Functional interaction between GCN5 and polyamines: a new role for core histone acetylation

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Polyamines are organic polycations essential for a wide variety of cellular functions, including nuclear integrity and chromosome condensation. Here we present genetic evidence that depletion of cellular polyamines partially alleviates the defects in *HO* and *SUC2* expression caused by inactivation of the GCN5 histone acetyltransferase. In addition, the combination of polyamine depletion and a *sin⁻* allele of the histone H4 gene leads to almost complete bypass of the transcriptional requirement for GCN5. In contrast, polyamine depletion does not alter the transcriptional requirements for the SWI/SNF chromatin remodeling complex nor does depletion lead to global defects in transcriptional regulation. In addition to these genetic studies, we show that polyamines facilitate oligomerization of nucleosomal arrays *in vitro*, and that polyamine-mediated condensation requires intact core histone N-terminal domains and is inhibited by histone hyperacetylation. Our studies suggest that polyamines are repressors of transcription *in vivo*, and that one role of histone hyperacetylation is to antagonize the ability of polyamines to stabilize highly condensed states of chromosomal fibers.
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Introduction

Polyamines are small, ubiquitous organic polycations that have been implicated in a wide variety of physiological functions including protein translation, membrane stabilization and cell proliferation (reviewed in Tabor and Tabor, 1984). As one might expect, biosynthesis of polyamines is essential for viability of both prokaryotic and eukaryotic cells. The most common polyamines are putrescine, spermidine and spermine, which contain two, three or four charged amine groups, respectively. Eukaryotic organisms contain all three of these amines at abundant levels (high micromolar to millimolar). Much attention has focused on the roles of polyamines in various disease processes. For instance, polyamines have been implicated in autoimmune disorders such as systemic lupus erythematosus (Brooks, 1994). Polyamines also accumulate in cancer cells, and

high levels of polyamines are found in the urine from cancer patients (reviewed in Russel and Duri, 1978; Chanda and Ganguly, 1988). Increased levels of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of polyamines, are associated with many types of cancer (Pegg, 1988), and overproduction of ODC can lead to acquisition of the transformed cell phenotype (Tabib and Bachrach, 1998). These observations have led to the development of inhibitors of polyamine biosynthesis, and several such drugs have been used successfully in the treatment of some cancers and protozoan diseases, particularly African trypanosomiasis (McCann *et al.*, 1987; Fairlamb, 1990a,b). In terms of polyamine function in the nucleus, several studies have implicated polyamines in the formation of higher order chromosomal fibers *in vitro* and *in vivo* (Belmont *et al.*, 1989; Belmont and Bruce, 1994), and spermidine and spermine have been shown to facilitate condensation of chromatin fragments *in vitro* (Colson and Houssier, 1989). Spermidine, spermine and related analogs have also been shown to interact specifically with nucleosome core particles and DNA *in vitro* (Morgan *et al.*, 1989).

Higher order folding of chromatin requires both cation-dependent charge neutralization and the flexible N-terminal domains of the core histones (reviewed in Fletcher and Hansen, 1996). These 25–40 amino acid N-terminal ‘tails’ are exposed at the surface of the nucleosome core particle (van Holde, 1988; Luger *et al.*, 1997) and contain the sites for post-translational histone acetylation. Recent studies have used nucleosomal array model systems to define the roles of the core histone N-termini and histone acetylation in chromatin condensation (Garcia-Ramirez *et al.*, 1992, 1995; Tse and Hansen, 1997; Tse *et al.*, 1998b). The DNA template for reconstitution of model arrays is composed of 12 tandem repeats of a 208 bp 5S rRNA gene from *Lytechinus variegatus* (the 208-12 template). When these nucleosomal arrays are incubated in physiological mixtures of monovalent and divalent salt, they both fold extensively and oligomerize (Schwarz and Hansen, 1994). Importantly, although oligomerization has commonly been referred to throughout the literature as ‘precipitation’ or ‘aggregation’, and as such is often dismissed as biologically irrelevant, it has been shown recently that the oligomerization transition is a highly cooperative and fully reversible process (Schwarz *et al.*, 1996) that shares many characteristics known to be involved in chromosomal fiber formation *in vivo* (reviewed extensively in Fletcher and Hansen, 1996; also see Sen and Crothers, 1986; Widom, 1986; Belmont and Bruce, 1994). Furthermore, both higher order folding and oligomerization are absolutely dependent on the presence of the N-terminal domains (Tse and Hansen, 1997) and are diminished by histone hyperacetylation (Tse *et al.*, 1998b), consistent with a fundamental role for the core histone

N-termini in all steps leading to chromosomal fiber condensation (reviewed in Fletcher and Hansen, 1996; Hansen, 1997; Hansen *et al.*, 1998; Luger and Richmond, 1998).

Gcn5p is the founding member of a growing family of histone acetyltransferases (Neuwald and Landsman, 1997), which includes several proteins previously identified as transcriptional coactivators. The *GCN5* gene was identified initially as a positive regulator of amino acid biosynthetic genes (Georgakopoulos and Thireos, 1992), and subsequently as a putative transcriptional adaptor (Marcus *et al.*, 1994). More recent studies have shown that Gcn5p has intrinsic histone acetyltransferase activity (Brownell *et al.*, 1996), and that it is the catalytic subunit of several, distinct histone acetyltransferase complexes (Grant *et al.*, 1997; Pollard and Peterson, 1997; Ruiz-Garcia *et al.*, 1997; Saleh *et al.*, 1997), and that Gcn5p preferentially acetylates condensed nucleosomal arrays when assayed under optimal conditions *in vitro* (Tse *et al.*, 1998a). *GCN5*-dependent histone acetylation is recruited to the promoter region of a small number of genes in yeast, and has been shown to precede and be required for activated transcription (Kuo *et al.*, 1998; Krebs *et al.*, 1999). Gcn5p activity may disrupt a domain of condensed chromatin surrounding the target gene, or acetylation may promote or inhibit the binding of non-histone proteins to the promoter region.

Here we describe genetic and biochemical studies that indicate a functional link between *GCN5*-dependent histone acetylation and polyamine function *in vivo* and *in vitro*. We have isolated the *ARG3* gene as a multicopy suppressor of the transcriptional defects caused by a mutation in *GCN5*. Overexpression of Arg3p appears to partially restore transcriptional activity in a *gcn5* mutant by limiting the availability of ornithine for polyamine biosynthesis. Consistent with this apparent link, a deletion of the *SPE1* gene, which encodes the rate-limiting enzyme for polyamine biosynthesis (ODC), also partially alleviates the defects in *HO* and *SUC2* expression of a *gcn5* mutant. Furthermore, we find that the combination of a *spe1* deletion and a *sin⁻* allele of the histone H4 gene leads to almost complete bypass of the transcriptional requirement for *GCN5*. In complementary *in vitro* studies, we find both that polyamines facilitate the reversible oligomerization of nucleosomal arrays, and that polyamine-dependent oligomerization requires the core histone N-terminal tails and is diminished by histone acetylation. These studies support the idea that polyamines contribute to transcriptional repression *in vivo* by stabilizing condensed chromatin fibers, and that histone acetyltransferases, such as Gcn5p, promote transcriptional induction in part by counteracting polyamine-dependent chromatin condensation.

