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DNA-binding properties of the yeast SWI/SNF complex

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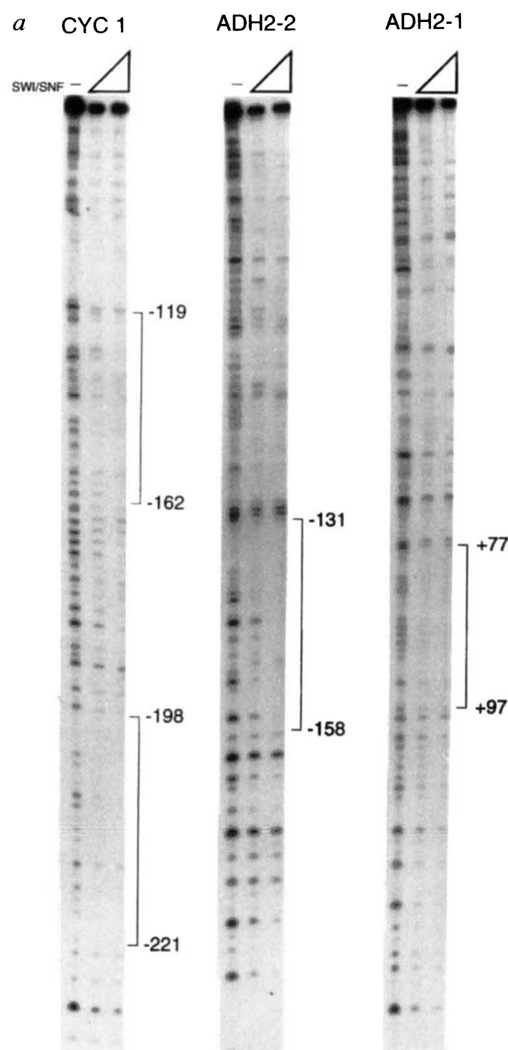
THE SWI/SNF complex is required for the enhancement of transcription by many transcriptional activators in yeast[1,2]. Genetic and biochemical studies indicate that the complex facilitates activator function by antagonizing chromatin-mediated transcriptional repression[3–6]. The absence of known DNA-binding motifs in several SWI/SNF subunits and the failure to identify SWI/SNF-dependent DNA-binding activities in crude yeast extracts have led to the belief that the complex does not bind DNA[7,8]. Here we show that the SWI/SNF complex has a high affinity for DNA and that its DNA-binding properties are similar to those of proteins containing HMG-box domains[9]. The complex interacts with the minor groove of the DNA helix, binds synthetic four-way junction DNA, and introduces positive supercoils into relaxed plasmid DNA. These properties are likely to be important in the remodelling of chromatin structure by the SWI/SNF complex.

A gel retardation assay was used to test whether the purified yeast SWI/SNF complex binds DNA. The data in Fig. 1 show that the SWI/SNF complex binds with high affinity to promoter sequences from both SWI-dependent (*SUC2* and *ADH2*) and SWI-independent (*CYC1*) genes (Fig. 1*a*). Based on the concentration of SWI/SNF complex needed to bind half the probe DNA, we estimate the apparent binding constant to be $1\text{–}9 \times 10^{-9}$ M (this value assumes 1–3 binding sites per probe molecule). We also found that the complex bound equally well to both promoter and coding-region sequences derived from the SWI-dependent gene, *HO*, demonstrating that the complex has no intrinsic affinity for regulatory sequences (results not shown). In all DNA-binding experiments, the addition of ATP had no effect on the formation of protein–DNA complexes (not shown). Although the complex does appear to be rather promiscuous for high-affinity DNA binding, it has some sequence specificity. For instance, all binding reactions contain a 1,000-fold molar excess of the double-stranded DNA poly(dG-dC)·poly(dG-dC). In addition, the complex binds poorly to the upstream activation sequence of the SWI-dependent *INO1* gene (Fig. 1*a*) and the 154-base-pair (bp) GAL4-binding sequence used in our previous study[6] (data not shown). Furthermore, binding of the complex to *ADH2* and *CYC1* DNA probes resulted in protection of specific sequences from DNase (Fig. 2*a*). As in the gel retardation assays, addition of ATP to footprinting reactions had no effect (data not shown).

