Chromatin-remodelling complexes of the SWI/SNF family function in the formation of nucleosome-depleted, transcriptionally active promoter regions (NDRs). In the yeast *Saccharomyces cerevisiae*, the essential SWI/SNF complex RSC contains 16 subunits, including the ATP-dependent DNA translocase Sth1. RSC removes nucleosomes from promoter regions and positions the specialized +1 and −1 nucleosomes that flank NDRs. Here we present the cryo-electron microscopy structure of RSC in complex with a nucleosome substrate. The structure reveals that RSC forms five protein modules and suggests key features of the remodelling mechanism. The body module serves as a scaffold for the four flexible modules that we call DNA-interacting, ATPase, arm and actin-related protein (ARP) modules. The DNA-interacting module binds extra-nucleosomal DNA and is involved in the recognition of promoter DNA elements that influence RSC functionality. The ATPase and arm modules sandwich the nucleosome disc with the Snf2 ATP-coupling (SnAC) domain and the finger helix, respectively. The translocase motor of the ATPase module engages with the edge of the nucleosome at superhelical location +2. The mobile ARP module may modulate translocase–nucleosome interactions to regulate RSC activity. The RSC–nucleosome structure provides a basis for understanding NDR formation and the structure and function of human SWI/SNF complexes that are frequently mutated in cancer.

We isolated endogenous RSC from *S. cerevisiae* by affinity purification of the tagged subunit Rsc2 (Extended Data Fig. 1a). A RSC–nucleosome complex was assembled in the presence of the ATPase transition-state analogue ADP-BeF₃ (Extended Data Fig. 1b). Cryo-electron microscopy (cryo-EM) analysis resulted in a reconstruction at approximately 15 Å resolution that revealed the nucleosome, four turns of DNA exiting from one side of the nucleosome and five RSC modules that we refer to as ATPase, ARP, body, arm and DNA-interaction module (DIM) (Fig. 1a, Extended Data Fig. 2). Focused 3D classification enabled modelling of the nucleosome, the associated ATPase and the ARP module. We also analysed the free RSC complex using cryo-EM. This resulted in structures of the body and arm modules at resolutions of 3.6 Å and 3.8 Å, respectively, which we could fit into the density of the RSC–nucleosome complex (Extended Data Figs. 3, 4a–c). This led to a structural model of the RSC–nucleosome complex that lacks only the DIM and agrees with lysine–lysine cross-linking information (Extended Data Fig. 1c, d, Supplementary Tables 1, 2).

**RSC structure**

The structure reveals the intricate architecture of RSC (Fig. 1, Supplementary Video 1). The body module contains subunits Rsc4, Rsc6, Rsc8, Rsc9, Rsc30 and Htl1 and the N-terminal region of Sth1 (Extended Data Fig. 5a, Extended Data Table 1). The ARP module is flexibly tethered to the body and comprises the helicase-SANT associated (HSA) region of Sth1, the actin-related proteins Arp7 and Arp9, and subunit Rtt102. The C-terminal region of Sth1 extends from the HSA region and forms the ATPase module (Extended Data Fig. 6a). The arm module protrudes from the body and contains subunit Sth1 and parts of Rsc8, Npl6 and Rsc9 (Extended Data Fig. 5a). The arm and body modules are tightly connected by two copies of Rsc8 that adopt different structures (Extended Data Fig. 6b). The N-terminal Swi3p, Rsc8p and Moira (SWIRM) domains of the two Rsc8 copies reside in the arm, whereas the SANT domains and one of the ZZ zinc-finger domains and the long C-terminal helices reside in the body.

RSC also contains six domains that are implicated in interactions with histone tails. The N-terminal bromodomain in Rsc58 locates to the surface of the body (Extended Data Fig. 6c). The five other domains are mobile and include a bromodomain in Sth1, two bromodomains in Rsc2, a bromo-adjacent homology (BAH) domain in Rsc2 that binds histone H3, and a tandem bromodomain in Rsc4 that interacts with acetylated H3 tails, in particular acetylated lysine K14. RSC also contains five putative DNA-binding domains, four of which are mobile. These include the zinc-finger domains in subunits Rsc3 and Rsc30, an RFX domain in subunit Rsc9 and a ZZ zinc-finger domain in one of the two Rsc8 subunits. In summary, RSC consists of five modules and nine flexibly connected domains, of which some are involved in substrate selection.
A TPase and translocation

RSC engages in multivalent interactions with its substrate, contacting both DNA and histones (Fig. 1). The ATPase and arm modules interact with the nucleosome, whereas the DIM engages with DNA exiting from the nucleosome. The ATPase module binds the edge of the nucleosome, contacting both DNA gyres in a conformation poised for translocation activity (Extended Data Fig. 6d). The two lobes of the ATPase motor domain contact one gyre at superhelical location (SHL) +2 and adopt the same relative orientation as in the structure of the related Snf2 ATPase bound to a nucleosome\textsuperscript{17}. The N-terminal ATPase lobe 1 also binds the second DNA gyre around SHL −6 (Fig. 2), towards which the N-terminal tail of histone H3 protrudes (Extended Data Fig. 4d). Considering the known directionality of the translocase\textsuperscript{18}, this led to a model in which the RSC ATPase motor pumps DNA towards the nucleosome dyad and along the octamer surface in the exit direction, which corresponds to the upstream direction of transcription, thus liberating more promoter DNA.

The ARP module couples RSC ATPase activity to DNA translocation and regulates the remodelling activity\textsuperscript{5,19}. Our results suggest that this regulation involves changes in the position of the mobile ARP module that influence the conformation and mobility of the ATPase lobe 1 and its interactions with both DNA gyres (Fig. 2). These changes are probably transmitted through the hinge region between the HSA region and lobe 1 that includes the post-HSA region of Sth1. Mutations of the post-HSA region increase ATPase activity and DNA translocation, suggesting that the hinge acts as a throttle for the ATPase\textsuperscript{4,5,20}. The ARP module adopts a defined position in the RSC–nucleosome complex, but it is mobile in the free RSC structure. We propose that the position of the mobile ARP module influences the motility of the bilobal ATPase motor and thereby controls translocation activity of RSC (Fig. 2).
Nucleosome and DNA recognition

The arm module and its finger helix may also contribute to substrate selection. RSC preferentially recognizes nucleosomes that contain the histone variant H2A.Z. Such nucleosomes show a more extended acidic patch and may have increased affinity for the basic RSC finger. The arm module may also contact the unique C-terminal tail of H2A.Z that protrudes near Sfh1 (Extended Data Fig. 4d). The arm–octamer interaction may also explain why ubiquitination of histone H2B counteracts RSC function. The ubiquitin moiety is linked to H2B residue K123 (K120 in human) and, although flexible, can adopt a position that sterically interferes with the arm–octamer interaction (Extended Data Fig. 4f).

RSC binds both the nucleosome and the DNA that exits from the nucleosome (Extended Data Fig. 7a). The DIM contacting DNA around 20–40 base pairs (bp) upstream of SHL –7 of the nucleosome. This is in agreement with RSC protecting around 50 bp of extra-nucleosomal DNA from nuclease digestion. The DIM–DNA contact also explains how RSC recognizes specific DNA elements that are enriched in promoters. According to our cryo-EM and cross-linking results, the DIM contains parts of RSC subunits Rsc2, Rsc3 and Rsc30 (Extended Data Fig. 1d). Rsc3 and Rsc30 recognize a CGCG DNA element located upstream of the transcription start site, probably via their zinc cluster domains.

