# A chromatin remodelling complex involved in transcription and DNA processing

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**The packaging of the eukaryotic genome in chromatin presents barriers that restrict the access of enzymes that process DNA[1,2]. To overcome these barriers, cells possess a number of multi-protein, ATP-dependent chromatin remodelling complexes, each containing an ATPase subunit from the SNF2/SWI2 superfamily[3,4]. Chromatin remodelling complexes function by increasing nucleosome mobility and are clearly implicated in transcription[5–7]. Here we have analysed SNF2/SWI2- and ISWI-related proteins to identify remodelling complexes that potentially assist other DNA transactions. We purified a complex from *Saccharomyces cerevisiae* that contains the Ino80 ATPase[8]. The INO80 complex contains about 12 polypeptides including two proteins related to the bacterial RuvB DNA helicase[9–11], which catalyses branch migration of Holliday junctions. The purified complex remodels chromatin, facilitates transcription *in vitro* and displays 3′ to 5′ DNA helicase activity. Mutants of *ino80* show hypersensitivity to agents that cause DNA damage, in addition to defects in transcription[8]. These results indicate that chromatin remodelling driven by the Ino80 ATPase may be connected to transcription as well as DNA damage repair.**

We searched the *Saccharomyces* genome database for genes that were highly related to *Drosophila ISWI*, which codes for the ATPase subunit of the nucleosome remodelling factor NURF[12]. We found *SWI2/SNF2*, *STH1*, *ISWI*, *ISW2*, *CHD1* and the three open reading frames (ORFs) *YFR038W*, *YGL150C* and *YDR334W*. Ebbert *et al.*[8] identified *YGL150C* in a genetic screen for mutants affecting inositol biosynthesis. Previously we had called it *ARI1* (for *ATPase Related to ISWI*); hereafter, we use the *INO80* (ref. 8) nomenclature for this gene. Typical of SNF2/SWI2 family proteins, the conserved ATPase domain of Ino80 comprises a large proportion of the coding region. Beyond the ATPase domain, Ino80 does not possess the SANT domain found in Isw1 and Isw2 (ref. 13); however, sequence comparison of *INO80* and its counterparts from human (*hINO80*) and *Drosophila* (*dINO80*) reveals two additional conserved regions, the TELY motif at the amino terminus and the GTIE motif at the carboxy terminus.

To analyse Ino80 biochemically, we engineered a double-Flag tag at the C terminus of the chromosomal *INO80* gene by homologous recombination. The behaviour of the strain carrying INO80–Flag was identical to wild type under all conditions tested. Immunostaining showed that Ino80–Flag is localized unevenly in the nucleus and is associated with the chromosomes of dividing cells (data not shown). Gel-filtration chromatography of INO80–Flag cell extracts shows the bulk of Ino80 in a high relative molecular mass ($M_r$) fraction between 1,000K and 1,500K (Fig. 1a), with no detectable material in smaller fractions. Therefore most, if not all, of Ino80 is present in a large complex. The high $M_r$ of native Ino80 is partly consistent with a previous study, which found both high and low $M_r$ fractions[8]; the presence of a low $M_r$ species, presumably uncomplexed or partially complexed Ino80, may be an artefact of overexpression.

We purified the complex of proteins associated with Ino80, the 'INO80 complex' (INO80.com), by immunoprecipitation with anti-Flag agarose. The purified INO80 complex contains 12 principal polypeptides, most of which, with notable exceptions, are present at roughly equivalent stoichiometry (Fig. 1b). We sequenced the peptides of individual protein bands by microcapillary reverse-phase high performance liquid chromatography nano-electrospray ion trap mass spectrometry. Data identifying seven proteins in the complex are given in Supplementary Information. Actin (Act1) and three actin-related proteins, Arp4, Arp5 and Arp8, are associated with the complex in addition to Ino80. β-Actin is a functional component of the mammalian BAF complex[14], and Arp7 and Arp9 are shared by the yeast SWI/SNF and RSC complexes[15]. Actin and Arp4 are also contained in the yeast NuA4 histone acetyltransferase complex[16]. Two other intensely staining polypeptides of the INO80 complex, Rvb1 and Rvb2, are encoded by the ORFs *YDR190C* and

