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Germinating conidiospores of *Aspergillus* amino acid auxotrophs are hypersensitive to heat shock, oxidative stress and DNA damage

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Abstract Germinating conidiospores (conidia) of *Aspergillus nidulans* amino acid-requiring strains are hypersensitive to heat, oxidative stress, UV radiation and chemical mutagens when compared with other strains. They also showed an increased mutation rate. Sensitivity to stress conditions has been correlated with an abnormal RAS/cAMP pathway in mutants of *S. cerevisiae*. We suggest that the RAS/cAMP pathway is defective in germinating conidia of *Aspergillus* amino acid auxotrophs and that this is responsible for suppressing DNA repair and conferring sensitivity to oxidative stress and heat shock.

Key words: DNA repair; Heat shock; Oxidative stress; Cancer; cAMP/RAS; *Aspergillus nidulans*

1. Introduction

The eukaryote microorganisms are attractive systems for the analysis of the mechanisms of DNA repair, and many DNA repair mutants have been isolated in the yeasts and filamentous fungi. Generally they are isolated on the basis of their sensitivity to radiation or mutagens since it is predicted that mutants with defective DNA repair will be more susceptible than wild-type to DNA damaging agents.

Many *Aspergillus* genes conferring sensitivity to UV radiation (*uvs* mutants) and methyl methanesulphonate (MMS) (*mus*, mutagen-sensitive) have been isolated and allocated to complementation groups [1,2]. However, it has also been shown that other mutants with less obvious defects in DNA repair are also sensitive to mutagenic agents [3,4]. Kafer [3] showed that all the amino acid auxotrophs of *Aspergillus nidulans* tested were more sensitive to the chemical mutagen MMS than wild-type. Furthermore allelic mutations in a suppressor gene, *sms*A (suppressor of MMS sensitivity), reversed the MMS sensitivity in double mutant strains without affecting the specific requirement for the amino acid. We show here that germinating conidia of the amino acid auxotrophs are also more sensitive to UV radiation and the chemical mutagen 4-nitroquinolene 1-oxide which has been used previously to induce mutations in conidia of *A. nidulans* [5]. In addition they are more sensitive than wild-type to heat shock and hydrogen peroxide-induced oxidative stress.

2. Materials and methods

Routine growth and maintenance of *Aspergillus* cultures was as described previously [6]. The genotypes of *Aspergillus* strains were; R21, *y*A2 *paba*A1 (no amino acid requirement): R153, *w*A3;*pyro*A4 (no amino acid requirement): *y*A2 *paba*A1; *lys*B5 (requires lysine): *y*A2 *paba*A1; *meth*G1 (requires methionine): *y*A2 *paba*A1 *pro*A1 (requires proline): *y*A2 *paba*A1; *tyr*B1 (requires tyrosine): G034, *bi*A1; *arg*B2 (requires arginine): G421, *acr*A1; *uvs*B312 *pyro*A4; *ribo*B2 (UV sensitive): G423, *an*A1 *y*A2; *acr*A1; *uvs*H311; *fw*A2 (UV sensitive): and FGSC A395, *bi*A1; *sms*A61 *s*B3; *lys*D20 T1(III;VII) (requires lysine).

Suspensions of conidia were prepared freshly in Tween-saline (0.08%, w/v NaCl and Tween-80 diluted 2.5×10^{-4}, v/v). Pregerminated conidia were prepared by shaking vigorously 2.5×10^5 conidia for 6 h at 37°C in 20 ml YEG [7] medium (0.5% yeast extract, 2% glucose, 0.2% Tween-80), supplemented with the appropriate growth factors. Conidia were irradiated in darkness with UV (254 nm) in open Petri dishes 6 cm below the source (Camag Universal-UV-Lampe). Hydrogen peroxide treatment was for 10 min and the cells were pelleted and resuspended in 200 μl YEG medium. Cells were exposed to the chemical mutagen 4-nitroquinolene 1-oxide [8] at a final concentration of 1 μg per ml at 37°C for various times and then the solution neutralised by the addition of an equal volume of 5% sodium thiosulphate. In all cases appropriate dilutions were made in Tween-saline, and 100 μl spread on each of five replicate plates of fully supplemented malt extract agar medium containing 0.01% Triton X-100 to restrict colony growth [5]. Colony counts were made after 24 h at 37°C.

