The SWI/SNF complex acts to constrain distribution of the centromeric histone variant Cse4

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In order to gain insight into the function of the *Saccharomyces cerevisiae* SWI/SNF complex, we have identified DNA sequences to which it is bound genome-wide. One surprising observation is that the complex is enriched at the centromeres of each chromosome. Deletion of the gene encoding the Snf2 subunit of the complex was found to cause partial redistribution of the centromeric histone variant Cse4 to sites on chromosome arms. Cultures of *snf2Δ* yeast were found to progress through mitosis slowly. This was dependent on the mitotic checkpoint protein Mad2. In the absence of Mad2, defects in chromosome segregation were observed. In the absence of Snf2, chromatin organisation at centromeres is less distinct. In particular, hypersensitive sites flanking the Cse4 containing nucleosomes are less pronounced. Furthermore, SWI/SNF complex was found to be especially effective in the dissociation of Cse4 containing chromatin *in vitro*. This suggests a role for Snf2 in the maintenance of point centromeres involving the removal of Cse4 from ectopic sites.

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Introduction

The accurate segregation of eukaryotic chromosomes is required to ensure that genetic information is inherited by both daughters following cell division. This fundamental process is orchestrated via specialised chromatin structures termed centromeres. Centromeres serve as the point of attachment for kinetochore proteins, which themselves interact with microtubules during mitosis. In budding yeast, CEN DNA is made up of a 125-bp sequence consisting of three conserved DNA elements, CDEI, CDEII and CDEIII (Hegemann and Fleig, 1993; Sorger *et al*, 1995; Cheeseman *et al*, 2002; Cleveland *et al*, 2003). Each of these elements is bound by sequence-specific DNA-binding proteins that serve to nucleate the assembly of kinetochores. Kinetochores consist of inner, central and outer complexes, the latter of which provide sites of microtubule attachment (reviewed by Hyman and Sorger, 1995). Budding yeast centromeres differ from those in many other species in that the centromeric core is restricted to a nuclease-resistant region spanning ~200 bp (Furuyama and Biggins, 2007). This core is flanked on either side by nuclease hypersensitive sites, which are in turn flanked by arrays of positioned nucleosomes (Bloom and Carbon, 1982; Funk *et al*, 1989; Glowczewski *et al*, 2000).

A common feature of centromeric chromatin is the presence of a centromere-specific variant of histone H3 that has a key role in centromere function. In humans, this variant is known as CenpA, Cid in *Drosophila melanogaster*, Cnp1 in *Schizosaccharomyces pombe* and Cse4 in *Saccharomyces cerevisiae* (Bloom and Carbon, 1982; Palmer *et al*, 1987; Funk *et al*, 1989; Schulman and Bloom, 1991; Sullivan *et al*, 1994; Glowczewski *et al*, 2000). These centromeric histone variants have considerable homology with histone H3 (c60% identity within the histone fold region) (Sullivan *et al*, 1994) and like histone H3 are predicted to adopt a histone fold structure and to interact with histone H4.

The *S. cerevisiae CSE4* gene has been identified in several screens for mutants defective in chromosome segregation (Smith *et al*, 1996; Baker *et al*, 1998). Cse4 is found to be physically associated with centromeric chromatin both by chromatin immunoprecipitation (ChIP) and immunofluorescence (Meluh *et al*, 1998). Cse4 differs from centromeric variants in other species in that it includes an N-terminal extension in addition to a core domain with homology to H3. It is nonetheless found to physically and genetically associate with histone H4 (Glowczewski *et al*, 2000; Camahort *et al*, 2009). Despite some recent reports to the contrary, it remains possible that Cse4 and other centromeric histone variants function as components of an octameric nucleosome-related structure (Camahort *et al*, 2009). In *S. cerevisiae*, it is known

that the inner kinetochore protein Scm3 interacts with Cse4 and is required for recruitment of Cse4 to centromeres (Camahort *et al*, 2007; Mizuguchi *et al*, 2007; Stoler *et al*, 2007; Zhang *et al*, 2007). Mislocalisation of Cse4 has been reported to cause chromosome loss (Au *et al*, 2008) and in humans this mislocalisation is correlated with colorectal cancer (Tomonaga *et al*, 2003). However, overexpression of Cse4 does not necessarily result in mislocalisation to sites out with centromeres (Crotti and Basrai, 2004) raising the possibility that additional mechanisms exist to remove Cse4 that has been misincorporated outwith centromers. One such mechanism involves the ubiquitin-mediated proteolysis of ectopically located Cse4 (Collins *et al*, 2005).

The structure of chromosomes is regulated throughout their length using a diverse range of strategies. These include the post-translational modification of histones (Kouzarides, 2007) and the action of ATP-dependent chromatin remodelling enzymes (Becker and Horz, 2002). ATP-dependent remodelling enzymes share homology with ATP-dependent DNA translocases and can be classified into many evolutionary conserved subfamilies based on this homology (Flaus *et al*, 2006). One of the first of these proteins to be characterised was the yeast Snf2 protein (Laurent *et al*, 1991; Hirschhorn *et al*, 1992). The Snf2 protein has since been shown to function as the catalytic subunit of the 11 subunit SWI/SNF complex (Smith *et al*, 2003). This complex has been shown to be capable of disrupting, repositioning or displacing histones *in vitro* and *in vivo* (Côté *et al*, 1994; Owen-Hughes *et al*, 1996; Whitehouse *et al*, 1999; Becker and Horz, 2002).

