

Two Functionally Distinct Forms of the RSC Nucleosome-Remodeling Complex, Containing Essential AT Hook, BAH, and Bromodomains

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Summary

RSC is an essential 15 protein nucleosome-remodeling complex from *S. cerevisiae*. We have identified two closely related RSC members, Rsc1 and Rsc2. Biochemical analysis revealed Rsc1 and Rsc2 in distinct complexes, defining two forms of RSC. Genetic analysis has shown that Rsc1 and Rsc2 possess shared and unique functions. Rsc1 and Rsc2 each contain two bromodomains, a bromo-adjacent homology (BAH) domain, and an AT hook. One of the bromodomains, the BAH domain, and the AT hook are each essential for Rsc1 and Rsc2 functions, although they are not required for assembly into RSC complexes. Therefore, these domains are required for RSC function. Additional genetic analysis provides further evidence that RSC function is related to transcriptional control.

Introduction

Chromatin structure plays significant and dynamic roles in transcriptional regulation (reviewed in Owen-Hughes and Workman, 1994; Workman and Kingston, 1998). Nucleosomes, the basic units of chromatin structure, have been shown to impair transcription initiation by preventing the binding of transcription factors to promoter elements or by impeding transcriptional elongation by RNA polymerases (Workman and Kingston, 1998). The ability of nucleosomes to repress transcription is potentiated by protein complexes that can either modify histones or alter nucleosomal structure. Such complexes contain one of two general classes of factors. The first class controls the acetylation state of histones by either acetylation or deacetylation of histone

amino-terminal “tails” (Grunstein, 1997). Histone hypoacetylation is correlated with transcriptional repression, whereas histone hyperacetylation is correlated with activation. Several complexes have been identified that display either histone acetyltransferase (HAT) or histone deacetylase (HDAC) activity, and their important roles in transcriptional control have been demonstrated (reviewed in Grunstein, 1997; Kuo and Allis, 1998). HAT complexes include the yeast complex SAGA (containing certain Spt proteins, Ada proteins, Taf proteins, and the Gcn5 acetyltransferase) and a complex of similar composition (named PCAF) from mammalian cells (Grant et al., 1998; Ogryzko et al., 1998).

The second class of factors is composed of nucleosome-remodeling complexes that utilize ATP hydrolysis (Cairns, 1998). This class includes the “SWI/SNF-related” factors, named after the *S. cerevisiae* SWI/SNF complex. SWI/SNF has been shown to control transcription of a subset of *S. cerevisiae* genes via its nucleosome-remodeling activity (reviewed in Peterson and Tamkun, 1995; Kingston et al., 1996). All of the SWI/SNF-related complexes are composed of 8–15 proteins, are related compositionally, and display ATP-dependent remodeling of nucleosomes in vitro (Workman and Kingston, 1998). Many fundamental questions remain about SWI/SNF-related complexes, including their composition, roles in vivo, regulation, and mechanism of action.

The *S. cerevisiae* RSC complex was identified and purified biochemically based on the homology of some of its components to SWI/SNF proteins and by virtue of its nucleosome-remodeling activity (Cairns et al., 1996). Recent findings suggest that RSC may play a more widespread role than SWI/SNF, as only RSC is essential for growth (Laurent et al., 1992; Cairns et al., 1996; Cao et al., 1997; Treich and Carlson, 1997). In addition, RSC is approximately ten times more abundant than SWI/SNF (Cairns et al., 1996). RSC function is required for normal cell cycle progression (Tsuchiya et al., 1992; Laurent et al., 1992; Cao et al., 1997; Du et al., 1998), and certain *rsc* mutations affect the transcription of the *CHA1* gene and certain genes involved in early meiosis (Moriera and Holmberg, 1999; Yukawa et al., 1999). However, the precise role and scope of RSC in transcription or in other chromatin-related processes in vivo has not yet been established, and we know little about how individual components of RSC contribute to RSC function. In this work, we have identified and characterized two related components of RSC, Rsc1 and Rsc2.

Results

Identification of Rsc1 and Rsc2

RSC was previously purified to homogeneity from yeast extracts (Figure 1A; Cairns et al., 1996). Peptides from Rsc1 (formerly p110/112) and Rsc2 were isolated and analyzed by MALDI-TOF mass spectrometry and limited Edman sequencing (Erdjument-Bromage et al., 1994; Cairns et al., 1996). Mass fingerprinting with 12 peptides

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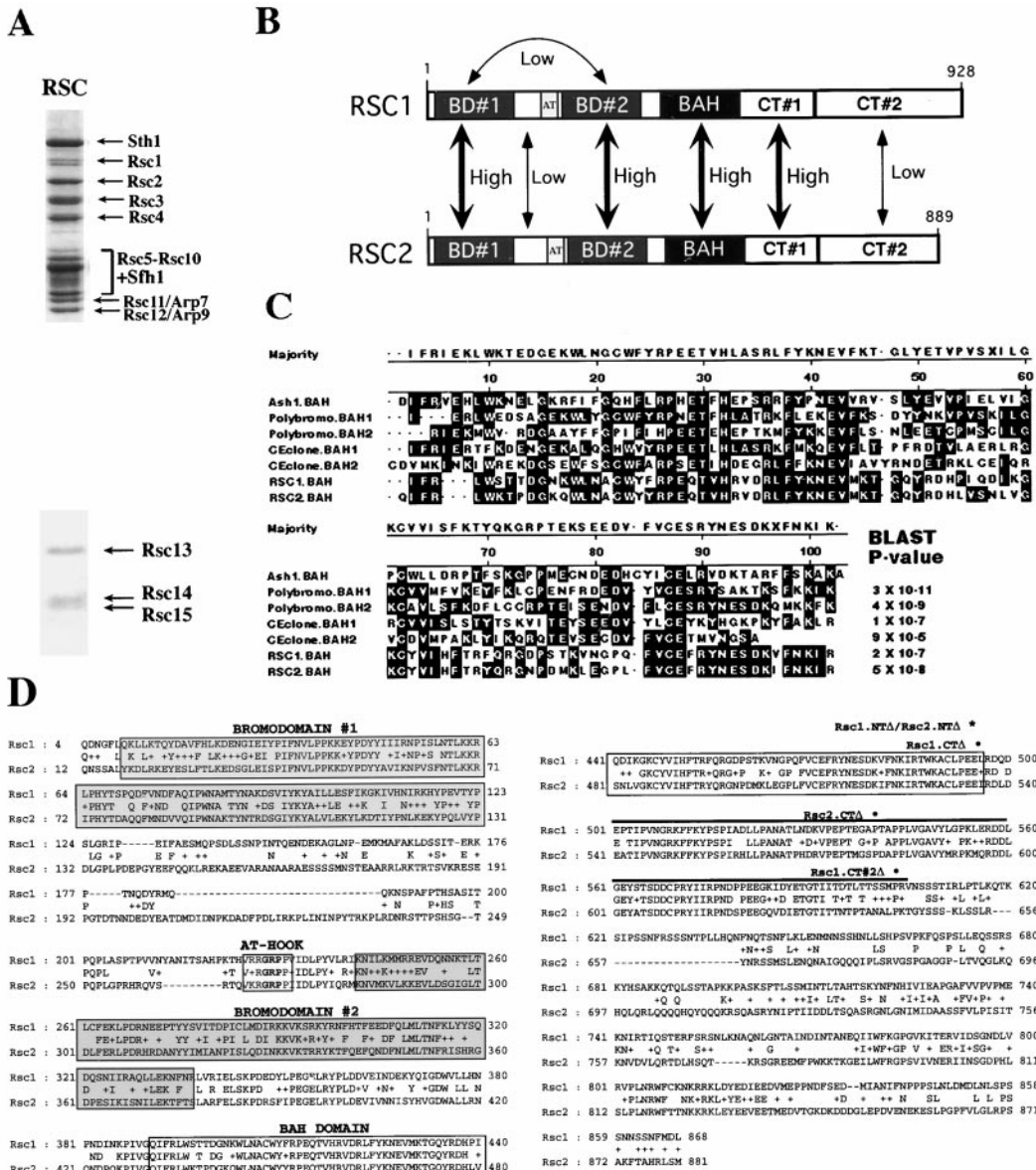