3. Results and discussion

Conidia of *A. nidulans* are uninucleate and haploid, so loss-of-function mutations are expressed. Germinating conidia of amino acid auxotroph strains were found to be more sensitive to heat shock than other strains (Fig. 1A) when tested in a rich medium which contained the required amino acid. They are also considerably more sensitive to oxidative stress than wild-type when treated with hydrogen peroxide (Fig. 1B). In both cases the conidia were treated after shaking vigorously for 6 h at 37°C in a rich medium to a stage where they were germinating.

Since it has been reported that conidia of amino acid auxotrophs are more sensitive to MMS than are wild-type conidia it was important to investigate whether this was specific to MMS or a more general phenomenon related to the sensitivity to heat shock and oxidative stress reported above. We were surprised to find that germinating conidia of amino acid auxotrophs are also more sensitive than wild-type conidia (R21, *y*A2 *paba*A1 or R153, *w*A3;*pyro*A4) to UV irradiation (Fig. 1C) and the chemical mutagen NQO (Fig. 1D). Spores were more sensitive to killing by UV whether they were irradiated immediately after harvesting from Petri dishes (dormant spores) or after pre-incubation in a rich medium for 6 h to allow germination. Auxotrophs with different spore colours were also used to eliminate the possibility of differential UV absorption by the pigments present in the spore wall. We found no differences

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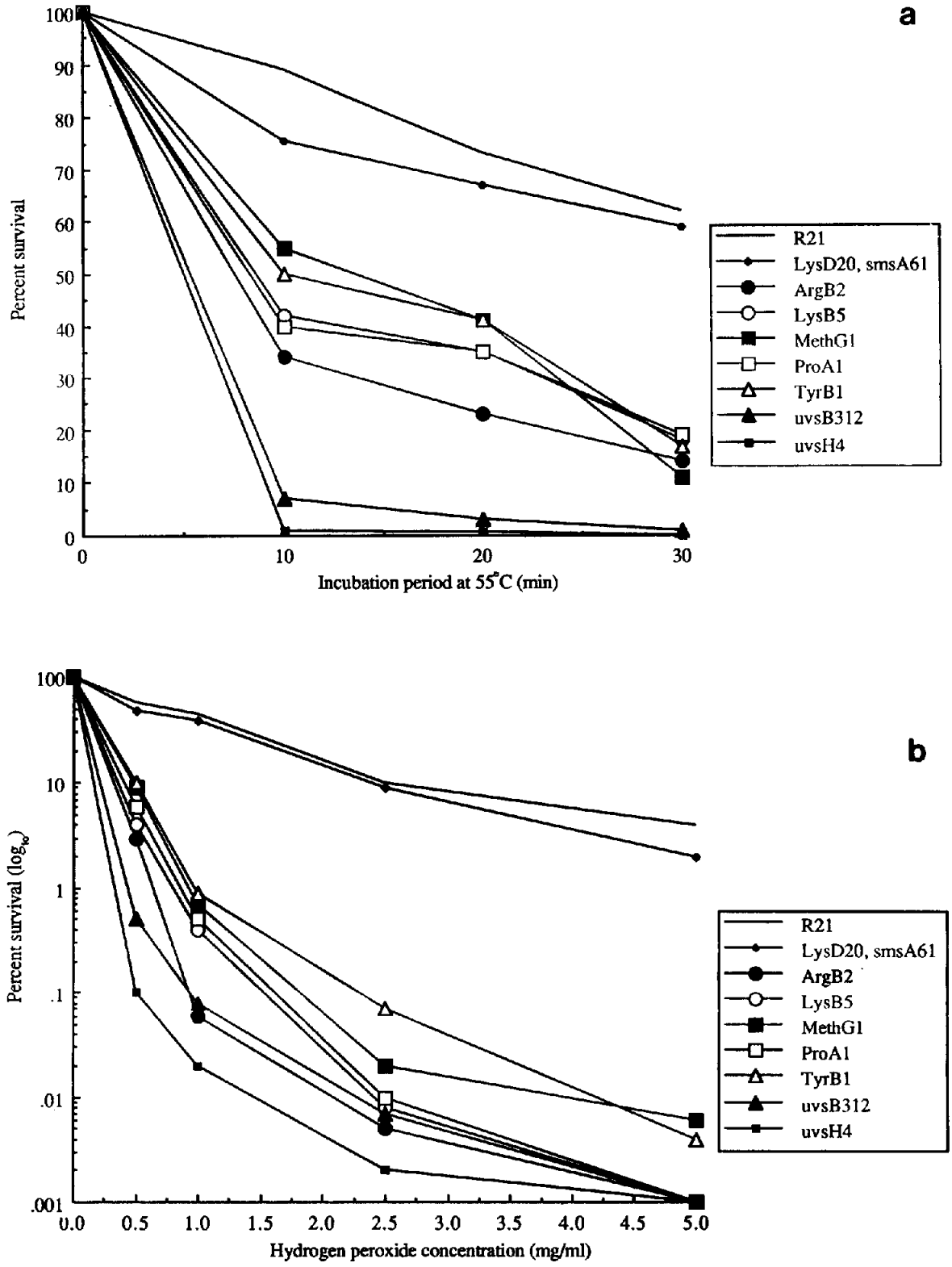

