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Short Communication

Physical mapping and cloning of *RAD56*

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ABSTRACT

Here we report the physical mapping of the *rad56-1* mutation to the *NAT3* gene, which encodes the catalytic subunit of the NatB N-terminal acetyltransferase in *Saccharomyces cerevisiae*. Mutation of *RAD56* causes sensitivity to X-rays, methyl methanesulfonate, zeocin, camptothecin and hydroxyurea, but not to UV light, suggesting that N-terminal acetylation of specific DNA repair proteins is important for efficient DNA repair.

1. Introduction

The genome is constantly challenged by endogenous and exogenous DNA damage. To counteract genotoxic insults, cells have evolved a number of DNA repair pathways. Each of these pathways can repair a defined subset of DNA lesions and consequently mutants in each pathway are sensitive to a specific set of genotoxic agents. The sensitivity of mutants to specific types of DNA damage has been applied to identify and clone genes responsible for each of the DNA repair pathways that we know today.

Many of the early genetic screens for DNA repair mutants were performed in bacteria and yeast, and the identified DNA repair pathways later turned out to be conserved in multicellular organisms. In yeast *Saccharomyces cerevisiae*, genetic loci which confer X-ray but not UV sensitivity when mutant were numbered *rad50* and upwards (Game and Cox, 1971). One such study sorted the *RAD50–RAD57* genes into eight complementation groups (Game and Mortimer, 1974). Today all of these genes have been mapped physically to specific loci except for *RAD56*, for which the best mapping data place it on the right arm of chromosome 16 distal to *aro7* (Mortimer et al., 1989), or further from the centromere with a genetic distance of 0.8 cM or less from *SGE1* (Amakasu et al., 1993). In this study, we map and clone *RAD56* and we sequence the original X-ray sensitive *rad56-1* mutant allele. The *rad56-1* mutant has a 1 base pair deletion of an A at position 639 in the *NAT3* gene leading to a truncated Nat3 protein.

2. Results

2.1. Linkage analysis of rad56-1

The *rad56-1* allele originally isolated by Richard Snow at the University of California, Davis, as Mutant RS31 segregates as a single recessive mutation resulting in X-ray sensitivity (Game and Mortimer, 1974). The *rad56-1* mutation was subsequently reported to cause sensitivity to bleomycin and to exhibit reduced reversion mutagenesis upon ethyl methanesulfonate treatment (Moore, 1978; Prakash and Higgins, 1982). Initially, we confirmed the mapping of the *rad56-1* mutation to chromosome 16 by chromosome-scale genetic mapping (data not shown) (Reid et al., 2008). To genetically map the *rad56-1* mutation with greater accuracy, we crossed the mutant strain to 35 strains harboring gene deletions along the right arm of chromosome 16 and subjected the diploids to sporulation and tetrad dissection. The resulting spores were scored for sensitivity to 0.03% methyl methanesulfonate (MMS) to follow the segregation of the *rad56-1* allele. The resulting linkage analysis points to a region around position 790 kbp on chromosome 16 as the approximate physical location of *rad56-1* (Fig. 1A).

2.2. Cloning of RAD56 by complementation

Using a tiling array of genomic clones selected based on the linkage analysis to cover the region of chromosome 16 harboring

Abbreviations: MMS, methyl methanesulfonate; YFP, yellow fluorescent protein; NBRP, National Bioresource Project Japan; cM, centimorgan; T, tetratype; NPD, non-parental ditype; YPD, yeast extract peptone dextrose; SC, synthetic complete; bp, base pair; AU, arbitrary units.