Figure 1. RSC Complex Contains Two Highly Similar Proteins, Rsc1 and Rsc2

(A) SDS-PAGE analysis of purified RSC complex. Fraction #36 from from Mono S (Cairns et al., 1996), separated by SDS-PAGE and stained with Coomassie dye. Rsc1 appears as either a single band or a doublet depending upon the preparation suggesting that it is susceptible to proteolysis.

(B) Rsc1 and Rsc2 are highly similar, and regions of high similarity and low similarity between the two proteins are indicated.

(C) Alignments of BAH domains. Alignment of seven BAH domains: the Ash1 protein from *Drosophila* (Tripoulas et al., 1996), ORF C26C6 from *C. elegans* (function unknown), polybromo, and Rsc1 and Rsc2.

(D) Alignment of Rsc1 and Rsc2 and the domains that were deleted or mutated in Rsc1 or Rsc2 derivatives. Boxes envelop the two bromodomains (BD#1 and BD#2, dark shading), the AT hook motif (light shading), and the BAH domain (no shading). For the bromodomains and the BAH domain, the boxes also indicate the endpoints for the deletion derivatives. The residues in the AT hook that were changed to alanines are shown in boldface (GRP). A bar is present over the section of the C terminus that is highly similar between Rsc1 and Rsc2 (CT#1), whereas CT#2 has only limited similarity. The locations where C-terminal deletion constructs terminate are shown as bullets (·), and the location where the N-terminal deletions initiate is shown with an asterisk (*).

from Rsc1 uniquely identified the open reading frame (ORF) YGR056W; a finding confirmed by Edman sequencing of the peptides NAQNLGNTAINDINTANE and FFKYPSPIAHLLPANA, which correspond to residues 511–526 and 759–776, respectively. Mass fingerprinting

with 12 peptides from Rsc2 uniquely identified the ORF YLR357W; a finding confirmed by Edman sequencing of the peptide STTPSHSGTPQPL, which corresponds to residues 241–253. Rsc1 and Rsc2 are not members of yeast RNA polymerase II holoenzyme or any of the

basal transcription factor complexes or purified SWI/SNF (data not shown).

Rsc1 and Rsc2 Are Highly Similar, Contain Two Bromodomains, a BAH Domain, and an AT Hook, and Resemble the Metazoan Protein Polybromo

Sequence comparisons using the algorithm BLAST (Altschul et al., 1990) reveal Rsc1 and Rsc2 as highly similar proteins (BLAST p value 2×10^{-288} , 45% identical or 62% similar). We can divide their regions of similarity into six sections: two bromodomains (BD#1 and BD#2), an AT hook motif, a BAH region (see below), and two regions in their carboxyl termini: a region of high identity (CT#1) and a region of moderate identity (CT#2) (Figure 1). The *S. cerevisiae* genome does not encode another protein with a domain structure similar to Rsc1 and Rsc2. It is probable that one homolog arose from the other through an ancient chromosomal duplication (Wolfe and Shields, 1997).

The bromodomain is a 110-amino acid motif found in many proteins important for the regulation of transcription and chromatin structure, including many with HAT activity and the ATPase subunits of SWI/SNF-related nucleosome-remodeling complexes (Jeanmougin et al., 1997; Winston and Allis, 1999). Bromodomains bind to the amino-terminal tails of histones H3 and H4 (Ornaghi et al., 1999) and function as acetyl-lysine binding motifs (Dhalluin et al., 1999). In Gcn5 (Candau et al., 1997) and Snf2/Swi2 (Laurent et al., 1992), the bromodomain does not play a significant role, while in at least one other protein, Sth1 (the ATPase present in RSC), the bromodomain has an important function (Du et al., 1998).

The BAH (bromo-adjacent homology) region is a domain of unknown function first identified in the chicken polybromo protein (Nicolas and Goodwin, 1996). A novel family of genes in *S. pombe*, *C. elegans*, *Drosophila*, and human cells contain multiple bromodomains and one or more BAH domains, including the metazoan protein polybromo (Nicolas and Goodwin, 1996) (Figure 1C and data not shown). Rsc1 and Rsc2 are the only proteins in *S. cerevisiae* with a BAH domain that is highly similar to those present in polybromo. However, other searches suggest that a BAH-related domain may be present in the amino terminus of the yeast ORC1 protein (Callebaut et al., 1999), which serves an important role in establishing silencing at the mating type loci.

The AT hook is a short DNA-binding motif first found in the high-mobility group chromosomal protein HMG-I(Y) (Reeves and Nissen, 1990; Aravind and Landsman, 1998). The motif appears to be limited to transcription factors and proteins that affect chromatin structure (such as human trithorax [ALL-1/HRX], TAF250, and *Drosophila* Ash1) and is named for its preferred association with the minor groove of AT-rich DNA. The center of the motif consists of a glycine-arginine-proline tripeptide (GRP) that is both necessary and sufficient for DNA binding (Huth et al., 1997). As AT hooks display limited sequence specificity, they appear to collaborate with other DNA-binding domains to assist in binding to particular sequences or regions. Later, we show to what extent each of the three functional domains present in Rsc1 and Rsc2 (bromodomains, a BAH domain, or the AT hook) is essential for Rsc1 and Rsc2 function.