Fig. 1. The effect of (a) heat-shock, (b) oxidative stress, (c) UV radiation, and (d) 4-nitroquinolene 1-oxide, on the survival of germinating conidia of *A. nidulans*. Freshly prepared suspensions of conidia were pre-germinated by shaking vigorously for 6 h at 37°C in 20 ml YEG medium [7] supplemented with the appropriate growth factors. The pre-germinated conidia were subjected to heat shock (55°C), hydrogen peroxide for 10 min, UV radiation (254 nm) or 4-nitroquinolene 1-oxide (1 μg/ml). Dilutions were spread on each of five replicate plates of fully supplemented malt extract agar medium containing 0.01% Triton X-100 to restrict colony growth [5]. Colony counts were made after 24 h at 37°C. Each point is the mean of five replicates.

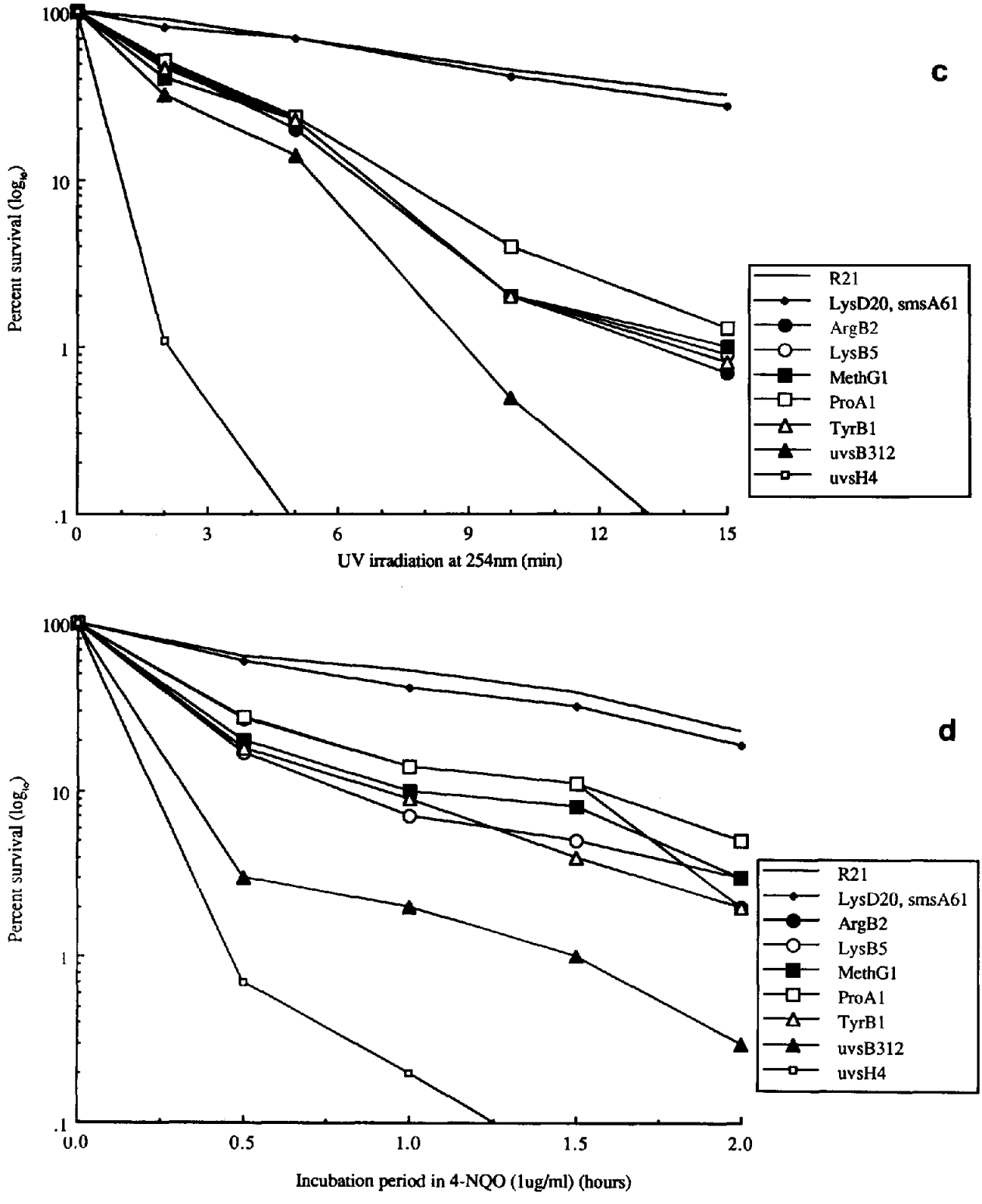

Fig. 1 (*continued*).

between auxotrophs with white, yellow or green spore colour. The UV-sensitive mutants *uvs*B and *uvs*H, which are known to be defective in DNA repair, were most sensitive to heat shock, oxidative stress and 4-nitroquinolene 1-oxide. The sensitivities of the amino acid auxotrophs to stress conditions and mutagens were in general intermediate between the wild-type and the *uvs* mutant strains.

Similar results were obtained with strains requiring other amino acids, but not with strains requiring other growth factors. For example the strain FGSC446 (*su*A1*ad*E20 *paba*A1

*y*A2 *ad*E20; *acr*A1; *gal*A1; *pyro*A4; *fac*A303; *lac*A1 *s*B3; *cho*A1 *nic*B8; *ribo*B2 *cha*A1), which has multiple requirements (but not for amino acids), was not hypersensitive to UV radiation. The effect appears to be specific to amino