Subunit Rtt102 Controls the Conformation of the Arp7/9 Heterodimer and Its Interactions with Nucleotide and the Catalytic Subunit of SWI/SNF Remodelers*

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Chromatin-remodeling complexes are assembled around a catalytic subunit that contains a central ATPase domain and flanking sequences that recruit auxiliary subunits. The catalytic subunits of SWI/SNF remodelers recruit Arp7/9 through a helicase/SANT-associated (HSA) domain N-terminal to the ATPase domain. Arp7/9-containing remodelers also carry the auxiliary subunit Rtt102, but the role of this subunit is poorly understood. Here, we show that Rtt102 binds with nanomolar affinity to the Arp7/9 heterodimer and modulates its conformation and interactions with the ATPase subunit and nucleotide. When bound to Rtt102, Arp7/9 interacts with a shorter segment of the HSA domain. Structural analysis by small-angle x-ray scattering further shows that when bound to Rtt102, the complex of Arp7/9 with the catalytic subunit assumes a more stable compact conformation. We also found that Arp7, Arp9, and Arp7/9 interact very weakly with ATP, but Rtt102 promotes high-affinity ATP binding to a single site in the heterodimer. Collectively, the results establish a function for subunit Rtt102 as a stabilizing factor for the Arp7/9 heterodimer, enhancing its interaction with nucleotide and controlling the conformation of SWI/SNF remodelers in an Arp7/9-dependent manner.

In eukaryotic cells, DNA is wrapped around histone octamers (consisting of two copies of core histones H2A, H2B, H3, and H4) to form the nucleosome, which constitutes the basic building block of chromatin (1). Cells use chromatin-remodeling complexes (remodelers) to reversibly alter chromatin structure, including histone octamer sliding, DNA looping, and histone substitution (2, 10, 11). N- and C-terminal to the recA domains, the sequences of the catalytic subunits of the four families of remodelers diverge. These divergent regions frequently serve as a platform for recruitment of other remodeler subunits, generally known as auxiliary subunits. Auxiliary subunits serve diverse functions, including histone recognition and modulation of the ATPase activity of the catalytic subunit (2).

Two families of remodelers, SWI/SNF and INO80, contain among their auxiliary subunits actin and actin-related proteins (Arps)3 (2, 12–14). In the cytoplasm, actin and Arps are part of the actin cytoskeleton that plays crucial roles in cell motility, endocytosis, and the maintenance of cell morphology (15). In the nucleus, actin and Arps form part of large macromolecular complexes that are generally involved in the regulation of gene expression (16, 17). The specific role of actin and Arps within remodelers is not fully understood, yet emerging evidence suggests that they may regulate the ATPase activity of remodelers (12, 18–20) and may also play a role in nucleosome targeting (21–23). Actin and Arps are recruited to remodelers through interaction with an ~80-amino acid (aa) region N-terminal to the recA domains, known as the helicase/SANT-associated (HSA) domain (19). The HSA domain of the two INO80 family remodelers (INO80 and SWR1) recruits actin and Arp4, as well as Arp8 in the case of INO80 (12, 19, 24). In SWI/SNF family remodelers (RSC and SWI/SNF), the HSA domain binds Arp7

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3 The abbreviations used are: Arp, actin-related protein; aa, amino acid; HSA, helicase/SANT-associated; RSC, remodel the structure of chromatin; mHSA, minimal HSA; pHSA, post-HSA; MBP, maltose-binding protein; TEV, tobacco etch virus; ITC, isothermal titration calorimetry; e-ATP, 1,6-etheno-ATP; SAXS, small-angle x-ray scattering; P1, protrusion-1.
and Arp9 (19). Arp7/9-containing remodelers also carry subunit Rtt102 (25), which co-purifies with the Arps (19). In a recent structure of a quaternary complex of Arp7/9 with Rtt102 and the HSA domain of Snf2 (the catalytic subunit of SWI/SNF), Rtt102 is mostly disordered but seems to interact only with the Arps and not the HSA domain (26). However, it is unknown whether Rtt102 alters the way in which Arp7/9 interacts with the catalytic subunit and whether it affects the conformation of the catalytic subunit. Here, we present a quantitative and structural analysis of the interaction of Rtt102 with Arp7/9, revealing profound effects of this subunit on the conformation and nucleotide affinity of Arp7/9, which in turn affect the conformation of SWI/SNF family remodelers.