Results

Multicopy *ARG3* alleviates transcriptional defects due to a *gcn5* mutation

We have used a genetic screen to identify genes that when present in high copy number rescue the transcriptional defects caused by a mutation in the yeast *GCN5* gene, which encodes the catalytic subunit of at least three histone acetyltransferase complexes (Grant *et al.*, 1997; Pollard and Peterson, 1997; Saleh *et al.*, 1997). The yeast strain

Fig. 1. Multicopy *ARG3* partially alleviates the defect in *HO-LacZ* expression due to mutations in *GCN5*. Expression of a chromosomal *HO-LacZ* fusion was measured by liquid β -galactosidase assays in wild-type (CY432), *gcn5⁻* (CY563) and the *gcn5⁻* (CY563) strain harboring a high copy plasmid containing the *ARG3* ORF (pARG3). Assays were performed with cells grown in the presence or absence of additional ornithine in the growth media. Assays were performed in triplicate and the standard error was <20%.

that we have used for this hunt harbors a null allele of *GCN5* and an *HO-LacZ* fusion gene integrated at the chromosomal *ho* locus. Because *HO-LacZ* expression requires the *GCN5* product (Pollard and Peterson, 1997; Perez-Martin and Johnson, 1998), this *gcn5⁻* strain is white in β -galactosidase filter assays. A high copy (100–200 copies/cell) yeast genomic library (Nasmyth and Reed, 1980) was introduced into this *gcn5⁻* strain. Of the initial 2500 transformants (approximately one genome equivalent), 60 candidates were isolated that were blue in β -galactosidase filter assays (expressing *HO-LacZ*). Of these 60 initial positives, 30 plasmids were able to re-confer suppression of the *gcn5⁻* phenotype following recovery from yeast and passage through bacteria. From this pool, PCR analysis identified one plasmid that contained the *GCN5* gene; this isolate was eliminated from further characterization. After partial sequencing of the genomic inserts of five of the most potent suppressors, we found that two of the plasmids contained overlapping restriction fragments harboring the *ARG3* locus as well as several additional genes. To confirm that *ARG3* was responsible for high copy suppression, the *ARG3* gene was subcloned into the high copy vector and introduced into the *gcn5⁻* strain. The isolated *ARG3* gene was able to suppress the defect in *HO-LacZ* expression to a similar level to that of the original, larger clone (data not shown; see Figure 1). In addition, multicopy *ARG3* also alleviated the defect in *HO-LacZ* expression due to a deletion of *ADA2* or *ADA3*, two other components of *ADA-GCN5* acetyltransferase complexes (data not shown) (Grant *et al.*, 1997; Pollard and Peterson, 1997; Saleh *et al.*, 1997).

Fig. 2. Polyamine depletion partially alleviates *gcn5* Δ transcriptional defects. (A) The biosynthetic pathway of polyamine production in *Saccharomyces cerevisiae*. (B) Depletion of intracellular polyamines. *HO-LacZ* expression in wild-type (CY773), *spe1* Δ (CY765), *gcn5* Δ (CY761) or a *spe1* Δ *gcn5* Δ (CY769) double mutant, grown in YPD rich media or polyamine-free media for 4 days. β -galactosidase assays were performed in triplicate and the standard error was <20%. (C) Polyamine add back. The indicated concentrations of spermidine were added to cultures of *gcn5* Δ (CY761) or *spe1* Δ *gcn5* Δ (CY769) double mutants after 4 days of polyamine depletion. *HO-LacZ* expression was measured as described in Figure 1. (D) *SUC2* expression. Levels of invertase activity (*SUC2* expression) in wild-type (CY773), *spe1* Δ (CY765), *gcn5* Δ (CY761) or *spe1* Δ *gcn5* Δ (CY769) double mutants were determined after 4 days growth in polyamine-free media. Invertase activities from three independent cultures were averaged and the standard error was <20%. *HO-LacZ* was measured in parallel and is shown for comparison.

The *ARG3* gene encodes ornithine transcarbamoylase, a mitochondrial enzyme responsible for the conversion of ornithine into citrulline, one step of the urea cycle. The only other use of ornithine pools in the cytoplasm is for production of intracellular polyamines (see Figure 2A). To explain our genetic results, we hypothesized that overexpression of *ARG3* may deplete the ornithine pool, thereby limiting the production of polyamines, and that depletion of cellular polyamines may be responsible for the suppression of *gcn5* Δ transcriptional defects due to their effects on chromatin structure. To test this possibility, we supplemented the growth media with ornithine, then re-evaluated the ability of multicopy *ARG3* to alleviate the *HO-LacZ* defect in the *gcn5* mutant (Figure 1). The *gcn5* mutant has 3% of the wild-type level of *HO-LacZ*

expression. In the presence of multicopy *ARG3*, expression is enhanced >3-fold, to 10% of the wild-type level. This effect of multicopy *ARG3* on *HO-LacZ* expression is similar in magnitude to the suppression observed previously for semi-dominant mutations in the genes encoding histone H3 or H4 (Pollard and Peterson, 1997; see also Figure 5). When additional ornithine is present in the growth media, the effect of multicopy *ARG3* is decreased such that *HO-LacZ* expression is enhanced only 1.5-fold in the *gcn5* mutant (Figure 1). In contrast, addition of ornithine increases expression of *HO-LacZ* 1.4-fold in the wild-type strain. These data are consistent with our hypothesis that multicopy *ARG3* leads to a depletion of ornithine pools, and that this depletion is required to observe suppression of the *gcn5* mutant phenotype.

Polyamine depletion alleviates *gcn5*[−] transcriptional defects

Because ornithine is the sole precursor for polyamine biosynthesis (Figure 2A), depletion of cytosolic ornithine would result in decreased levels of polyamines. To test directly whether polyamine depletion can alleviate transcriptional defects due to a deletion of *GCN5*, we generated a congeneric set of strains that harbor deletions of both *GCN5* and *SPE1*. *SPE1* encodes ODC, which converts ornithine to putrescine (Figure 2A) (Schwartz *et al.*, 1995). In *spe1* mutants, polyamines can not be synthesized, and cellular levels of polyamines decline during growth in polyamine-free media (PFM) (Schwartz *et al.*, 1995). Following 5–6 days of growth in PFM, *spe1* cells arrest reversibly in the G₁ phase of the cell cycle (Balasundaram *et al.*, 1991).

Wild-type, *spe1*, *gcn5* and *spe1 gcn5* mutants were grown in PFM for 4 days to deplete polyamine pools, then expression of *HO-LacZ* was analyzed (Figure 2B). Following 4 days of depletion, *spe1* mutants grow slowly, but contain sufficient levels of polyamines to perform essential functions. In the presence of *SPE1*, growth in PFM had little effect on *HO-LacZ* expression in either a *GCN5*⁺ or *gcn5*[−] strain. In contrast, expression in the *gcn5 spe1* double mutant was increased nearly 6-fold in comparison with a *gcn5* strain grown in PFM (3.7 versus 21% of the wild-type level). Furthermore, the effect of the *spe1* mutation was nearly eliminated by growth in rich media [Figure 2B, yeast extract/peptone/dextrose (YPD)] or by addition of spermidine to the PFM (see Figure 2C). Thus, depletion of cellular polyamines can partially alleviate the defect in *HO-LacZ* expression due to deletion of *GCN5*.

The *GCN5* gene product is also required for full expression of the *SUC2* gene (Pollard and Peterson, 1997). To test whether polyamine depletion has a more general effect on *GCN5*-dependent transcription, we assayed expression of *SUC2* after depletion of polyamines (Figure 2D). After 4 days of growth in PFM, the *gcn5 spe1* double mutant had nearly a 3-fold higher level of *SUC2* expression than the level observed in the *gcn5* single mutant (8 versus 20% of wild-type levels). In contrast, *SUC2* expression in the *GCN5 spe1* single mutant was decreased to 82% of the wild-type level. Thus, depletion of polyamines partially alleviates both the *HO-LacZ* and *SUC2* transcriptional defects caused by loss of *GCN5* function.

Recently we have found that growth of *gcn5* mutants is extremely sensitive to low concentrations of *t*-butylhydroperoxide (tBOOH) which is a stable inducer of the oxidative stress response in yeast (Kuge and Jones, 1994). The inability of *gcn5* mutants to grow on this medium suggests that *GCN5* might be required for expression of one or more genes induced by oxidative stress. To test whether a deletion of *SPE1* might alleviate this growth defect, serial dilutions of wild-type, *spe1*, *gcn5* and *gcn5 spe1* cells were spotted onto media that contained or lacked tBOOH (Figure 3). Whereas the *gcn5* mutant shows a severe growth defect on plates containing tBOOH, the *gcn5 spe1* double mutant grows nearly as well as the wild-type strain (upper panel). Thus, polyamine depletion alleviates many of the phenotypes of a *gcn5* mutant.