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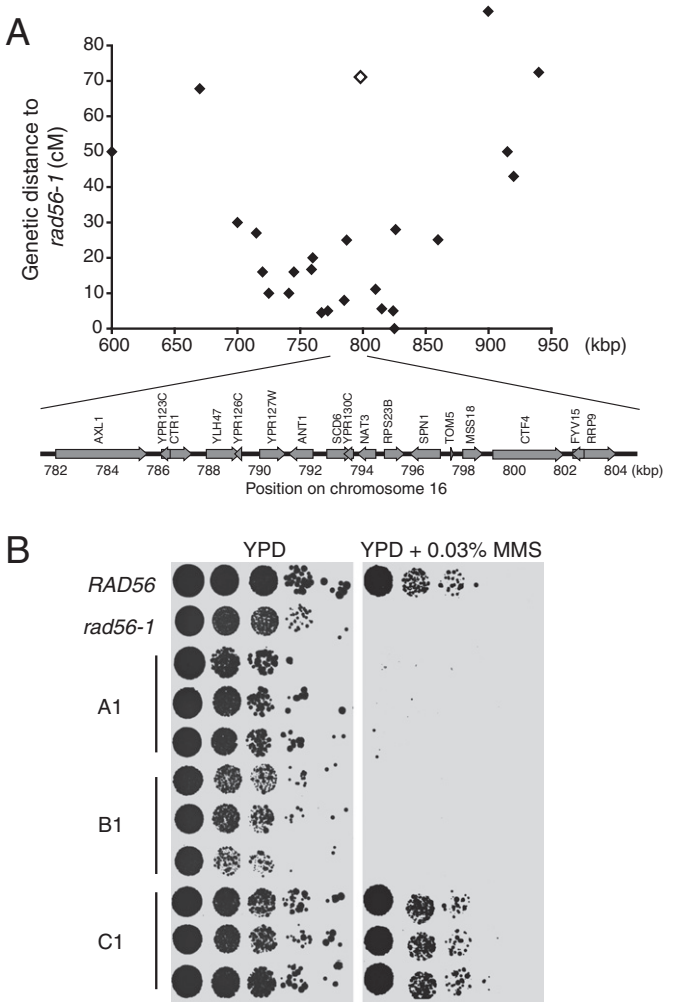

Fig. 1. Mapping and cloning of *RAD56*. (A) Linkage analysis for *rad56-1*. The *rad56-1* mutant strain (BY25622) was mated to selected strains from the yeast gene disruption collection harboring gene deletions along the right arm of chromosome 16 (Winzeler et al., 1999) using standard media and genetic techniques (Sherman et al., 1986). The obtained diploid strains were sporulated and the tetrads scored for MMS sensitivity and G418 resistance to calculate the genetic distance using Perkin's formula: Map Units (cM) = 50 × (T + 6NPD) divided by total tetrads (Table 1) (Perkins, 1949). The open diamond represents the cross made with the *tom5*Δ strain. However, we were unable to confirm the *KanMX*-marked *TOM5* deletion of this strain, suggesting that the *KanMX* marker in this strain is inserted somewhere else in the genome and therefore unlinked to *rad56-1*. (B) Cloning of *RAD56* by complementation. Selected clones A1 (YGPM7h11), B1 (YGPM25p18), and C1 (YGPM3m18) spanning regions 772318-784604, 783375-794337 and 793235-804822 on chromosome 16, respectively, from a yeast genomic tiling collection (YSC4613, Open Biosystems) were transformed into a wild-type (DP6-6A) or a *rad56-1* mutant strain (DP5-2D) and 10-fold serial dilutions of single transformants plated on yeast extract peptone dextrose (YPD) or YPD containing 0.03% MMS and incubated for 3 days at 30 °C. Clone C1 complemented the MMS sensitivity of the *rad56-1* strain (Table 2).

RAD56, we identified a single clone C1, which complements the MMS sensitivity of the *rad56-1* strain (Fig. 1B). This clone contains the full or partial open reading frames of *SCD6*, *YPR130C*, *NAT3*, *RPS23B*, *YPR133C*, *TOM5*, *MSS18*, *CTF4*, *FYV15*, and *RRP9*. By subcloning, we constructed a minimal complementing vector pDP2 containing only the *NAT3* gene (Fig. 2A). Sequencing of the genomic *NAT3* locus in the *rad56-1* strain revealed a 1 base pair deletion of an A at position 639 leading to a truncated Nat3-V214STOP protein (Fig. 2B), which presumably leaves the catalytic N-acetyltransferase domain intact.