**EXPERIMENTAL PROCEDURES**

**Proteins**—All of the protein constructs (see Fig. 1A for a detailed definition of ATPase constructs) for this study were amplified by PCR from a *Saccharomyces cerevisiae* genomic DNA library (Novagen). The UniProt accession codes for the proteins expressed are as follows: Arp7, Q12406; Arp9, Q05123; Rtt102, P53330; Sth1, P32597; and Snf2, P22082. Table 1 lists the proteins expressed, expression plasmids, cloning sites, and purification tags. For Arp9, the 5′-intron was removed. Most proteins were expressed in *Escherichia coli* BL21 (DE3)-RIL cells (Stratagene) carrying additional plasmids for coexpression of two chaperones (cells were a generous gift of Dr. T. Sitar, Max Planck Institute). Proteins fused to maltose-binding protein (MBP) were expressed in *Escherichia coli* BL21(DE3) cells (New England Biolabs). Transformed cells were grown in Terrific Broth supplemented with 0.4% glycerol and antibiotics at 37°C to A = 2. Expression was induced with 1 mM isopropyl β-D-thiogalactopyranoside, and cells were grown for 14 h at 18°C. Ternary and quaternary complexes were co-purified from mixed pellets (Table 1). For protein species bearing a His₆ affinity tag, cells were resuspended in lysis buffer (20 mM Tris (pH 8.0), 300 mM NaCl, 5% glycerol, and 4 mM benzamidine), lysed using a Microfluidizer apparatus (Microfluidics), and clarified by centrifugation. The lysates were purified on a nickel-nitrilotriacetic acid affinity column (Qiagen) and washed extensively with lysis buffer supplemented with 30 mM imidazole. Proteins were eluted with 250 mM imidazole, and the His tag was cleaved using tobacco etch virus (TEV) or thrombin protease at 4°C in sample buffer (20 mM HEPES (pH 7.8), 200 mM NaCl, 5% glycerol, and 1 mM DTT). Remaining uncleaved protein was removed by repurification through the nickel-nitrilotriacetic acid affinity column. The proteins were concentrated and additionally purified through a Superdex-200 gel filtration column (GE Healthcare) equilibrated with sample buffer. For protein species bearing an MBP affinity tag, cells were resuspended, lysed, and clarified as described above, followed by purification on an amylose affinity column (New England Biolabs). Proteins were eluted with 10 mM maltose, concentrated, and purified by gel filtration chromatography as described above. For the Rtt102-Arp7/9-HSA complex, the MBP tag on Sth1 was cleaved with TEV protease prior to gel filtration.

**Circular Dichroism**—The far-UV spectra of Arp7, Arp9, Arp7/9, and rabbit skeletal actin were recorded at 25°C using an Aviv Biomedical model 410 circular dichroism spectrometer. The protein concentration was 5 μM in all experiments. The buffer conditions were 10 mM phosphate (pH 8.0), 150 mM KF, 5% glycerol, and 0.5 mM DTT for the Arps and 5 mM phosphate (pH 8.5), 0.2 mM CaCl₂, 0.2 mM ATP, and 0.5 mM DTT for actin.

**Isothermal Titration Calorimetry (ITC)**—ITC experiments were performed using a MicroCal VP-ITC calorimeter at 20°C, with a duration of 20 s for each injection and an interval of 5 min between injections. Prior to each experiment, proteins were

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**Table 1**

| Proteins and complexes | Vectors (restriction sites) | Purification tag (N- or C-terminal) | Cleavage site |
|------------------------|-----------------------------|------------------------------------|--------------|
| **Monomeric species**  |                             |                                    |              |
| Arp7                   | pRSF-Duet1 (NcoI, Sall)     | His₆ (C)                           | Thrombin     |
| Arp9                   | pRSF-Duet1 (NcoI, Sall)     | His₆ (C)                           | Thrombin     |
| Rtt102                 | pRSF-Duet1 (BamHI, Sall)    | His₆ (N)                           | TEV          |
| Sth1(301–1097)         | pRSF-Duet1 (BamHI, Sall)    | His₆ (N)                           | TEV          |
| MBP-Sth1(301–364)      | pMAL-c2X (BamHI, Sall)      | MBP (N)                            | TEV          |
| MBP-Sth1(301–387)      | pMAL-c2X (BamHI, Sall)      | MBP (N)                            | TEV          |
| MBP-Snfl(578–642)      | pMAL-c2X (BamHI, Sall)      | MBP (N)                            | TEV          |
| MBP-Snfl(578–665)      | pMAL-c2X (BamHI, Sall)      | MBP (N)                            | TEV          |

| **Dimeric species**    |                             |                                    |              |
| Arp7/9                 | pRSF-Duet1 (Ndel, Xhol)/(NcoI, Sall) | Arp7-His₆ (C) | Thrombin |