NDR formation

The results further elucidate the formation of NDRs. In S. cerevisiae, the DNA linker length between two nucleosomes is only about 23 bp on average. Steric considerations predict that RSC can bind to DNA as observed in our structure only at sites in chromatin where the length of the DNA linking two nucleosomes is at least 40–50 bp (Extended Data Fig. 7b). This may explain why RSC is targeted preferentially to promoter regions, which are intrinsically depleted of nucleosomes, and also why two closely spaced nucleosomes impair binding of the RSC-related complex SWI/SNF.

Nucleosome and DNA recognition

The arm module and its finger helix may also contribute to substrate selection. RSC preferentially recognizes nucleosomes that contain the histone variant H2A.Z. Such nucleosomes show a more extended acidic patch and may have increased affinity for the basic RSC finger. The arm module may also contact the unique C-terminal tail of H2A.Z that protrudes near Sfh1 (Extended Data Fig. 4d). The arm–octamer interaction may also explain why ubiquitination of histone H2B counteracts RSC function. The ubiquitin moiety is linked to H2B residue K123 (K120 in human) and, although flexible, can adopt a position that sterically interferes with the arm–octamer interaction (Extended Data Fig. 4f).

RSC binds both the nucleosome and the DNA that exits from the nucleosome (Extended Data Fig. 7a). The DIM contacting DNA around 20–40 base pairs (bp) upstream of SHL –7 of the nucleosome. This is in agreement with RSC protecting around 50 bp of extra-nucleosomal DNA from nuclease digestion. The DIM–DNA contact also explains how RSC recognizes specific DNA elements that are enriched in promoters. According to our cryo-EM and cross-linking results, the DIM contains parts of RSC subunits Rsc2, Rsc3 and Rsc30 (Extended Data Fig. 1d). Rsc3 and Rsc30 recognize a CGCG DNA element located upstream of the transcription start site, probably via their zinc cluster domains.

NDR formation

The results further elucidate the formation of NDRs. In S. cerevisiae, the DNA linker length between two nucleosomes is only about 23 bp on average. Steric considerations predict that RSC can bind to DNA as observed in our structure only at sites in chromatin where the length of the DNA linking two nucleosomes is at least 40–50 bp (Extended Data Fig. 7b). This may explain why RSC is targeted preferentially to promoter regions, which are intrinsically depleted of nucleosomes, and also why two closely spaced nucleosomes impair binding of the RSC-related complex SWI/SNF.

Nucleosome and DNA recognition

The arm module and its finger helix may also contribute to substrate selection. RSC preferentially recognizes nucleosomes that contain the histone variant H2A.Z. Such nucleosomes show a more extended acidic patch and may have increased affinity for the basic RSC finger. The arm module may also contact the unique C-terminal tail of H2A.Z that protrudes near Sfh1 (Extended Data Fig. 4d). The arm–octamer interaction may also explain why ubiquitination of histone H2B counteracts RSC function. The ubiquitin moiety is linked to H2B residue K123 (K120 in human) and, although flexible, can adopt a position that sterically interferes with the arm–octamer interaction (Extended Data Fig. 4f).

RSC binds both the nucleosome and the DNA that exits from the nucleosome (Extended Data Fig. 7a). The DIM contacting DNA around 20–40 base pairs (bp) upstream of SHL –7 of the nucleosome. This is in agreement with RSC protecting around 50 bp of extra-nucleosomal DNA from nuclease digestion. The DIM–DNA contact also explains how RSC recognizes specific DNA elements that are enriched in promoters. According to our cryo-EM and cross-linking results, the DIM contains parts of RSC subunits Rsc2, Rsc3 and Rsc30 (Extended Data Fig. 1d). Rsc3 and Rsc30 recognize a CGCG DNA element located upstream of the transcription start site, probably via their zinc cluster domains.
and Arp9, and putative counterparts of subunits Rsc2, Rsc4 and Rsc9 (Extended Data Table 1). Projection of the homologous regions onto the RSC structure reveals that the ATPase, ARM and arm modules and a large part of the body are conserved in PBAF (Extended Data Fig. 5b). Although PBAF apparently lacks the DIM, it contains 12 putative DNA-binding domains that may mediate DNA recognition (Extended Data Table 1). Owing to these similarities, the RSC structure can be used to locate protein sites in PBAF that are mutated in human cancers (Extended Data Fig. 5b). Most mapped mutations are predicted to destabilize protein folds. However, mutations are particularly enriched within the ATPase, ARM and arm modules that surround and contact the nucleosome, suggesting that they cause functional defects. Sequence and domain conservation further suggest that the architecture of the RSC-related yeast SWI/SNF complex and its human counterpart BAF are also similar (Extended Data Fig. 8, Extended Data Table 1). Comparison of our structure with nucleosome complex structures of other families of chromatin remodelers reveals marked differences (Extended Data Fig. 5c).

While this manuscript was under review, three related structures were reported: two structures of RSC–nucleosome complexes29,30 and a structure of the yeast SWI/SNF–nucleosome complex31. Together, these structures provide a basis for understanding the multiple functions of SWI/SNF family remodelers in chromatin biology.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2088-0.

