Saccharomyces cerevisiae Rad16 mediates ultraviolet-dependent histone H3 acetylation required for efficient global genome nucleotide-excision repair

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In yeast, global genome nucleotide-excision repair (GG-NER) requires a protein complex containing Rad7 and Rad16. Rad16 is a member of the switch/sucrose nonfermentable superfamily, and it is presumed that chromatin remodelling is its primary function during repair. We show that *RAD16* is required for ultraviolet-dependent hyperacetylation of histone H3 (Lys 9 and Lys 14) at the *MFA2* promoter and throughout the genome. The yeast repressor complex Ssn6–Tup1 represses many genes including *MFA2*. *TUP1* deletion results in constitutive hyperacetylation of histone H3, nucleosome disruption and derepression of gene transcription in Tup1-regulated genes. GG-NER in the *MFA2* promoter proceeds more rapidly in *tup1Δ* α -cells compared with wild type, even when transcription is inhibited. We show that elevated histone H3 acetylation levels in the *MFA2* promoter in *tup1Δ* α -cells result in Rad7- and Rad16-independent GG-NER, and that Rad16 mediates the ultraviolet-induced acetylation of histone H3, necessary for efficient GG-NER.

Keywords: chromatin; histone acetylation; *MFA2*; NER; yeast

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INTRODUCTION

Nucleotide-excision repair (NER) is a fundamental cellular process. The excision of lesions from non-transcribed regions of the genome involves global genome NER (GG-NER), which requires a specific subset of NER proteins (Friedberg *et al*, 2005). Various studies have provided a detailed mechanistic understanding of some of the core enzymatic activities associated with NER, but a precise mechanistic understanding of how it functions in relation to chromatin structure is lacking.

In eukaryotes, DNA associates with histones to form nucleosomes. The nucleosomal arrays are compacted further into higher order chromatin structures. Chromatin influences how proteins

functionally interact with regulatory regions in the DNA to facilitate events such as transcription, replication, recombination and repair (Wolffe, 2000; Ataian & Krebs, 2006). This is achieved primarily by the activity of two main processes: histone modification and chromatin remodelling. Both processes have been studied for their roles in gene regulation (Wu & Grunstein, 2000; Waterborg, 2002), and more recently in NER (Yu *et al*, 2005; Gong *et al*, 2006).

In *Saccharomyces cerevisiae*, Rad7 and Rad16 form a protein complex that is specifically required for GG-NER (Verhage *et al*, 1996; Reed *et al*, 1999; Li & Smerdon, 2004). Rad16 shares marked homology with Snf2, the catalytic subunit of the switch/sucrose nonfermentable (SWI/SNF) chromatin-remodelling complex (Bang *et al*, 1992). SWI/SNF superfamily proteins show ATPase activity that is stimulated by DNA or chromatin (Eisen *et al*, 1995; Whitehouse *et al*, 1999), and all SWI/SNF-like proteins generate superhelical tension in linear DNA fragments through a DNA translocase activity associated with their ATPase function (Havas *et al*, 2000; Van Komen *et al*, 2000). It is now established that the generation of superhelicity in DNA is a fundamental mechanistic paradigm common to SWI/SNF-like chromatin remodelling complexes for altering chromatin structure (Havas *et al*, 2000). We showed previously that generation of superhelical torsion in DNA by the Rad16 component of a GG-NER complex is needed to excise DNA damage during GG-NER *in vitro* (Yu *et al*, 2004). Although these experiments indicated a fundamental activity of the GG-NER complex during NER, they were unable to provide information on how the GG-NER complex functioned on chromatin, as the experiments were conducted on naked DNA.