| **Trimeric species (mixed pellets of separately expressed proteins and binary complexes)** |
|---------------------------------------------|---------------------------------------------|-----------------|
| Arp7/9-Sth1(301–387)                       | pRSF-Duet1 (Ndel, Xhol)/(NcoI, Sall), pMAL-c2X(BamHI, Sall) | Stth1-MBP (N) | TEV |
| Rtt102-Arp7/9                             | pRSF-Duet1 (BamHI, Sall), pRSF-Duet1 (Ndel,Xhol)/(NcoI, Sall) | Rtt102-His₆ (N) | TEV |
| Arp7/9-Sth1(301–1097)                     | pRSF-Duet1 (Ndel, Xhol)/(NcoI, Sall), pRSF-Duet1 (BamHI, Sall) | Stth1-His₆ (N) | TEV |

| **Quaternary species (mixed pellets of separately expressed proteins and binary complexes)** |
|-------------------------------------------------|---------------------------------|-----------------|
| Rtt102-Arp7/9-Sth1(301–387)                    | pET-Duet1 (Ndel, Xhol), pRSF-Duet1 (Ndel, Xhol)/(NcoI, Sall), pMAL-c2X (BamHI, Sall) | Sth1-MBP (N) | TEV |
| Rtt102-Arp7/9-Sth1(301–1097)                   | pET-Duet1 (Ndel, Xhol), pRSF-Duet1 (Ndel, Xhol)/(NcoI, Sall), pRSF-Duet1 (BamHI, Sall) | Sth1-His₆ (N) | TEV |
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extensively dialyzed in sample buffer. For each experiment (as indicated in the figures), the concentration of the protein being titrated was 50–80 μM, and that of the binding partner in the cell was ~10-fold lower. For ATP-binding experiments, 2.5 mM MgCl₂ was added to the sample buffer, and the concentration of ATP was 50 μM (for the complexes) or 500 μM (for the individual Arps). The heats of binding were corrected for the small exothermic heats of injection resulting from injecting proteins into buffer. Data were analyzed using the Origin program (OriginLab Corp.).

Nucleotide Exchange Assay—Rtt102-Arp7/9 at 50 μM was extensively dialyzed in sample buffer supplemented with 2.5 mM MgCl₂ and 50 μM 1,6-etheno-ATP (ε-ATP; Invitrogen). The exchange reaction was initiated by adding 0.6 mM ATP or ADP and was monitored by measuring the decrease in ε-ATP fluorescence at 410 nm (with excitation at 355 nm) with a Cary Eclipse fluorescence spectrophotometer (Varian) for 5 min. The exchange reactions were corrected for dilution resulting from the addition of ATP or ADP to buffer and normalized to the fluorescence recorded before the addition of nucleotide.

Small-angle X-ray Scattering (SAXS)—Data were collected at Cornell High Energy Synchrotron Source (CHESS) beamline F2 at 20 °C using 30-s exposures. Prior to each experiment, samples were extensively dialyzed in sample buffer. To minimize radiation damage, samples were continuously oscillated inside the cuvette during data collection. To control for potential aggregation effects, samples were analyzed at three different concentrations (1.0, 0.5, and 0.25 dilutions of the maximal concentration). The scattering profile and Guinier plot of each sample were determined as the average of 10 independent scans subtracted from that of 10 sample scans with the program BioXTAS RAW (27, 28). Distance distribution functions were calculated using the program GNOM (29). Ab initio envelopes were generated by calculating 20 independent models with the program DAMMIF (30), which were then averaged with the program DAMAVER (31) to produce a single average envelope.

RESULTS

Bacterially Expressed Arp7, Arp9, and Arp7/9 Are Properly Folded—The experiments performed here required large amounts of pure proteins. Although E. coli-expressed actin is not properly folded, some nuclear Arps, including the Arp7/9 heterodimer, have been successfully expressed in this system (21, 22, 32, 33). However, Arp7 and Arp9 have not been expressed separately. Here, we established the large-scale bacterial expression of Arp7 and Arp9, both individually and as a heterodimer (Fig. 1B). To verify that the proteins were properly folded, we first analyzed their secondary structure content by CD using muscle-purified actin as a control. The CD spectra of Arp7, Arp9, Arp7/9, and actin were all very similar (Fig. 1C) and displayed the characteristic profile of a mixed α/β-fold, as expected for this family of proteins (26, 34). We also collected SAXS data from Arp7, Arp9, and Arp7/9 and calculated their Kratky plots (Fig. 1D). These plots were all bell-shaped, which is characteristic of folded globular proteins (35). We thus concluded that bacterially expressed Arp7, Arp9, and Arp7/9 are properly folded.