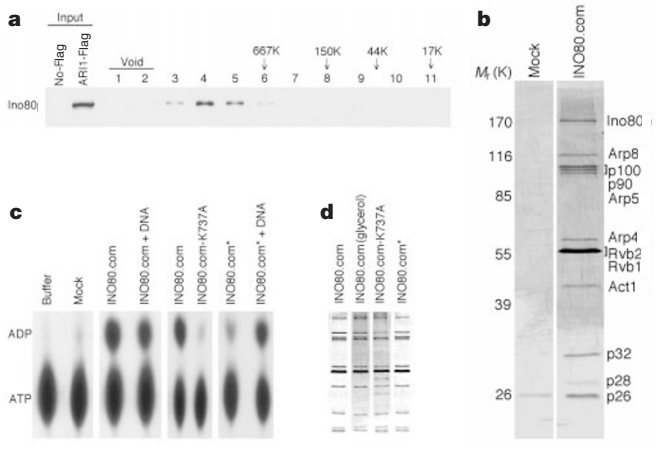


**Figure 1** Purification and characterization of the INO80 complex. **a**, Superose-6 chromatography of whole-cell extract from INO80–Flag cells. Fractions containing Ino80 were detected by western blotting with anti-Flag antibody. **b**, SDS–PAGE and silver staining showing immunoaffinity-purified INO80 complex (INO80.com). Mock purification eluted only the IgG light chain. **c**, Thin-layer chromatography analysis showing ATPase activity of INO80 complexes INO80.com, INO80.com-K737A and INO80.com* (see text for details). DNA was double-stranded ΦX174 RF DNA. **d**, SDS–PAGE and silver staining showing composition of INO80 complexes.

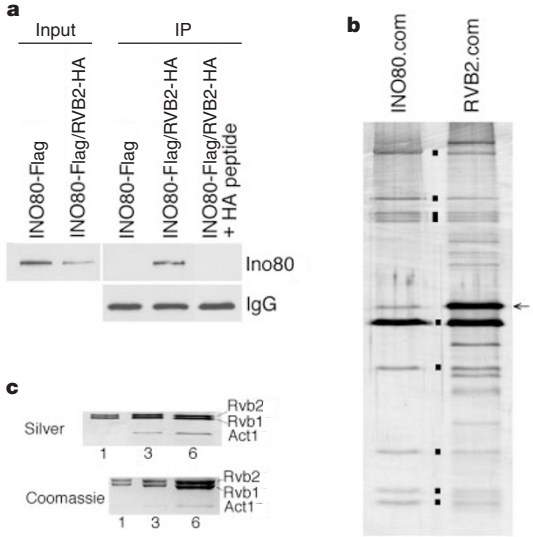


**Figure 2** Rvb1 and Rvb2 are subunits of the INO80 complex. **a**, Western blots showing immunoprecipitation of Ino80 with Rvb2–HA using anti-HA antibody and detection with anti-Flag antibody. **b**, SDS–PAGE and silver stain showing the RVB2 complexes (RVB2.com). Filled dots indicating bands shared with INO80.com. Note the extra bands in RVB2.com. Arrow indicates Rvb2–Flag. **c**, SDS–PAGE showing stoichiometry of Rvb1 and Rvb2 in the INO80 complex. Increasing amounts of INO80 complex (1×, 3× and 6×) were analysed by SDS–PAGE.