Recently, we reported that the histone acetyl transferase Gcn5 facilitates efficient NER at *MFA2* (Teng *et al*, 2002; Yu *et al*, 2005). Our study showed that histone H3 acetylation (H3Ac), previously linked only to the regulation of gene transcription, can also facilitate GG-NER at this locus after the exposure of cells to ultraviolet light (Yu *et al*, 2005). We also showed that histone H3 is hyperacetylated throughout the genome after ultraviolet irradiation, but this is not significantly reduced in the *gcn5* histone acetyl transferase mutant strain, possibly because specific histone acetyl transferases affect H3Ac in certain regions of the genome following ultraviolet irradiation. Surprisingly, we found

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that ultraviolet irradiation-induced H3Ac occurred independently of functional NER. Here, we report that ultraviolet irradiation-induced H3Ac depends on Rad16. Furthermore, by examining events at the *MFA2* gene, we show that it is possible to eliminate the requirement for Rad7 and Rad16 during GG-NER by constitutively elevating the levels of H3Ac in the *MFA2* gene.

MFA2 is an a-mating-type-specific gene, which is transcriptionally active in a-mating-type cells and silent in α -mating-type cells. The transcriptional regulation of this gene involves the yeast general repressor complex Ssn6–Tup1 (Keleher *et al*, 1992). Previously, we identified four positioned nucleosomes at *MFA2* in α -cells where the gene is repressed (Teng *et al*, 2001). In *TUP1*-regulated genes, including *MFA2*, deletion of *TUP1* results in the disruption of positioned nucleosomes, derepression of gene transcription and elevated histone acetylation (Cooper *et al*, 1994; Bone & Roth, 2001; Malave & Dent, 2006). We show that Rad16 mediates ultraviolet irradiation-induced H3Ac, which results in the chromatin remodelling required for efficient GG-NER. We also discuss possible mechanisms relating to how this is achieved.

RESULTS

UV-dependent histone H3 acetylation requires Rad16

Ultraviolet irradiation stimulates histone H3 hyperacetylation in the regulatory region of *MFA2* and throughout the genome in wild-type cells. This established that, in yeast, repressed chromatin is specifically hyperacetylated in response to ultraviolet irradiation. We showed that although ultraviolet irradiation-induced hyperacetylation of histone H3 is required for efficient NER, functional NER is not required to observe H3Ac following ultraviolet irradiation (Yu *et al*, 2005). Fig 1 shows that most ultraviolet irradiation-induced H3Ac (Lys 9 and Lys 14), at the regulatory region of the *MFA2* gene (Fig 1A) and in the genome overall (Fig 1B), requires the GG-NER factor Rad16. We surmised that Rad16-dependent acetylation of histone H3 in response to ultraviolet irradiation could represent an important stage during GG-NER in chromatin. To investigate this, we measured GG-NER in the *MFA2* gene of *TUP1*-deleted cells in which H3Ac is constitutively elevated, even in the absence of ultraviolet irradiation.

TUP1 deletion alters gene expression of *MFA2*

Fig 2A shows northern blots detecting the level of *MFA2* messenger RNA in wild-type and mutant cells. *MFA2* mRNA is detected in wild-type α -cells, but not in wild-type or *rad16* Δ α -cells. Deletion of *TUP1* activates *MFA2* transcription in α -cells to a level similar to that in wild-type α -cells. Deletion of *RAD16* in a *tup1* Δ α -strain has no effect on the level of *MFA2* mRNA. However, a TATA box mutation in the promoter of *MFA2* abolishes the transcription induced by *TUP1* deletion. These observations confirm that we can exclusively examine GG-NER in the promoter of *MFA2* in subsequent experiments.

TUP1 deletion elevates histone H3 acetylation at *MFA2*

We measured levels of H3Ac in the *MFA2* promoter in the absence of ultraviolet light in wild-type α - and α -, *tup1* Δ α -, *tup1* Δ *rad16* Δ α - and *tup1* Δ *mfa2*^{TATA} α -cells (Fig 2B). The elevated level of H3Ac in the wild-type α -strain reflects the transcriptionally active status of *MFA2*. A tenfold elevation in the constitutive levels of H3Ac is seen in the *tup1* Δ α - and the *tup1* Δ *rad16* Δ α -cells, and importantly in the transcription