The Full HSA Domain Is Required for Binding of Arp7/9 in the Absence of Rtt102—The minimal HSA domain (i.e. the minimal fragment required for binding of the Arps) was previously defined as comprising Sth1 Val-301–His-359 (corresponding to Snf2 Ala-579–His-637) (19), yet secondary structure prediction suggested that this region formed part of a single long helix extending to Sth1 Leu-381 (Snf2 Leu-659). While the minimal HSA domain is poorly conserved, extending the domain to the end of this predicted helix would include 22 additional amino acids that are more highly conserved (Fig. 1A). To test whether this 22-aa region is important for Arp7/9 binding, we made two constructs of both Sth1 and Snf2, termed mHSA (for minimal HSA) and HSA, which either included or lacked this region, respectively (Fig. 1A). To increase their solubility, these constructs were expressed as fusion proteins with MBP. We used ITC to analyze the interaction of the Arp7/9 heterodimer with the mHSA and HSA fragments of Sth1 and Snf2 (Fig. 1, E and F). ITC experiments for this study were repeated three times (the values given in the figures and the thermodynamic parameters listed in Table 2 correspond to the average of these three measurements). We found that the HSA construct of Sth1 bound to Arp7/9 with ~25-fold higher affinity than the mHSA construct ($K_D = 25 \text{ versus } 620 \text{ nm}$). The difference in affinity for the equivalent constructs of Snf2 was even greater, ~130-fold ($K_D = 6.4 \text{ versus } 820 \text{ nm}$). This was a surprising finding in light of the recent crystal structure of the quaternary complex of Rtt102-Arp7/9 with the HSA fragment of Snf2, in which the Arps do not appear to make direct contacts with the C-terminal 22 amino acids of the HSA helix (26). We also found that heterodimerization is required for binding of the Arps to the HSA domain, because neither Arp7 nor Arp9 individually bound to the HSA fragment of Snf2 (Fig. 1F).

Rtt102-Arp7/9 Interacts with a Shorter Segment of the HSA Domain Compared with Arp7/9 Alone—Arp7/9-containing remodelers also carry subunit Rtt102 (25), which biochemical (19) and structural (26) studies suggest interacts with the Arps but not with the HSA domain. We asked whether binding of Rtt102 to the Arps affects their interactions with the HSA domain. To test this possibility, we first analyzed the interaction of the Arps with Rtt102 by native gel electrophoresis (Fig. 2A). Alone, neither Rtt102 nor Arp7 entered the gel, whereas Arp9 and the Arp7/9 heterodimer entered the gel but ran as two separate species. Because these proteins ran as single bands on regular gel electrophoresis (Fig. 1B), the presence of multiple bands in the native gel is consistent with structural heterogeneity or lack of stability. In contrast, the ternary complex Rtt102-Arp7/9 ran as a single band in the native gel (Fig. 2A), suggesting that Rtt102 stabilizes the Arp7/9 heterodimer. Next, we analyzed the interaction of Rtt102 with the Arps using ITC (Fig. 2B). Rtt102 did not bind Arp7 and bound Arp9 with relatively weak affinity (780 nM). In contrast, Rtt102 bound with high affinity to the Arp7/9 heterodimer ($K_D = 2.7 \text{ nm}$). These results are consistent with the crystal structure (26) in which Rtt102 interacts mostly with Arp9 but also contacts Arp7, forming a bridge across the Arp7-Arp9 interface.

Next, we analyzed the interaction of co-purified Rtt102-Arp7/9 with the mHSA and HSA fragments of Sth1 and Snf2 (Fig. 2, C and D). To our surprise, Rtt102 enhanced the affinity...
of Arp7/9 for the mHSA fragment of Sth1 by 5-fold ($K_D = 120$ nM versus 620 nM) and that of Snf2 by 37-fold ($K_D = 22$ nM versus 820 nM). However, in stark contrast to what was observed in the absence of Rtt102 (Fig. 1, E and F), the Rtt102-Arp7/9 ternary complex bound to the HSA fragment of Sth1 with only ~2.7-fold greater affinity compared with the mHSA fragment ($K_D =$