Here, we find that the ATP-dependent SWI/SNF chromatin remodelling complex has a role in maintaining the centromeric distribution of Cse4. SWI/SNF also contributes to the generation of nuclease hypersensitive sites flanking centromeres and acts to dissociate Cse4 containing chromatin *in vitro*. Our data support a model in which SWI/SNF acts to influence Cse4 distribution via the removal of misincorporated Cse4 rather than via directing incorporation at centromeres.

Results

Snf2 binding is enriched at yeast centromeres

In order to gain insight into the regions of the yeast genome with which the SWI/SNF complex is associated, we isolated DNA fragments bound by Snf2 using ChIP. This DNA was then amplified and hybridised to tiling microarrays with 32 bp resolution. Analysis of these data resulted in the observation of enrichment at several previously reported locations including promoters, Swi4-binding sites and coding regions (Cosma *et al*, 1999; Schwabish and Struhl, 2007; Venters and Pugh, 2009). It was also apparent that there was an enrichment of Snf2 within the vicinity of the centromere of each chromosome. Figure 1A shows the enrichment of Snf2 for all yeast chromosomes aligned by their CDEII element. A clear enrichment centred over the centromere spanning ∼1000 bp was observed. The enrichment within the vicinity of centromeres is also apparent for individual chromosomes as illustrated by the example of CEN16 (Figure 1B).

To gain confidence that the centromeric distribution we observed was not specific to the array format we were using, we also applied DNA fragments bound by Snf2 to Affymetrix
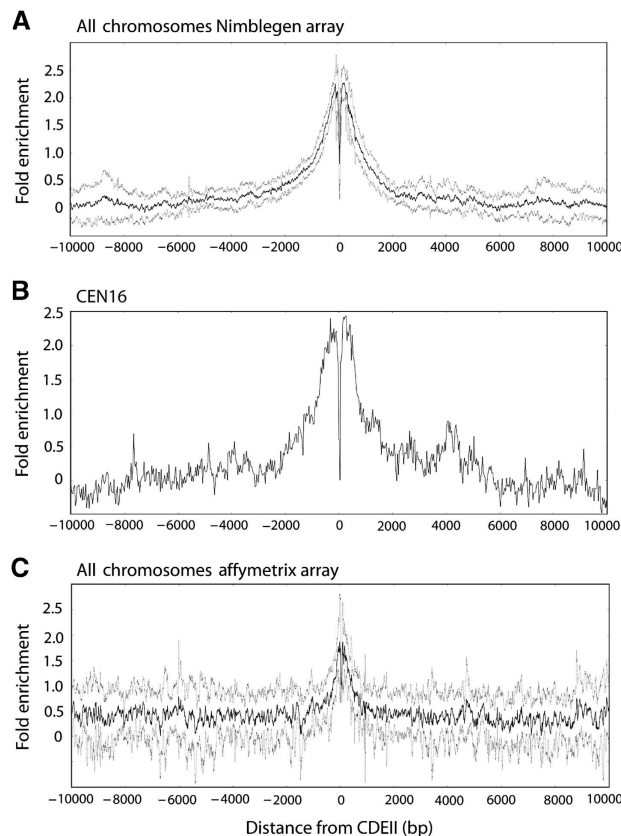

Figure 1 Centromeres are enriched for Snf2. (**A**) Enrichment of Snf2-myc associated DNA fragments (solid line) was calculated by averaging enrichment from all chromosomes following hybridisation to Nimblegen tiling arrays aligned by the position of their CDEII element, standard deviation is depicted as dotted line (**B**). Input normalised signal for Snf2-myc enrichment at a region spanning 10 000 bp ± the CDEII element of chromosome 16. (**C**) As in (**A**) but hybridisation was carried out on an Affymetrix tiling array.

tiling arrays comprised of 25 base oligos tiled every 5 bp (Figure 1C). A similar pattern of centromeric enrichment was also observed using this platform and was confirmed by quantitative real-time PCR (data not shown). As a role for Snf2 in centromere function had not, to our knowledge been proposed previously, we next sought to investigate the role of Snf2 in maintaining centromere integrity and chromosome segregation.

Snf2 is required to maintain the distribution of the centromeric histone variant Cse4

The point centromeres of *S. cerevisiae* are occupied by a single chromatin particle containing the centromeric histone variant Cse4. To investigate the genomewide localisation of Cse4, ChIP was used to enrich for bound DNA fragments. Hybridisation to microarrays enabled the distribution to be measured. Consistent with previous reports, Cse4 was enriched at the centromere of each chromosome with sites on chromosome arms observed occasionally (Figure 2A and C; Supplementary Figure S1). To investigate the role of SWI/SNF in maintaining this distribution, ChIP-chip was also performed in a *snf2* deletion strain (Figure 2B and D; Supplementary Figure S1). This shows a reduction in the enrichment at centromeres and considerably increased Cse4 binding at sporadic locations along chromosome arms.
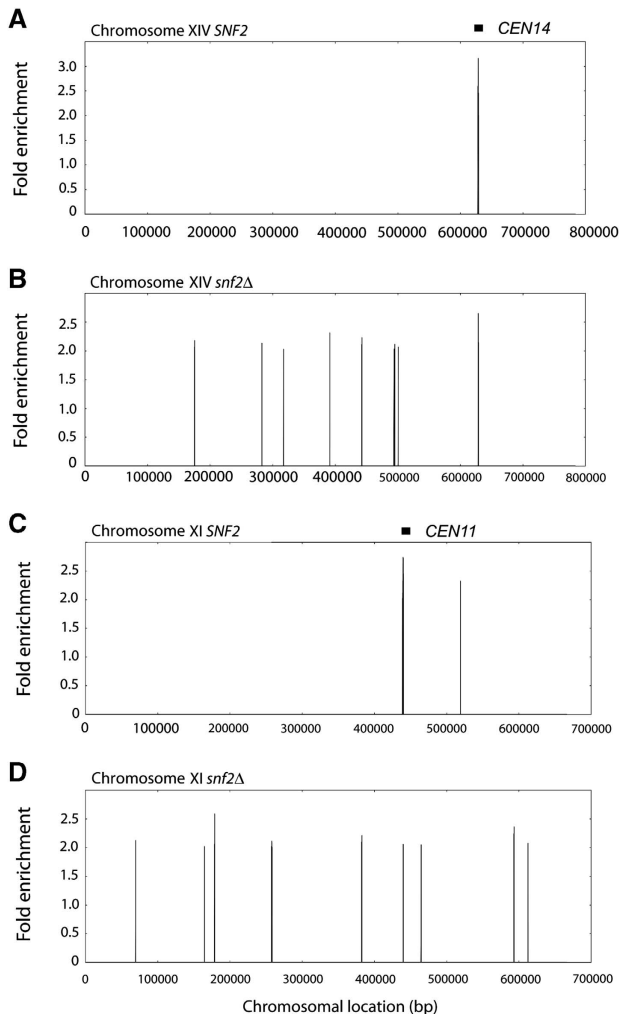
Figure 2 Deletion of Snf2 alters the genomewide distribution of Cse4. Cse4 is predominantly enriched at centromeres in a $SNF2^+$ typical enrichments for chromosomes 14 and 11 (**A**, **C**). In a *snf2*Δ, Cse4 is redistributed across the entire chromosome arms at ectopic sites as well as centromeres (**B**, **D**).

