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DUN1 defines one branch downstream of *RAD53* for transcription and DNA damage repair in *Saccharomyces cerevisiae*

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First published online 3 November 2000

The checkpoint pathway mediates multiple cellular responses to DNA damage (transcriptional induction, cell cycle arrest and DNA repair) that are required for survival. We have previously demonstrated [1] that the checkpoint genes, *RAD9, RAD24, RAD17, MEC3, RAD53* and *MEC1* are required for a normal DNA damage response.

Of the genes known to function in the cellular responses to DNA damage one, *DUN1*, encodes a protein kinase whose phosphorylation is *RAD53*-dependent [2]. *DUN1* was isolated as a mutant allele unable to induce the expression of ribonucleotide reductase (RNR) genes after treatment with both MMS (methyl methane sulphonate) or HU (hydroxyurea) [2].

We have previously reported [1] that the DNA-damage checkpoint genes control the UV-dependent transcriptional induction of a large regulon of genes, termed the DNA Damage Regulon (DDR), involved in nucleotide synthesis, replication, nucleotide excision, mismatch and recombinational repair. We were interested in defining the role of *DUN1* in this transcriptional response. Therefore, we analysed UV damage-dependent induction of representative genes from the DDR in *dun1*Δ cells.

We used a protocol that examines the transcriptional response to DNA damage independently of cell cycle regulation by arresting cells in G_1 and maintaining this arrest for the duration of the experiment [1]. Wild type (FF181268, *Mata; bar1::LEU2; leu2; ura3; trp1; his7; lys1*) and *dun1*Δ (FFD1 *Mata; bar1::LEU2; dun1::LEU2; leu2; ura3; trp1; his7*) cells were grown to logarithmic phase and arrested in G_1 with the mating pheromone α factor. Once G_1 arrest was complete, the cells were UV-irradiated, harvested at various times after the UV irradiation and processed for mRNA extraction. The mRNA was Northern-blotted and probed as described in Fig. 1. Experiments were controlled by parallel mock treatments without the damaging agent. We used probes for transcripts from groups of genes previously defined as being dependent on the DNA damage checkpoint pathway [1]. These transcripts represent distinct functional groups: *RNR3* (nucleotide biosynthesis), *RAD27* (nucleotide excision repair), *RAD51* (recombinational repair), *CDC9* (general DNA metabolism), *PMS1* (mismatch repair) and *RAD53* (transduction of the DNA damage signal).

DUN1 is considered to be a transcriptional regulator of DNA damage-specific induction of the three *RNR* genes [2]. Our data extend this observation by demonstrating that CDC9 and *PMS1* also show significant *DUN1*-dependent transcriptional induction after DNA damage (Fig. 1). However, examination of other transcripts known to be inducible after damage in a checkpoint pathway-specific fashion revealed that *DUN1* does not play a major role in the damage-specific induction of the *RAD51* and *RAD54* transcripts and only a partial role in the induction of the *RAD27* transcript (four-fold in the *dun1*Δ mutant versus eight-fold in the wild type) decreased in *dun1*Δ cells (Fig. 1). Thus the *DUN1*-regulated transcriptional response to DNA damage can be classified into three categories, (a) largely *DUN1*-dependent (note that there is still some residual induction of *RNR* genes in *dun1*Δ cells, see Fig. 1 and [2], (b) partially *DUN1*-depen-