1. Lorch, Y. & Kornberg, R. D. Chromatin-remodeling for transcription. Q. Rev. Biophys. 50, e5 (2017).
2. Clapier, C. R., Iwasa, J., Cairns, B. R. & Peterson, C. L. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. Nat. Rev. Mol. Cell Biol. 18, 407–422 (2017).
3. Cairns, B. R. et al. RSC, an essential, abundant chromatin-remodeling complex. Cell 87, 1249–1260 (1996).
4. Saha, A., Wittmeyer, J. & Cairns, B. R. Chromatin remodeling by RSC involves ATP-dependent DNA translocation. Genes Dev. 16, 2120–2134 (2002).
5. Clapier, C. R. et al. Regulation of DNA translocation efficiency within the chromatin remodeler RSC/Sth1 potentiates nucleosome sliding and ejection. Mol. Cell 62, 453–461 (2016).
6. Krietenstein, N. et al. Genomic nucleosome organization reconstituted with pure proteins. Cell 167, 709–712 (2016).
7. Klein-Brill, A., Joseph-Strauss, D., Appleborm, A. & Friedman, N. Dynamics of chromatin and transcription during transient depletion of the RSC chromatin remodeling complex. Cell Rep. 26, 279–292 (2019).
8. Kubik, S. et al. Nucleosome stability distinguishes two different promoter types at all protein-coding genes in yeast. Mol. Cell 60, 422–434 (2015).
9. Ramachandran, S., Zentner, G. E. & Henikoff, S. Asymmetric nucleosomes flank promoters in the budding yeast genome. Genome Res. 25, 381–390 (2015).
10. Badis, G. et al. A library of yeast transcription factor motifs reveals a widespread function for Rsc2 in targeting nucleosome exclusion at promoters. Mol. Cell 32, 878–887 (2008).
11. Lorch, Y., Maier-Davis, B. & Kornberg, R. D. Role of DNA sequence in chromatin remodeling and the formation of nucleosome-free regions. Genes Dev. 28, 2492–2497 (2014).
12. Kubik, S. et al. Sequence-directed action of RSC remodeler and general regulatory factors modulates +1 nucleosome position to facilitate transcription. Mol. Cell 71, 89–102 (2018).
13. Palice, J. L. & Kadoch, C. Composition and function of mammalian SWI/SNF chromatin remodeling complexes in human disease. Cold Spring Harb. Symp. Quant. Biol. 81, 53–60 (2016).
14. Chambers, A. L., Pearl, L. H., Oliver, A. W. & Downs, J. A. The BH4 domain of Rsc2 is a histone H3 binding domain. Nucleic Acids Res. 41, 9168–9182 (2013).
15. Kasten, M. et al. Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 lys14. EMBO J. 23, 1348–1359 (2004).
16. VanDemark, A. P. et al. Autoregulation of the Rsc4 tandem bromodomain by Gcn5 acetylation. Mol. Cell 27, 817–828 (2007).
17. Li, M. et al. Mechanism of DNA translocation underlying chromatin remodelling by Snf2. Nature 567, 409–413 (2019).
18. Saha, A., Wittmeyer, J. & Cairns, B. R. Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. Nat. Struct. Mol. Biol. 12, 747–755 (2005).
19. Schubert, H. L. et al. Structure of an actin-related subcomplex of the SWI/SNF chromatin remodeler. Proc. Natl Acad. Sci. USA 110, 3345–3350 (2013).
20. Szerlong, H. et al. The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases. Nat. Struct. Mol. Biol. 15, 469–476 (2008).
21. Sen, P. et al. The Snf4 domain of SWI/SNF is a histone anchor required for remodeling. Mol. Cell. Biol. 33, 360–370 (2013).
22. Lorch, Y., Maier-Davis, B. & Kornberg, R. D. Histone acetylation inhibits RSC and stabilizes the +1 nucleosome. Mol. Cell. 72, 594–600 (2018).
23. Cakiroglu, A. et al. Genome-wide reconstitution of chromatin transactions reveals that RSC preferentially disrupts H2A.Z-containing nucleosomes. Genome Res. 29, 988–998 (2019).
24. Suto, R. K., Clarkson, M. J., Tremethick, D. J. & Luger, K. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. Nat. Struct. Mol. Biol. 33, 838–843 (2019).
25. Materne, P. et al. Histone H2B ubiquitylation represses gametogenesis by opposing RSC-dependent chromatin remodeling at the ste11 master regulator locus. eLife 8, e43500 (2019).
26. Brahma, S. & Henikoff, S. RSC-associated subnucleosomes define MNase-sensitive promoters in yeast. Mol. Cell 73, 238–249 (2019).
27. Brogaard, K., Xi, L., Wang, J. P. & Widom, J. A map of nucleosome positions in yeast at base-pair resolution. Nature 486, 496–501 (2012).
28. Dechassa, M. L. et al. SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. Mol. Cell 38, 590–602 (2010).
29. Patel, A. B. et al. Architecture of the chromatin remodeler RSC and insights into its nucleosome engagement. eLife 8, e45449 (2019).
30. Ye, Y. et al. Structure of the RSC complex bound to the nucleosome. Science 366, 638–643 (2019).
31. Han, Y., Reyes, A. A., Malik, S. & He, Y. Cryo-electron microscopy structure of a nucleosome-bound SWI/SNF chromatin remodeling complex. Preprint at https://www.biorxiv.org/content/10.1101/805184v1 (2019).

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2020.
Preparation of RSC complex

The yeast *Saccharomyces cerevisiae* contains two isoforms of RSC that comprise either the subunit Rsc1 or its homologue Rsc2 (Extended Data Table 1). We isolated the Rsc2-containing 16-subunit isoform.

Preparation of nucleosome substrates

*Xenopus laevis* histones were expressed and purified as described, with several modifications. All purification procedures were performed at 4 °C unless stated otherwise. Six-hundred grams of yeast granules were lysed by cryo-milling (Splex Freezer/Mill 6875D) and stored at −80 °C. Yeast powder was thawed at 30 °C, diluted with 100 ml 1× lysis buffer and cleared by centrifugation (25,200 g). The supernatant was centrifuged at 4 °C unless stated otherwise. Six-hundred grams of yeast granules were lysed by cryo-milling (Splex Freezer/Mill 6875D) and stored at −80 °C. Yeast powder was thawed at 30 °C, diluted with 100 ml 1× lysis buffer and cleared by centrifugation (25,200 g). The supernatant was centrifuged at 4 °C unless stated otherwise.

Cryo-EM analysis of RSC–nucleosome complex

RSC–nucleosome complex was adsorbed to a thin carbon film before plunge freezing as described, with minor modifications. A small, thin (~3.1 nm) carbon film was floated from the mica sheet onto a 50 μl drop of sample and incubated for 2–3 min. The carbon film was recovered and immediately used for cryo-EM sample preparation. Typical yields were 0.2–0.3 mg from 600 g yeast granules.
and comparison with low-pass-filtered high-resolution structures (for example, the nucleosome) because the software-generated values were overestimated for the density in this resolution range. Further processing of the particles revealed great flexibility and dynamics which could not be resolved by focused 3D classifications and refinements.

The particles corresponding to the RSC–nucleosome map were re-extracted centred on the nucleosome with a box mainly including the nucleosome and the Sth1 ATPase module. Global 3D classification resulted in a good class that revealed the Sth1 ATPase subunit bound to the nucleosome. Focused 3D refinement and postprocessing with automatic $B$-factor determination in RELION excluding the ATPase density provided a nucleosome map (map 2) at a resolution of 3.6 Å (gold-standard Fourier shell correlation (FSC) 0.143 criterion) and a $B$-factor of −155 Å$^2$ (Extended Data Fig. 2). Improvement of the ATPase density turned out to be very difficult and showed its highly dynamic nature in this sample. A strategy of focused 3D classification without image alignment on the ATPase part, followed by a global 3D refinement and additional focused 3D classification on the ATPase-nucleosome density led to the best results. A focused 3D classification and postprocessing with automatic $B$-factor determination in RELION resulted in an overall resolution of the ATPase–nucleosome map (map 3) of 4.3 Å (FSC 0.143 criterion) and $B$-factor of −186 Å$^2$ (Extended Data Fig. 2). Final focused maps were combined using the Frankenmap tool distributed with Warp (map 7) (Extended Data Fig. 2). Masks encompassing the regions of interest were created with UCSF Chimera and RELION.

**Cryo-EM analysis of the free RSC complex**

Freshly purified RSC complex was mixed with ADP-BeF$_3$ to a final concentration of 1 mM and incubated for 15 min on ice. BS3 (bis(sulfosuccinimidyl(suberate)) cross-linker (Thermo Fischer Scientific) was added to a final concentration of 1 mM, incubated on ice for 30 min before quenching with Tris-HCl, pH 7.5, and ammonium bicarbonate at a final concentration of 100 mM and 20 mM, respectively. After size exclusion chromatography using a Sepharose 6 Increase 3.2/300 column (GE Healthcare) pre-equilibrated in gel filtration buffer (50 mM HEPS pH 7.6, 150 mM potassium acetate, 4 mM MgCl$_2$, 1 mM DTT), peak fractions were immediately applied to cryo-EM grids. Four microlitres of sample were applied to glow-discharged (Pelco easiGlow) R2/2 gold grids (Quantifoil). Grids were blotted and vitrified as described above.

Cryo-EM data was collected as described above, with small modifications. The energy filter slit width was set to 20 eV. Micrographs for the two 0°-tilt datasets were collected at a dose rate of 4.88 e$^-$/Å$^2$/s for 8 s resulting in a total dose of 39 e$^-$/Å$^2$ and a dose rate of 5.02 e$^-$/Å$^2$/s over 9 s resulting in a total dose of 45.2 e$^-$/Å$^2$, respectively, and fractionated over 40 frames. The third, 25° tilted dataset was acquired in 44 frames at a dose rate of 4.99 e$^-$/Å$^2$/s for 11 s resulting in a total dose of 54.9 e$^-$/Å$^2$.