In principle, such a redistribution might in part result from increased expression of Cse4 in a *snf2* mutant. However, western blotting of whole-cell extracts indicates that the total amount of Cse4 present varies little when *SNF2* is deleted (Supplementary Figure S2). However, it was notable that the proportion of Cse4 associated with chromatin increases in a *snf2* mutant and that this effect could also be observed following induction of a *SNF2* degron (Supplementary Figure S2). This is consistent with the genomewide location analysis, which also indicates an overall increase in Cse4-enriched sites in a *snf2* mutant (Supplementary Figure S1).

The mitotic checkpoint is unsatisfied in the absence of Snf2

As Cse4 is required for centromere function, it might be anticipated that *snf2* mutant strains display a chromosome segregation defect. In order to investigate this further, the fate of an individual centromere was monitored by fluorescence microscopy (Michaelis *et al*, 1997). The number of copies of CEN5 visualised as a result of tagging with tet-GFP was compared with spindle poles with the ratio of the two signals

proving a means to assess aneuploidy. In a *snf2*Δ strain, there was not a major increase in aneuploidy for *CEN5* (Figure 3A). However, in synchronised cultures of *snf2*Δ strains, it is clear that chromosome segregation does not go to completion at the same rate as for the wt strain (Figure 3B). Separation events are observed, but the distance between centromeres oscillates between 1 and 2 μm for a prolonged period prior to anaphase (Supplementary Figure S3A). One possible explanation for this is that the mitotic checkpoint is abrogated. Defects in the localisation of Cse4 might be anticipated to have this effect as conditional alleles of *CSE4* have been observed to have the mitotic checkpoint unsatisfied (Glowczewski *et al*, 2000). To investigate this further, the effect of inactivating the mitotic checkpoint through deletion of *MAD2* was investigated. Scoring the proportion of cells in metaphase by fluorescent microscopy indicated a reduction from 31% in *snf2*Δ to 21% *snf2*Δ *mad2*Δ consistent with unsatisfied mitotic checkpoint in the absence of Snf2. This was confirmed by fluorescent-activated cell sorting (FACS) analysis, which indicated a reduction in the proportion of cells with a 2C DNA content from 86% in a *snf2*Δ strain to 67% in a *snf2*Δ *mad2*Δ Figure 3C. Furthermore, chromosome missegregation was more pronounced in the absence of Snf2 and Mad2 as observed by monitoring segregation of CEN5 (Figure 3A) as an increase in the proportion of cells with DNA content other than 1 or 2C in FACS analysis (Figure 3C and D). Finally, degron-mediated depletion of Snf2 was observed to cause a delay in chromosome segregation (Supplementary Figure S3B and C) similar to that observed following deletion of *SNF2* (Figure 3B). This delay was reduced following deletion of *MAD2* (Supplementary Figure S3B and C). In concert, these observations are consistent with mislocalisation of Cse4 causing a proportion of cells to arrest at the mitotic checkpoint until centromere function can be restored. This may contribute to the previously reported slow growth of *snf2* mutants.

Snf2 contributes to nucleosome organisation at centromeres

How does SWI/SNF act to maintain Cse4 localisation? One possibility is that SWI/SNF directs incorporation of Cse4 at centromeres, perhaps in a manner similar to the way the Swr1 complex directs incorporation of the histone variant Htz1 (Mizuguchi *et al*, 2004). However, we do not find that Cse4 is generally colocalised with SWI/SNF at all sites in the genome (not shown). Furthermore, SWI/SNF-related complexes have a stronger activity in the destabilisation of nucleosomes than in their assembly (Owen-Hughes *et al*, 1996; Bruno *et al*, 2003; Lorch *et al*, 2006; Dechassa *et al*, 2010).

To investigate the effect of the SWI/SNF complex on centromeric chromatin organisation, we mapped the locations of nucleosomes genomewide using tiled microarrays. In a wild-type ($SNF2^+$) strain, nucleosome alignment for each chromosome with respect to the CDEII element provides a means of viewing the average chromatin organisation at the 16 *S. cerevisiae* centromeres. Prominent features include a protected region of some 177 bp spanning the centromere flanked by hypersensitive sites on either side (Figure 4). This is consistent with previous studies of chromatin organisation at centromeres (Furuyama and Biggins, 2007). To investigate the role of Snf2 in this region, chromatin was also mapped in

a *snf2*Δ strain. In this case, the hypersensitive sites flanking the centromeric nucleosome are less prominent (Figure 4A). In contrast, hypersensitive sites flanking ARS sequences are not affected (Figure 4B). This supports a role for SWI/SNF in the organisation of chromatin adjacent to centromeres.