Fig 1 | Histone acetylation in wild-type and *rad16* Δ cells after ultraviolet irradiation. (A) Chromatin immunoprecipitation analysis of histone H3 acetylation (H3Ac) level in the *MFA2* promoter using H3Ac (Lys 9 and Lys 14) antibodies. U: untreated samples; 0: cells received 150 J/m² of ultraviolet without repair; 1, 2 or 4 cells being irradiated with ultraviolet and then were allowed to repair in YPD for these hours. (B) Western analysis of H3Ac (Lys 9 and Lys 14) level in whole-cell extracts. Cyclophilin A (CyP) was used as a control. Acetylation is presented as the fold increase relative to unirradiated cells. WT, wild type.

inhibited TATA box-mutated *tup1* Δ *mfa2*^{TATA} α -cells, even in the absence of ultraviolet light. Thus, absence of the Tup1 repressor results in constitutively higher levels of H3Ac in the *MFA2* promoter, even when transcription is inhibited.

TUP1 deletion enhances CPD repair in *MFA2*

We examined the repair of cyclobutane pyrimidine dimers (CPDs) in the promoter region of *MFA2* in wild-type, *tup1* Δ and TATA box-mutated *tup1* Δ *mfa2*^{TATA} α -cells. Typical gels to analyse this region are shown in supplementary Figs 1, 2 online. Repair was expressed as the time taken to remove 50% (*T*_{50%}) of the CPDs at specific positions (Fig 3). NER in *tup1* Δ cells is enhanced for all detectable CPDs in both strands (Fig 3A). In wild-type α -cells, in which *MFA2* is repressed, the overall repair rate of CPDs was *T*_{50%} 3.3 ± 0.2 h for the transcribed strand and *T*_{50%} 3.7 ± 0.3 h for the non-transcribed strand. In the *tup1* Δ α -strain, repair of the transcribed and non-transcribed strands had a *T*_{50%} of 1.4 ± 0.2 and 1.5 ± 0.2 h, respectively.

GG-NER is unaltered when transcription is inhibited

The deletion of *TUP1* results in histone H3 modification, chromatin structural changes and transcription activation in the

Fig 2 | Transcription and constitutive H3 acetylation (Lys 9 and Lys 14) level at *MFA2*. (A) *MFA2* messenger RNA is detected by northern blots in wild-type (WT) α - and α -cells, *rad16* Δ , *tup1* Δ , *tup1* $\Delta*rad16* Δ and the *MFA2* TATA box mutant *tup1* $\Delta*mfa2*^{TATA} (*t.m*^{TATA}) α -cells, using *ACT1* as an internal control. (B) The level of H3Ac (Lys 9 and Lys 14) in the *MFA2* promoter in wild-type α - and α -, *tup1* Δ , *tup1* $\Delta*rad16* Δ and *tup1* $\Delta*mfa2*^{TATA} (*t.m*^{TATA}) α -cells is presented as the fold increase relative to that in wild type α -cells, in which *MFA2* is repressed. H3Ac, histone H3 acetylation.$$$$

MFA2 gene. As gene expression could influence NER in the region, we inhibited *MFA2* transcription by mutating its TATA box to eliminate any effect of transcription on repair (Fig 2A). NER in the *tup1* $\Delta*mfa2*^{TATA} strain is not significantly altered compared with *tup1* Δ cells, and remains much faster than that in wild-type α -cells (Fig 3A; supplementary Fig 2 online). The average $T_{50\%}$ for CPDs is 1.6 ± 0.2 h for the transcribed strand and 1.7 ± 0.2 h for the non-transcribed strand. Therefore, *tup1* Δ cells show enhanced lesion removal by GG-NER in the *MFA2* promoter, and this is not affected following the inhibition of transcription.$