Pre-processing and particle picking were carried out as described above and resulted in 363,824 particles from the first dataset (3,284 micrographs), 170,028 particles from the second dataset (1,216 micrographs) and 475,168 particles from the tilted dataset (3,158 micrographs). Particles were processed with global 3D classifications using RELION 3 and a negative stain reconstruction of the RSC complex as a first reference to obtain an improved initial reference. All 1,009,020 particles were newly extracted and bad particles were sorted out in multiple rounds of global 3D classifications in combination with global 3D refinements. The best resulting class was refined with a mask excluding the flexible DIM. Particles corresponding to this reconstruction were subjected to CTF refinement and Bayesian polishing in RELION. Using focused 3D refinements, the maps for the arm module (map 4), and body1 (map 5) and body2 (map 6) submodules were further improved. Post-processing with automatic $B$-factor determination in RELION resulted in overall resolutions of 3.8 Å, 3.6 Å and 3.6 Å, respectively, and $B$-factors of −156 Å$^2$, −106 Å$^2$ and −100 Å$^2$, respectively (Extended Data Fig. 3). Final focused maps were combined with Warp (map 8).

Post-processing with automatic $B$-factor determination in RELION resulted in overall resolutions of 3.6 Å and a $B$-factor of −106 Å$^2$ (Extended Data Fig. 3).

**Structural modelling**

Previous electron microscopy studies revealed the overall shape of RSC, but did not allow any molecular modelling of the yeast Snf2 bound to the nucleosome. The final map 7 was created with the local resolution tool from RELION and a $B$-factor of −150 Å$^2$. The structure of the yeast Snf2 bound to the nucleosome in the ADP-BeF$_3$ state (Protein Data Bank (PDB) code 5Z3U) was used as basis for modelling. Published data together with the close homology between Sth1 and Snf2 (Extended Data Fig. 8) suggest that Sth1 also binds at CTH+2. The modeller and the nucleosome part were fitted separately. The X. laevis histones and Widom 601 sequence for PDB 5Z3U were the same as used in our study. The nucleosome structure was rigid-body fitted into our cryo-EM map in UCSF Chimera and the entry side DNA and histone tails trimmed according to the density in COOT. DNA sequence information was not resolved and assumed to match the 5Z3U template. Due to lower resolution, amino acid side chains of residues 15–22 of H4 (chain B) were stubbed in COOT. The nucleosome structure was flexibly fitted using Nanmodator and real space refined in PHENIX with secondary structure restraints (including base pairing and base stacking restraints).

High conservation of amino acids between Sth1 and Snf2 ATPase domains (Extended Data Fig. 8) allowed for generation of a Sth1 homology model with Rosetta. The homology model was trimmed according to the density in COOT, the brace-II helix was removed, and amino acid side chains were stubbed owing to the lower resolution of the map area before rigid-body docking using UCSF Chimera. Additional real space refinement with secondary structure restraints (including base pairing and base stacking restraints) was performed in PHENIX. Density for the Sth1 SnAC domain was clearly observed at lower contour level. However, the local resolution was not sufficient to build a model for the SnAC structure or to further investigate its interactions with the nucleosome. The overhanging exit side DNA was modelled by generating a bend DNA following the density in map 1 using 3D-DART. The DNA duplex was connected to the nucleosomal Widom 601 DNA and geometry optimized with base pairing and base stacking restraints in PHENIX.

Map 1 allowed for the rigid-body docking of the crystal structure of the ARP module bound to the Snf2 HSA region (PDB code 4I6M) using UCSF Chimera. The amino acid residues of the Snf2 HSA helix were mutated to the ones from Sth1 according to sequence alignment (Extended Data Fig. 8) starting at the C terminus, ignoring gaps, and stubbed. The model for the Sth1 HSA helix is thus an extrapolation based on the strong $\alpha$-helical secondary structure prediction and the register might differ slightly. We next aligned the focused refined maps 4–6 and the combined map 8 to map 1 and used them for model building of the RSC core. SWISS-MODEL was used to generate homology models for the Rsc58 N-terminal bromodomain (PDB code 3LJW), the Rsc6 SWIB domain (PDB code IUIHR), the Rsc8 SWIRM (PDB code 2F3Q), SANT (PDB code 2YSU) and ZZ zinc-finger domains (PDB code 1ITO), the Rsc9 armadillo-like domain (PDB code 4V3Q) and the Sfh1 RPTI and RPT2 domains (PDB code 6AXS). The homology models were rigid-body placed using UCSF Chimera and manually adjusted and rebuilt in COOT.

The quality of the maps allowed for de novo building of the other model parts (Supplementary Table 1). Modelling was guided and validated by BS3 cross-linking data visualized with xVIs and secondary structure predictions performed with Quick2D and PSIPRED. Amino acid residues connecting the domains of the two Rsc8 subunits could not be modelled. For clarification, they were placed into a single chain (chain L) clustered by proximity. The Sfh1 C-terminal finger helix was built into the density of map 7. A polyalanine model was placed into
density that could not be assigned to any RSC subunit (chain X). Bulky amino acid side chain density in the maps 4–8 enabled us to assign the sequence registers, however in some regions register shifts cannot be entirely excluded. The modelled RSC subunits Rsc4, Rsc58, Rsc6, Rsc8, Rsc9, Npl6, Htl1, Shf1 and Shh1 (residues 48–297) together with the polyalanine chain were applied to several rounds of real-space refinement and geometry optimisation using PHENIX44, and flexible fitting with Namdinator48 against the combined map 8. MolProbity49 was used to flip and optimise Asn, Gln and His side chains. The C-terminal finger helix of Shf1 was real space refined with PHENIX against map 7. The final structure displayed excellent stereochemistry as shown by MolProbity (Supplementary Table 2, map7 ATPase + nucleosome, map8 (RSC core)). The slightly worse model statistics of the full structure arise from outliers in the ARP module crystal structure that was rigid-body docked due to insufficient density information. Figures were created using PyMol84, UCSF Chimera43 and UCSF ChimeraX65. The angular distribution plots were generated using the AngularDistribution tool distributed with Warp48.

The RSC structure and density assignments are consistent with a large amount of biochemical and genetic data, including the known requirement of the Rsc4 C-terminal region for cell growth15, the interaction between Rsc6 and Rsc864, the lethality of Rsc58 truncation45, and the known interaction between Rsc3 and Rsc3046. Sites of missense mutations found in human cancers were derived from the cbio cancer genomics portal (cbioPortal)70 and mapped onto the RSC structure for residues that are identical in its human counterpart PBAF using MSAProbs60,71. MSAPobs and Aline72 were used to map conservation across species.

