA interaction needed to be further investigated using a targeted mutagenesis approach followed by in vivo and in vitro analyses. In fact, the α-helix of Sgf11 ZnF is also involved in a protein-protein interaction with Ubp8, and some residues identified in our DNA binding CSP experiments belong to this interface, such as Leu-93, which is facing helix 12 of UBP8 (5).

Strikingly, two conserved arginine residues within the Sgf11 helix, namely Arg-84 and Arg-91, as well as the conserved Arg-78, which is located within the β-strand region, build up a pos-
itively charged cluster opposite the hydrophobic interface to Ubp8. The C-terminal arginine residues (Arg-95, Arg-98, and Arg-99) further extend this patch. Even though these three arginines are not conserved in Sgf11 homologs, additional basic residues are always found in the C-terminal parts of these ZnF (Fig. 1A). Because these positively charged residues could be potential interaction partners to the DNA backbone, we analyzed the effects of their substitution in vivo and in vitro.

We first analyzed whether the substitution of arginine residues within Sgf11 affects the deubiquitination activity of SAGA in vivo. As reported previously, we observed a strong accumulation of mono-ubiquitinated H2B after substituting Arg-84 by an alanine (Fig. 5C). We further show that the combined mutation of the arginine residues at the C-terminal end of Sgf11 ZnF (Arg-91, -95, -98, and -99) remarkably increases the level of ubiquitinated H2B in vivo. D, HA immunoprecipitations (IP) using protein extracts from the indicated strains were analyzed by Western blot (WB) with antibodies against Tafl2 and Gcn5, two subunits of SAGA, and with an anti-HA antibody to visualize the tagged versions of Sgf11. Note that the endogenous SGF11 gene is deleted in the two strains expressing an HA-tagged version of Sgf11.

We further analyzed the DNA affinity of two Sgf11 ZnF mutants in vitro by NMR. The substitution of Arg-84 by an alanine resulted in a strong reduction of affinity for DNA oligomers (Fig. 5B). The same behavior was also observed for the Sgf11-R95A-R98/R99 mutant lacking the last three C-terminal arginines (Fig. 5A).

Together, these results highlight the functional importance of a large positively charged patch formed by arginine residues at both ends of the ZnF helix for the interaction of Sgf11 with DNA, and they suggest a role of Sgf11 ZnF in the optimal positioning of the DUBm on the nucleosome particle.

**DISCUSSION**

Initially discovered as DNA binding domains, zinc fingers now emerge as multifunctional domains able to mediate a wide range of molecular interactions (29). Comparative analysis of the full proteome has shown that zinc fingers are present in a larger proportion in Eukaryota where they are involved in the regulation of numerous cellular processes, including gene expression (30). This is strikingly illustrated by the presence of seven zinc atoms within three out of four subunits constituting the active DUBm of the coactivator/epigenetic player SAGA. Most of the zinc coordination sites were found within the Ubp8 protease subunit (31), but two sites were also found in Sgf11 and

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**FIGURE 5. DNA binding through the ZnF of Sgf11 is required for SAGA DUB activity in vivo.** A and B, NMR titration experiments of Sgf11 ZnF mutants with the previously used 10-base DNA oligomer. The positions of mutations are highlighted in red in the ribbon representation of the Sgf11 ZnF structure. The substitution of α-helical arginine residues abrogates the ZnF-DNA interaction in vitro. C, measurement of ubiquitinated histone H2B levels in the indicated strains. Although the two C-terminal arginine residues (Arg-98 and Arg-99) seem to be dispensable for the deubiquitination activity, the substitution of α-helical arginine residues (Arg-84, Arg-91, and Arg-95) remarkably increases the level of ubiquitinated H2B in vivo. D, HA immunoprecipitations (IP) using protein extracts from the indicated strains were analyzed by Western blot (WB) with antibodies against Tafl2 and Gcn5, two subunits of SAGA, and with an anti-HA antibody to visualize the tagged versions of Sgf11. Note that the endogenous SGF11 gene is deleted in the two strains expressing an HA-tagged version of Sgf11.
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Sgf73, where they stabilize C2H2-like ZnF folds. The functional importance of these latter ZnF in the regulation of the protease activity early was recognized. Sgf73 ZnF is involved in protein-protein interaction with Ubp8, tethering the two domains of the protease into an active conformation (5, 6). The proximity of the Sgf11 ZnF with the catalytic site of Ubp8 suggests its direct involvement in protease activity and/or substrate recognition. Accordingly, mutations of Sgf11 ZnF residues that are located at the interface between Ubp8 and Sgf11 ZnF domain impaired Ubp8 activity (5). Moreover, the deletion of Sgf11 ZnF was shown to reduce the Ubp8 enzymatic activity by 2 orders of magnitude, an effect that was correlated to the destabilization of the catalytic site of Ubp8 (32). In addition, the presence of a conserved set of surface-located basic residues in the Sgf11 ZnF suggested that electrostatic interactions with the DNA backbone could be involved in substrate recognition (6).