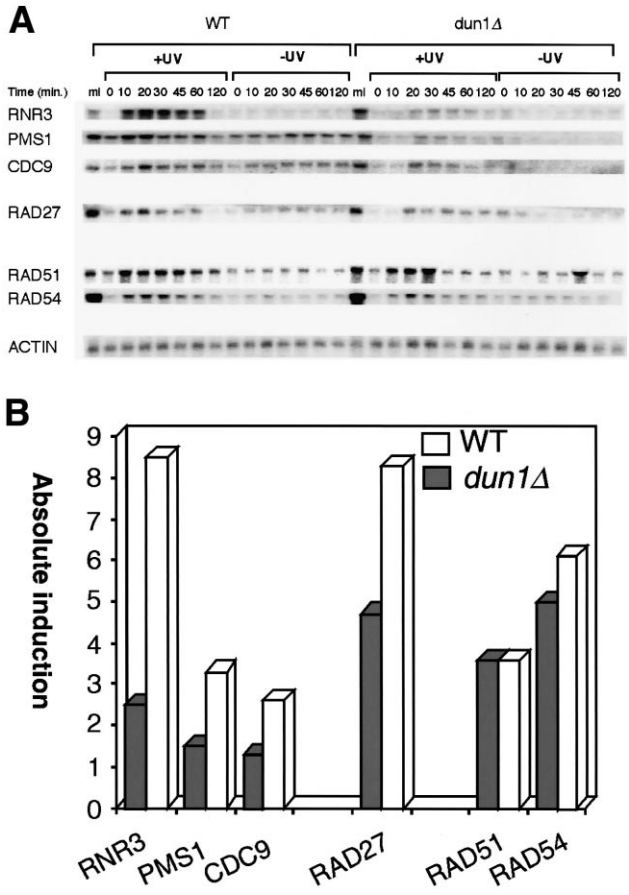

Fig. 1. Northern blot analysis following UV irradiation of G_1-synchronised cells. A: Cells were exponentially grown in YPD at 30°C and treated with 0.5 μg/ml of α factor for 2 h at 30°C. Growth arrest was monitored by phase contrast microscopy. After 2 h (when the number of unbudded cells with a 'shmoo' morphology was at least 95%) cultures were harvested, washed with saline 0.9% NaCl and resuspended at a final concentration of 1×10^7 cells in 100 ml of saline. 50 ml of cells were UV-irradiated at 30 J/m^2 and 50 ml were kept as a mock-treated control. After treatment cells were resuspended in YPD medium at 1×10^7 cells/ml plus 0.5 μg/ml α factor. Total RNA was analysed by Northern blot analysis [1] using probes for the genes indicated in the figure. Probes used were obtained by PCR, using oligonucleotides designed to anneal within the corresponding ORF in order to amplify fragments ranging from 1–1.8 kb. *ACT1* was used as a loading and general transcription control. B: Quantification was performed by using a phosphorimager (Molecular Dynamics Ltd., and normalised first to the actin loading control and second to the value measured at time 0) as previously described in [1].

dent and (c) *DUN1*-independent. Our results are consistent with a model in which the pathway leading to transcriptional induction of various genes diverges downstream of *RAD53*. No doubt reflecting differential requirements for transcription activators or repressors in the various promoters; some that are effective targets of Dun1 and others that are not.

In addition to their roles in induction of the DDR and cell cycle arrest, the *RAD9, RAD24, MEC1* and *RAD53* genes are involved in non-homologous end-joining (NHEJ) of double strand breaks as we have recently reported in this journal [3]. Therefore, we investigated whether the *DUN1* gene also participates in NHEJ processes. We utilised a previously described plasmid re-ligation assay [3,4], cells were grown to log phase in minimal YNB medium and transformed either with *Bam*HI linearised or with the non-digested pRS315 plasmid. The efficiency of plasmid re-ligation after transformation was determined as the number of transformants obtained with the digested plasmid relative to the number of transformants obtained with the non-digested one. We obtained that in *dun1*Δ cells there is a significant reduction in the efficiency of re-ligation compared to wild type cells (33 compared to 100%). This reduction is comparable with that obtained with a *rad9*Δ (FF181270, *Mata; bar1::LEU2; rad9::URA3; leu2; ura3; trp1; his7; lys1*) mutant (25%), but less than the previously reported reduction obtained with a *rad24*Δ-*rad9*Δ double mutant (6%) [3]. Plasmids recovered from transformed *dun1*Δ cells could be re-linearised with *Bam*HI (data not shown) indicating that although in the absence of Dun1 repair is less efficient, it remains error free. Similarly, although the level of repair observed in *rad24*Δ-*rad9*Δ cells is low [3], it is also error free. The major pathway of error free NHEJ is dependent on Ku proteins in budding yeast [4] and it is this pathway that is regulated by the DNA damage checkpoint pathway ([2] and this work). Recently, the localisation of Ku and Sir proteins from telomeres to double strand breaks has been reported [5,6]. It is possible that *DUN1* might participate in this process. Alternatively, or in addition, Dun1 might directly activate other components of the NHEJ machinery or be involved in the transcriptional activation of genes encoding rate limiting components of NHEJ (note that *YKU70, YKU80, LIG4* and *LIF4* are not members of the DDR, M.A. de la T.-R., unpublished data).

Our data are consistent with a role for *DUN1* as a component of a branch of the DNA damage checkpoint pathway that functions downstream of *RAD53* and *RAD9* and *RAD24* epistasis groups. This role is clearly not limited to transcriptional regulation of *RNR* genes after DNA damage, as in addition to being involved in damage-dependent transcriptional induction of other members of the DDR (Fig. 1), *DUN1* also has an important role in NHEJ. It seems unlikely that Dun1 directly participates in the enzymology of these repair pathways, rather it appears most likely that Dun1 regulates these responses by targeting factors required for their efficient operation. Similarly, Dun1 must also target factors required for a complete transcriptional response to DNA damage and a normal cell cycle arrest after DNA damage.

The participation of the Dun1 protein kinase in a wide range of cellular responses to DNA damage suggests that rather than being required as a component of all these processes, it more likely regulates factors required for these responses. Furthermore, because phenotypes can readily be detected in cells harbouring null alleles of *DUN1*, this indicates that *DUN1* can not be simply redundant with other components of the checkpoint pathway. Rather a degree of specificity must exist towards at least some of its targets.

Acknowledgements: We thank Francis Fabre for providing FF181268, FF181270 and FFD1 strains and Dr Enric Herrero for reading the manuscript and helpful discussions.

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PII: S0014-5793(00)02198-0