FIGURE 1. The full HSA domain is required for binding of Arp7/9 in the absence of Rtt102. A, domain architecture of Sth1 (catalytic subunit of the RSC complex) and definition of Sth1 and Snf2 (catalytic subunit of the SWI/SNF complex) constructs made for this study. recA-N and recA-C, N- and C-terminal recA domains of the ATPase, respectively; B, bromodomain. Also shown is a sequence alignment of the HSA–pHSA regions of yeast Sth1 (UniProt P32597), yeast Snf2 (UniProt P22082), Drosophila BRM (UniProt P25439), and human BRG1 (UniProt P51532). The blue background highlights sequence conservation, with dark blue indicating the highest conservation. Secondary structure prediction with the program Jpred 3 (50) identified two long helices within this region (indicated above). The helix of the HSA domain was confirmed by the crystal structure of the Rtt102-Arp7/9-HSA complex (26). B, SDS-PAGE (12%) analysis of the following proteins and complexes: Rtt102, actin, Arp7, Arp9, Arp7/9, and Rtt102-Arp7/9. C, comparison of the CD spectra of actin, Arp7, Arp9, and Arp7/9 (color-coded). D, comparison of the Kratky plots of Arp7, Arp9, and Arp7/9 (color-coded) collected at ~3 mg/mL. E and F, heats of injection and binding isotherms for the ITC titrations of the mHSA and HSA domains of Sth1 (E) and Snf2 (F) into Arp7/9. The average binding affinities and stoichiometries of the fits from three independent experiments are indicated (refer to Table 2 for the average thermodynamic parameters of the titrations).
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TABLE 2
Thermodynamic parameters of the fits of ITC titrations

| Proteins | $K_D$ ($\mu M$) | $\Delta H$ (kcal/mol) | $\Delta S$ (kcal/mol) | $n$ |
|----------|----------------|-----------------------|-----------------------|-----|
| Arp7 Snf(578–665) (HSA) | No binding |  |  |  |
| Arp9 Snf(578–665) (HSA) | No binding |  |  |  |
| Arp7/9 |  |  |  |  |
| Sth(301–364) (mHSA) | 0.62 ± 0.03 | -31.6 ± 0.2 | 23.1 ± 0.1 | 1.29 ± 0.2 |
| Sth(301–387) (HSA) | 0.025 ± 0.010 | -51.6 ± 1.4 | 41.3 ± 1.5 | 0.86 ± 0.07 |
| Snf(578–842) (mHSA) | 0.82 ± 0.16 | -27.1 ± 0.6 | 18.9 ± 0.6 | 1.34 ± 0.14 |
| Snf(578–665) (HSA) | 0.0064 ± 0.0008 | -45.9 ± 4.4 | 34.8 ± 4.4 | 1.09 ± 0.20 |
| Rtt102 |  |  |  |  |
| Arp7 | No binding |  |  |  |
| Arp9 | 0.78 | -59.7 | 51.5 | 0.815 |
| Arp7/9 | 0.0027 ± 0.0008 | -67.0 ± 4.0 | 55.4 ± 4.1 | 1.09 ± 0.02 |
| Rtt102-Arp7/9 |  |  |  |  |
| Sth(301–364) (mHSA) | 0.12 ± 0.02 | -37.0 ± 4.8 | 27.5 ± 4.7 | 1.13 ± 0.11 |
| Sth(301–387) (HSA) | 0.045 ± 0.007 | -55.3 ± 0.5 | 45.4 ± 0.6 | 0.93 ± 0.03 |
| Sth(301–1097) (HSArecA) | 0.075 ± 0.005 | -39.4 ± 0.9 | 29.8 ± 0.9 | 0.78 ± 0.13 |
| Sth(388–1097) (pHSArecA) | No binding |  |  |  |
| Snf(578–842) (mHSA) | 0.022 ± 0.001 | -25.5 ± 0.5 | 15.2 ± 0.5 | 1.09 ± 0.02 |
| Snf(578–665) (HSA) | 0.026 ± 0.011 | -39.0 ± 3.7 | 28.8 ± 3.4 | 0.99 ± 0.07 |
| ATP |  |  |  |  |
| Arp7 | Weak binding (data not fitted) |  |  |  |
| Arp9 | Weak binding (data not fitted) |  |  |  |
| Arp7/9 | 4.27 | -17.9 ± 1.6 | -8.8 ± 1.7 | 0.70 ± 0.05 |
| Rtt102-Arp7/9 | 0.20 ± 0.08 | -17.9 ± 1.6 | -8.8 ± 1.7 | 0.70 ± 0.05 |
| Rtt102-Arp7/9-HSA | 0.094 ± 0.02 | -12.9 ± 0.4 | 3.5 ± 0.4 | 0.59 ± 0.04 |

45 versus 120 nM) and with nearly identical affinities compared to the HSA and mHSA fragments of Snf2 ($K_D = 26$ versus 22 nM). It thus appears that the 22-aa C-terminal portion of the HSA domain, which adds dramatically to the affinity of the Arp7/9 heterodimer, contributes little to the interaction when Rtt102 is bound to the Arps (Fig. 2, C and D, and Table 2). These data indicate that in the absence of Rtt102, Arp7/9 adopts a more “relaxed” or extended conformation, making contacts with the whole HSA domain, whereas binding of Rtt102 stabilizes a more compact conformation of the Arps, in which they interact mostly with the mHSA region, as observed in the crystal structure (26).