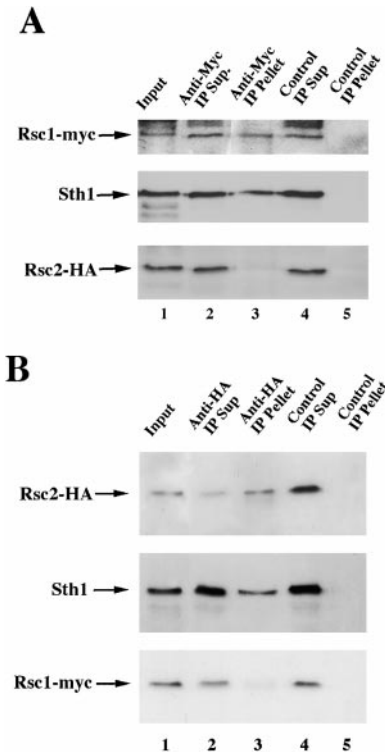


Figure 2. Rsc1 and Rsc2 Are Present in Separate RSC Subcomplexes

Whole-cell extracts were prepared from YBC681 (*rsc1Δ rsc2Δ*, p424.RSC1.3XMYC) transformed with pRSC2.2XHA.

(A) Immunoprecipitation of Rsc1-myc protein with anti-myc antibodies coprecipitates Sth1 protein but not Rsc2-HA protein. The following were separated in a SDS-6% acrylamide gel and immunoblotted: one-fourth of the input (100 μg, lane 1), half of the anti-myc IP supernatant (lane 2), half of the anti-myc IP pellet (lane 3), half of the control (protein G-agarose beads alone) IP supernatant (lane 4), or half of the control IP pellet (lane 6).

(B) Immunoprecipitation of Rsc2-HA protein coprecipitates Sth1 protein but not Rsc1-myc protein. The following were separated in a SDS-7.5% acrylamide gel and immunoblotted: one-fourth of the input (50 μg, lane 1), half of the anti-HA IP supernatant (lane 2), half of the anti-HA IP pellet (lane 3), half of the control (protein G-agarose beads alone) IP supernatant (lane 4), or half of the control IP pellet (lane 5). Immunoprecipitation of Rsc1-myc and of Rsc2-HA also coimmunoprecipitated the two other RSC proteins tested, Rsc6 and Arp9 (data not shown).

Rsc1 and Rsc2 Are Present in Distinct Forms of RSC

The presence of two closely related RSC members raised the possibility that they might not both be present in the same complex, but rather might exist in distinct forms of RSC. To test this possibility, we performed coimmunoprecipitation experiments using myc-tagged Rsc1 and HA-tagged Rsc2. Although anti-myc antibodies were able to immunoprecipitate Rsc1-myc protein and coimmunoprecipitate the RSC component Sth1, they were unable to coimmunoprecipitate Rsc2-HA (Figure 2A). Similarly, anti-HA antibodies could immunoprecipitate Rsc2-HA and coimmunoprecipitate Sth1 but could not coimmunoprecipitate Rsc1-myc (Figure 2B). These results demonstrate that Rsc1 and Rsc2 are present in separate forms of RSC. Previous work (Cairns et

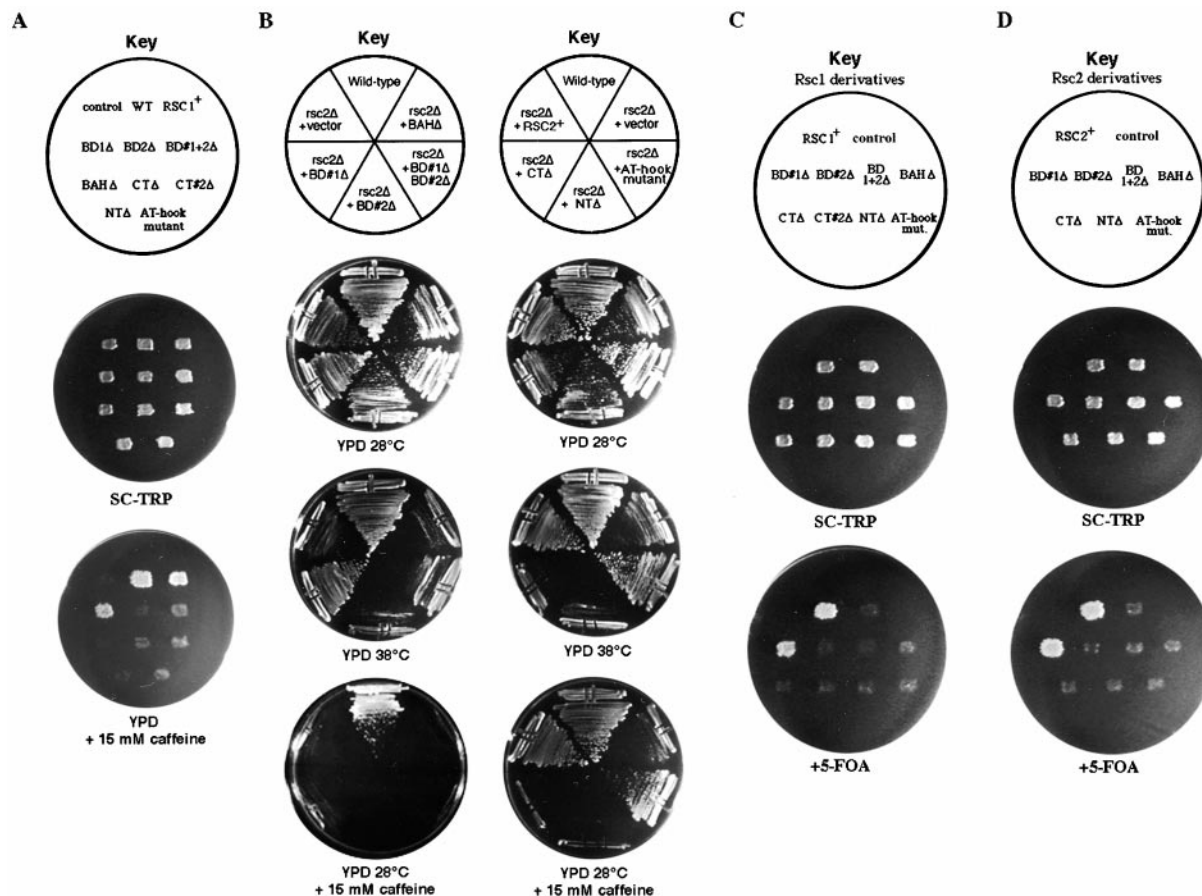


Figure 3. Phenotypes Conferred by Domain Mutations in Rsc1 or Rsc2

(A) Growth of strains bearing wild-type *RSC1* or *rsc1* domain deletion mutations (borne on *TRP1 CEN* plasmids). All strains are *rsc1Δ* (YBC622) transformants except WT (FY120). Control: empty vector.