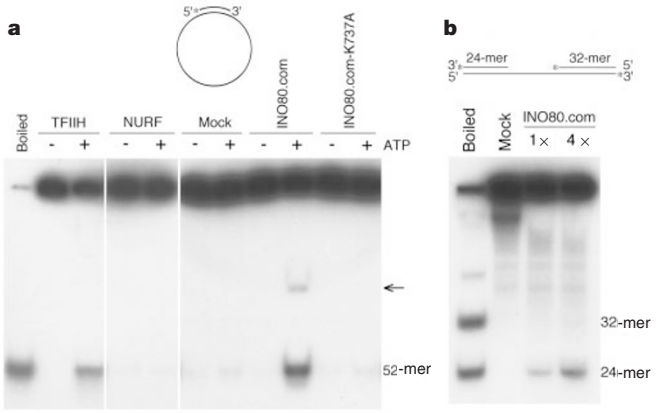


**Figure 3** INO80 complex has 3′ to 5′ specific helicase activity. **a**, Native PAGE (10%) showing the displacement of a $^{32}$P-labelled 52-mer annealed to circular single-stranded ΦX174 DNA on incubation with the indicated reagents. (INO80.com also produces a weak band (arrow), which may be a metastable intermediate.) **b**, Native PAGE (10%) showing the displacement of the $^{32}$P-labelled 24-mer or 32-mer. 1× and 4× refer to concentrations of INO80 complex. All reactions contained ATP. Structures of substrates are shown on top and the $^{32}$P-label is indicated by an asterisk.

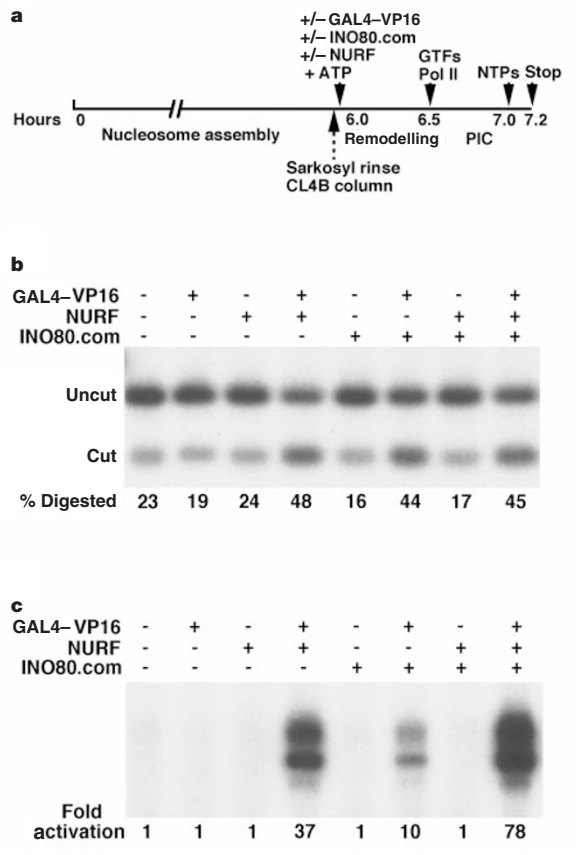


**Figure 4** INO80 complex remodels and promotes transcription from a chromatin template. **a**, Protocol. **b**, Southern blots showing that both NURF and the INO80 complex enhance digestion of a *Pst*I–*Cla*I fragment (uncut) by *Bam*HI (cut). **c**, Transcription analysis of remodelled chromatin templates. Primer extension of transcripts from the E4 promoter (+99 to +72 primer). ATP was present in all reactions and was needed for the remodelling step.

*YPL235W*, respectively. Rvb1 and Rvb2 were previously identified as 'RuvB-like' proteins (scRUVBL1/scTIP49a and scRUVBL2/scTIP49b) sharing homology to bacterial RuvB[9,10], the Holliday junction DNA helicase[17]; both *RVB1* and *RVB2* genes are essential in yeast[9,11]. The first RuvB-like protein (TIP49/rTIP49a) was identified in rat[10], and a pair of RuvB-like proteins (hTIP49a/RUVBL1/TIP49 and hTIP49b/TIP48) are found in human and are associated in large complexes[9,11,18,19].

Like other ATP-driven chromatin remodelling complexes, the INO80 complex has ATPase activity (Fig. 1c, INO80.com). The ATPase activity was intrinsically high, however, and was not further stimulated by exogenous DNA (Fig. 1c, INO80.com+DNA). We ascribe the bulk of this ATPase activity to Ino80, as the complex immunopurified from a strain carrying a K737A substitution in the ATP-binding site retained the same subunit composition (Fig. 1d, INO80.com-K737A), but showed substantially reduced activity (Fig. 1c, INO80.com-K737A). The remaining activity (~5%) may be derived from other subunits of the complex, or from the K737A protein. The inability to stimulate ATPase activity by exogenous DNA might be caused by the presence of endogenous DNA associated with the INO80 complex. We detected DNA of heterogeneous size in the purified preparation, and effectively removed the nucleic acid by extensive digestion with DNase I (data not shown). DNase I digestion had no observable effects on the polypeptide composition of the INO80 complex (Fig. 1d, INO80.com*), but the intrinsic ATPase activity of the INO80 complex was substantially reduced (Fig. 1c, INO80.com*). We could then stimulate the ATPase activity of the DNase I-treated complex with exogenous DNA (Fig. 1c, INO80.com*+DNA), or with nucleosomes (data not shown). Thus, like the related remodelling complexes SWI/SNF[20] and RSC[21], the INO80 complex shows DNA-dependent ATPase activity.

