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Rad52 multimerization is important for its nuclear localization in *Saccharomyces cerevisiae*

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ABSTRACT

Rad52 is essential for all homologous recombination and DNA double strand break repair events in *Saccharomyces cerevisiae*. This protein is multifunctional and contains several domains that allow it to interact with DNA as well as with different repair proteins. However, it has been unclear how Rad52 enters the nucleus. In the present study, we have used a combination of mutagenesis and sequence analysis to show that Rad52 from *S. cerevisiae* contains a single functional pat7 type NLS essential for its nuclear localization. The region containing the NLS seems only to be involved in nuclear transport as it plays no role in repair of MMS-induced DNA damage. The NLS in Rad52 is weak, as monomeric protein species that harbor this NLS are mainly located in the cytosol. In contrast, multimeric protein complexes wherein each subunit contains a single NLS_{Rad52} sort efficiently to the nucleus. Based on the results we propose a model where the additive effect of multiple NLS_{Rad52} sequences in a Rad52 ring-structure ensures efficient nuclear localization of Rad52.

1. Introduction

The integrity of the genome is constantly challenged by DNA damage induced by reactive metabolic intermediates and environmental agents. Among the different types of DNA lesions that can occur, DNA double strand breaks are particularly dangerous, as they may cause cell death or provoke genomic rearrangements. In *Saccharomyces cerevisiae*, DNA double strand breaks are mainly repaired by pathways that involve homologous recombination (HR). HR depends on the genes of the *RAD52* epistasis group, *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *RDH54*, *RFA1*, *MRE11* and *XRS2* [1]. Among these, mutations in *RAD52* show the most severe phenotype, reflecting the involvement of this gene in multiple HR pathways. The importance of Rad52 is stressed by the fact that it is conserved from yeast to human.

Several biochemical properties of Rad52 are germane for its HR role, including DNA binding and an ability to interact with the Rad51 recombinase, Rad59 protein, and the single-strand DNA binding protein RPA. These attributes enable Rad52 to promote the annealing of RPA-coated ssDNA and to function with Rad51 in the displacement of RPA from ssDNA [2–9]. The highly conserved N-terminus of Rad52 contains domains that allow it to self-associate and form ring-structures, to bind Rad59, to bind DNA and to facilitate DNA annealing (Fig. 1) [7,2,8–11]. The middle- and C-terminal regions of yeast and human Rad52 proteins have been shown to contain the RPA and Rad51 interaction domains, respectively, but are otherwise not well conserved in primary sequence [12,13,2,14,15]

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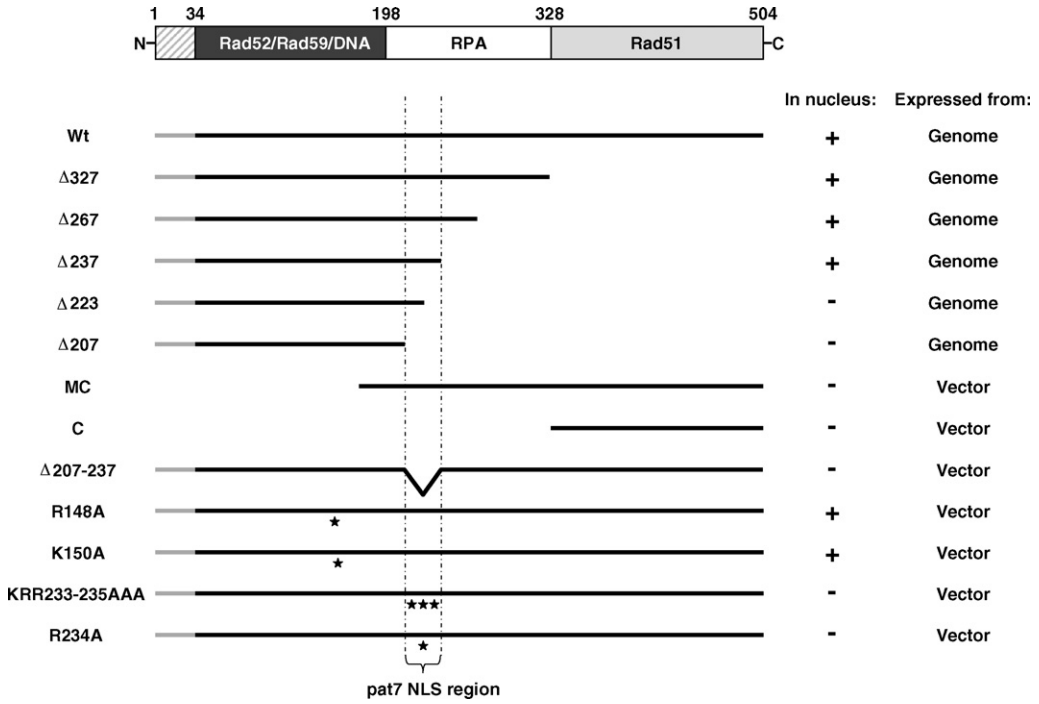