(B) Growth of strains bearing wild-type *RSC2* or *rsc2* domain deletion mutations (borne on *TRP1 CEN* plasmids). All strains are *rsc2Δ* (YBC82) transformants except WT (FY120). Control: empty vector.

(C) A strain (YBC800; *rsc1Δ rsc2Δ*) harboring the *URA3*-marked *RSC2*⁺ plasmid p316.RSC2.2XHA was transformed with plasmids bearing *rsc1* derivatives and tested for their ability to lose the *RSC2*⁺ *URA3* plasmid, revealed by growth on media containing 5-FOA. *RSC1*: YBC800 containing the wild-type allele (p314.RSC1.3XMYC). Control: empty vector.

(D) A strain (YBC803, *rsc1Δ rsc2Δ*) harboring the *URA3*-marked *RSC1*⁺ plasmid p316.RSC1.3XMYC was transformed with plasmids bearing *rsc2* derivatives and tested as in (C). Control: empty vector.

al., 1996) also defined two forms of RSC that differ by the presence or absence of another RSC component, Rsc3. Anti-Rsc3 antibodies will be required to determine if Rsc3 is specific to either the Rsc1 or Rsc2 form of RSC.

rsc1Δ and *rsc2Δ* Mutations Confer Specific Growth Defects and Are Lethal in Combination with Each Other

To investigate their functional roles, we constructed strains bearing deletions of *RSC1* or *RSC2*. In case the mutations caused inviability, we replaced one homolog in a diploid cell with the *HIS3* gene and then determined the mutant phenotype in haploids after tetrad analysis. Both *rsc1Δ::HIS3* and *rsc2Δ::HIS3* are viable, although slightly slower growing than wild-type.

We find that *rsc1Δ* strains, but not *rsc2Δ* strains, grow poorly on media containing 150 mM hydroxyurea (an inhibitor of ribonucleotide reductase; data not shown). In contrast, we find that *rsc2Δ* mutants are more sensitive to elevated temperature than are *rsc1Δ* mutants. In

addition, both *rsc1Δ* and *rsc2Δ* strains grow very poorly on media containing caffeine (which effects osmotic stability and cAMP signaling) or on media containing 1.0 M NaCl (data not shown). Although we do not understand all the effects that caffeine, high salt, and elevated temperature have on cell function, the *rsc1Δ* and *rsc2Δ* mutant phenotypes demonstrate that Rsc1 and Rsc2 are both required for certain functions, yet distinctly required for others.

As Rsc1 and Rsc2 are highly similar, they may also be redundant for certain functions. To address this possibility, we attempted to construct a *rsc1Δ rsc2Δ* double mutant. However, sporulation and tetrad dissection of a diploid heterozygous at both the *RSC1* and *RSC2* loci failed to yield *rsc1Δ rsc2Δ* haploid segregants, suggesting that a *rsc1Δ* and *rsc2Δ* double mutant is inviable. This result was verified by demonstrating that a *rsc1Δ rsc2Δ* strain harboring a *URA3*-marked plasmid bearing either *RSC1* or *RSC2* is unable to lose the plasmid on media containing 5-FOA.

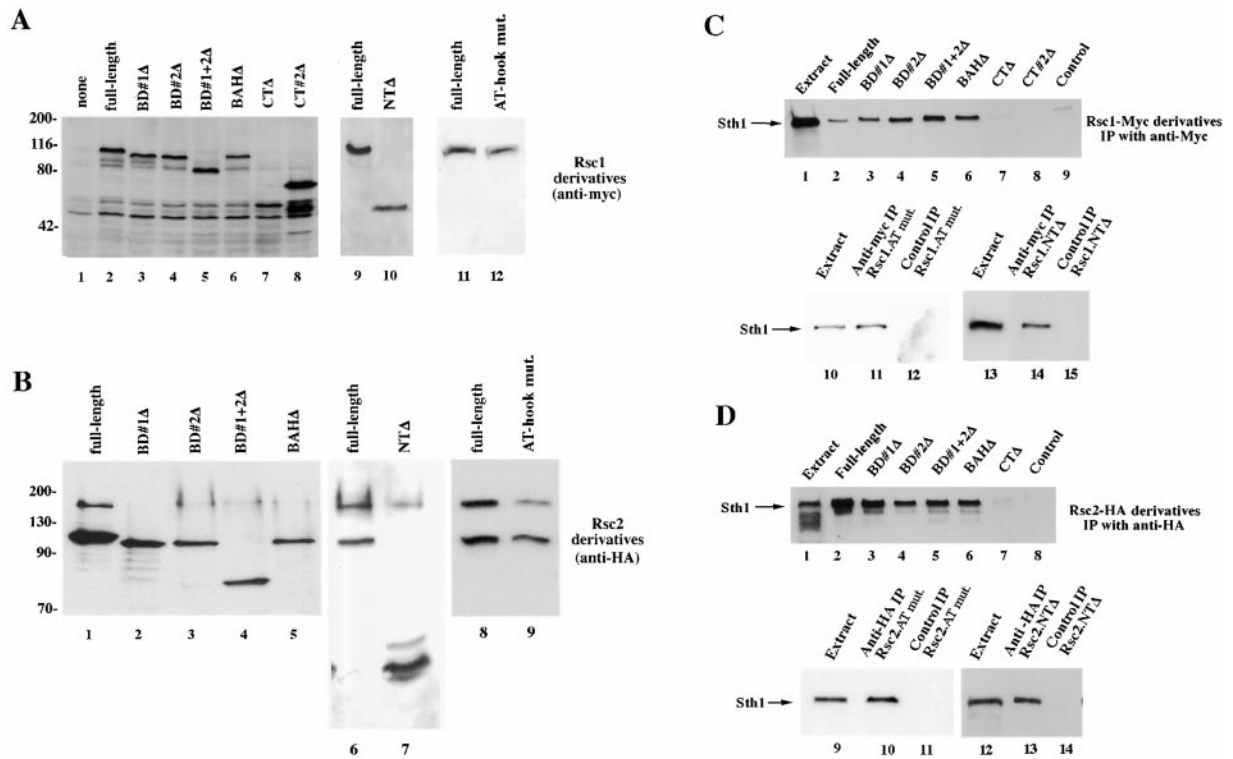


Figure 4. Expression of Mutant Rsc1 and Rsc2 Derivatives, and Their Ability to Assemble into RSC Complex