Among the polypeptides associated with Ino80, Rvb1 and Rvb2 are of special interest. To confirm that these proteins are subunits of the INO80 complex, we expressed Rvb2 from a plasmid carrying *RVB2* tagged with a haemagglutinin A (HA) epitope in INO80–Flag cells. Immunoprecipitation with anti-HA antibody revealed a specific interaction with Ino80 (Fig. 2a), strongly suggesting that this RuvB-like protein (and probably Rvb1 as well) is a true component of the INO80 complex. To assess whether Ino80 forms one complex or several complexes each containing a different subset of the 11 associated polypeptides, we analysed the proteins associating with Rvb2 in a wild-type strain transformed with a RVB2–Flag construct. Immunopurification of Rvb2–Flag revealed that 11 out of the 12 proteins of the INO80 complex co-purify with Rvb2 (Fig. 2b, filled dots). The twelfth polypeptide, Arp4, may be obscured by the co-migrating Rvb2–Flag protein band (Fig. 2b, arrow). Moreover, glycerol gradient centrifugation of the original, immunopurified INO80 complex (from INO80–Flag cells) showed that all of the 12 polypeptides sedimented together in a high $M_r$ fraction (Fig. 1d, INO80.com (glycerol)). These data suggest that Ino80 is contained in a single protein complex of about 12 distinct proteins. In addition, a number of new proteins co-purified with Rvb2–Flag (Fig. 2b). The presence of these polypeptides, which are evidently not part of the INO80 complex, may expand the functions of Rvb2 and explain why, in contrast to *ino80* mutants, *rvb2* mutants are lethal[11].

The staining intensity of Rvb1 and Rvb2 protein bands in the INO80 complex is unusually high in relation to other polypeptides (Fig. 1b). Analysis on a higher resolution gel revealed a closely spaced doublet band corresponding to the 50K Rvb1 and 52K Rvb2 polypeptides (Fig. 2c). We determined the stoichiometry of the RuvB-like proteins by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and densitometry after staining with silver or Coomassie blue (Fig. 2c). The molar ratios for Rvb1 and Rvb2 relative to Act1 are 6.5 and 5.6 (silver), and 6.3 and 6.7 (Coomassie), respectively, whereas the ratios relative to Ino80 are 5.3 and 5.6 (Coomassie). To a first approximation, both Rvb1 and Rvb2 show 6:1 stoichiometry compared with other polypeptides in the INO80 complex. The correspondence between these values and the known double hexamer composition of bacterial RuvB is striking[17].

Given that bacterial RuvB[17] is an ATP-dependent DNA helicase, we investigated whether the INO80 complex containing Rvb1 and Rvb2 subunits has helicase activity. For these experiments and the *in vitro* transcription studies below, we used the INO80 complex without DNase I treatment because of difficulty in eliminating residual DNase I after digestion. We found that the INO80 complex was able to displace a radiolabelled 52-nucleotide DNA strand from a duplex formed by annealing the oligonucleotide to single-stranded ΦX174 DNA (Fig. 3a). Primer displacement was ATP-dependent, as noted for other helicases such as TFIIH. In contrast, *Drosophila* NURF showed no evidence of helicase activity, despite its potency in chromatin remodelling. The Ino80–K737A mutant complex failed to show DNA helicase activity (Fig. 3a), indicating that Rvb1 and Rvb2 may be functionally coupled to the Ino80 ATPase. (The ability of RuvB-like proteins to function independently as helicases is a subject of debate[9,11,19].) To examine the directionality of helicase action, we used a substrate consisting of 24-mer and 32-mer oligonucleotides annealed to the corresponding ends of linear, single-stranded ΦX174 DNA (Fig. 3b, top). The INO80 complex was able to displace only the 24-mer, indicating a 3′ to 5′ helicase activity (Fig. 3b). The directional helicase activity of the INO80 complex was also ATP dependent (data not shown).