SWI/SNF destabilises Cse4 containing nucleosomes

The SWI/SNF complex has been implicated in the removal of nucleosomes at a number of yeast genes (Barbaric *et al*, 2007; Schwabish and Struhl, 2007; Bryant *et al*, 2008; Shivaswamy
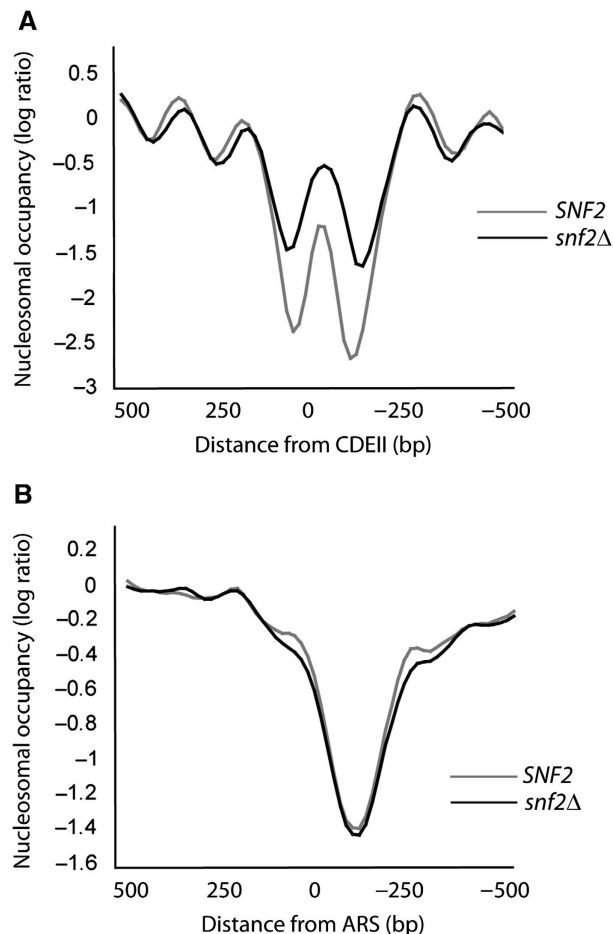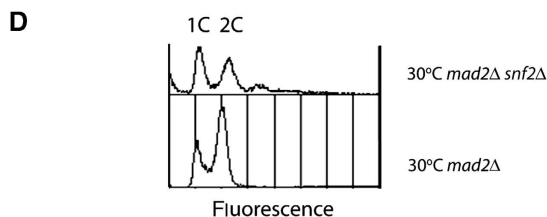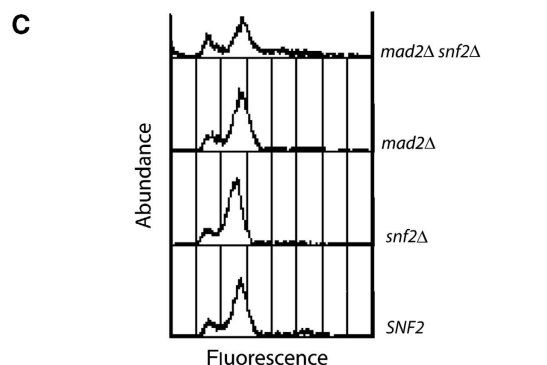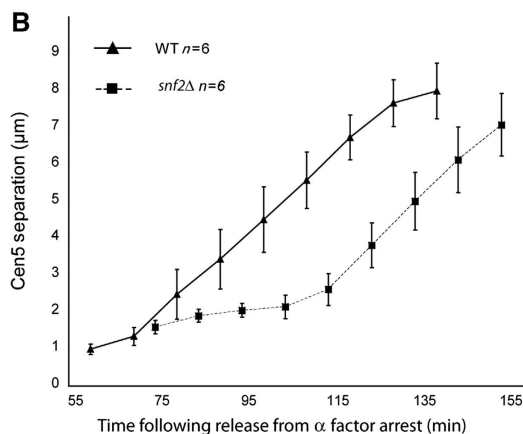
Figure 3 Chromosome segregation defects in Snf2 mutants. (**A**) Anueploidy for *CEN5* was assessed by microscopy in a strain in which both *CEN5* and Spc42 were fluorescently tagged. Cells in which the ratio of *CEN5*:Spc42 foci was not 1:1 were scored as anueploid. Cells were cultured at 20°C. (**B**) Metaphase-to-anaphase progression was monitored via the separation between fluorescently tagged CEN5 loci. Cultures were arrested using α factor then released and grown at 30°C. The separation between fluorescently tagged CEN5 loci was measured by microscopy at various times following α factor induced cell-cycle arrest. Fisher's test *P*-values are indicated on the graph. (**C**) Asynchronously grown liquid cultures grown at 20°C were analysed using FACS. The abundance of cells with different DNA content is indicated. In the absence of Snf2 and Mad2, an increase in the proportion of cells with low and high DNA content was observed. (**D**) Cultures were grown at 20°C then shifted to 30°C for 3 h and subject to FACS as above. Polyploidy in the *mad2*Δ *snf2*Δ strain is more pronounced following growth at the higher temperature.
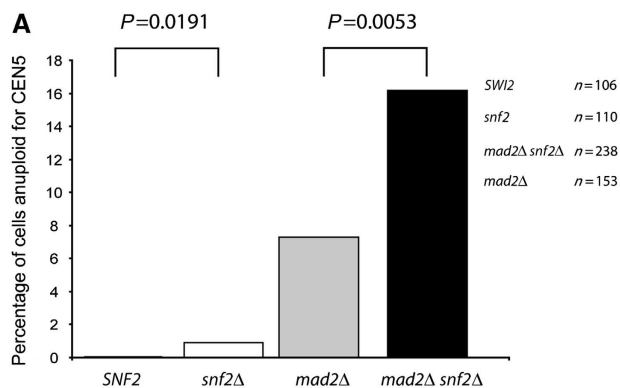