Fig. 3. Polyamine depletion alleviates the sensitivity of *gcn5* mutants to oxidative stress. Strains were grown to an OD₆₀₀ of 1.0 and diluted to a concentration of 2×10^5 cells/ml in phosphate-buffered saline. A 5 μ l aliquot of 4-fold dilutions was spotted onto plates in the absence or presence of 200 μ M *t*-butylhydroperoxide (−/+ tBOOH). The spots were allowed to dry and the plates were incubated at 30°C for 3 days. Strains used were wild-type (CY773), *spe1* Δ (CY765), *gcn5* Δ (CY761), *swi2* Δ (CY778), *spe1* Δ *gcn5* Δ (CY769) and *spe1* Δ *swi2* Δ (CY777).

Genetic relationship among polyamines, GCN5 and SWI/SNF complex

GCN5 is required for expression of many of the same genes that require the SWI/SNF chromatin remodeling complex (Pollard and Peterson, 1997). In addition, *GCN5* and SWI/SNF subunit genes show similar genetic interactions with chromatin components (Pollard and Peterson, 1997; Perez-Martin and Johnson, 1998). For instance, semi-dominant *sin*[−] alleles of the genes encoding histones H3 and H4 can partially alleviate the transcriptional defects of *gcn5* or *swi/snf* mutations. Due to the similarities between these two groups of mutants, we tested whether polyamine depletion could alleviate the defects in growth and transcription due to a deletion of the *SWI2/SNF2* gene, which encodes the catalytic subunit of the SWI/SNF complex. First, we tested whether a deletion of *SPE1* could alleviate the slow growth of a *swi2* mutant on media containing tBOOH (Figure 3, lower panel). Unlike the case for *gcn5*, deletion of *SPE1* did not alleviate the slow growth of a *swi2* mutant, in fact the *swi2 spe1* double mutant was more sensitive to this inducer of oxidative stress. Next, we analyzed expression of the *HO-LacZ* fusion gene in an isogenic set of *SWI*⁺ *SPE*⁺, *spe1*, *swi2* and *swi2 spe1* strains (Figure 4A). A *swi2* deletion decreases *HO-LacZ* expression to 0.7% of the wild-type level; this transcriptional defect is not suppressed by depletion of polyamines (0.9% of wild type; Figure 4A). Importantly, these results demonstrate that the ability of *spe1* to suppress the transcriptional defects of a *gcn5* mutant is not due to stabilization or enhanced translation of a low level of lacZ transcripts. These results also

Fig. 4. Polyamine depletion does not alleviate the requirement for the SWI/SNF chromatin remodeling complex. **(A)** *HO-LacZ* expression in wild-type (CY773), *spe1* Δ (CY765), *gcn5* Δ (CY761), *swi2* Δ (CY778), or *spe1 gcn5* Δ (CY769) and *spe1 swi2* Δ (CY777) double mutants grown in PFM for 4 days. **(B)** Growth rates of yeast strains in PFM. *Spe1*⁻, *spe1*⁻ *gcn5*⁻ and *spe1*⁻ *swi2*⁻ cells were grown in normal or polyamine-free media, with appropriate dilution to maintain an OD₆₀₀ between 0.05 and 0.8. Doubling times were calculated after growth in culture at the times indicated.

indicate that depletion of polyamines does not bypass requirements for all types of chromatin remodeling complexes.

One possibility that we considered is that the inability of a *spe1* mutation to alleviate the phenotypes of a *swi2* mutant is due to the slow growth of this strain, which might delay the kinetics of polyamine depletion. To investigate this possibility, we determined the growth rates of *spe1*, *gcn5 spe1* and *swi2 spe1* cells grown in media that contain (SD) or lack (PFM) polyamines (Figure 4B). Similarly to previous studies, we found that the growth rate of *spe1* cells began to slow dramatically after ~3 days of growth in PFM, and cells stopped dividing after 5 days of growth (Figure 4B and data not shown). Likewise, the *gcn5 spe1* double mutant showed similar kinetics of growth arrest in PFM. Importantly, the growth rate of the *swi2 spe1* double mutants also began to decline after 3 days of culturing in PFM and growth arrest was achieved after 4–5 days (Figure 4B and data not shown). Thus, the

Fig. 5. Polyamine depletion and histone *sin* mutations are additive for alleviation of *gcn5* transcription defects. *HO-LacZ* expression in wild-type (CY773), *spe1* Δ (CY765), *gcn5* Δ (CY761) or *spe1 gcn5* Δ (CY769) double mutants grown in polyamine-free media. Strains harbor either a wild-type copy of histone H4 or a semi-dominant (*sin*⁻) allele of histone H4 on ARS/CEN plasmids (Kruger *et al.*, 1995; Wechsler *et al.*, 1997).

slow growth of the *swi2* mutant does not appear to slow the kinetics of polyamine depletion.

Polyamines and an intact histone octamer contribute equally to transcriptional repression in the absence of GCN5

The genetic interactions between *GCN5* and histone H4 (Pollard and Peterson, 1997; Perez-Martin and Johnson, 1998) indicate that one role for *GCN5* is to antagonize transcriptional repression mediated by nucleosomes. To examine the genetic relationship between polyamines and histones, we introduced a low copy plasmid containing either a wild-type copy of *HHF2* (histone H4) or a semi-dominant *sin*⁻ allele (Kruger *et al.*, 1995; Wechsler *et al.*, 1997) of *HHF2* (*hhf2-7*) into the *gcn5* and *gcn5 spe1* mutant strains. The semi-dominant histone H4 allele leads to a 4.5-fold increase in *HO-LacZ* expression in the *gcn5* mutant (Figure 5), which is similar to levels of suppression seen with polyamine depletion (Figure 2B). However, when the *spe1* deletion is combined with a *sin*⁻ *HHF2* allele, *HO-LacZ* expression increases 14-fold to nearly 60% of the wild-type level (Figure 5). Thus, the combination of polyamine depletion and a *sin*⁻ allele of the histone H4 gene almost completely alleviates the *GCN5* dependence of *HO-LacZ* expression. Furthermore, these data suggest that polyamines and the SIN domain of histone H4 act independently to contribute to transcriptional repression.

Depletion of polyamines does not lead to global transcriptional defects

Our genetic studies indicate that polyamines behave formally as a repressor of transcription, and one role of *GCN5* is to antagonize these repressive effects. One possibility is that polyamines control expression of a small subset of genes, perhaps only those genes regulated by *GCN5* (e.g.

Fig. 6. Polyamine depletion does not lead to global changes in gene expression. *Spe1⁻* or *spe1⁻ gcn5⁻* cells were grown in minimal medium that contains (SD) or lacks polyamines (PFM) for 72 h, and then either not treated or treated with 1 mM copper sulfate for 20 min (-/+ Cu). A single nylon membrane containing mRNA from these cells was hybridized with radiolabeled probes as indicated.

HO and *SUC2*). Alternatively, the presence or absence of polyamines may exert a global influence on gene expression. To test these possibilities, we investigated the expression of four different genes after *SPE1⁺* or *spe1⁻* cells were grown in PFM for 4 days. Cells were either harvested immediately for RNA isolation, or treated for 20 min with copper to induce expression of the *CUP1* and *SSA4* genes. Figure 6 shows the Northern blot analysis of *SSA4*, *CUP1*, *DED1* and *ADH1* expression after polyamine depletion. In the case of the constitutively expressed genes, *DED1* and *ADH1*, polyamine depletion did not increase or decrease the steady-state level of RNA. Likewise, polyamine depletion did not increase the basal, uninduced level of *SSA4* or *CUP1* expression, nor did depletion alter the response of *SSA4* or *CUP1* to copper treatment. Thus, for the four genes tested, polyamine depletion did not influence either the basal or induced level of gene expression. These results are consistent with our analysis of *HO* and *SUC2* expression, where no significant changes were observed unless *GCN5* was inactivated (Figure 2). These results reinforce the specificity of the genetic interactions between *GCN5* and polyamines, and are consistent with the view that polyamines may inhibit expression of only a small subset of genes.