(A) Expression of Rsc1 derivatives. YBC622 (*rsc1Δ*) was transformed with one of a series of plasmids directing the synthesis of myc-tagged Rsc1 mutant derivatives. Extracts (100 μ g) were separated on a SDS-7.5% acrylamide gel and immunoblotted with anti-myc antibodies. (B) Expression of Rsc2 derivatives. YBC82 (*rsc2Δ*) was transformed with one of a series of plasmids directing the synthesis of HA-tagged Rsc2 mutant derivatives. Extracts (100 μ g) were separated on a SDS-7.5% acrylamide gel and immunoblotted with anti-HA antibodies. (C) Assembly of Rsc1 derivatives into RSC complex. YBC622 (*rsc1Δ*) was transformed with one of a series of plasmids directing the synthesis of myc-tagged Rsc1 mutant derivatives. Extracts were utilized in immunoprecipitation experiments using anti-myc antibodies (except control). For each, half of the IP pellet derived from an IP with 400 μ g of whole-cell extract was loaded on a SDS-7.5% acrylamide gel and immunoblotted with affinity-purified anti-Sth1 antibodies. Extract, 100 μ g of an extract from YBC622 transformed with pRSC1.3XMYC. Control, half of the IP pellet of the pRSC1.3XMYC extract treated with protein G-agarose beads only. (D) YBC82 (*rsc2Δ*) was transformed with one of a series of plasmids directing the synthesis of HA-tagged Rsc2 mutant derivatives. Extracts were utilized in immunoprecipitation experiments using anti-HA antibodies (except control). For each, half of the IP pellet derived from an IP with 200 μ g of whole-cell extract was loaded on a SDS-7.5% acrylamide gel and immunoblotted with affinity-purified anti-Sth1 antibodies. Extract, 100 μ g of an extract from YBC82 transformed with pRSC2.2XHA. Control, half of the IP pellet of the pRSC2.2XHA extract treated with protein G-agarose beads only. For both Rsc1 and Rsc2, all derivatives that coprecipitate Sth1 also coprecipitate Arp9 and Rsc6, and those that fail to coprecipitate Sth1 also fail to coprecipitate Arp9 and Rsc6 (data not shown).

All Rsc1 and Rsc2 Functions Require Bromodomain #2, the BAH, and the C-Terminal Region, whereas Only a Subset Require Bromodomain #1 or the AT Hook

The *rsc1Δ* and *rsc2Δ* mutant phenotypes allow us to determine the requirement for each of the domains in Rsc1 and Rsc2 function. For both Rsc1 and Rsc2, we prepared epitope-tagged derivatives lacking either BD#1, BD#2, both BD#1 and BD#2, the BAH domain, the amino terminus (NT), or the C terminus (Figure 1D). A site-directed mutation in the AT hook of Rsc1 and Rsc2 was prepared by replacing the central conserved GRP motif with three alanines (Figure 1D). All derivatives were placed on a low copy number plasmid and were produced at levels within 2-fold of the epitope-tagged full-length protein (Figures 4A and 4B).

For functional analysis, plasmids encoding each derivative were used to transform a *rsc1Δ* mutant or a *rsc2Δ* mutant, and the mutant phenotypes were assessed (Figures 3A and 3B). This analysis demonstrated

that, for both Rsc1 and Rsc2, BD#2, the BAH region, and C terminus are absolutely required for function. That is, the *rsc1* and *rsc2* mutants lacking these regions have phenotypes identical to the null mutations. In contrast, BD#1 is not required for any of the Rsc1 functions tested, whereas BD#1 is required for Rsc2 function with caffeine (Figure 3B). In addition, the AT hook is required for Rsc1 functions (Figure 3A), but not for Rsc2 functions (Figure 3B). To determine whether each derivative could confer viability to a *rsc1Δ rsc2Δ* strain, plasmids encoding each derivative were used to transform a *rsc1Δ rsc2Δ* strain that already contained a *URA3*-marked *RSC1* plasmid, or a *URA3*-marked *RSC2* plasmid. Importantly, we find that Rsc1 or Rsc2 derivatives that lack either BD#2, the AT hook, the BAH domain, or the C terminus do not suppress the *rsc1Δ rsc2Δ* double mutant lethality (Figure 3C). For example, in a *rsc1Δ* strain, the Rsc2 BD#2, AT hook, BAH domain, and C terminus are all essential for viability. In contrast, BD#1 is not required for growth ability. Remarkably, although the AT hook is not required

Table 1. Double Mutant Combinations Involving *rsc1Δ*, *rsc2Δ*, and Mutations in Other Chromatin-Remodeling Factors

Relevant Genotype	Growth Phenotype				
	Rich Complete	15 mM Caffeine	2% Formamide	Galactose	150 mM Hydroxyurea
Wild type ^a	+	+	+	+	+
<i>rsc1Δ</i> ^b	+/-	-	+/-	+/-	-/+
<i>rsc2Δ</i> ^c	+/-	-	-/+	+	+/-
<i>spt20Δ</i> ^d	+/-	+/-	+/-	-/+	-/+
<i>gcn5Δ</i> ^e	+	+/-	-/+	+/-	+
<i>spt3Δ</i> ^f	+	+/-	+	+/-	+
<i>sin3Δ</i>	+	+/-	+	+	+
<i>snf2Δ</i> ^h	+ ¹	+/-	+	+/-	+/-
<i>rsc1Δ rsc2Δ</i> ⁱ	inviabile				
<i>rsc1Δ spt20Δ</i> ^j	-/+	-	-/+	-	-
<i>rsc2Δ spt20Δ</i> ^k	inviabile				
<i>rsc1Δ gcn5Δ</i>	+/-	-	-/+	-/+	-
<i>rsc2Δ gcn5Δ</i> ^m	-	-	-	-	-
<i>rsc1Δ spt3Δ</i> ⁿ	-/+	-	-/+	-/+	-
<i>rsc2Δ spt3Δ</i> ^o	-/+	-	-	-	-
<i>rsc1Δ sin3Δ</i> ^p	-/+	-	ND	-/+	-/+
<i>rsc2Δ sin3Δ</i> ^q	-/+	-	-	-/+	-/+
<i>rsc1Δ snf2Δ</i> ^r	-/+	-	-/+	-	-
<i>rsc2Δ snf2Δ</i> ^s	-/+	-	-	-	-

Scoring: (wild-type growth) + > +/- > -/+ > - (extremely slow growth).

Strains: ^aFY120; ^bYBC622; ^cYBC82; ^dFY1106 (*psi*⁻); ^eFY1294; ^fFY403; ^gYBC634; ^hYBC28; ⁱYBC643; ^jYBC804 (*psi*⁻); ^kYBC660 (*psi*⁻); ^lYBC699; ^mYBC701; ⁿYBC724; ^oYBC796; ^pYBC702; ^qYBC703; ^rYBC721; ^sYBC705.

¹ *snf2Δ* strains that are Trp⁺ and Leu⁺ (YBC28) grow well.

ND, not determined.

for Rsc2-specific functions, the AT hook must be present in either Rsc1 or Rsc2 for cell viability (Figures 3C and 3D). These results demonstrate that the two bromodomains in Rsc1 and Rsc2 are functionally distinct, provide strong evidence for an essential AT hook in yeast, and demonstrate a functional requirement for a BAH domain.