Figure 4 Snf2-dependent alterations to chromatin structure adjacent to centromeres. Chromatin structure was mapped genomewide by hybridising nucleosomal DNA to Affymetrix tiling arrays. (**A**) Average nucleosome occupancy at all centromeres was aligned by the CDEII element to illustrate the arrangement of chromatin at the centromeres of the 16 chromosomes. Wild-type nucleosome positioning was assessed using data from Lee *et al* (2007). The data for the *snf2* deletion is the average of two repeats made using the same arrays. It is apparent that there are changes to chromatin flanking the centromere and that in particular the nuclease hypersensitive sites flanking centromeres are less pronounced. To illustrate that not all hypersensitive sites in the genome are altered in a *snf2*Δ strain chromatin was also aligned to ARS elements (**B**). Snf2 appears to have a minor role in chromatin organisation at these sites.
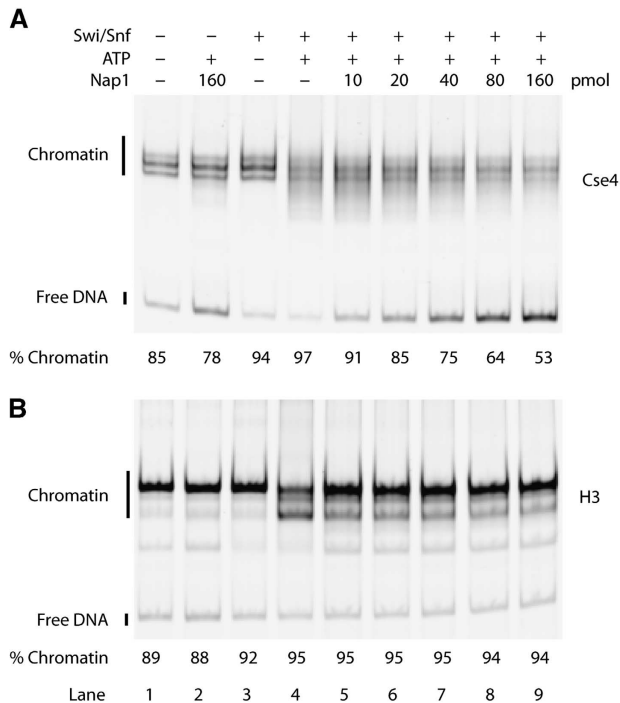
Figure 5 SWI/SNF can dissociate Cse4 containing nucleosomes. Chromatin was assembled onto a DNA fragment consisting of the 601 positioning sequence flanked by 42 and 47 bp linker DNA by salt dialysis using either yeast octamers containing Cse4 (**A**) or octamers containing Histone H3 (**B**). In all, 1 pmol of nucleosomes were incubated with SWI/SNF (0.5 pmol), Nap1 as indicated in the presence or absence of 1 mM ATP as indicated. Increasing amounts of Nap1 in the presence of SWI/SNF cause the Cse4 chromatin to dissociate, resulting in an increase in the free DNA present (**A**, lanes 5–9).