Four observations indicate that this DNA-binding activity is due to SWI/SNF complex. First, SWI/SNF complex can be fractionated on a native-DNA cellulose column (Fig. 1 legend). Second, DNA binding activity co-elutes with SWI/SNF complex from the final gel filtration column (data not shown). Third, fractions from a mock SWI/SNF purification do not contain detectable DNA-binding activity (data not shown). Fourth, addition of a monoclonal antibody directed against an engineered epitope at the C



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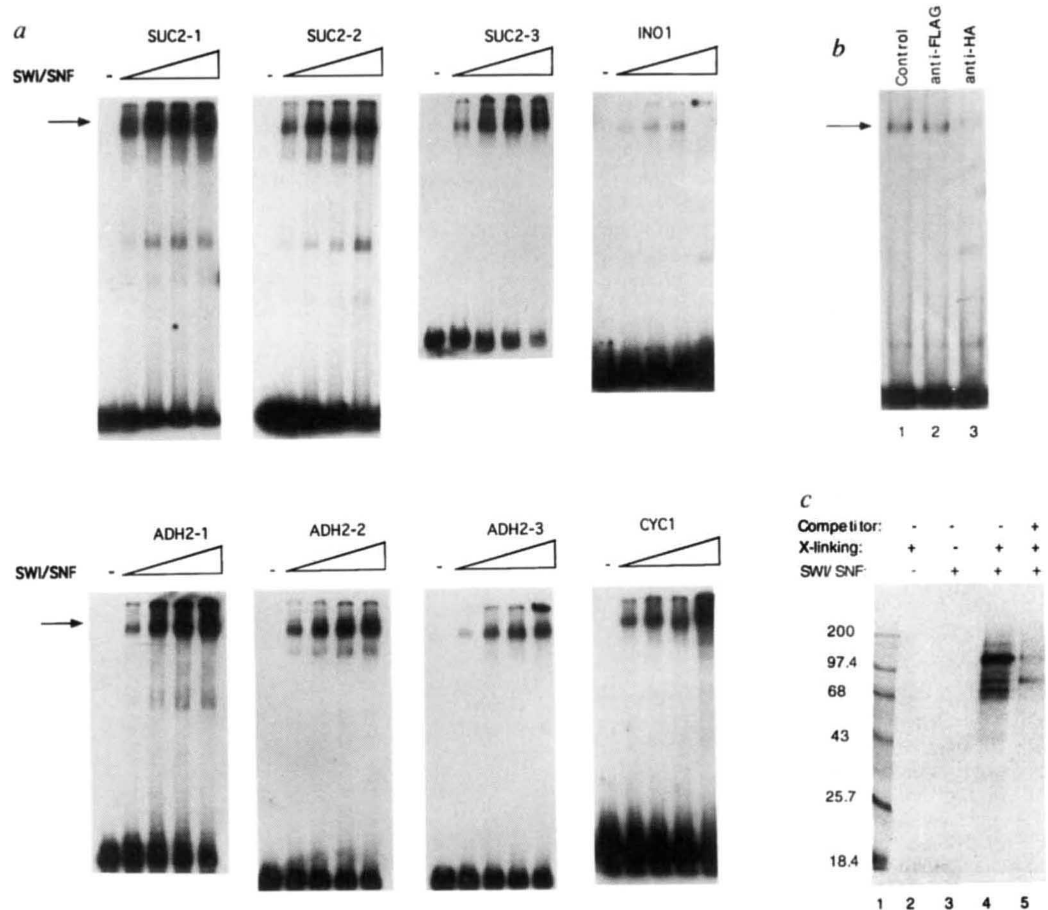

FIG. 1 The yeast SWI/SNF complex binds to DNA. *a*, Gel retardation analysis. Each panel shows a set of parallel gel retardation experiments in which increasing amounts of SWI/SNF complex (1.5 to 6.0 nM) were incubated with 0.1 nM ^{32}P-labelled promoter sequences derived from both SWI-dependent (*SUC2*, *ADH2*, *INO1*) and SWI-independent (*CYC1*) genes. Probes used in these experiments include: SUC2-1, SUC2-2 and SUC2-3, which contain bases −171 to +14, −171 to −386 and −386 to −712, respectively, of the *SUC2* promoter; INO1 encompasses −126 to −333 of the *INO1* promoter; ADH2-1, ADH2-2 and ADH2-3 contain −54 to +116, −182 to −54, and −413 to −182, respectively, of the *ADH2* promoter; CYC1 encompasses −249 to +11 of the *CYC1* promoter. *b*, Antibody supershifts. DNA-binding reactions were supplemented with 80 ng anti-FLAG monoclonal antibody (lane 2), 80 ng 12CA5 monoclonal antibody (lane 3) or received no antibody (lane 1). *c*, UV crosslinking of the SWI/SNF complex to the SUC2-3 probe. Lane 1, no UV; lane 2, no SWI/SNF; lane 3, +UV, +6 nM SWI/SNF; lane 4, identical to lane 3 but reactions contained a 20-fold molar excess of unlabelled probe.