Rad7- and Rad16-independent GG-NER in *MFA2*

The Rad7–Rad16 complex is generally considered to be essential for GG-NER in *S. cerevisiae* (Verhage et al, 1994; Reed et al, 1999). Rad7 and Rad16 are required for CPD removal from the non-transcribed strand of *MFA2* in both wild-type α - and α -cells (Teng et al, 1997). If the ultraviolet irradiation-induced Rad16-dependent H3Ac has a significant role in NER, one might predict Rad7- and Rad16-independent GG-NER in genomic regions where there are constitutively elevated levels of H3Ac, such as at *MFA2* in *tup1* Δ α cells; Figs 3B,4A show that this is indeed the case. In the *rad7* Δ and *rad16* Δ mutants, GG-NER is completely defective in the *MFA2* promoter, whereas in the *tup1* $\Delta*rad7* Δ and the *tup1* $\Delta*rad16* Δ mutant, repair is restored even in the absence of functional transcription in the *tup1* $\Delta*rad16* $\Delta*mfa2*^{TATA} and *tup1* Δ *rad7* $\Delta*mfa2*^{TATA} strains (data not shown). Rad7 and Rad16 are no longer required for GG-NER in the region (Figs 3B,4A). We confirmed the absence of CPD repair in the *tup1* $\Delta*rad14* Δ mutant$$$$$$

Fig 3 | Removal of CPD from the promoter of *MFA2*. (A) Time to remove 50% of the initial CPDs ($T_{50\%}$) at the *MFA2* promoter in wild-type (WT), *tup1* Δ and *tup1* $\Delta*mfa2*^{TATA} α -strains. $T_{50\%}$ of a single CPD or a group of close CPDs with a similar repair rate was calculated or extrapolated as described by Teng et al (2002) from typical gels shown in supplementary Figs 1,2 online. The $T_{50\%}$ of slowly repaired or unrepaired CPDs ($T_{50\%} \geq 6$ h) is shown at the same level (6 h). The dotted ellipses represent the positioned nucleosomes. The diagram below the graph indicates the start of the *MFA2* coding region (arrow) and its regulating elements (TATA box- and Mcm1-binding site). (B) Time to remove 50% of the initial CPDs ($T_{50\%}$) at the *MFA2* promoter in *tup1* Δ , *rad16* Δ and *tup1* $\Delta*rad16* Δ α -strains. See Fig 3A legend for labels. CPD, cyclobutane pyrimidine dimer; MCM, minichromosome maintenance protein; NTS, non-transcribed strand; TS, transcribed strand; WT, wild type.$$

in the region examined (data not shown), indicating that the enhanced CPD repair seen in the absence of Tup1 is unequivocally through Rad7- and Rad16-independent GG-NER.

Rad16-independent GG-NER can occur in other genes

We examined NER in other Tup1-regulated genes (Fig 4B) to determine whether the effect of Tup1 deletion on GG-NER is unique to *MFA2*. In *rad16* Δ and *tup1* $\Delta*rad16* Δ mutants, no CPD removal was detected up to 3 h after ultraviolet irradiation in the non-transcribed strand of *RPB2*, the transcription of which is not regulated by Tup1. However, for the Tup1-regulated genes, *STE2* and *BAR1*, we detected GG-NER in regions of the promoters in the$

Fig 4 | Typical sequencing gels showing the repair of ultraviolet-induced CPDs at nucleotide resolution. (A) Repair of ultraviolet-induced CPDs in the non-transcribed strand (NTS) of the *Hae*III restriction fragment (–516 to +83) of *MFA2* in *rad16Δ*, *tup1Δrad16Δ* and *tup1Δrad7Δ* α -strains. Reference sequences for the combined A and G or C and T are indicated. Lane 0 has DNA from cells receiving 150 J/m² ultraviolet and extracted immediately after irradiation. Lanes numbered 1, 2, 3 and 4 contain DNA from cells that received ultraviolet light but that were incubated afterwards in medium for these hours before DNA extraction. *MFA2* upstream activating sequences (Mcm1-binding site and TATA box) and the start of the coding region of *MFA2* (arrow) are indicated. (B) Repair of ultraviolet-induced CPDs in the NTS of *MFA2*, *STE2* and *BAR1* promoters, and the NTS of the *RPB2* gene in *rad16Δ* and *tup1Δrad16Δ* α -strains. See (A) for other labels. The brackets indicate the regions in which Rad16-independent GG-NER is observed. The numbers mark the relative nucleotide positions referring to the start of the coding region ATG as +1 for each related gene. Mcm1-binding sites in the promoter regions of *MFA2*, *STE2* and *BAR1* are indicated by grey boxes. CPD, cyclobutane pyrimidine dimers; GG-NER, global genome repair; MCM, minichromosome maintenance protein; U, untreated samples.