The Binding Site for Rtt102-Arp7/9 Is Fully Contained within the HSA Domain of the ATPase—It is unclear whether the poorly conserved HSA domain accounts for all of the interactions linking Rtt102-Arp7/9 to SWI/SNF remodelers, as concluded by a previous study using pulldown experiments with yeast extracts and co-purification of coexpressed proteins (19). One important observation of that study was that the catalytic subunits of actin/Arp-containing remodelers also share two additional highly conserved regions, the post-HSA (pHSA) domain and protrusion-1 (P1). The pHSA domain is an ∼40-aa region found between the HSA domain and the first recA domain, whereas P1 is an ∼50-aa region between the two recA domains (Fig. 1A). Notably, dual deletion of Arp7/9 in yeast is lethal, but mutations in the pHSA or P1 region of Sth1 suppress lethality (19), raising the possibility that the Arps could interact with these two regions. To test this possibility, we measured binding of Rtt102-Arp7/9 to Sth1 constructs HSAreA and pHSArecA, extending from the HSA or pHSA domain to the end of the recA domains, respectively (Fig. 1A). Rtt102-Arp7/9 bound to HSAreA with a $K_D$ of 75 nM, i.e., with similar affinity and thermodynamic parameters as for the isolated HSA domain ($K_D = 45$ nM), but did not bind to pHSArecA, which lacks the HSA domain (Fig. 2, E and F). These results suggest that the ATPase domain and specifically the conserved pHSA and P1 regions do not directly contribute to the affinity of Sth1 for Rtt102-Arp7/9.

Binding of Rtt102 Results in a More Compact and Stable Conformation of the Arp7/9-ATPase Complex—Given its effect on the conformation of the Arp7/9 heterodimer, we asked whether Rtt102 also affects the conformation of the ATPase subunit. To test this possibility, we used SAXS to examine the overall conformation and stability of the Arp7/9-HSAreA complex of RSC in the presence and absence of Rtt102. As a reference, we also analyzed the Rtt102-Arp7/9-HSA complex. Scattering data collected at several concentrations of the three samples showed a linear dependence of the scattering intensity on protein concentration (Fig. 3, A–C), which is indicative of lack of aggregation. However, more careful examination using Guinier analysis (36) revealed linear plots for the two complexes containing Rtt102, consistent with monodisperse species, whereas that of Arp7/9-HSAreA was slightly nonlinear, which probably reflects weak aggregation (Fig. 3D). The scattering data were used to calculate pair distance distribution functions, $P(r)$, of the complexes. The $P(r)$ function provides a direct measure of the mass distribution within the complexes (35). In the absence of Rtt102, the $P(r)$ function of Arp7/9-HSAreA reached a maximum at ∼55 Å and decreased gradually to a maximal distance, $D_{max}$ of ∼285 Å (Fig. 3E). Although this behavior might be indicative of an extended conformation, the nonlinearity of the Guinier plot for this complex precludes an accurate analysis of particle dimensions. Conversely, in the presence of Rtt102, the $P(r)$ function showed a maximum at ∼70 Å and $D_{max}$ of ∼185...
Å, giving rise to a more bell-shaped function that is indicative of a more globular and compact complex. Consistent with these observations, the Kratky plots of complexes containing Rtt102 were bell-shaped, as expected for properly folded complexes, but that of Arp7/9-HSArecA had a less globular profile (Fig. 3F). Consistent with its stabilizing effect, the presence of Rtt102 reduced the radius of gyration, $R_g$, a measure of the mass distribution around the center of mass (35), of Arp7/9-HSArecA from 86.0 to 61.5 Å (Fig. 3E). Although this difference could be due in part to weak aggregation of the Arp7/9-HSArecA complex, it appears reasonable to conclude that Rtt102 stabilizes this complex and may also induce a conformational change, consistent with the binding of Rtt102-Arp7/9 to a shorter segment of the HSA domain than Arp7/9 alone (Figs. 1 and 2).