Preparation of cross-linking samples for mass spectrometry

RSC–nucleosome complex was prepared as described above. The cross-linking reaction was performed with BS3 cross-linker (Thermo Fischer Scientific) at a final concentration of 1 mM on ice for 30 min before quenching with Tris-HCl, pH 7.5, and ammonium bicarbonate at a final concentration of 100 mM and 20 mM, respectively. The cross-linked sample was applied to a 10%–25% sucrose gradient as described above (no glutaraldehyde in the heavy solution) and protein containing fractions were pooled (~800 μl - 50 μg complex) and applied to in-solution digest. 150 μl of urea buffer (8 M urea, 50 mM NH4HCO3, pH 8) and 60 μl 0.1 M DTT (in 50 mM NH4HCO3, pH 8) were added to reduce the sample for 30 min at 37 °C, 300 rpm. The sample was alkylated with 60 μl 0.1 M iodoacetamide (in 50 mM NH4HCO3, pH 8) for 30 min at 37 °C, 300 rpm in the dark. The reaction was quenched by addition of 60 μl 0.4 M iodoacetamide (in 50 mM NH4HCO3, pH 8) for 30 min at 37 °C, 300 rpm in the dark. The sample was digested for 30 min at 37 °C with 0.5 μl Pierce Universal Nuclease (250 U μl−1) in the presence of 1 mM MgCl2. The final sample volume was adjusted to 1200 μl with 50 mM NH4HCO3, pH 8 resulting in a final urea concentration of 1 M. Trypsin digest was performed overnight at 37 °C with 2.5 μg trypsin (Promega, V5111). Tryptic peptides were desalted with C18 spin columns (Harvard Apparatus 74-4601), lyophilized and dissolved in 30% (v/v) acetonitrile and 0.1% (v/v) trifluoroacetic acid. The peptide mixture was separated on a SuperPlex Peptide 3.2/300 (GE Healthcare) column run at 50 μl min−1 with 30% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid. Cross-linked species are enriched by size exclusion chromatography based on their higher molecular weight compared to linear peptides. Therefore 50 μl fractions were collected from a 1.0 ml post-injection. Fractions from 1.0–1.6 ml post-injection were dried in a speed vac and dissolved in 5% (v/v) acetonitrile, 0.05% (v/v) trifluoroacetic acid and subjected to liquid chromatography with mass spectrometry (LC–MS/MS).

LC–MS/MS analysis and cross-link identification

LC–MS/MS analyses were performed on a Q Exactive HF-X hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific) coupled to a Dionex Ultimate 3000 RSLCnano system. The peptide mixtures from in-solution digest were loaded on a Pepmap 300 C18 column (Thermo Fisher) at a flow rate of 10 μl min−1 in buffer A (0.1% (v/v) formic acid) and washed for 3 min with buffer A. The sample was separated on an in-house packed C18 column (30 cm; ReproSil-Pur 120 Å, 1.9 μm, C18-AQ; inner diameter, 75 μm) at a flow rate of 300 nl min−1. Sample separation was performed over 120 min using a buffer system consisting of 0.1% (v/v) formic acid (buffer A) and 80% (v/v) acetonitrile, 0.08% (v/v) formic acid (buffer B). The main column was equilibrated with 5% B, followed by sample application and a wash with 5% B. Peptides were eluted by a linear gradient from 15–48% B. The gradient was followed by a wash step at 95% B and re-equilibration at 5% B. Eluting peptides were analysed in positive mode using data-dependent top-30 acquisition methods. MS1 and MS2 resolution were set to 120,000 and 30,000 FWHM, respectively. Precursors selected for MS2 were fragmented using 30% normalized, higher-energy collision-induced dissociation fragmentation. Allowed charge states of selected precursors were +3 to +7. Further MS/MS parameters were set as follows: isolation width, 1.4 m/z; dynamic exclusion, 10 s; max. injection time (MS1/MS2), 60 ms/200 ms. The lock mass option (m/z 443.12002) was used for internal calibration. All measurements were performed in duplicates. The .raw files of all replicates were searched by the software pLink 2, v.2.3.12 against a customized protein database containing the expressed proteins and protein–protein cross-links were filtered with 1% FDR. Cross-links appearing less than three times were excluded to increase confidence and plotted using xViz37 and xNET38.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The coordinate file for the RSC–nucleosome-remodeling complex was deposited with the Protein Data Bank with accession code 6TDA. The cryo-EM maps used for model building were all deposited with the Electron Microscopy Data Base with accession code EMDB-10465.