The BAH and Bromodomains Have No Role in RSC Assembly, whereas the C Terminus Is Necessary and Sufficient for RSC Interaction

To examine the ability of Rsc1 and Rsc2 derivatives to assemble into RSC, we tested for interactions with other RSC components by coimmunoprecipitation. Immunoprecipitation of Rsc1 or Rsc2 derivatives was effective in coimmunoprecipitating Sth1 (Figure 4) as well as Rsc6 and Arp9 (data not shown) from extracts of the bromodomain deletion mutants, the BAH deletion mutant, and the AT hook mutant. In contrast, immunoprecipitation of the C-terminal deletions failed to coimmunoprecipitate other RSC components. The C terminus itself is sufficient for assembly, as Rsc1 and Rsc2 derivatives containing only the C terminus (Rsc1.NTΔ and Rsc2.NTΔ) coimmunoprecipitate RSC components (Figures 4C and 4D). Together, these results strongly suggest that the C-terminal portion of Rsc1 or Rsc2 is necessary and sufficient for assembly into RSC and that the other domains of Rsc1 and Rsc2 must have important alternative functions.

Phenotypes Conferred by Combining *rsc1Δ* or *rsc2Δ* Mutations with Mutations in Genes Encoding SAGA or SWI/SNF Complex Members

Recent studies have established that SWI/SNF and the SAGA complex control transcription of partially overlapping sets of genes and that this overlap is evidenced by

the requirement for either SWI/SNF or SAGA for viability (Roberts and Winston, 1997; Holstege et al., 1998; Biggar and Crabtree, 1999; Pollard and Peterson, 1998; Sudarsanam et al., 1999). To determine whether RSC might contribute to transcription in a similar fashion, we combined *rsc1Δ* or *rsc2Δ* mutations with mutations in SAGA-encoding genes. We tested three different classes of SAGA mutants: *spt20Δ*, believed to abolish all SAGA function; *gcn5Δ*, required for SAGA's HAT activity; and *spt3Δ*, required for a HAT-independent activity of SAGA (Roberts and Winston, 1997; Grant et al., 1998; Sterner et al., 1999). Double mutant analysis (Table 1) revealed two clear trends. First, the *rsc1Δ* and *rsc2Δ* mutations showed the same pattern of synthetic phenotypes with the different classes of SAGA mutations as was previously observed for *swi/snf* mutations: lethality in combination with *spt20Δ* and less severe growth defects in combination with *spt3Δ* and *gcn5Δ*. These double mutant phenotypes are specific for defects in SAGA function and not all *spt* mutants, as combining a *rsc1Δ* or *rsc2Δ* mutation with a mutation in *SPT16* (which encodes a protein not present in SAGA) did not cause synthetic phenotypes. Second, double mutants containing *rsc2Δ* mutations displayed a more severe growth defect than those containing *rsc1Δ* mutations. This difference may reflect the greater abundance of Rsc2 protein compared to Rsc1 or a greater reliance of the Rsc1-containing RSC complex on SAGA function. Taken together, these results provide genetic evidence that RSC and SAGA are functionally linked and provide further evidence that RSC regulates transcription via chromatin remodeling.

To determine whether there are cellular processes that require either RSC or SWI/SNF function, we constructed *rsc1Δ snf2Δ* and *rsc2Δ snf2Δ* double mutants. We find that both *rsc1Δ snf2Δ* and *rsc2Δ snf2Δ* double

mutants grow very slowly (Table 1). This result shows that the modest defects in RSC function conferred by lack of Rsc1 or Rsc2 make yeast cells much more reliant on SWI/SNF function for growth ability, and vice versa.

Discussion

Here, we have identified and characterized Rsc1 and Rsc2, two previously unstudied members of the RSC nucleosome-remodeling complex. These studies have led to three sets of discoveries. First, Rsc1 and Rsc2 are homologs that identify distinct forms of RSC complexes. Second, Rsc1 and Rsc2 are partially redundant, as *rsc1Δ rsc2Δ* double mutants are inviable while *rsc1Δ* and *rsc2Δ* single mutations are viable and cause certain distinct phenotypes. Finally, Rsc1 and Rsc2 contain three motifs required for their function: bromodomains, a BAH domain, and an AT hook motif. Analysis of mutants lacking each motif has demonstrated that they are not required for assembly of Rsc1 or Rsc2 into RSC complexes; therefore, they are likely required for functions of the complexes themselves. Our demonstration of Rsc1 and Rsc2 in separate RSC subcomplexes seems likely to underlie the phenotypic differences of *rsc1Δ* and *rsc2Δ* mutants, as each complex may regulate both unique and common gene targets. At least one of these common genes is likely essential for viability, as a *rsc1Δ rsc2Δ* double mutant is inviable.

The Two Bromodomains within Rsc1 and Rsc2 Play Different Roles

Our studies of the bromodomains in Rsc1 and Rsc2 have provided strong evidence that one of the bromodomains (BD#2) in each protein is essential for protein function. The bromodomain from the protein PCAF has been shown to bind acetyl-lysine, suggesting that bromodomains anchor proteins (or protein complexes) to acetylated chromatin (Dhalluin et al., 1999). Other recent experiments suggest that bromodomains can bind to the amino-terminal tails of histones H3 and H4 (Ornaghi et al., 1999), the recurrent targets of modification by acetylases and deacetylases. Taken together, these studies suggest that bromodomains could serve one or more roles in RSC and other bromodomain-containing complexes. For example, bromodomain/histone tail interactions could regulate RSC activity. However, given the likely roles of RSC in nucleosome remodeling and transcriptional control, we favor the possibility that bromodomain/histone tail interactions are important for maintaining RSC at acetylated nucleosomes and in maintaining the promoter's remodeled state.

The BAH and AT Hook Motifs Are Also Required for Rsc1 and Rsc2 Functions

Here, we demonstrate that the BAH domain is essential for all functions of Rsc1 and Rsc2. Like BD#2, the BAH domains and AT hooks of Rsc1 and Rsc2 are partially redundant for viability and, therefore, may help RSC associate with essential genes. Interestingly, the AT hook is required for most Rsc1 functions tested but not for the Rsc2-specific functions tested. Many proteins with AT hooks also contain bromodomains, and the AT hook often precedes the bromodomain by 20–40 amino

acids (i.e., Snf2/Swi2 and hBrm). We propose that AT hooks may collaborate with bromodomains to specify interactions with particular nucleosomes. In mammalian hBrm, the ATPase for one of the two human SWI/SNF complexes, the AT hook, has been shown to bind DNA and to affect association of the complex with chromatin (Bourachot et al., 1999). For RSC, the ATPase Sth1 lacks an AT hook, and therefore this function may be provided by Rsc1/Rsc2.