In previous work, *ino80* mutants exhibited reduced transcription of a number of reporter constructs[8]. In concurrence, we found reduced RNA levels for the endogenous *INO1*, *PHO5* and *Ty1* genes in *ino80* mutant cells but not for *INO2*, *PHO8* and *ACT1*, and normal activation but retarded attenuation of *HSP26* (data not shown). We also investigated the ability of the INO80 complex to affect transcription *in vitro* by reconstituting plasmid DNA carrying five GAL4-binding sites upstream of the adenovirus E4 core promoter into chromatin[22]. We incubated the chromatin template with a saturating amount of the GAL4–VP16 transactivator, INO80 complex and ATP (Fig. 4a), and measured remodelling activity by the change in accessibility to *Bam*HI digestion at a site upstream (−46) of the E4 TATA box. The INO80 complex displayed activity (44% digestion) comparable to the remodelling activity of NURF (48% digestion), whereas control reactions lacking the GAL4–VP16 transactivator, the INO80 complex or both, gave substantially lower cleavage (16–23% digestion) (Fig. 4b). We reproducibly observed a 10-fold transcriptional activation of chromatin remodelled by a saturating amount of the INO80 complex (Fig. 4a, c), which is lower than the 37-fold activation typically observed for NURF (Fig. 4c). Curiously, the combined actions of NURF and the INO80 complex, while creating no greater accessibility (45% digestion), resulted in 78-fold activation; the synergistic activation may reflect differences in the respective remodelling mechanisms.

To explore further the physiological functions of *INO80*, we investigated the phenotype of the *ino80* null mutant, and found it to be sensitive to hydroxyurea (100 mM), and the alkylating agent methyl methanesulphonate (MMS; 0.025%) (Fig. 5a). Both agents affect DNA metabolism: hydroxyurea inhibits DNA synthesis by reducing the dNTP pool; and MMS induces single- and double-stranded breaks in DNA. The *ino80* mutant also showed sensitivity to ultraviolet and ionizing radiation, which cause DNA damage. By analysis of serially diluted cultures, the *ino80* mutant was as sensitive to ultraviolet as a *rad9* null mutant in the same genetic background; *RAD9* is a cell-cycle checkpoint gene required for induction of genes that repair DNA damage[23] (Fig. 5b). The *ino80* mutant also showed sensitivity to ionizing radiation, although the degree of sensitivity was intermediate when compared with the *rad9* mutant (Fig. 5c). In contrast, a *snf2* null mutant in the same genetic background was insensitive to identical levels of hydroxyurea and MMS, and insensitive to ultraviolet or ionizing radiation (Fig. 5). The sensitivity to hydroxyurea, MMS, ultraviolet and ionizing radiation is due to the loss of *INO80*, as the null phenotype can be rescued with a plasmid carrying the wild-type gene (Fig. 5a, pINO80; and data not shown). Moreover, a K737A substitution in the ATP-binding motif abolished the ability to rescue the mutant phenotypes, indicating the importance of ATP-binding to *INO80* function *in vivo* (Fig. 5a, pINO80-K737A; and data not shown). These results indicate that *INO80* is involved, directly or indirectly, in the processing of DNA damage.

The INO80 complex might function indirectly in DNA repair by facilitating transcription of genes induced by DNA damaging