32. Caims, B. R. et al. Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol. Cell 4, 715–723 (1999).
33. Rigaut, G. et al. A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. 17, 1030–1032 (1999).
34. Lorch, Y. & Kornberg, R. D. Isolation and assay of the RSC chromatin-remodeling complex from Saccharomyces cerevisiae. Methods Enzymol. 377, 316–322 (2003).
35. Luger, K., Rechsteiner, T. J. & Richmond, T. J. Expression and purification of recombinant histones and nucleosome reconstitution. Methods Mol. Biol. 110, 1–16 (1999).
36. Dyer, P. N. et al. Reconstitution of nucleosome core particles from recombinant histones and DNA. Methods Enzymol. 375, 23–44 (2003).
37. Mascell, D. P. et al. Structural basis for retroviral integration into nucleosomes. Nature 523, 366–369 (2015).
38. Lowary, P. T. & Widom, J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. J. Mol. Biol. 276, 19–42 (1998).
39. Kastner, B. et al. Grafix: sample preparation for single-particle electron cryomicroscopy. Nat. Methods 5, 53–55 (2008).
40. Stark, H. Grafix: stabilization of fragile macromolecular complexes for single particle cryo-EM. Methods Enzymol. 481, 109–126 (2010).
41. Tegunov, D. & Cramer, P. Real-time cryo-EM data pre-processing with Warp. Nat. Methods 16, 1146–1152 (2019).
42. Zivanov, J. et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3. Elife 7, e42166 (2018).
43. Pottersen, E. F. et al. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612 (2004).
44. Asturias, F. J., Chung, W. H., Kornberg, R. D. & Lorch, Y. Structural analysis of the RSC chromatin-remodeling complex. Proc. Natl Acad. Sci. USA 104, 13477–13480 (2007).
45. Chaban, Y. et al. Structure of a RSC-nucleosome complex and insights into chromatin remodeling. Nat. Struct. Mol. Biol. 15, 1272–1277 (2008).
46. Leschziner, A. E. et al. Conformational flexibility in the chromatin remodeler RSC observed by electron microscopy and the orthogonal tilt reconstruction method. Proc. Natl Acad. Sci. USA 104, 4913–4918 (2007).
47. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D 66, 486–501 (2010).
48. Kidmose, R. T. et al. Namdinator—automatic molecular dynamics flexible fitting of structural models into cryo-EM and crystallography experimental maps. IUCrJ 6, 526–531 (2019).
71. Liu, Y., Schmidt, B. & Maskell, D. L. MSAProbs: multiple sequence alignment based on pair
68. Angus-Hill, M. L. et al. A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the
67. Taneda, T. & Kikuchi, A. Genetic analysis of RSC58, which encodes a component of a
69. Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring
66. Treich, I., Ho, L. & Carlson, M. Direct interaction between Rsc6 and Rsc8/Swh3,two
74. Combe, C. W., Fischer, L. & Rappsilber, J. xiNET: cross-link network maps with residue
59. Grimm, M., Zimniak, T., Kahraman, A. & Herzog, F. xVis: a web server for the schematic
65. Goddard, T. D. et al. UCSF ChimeraX: Meeting modern challenges in visualization and
62. Jones, D. T. A protein secondary structure prediction based on position-specific scoring
61. Buchan, D. W. A. & Jones, D. T. The PSIPRED protein analysis workbench: 20 years on.
54. Bienert, S. et al. The SWISS-MODEL repository-new features and functionality.
53. Waterhouse, A. et al. SWISS-MODEL: homology modelling of protein structures and
52. van Dijk, M. & Bonvin, A. M. 3D-DART: a DNA structure modelling server.
51. Raman, S. et al. Structure prediction for CASP8 with all-atom refinement using Rosetta.
49. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular
Bioinformatics
Mol. Genet. Genomics
271
3745 (1998).
50. Song, Y. et al. High-resolution comparative modeling with RosettaCM. Structure 21,
1735—1742 (2013).
58. van Dijk, M. & Bovim, A. M. 3D-DART: a DNA structure modelling server. Nucleic Acids
Res. 37, W235–W239 (2009).
56. Waterhouse, A. et al. SWISS-MODEL, homology modelling of protein structures and
complexes. Nucleic Acids Res. 46, W296–W303 (2018).
55. Bienert, S. et al. The SWISS-MODEL repository-new features and functionality. Nucleic Acids
Res. 45, D313–D319 (2017).
53. Waterhouse, A. et al. SWISS-MODEL, homology modelling of protein structures and
complexes. Nucleic Acids Res. 46, W296–W303 (2018).
52. van Dijk, M. & Bonvin, A. M. 3D-DART: a DNA structure modelling server.
51. Raman, S. et al. Structure prediction for CASP8 with all-atom refinement using Rosetta.
50. Song, Y. et al. High-resolution comparative modeling with RosettaCM. Structure 21,
1735—1742 (2013).
49. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular
Bioinformatics
Mol. Genet. Genomics
3745 (1998).
48. Charlop-Powers, Z., Zeng, L., Zhang, Q. & Zhou, M. M. Structural insights into selective
histone H3 recognition by the human Polybromo bromodomain 2. Cell Res. 20, 529–538
(2010).
47. Legge, G. B. et al. ZZ domain of CBP: an unusual zinc finger fold in a protein interaction
module. J. Mol. Biol. 343, 1081—1093 (2004).
46. Reichen, C. et al. Structures of designed armadillo-repeat proteins show propagation of
inter-repeat interface effects. Acta Crystallogr. D 72, 168—175 (2016).
45. Grimm, M., Zimniak, T., Kahraman, A. & Herzog, F. xVis: a web server for the schematic
visualization and interpretation of crosslink-derived spatial restraints. Nucleic Acids Res.
43, W362–W365 (2015).
44. Zimmermann, L. et al. A completely reimplemented mpi bioinformatics toolkit with a new
HIghPer server at its core. J. Mol. Biol. 430, 2237—2243 (2018).
43. Buchars, D. W. A. & Jones, D. T. The PSIPRED protein analysis workbench: 20 years on.
Nucleic Acids Res. 47, W402–W407 (2019).
42. Jones, D. T. Protein secondary structure prediction based on position-specific scoring
matrices. J. Mol. Biol. 292, 195—202 (1999).
41. Williams, C. J. et al. MolProbity: More and better reference data for improved all-atom
structure validation. Protein Sci. 27, 293—315 (2018).
40. Schrodinger, LLC. The PyMOL Molecular Graphics System version 1.8 (2015).
39. Goddard, T. D. et al. UCSF ChimeraX: Meeting modern challenges in visualization and
analysis. Protein Sci. 27, 14—15 (2018).
38. Tresch, I., Ho, L. & Carlson, M. Direct interaction between Rsc6 and Rsc8/Swh3, two
proteins that are conserved in SWI/SNF-related complexes. Nucleic Acids Res. 26, 3739—
3745 (1998).
37. Taneda, T. & Kikuchi, A. Genetic analysis of RSC58, which encodes a component of a
yeast chromatin remodeling complex, and interacts with the transcription factor Swi6. Mol.
Genet. Genomics 271, 479—489 (2004).
36. Angus-Hill, M. L. et al. A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the
chromatin remodeler RSC in gene expression and cell cycle control. Mol. Cell 7, 741—751
(2001).
35. Cerami, E. et al. The cBio cancer genetics portal: an open platform for exploring
multidimensional cancer genomics data. Cancer Discov. 2, 401—404 (2012).
34. Gao, J. et al. Integrative analysis of complex cancer genomics and clinical profiles using
the cBioPortal. Sci. Signal. 6, p61 (2013).
33. Liu, Y., Schmidt, B. & Maskell, D. L. MSAProbs: multiple sequence alignment based on pair
hidden Markov models and partition function posterior probabilities. Bioinformatics 26,
1958—1964 (2010).
32. Bond, C. S. & Schütte-lof, A. W. ALINE. A WYSIWYG protein-sequence alignment editor
for publication-quality alignments. Acta Crystallogr. D 65, S10—S12 (2009).
31. Yang, B. et al. Identification of cross-linked peptides from complex samples. Nat.
Methods 9, 804—806 (2012).
30. Combe, C. W., Fischer, L. & Rappsilber, J. xiNET: cross-link network maps with residue
resolution. Mol. Cell. Proteomics 14, 1137—1147 (2015).
29. Yan, Z. et al. PBAF chromatin-remodeling complex requires a novel specificity subunit,
BAF200, to regulate expression of selective interferon-responsive genes. Genes Dev. 19,
1662—1667 (2005).
28. Wang, W. et al. Architectural DNA binding by a high-mobility-group/kinase-like subunit
in mammalian SWI/SNF-related complexes. Proc. Natl Acad. Sci. USA 97, 13015—13020
(2000).
27. Nicolas, R. H. & Goodwin, G. H. Molecular cloning of polybromo, a nuclear protein
containing multiple domains including five bromodomains, a truncated HMGA-box, and
two repeats of a novel domain. Genes 176, 233—240 (1996).
26. Willhoft, O. et al. Structure and dynamics of the yeast SWR1-nucleosome complex.
Science 362, eaat7716 (2018).
25. Ayala, R. et al. Architecture and regulation of the human INO80-nucleosome complex.
Nature 556, 391—395 (2018).
24. Sundaramoorthy, R. et al. Structure of the chromatin remodeler enzyme Chd1 bound to
a ubiquitylated nucleosome. eLife 7, e35720 (2018).
23. Yan, L., Wu, H., Li, X., Gao, N. & Chen, Z. Structures of the ISWI-nucleosome complex
reveal a conserved mechanism of chromatin remodeling. Nat. Struct. Mol. Biol. 26,
258—266 (2019).
22. Roberts, X. & Gouet, P. Deciphering key features in protein structures with the new
ENDscript server. Nucleic Acids Res. 42, W320—W324 (2014).

Acknowledgements We thank current and former members of the Craster Laboratory,
including S. Osman, G. Kocik, P. Seweryn, S. Schibbach, S. Neyer and H. Hillen. F.R.W.
was supported by a Boehringer Ingelheim Fonds PhD fellowship. H.U. was supported by the
Deutsche Forschungsgemeinschaft (DFG) and the European Research Council Advanced Investigator Grant TRANSREGULON (grant agreement no. 693023), and the
Volkswagen Foundation.

Author contributions F.R.W. carried out all experiments and data analysis unless stated
otherwise. C.D. assisted with data collection and model building. A.S. and H.U. carried out
cross-linking and mass spectrometry analysis. H.W. helped with nucleosome biochemistry. D.T.
helped with cryo-EM data processing. P.C. designed and supervised the project. F.W. and C.D.
wrote the manuscript with input from all authors.

Competing interests The authors declare no competing interests.

Additional information Supplementary information is available for this paper at https://doi.org/10.1038/s41586-020-
2088-0.

Correspondence and requests for materials should be addressed to P.C.

Peer review information Nature thanks Blaine Bartholomew and the other, anonymous,
reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Preparation and characterization of RSC–nucleosome complex. 