tup1Δrad16Δ strain, whereas GG-NER was absent in the *rad16Δ* mutant. In the regions where Rad7- and Rad16-independent GG-NER occurred in *STE2* and *BAR1* (see bracketed regions in Fig 4B), lesion removal occurred at a similar level to that observed in the *MFA2* gene. Therefore, the suppression of Rad16 required for GG-NER extends to other Tup1-regulated genes.

DISCUSSION

The Rad16 protein shares significant homology with the Snf2 protein—the catalytic subunit of the SWI/SNF chromatin remodelling complex (Bang et al, 1992)—and Snf2 contains conserved motifs found in a large family of ATPases thought to be involved in chromatin remodelling activities (Eisen et al, 1995). Studies with *rad7Δ* and *rad16Δ* mutants have indicated that they have almost identical, intermediate ultraviolet light sensitivity and the same molecular repair defect; failure to perform GG-NER. Given the homology of Rad16 to other chromatin remodelling proteins and

the finding that GG-NER operates on transcriptionally repressed regions of the genome, it is not surprising that the role of Rad7 and Rad16 has long been thought to include a chromatin remodelling activity; however, to date none of the studies have suggested a role for these proteins in this process. Rad7 and Rad16 were not required in an *in vitro*-reconstituted GG-NER system; although, adding the purified Rad7–Rad16 complex moderately stimulated incision (Guzder et al, 1997). The same group also showed that the Rad7–Rad16 complex could bind to damaged DNA. Furthermore, the ATPase activity of Rad16 was attenuated at increasing levels of DNA damage (Guzder et al, 1998). The authors' interpretation of this result suggested a DNA scanning function for Rad7–Rad16, possibly using the ATPase motors of Rad16 during DNA-damage recognition. Our *in vitro* studies, using yeast whole-cell extracts (WCE), also indicated that Rad7 and Rad16 are not required in order to observe damage-dependent incision during global genome repair, but are required to detect efficient

excision of the damaged DNA (Reed *et al*, 1998). We also showed that a complex containing Rad7–Rad16–Abf1 generates negative supercoiling in DNA through the activity of Rad16 (Yu *et al*, 2004). This showed that the supercoiling generated in the DNA by this complex is required for damage excision on a template that was not assembled into nucleosomes and that the core activity of the ATPases in Rad16 generates superhelical torsion in the DNA that facilitates excision. However, these studies were not able to determine whether or how Rad16 and the GG-NER complex function on chromatin.

Our results provide an insight into how Rad16 functions during NER in chromatin. We have shown, that although functional NER is not required to observe the ultraviolet irradiation-induced acetylation of histone H3, Rad16 is necessary. Significantly, neither Rad7 nor Rad16 is required for GG-NER in non-transcribed DNA at specific promoters in which constitutively high levels of H3Ac occur. The results indicate that Rad16 activity during GG-NER mediates H3Ac, which results in changes in chromatin structure. Below we describe a possible mechanism for this.