Fig. 1 – Functional map of *Saccharomyces cerevisiae* Rad52 and an overview of the cellular localization of all Rad52 mutants. A schematic representation of Rad52 from *S. cerevisiae* is presented in the top. The hatched region covering aa residues 1–33 is not expressed. The dark region spanning aa residues 34–198 corresponds to the region of Rad52 that is highly evolutionary conserved. The regions in Rad52 that are involved in protein–protein interactions and in binding to DNA are indicated. A diagram showing all individual Rad52 deletion and mutated species relative to wild-type Rad52 is presented below. All Rad52 species are C-terminally extended by YFP (not shown in the figure). Rad52-YFP (Wt), Rad52-Δ327-YFP (Δ327), Rad52-Δ267-YFP (Δ267), Rad52-Δ237-YFP (Δ237), Rad52-Δ223-YFP (Δ223), Rad52-Δ207-YFP (Δ207), Rad52MC-YFP (MC), Rad52C-YFP (C), Rad52-Δ207-237-YFP (Δ207-237), Rad52-R148A-YFP (R148A), Rad52-K150A-YFP (K150A), Rad52-KRR233-235AAA-YFP (KRR233-235AAA) and Rad52-R234A-YFP (R234A). The position of single aa residue substitutions are indicated with stars and the pat7 NLS region is indicated below the figure. To the right, the cellular localization of individual Rad52 species and whether it is expressed from the genomic *RAD52* locus or from a vector is shown. Nuclear and cytosolic localization of a given Rad52 species is indicated by (+) and (–), respectively.

(Krejci et al., submitted). Notably, it has remained unclear how Rad52 is transported into the nucleus.

Most nuclear proteins larger than 40–60 kDa require active transport to enter the nucleus [16]. This transport is facilitated by nuclear transport receptors, importins, which recognize nuclear localization signals, NLSs, which are typically composed by clusters of basic amino acid (aa) residues [16]. The complex of a cargo protein and a nuclear transport receptor is then shuttled from the cytosol into the nucleus through the nuclear pore complex by forming transient interactions with nucleoporins that line the channel of the pore. In a previous search for NLS motifs in DNA repair proteins, no putative NLS in *S. cerevisiae* Rad52 was identified and it was proposed that Rad52 is escorted into the nucleus via an interaction with another protein factor that harbors such a transport signal [17]. We have located the region in Rad52 required for its nuclear localization. Combining these domain mapping results with a complementary sequence analysis, we have identified a single "pat7" type NLS in Rad52 and shown that it is essential and sufficient for efficient Rad52 transport into the nucleus. Interestingly, the functionality of this NLS seems to be dependent on Rad52 oligomerization being mediated by the N-terminus of the protein.

2. Materials and methods

2.1. Genetic methods and strains

All media were prepared as described by Sherman [18] with minor modifications as the synthetic medium contained twice the amount of leucine (60 mg/L). All strains are isogenic to W303 [19] except they are *RAD5* [20,21], and ADE2 (see Table 1). Integrated *RAD52* mutants were constructed and fused to *YFP* using the cloning-free PCR-based allele replacement method previously described by Erdeniz and colleagues [22,23]. Correct integration of the mutations were verified by PCR and sequencing (MWG-Biotech AG).

2.2. Plasmid construction

2.2.1. Plasmids expressing MmRad52-YFP and KlRad52-YFP

Plasmids were constructed from the CEN6-based plasmid pWJ1213 [24] by replacing *S. cerevisiae RAD52* with *RAD52* from *Kluveromyces lactis* and *Mus musculus* preserving the *S. cerevisiae RAD52* promoter. Both genes are lacking stop codons