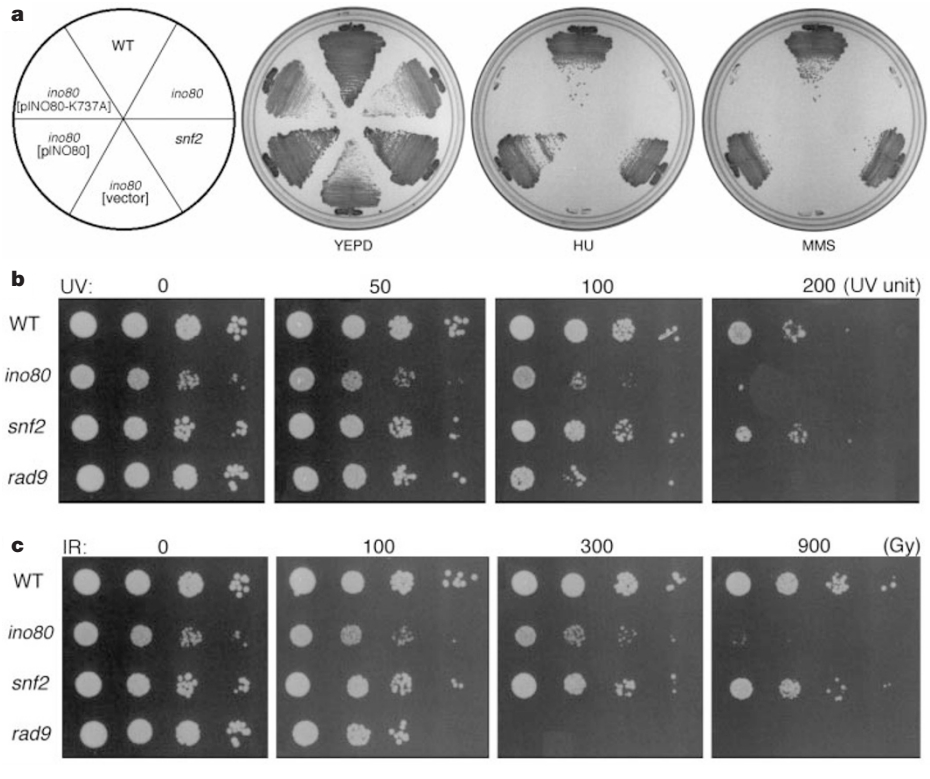


**Figure 5** Analysis of *ino80* mutant phenotypes. **a**, Strains used are wild type (WT), *ino80* null mutant (*ino80*), *snf2* null mutant (*snf2*), *ino80* with empty vector (*ino80*[Vector]), *ino80* with vector containing the wild-type *ino80* gene (*ino80*[pINO80]) and *ino80* with vector containing the K737A mutant (*ino80*[pINO80–K737A]). HU, hydroxyurea; MMS, methyl methanesulphonate. **b**, Serial dilutions of cells from WT, *ino80*, *snf2* and *rad9* null mutant (*rad9*) strains were given increasing doses of ultraviolet (UV) as indicated. **c**, Similar to **b**, stains were subjected to ionizing radiation (IR) at the indicated doses.

agents; however, we have found normal induction of the ribonucleotide reductase genes *RNR1* and *RNR3* (which are the downstream targets of the DNA damage checkpoint gene *MEC1*; ref. 24) after treatment of *ino80* cells with hydroxyurea (data not shown). Alternatively, chromatin remodelling by the INO80 complex might directly facilitate an aspect of DNA metabolism involving replication, recombination or repair, processes that are intimately connected[25]. The INO80 complex might either promote site recognition by the replication or repair machinery, or assist progression of the machinery through chromatin. In *Escherichia coli*, the RuvAB complex binds to Holliday junctions and promotes branch migration, the motive force being provided by the helicase activity of the two hexameric RuvB rings[17]. The RuvAB complex also has a role in the generation and prevention of double-stranded breaks at arrested replication forks through its actions on potential Holliday junction intermediates[26]. The yeast Rvb1 and Rvb2 hexamers in the INO80 complex might function similarly, but with the added coupling to chromatin remodelling proteins as an adaptation to the chromatin environment. Our results, together with the recent findings of human RuvB-like proteins being associated with c-Myc[18] and being contained in the multisubunit TIP60 HAT complex[19], underscore the linkage of this class of DNA helicases with diverse aspects of chromatin metabolism. □

## Methods

### Bioinformatics and gene cloning

We carried out the yeast gene search in the *Saccharomyces* genome database. A *hINO80* complementary DNA was cloned by filling a gap between the two expressed sequence tags using PCR, and was found to be nearly identical to a complementary DNA encoding the uncharacterized KIAA1259 protein. *dINO80* was predicted from genomic sequences.