Polyamines facilitate reversible oligomerization of nucleosomal arrays

How do polyamines act to inhibit transcription *in vivo*? One possibility is that polyamines stabilize or facilitate the formation of higher order condensed chromatin structures. In this view, *GCN5*-dependent acetylation would antagonize the repressive effects of polyamines by de-

stabilizing condensed chromatin domains. This could occur either if *GCN5* directly acetylates the polyamines themselves, or if *GCN5*-dependent acetylation of the core histone N-termini counteracts the ability of polyamines to cause chromatin condensation. Two different polyamine acetylases have been described, the cytoplasmic spermidine/spermine *N*¹-acetyltransferase and the nuclear spermidine *N*⁸-acetyltransferase (reviewed in Morgan, 1998). Polyamines can also be acetylated by crude preparations of nuclear histone acetyltransferases (Wong *et al.*, 1991). Polyamine acetylation initiates the catabolism of polyamines *in vivo* and it may also facilitate their transport across nuclear or plasma membranes. As is the case for histones, acetylation of polyamines neutralizes positive charge and is expected to disrupt the binding of polyamines to negatively charged binding sites (i.e. chromatin). We find, however, that native *GCN5*-containing histone acetyltransferase complexes (Pollard and Peterson, 1997) are unable to incorporate [³H]acetate into putrescine, spermidine or spermine (data not shown). Therefore, to investigate polyamine effects on chromatin structure, we have used defined model systems to determine directly whether polyamines can facilitate condensation of nucleosomal arrays *in vitro*.

The DNA template for reconstitution of model arrays is composed of 11–12 head-to-tail repeats of a 208 bp 5S rRNA gene from *Lytechinus variegatus* (the 208-11 and 208-12 templates). Each repeat can rotationally and translationally position a nucleosome after *in vitro* reconstitution with purified histone octamers (Hansen and van Holde, 1991). When these model nucleosomal arrays are incubated in low salt TE buffer, they assume an extended structure that sediments at 28S. Addition of monovalent cations (Na⁺) induces folding of the arrays to an intermediate 40S level, whereas 1–2 mM Mg²⁺ results in formation of extensively folded structures that sediment at 55S and are compacted to the same degree as 30 nM chromatin fibers (reviewed in Fletcher and Hansen, 1996). In addition to these intramolecular folding reactions, divalent cations (e.g. >2 mM Mg²⁺) can induce nucleosomal arrays to oligomerize reversibly (Schwarz *et al.*, 1996). The oligomerization transition is highly cooperative and generates relatively defined, soluble structures that sediment at >500S. Notably, there is substantial evidence that this process is related to the long range fiber-fiber interactions that stabilize higher order chromosomal domains such as chromonema fibers (Widom, 1986; Belmont and Bruce, 1994; Schwarz *et al.*, 1996; reviewed in Fletcher and Hansen, 1996).

We first used the 208-11 nucleosomal arrays to investigate the ability of spermidine to facilitate intermolecular oligomerization (Figure 7). Nucleosomal arrays were reconstituted with three different sources of histone octamers: (i) chicken erythrocyte histone octamers; (ii) recombinant *Xenopus* histone octamers; and (iii) recombinant histone octamers that contain a *Xenopus* H3–H4 tetramer and yeast H2A–H2B dimers. The *Xenopus*–yeast hybrid octamers display histone N-terminal domains that are nearly identical in sequence to those of bulk yeast chromatin. Increasing concentrations of spermidine were added to each of these nucleosomal arrays in TE buffer, and oligomerization was measured by determining either the decrease in radioactivity or the decrease in *A*₂₆₀ of the

sample following centrifugation in a microcentrifuge (Schwarz and Hansen, 1994; Schwarz *et al.*, 1996). For all three types of nucleosomal arrays, oligomerization was first detected at 50–125 μM spermidine and was >90% complete in TE buffer containing 250 μM spermidine (Figure 7A). Interestingly, we reproducibly observed that the *Xenopus*–yeast hybrid octamer arrays oligomerize at lower spermidine concentrations than the other array types.

The sharpness of the decrease in array concentration with increasing spermidine concentration indicates that spermidine-induced oligomerization is cooperative, as is the case in MgCl_2 (Schwarz *et al.*, 1996). However, induction of a similar extent of oligomerization requires 2–5 mM MgCl_2 . Thus, spermidine is ~20-fold more effective than Mg^{2+} at promoting oligomerization of nucleosomal arrays *in vitro*.

Divalent cations such as Mg^{2+} promote intramolecular folding of nucleosomal arrays prior to induction of oligomerization (Schwarz and Hansen, 1994; Schwarz *et al.*, 1996). To determine the effectiveness of polyamines at promoting array folding, reconstituted arrays were incubated in the presence of spermidine, and samples were analyzed by a sedimentation velocity experiment in the analytical ultracentrifuge (Hansen *et al.*, 1997). The integral distributions of sedimentation coefficients obtained after analysis of the data by the method of van Holde and Weischet (1978) are shown in Figure 7B. In the absence of spermidine, arrays reconstituted with chicken octamers sedimented as a nearly homogeneous population, having an $S_{\text{midpoint}} = 28\text{S}$, indicating that the arrays were saturated with histone octamers and unfolded under these conditions (Hansen *et al.*, 1989). In the presence of 100 μM spermidine (i.e. the greatest concentration that could be tested prior to induction of oligomerization; see Figure 7A), the sedimentation coefficient distribution ranged from 30 to 40S (Figure 7B), indicating only a small degree of spermidine-induced folding. Furthermore, the extent of folding induced by 100 μM spermidine is substantially less than that observed in Mg^{2+} immediately prior to oligomerization (Schwarz and Hansen, 1994; Schwarz *et al.*, 1996). Consequently, we next tested whether addition of spermidine altered the extent of array folding observed in the presence of mono- and divalent cations. Reconstituted arrays sedimented between 30 and 50S in buffer that contained 100 mM NaCl and 2 mM MgCl_2 (Figure 7B), consistent with previous observations of an equilibrium between extended and extensively folded structures under these conditions (Schwarz and Hansen,

Fig. 7. Polyamines promote cooperative oligomerization of nucleosomal arrays *in vitro*. (A) Oligomerization assays of saturated nucleosomal arrays. ^{32}P -labeled nucleosomal arrays were reconstituted with the indicated sources of histone octamers, incubated in 10 mM Tris pH 7.5, 1 mM EDTA and increasing concentrations of spermidine, sedimented in a microcentrifuge, and the percentage of the arrays remaining in the supernatant was measured by scintillation counting. The *Xenopus*–yeast hybrid octamers contained recombinant *Xenopus* H3–H4 tetramers and recombinant yeast H2A–H2B dimers. (B) Sedimentation velocity experiment. Reconstituted arrays were sedimented in an XL-A (Beckman) analytical ultracentrifuge equipped with scanner optics. Analysis was performed in either 10 mM Tris pH 7.5, 1 mM EDTA $\pm$ 100 μM spermidine, or in 10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl, 2 mM MgCl_2 $\pm$ 250 or 500 μM spermidine. The integral distribution of sedimentation coefficients is shown. The boundary fraction (y-axis) indicates the fraction of the sample that has an $S_{20,w}$ value equal to or less than the $S_{20,w}$ value indicated on the x-axis. (C) Hyperacetylation of core histones inhibits polyamine-induced oligomerization of nucleosomal arrays. Oligomerization assays of 208–12 nucleosomal arrays in TE buffer and increasing spermidine concentrations. Non-radioactive, saturated 12mer reconstituted arrays were prepared using chicken or HeLa hypoacetylated core histones, trypsinized chicken histones, hyperacetylated HeLa histones or DNA template in the absence of histones. Oligomerization was assayed as in (A), except that the percentage of array remaining in the supernatant was assayed by A_{260} .