Figure 6 Deletion of Snf2 results in mislocalisation of Cse4 to sites normally bound by SWI/SNF. The top 99 sites showing enrichment for Cse4 in a *snf2*Δ along with a control set of randomly selected sites were selected. These sites were then scored for enrichment of Snf2 in a wt strain. It can be seen that there is a significantly increased enrichment for Snf2 at the sites to which Cse4 becomes mislocalised (0.4-fold) in comparison to randomly selected sites (0.05-fold). This enrichment is not observed when comparing the enrichment of Snf2 at ectopic Cse4 sites in a wt strain. These observations support a role for SWI/SNF in the removal of Cse4 from sites on chromosome arms.

and Iyer, 2008; Gkikopoulos *et al*, 2009; Takahata *et al*, 2009) and to be capable of destabilising nucleosomes *in vitro* (Owen-Hughes *et al*, 1996; Bruno *et al*, 2003; Lorch *et al*, 2006; Dechassa *et al*, 2010). If SWI/SNF were to have a role in removing Cse4 from noncentromeric sites, it would be anticipated that SWI/SNF should be able to remove Cse4 containing nucleosomes from DNA. To test this, histone octamers were prepared using recombinant yeast histones H4, H2A and H2B and either histone H3 or Cse4. Octamers prepared in this way could be used to assemble chromatin particles on short DNA fragments that are likely to assume a nucleosomal organisation (Camahort *et al*, 2009). Chromatin prepared in this way was used as a substrate for remodelling assays using SWI/SNF complex in the presence or absence of the histone chaperone Nap1. The ATP-dependent action of SWI/SNF in the presence of increasing concentrations of the histone chaperone Nap1 resulted in a reduction in the proportion of DNA assembled as Cse4 containing chromatin and an increase in the amount of free DNA (Figure 5A). This shows that the SWI/SNF complex is capable of destabilising chromatin that contains Cse4. Furthermore, as nucleosomes assembled using H3 were not destabilised following remodelling with SWI/SNF and Nap1, it appears that Cse4 containing chromatin is especially susceptible to SWI/SNF driven dissociation (Figure 5B). This property of Cse4 containing nucleosomes is not unique to the 601 DNA fragment as similar observations were made with nucleosomes assembled onto a DNA fragment derived from the MMTV promoter
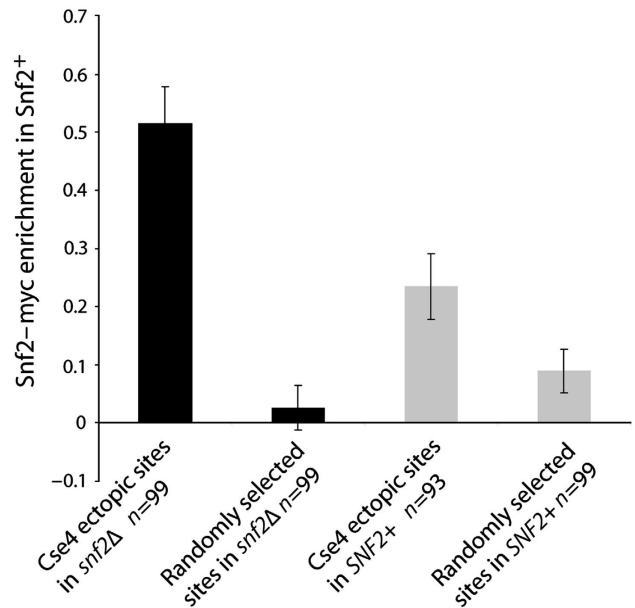
(Supplementary Figure S4). The susceptibility of Cse4 containing nucleosomes to dissociation is specific to a subset of remodelling enzymes as Chd1 was found not to function in this assay (Supplementary Figure S4).

The observation that SWI/SNF is capable of directing dissociation of Cse4 containing chromatin supports a role for SWI/SNF in the removal of Cse4 from ectopic locations along chromosome arms. If this is the case then it might be anticipated that deletion of *SNF2* would result in accumulation of Cse4 at sites previously bound by Snf2. This is, in fact, what we observe. In a *snf2*Δ strain, the strongly enriched ectopic Cse4 sites are found to be enriched for Snf2 in a wild-type strain when compared with an equal number of randomly selected sites (Figure 6).

Discussion

Following the observation of Snf2 enrichment at centromeres, we discovered that the SWI/SNF complex has an unanticipated role in determining the localisation of the centromeric histone variant Cse4. SWI/SNF was also found to have a role in the maintenance of nuclease hypersensitive sites flanking centromeres and to be capable of removing Cse4 containing chromatin from DNA *in vitro*. These observations support a model in which SWI/SNF acts to maintain the organisation of *S. cerevisiae* point centromeres by removing Cse4 misincorporated at ectopic locations.

Although we show that Snf2 is enriched at centromeres we do not fully understand how it is recruited. One possibility is that the recently reported interactions between components

of the SWI/SNF complex and the kinetochore component Ndc80 have a role in this process (Wong *et al*, 2007). However, we have found that Snf2 is still recruited to centromeres when conditional Ndc80 mutants are grown at the nonpermissive temperature (Gkikopoulos, data not shown). This raises the possibility that additional mechanisms of centromeric recruitment exist. One attractive possibility is that Cbf1 acts to recruit SWI/SNF. Consistent with this, we have observed that the enrichment of Snf2 is greater in the vicinity of Cbf1-binding sites in comparison to randomly selected sites genomewide (Supplementary Figure S5).

The SWI/SNF complex is not the only chromatin remodelling complex implicated in centromere function. In budding yeast, the multi-subunit RSC complex is closely related to SWI/SNF but known to perform distinct functions (Laurent *et al*, 1992; Cairns *et al*, 1996). Mutations to RSC components also activate the mitotic checkpoint and influence chromatin structure within the vicinity of centromeres (Tsuchiya *et al*, 1992, 1998; Angus-Hill *et al*, 2001; Hsu *et al*, 2003; Yamada *et al*, 2008) and cause changes in chromosome ploidy (Campsteijn *et al*, 2007). However, depletion of RSC has been reported not to affect the recruitment of Cse4 to centromeres (Hsu *et al*, 2003). Furthermore, RSC has additional roles at sites of cohesion along chromosome arms (Huang *et al*, 2004) perhaps contributing to it's role in chromosome transmission (Wong *et al*, 2002). It is notable that Rsc3 is enriched at centromeres in a similar way to Snf2 (Venters and Pugh, 2009; data not shown) raising the possibility that both RSC and SWI/SNF function in a partially redundant way to destabilise Cse4 containing nucleosomes. Consistent with this, we find that RSC, like SWI/SNF can destabilise Cse4 containing nucleosomes *in vitro* (data not shown).

An assortment of different ATP-dependent chromatin remodelling enzymes have been implicated in centromere function. For example, the Snf2-related ATPase, PICH, is recruited to centromeres and required for recruitment of Mad2 to kinetochores (Baumann *et al*, 2007). Other Snf2 family proteins have been proposed to influence the distribution of centromeric histone variants. These include the fission yeast Chd1 homologue, Hrp1, which has been shown to have a role in loading the centromeric histone variant Cnp1 at centromeres (Walfridsson *et al*, 2005). Related observations have been made in Chicken and Human cells (Okada *et al*, 2009), but it has more recently been reported that Chd1 has no role in CenpA deposition in Drosophila (Podhraski *et al*, 2010). Another nucleosome spacing complex, RSF, has been shown to influence CenpA distribution in HeLa cells (Perpelescu *et al*, 2009). In most of these cases, a role for remodelling enzymes in directing deposition has been proposed. In contrast, we proposed that SWI/SNF acts to maintain the distribution of Cse4 by removing it from ectopic locations.