METHODS. DNA-binding assays (15 μl) contained DNA-binding buffer (4 mM Tris-Cl, pH 7.4, 5mm $MgCl_2$, 75 mM NaCl, 1 mM DTT, 0.05 mg ml^{-1} BSA, 4% glycerol), 300 ng poly(dG-dC)·poly(dG-dC), 0.1 nM end-labelled ^{32}P-probe DNA, and 1.5 to 6 nM SWI/SNF complex. Protein–DNA complexes were resolved on 4% polyacrylamide (80:1 acrylamide:bisacrylamide ratio)/0.4× TBE gels, and analysed by autoradiography. SWI/SNF complex was purified as described[6], with an additional DNA-cellulose column step. Mock SWI/SNF purifications used strain CY340, isogenic to strain CY396 (ref. 6), which does not express the SWI2-HA-6HIS fusion. Fractions from the Mono-Q step were diluted to 100 mM NaCl and loaded onto a 0.5 ml DNA–cellulose column pre-equilibrated in DNA-binding buffer. Bound protein was eluted with DNA-binding buffer containing 300 mM NaCl, and then subjected to gel filtration as described[6]. UV crosslinking: the SUC2-3 probe was internally labelled with [α-^{32}P]dCTP, and bromodeoxyuridine was incorporated in place of dTTP to increase the efficiency of crosslinking. Standard DNA-binding reactions were UV-irradiated as described[25], treated with 1 unit DNase I for 30 min at 37 °C and analysed on 10% SDS–PAGE.

b

ADH2-1	$^{+77}$ TTCCAAAGCCAAAGCCCAACG^{+97}
ADH2-2	$^{-158}$AAATAGAGTGCCAGTAGCGACTTTTTTC^{-131}
CYC1-a	$^{-162}$ACGACACATGATCATATGGCATGCATGTGCTCTGTATGTATAT^{-119}
CYC1-b	$^{-221}$ TAGCGTGGATGGCCAGGCAACTTT^{-198}

FIG. 2 *a*, Footprinting analysis of the yeast SWI/SNF complex. DNAse I digestion patterns of the CYC1, ADH2-1 and ADH2-2 probes, either in the absence (lanes 1, 4 and 7) or in the presence of 6 nM (lanes 2, 5 and 8) or 12 nM (lanes 3, 6 and 9) SWI/SNF complex. *b*, Sequence comparison of SWI/SNF binding sites.

METHODS. Gel retardation assays were scaled up by a factor of two. Binding reactions were incubated for 30 min, followed by DNase I digestion (0.03 U) for 1 min at room temperature. Reactions were stopped by the addition of 5 volumes of STOP (5 mM Tris, pH 7.5, 175 mM NaCl, 10 mM EDTA, 3.5 M urea, 1% SDS), phenol/chloroform-extracted and ethanol-precipitated. Samples were analysed on 8% polyacrylamide/8M urea sequencing gels.

terminus of SWI2 disrupts and supershifts the DNA–protein complex (Fig. 1*b*, lane 3). Addition of a control antibody had no effect (Fig. 1*b*, lane 2). Ultraviolet crosslinking experiments were done to identify which of the 11 polypeptides[6,10,11] of the SWI/SNF complex contact DNA. Three polypeptides were consistently crosslinked that corresponded to the 150K SWI1 polypeptide (M_r 150,000) and to two (p68 and p78) components of the complex that have not yet been cloned (Fig. 1*c*).

The DNA-binding properties of the SWI/SNF complex share many features of HMG-box domains. Like several HMG-box-containing polypeptides[9], the SWI/SNF complex does not appear to recognize a consensus DNA sequence (Fig. 2*b*). The minor-groove-binding drugs distamycin A (Fig. 3a) and chromomycin A3 (data not shown), compete with SWI/SNF complex for DNA binding suggesting that, like HMG-box proteins[12–14], the complex interacts (at least in part) with the minor groove. We also found that the SWI/SNF complex, like the HMG-box-domain-containing polypeptides HMG-1 (ref. 15) and UBF (ref. 16), exhibited a DNA-length dependence in binding assays (Fig. 3*b*).