1994; Tse *et al.*, 1998b). Importantly, addition of 250 or 500 μM spermidine did not alter the sedimentation coefficient distribution observed in 100 mM NaCl/2 mM MgCl_2 alone (Figure 7B), indicating that spermidine has no additional effects on folding other than those due to the inorganic cations themselves. It should be noted that ~ 1 mM spermidine was required to induce oligomerization in the presence of 100 mM NaCl (data not shown), consistent with previous effects of NaCl on Mg^{2+} -dependent oligomerization (Schwarz *et al.*, 1996). Taken together, the data in Figure 7 indicate that polyamines are considerably more effective at facilitating oligomerization rather than folding of nucleosomal arrays.

Core histone acetylation inhibits spermidine-mediated oligomerization

Previous studies have shown that oligomerization of nucleosomal arrays requires intact core histone N-terminal tails (Schwarz *et al.*, 1996; Tse and Hansen, 1997), and is diminished by histone hyperacetylation (Ridsdale *et al.*, 1990; Tse *et al.*, 1998b). To investigate the role of the histone tails and acetylation on spermidine-induced chromatin condensation, we reconstituted nucleosomal arrays with three different sources of histone octamers: (i) hypoacetylated histone octamers isolated from HeLa cells; (ii) trypsinized chicken histone octamers that lack most of their N- and C-terminal tails; and (iii) hyperacetylated histone octamers isolated from butyrate-treated HeLa cells. These different types of reconstituted arrays were incubated in TE buffer containing increasing concentrations of spermidine, and oligomerization was analyzed by the microcentrifuge assay.

Figure 7C shows a representative set of oligomerization profiles of nucleosomal arrays reconstituted with different types of histone octamers. Similarly to the experiment shown in Figure 7A, 200 μM spermidine was required for complete intermolecular association of the hypoacetylated nucleosomal arrays. In contrast, trypsinized nucleosomal arrays behaved like histone-free naked DNA, requiring >500 μM spermidine to oligomerize completely. The hyperacetylated arrays required 50% higher spermidine concentrations to achieve the same level of intermolecular association as observed for the hypoacetylated arrays (Figure 7C). Thus, intact core histone termini are required for spermidine to promote efficient intermolecular association of nucleosomal arrays, and histone acetylation partially disrupts this spermidine-dependent condensation reaction.

Discussion

Polyamines are essential for maintaining higher order organization of chromatin *in vivo* and *in vitro* (Belmont *et al.*, 1989; Belmont and Bruce, 1994), but their role in the regulation of transcription has not been investigated thoroughly. We have shown that depletion of cellular polyamines partially alleviates many of the transcriptional defects due to loss of the Gcn5p histone acetyltransferase. In contrast, depletion of polyamines does not lead to global changes in transcription, nor does depletion alleviate the need for the SWI/SNF chromatin remodeling complex. Recently, the *SPE3* gene, which encodes spermidine synthase, was isolated in a genetic screen for mutations

that alleviate mitotic repression of sporulation-specific genes (Friessen *et al.*, 1998). As is the case for total polyamine depletion (i.e. *spe1⁻*), the inability to synthesize spermidine resulted in gene-specific defects in transcriptional repression. For instance, basal expression from a UAS-less *CYC1-lacZ* fusion gene was not affected by a deletion of *SPE3*, but repression mediated by an $\alpha 2$ -Mcm1p operator or a NRE^{DIT} mitotic repression element is weakened considerably (Friessen *et al.*, 1998). Likewise, our genetic studies support the view that polyamines are gene-specific repressors of transcription *in vivo*, and that one role of the Gcn5p histone acetyltransferase is to antagonize polyamine function.

In order to understand better the mechanistic relationship between polyamines and histone acetylation, we tested whether polyamines could promote higher order folding and/or oligomerization of nucleosomal arrays. We found that polyamines potently induce cooperative oligomerization of nucleosomal arrays, relative to their effects on higher order folding. Furthermore, this polyamine-mediated oligomerization reaction requires intact core histone tails and is inhibited by histone hyperacetylation. These results suggest that one mechanism through which polyamines repress transcription is by stabilizing higher order chromosomal fiber structure, and that *GCN5*-dependent histone acetylation counteracts polyamine-induced chromatin condensation, leading to enhancement of transcription.

Isolation of ARG3 as a multicopy suppressor of a gcn5 mutant

Our multicopy suppressor screen was initially designed to identify factors that, when overexpressed, might compensate for inactivation of *GCN5*. This potential class of suppressor was not obtained in this screen, either because it does not exist, or because the screen was not saturated. Consistent with the latter possibility, we only screened 2500 transformants, which is at least 10-fold fewer than would be required to approach saturation. Although we screened a minimum number of transformants, we were able to isolate independently two different library plasmids that contained the *ARG3* gene. Multicopy *ARG3* leads to a 30% increase in *HO-LacZ* expression in a *gcn5* mutant, similar to suppression observed with semi-dominant *sin⁻* alleles of histones H3 or H4 (Pollard and Peterson, 1997). *ARG3* encodes a metabolic enzyme, so it was not obvious why multicopy *ARG3* should alleviate transcriptional defects due to loss of Gcn5p. Given the role of Arg3p (ornithine transcarbamoylase) in the urea cycle, we hypothesized that overexpression of Arg3p activity might deplete cytoplasmic levels of ornithine, the primary precursor for the production of polyamines. Consistent with this view, addition of ornithine to the growth medium diminished the ability of multicopy *ARG3* to alleviate the defect in *HO-LacZ* expression due to inactivation of *GCN5*. Furthermore, eliminating the biosynthesis of polyamines by deleting the *SPE1* gene also allowed transcription in the absence of *GCN5*. These genetic studies are consistent both with polyamines playing a negative role in transcription and with Gcn5p antagonizing this function of polyamines.

Hierarchy of chromatin remodeling

Genetic studies have indicated a functional relationship between the GCN5 histone acetyltransferase and the SWI/SNF chromatin remodeling complex (Pollard and Peterson, 1997; Roberts and Winston, 1997). Both GCN5 and SWI/SNF are required for expression of a similar set of genes *in vivo*, and past studies have shown that they share similar genetic interactions with chromatin components. For instance, deletion of the *SIN1* gene, which encodes an HMG1-like protein, or single amino acid changes in histones H3 or H4, partially alleviates the defects in growth and transcription due to inactivation of either GCN5 or SWI/SNF (Kruger and Herskowitz, 1991; Kruger *et al.*, 1995; Pollard and Peterson, 1997; Perez-Martin and Johnson, 1998). In contrast, depletion of polyamines by either multicopy *ARG3* or a *spe1* deletion partially alleviates only the transcriptional requirements for GCN5; depletion of polyamines does not allow transcription in the absence of SWI/SNF. If GCN5 and SWI/SNF function in the same pathway to facilitate transcription, then these results suggest that SWI/SNF can function downstream of polyamines and GCN5 (i.e. GCN5⁻/polyamines⁻/SWI/SNF⁻/histones⁻/transcription). Furthermore, this epistasis pathway suggests that one function of polyamines might be to inhibit the function of SWI/SNF. This genetic model is consistent with the observation that mammalian SWI/SNF complex appears to be excluded from highly condensed regions of chromatin *in vivo* (Reyes *et al.*, 1997).