### Manipulation of yeast

All strains (see Supplementary Information) are in the S288C background. The chromosomal *INO80* gene was tagged at the extreme C terminus by integrating a pRS406 plasmid containing the *Xho*I–*Hin*dIII fragment of *INO80* in which a double Flag sequence was introduced right before the stop codon (pRS406–INO80/2Flag). The URA3 marker was 'popped out' through homologous recombination. The tagged INO80–Flag strain was confirmed by Southern blotting, PCR and sequencing. The *RVB2* ORF was tagged with HA at the C terminus in a low-copy plasmid pYX112 (pRVB2–HA). Similarly, the *INO80* ORF was cloned into pYX112 (pINO80) and a single amino-acid substitution (K737A) was then made (pINO80–K737A). For purification of INO80.com-K737A and RVB2.com, we cloned fragments of ORFs with native promoters (to −500 and to −1,000, respectively) into a modified pRS416 plasmid, which contains a double Flag sequence. The *ino80* strain was created using the PCR deletion method and found to be viable; however, in a W303 strain background, *INO80* is essential and *ino80* temperature-sensitive mutants showed a G2/M arrest phenotype upon temperature shift (unpublished data). The *snf2* strain was a gift from F. Winston (Harvard University) and the *rad9* strain was from Research Genetics. Phenotypic analysis was done by subjecting cells to 100 mM hydroxyurea, 0.025% MMS, ultraviolet in a Stratalinker or ionizing radiation using a Cs-137 gamma ray source on YEPD plates. We incubated plates at 30 °C for 2–3 days and then scored.

### Protein techniques

Four litres of INO80–Flag cells were grown to saturation for 2 days at 30 °C in YEPD. Whole-cell extracts were prepared essentially as described[13]. About 40 ml of extract in Buffer H-0.3 (ref. 13) was routinely obtained. Direct immuno-affinity purification of the INO80 complex was done essentially as described[13]. To remove associated DNA, DNase I (50 μg) in buffer H-0.1 was added to beads after washing with buffer H-0.15 and allowed to digest for 30 min at 24 °C. The yield was roughly 10 μg protein complex per litre of cells. INO80.com-K737A and RVB2.com were similarly purified from epitope-tagged cell extracts. We carried out mock purification using extract made from wild-type cells. Gel-filtration chromatography was on a Superose-6 column in buffer H-0.3. 17–35% glycerol gradient sedimentation was in buffer H-0.1. Silver and Coomassie stained gels were scanned and quantified with NIH Image software. Peptide sequencing was done at the Harvard Microchemistry Facility on a Finnigan LCQ quadrupole ion trap mass spectrometer.

### *In vitro* activity assays

The ATPase and restriction accessibility assays were carried out as described[13,27]. Chromatin transcription was done essentially as described[22], except that GAL4–VP16 was used as transactivator and that purified *Drosophila* RNA polymerase II and purified and recombinant general transcription factors were used instead of nuclear extract. For the helicase assay, a $^{32}$P-labelled 52-mer was annealed to single-stranded ΦX174 DNA at position 702–753 (ref. 9). Reactions were carried out in 10 μl for 30 min at 30 °C and included 20 ng of complexes (1×). TFIIH[28] and NURF[29] were purified as described. The substrate for directional helicase activity was prepared essentially as described[30] except that dGTP was used to blunt ends after cutting with *Hap*II of the helicase substrate (see above) and filling-in with $^{32}$P-dCTP, resulting in the generation of a 24-mer and a 32-mer.