The $P(r)$ function for the highly globular complex of Rtt102-Arp7/9-HSA had a symmetric appearance, with maximum at $\sim 60 \text{ Å}$, $D_{max} \sim 130 \text{ Å}$, and $R_g = 46.9 \text{ Å}$ (Fig. 3E). This radius of gyration is larger than that calculated from the coordinates of

**FIGURE 2.** Rtt102-Arp7/9 interacts with a shorter segment of the HSA domain compared with Arp7/9 alone. A, native PAGE analysis (6%, pH 8.8) of recombinant proteins and complexes (as indicated). B, heats of injection and binding isotherms for the ITC titrations of Rtt102 into Arp7 (green), Arp9, and Arp7/9. C and D, titrations of the mHSA and HSA domains of Sth1 (C) and Snf2 (D) into Rtt102-Arp7/9. E, titration of Rtt102-Arp7/9 into the HSArecA and pHSArecA (green) fragments of Sth1. The average binding affinities and stoichiometries of the fits from three independent experiments are indicated. F, thermodynamic parameters of the interactions of Rtt102-Arp7/9 with the mHSA, HSA, and HSArecA fragments of Sth1. Error bars represent the S.D. from three independent experiments. Refer to Table 2 for further details.
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The crystal structure (37), 32.3 Å, which is likely due to the absence of several disordered regions in the structure. Specifically, the complex analyzed by SAXS contains 270 residues more than revealed by the crystal structure, including 103 residues of Rtt102 and several loops of the Arps that are disordered in the structure (26).

Using the data collected at the highest sample concentration (~10 mg/ml), which produced stronger scattering, we calculated low-resolution envelopes of the two Rtt102-containing complexes by averaging 20 ab initio structures (Fig. 3G). The envelope of Rtt102-Arp7/9-HSA could be superimposed only onto one side of that of Rtt102-Arp7/9-HSAreCA. The large portion of the Rtt102-Arp7/9-HSAreCA envelope unaccounted for by this superimposition is predicted to belong to the recA domains. The size and shape of the envelopes are generally consistent with information available about the structures of the individual components, i.e. the crystal structures of Rtt102-Arp7/9-HSA (26) and the related ATPase domain of Rad54 (9) (Fig. 3H). A definitive fit of these structures into the envelopes is not proposed because of ambiguities about the overall orientation of the domains and the fact that the structures do not account for large segments of the proteins in the envelopes. As stated above, in the absence of Rtt102, the Arp7/9-HSAreCA ternary complex is weakly aggregated and heterogeneous; thus, a reliable ab initio envelope of this complex could not be calculated (35).

Interaction with Rtt102 and the HSA Domain Increases the Affinity of Arp7/9 for ATP—Cytoskeletal actin and Arps bind and hydrolyze ATP, which regulates their transition in and out of filamentous networks (15, 34). Because nuclear Arps share the actin fold and the nucleotide-binding pocket is particularly well conserved, they could be expected to also bind and hydrolyze ATP. Arp4 and Arp8 have been shown to co-purify with ATP, although like actin (15, 34), they had no detectable ATPase activity in isolation (21, 22, 32). Surprisingly, however, genetic data show that mutating the nucleotide-binding pocket of Arp7 or Arp9 has no effect on yeast survival, which led to the suggestion that they do not bind ATP (13). The structure of Rtt102-Arp7/9-HSA was also determined in the absence of nucleotide (26). Although these results could be interpreted as evidence that Arp7/9 does not bind ATP, this has not been formally investigated. Here, we quantitatively analyzed the binding of ATP to Arp7, Arp9, and their heterodimer in the presence and absence of Rtt102. Individually, Arp7 and Arp9 appeared to interact with ATP, albeit very weakly (Fig. 4A). The titration of an ~70-fold molar excess of ATP produced a more pronounced isotherm for Arp7 than for Arp9 (estimated \( K_D = 12 \) and 44 µM, respectively), indicating that ATP has a slight preference for Arp7. Because, these affinities appeared too low for this family of proteins (21), we decided to test whether the affinity for the nucleotide increased for the heterodimer. However, ATP also bound with relatively weak affinity to Arp7/9 (\( K_D = 4.3 \) µM), and the stoichiometry converged to ~1.8 (Fig. 4B), possibly reflecting the presence of two weak binding sites within the heterodimer. Remarkably, when we titrated ATP into Rtt102-Arp7/9, the affinity increased by >20-fold (\( K_D = 200 \) nM) (Fig. 4C), which, together with other data presented here, suggests a stabilizing effect of Rtt102 on Arp7/9. In this case, the stoichiometry of the interaction converged to 0.7, indicating that the nucleotide binds to a single site within the Rtt102-Arp7/9 complex. Because Arp7 individually displayed higher affinity for ATP compared with Arp9 (Fig. 4A), it is possible that this site resides within Arp7. This is also consistent with the crystal structure of Rtt102-Arp7/9-HSA (26), in which the ATP-binding cleft of Arp7 displays a relatively closed conformation resembling that of actin, whereas Arp9 displays a wide-open cleft, which is also less conserved at the sequence level (Fig. 4D).