Roles for GCN5 in transcriptional regulation

A deletion of the *GCN5* gene leads to defects in expression of a subset of yeast genes (Georgakopoulos and Thireos, 1992; Pollard and Peterson, 1997; Holstege *et al.*, 1998). Likewise, single amino acid changes that eliminate the *in vitro* GCN5-dependent histone acetyltransferase activity lead to similar transcriptional defects (Wang *et al.*, 1997; Kuo *et al.*, 1998), indicating that the enzymatic activity of GCN5 is crucial for its function *in vivo*. Chromatin immunoprecipitation studies have shown that GCN5-dependent histone acetyltransferase activity is recruited to at least two target genes, *HIS3* and *HO*, where the acetylation of nucleosomes upstream of the coding region appears to be required for optimal transcription *in vivo* (Kuo *et al.*, 1998; Krebs *et al.*, 1999). How does GCN5-dependent histone acetylation enhance transcription? One possibility is that histone acetylation directly causes nucleosome disruption, such that key transcriptional regulators are able to access their binding sites more effectively. Alternatively, histone acetylation may disrupt localized condensation of chromatin that surrounds a GCN5-dependent gene, and the subsequent, unfolded nucleosomal array is more permissive for binding of transcription factors or for recruitment of additional chromatin remodeling factors (e.g. SWI/SNF). Both of these models are consistent with the observation that GCN5 action is required for the binding of the SWI4/SWI6 activator to the *HO* upstream regulatory region (Cosma *et al.*, 1999). However, our studies presented here are consistent with the latter model, whereby GCN5-dependent histone acetylation disrupts the ability of polyamines to stabilize the fiber-fiber interactions that contribute to chromatin-mediated repression. Consistent with this view, depletion of polyamines alleviates *gcn5⁻* transcriptional defects, and polyamine-depend-

ent oligomerization of nucleosomal arrays *in vitro* is inhibited by histone hyperacetylation. Furthermore, Gcn5p has recently been shown to prefer condensed nucleosomal arrays as substrates for acetylation *in vitro* (Tse *et al.*, 1998a). Since only partial depletion of polyamines can be achieved *in vivo* due to their roles in essential cellular processes, it may be that disruption of polyamine function is a primary role for GCN5-dependent histone acetylation.

Targeting of polyamine-mediated transcriptional repression by histone deacetylase activity?

Our *in vitro* studies indicate that polyamine-dependent chromatin condensation is most effective with a hypoacetylated nucleosomal array substrate. Nucleosomal arrays reconstituted with intact, hypoacetylated histone octamers fully oligomerize at 200 μ M spermidine, whereas 50% more spermidine is required to condense hyperacetylated nucleosomal arrays. These results suggest that the contribution of polyamines to transcriptional regulation *in vivo* may also be controlled by the balance between histone acetylation and deacetylation reactions. Hypoacetylated chromatin domains are often correlated with transcriptional inactivity (Allfrey *et al.*, 1964), and the propensity of such hypoacetylated domains to undergo intramolecular and intermolecular condensation reactions plays a dominant role in transcriptional repression *in vitro* (Hansen and Wolffe, 1992; Tse *et al.*, 1998b), and probably *in vivo*. In many cases, the hypoacetylated state of a promoter region is established by the recruitment of gene-specific histone deacetylase complexes (Kadosh and Struhl, 1997, 1998; Rundlett *et al.*, 1998). Although polyamines are ubiquitous components of chromatin, the targeting of gene-specific deacetylase activity might lead indirectly to the selective interaction of polyamines with the surrounding, hypoacetylated chromatin fiber. Furthermore, the resulting polyamine-dependent condensation of the chromatin fiber may play a significant role in the mechanism whereby a targeted deacetylase promotes transcriptional repression.

Materials and methods

Media, strains and plasmids

Cultures were grown at 30°C in YPD medium (2% yeast extract, 1% bacto-peptone with 2% glucose), unless specified. S (minimal) medium contains 6.7 g/l yeast nitrogen base without amino acids (Difco Laboratories) and is supplemented with the required amino acids. PFM is S medium that has been filter sterilized instead of autoclaving. Cultures grown for depletion of polyamines were grown in glassware that was previously acid washed to remove residual amines from containers. Depletion of polyamines was achieved by dilution of cultures that had been grown to saturation in rich medium (YPD) into PFM. Cultures were then grown for 4 days in PFM with successive 1/100 dilutions into fresh media every 20 h. Strains are described in Table I. Other general yeast manipulations were performed as described (Guthrie and Fink, 1991).

Overexpression plasmids containing a yeast genomic library have been described (Nasmyth and Reed, 1980). Yeast transformations were performed by the lithium acetate method described previously (Geitz and Scheidl, 1991). Sequencing of isolated clones was performed using the automated dideoxy sequencing method of Promega®. Plasmids were prepared by alkaline lysis, and sequencing primers were complementary to the 5' and 3' ends of the *Bam*HI site of the vector, reading into inserts. Subcloning of the *ARG3* open reading frame (ORF) (pARG3) was achieved by a *Bst*BI digest of the isolated clone, which released a large 6.5 kb fragment and two smaller fragments of 1.6 kb and 800 bp. The large 6.5 kb fragment contained the yRp7 vector with an intact

Table I. Strain list

Strain	Relevant genotype	Comments/source
CY432	<i>a</i> <i>SWT</i> ⁺ <i>HO::lacZ</i>	Pollard and Peterson (1997)
CY433	α <i>swi10-28(snf5⁻) HO::lacZ</i>	Pollard and Peterson (1997)
CY434	α <i>swi9-289(gcn5) HO::lacZ</i>	Pollard and Peterson (1997)
CY435	α <i>swi7-288 (ada3) HO::LacZ</i>	Pollard and Peterson (1997)
CY436	α <i>swi8-299 (ada2) HO::LacZ</i>	Pollard and Peterson (1997)
CY561	<i>a</i> <i>ada2Δ::URA3-hisG</i>	isogenic to CY432 (Pollard and Peterson, 1997)
CY562	<i>a</i> <i>ada3::URA3-hisG</i>	isogenic to CY432 (Pollard and Peterson, 1997)
CY563	<i>a</i> <i>gcn5Δ::URA3-hisG</i>	isogenic to CY432 (Pollard and Peterson, 1997)
CY761	α <i>HO::LacZ gcn5Δ::URA3-hisG</i>	segregant 1c CY563 × CY763
CY763	α <i>spe1Δ::LEU2</i>	H.Tabor 359
CY765	α <i>HO::LacZ spe1Δ::LEU2</i>	segregant 2a CY563 × CY763
CY769	<i>a</i> <i>HO::LacZ spe1Δ::LEU2 gcn5Δ::URA3-hisG</i>	segregant 2c CY563 × CY763
CY771	α <i>SPE1 SPE2 SPE3 GCN5</i>	H.Tabor 2602
CY773	<i>a</i> <i>HO::LacZ SPE1 GCN5</i>	segregant 1d CY563 × CY763
CY776	<i>a</i> <i>HO::LacZ spe1Δ::LEU2</i>	segregant 3a CY563 × CY763
CY777	<i>a</i> <i>HO::LacZ spe1Δ::LEU2 swi2Δ::TRP1</i>	CY776 with <i>swi2Δ::TRP1</i> cassette (J.Krebs, unpublished)
CY778	<i>a</i> <i>HO::LacZ swi2Δ::TRP1</i>	CY773 with <i>swi2Δ::TRP1</i> cassette

ARG3 ORF. This fragment was separated by agarose electrophoresis, isolated and re-ligated before transforming into bacteria for large-scale purification and re-transformation into yeast strains.

***β*-galactosidase and invertase assays**

Filter *β*-galactosidase assays were performed on cells grown overnight on filters overlaid on agar media as described previously (Andrews and Herskowitz, 1989). Liquid *β*-galactosidase assays were performed on cells grown to mid-log phase as described previously (Stern *et al.*, 1984). *β*-galactosidase units were calculated as Miller units. For invertase assays, cultures were grown to mid-log phase in media containing 2% glucose, then derepressed for *SUC2* transcription by washing cells in water and growing for 2–3 h in media containing 0.05% glucose. Invertase assays were performed as described (Celenza and Carlson, 1984). Invertase units were calculated as μ mol of glucose/min/mg dry weight of cells. Assays were performed in triplicate, with results averaged and error values were within 20% of the mean for both *β*-galactosidase and invertase assays.

RNA analysis

Strains were grown in SD medium or PFM as indicated in the legend to Figure 6. Total yeast RNA was isolated from cells using a glass bead/phenol method (Jensen *et al.*, 1983). Eight microliters of dimethylsulfoxide, 1.6 μ l of 0.1 M phosphate buffer pH 6.5 and 2.5 μ l of 6.6 M deionized glyoxal were added to 5 μ l of RNA sample containing 8 μ g of RNA. The mixture was heated to 50°C for 15 min. Then 8 μ l of RNA loading dye were added (50% glycerol, 10 mM phosphate buffer pH 6.5, 0.4% bromophenol blue) and the sample subjected to electrophoresis in a 1.2% agarose gel buffered by 10–15 mM phosphate buffer pH 6.5. The RNA was blotted onto a 'GeneScreen' membrane (NEN Research Products) and fixed with UV light, using a 'Stratalinker' (Stratagene).