Speculation

We think it unlikely that Rad16 has a direct role in histone acetylation, either at *MFA2* or globally. Indeed, we have shown previously that H3Ac at *MFA2* is catalysed by the histone acetyl transferase Gcn5 (Teng *et al*, 2002; Yu *et al*, 2005). It is also evident that H3Ac is not stimulated by NER *per se*, as acetylation occurs in the absence of NER in *rad4Δ* or *rad14Δ* strains (Yu *et al*, 2005). Therefore, it is clear that the ultraviolet irradiation-induced H3Ac is specifically mediated by Rad16. We are now performing experiments to examine the effect of Rad16 on the ability of the Gcn5 histone acetyl transferase to bind to and acetylate histone H3 in the promoter of *MFA2*—and possibly other histone acetyl transferases in other regions of the genome—in response to ultraviolet irradiation. We speculate that Rad16 generates superhelical torsion in DNA through the activity of its ATPase motors, and that this mediates incremental changes in chromatin in response to ultraviolet irradiation. This might alter the ability of histone acetyl transferases such as Gcn5 to induce further changes in chromatin structure through histone acetylation necessary for efficient NER. Encoded within the *RAD16* gene are two distinct biochemical functions: an ATPase activity able to generate superhelical torsion in DNA and an E3 ubiquitin ligase activity, the substrates of which include but might not be limited to the NER factor Rad4 (Gillette *et al*, 2006). In cells, both these activities are inactivated in Rad7-deleted cells. We showed that constitutively elevated H3Ac levels in the *MFA2* promoter are sufficient to rescue GG-NER in both Rad7- and Rad16-deleted cells. Although this observation does not prove that the function of the GG-NER complex is necessary for H3Ac, our future work will focus on how the biochemical activities of Rad16 and other components of the GG-NER complex contribute to triggering histone acetyl transferase activity and chromatin remodelling in response to ultraviolet irradiation in yeast.

METHODS

Details of the yeast strains and the methods are given in the supplementary information online.

Ultraviolet treatment of yeast cells, DNA isolation and high-resolution mapping of CPD sites. These were carried out as

described by Teng *et al* (1997). In brief, exponential phase yeast cells were resuspended at 2×10^7 cells/ml in pre-chilled PBS and irradiated with 254 nm ultraviolet light and followed by incubation in YPD at 30 °C for various repair times. DNA was isolated and digested with *HaeIII*, and cleaved specifically at CPD sites using T4 endonuclease V (T4 endo V; Epicentre Biotechnologies, Madison, WI, USA). The purified DNA fragments were end-labelled with [α - 32 P]dATP and resolved by electrophoresis on polyacrylamide gel.

RNA isolation and northern blot analysis. These were carried out as described previously (Teng *et al*, 2002; Yu *et al*, 2005).

Chromatin immunoprecipitation. This was carried out as described by Yu *et al* (2005). In brief, 100 ml of exponential phase yeast cells was crosslinked by formaldehyde. Washed cells were lysed by vortexing with glass beads. The lysate was sonicated to generate DNA fragments ranging from 200 to 800 bp in size. Histone H3 acetyl antibody (at Lys 9 and Lys 14; Upstate Biotechnology, Millipore, MA, USA) was used to immunoprecipitate sheared chromatin and input samples were used as controls. Quantitative PCR was carried out in real time to analyse the enrichment of DNA fragments after immunoprecipitation.

Western blot analysis. This was carried out according to as in Reed *et al* (1999). In brief, ultraviolet-treated and untreated cells were washed with 10 ml of dialysis buffer (20 mM HEPES-KOH, 10 mM MgSO₄, 10 mM EGTA, 20% glycerol and 5 mM dithiothreitol) with 100 μ l of 100 $\times$ protease inhibitor (100 mM phenylmethylsulphonyl fluoride, 30 mg/ml benzamidin, 100 μ g/ml pepstatin, 100 μ g/ml leupeptin, 100 μ g/ml chymostatin and 100 μ g/ml antipain) and resuspended in 0.5 ml of dialysis buffer with protease inhibitor in 2 ml eppendorf tubes. Cells were lysed by vortexing with glass beads. The supernatant phase was collected as the WCE for western blot. Protein electrophoresis on 6% polyacrylamide-SDS gel and western blotting were carried out according to the standard procedure.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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