One striking feature of HMG-box domains is their ability to recognize structured DNA[9] such as cruciforms and four-way

junctions[17,18]. As shown in Fig. 3*c*, *d*, the SWI/SNF complex binds with high affinity (apparent binding constant, 1 nM) to synthetic four-way-junction (4WJ) DNA but not to the duplex 'arms'. Binding to the synthetic 4WJ was also sensitive to low concentrations of distamycin (data not shown). The recognition of structured DNA may be crucial for SWI/SNF function: we found that 6 nM synthetic 4WJ was as effective as double-stranded plasmid DNA[6] in stimulating the ATPase activity of the complex (> 6-fold), whereas the same concentration of the duplex 'arms' was completely ineffective. Interestingly, the structure formed in synthetic 4WJ DNA may mimic the crossover point where DNA enters and exits the nucleosome[19].

We also found that the SWI/SNF complex can introduce supercoils into circular DNA in the presence of bacterial topoisomerase I (Fig. 4). SWI/SNF complex is extremely active in this assay as it is able to drive a large change in linking number (> 10) even at a 1:1 ratio of SWI/SNF to plasmid (Fig. 4*a*, lanes 7 and 8). By comparison, the binding of about 200 molecules of HMG-1 per plasmid is required to introduce one superhelical turn[20]. The SWI/SNF complex did not introduce supercoils in the absence of topoisomerase I, nor did this activity require ATP. The observation that eukaryotic topoisomerases are able to remove SWI/SNF-induced supercoils (Fig. 4*b*; compare lanes 6 to 8 and 9) where *E. coli* topoisomerase I has no effect (Fig. 4*b*; compare lanes 6 and 7), indicates that the SWI/SNF complex introduces only positive supercoils in this assay. The electrophorectic mobility of the SWI/SNF-induced supercoils on a two-dimensional chloroquine gel is also consistent with positive supercoils (data not shown). In contrast, several HMG-box-containing polypeptides, including HMG-1 (ref. 20), mtTF1 (ref. 21) and ABF2 (ref. 22), induce negative supercoils into DNA in the presence of topoisomerase I.

What is the basis of the SWI/SNF complex-induced supercoiling? One possibility is that the SWI/SNF complex wraps the DNA template in a right-handed direction, introducing positive supercoils. But wrapping is unlikely because SWI/SNF binding does not induce the periodic hypersensitivity to DNase I that is typically observed for such DNA–protein complexes (Fig. 2). Alternatively, SWI/SNF may not wrap DNA but may use the