DNA fragments to be labeled were generated by PCR or restriction digestion and purified from an agarose gel. A 50 ng aliquot of probe DNA was labeled with [³²P]dCTP using a 'Megaprime' Kit (Amersham). The probe was heated to 100°C for 5 min before addition to the hybridization tube.

Blots were pre-hybridized for 1–3 h at 42°C in 10 ml of hybridization solution (2.5 ml of 20× SSPE, 0.5 ml of 100× Denhardt's, 0.1 g of SDS, 5 ml of formamide, 2 ml of 50% dextran sulfate and 100 μ l of 10 mg/ml heat-denatured, sonicated salmon sperm DNA). The heat-denatured probe was added directly to the pre-hybridization solution and hybridization was left to proceed for 14–20 h at 42°C. Blots were washed using two 45 min washes at 42°C in 2× SSPE, followed by a 30 min wash at 65°C in 0.2× SSPE/0.5% SDS.

Reconstitution of nucleosomal arrays

Histones were purified to apparent homogeneity from chicken erythrocytes as described (Hansen *et al.*, 1989) with one minor modification. Following elution from hydroxylapatite, 10 ml of isolated histones were then passed over an equilibrated S-300 column in 90 mM phosphate, 2.2 M NaCl in order to remove residual nucleases. Recombinant yeast and *Xenopus* histones were purified from bacteria and histone octamers

were refolded by following the procedures of Luger *et al.* (1999). Reconstitutions of histone octamers onto 208–12 array templates were performed by salt gradient dialysis using a ratio of histone octamer to 5S rRNA repeat of 1.0–1.4 (Hansen *et al.*, 1989). The extent of saturation of reconstitutions was assayed by *Eco*RI analysis (Logie and Peterson, 1999) or by sedimentation velocity in the analytical ultracentrifuge. Buffers used for reconstitution with hyperacetylated histones also contained 3 mM butyrate and those using trypsinized histones contained 1 mM dithiothreitol.

Oligonucleosome self-association assays

Formation of oligomeric array structures was measured using the intermolecular association assay described previously (Schwarz and Hansen, 1994). Briefly, reconstituted nucleosomal arrays (30 μ l) were mixed with polyamine solutions (30 μ l), then incubated at room temperature for $\geq$ 5 min and centrifuged in an Eppendorf microcentrifuge at 13 500 r.p.m. for 10 min. The absorbance of the supernatant was determined at 260 nm. Alternatively, nucleosomal arrays were ³²P-end-labeled (Logie and Peterson, 1999) and intermolecular association was monitored by scintillation counting of the centrifuged supernatant. Data are expressed as the percentage of sample remaining in the supernatant after centrifugation.

Analytical ultracentrifugation analysis

Sedimentation velocity experiments were carried out in a Beckman XL-A analytical ultracentrifuge as described previously (Schwarz and Hansen, 1994). Digitized scans were analyzed by the method of van Holde and Weischet (1978) to determine the integral distribution of sedimentation coefficients.

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References

- Allfrey,V.G., Faulkner,R. and Mirsky,A.E. (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl Acad. Sci. USA*, **51**, 786–793.
- Andrews,B. and Herskowitz,I. (1989) Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. *Cell*, **57**, 21–29.
- Balasundaram,D., Taylor,C.W. and Tabor,H. (1991) Spermidine or spermine is essential for the aerobic growth of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **88**, 5872–5876.

- Belmont, A.S. and Bruce, K. (1994) Visualization of G₁ chromosomes: a folded, twisted, supercoiled chromonema model of interphase chromatid structure. *J. Cell Biol.*, **127**, 287–302.
- Belmont, A.S., Braumfeld, M.B., Sedat, J.W. and Agard, D.A. (1989) Large-scale chromatin structural domains within mitotic and interphase chromosomes *in vivo* and *in vitro*. *Chromosoma*, **98**, 129–143.
- Brooks, W.H. (1994) A model for systemic lupus erythematosus based on chromatin disruption by polyamines. *Med. Hypotheses*, **43**, 403–408.
- Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmonson, D.G., Roth, S.Y. and Allis, C.D. (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843–851.
- Celenza, J.L. and Carlson, M. (1984) Cloning and genetic mapping of *SNF11*, a gene required for expression of glucose repressible genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **4**, 49–53.
- Chanda, R. and Ganguly, A.K. (1988) Polyamines in relation to human breast, rectal and squamous cell carcinoma. *Cancer Lett.*, **39**, 311–318.
- Colson, P. and Houssier, C. (1989) Polyamine addition to preparation media induces chromatin condensation, irreversibly at low ionic strength. *FEBS Lett.*, **257**, 141–144.
- Cosma, M.P., Tanaka, T. and Nasmyth, K. (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell*, **97**, 299–311.
- Fairlamb, A.H. (1990a) Future prospects for the chemotherapy of human trypanosomiasis. 1. Novel approaches to the chemotherapy of trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, **84**, 717–720.
- Fairlamb, A.H. (1990b) Trypanothione metabolism and rational approaches to drug design. *Biochem. Soc. Trans.*, **18**, 1279–1284.
- Fletcher, T.M. and Hansen, J.C. (1995) Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. *J. Biol. Chem.*, **270**, 25359–25362.
- Fletcher, T.M. and Hansen, J.C. (1996) The nucleosomal array: structure/function relationships. *Crit. Rev. Eukaryot. Gene Expr.*, **6**, 149–188.
- Friesen, H., Tanny, J. and Segall, J. (1998) *SPE3*, which encodes spermidine synthase, is required for full repression through NRE^{DTT} in *Saccharomyces cerevisiae*. *Genetics*, **150**, 59–73.
- Garcia-Ramirez, M., Dong, F. and Ausio, J. (1992) Role of the histone 'tails' in the folding of oligonucleosomes depleted of histone H1. *J. Biol. Chem.*, **267**, 19587–19595.
- Garcia-Ramirez, M., Rocchini, C. and Ausio, J. (1995) Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.*, **270**, 17923–17928.
- Geitz, R.D. and Scheistl, R.H. (1991) Applications of high efficiency lithium acetate transformation of intact yeast cells using single stranded nucleic acids as carrier. *Yeast*, **7**, 253–263.
- Georgakopoulos, T. and Thireos, G. (1992) Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.*, **11**, 4145–4152.
- Grant, P.A. et al. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.*, **11**, 1640–1650.
- Guthrie, C. and Fink, G.R. (1991) Guide to yeast genetics and molecular biology. *Methods Enzymol.*, **194**, 1–933.
- Hansen, J.C. (1997) The core histone amino-termini: combinatorial interaction domains that link chromatin structure with function. *ChemTracts: Biochem. Mol. Biol.*, **11**, 56–69.
- Hansen, J.C. and van Holde, K.E. (1991) The mechanism of nucleosome assembly onto oligomers of the sea urchin 5S DNA positioning sequence. *J. Biol. Chem.*, **266**, 4276–4282.
- Hansen, J.C. and Wolffe, A.P. (1992) Influence of chromatin folding on transcription initiation and elongation by RNA polymerase III. *Biochemistry*, **31**, 7977–7988.
- Hansen, J.C., Ausio, J., Stanik, V.H. and van Holde, K.E. (1989) Homogeneous reconstituted oligonucleosomes, evidence for salt-dependent folding in the absence of histone H1. *Biochemistry*, **28**, 9129–9136.
- Hansen, J.C., Kreider, J.I., Demeler, B. and Fletcher, T.M. (1997) Analytical ultracentrifugation and agarose gel electrophoresis as tools for studying chromatin folding in solution. *Methods*, **12**, 62–72.
- Hansen, J.C., Tse, C. and Wolffe, A.P. (1998) Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry*, **37**, 17637–17641.
- 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*, **95**, 717–728.
- Jensen, R., Sprague, G.F., Jr and Herskowitz, I. (1983) Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating-type locus. *Proc. Natl Acad. Sci. USA*, **80**, 3035–3039.
- Kadosh, D. and Struhl, K. (1997) Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell*, **89**, 365–371.
- Kadosh, D. and Struhl, K. (1998) Histone deacetylase activity of Rpd3 is important for transcriptional repression *in vivo*. *Genes Dev.*, **12**, 797–805.
- Krebs, J., Kuo, M.-H., Allis, C.D. and Peterson, C.L. (1999) Cell cycle regulated histone acetylation required for expression of the yeast *HO* gene. *Genes Dev.*, **13**, 1412–1421.
- Kruger, W. and Herskowitz, I. (1991) A negative regulator of *HO* transcription, SIN1 (SPT2), is a nonspecific DNA-binding protein related to HMG1. *Mol. Cell. Biol.*, **11**, 4135–4146.
- Kruger, W., Peterson, C.L., Sil, A., Coburn, C., Arents, G., Moundrianakis, E.N. and Herskowitz, I. (1995) Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. *Genes Dev.*, **9**, 2770–2779.
- Kuge, S. and Jones, N. (1994) YAP1 dependent activation of TRX2 is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides. *EMBO J.*, **13**, 655–664.
- Kuo, M.-H., Zhou, J., Jambeck, P., Churchill, M.E.A. and Allis, C.D. (1998) Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes *in vivo*. *Genes Dev.*, **12**, 627–639.
- Logie, C. and Peterson, C.L. (1999) Purification and biochemical properties of yeast SWI/SNF complex. *Methods Enzymol.*, **304**, 726–741.
- Luger, K. and Richmond, T.J. (1998) The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.*, **8**, 140–146.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, **389**, 251–260.
- Luger, K., Rechsteiner, T.J. and Richmond, T.J. (1999) Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol.*, **304**, 3–19.
- Marcus, G.A., Silverman, N., Berger, S.L., Horiuchi, J. and Guarente, L. (1994) Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. *EMBO J.*, **13**, 4807–4815.
- McCann, P.P., Pegg, A.E. and Sjoerdsma, A. (1987) *Inhibition of Polyamine Metabolism*. Academic Press, San Diego, CA.
- Morgan, D.M.L. (1998) *Methods in Molecular Biology: Polyamine Protocols*. Humana Press, Totowa, NJ.
- Morgan, J.E., Calkins, C.C. and Matthews, H.R. (1989) Discovery and mapping of discrete binding sites on nucleosome core particles for a photoaffinity derivative of spermine. *Biochemistry*, **28**, 5095–5106.
- Nasmyth, K.A. and Reed, S.I. (1980) Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. *Proc. Natl Acad. Sci. USA*, **77**, 2119–2123.
- Neuwald, A.F. and Landsman, D. (1997) GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem. Sci.*, **22**, 154–155.
- Pegg, A.E. (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.*, **48**, 759–774.
- Perez-Martin, J. and Johnson, A.D. (1998) Mutations in chromatin components suppress a defect of Gcn5 protein in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **18**, 1049–1054.
- Pollard, K.J. and Peterson, C.L. (1997) Role for *ADA/GCN5* products in antagonizing chromatin-mediated transcriptional repression. *Mol. Cell. Biol.*, **17**, 6212–6222.
- Reyes, J.C., Muchardt, C. and Yaniv, M. (1997) Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. *J. Cell Biol.*, **137**, 263–274.
- Riddsdale, J.A., Hendzel, M.J., Delcuve, G.P. and Davie, J.R. (1990) Histone acetylation alters the capacity of the H1 histones to condense transcriptionally active/competent chromatin. *J. Biol. Chem.*, **265**, 5150–5156.
- Roberts, S.M. and Winston, F. (1997) Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada and Gcn5 proteins, with the Swi/Snf and Srb/mediator complexes. *Genetics*, **147**, 451–465.
- Ruiz-Garcia, A.B., Sendra, R., Pamblanco, M. and Tordera, V. (1997) Gcn5p is involved in the acetylation of histone H3 in nucleosomes. *FEBS Lett.*, **403**, 186–190.

- Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M. and Grunstein, M. (1998) Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. *Nature*, **392**, 831–835.
- Russel, D.H. and Duri, B.G.M. (1978) Polyamines and their accumulation in tumor cells. *Prog. Cancer Res. Ther.*, **8**, 15–41.
- Saleh, A., Lang, V., Cook, R. and Brandl, C.J. (1997) Identification of native complexes containing the yeast coactivator/repressor proteins NGG1/ADA3 and ADA2. *J. Biol. Chem.*, **272**, 5571–5578.
- Schwartz, B., Hittleman, A., Daneshvar, L., Basu, H.S., Marton, L.J. and Feuerstein, B.G. (1995) A new model for disruption of the ornithine decarboxylase gene, *SPE1*, in *Saccharomyces cerevisiae* exhibits growth arrest and genetic instability at the MAT locus. *Biochem. J.*, **312**, 83–90.
- Schwarz, P.M. and Hansen, J.C. (1994) Formation and stability of higher order chromatin structures. *J. Biol. Chem.*, **269**, 16284–16289.
- Schwarz, P.M., Felthaus, A., Fletcher, T.M. and Hansen, J.C. (1996) Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. *Biochemistry*, **35**, 4009–4015.
- Sen, D. and Crothers, D.M. (1986) Condensation of chromatin; role of multivalent cations. *Biochemistry*, **25**, 1495–1503.
- Stern, M., Jensen, R. and Herskowitz, I. (1984) Five *SWI/SNF* genes are required for expression of the *HO* gene in yeast. *J. Mol. Biol.*, **178**, 853–868.
- Tabib, A. and Bachrach, U. (1998) Polyamines induce malignant transformation in cultured NIH 3T3 fibroblasts. *Int. J. Biochem. Cell Biol.*, **30**, 135–146.
- Tabor, C.W. and Tabor, H. (1984) Polyamines. *Annu. Rev. Biochem.*, **53**, 749–790.
- Tse, C. and Hansen, J.C. (1997) Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. *Biochemistry*, **36**, 11381–11388.
- Tse, C., Ruiz-Garcia, A.B., Georgieva, E.I., Sendra, R. and Hansen, J.C. (1998a) Gcn5p, a transcription-related histone acetyltransferase, acetylates nucleosomes and folded nucleosomal arrays in the absence of other protein subunits. *J. Biol. Chem.*, **273**, 32388–32392.
- Tse, C., Sera, T., Wolffe, A.P. and Hansen, J.C. (1998b) Disruption of higher order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. *Mol. Cell. Biol.*, **18**, 4629–4638.
- van Holde, K.E. (1988) *Chromatin*. Springer-Verlag, New York, NY.
- van Holde, K.E. and Weisheit, W.O. (1978) Boundary analysis of sedimentation-velocity experiments with monodisperse and paucidisperse solutes. *Biopolymers*, **17**, 1387–1403.
- Wang, L., Mizzen, C., Ying, C., Candau, R., Barlev, N., Brownell, J., Allis, C.D. and Berger, S.L. (1997) Histone acetyltransferase activity is conserved between yeast and human GCN5 and is required for complementation of growth and transcriptional activation. *Mol. Cell. Biol.*, **17**, 519–527.
- Wechsler, M.A., Kladde, M.P., Alfieri, J.A. and Peterson, C.L. (1997) Effects of Sin[−] versions of histone H4 on yeast chromatin structure and function. *EMBO J.*, **16**, 2086–2095.
- Widom, J. (1986) Physicochemical studies of the folding of the 100 Å nucleosome filament into the 300 Å filament. Cation dependence. *J. Mol. Biol.*, **190**, 411–424.
- Wong, L.-J.C., Sharpe, D.J. and Wong, S.S. (1991) High-mobility group and other nonhistone substrates for nuclear histone N-acetyltransferase. *Biochem. Genet.*, **29**, 461–475.

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