The accumulation of Cse4 at ectopic locations has been observed previously (Camahort *et al*, 2009; Lefrancois *et al*, 2009). What defines these ectopic locations is not clear. One possibility is that Cse4 is incorporated at sites of high histone turnover (Dion *et al*, 2007). We find a 10% coincidence between sites of ectopic Cse4 and hot nucleosomes. This is statistically highly significant, suggesting that histone turnover contributes at a subset of cases. However, additional factors are likely to be involved at locations where there is no coincidence between Cse4 incorporation and histone turnover.

In vitro we found that the combined action of SWI/SNF and Nap1 acted to preferentially destabilise Cse4 containing nucleosomes. Our favoured explanation for how this might occur is that Cse4 containing nucleosomes are inherently less stable than H3 containing nucleosomes. We are not aware of any evidence to suggest that Nap1 normally participates in this process, but it is possible that a spectrum of nucleosome destablising activities may be effective in removing Cse4 containing nucleosomes from sites along chromosome arms. This raises the question of what stabilises Cse4 deposited at the centromere? A possible explanation for this is that association of kinetochore proteins prevents Cse4 containing chromatin from docking within the nucleosome-binding cleft in the SWI/SNF complex (Smith *et al*, 2003; Dechassa *et al*, 2008). Consistent with this, previous studies indicate that the Ndc10 kinetochore components are required for localisation of Cse4 to centromeres (Ortiz *et al*, 1999; Pearson *et al*, 2003). Alternatively, if Scm3 remains associated with Cse4 at centromeres, the resulting chromatin particle may not be a good substrate for SWI/SNF (Mizuguchi *et al*, 2007). The recruitment of Cse4 to centromeres is unlikely by itself to be sufficiently accurate to specify a single nucleosome/chromatin particle. However, the action of SWI/SNF and possibly additional nucleosome dissociating activities could help to remove Cse4 that happens to be deposited at locations along chromosome arms. The degradation of ectopic Cse4 provides an additional means of regulating the localisation of Cse4 (Hewawasam *et al*, 2010; Ranjitkar *et al*, 2010).

The presence of hypersensitive sites flanking the centromere might be anticipated to alter the net contribution of a yeast centromere to DNA topology. For example, if a functional centromere consists of one left-handed nucleosome flanked by two nucleosome-depleted regions and inactivation of the centromere results in loss of the nucleosome-free regions, this could result in a net change in linking number of $+2$. Such a change in linking number has been observed, but has been interpreted as evidence that the centromeric chromatin particle constrains a positive supercoil (Furuyama and Henikoff, 2009).

It is not clear whether the function of SWI/SNF in removing ectopic Cse4 is restricted to organisms with point centromeres. However, it is notable that human SWI/SNF B (PBAF) localises to kinetochores in metaphase (Xue *et al*, 2000). Furthermore, deletion of BRG1 results in the dissolution of pericentromeric heterochromatin, and appearance of micronuclei and an increase in cells undergoing aberrant mitoses (Bourgo *et al*, 2009). Further study will be required to establish whether these observations reflect and activity of BRG1 related to that of SWI/SNF at budding yeast centromeres.

Our study provides an example of a SWI/SNF-related complex functioning to maintain a nucleosome-depleted region. This adds to a list of specific examples in which SWI/SNF has been linked to nucleosome removal (Barbaric *et al*, 2007; Schwabish and Struhl, 2007; Bryant *et al*, 2008; Shivaswamy and Iyer, 2008; Gkikopoulos *et al*, 2009; Takahata *et al*, 2009) and general correlations between Snf2 localisation and histone occupancy genomewide (Gkikopoulos, data not shown; Dion *et al*, 2007; Venters and Pugh, 2009). It is important to point out that while this

manuscript has focused on the Snf2-dependent changes to chromatin structure at centromeres, additional changes are evident elsewhere in the genome in our data set (data not shown). The closely related RSC complex has also been shown to have a role in the generation of nucleosome-free regions at a significant number of promoters genomewide (Badis *et al*, 2008; Hartley and Madhani, 2009; Venters and Pugh, 2009). Regions of high nucleosome turnover that show net depletion of histones are a common feature of many eukaryotic genomes (reviewed by Radman-Livaja and Rando, 2010). Our observations linking chromatin disassembly to the distribution of Cse4 lend support to the attractive notion that nucleosome-depleted regions may act to punctuate the genomewide distribution of chromatin marks (Dion *et al*, 2007).

Materials and methods

Yeast growth and manipulation

Standard procedures were used for culture and manipulation of yeast (Amberg *et al*, 2005). Strains used in this study are listed in Supplementary Table S1. Constructs of CEN5-tetOs were previously described (Tanaka *et al*, 2000). *snf2Δ and mad2Δ* mutants were constructed according to a PCR-based protocol (Longtine *et al*, 1998). Deletion of *SWI2* was always carried out as the last step in all *snf2Δ* strains used in this study. *CSE4* was tagged with 3xmyc using the plasmid pYM5 as previously described (Knop *et al*, 1999). For the construction of the swi2-degron strains, an auxin-based system was used as previously described (Nishimura *et al*, 2009). *SWI2* was tagged with 9xmyc using the plasmid pYM20 as previously described (Janke *et al*, 2004). To deplete Snf2-aid, starter cultures were grown at 25°C for at least 8 h in YPA medium supplemented with 2% galactose and 2% raffinose. Subsequently, cultures were incubated at 30°C and Snf2-aid depletion was induced by addition of IAA at 2 mM final concentration.