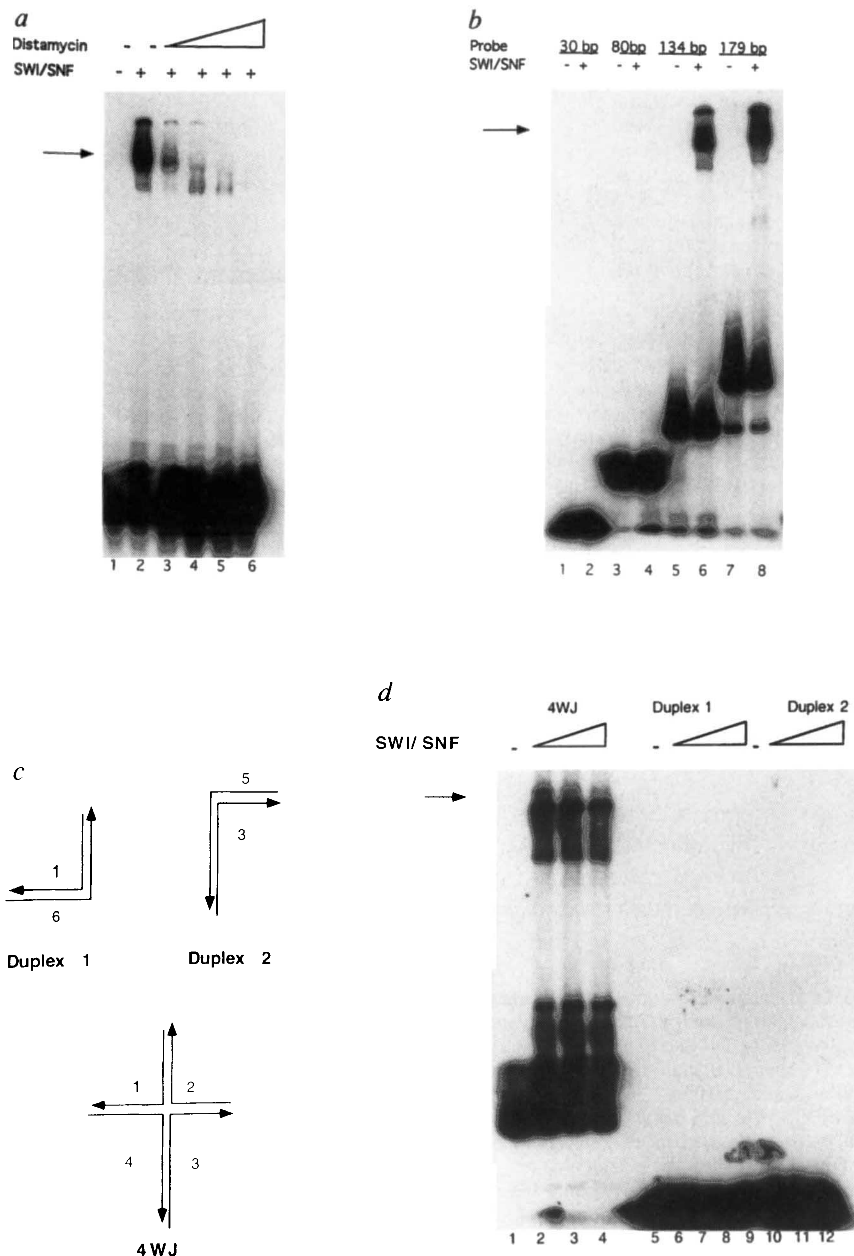

FIG. 3 The SWI/SNF complex has similar DNA-binding properties to HMG-box domains. *a*, The minor-groove-binding reagent distamycin A competes with the SWI/SNF complex for binding to DNA. Increasing amounts of distamycin A were added to DNA-binding reactions, which contained 0.1 nM labelled SUC2-3 fragment and 3 nM SWI/SNF complex, and its effect on binding assessed by gel retardation analysis. Lanes 1, probe DNA alone; 2, probe DNA and the SWI/SNF complex; 3–6, constant amount of SWI/SNF complex with increasing amounts of distamycin A (concentrations used were: lane 3, 0.1 μM (molar ratio of reagent per bp, 0.4); lane 4, 1 μM (molar ratio, 4); lane 5, 10 μM (molar ratio, 40); lane 6, 100 μM (molar ratio, 400). *b*, Binding of SWI/SNF complex to DNA depends on the length of DNA. The complex (3 nM) was tested for binding to the −129 to −158 sequence of the *ADH2* promoter (Fig. 2*b*) which was contained on DNA fragments of different sizes. All DNA probes were present at 0.1 nM in the binding reactions. *c* and *d*, The SWI/SNF complex binds to synthetic cruciform DNA: *c*, the synthetic cruciform DNA (4WJ) and the control duplexes whose sequences are the same as each 'half' of the synthetic cruciform DNA. Numbers 1–6 denote oligonucleotides that were annealed to form the 4WJ (1–4) and control duplexes (1,6 and 5,3)[26]. *d*, a gel retardation analysis of DNA-binding reactions in which the complex (lanes 2, 6, 10: 1.5 nM; lanes 3, 7, 11: 3 nM) was incubated with 0.1 nM cruciform DNA (lanes 2–4) or with 0.1 nM duplex molecules (lanes 6–8 and 10–12).

METHODS. To prepare probes for the length-dependence experiment, oligonucleotides containing bases −129 to −158 of the *ADH2* promoter were annealed to produce the 30-bp probe. The 80-bp probe was generated by PCR amplification of the −89 to −168 sequence of the *ADH2* promoter. Digestion of plasmid pHH26 with *Sal*I or *Eco*RI/*Hin*dIII released fragments of 134 bp and 179 bp, respectively, which contained the −129 to −158 sequence.