**a.** Preparation of endogenous Rsc2-containing isoform of the RSC complex from *S. cerevisiae*. Analysis of purified RSC by size-exclusion chromatography and SDS–PAGE showed high purity and homogeneity with stoichiometric subunits as assessable by Coomassie stain. Subunit identity was confirmed by mass spectrometry. The table shows the expected molecular weights of the RSC subunits. For gel source data, see Supplementary Fig. 1.

**b.** Assembly of the RSC–nucleosome complex. SDS–PAGE analysis of fractions 7–20 of a 10–25% sucrose-gradient ultracentrifugation. Complex formation was successful as demonstrated by the comigration of histones with the RSC complex. The unbound over-stoichiometric nucleosomes only migrated to fractions 7 and 8 (black arrow). Fraction 16 in the presence of cross-linker was used for cryo-EM grid preparation (dashed box).

**c.** Location of cross-linking sites mapped onto the structure. BS3 cross-links that appeared at least in triplicates were mapped onto the RSC–nucleosome structure. Lysine residues involved in the cross-linking network are shown as blue spheres and cross-linked residues are connected with lines indicating permitted (blue) and non-permitted (red) cross-linking distances. Of the mapped cross-links, 87.5% are within the permitted cross-linking distance, which was set to 30 Å. The remaining 12.5% of non-permitted cross-links probably reflect ambiguity caused by the presence of two identical Rsc8 subunits in the structure as well as flexibility of the complex in buffer or arise from technical errors.

**d.** The cross-linking network between subunits of the RSC–nucleosome complex. Subunits are coloured as in Fig. 1. Cross-links with a score above 2.5 are shown. A comprehensive list of cross-links is presented in Supplementary Data 1. Cross-linking mass-spectrometry experiments were performed in duplicates with similar results.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Cryo-EM analysis of the RSC–nucleosome complex.

a, Representative cryo-EM micrograph of the RSC–nucleosome complex shows homogeneously distributed individual particles. b–d, Two-dimensional class averages of the RSC–nucleosome complex (b), the ATPase–nucleosome subcomplex (c) and the nucleosome subcomplex (d). e, FSC plots reveal the overall resolutions of the cryo-EM reconstructions. f, Cryo-EM processing workflow for the reconstructions of the RSC–nucleosome complex, the ATPase–nucleosome subcomplex and the nucleosome subcomplex. Particle distribution after 3D classifications is indicated below the corresponding map. g, Local resolution estimation of the combined ATPase–nucleosome map as implemented in RELION42. We note that the resolution of the peripheral area with the ATPase module is overestimated. h–j, Angular distribution plot for all particles contributing to the final reconstructions of the RSC–nucleosome complex (h), the ATPase–nucleosome subcomplex (i) and the nucleosome subcomplex (j).
Extended Data Fig. 3 | Cryo-EM analysis of the free RSC complex.

**a**, Representative cryo-EM micrograph of the free RSC complex shows homogeneously distributed individual particles. **b**, Two-dimensional class averages of the free RSC complex. **c**, Cryo-EM processing workflow for the reconstruction of the free RSC complex. Particle distribution after 3D classifications is indicated below the corresponding map. The final maps after focused 3D refinement and masks are depicted in colour. Views are generally rotated by 180° with respect to Fig. 1c, right. **d**, Angular distribution plot for all particles contributing to the final reconstruction of the free RSC complex. **e**, Two views of the combined RSC core map coloured according to the local resolution as implemented in RELION. **f**, FSC plots of the maps used for model building of the RSC core complex.
Extended Data Fig. 4 | Cryo-EM densities for selected RSC regions. a–c. Examples of map quality. a. Close-up of the Rsc4 β-sheet shows clear separation of individual strands. b. The high quality of the map for the ZZ zinc-finger of Rsc8 allowed backbone tracing and placement of side chains as well as for the zinc ion. c. Coiled-coil helices of the two Rsc8 subunits with density for one helix. d. View along the exit DNA in the direction of the nucleosome showing the lowpass-filtered maps for the modules ATPase, ARP, DIM, arm and body, and the nucleosome. At the site where the H2A C-terminal tail protrudes from the nucleosome near Sfh1, there is low-resolution density connecting the arm module and the nucleosome. Density bridging form the ARP module to the exit DNA close to the H3 histone tail can be observed. e. Density representing the finger helix (green) at the acidic patch of the nucleosome (indicated by H2A in yellow). Side-chain density is visible for conserved arginine residues. f. Interaction of RSC with the nucleosome is sterically impaired by the flexibly bound ubiquitin moiety at H2B lysine 123 (K120 in human). The Sfh1 finger helix and the ubiquitin moiety (ubiquitylated nucleosome PDB code 6NOG) overlap after superposition of nucleosomes.
Extended Data Fig. 5 | Structure of RSC body and arm modules, cancer mutations and remodeler families. a, Cartoon representation of RSC core viewed as in Fig. 1. Important structural elements are labelled. b, Conservation between SWI/SNF complexes RSC (yeast) and PBAF (human). Residues that are identical (blue) or conserved (light blue) in human PBAF highlighted on the RSC structure (grey). Purple spheres depict identical residues that show missense mutations in various cancers. c, Comparison of overall structure of RSC with complexes of INO80 (yeast INO80) and CHD (yeast CHD1) families. ATPase motor domains are shown in orange, DNA is shown in blue. With regard to the INO80 family, the ATPase of the SWR1 complex also binds SHL +2 (ref. 84), whereas the ATPase of the INO80 complex binds SHL −6 (refs. 82,83). The ARP module of INO80 contacts exit DNA, which is not the case in RSC. The INO80 complex also contacts both faces of the histone octamer82, resembling the sandwiching interactions made by RSC on a topological level. With respect to the CHD family, the ATPase motor of yeast Chd1 also binds SHL +2, but its DNA-binding region engages with exit DNA near the nucleosome, leading to a different DNA trajectory83,86. With respect to the ISWI family, the ATPase motor binds SHL +2 (ref. 85), but other interactions have not been structurally resolved (not shown).
Extended Data Fig. 6 | Course of polypeptide chains of architectural subunits Sth1, Rsc8 and Rsc58 and ATPase–nucleosome interactions. 

a, Back view of RSC. The Sth1 subunit of RSC starts with its N terminus in the body module and tracks through it, turning around with a contact helix and loop. Forming the central helix I, the hook and the central helix II it folds back and forth tightly interweaving the body module before it exits with its HSA region through the ARP module to build the ATPase module. 
b, RSC with the domains of the two Rsc8 subunits highlighted in blue. Both Rsc8 start N-terminal with their SWIRM domains in the arm module where they support the two repeat domains of Sfh1 in a similar manner. They then follow distinct paths through the arm towards the body module where they contribute with both their SANT and ZZ zinc-finger domains. Here the two domains of each subunit form different contacts with various interactions partners and whereas one ZZ zinc-finger domain is tightly packed at the body and DNA-interaction module interface, the other seems to extend from the body, presumably as additional interaction surface. Both Rsc8 subunits unite again with their C-terminal long helices in a coiled-coil fold on the opposite side of the body module. 
c, Rsc58 N-terminal bromodomain attaches to the top of the body module. Then, Rsc58 follows an interwound path through the body module via the central and connector loop. It turns back, docking to the body with a three-helix bundle and stabilizing the module with its C-terminal end. 
d, Contacts of Sth1 ATPase motor (orange) with the nucleosome. View as in Fig. 1c, left, but rotated by 45° around a horizontal axis. Arrows indicate directionality of DNA translocation.
**Extended Data Fig. 7| DNA recognition and NDR formation. a,** Space-filling RSC–nucleosome structure with DIM (green) and SnAC (orange) densities. View on the top as in Fig. 1c, left, but rotated by 90° around the vertical and horizontal axis. Arrows indicate directionality of DNA translocation. Number of upstream DNA base pairs relative to SHL −7 is provided. **b,** Schematic of a promoter before (top) and after (bottom) RSC remodelling shows NDR formation by sliding the flanking −1 and +1 nucleosomes away from the NDR centre. Arrows indicate the transcription start site.
Extended Data Fig. 8 | Sequence alignments for the Sth1 ATPase domain and HSA region. a, Sequence alignment of the S. cerevisiae Sth1 ATPase domain to the homologous Snf2 ATPase domain of the same organism. Secondary structure elements are represented in orange according to the cryo-EM structure of the Snf2 ATPase (PDB entry 5Z3U)17. Residues modelled in the Snf2 structure are topped by a black line with helical regions shown as cylinders and sheet regions as arrows. The Sth1 residues modelled in this work are indicated with a black dashed line below. ATPase motifs are underlined. Invariant residues are coloured in dark blue and conserved residues in light blue. The alignment was generated with MSAProbs71 within the MPI Bioinformatics Toolkit60 and visualized using ESPript88.