As an alternative method to explore the binding of ATP to Rtt102-Arp7/9, we monitored the exchange of ε-ATP, an ATP analog whose fluorescence increases when bound to protein. In these experiments, ε-ATP prebound to Rtt102-Arp7/9 was competed out with ATP (or ADP), which produced a fluorescence decrease. The addition of a 12-fold molar excess of ATP led to a sharp decrease in fluorescence, whereas ADP was 5-fold less efficient in displacing ε-ATP (Fig. 4E). This confirms that Rtt102-Arp7/9 binds nucleotide, with a clear preference for ATP versus ADP.

In light of these results, we also tested whether the HSA domain alters the affinity of the Arps for nucleotide. Indeed, the HSA domain promoted tighter binding of ATP to the Arps (Fig. 4F), albeit to a lesser extent than Rtt102 (\( K_D = 827 \) versus 200 nM). Interestingly, the tightest binding of ATP (\( K_D = 94 \) nM) was observed for the Rtt102-Arp7/9-HSA quaternary complex (Fig. 4G), and as determined above, the nucleotide appeared to bind to a single site in this complex.

DISCUSSION

Chromatin remodelers are large complexes, containing up to 17 protein subunits organized around a central ATPase subunit. The role of many of these subunits remains unknown, but it is becoming increasingly clear that they participate in modulation of the catalytic activity of the ATPase and targeting of remodelers to nucleosomes (2, 38). For example, in human and yeast SWI/SNF remodelers, the auxiliary subunits Snf5 and Swi3 ensure nucleosome occupancy at promoters (39) and tar-

FIGURE 3. Binding of Rtt102 stabilizes a compact conformation of the Arp7/9-ATPase complex. A–C, x-ray scattering data collected at three different concentrations from the RSC subcomplexes Rtt102-Arp7/9-HSA, Rtt102-Arp7/9-HSAreCA, and Arp7/9-HSAreCA, respectively. Scattering intensities are plotted versus momentum transfer, and the dependence of I on sample concentration is shown in the insets. D, Guinier plots of the three complexes calculated from the scattering data at the highest sample concentration (color-coded). Data within the Guinier region (\( R_g < 1.3 \)) for the two Rtt102-containing complexes were linearly fitted (black lines). E, calculated P(\( R \)) functions of the three complexes (color-coded). Dashed lines indicate the calculated \( R_g \) values for each complex. F, Kratky plots of the three complexes (color-coded). G, comparison of the envelopes of Rtt102-Arp7/9-HSA (blue) and Rtt102-Arp7/9-HSAreCA (red) resulting from the averaging of 20 ab initio models. The average normalized spatial discrepancies of the ab initio models are 1.06 ± 0.06 and 0.99 ± 0.07 for the Rtt102-Arp7/9-HSA and Rtt102-Arp7/9-HSAreCA complexes, respectively. H, superimposition of the envelopes and comparison with the most closely related crystal structures (shown to scale) available for the individual components, i.e. Rtt102-Arp7/9-HSA (Protein Data Bank code 4I6M) (26) and the Rad54 ATPase domain (code 1Z6A) (9).
FIGURE 4. Interaction with Rtt102 and the HSA domain increases the affinity of Arp7/9 for ATP. A–C, ITC titrations of ATP into buffer (black), Arp7 (red), and Arp9 (blue) (A), Arp7/9 (B), and Rtt102-Arp7/9 (C). The average binding affinities and stoichiometries of the fits are indicated (refer to Table 2 for further details). D, comparison of the nucleotide-binding clefts of actin, Arp7, and Arp9. The structure of actin shown corresponds to that of its complex with gelsolin (Protein Data Bank code 3CI5) (51). The Arp7 and Arp9 structures are from the complex of Rtt102-Arp7/9-HSA (code 4I6M) (26) that lacks nucleotide. For this figure, the three proteins were superimposed to show the same orientation, and the nucleotide displayed in Arp7 and Arp9 is modeled according to its position in the actin structure. E, exchange of ε-ATP bound to Rtt102-Arp7/9 by the addition of ATP (orange) or ADP (green). Data were buffer-subtracted and normalized as described under “Experimental Procedures.” F and G, ITC titrations of ATP into Arp7/9-HSA and Rtt102-Arp7/9-HSA, respectively. The average binding affinities and stoichiometries of the fits are indicated (see also Table 2). H, diagram illustrating the incremental increase in nucleotide-binding affinity upon assembly of the Rtt102-Arp7/9-HSA quaternary complex (color-coded as in Figs. 1A and 3H).
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function will depend on interaction with both Rtt102 and the catalytic subunit.

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