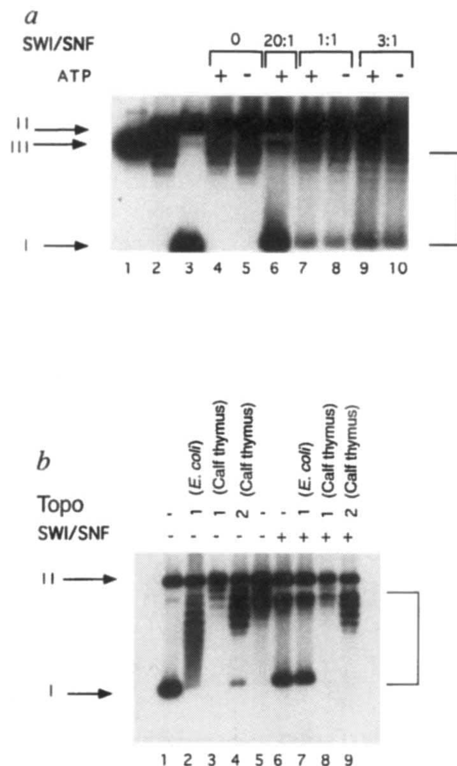

FIG. 4 SWI/SNF introduces positive supercoils into relaxed plasmid DNA in the presence of bacterial topoisomerase I. *a*, SWI/SNF induces supercoiling. Closed relaxed plasmid DNA (lane 2) was incubated with the SWI/SNF complex and bacterial topoisomerase I (topo I; 3 units). Molar ratios of SWI/SNF to plasmid are indicated above each set of lanes. A ratio of 20:1 corresponds to 3 nM SWI/SNF. Addition of ATP to a subset of the reactions is indicated. Arrows to the right denote topology standards, lane 1 contains linear plasmid (form III), lane 3 contains supercoiled plasmid (form I) and some nicked circles (comigrate with form II, closed relaxed DNA). The bracket to the right denotes SWI/SNF-induced topoisomers. *b*, SWI/SNF introduces positive supercoils. Plasmid DNA was supercoiled with SWI/SNF (3 nM) and bacterial topo I as in *a*. DNA was purified (lane 6) and retreated with either bacterial topo I (3 units; lane 7), calf thymus topo I (3 units; lane 8), or calf thymus topo II (7 units; lane 9). Lanes 1–4 show control reactions in which negatively supercoiled plasmid DNA (lane 1) was incubated with each topoisomerase under conditions identical to those for lanes 7–9. Lane 5 shows the starting relaxed substrate DNA. Reactions that contained calf thymus topo II contained 1 mM ATP.

METHODS. Supercoiling reactions (20 μl) contained 1× supercoiling buffer (20 mM HEPES, pH 7.5, 7 mM $MgCl_2$, 15 mM KCl, 0.5 mM DTT, 50 μg per ml BSA), 50 ng pJH28, SWI/SNF, and topoisomerases where indicated. Reactions were incubated for 45 min at 30 °C, stopped with 80 μl 1% SDS, 10 mM EDTA, 100 $\mu g\,ml^{-1}$ proteinase K, 50 $\mu g\,ml^{-1}$ tRNA, and incubated for 30 min at 37 °C. Samples were extracted with phenol/chloroform, ethanol-precipitated, and electrophoresed on 0.8% agarose gels without ethidium bromide and then Southern-blotted. DNA was purified and electrophoresed in the presence of chloroquine as described[27]. Blots were probed with pJH28 labelled with [α-^{32}P]dCTP by random priming. Plasmid pJH28 contains *SUC2* sequences from −1, 100 to +14 in plasmid pRS316.

energy of DNA binding to change the helical twist, resulting in an overwinding of the DNA. Changes in helical twist may destabilize histone–DNA[23] as well as histone–histone interactions[24], so the ability of the SWI/SNF complex to modulate both DNA structure and topology may be important in SWI/SNF-dependent disruption of nucleosome structure. □

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ERRATA

Crystal structure of a G-protein βγ dimer at 2.1 Å resolution

John Sondek, Andrew Bohm, David G. Lambright, Heidi E. Hamm & Paul B. Sigler

Nature **379,** 369–374 (1996)

In this title, a typographical error caused the substitution of a subscripted 'A' for a hyphen in 'G-protein'. The correct title is given here. □

Bifurcations of the Atlantic thermohaline circulation in response to changes in the hydrological cycle

Stefan Rahmstorf

Nature **378,** 145–149 (1995)

The shaded areas A and B of Fig. 1*a* of this Article were lost during printing. The correct figure is shown here. □