2.3. Phenotype of rad56 mutant strains

To verify the phenotype of the *rad56-1* mutation, we reintroduced the 1 base pair deletion into a wild-type strain of the W303 genetic background and generated as well a complete deletion of the predicted open reading frame of the *NAT3* gene. Both the *nat3-V214STOP* and *nat3*Δ strains exhibit MMS sensitivity comparable to that of the original *rad56-1* strain and the sensitivity is rescued by the *NAT3*-containing vector (Fig. 2A). In addition, the *rad56-1* mutant is also sensitive to the DNA damaging agents zeocin, which generates DNA double-strand breaks, hydroxyurea, which causes DNA replication stalling and collapse by depletion of dNTP pools through inhibition of ribonucleotide reductase (Reichard, 1988), and the topoisomerase I inhibitor camptothecin (data not shown).

To compare expression and cellular localization of wild-type Nat3 and the Nat3-1 mutant protein, we inserted the coding sequence for yellow fluorescent protein (YFP) at the 3′-end of *NAT3* or *nat3-1* as described (Eckert-Boulet et al., 2011). Both fusion proteins exhibit MMS sensitivity comparable to their untagged counterparts (Fig. 3A). Importantly, mutant and wild-type Nat3 exhibit comparable pancellular localization and fluorescence intensities (Figs. 3B and C), indicating that protein stability and localization are unaffected by the Nat3-1 truncation.

3. Discussion

Here we map the original *rad56-1* mutation to the *NAT3* gene, which encodes the catalytic subunit of the NatB N-terminal acetyltransferase (Polevoda and Sherman, 2003). Consistent with our findings, deletion mutants of *NAT3* have been reported to be sensitive to X-rays, (Bennett et al., 2001; Game et al., 2005), as have deletion mutants of *MDM20* (Game et al., 2005), which encodes the other subunit of the Nat3 acetyltransferase protein (Polevoda et al., 2003). In addition, *nat3* and *mdm20* deletion mutants have been shown to be sensitive to other DNA damaging agents such as bleomycin, camptothecin and hydroxyurea (Polevoda et al., 2003). This class of N-terminal acetyltransferases catalyzes acetylation of the amino-terminal methionine residues of all proteins beginning with Met-Asp or Met-Glu and of some proteins beginning with Met-Asn or Met-Met (Polevoda et al., 2009). The *rad56-1* mutation results in a truncated Nat3-V214STOP protein, which presumably leaves the N-acetyltransferase domain intact. Since the Nat3-1-YFP fusion protein exhibits protein levels and cellular localization similar to the wild-type protein, we speculate that the Nat3-1 truncation may impair interaction with its substrates. The complete deletion of *NAT3* is more sensitive to DNA damaging agents than the *nat3-V214STOP* allele (Fig. 2A).

The identification of the *rad56-1* mutation within *NAT3* is consistent with early mapping data that placed it ~44 cM distal to *aro7* on the right arm of chromosome 16 (Game and Mortimer, 1974). The genome sequence indicates a physical distance of ~119 kb between *aro7* and *NAT3*, giving a value of about 2.7 kb per cM, which is typical for *Saccharomyces*. However, a report of tight linkage between *SGE1* and *rad56-1* (Amakasu et al., 1993) is surprising, given that *SGE1* is located ~240 kb distal to *NAT3*. This could imply that there are strain differences involved, for example an inversion. Alternatively, given that no recombinants were observed among the 61 complete tetrads analyzed (Amakasu et al., 1993), it is possible that the *sge1*Δ *rad56-1* double mutant is inviable, resulting in only parental ditypes being seen in tetrads with four surviving spores.

The spectrum of DNA damage sensitivities and the genetic and physical interaction network for *nat3*Δ suggests that some aspect of homologous recombination is defective in this mutant. Accordingly, *NAT3* was previously reported to belong to a cluster of synthetic lethal interactions connecting subunits of the Nup84 nuclear pore subcomplex (*nup133*Δ) with a deletion of the *RAD27* nuclease encoding gene required for Okazaki fragment processing and maturation (Loeillet et al., 2005). This cluster includes essentially the entire *RAD52* epistasis group. However as expected, the functional genomic interaction network for Nat3 indicates that its function is required for a wide