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1. Kornberg, R. D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98,** 285–294 (1999).
2. Luger, K. & Richmond, T. J. DNA binding within the nucleosome core. *Curr. Opin. Struct. Biol.* **8,** 33–40 (1998).
3. Eisen, J. A., Sweder, K. S. & Hanawalt, P. C. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.* **23,** 2715–2723 (1995).
4. Peterson, C. L. Multiple SWItches to turn on chromatin? *Curr. Opin. Genet. Dev.* **6,** 171–175 (1996).
5. Armstrong, J. A. & Emerson, B. M. Transcription of chromatin: these are complex times. *Curr. Opin. Genet. Dev.* **8,** 165–172 (1998).
6. Kadonaga, J. T. Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines. *Cell* **92,** 307–313 (1998).
7. Workman, J. L. & Kingston, R. E. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* **67,** 545–579 (1998).
8. Ebbert, R., Birkmann, A. & Schüller, H. J. The product of the SNF2/SWI2 paralogue INO80 of *Saccharomyces cerevisiae* required for efficient expression of various yeast structural genes is part of a high-molecular-weight protein complex. *Mol. Microbiol.* **32,** 741–751 (1999).
9. Qiu, X. B. *et al.* An eukaryotic RuvB-like protein (RUVBL1) essential for growth. *J. Biol. Chem.* **273,** 27786–27793 (1998).
10. Kanemaki, M. *et al.* Molecular cloning of a rat 49-kDa TBP-interacting protein (TIP49) that is highly homologous to the bacterial RuvB. *Biochem. Biophys. Res. Commun.* **235,** 64–68 (1997).
11. Kanemaki, M. *et al.* TIP49b, a new RuvB-like DNA helicase, is included in a complex together with another RuvB-like DNA helicase, TIP49a. *J. Biol. Chem.* **274,** 22437–22444 (1999).
12. Tsukiyama, T., Daniel, C., Tamkun, J. & Wu, C. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodelling factor. *Cell* **83,** 1021–1026 (1995).
13. Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J. & Wu, C. Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodelling factors in *Saccharomyces cerevisiae*. *Genes Dev.* **13,** 686–697 (1999).
14. Zhao, K. *et al.* Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95,** 625–636 (1998).
15. Cairns, B. R., Erdjument-Bromage, H., Tempst, P., Winston, F. & Kornberg, R. D. Two actin-related proteins are shared functional components of the chromatin-remodelling complexes RSC and SWI/SNF. *Mol. Cell* **2,** 639–651 (1998).
16. Galarneau, L. *et al.* Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol. Cell* **5,** 927–937 (2000).
17. West, S. C. Processing of recombination intermediates by the RuvABC proteins. *Annu. Rev. Genet.* **31,** 213–244 (1997).
18. Wood, M. A., McMahon, S. B. & Cole, M. D. An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. *Mol. Cell* **5,** 321–330 (2000).
19. Ikura, T. *et al.* Link of TIP60 histone acetylase to DNA repair and apoptosis. *Nature* (submitted).
20. Côté, J., Quinn, J., Workman, J. L. & Peterson, C. L. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265,** 53–60 (1994).
21. Cairns, B. R. *et al.* RSC, an essential, abundant chromatin-remodelling complex. *Cell* **87,** 1249–1260 (1996).
22. Mizuguchi, G., Tsukiyama, T., Wisniewski, J. & Wu, C. Role of nucleosome remodelling factor NURF in transcriptional activation of chromatin. *Mol. Cell* **1,** 141–150 (1997).
23. Weinert, T. A. & Hartwell, L. H. Characterization of RAD9 of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. *Mol. Cell. Biol.* **10,** 6554–6564 (1990).
24. Huang, M. & Elledge, S. J. Identification of RNR4, encoding a second essential small subunit of ribonucleotide reductase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **17,** 6105–6113 (1997).
25. Haber, J. E. DNA recombination: the replication connection. *Trends Biochem. Sci.* **24,** 271–275 (1999).
26. Seigneur, M., Bidnenko, V., Ehrlich, S. D. & Michel, B. RuvAB acts at arrested replication forks. *Cell* **95,** 419–430 (1998).
27. Gdula, D. A., Sandaltzopoulos, R., Tsukiyama, T., Ossipow, V. & Wu, C. Inorganic pyrophosphatase is a component of the *Drosophila* nucleosome remodelling factor complex. *Genes Dev.* **12,** 3206–3216 (1998).
28. Hansen, S. K. & Tjian, R. TAFs and TFIIA mediate differential utilization of the tandem Adh promoters. *Cell* **82,** 565–575 (1995).
29. Tsukiyama, T. & Wu, C. Purification and properties of an ATP-dependent nucleosome remodelling factor. *Cell* **83,** 1011–1020 (1995).
30. Park, J. S., Choi, E., Lee, S. H., Lee, C. & Seo, Y. S. A DNA helicase from Schizosaccharomyces pombe stimulated by single-stranded DNA-binding protein at low ATP concentration. *J. Biol. Chem.* **272,** 18910–18919 (1997).


**Supplementary information** is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.


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