The solution structures of both ZnF were found to be very similar to those found in complex with the remaining subunits of DUBm, highlighting the autonomous folding properties that are generally expected for zinc fingers. Some local differences were nevertheless found, mostly within the zinc coordination sphere, where protein truncations previously led to alternative coordination patterns. The few extended C-terminal residues that were used in our constructs of Sgf73 ZnF allowed the description of the native geometry of the zinc-binding site, which involves the residues Cys-78, Cys-81, His-93, and His-97. We observed that the two histidine residues assume different protonation states with His-93 being protonated on its Nδ, although His-97 exhibits a Ne protonation. This mixed protonation state is highly uncommon for C2H2-like ZnF domains, where an Ne zinc coordination is sterically preferred, although described for the E3 ubiquitin ligase RING domains (33). This particular zinc coordination geometry may therefore be related to the role of this ZnF in protein-protein interactions within the DUBm. The distinct heteronuclear relaxation parameters measured for Sgf73 ZnF highlight a specific dynamic behavior that may be relevant to promote crucial protein-protein contacts within DUB, a hypothesis that deserves further investigation. Furthermore, the observation of line broadening for the neighboring Cys-98 may indicate the presence of a low populated alternative zinc binding geometry, highlighting the promiscuous metal binding properties of zinc fingers. In case of the Sgf11 ZnF, the major difference between our NMR structure and the DUBm x-ray structure is found in the torsion angles of Cys-76 (Fig. 2C). Although the structure from Samara et al. (6) is in agreement with our results, Köhler et al. (5) describe an uncommon geometry for the zinc coordination site leading to the formation of a disulfide bridge between Cys-76 and Cys-92.

Aiming at further deciphering the functional role of Sgf73 and Sgf11 ZnF, we investigated their DNA binding properties in solution by NMR supplemented by in vitro and in vivo mutational analyses. NMR titration experiments performed with either double-stranded DNA oligomers or nucleosomal DNA demonstrated the ability of Sgf11 ZnF to bind DNA, whereas no such interaction was evidenced for Sgf73. These experiments led to the identification of a consistent protein-DNA interface involving the C-terminal part of the ZnF α-helix and Asn-75, which is located in the loop between the two β-strands. The apparent higher affinity of the Sgf11 ZnF for nucleosomal DNA, evidenced by the slow exchange regime observed between the bound and free forms of the protein signal, suggests the presence of additional interaction sites between the histone-bound DNA and the protein, an hypothesis that is consistent with a more extended set of residues displaying chemical shifts perturbations (Fig. 4B). Alternatively, this observation may be explained by a preference in binding a specific DNA conformation that may only be present in nucleosomes such as an increased curvature. Such a bent DNA conformation is characterized by a widening of the major groove at the outer side of the DNA bend, a specific feature that might be crucial to properly house the whole ZnF α-helix. This interpretation would be consistent with the observation that NMR signals of C-terminal residues within the Sgf11 ZnF are shifted to different positions when either mononucleosomes or dsDNA are added to protein samples.