Rsc1 and Rsc2 are highly similar to the metazoan protein polybromo, which contains five bromodomains followed by two BAH regions and an HMG-like domain (Nicolas and Goodwin, 1996). Based on the presence of Rsc1 and Rsc2 in RSC, we propose that polybromo is the human ortholog of Rsc1 and Rsc2 and that it is likely to be present in human SWI/SNF complex.

Evidence for RSC in Transcriptional Control

Several experiments support a role for RSC in transcriptional regulation. First, certain *rsc* mutations cause a mild Spt⁻ phenotype, a mutant phenotype shared by many transcription factors (Cairns et al., 1998). Also, recent studies support for a role of RSC in transcription of the *CHA1* gene and certain early meiotic genes (Moriola and Holmberg, 1999; Yukawa et al., 1999). Our double mutant analysis presented here provides additional evidence that RSC regulates transcription via chromatin remodeling, as *rsc1Δ* or *rsc2Δ* mutations, in combination with mutations that either impair or abolish SAGA function, cause either inviability or strong mutant phenotypes.

Nucleosome-Remodeling Complexes in *S. cerevisiae*

In *S. cerevisiae*, recent studies have highlighted the complexity of transcriptional control by nucleosome-remodeling complexes. Yeast contains many nucleosome-remodeling complexes that may, like RSC, display interesting compositional heterogeneity (Tsukiyama et al., 1999). In combination with the other large transcription complexes that have been identified and studied, including SAGA, ADA, and two forms of RNA polymerase II holoenzyme (Hampsey, 1999), sorting out distinct and redundant roles for each presents a formidable and important challenge for future investigations.

Experimental Procedures

Media, Genetic Methods, and Strains

Rich media (YPD), synthetic complete (SC) media, and sporulation media were prepared as described in Rose et al. (1990). Standard procedures were used for transformations, sporulation, and tetrad analysis. *S. cerevisiae* strains are all isogenic derivatives of S288C. Due to space restrictions, full genotypes for yeast strains are available on request.

Plasmids

The pRS series of plasmids is described in Sikorski and Hieter (1989). A cosmid (pEGH119) containing the *RSC1* gene was the gift of H. Tettelin. A 7 kb *Cl*I fragment containing *RSC1* was cloned into the *Cl*I site of pRS316 (*URA3, CEN6*) to create pNCU.RSC1. A 5 kb *Cl*I/*Spe*I fragment of pNCU.RSC1 was cloned into the *Cl*I/*Spe*I sites of pRS314 to create pNCT.RSC1. Details for construction of a plasmid directing the synthesis of triple myc-tagged Rsc1 (pNCT.RSC1.3XMYC) are available on request. Plasmid pNCT.RSC1.3XMYC fully complements *rsc1Δ*.

Deletion derivatives of myc-tagged Rsc1 (except Rsc1.NT Δ) were constructed by PCR amplification of two DNA fragments from the 5' and 3' ends of RSC1.3XMYC, using *Pfu* polymerase and specific oligonucleotide primers (details available on request). The plasmids encoding RSC1 deletion derivatives lack the following amino acids: p314.RSC1.BD#1 Δ , K31-P123; p314.RSC1.BD#2 Δ , K242-L338; p314.RSC1.BD1+2 Δ , K31-L338; p314.RSC1.BAH Δ , N391-Q495; p314.RSC1.NT Δ , K31-Q495; p314.RSC1.CT Δ , L496-L911; p314.RSC1.CT#2 Δ , R604-L911. A high-copy (2 μ m origin) plasmid bearing RSC1 was prepared by cloning the ClaI/SacII fragment of p314.RSC1.3XMYC into the ClaI/SacII sites of pRS424 to afford p424.RSC1.3XMYC.

A cosmid (9638) containing the RSC2 gene was obtained from the ATCC (#71198). Construction of plasmids directing synthesis of double HA-tagged Rsc2 (p316.RSC2.2XHA [*URA3 CEN6*] and p314.RSC2.2XHA [*TRP1 CEN6*]) is available on request, as are procedures for preparing deletion derivatives. The plasmids encoding RSC2 deletion derivatives lack the following amino acids: p426.RSC2.BD#1 Δ and p314.RSC2.BD#1 Δ , K59-P126; p426.RSC2.BD#2 Δ and p314.RSC2.BD#2 Δ , K282-L378; p426.RSC2.BD1+2 Δ and p314.RSC2.BD#1+2 Δ , K59-L378; p426.RSC2.BAH Δ and p314.RSC2.BAH Δ , N431-Q535; p314.RSC2.NT Δ , K59-Q535; p314.RSC2.CT Δ , P580-S889.

Preparation of Whole-Cell Extracts

Strains were grown in 50 ml of selective media at 30°C with shaking to an OD₆₀₀ of 2.0. Cells were harvested, washed with 1 ml breaking buffer (50 mM Tris [pH 7.5], 12% glycerol, 500 mM NaCl, 0.1% Triton X-100, 0.5 mM DTT, 5 mM EDTA) with a protease inhibitor cocktail (0.0142 mg leupeptin, 0.0685 mg pepstatin A, 0.01 mg chymostatin, 8.5 mg PMSF, 16.5 mg benzamide), and pelleted by centrifugation at 8000 \times g. Cell pellets were resuspended in twice the cell volume of breaking buffer, placed in 0.8 ml microfuge tubes, and disrupted by bead beating with 0.4 ml of zirconia beads (0.5 mm diameter) at 4°C with three 5 min pulses, separated by 5 min of cooling on ice. Extracts were recovered with a 10 min spin at 10,000 rpm in a microfuge at 4°C.

Immunoprecipitation Analysis

Anti-myc or anti-HA antibodies were prebound to protein G-agarose beads at a density of 0.5 mg/ml in IP buffer (50 mM Tris-Cl [pH 7.5], 10% glycerol, 100 mM NaCl, 1 mM EDTA, 0.05% Tween-20) containing a protease inhibitor cocktail and were stored at 4°C as a 50% slurry with 0.02% sodium azide. Each immunoprecipitation mixture (100 μ l) contained 200 μ g of whole-cell extract (400 μ g for anti-myc immunoprecipitations) and 40 μ l of antibody beads (50% slurry) in IP buffer and was rotated in microfuge tubes at 4°C for 5 hr. Precipitates were recovered by centrifugation at 8000 \times g in a microfuge at 4°C, and the supernatants were isolated and stored at -80°C. Immune precipitates were washed three times with IP buffer containing 250 mM NaCl. SDS loading buffer (30 μ l) was added to the recovered precipitates, and they were heated at 94°C for 2 min before loading on an SDS-7.5% acrylamide gel. Immunoblotting utilized standard methods for chemiluminescent detection.

Isolation and Identification of Rsc1 and Rsc2

RSC purification, peptide sequencing, mass spectrometric analyses, and sequence comparisons were performed exactly as described previously (Cairns et al., 1996).