Chromatin immunoprecipitation

ChIP was performed using monoclonal anti-myc antibody (9E10—Millipore) and polyclonal anti-mouse Dynabeads (Dynal) as previously described (Gkikopoulos *et al*, 2009).

Chromatin isolation and western blotting

To examine Cse4 level in chromatin, extracts were prepared based on previously described protocols (Conradt *et al*, 1992; Donovan *et al*, 1997). In brief, after spheroplasting and regrowth, cells were washed three times with lysis buffer (0.4 M Sorbitol/150 mM potassium acetate/2 mM magnesium acetate/20 mM Pipes/KOH, pH 6.8/1 mM phenylmethylsulfonyl fluoride/10 μg/ml leupeptin/ 1 μg/ml pepstatin A/10 mM benzamidine). Cells were resuspended in lysis buffer at no more than 8×10^8 cells/ml and lysed by addition of Triton X-100 to a final concentration of 1%. The chromatin-enriched fraction was isolated after centrifugation for 15 min at 15 800 *g*, and the supernatant was carefully removed. To clean the samples from the DNA, extracts were incubated with three Kunitz units of DNase1. In all cases, lysates were separated into a supernatant and a pellet as described above after a 5-min incubation on ice. For the immunoblot analysis, pellet and supernatant samples were run on 4–12% NUPAGE gels. After that, proteins were transferred to nitrocellulose and blocked with 5% dry milk in Tris-buffered saline containing 0.1% Tween 20. Purified 9E10 (anti-myc) was used at 1:2000 dilution, and immunoreactive bands were visualised with enhanced chemiluminescence (Millipore) according to the manufacturer's instructions.

Microarray hybridisation and data analysis

For microarray hybridisations, DNA purified by ChIP was amplified using the WGA kit (Sigma). Products were applied to Nimblegen tiling arrays (ArrayExpress accession: A-MEXP-1949) according to the manufacturer's specifications except for the data shown in Figures 1C and 4 where Affymetrix PN 520055 arrays were used and processed according to Badis *et al* (2008). Data from hybridisations used in this study has been submitted to ArrayExpress; Accession numbers: E-TABM-1141, E-MEXP-3163. For the nucleosome analysis,

the raw data from Affymetrix GCOS software (.CEL format) were analysed with Affymetrix Tiling Analysis Software v1.1. A tiling analysis group (.TAG file) for a two-sample analysis containing the three nucleosomal experiments as the 'treatment' and the three whole-genome samples as the 'control' was created in the Tiling Analysis Software. The two samples were then quantile normalised and median centred to produce a .txt file. ARS coordinates were defined from SGD. Fold enrichment in Figure 1 was calculated by averaging the mean log2 enrichment ratio per bp across all chromosomes. For the purpose of plotting Figure 2, a threshold was selected and only enrichment values above the threshold were plotted on the graph. For the selection of ectopic sites in *SWI2 +*, a lower threshold to that applied in *snf2Δ* data set was used in order to select a similar number of sites. Enrichment values for Snf2-myc in Figure 6 were calculated by taking the mean signal of a 400-bp long window centred at the assigned location of either the ectopic or the randomly selected site and standard error was plotted.

Fluorescent microscopy techniques

Cultures were prepared for microscopy essentially as described previously (Dewar *et al*, 2004; Tanaka *et al*, 2005). For synchronisation with α factor, cells were grown at 25°C to an OD: 0.2–0.4 and cell synchrony was monitored my bright field microscopy over 2–3 h to achieve at least 90% arrested cells. Release from α factor arrest was achieved by washing cells with 2 × volumes distilled water and cells were subsequently grown at 30°C. Delta T-dishes and Delta T heater (Bioptechs Inc) were used for live cell microscopy at 30°C.

FACS

Cells were fixed using 70% ethanol, then stained with propidium iodide as described (Amberg *et al*, 2005). Aggregates of cells were disrupted by sonication prior to FACS analysis.

Purification of SWI/SNF and Nap1

SWI/SNF was purified from yeast whole-cell extract by tandem affinity purification (TAP) over two-affinity columns from a Snf6TAP tag strain (Puig *et al*, 2001). Nap1 was expressed from a pET15b vector (Novagen) in *Escherichia coli* and purified by cobalt affinity, anion exchange and gel filtration chromatography.

Nucleosome reconstitution and remodelling

Recombinant yeast histones (Cse4, H3, H4, H2A and H2B) were expressed and purified as described (Luger *et al*, 1999). Nucleosomes were assembled by mixing equimolar amounts of histone octamer and DNA in high salt and performing continuous dialysis into low salt. The DNA fragment used in the assembly has 42 and 47 nucleotides flanking a synthetically selected 601 nucleosome positioning sequence (Thastrom *et al*, 1999). The fragment was PCR amplified with primer labelled with Cy5 dye (Ferreira *et al*, 2007). In all, 1 pmol of Cse4 and H3 octamers assembled onto Cy5-labelled 42W47 DNA was incubated with SWI/SNF (0.5 pmol) and Nap1 (as indicated) in the presence of 1 mM ATP at 30°C for 1 h. The buffer used in reactions contained 50 mM NaCl, 50 mM Tris (pH 7.5) and 3 mM $MgCl_2$. The reactions were stopped using 500 ng of *Hin*dIII digested λ phage DNA and adding glycerol to 4% (V/V) and placed on ice. The samples were separated on TE (10 mM Tris–HCl pH 8.0, 1 mm EDTA), 5% polyacrylamide gels for 1 h at 300 V at 4°C. Gels were scanned in a Fuji FLA-5100 fluorescent scanner.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

Conflict of interest

The authors declare that they have no conflict of interest.

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