b, Sequence alignment of the HSA regions from S. cerevisiae homologues Sth1 and Snf2. Illustration and generation of the alignment as in a.
Extended Data Table 1 | Subunit composition of RSC and related chromatin remodelling complexes

| Module          | S. cerevisiae | S. pombe | D. melanogaster | H. sapiens |
|-----------------|---------------|----------|-----------------|------------|
| ATPase module   | RSC           | SWI/SNF  | RSC             | SWI/SNF    | PBAP      | BAP   | PBAF  | BAF   |
|                 | Sth1          | Snf2     | Snf21           | Snf22      | BRM       | BRM   | BRG1  | BRG1/BRM |
| Arp module      | Arp9          | Arp9     | Arp9            | Arp9       | β-actin   | β-actin | β-actin | β-actin |
|                 | Arp7          | Arp7     | Arp42           | Arp42      | BAP55     | BAP55  | BAF53A/B | BAF53A/B |
|                 | Rtt102        | Rtt102   |                 |            |           |       |       |       |
| arm module      | Sfh1          | Snf5     | Sfh1            | Snf2       | SNR1      | SNR1   | BAF47  | BAF47  |
|                 | Npl6          | Swp82    | Rsc7            | Snf59      |           |       |       |       |
| body module     | Rsc6          | Swp73    | Ssr3            | Ssr3       | BAP60     | BAP60  | BAF60A/B/C | BAF60A/B/C |
|                 | Rsc9          | Swi1     | Rsc9            | Sol1       | BAP170    | OSA   | BAF200 | BAF250A/B |
|                 | Htt1          |          |                 |            |           |       |       |       |
|                 | Rsc58         |          | Rsc58           |            |           |       |       |       |
|                 | Rsc4          |          | Rsc4            |            |           |       |       |       |
|                 | Rsc2 / Rsc1   |          | Polybromo       |            |           |       |       | BAF180 |
| DNA-interaction | Rsc8, Rsc30   |          |                 |            |           |       |       |       |
| Scaffold        | Ldb7          | BAP111   | BAP111          | BAF57      | BAF57     |
|                 |               | Snf11    |                 |            | BAF45A    | BAF45A/B/C |
|                 |               | Snf6     |                 |            | BRD7      | BRD9   |
|                 |               | Taf14    |                 |            | BCL11A/B  | BCL11A/B |
|                 |               | Tfg3     |                 |            | BCL7A/B/C | BCL7A/B/C |
|                 |               | Snf30    |                 |            |           |       |

Assignment to the structural modules based on the S. cerevisiae structure of RSC presented in this work. Subunits occurring together in the complex are separated by comma, a solidus indicates the use of one of the subunits. Subunits that could not be assigned to a module by homology are listed below. The PBAF subunit BAF200 probably corresponds to Rsc8, because it comprises an armadillo-repeat fold, and the BAF180 subunit comprises regions that resemble Rsc2 and Rsc4. Only the small RSC subunits Rsc58, Rtt102 and Htt1 lack obvious counterparts. PBAF subunits contain 12 DNA-binding domains located in subunits BAF180 (HMG box), BAF200 (AT-rich domain, two C2H2 zinc-fingers and RFX domain), BAF57 (HMG box) and BCL11A/B (six C2H2 zinc-fingers). The BAF subunit BAF250a is predicted to contain five armadillo repeats, and is probably the counterpart of Rsc9.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|-----|-----------|
| ✗   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ✗   | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ✗   | The statistical test(s) used AND whether they are one- or two-sided |
| ✗   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ✗   | A description of all covariates tested |
| ✗   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ✗   | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ✗   | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted |
| ✗   | Give P values as exact values whenever suitable. |
| ✗   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ✗   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ✗   | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy Information about availability of computer code

Data collection: FEI/EPU 1.10, plink 2 (version 2.3.1)

Data analysis: RELION 3.0, Warp 1.0.6/1.0.7, UCSF Chimera (version 1.12.1), UCSF ChimeraX (version 0.91), COOT (version 0.8.9.2), PHENIX (version 1.16), PyMoI (Schrodinger LLC version 1.8.4.1), Aline 1.0.0.23, XimENAlyzer 1.1.1, XmdvView (online version April 2019 - August 2019), 3D-DART (online version April 2019 - August 2019), MSA-Pros (online version 0.9.7), ESPrf (webservers July 2019 - August 2019), ROBETTA (online version February 2019 - August 2019), XwIs (online version February 2019 - August 2019), Quick2D (online version August 2018 - August 2019), PSSPRED (online version February 2019 - July 2019), Molprobity plugin (PHENIX), XNet (online version June 2019 - August 2019), SWISS-MODEL (online version October 2018 - August 2019), Molprobity (online version June 2019 - August 2019), Molprobity (online version June 2019 - August 2019)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy Information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The coordinate file for the RSC-nucleosome complex structure was deposited with the Protein Data Bank with accession code 6TDA. The cryo-EM density maps used for model building were all deposited with the Electron Microscopy Data Base (EMDB) with accession code EMD-10465.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences  Behavioural & social sciences  Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Structural data was collected on two independently prepared samples. No statistical methods were used to predetermine the sample size. For cryo-EM, the sample sizes were determined by available electron microscopy measuring time and density of the particles on the sample grids. Statistics regarding cryo-EM analysis have all been detailed in the methods section.

Data exclusions

No data were pre-excluded from the analyses. Low quality data from cryo-EM was sorted out in RELION to reach high resolution with criteria pre-established in RELION.

Replication

Cross-linking mass spectrometry experiments were performed in duplicates with reproducible results.

Randomization

Samples were not allocated to groups.

Blinding

Investigators were not blinded during data acquisition and analysis because it is not a common procedure for the methods employed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study; if you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | Antibodies            |
| ✓   | Eukaryotic cell lines |
| ✓   | Palaeontology         |
| ✓   | Animals and other organisms |
| ✓   | Human research participants |
| ✓   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✓   | ChIP-seq              |
| ✓   | Flow cytometry        |
| ✓   | MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Saccharomyces cerevisiae, RSC2-TAP-HIS3 yeast strain [YSC1177-YLR357W], Dharmacon TAP-tagged open reading frame (ORF) library

Authentication

N.A.

Mycoplasma contamination

Cells were not tested for mycoplasma.

Commonly misidentified lines

[See EUCAST register]