Intriguingly, a minor low populated set of correlation peaks that shows up in the oligonucleotide titration experiments resemble the chemical shift pattern of the Sgf11 complex with mononucleosomes. This observation suggests that observed chemical shift differences between the ZnF complexes with dsDNA oligomers and mononucleosomes do not arise from the histone proteins but rather from different DNA conformations. It may further indicate that the dsDNA oligomers contain low populated conformations that are present when the DNA is wrapped around the histone core. The ability to observe nucleosome-bound Sgf11 ZnF signals, despite the high molecular weight of the complex, is intriguing and suggests that the protein still assumes a high degree of motional freedom within the complex. This observation may also arise from the formation of an intermediate DNA complex where the Sgf11 ZnF exhibits significant flexibility, similar to that observed in the nonspecific lac repressor-DNA complexes (34). In the case of Sgf11 ZnF, it is expected that the remaining parts of the DUB will drive the transition from this flexible intermediate to a more rigid assembly, adequately positioned to perform its enzymatic activity. Although the characterization of this putative intermediate complex requires further investigation, both our NMR interaction and mutagenesis experiments identified a limited set of Sgf11 residues that are commonly involved in these complexes. Furthermore, a soft-docking procedure conducted using the HADDOCK program and NMR-derived distance restraints led to a model (Fig. 6) that is compatible with the one proposed by Samara et al. (6).

Interaction between C2H2 ZnF-containing proteins and DNA has been extensively studied since the discovery of this type of domain in the late 1980s. Specific DNA sequence recognition is achieved by the repetition of ZnF domains that make up specific contacts between groups of three adjacent DNA bases and amino acids located in the ZnF α-helix at positions −1, 2, 3 and 6 relatively to the helix N terminus (35). Remarkably, none of these residues in Sgf11 ZnF display chemical shift perturbations of backbone amides upon DNA interactions, suggesting a different mode of binding that would rather involve the C-terminal residues of the ZnF helix (corresponding to residue numbers above 6). A corresponding binding geometry was indeed already described for two proteins con-
containing four ZnF, namely the Wilms tumor suppressor protein (36) and the Ying-Yang 1 protein (37). In both proteins, specific DNA interactions are mediated by ZnF2–4, whereas the N-terminal ZnF was found to bind the DNA major groove in a reverse orientation, making DNA interactions via the C-terminal part of its α-helix. In both proteins, this N-terminal ZnF does not participate in a specific DNA recognition and does not contribute significantly to the overall binding affinity. The similarity between these C2H2 ZnF and the DNA binding interface found for the CCHC ZnF of Sgf11 is striking and suggests a similar function in the DNA binding process. This role would be to ensure an optimal positioning of the protein within a DNA complex via nonspecific interactions.

For Sgf11 ZnF, such a role in nonspecific DNA binding would be in perfect agreement with our mutational analysis. Indeed, we demonstrated that a stepwise substitution of arginine residues by alanine within the ZnF helix abrogates the DNA interaction (Fig. 5). The substitution of Arg-84 disrupted the DNA interaction...
binding in vitro and the DUBm activity in vivo. The same effect could be observed for a combined deletion of the nonconserved C-terminal arginines (Arg-98 and Arg-99) and the substitution of Arg-95 and Arg-91 with alanine residues. These data suggest the importance of an extended positively charged patch rather than arginine residues at specific positions.

Our mutagenesis and chemical shift data are consistent and further extend the model that has been proposed by Samara et al. (6) for the interaction between the DUBm and nucleosomes (Fig. 6). In this model, the nonspecific interaction of arginine side chains located at both ends of the ZnF helix with the DNA phosphate backbone (Fig. 6A) contributes to the proper positioning of the Ubp8 active site (encompassing catalytic residues Cys-146, His-427, and Asn-443) in close vicinity to the ubiquitinated lysine residue in H2B (Fig. 6B). Our model further suggests a second anchoring point for the DUBm complex to the nucleosomal DNA, including residues Phe-365–Lys-375 from Ubp8 (Fig. 6C). Within this loop, Arg-374 could also interact with the DNA backbone. In higher vertebrates, a second arginine residue extends this positively charged patch (5), which supports a possible participation of this Ubp8 loop in anchoring the DUBm to DNA.

In conclusion, the DNA binding properties of the Sgf11 ZnF reported in our study suggest a dual role for this domain where nonspecific DNA interactions are coupled to the recognition of ubiquitinated H2B by the SAGA DUBm for optimal deubiquitination activity. Such nonspecific couplings between chromatin components and complexes involved in chromatin modification may have evolved in higher eukaryotes enabling more sophisticated reading of the chromatin state and consequently more efficient transcription regulation processes. The presence of an additional zinc finger in ATX7NL3, the human ortholog of Sgf11, provides an example of this evolution where zinc fingers seem to play a key role.

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