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References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.
- Aravind, L., and Landsman, D. (1998). AT hook motifs in a wide variety of DNA-binding proteins. *Nucleic Acids Res.* 26, 4413-4421.
- Biggar, S.R., and Crabtree, G.R. (1999). Continuous and widespread role for Swi-Snf complex in transcription. *EMBO J.* 18, 2254-2264.
- Bourachot, B., Yaniv, M., and Muchardt, C.M. (1999). The activity of brm/Snf2a is dependent on a high-mobility group protein I/Y-like DNA binding domain. *Mol. Cell. Biol.* 19, 3931-3939.
- Cairns, B.R. (1998). Chromatin remodeling machines: similar motors, ulterior motives. *Trends Biochem. Sci.* 23, 20-25.
- Cairns, B.R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R.D. (1996). RSC, an essential, abundant chromatin remodeling complex. *Cell* 87, 1249-1260.
- Cairns, B.R., Erdjument-Bromage, H., Tempst, P., Winston, F., Kornberg, R.D. (1998). Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol. Cell* 2, 639-651.
- Callebaut, I., Courvalin, J.C., and Mornon, J.P. (1999). The BAH (bromo-adjacent homology) domain: a link between DNA methylation, replication, and transcriptional regulation. *FEBS Lett.* 446, 189-193.
- Candau, R., Zhou, J.X., Allis, C.D., and Berger, S.L. (1997). Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. *EMBO J.* 16, 555-565.
- Cao, Y., Cairns, B.R., Kornberg, R.D., and Laurent, B.L. (1997). Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. *Mol. Cell. Biol.* 17, 3323-3334.
- Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.-M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. *Nature* 399, 491-496.
- Du, J., Nasir, I., Benton, B.K., Kladden, M.P., and Laurent, B.C. (1998). Sth1p, a *Saccharomyces cerevisiae* Snf2p/Swi2p homolog, is an essential ATPase in RSC and differs from Snf/Swi in its interactions with histones and chromatin-associated proteins. *Genetics* 150, 987-1005.
- Erdjument-Bromage, H., Lui, M., Sabatini, D.M., Snyder, S.H., and Tempst, P. (1994). High-sensitivity sequencing of large proteins: partial structure of the rapamycin-FKBP12 target. *Protein Sci.* 3, 2435-2446.
- Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., III, and Workman, J.L. (1998). A subset of TAFII are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* 94, 45-53.
- Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature* 389, 349-352.
- Hampsey, M. (1999). Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol. Mol. Biol. Rev.* 62, 465-503.
- Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* 25, 717-728.
- Huth, J.R., Bewley, C.A., Nissen, M.S., Evans, J.N., Reeves, R., Gronenborn, A.M., Clore, G.M. (1997). The solution structure of an HMG-I(Y)-DNA complex defines a new architectural minor groove binding motif. *Nat. Struct. Biol.* 4, 657-665.
- Jeanmougin, F., Wurtz, J.M., Le Douarin, B., Chambon, P., and Losson, R. (1997). The bromodomain revisited. *Trends Biochem. Sci.* 22, 151-153.
- Kingston, R.E., Bunker, C.A., and Imbalzano, A.N. (1996). Repression and activation by multiprotein complexes that alter transcription. *Genes Dev.* 10, 905-920.
- Kuo, M.H., and Allis, D. (1998). Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* 20, 615-626.
- Laurent, B.C., Yang, X., and Carlson, M. (1992). An essential *Saccharomyces cerevisiae* gene homologous to SNF2 encodes a heliase-related protein in a new family. *Mol. Cell. Biol.* 12, 1893-1902.

- Moriera, J.M., and Holmberg, S. (1999). Transcriptional repression of the yeast *CHA1* gene requires the chromatin-remodeling complex RSC. *EMBO J.* *18*, 2836–2844.
- Nicolas, R.H., and Goodwin, G.H. (1996). Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG box, and two repeats of a novel domain. *Gene* *175*, 233–240.
- Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.J., Howard, B.H., Qin, J., Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. *Cell* *94*, 35–44.
- Ornaghi, P., Ballario, P., Lena, A.M., Gonzalez, A., and Filetici, P. (1999). The bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4. *J. Mol. Biol.* *287*, 1–7.
- Owen-Hughes, T., and Workman, J.L. (1994). Experimental analysis of chromatin function in transcriptional control. *Crit. Rev. Eukaryot. Gene Expr.* *4*, 403–441.
- Peterson, C.L., and Tamkun, J. (1995). The SWI/SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.* *20*, 143–146.
- Pollard, K.J., and Peterson, C.L. (1998). Chromatin remodeling: a marriage between two families. *Bioessays* *20*, 771–780.
- Reeves, R., and Nissen, M.S. (1990). The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. *J. Biol. Chem.* *265*, 8573–8582.
- Roberts, S., and Winston, F. (1997). Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/Mediator complexes. *Genetics* *147*, 451–465.
- Rose, M., Winston, F., and Hieter, P. (1990). *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
- Sikorski, R.S., and Hieter, P. (1989). A system of vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* *122*, 19–27.
- Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L., and Berger, S.L. (1999). Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. *Mol. Cell. Biol.* *19*, 86–98.
- Sudarsanam, P., Cao, Y., Wu, L., Laurent, B.C., and Winston, F. (1999). The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. *EMBO J.* *18*, 3101–3106.
- Treich, I., and Carlson, M. (1997). Interaction of a Swi3 homolog with Sth1 provides evidence for a Swi/Snf-related complex with an essential function in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* *17*, 1768–1775.
- Tripoulas, N., LaJeunesse, K., Gildea, J., and Shearn, A. (1996). The *Drosophila* ash1 gene product, which is localized at specific sites on polytene chromosomes, contains a SET domain and a PHD finger. *Genetics* *143*, 913–928.
- Tsuchiya, E., Uno, A., Masuoka, K., Kanemori, Y., Okabe, S., and Mikayawa, T. (1992). The *Saccharomyces cerevisiae* NPS1 gene, a novel CDC gene which encodes a 160 kDa nuclear protein involved in G2 phase control. *EMBO J.* *11*, 4017–4026.
- Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J., and Wu, C. (1999). Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev.* *13*, 686–697.
- Wolfe, K.H., and Shields, D.C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* *387*, 708–713.
- Winston, F., and Allis, C.D. (1999). The bromodomain: a chromatin-targeting module? *Nat. Struct. Biol.* *6*, 601–604.
- Workman, J., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* *67*, 545–579.
- Yukawa, M., Katoh, S., Miyakawa, T., and Tsuchiya, E. (1999). Nps1/Sth1p, a component of an essential chromatin-remodeling complex of *Saccharomyces cerevisiae*, is required for the maximal expression of early meiotic genes. *Genes Cells* *4*, 99–110.