acid-requiring mutants, which is in agreement with the results obtained previously by Kafer with MMS [3]. A mutation in the gene *sms*A which suppressed the MMS hypersensitivity of all amino acid auxotrophs tested also suppressed the UV hypersensitivity in a lysine requiring (*lys*D) double mutant (*lys*D *sms*A). More interestingly it also suppressed the hypersensitivity to heat and oxidative stress (Fig. 1A and B).

It is relatively easy to determine mutation frequency in *A. nidulans* since mutations in genes involved in the pathway of spore pigment synthesis give rise to easily recognised mutant colony colour phenotypes. We have determined the mutation frequency from yellow to white colony colour for the yellow strain R21, (*y*A2 *paba*A1) and a methionine-requiring strain (*y*A2 *paba*A1, *meth*G), which are isoallelic for *y*A2. The mutation frequency for the amino acid auxotroph was 51 in 28,000, compared with 7 in 28,000 colonies for the wild-type (R21) at the same dose of UV radiation. The decreased viability of the auxotroph conidia after UV irradiation is therefore associated with an increased mutation frequency.

Hypersensitivity to stress conditions is characteristic of a hyperactive RAS/cAMP pathway in mutants of *S. cerevisiae* [9] where high cAMP-PK activity has been correlated with the repression of some stress-protective proteins and other effects on metabolic pathways and gene transcription [10]. The detection of adequate levels of nitrogen, phosphate and sulphate is dependent on the activity of cAMP-PK. Genes involved in the nitrogen signalling pathway include *CDC*33 [11], the gene for a protein synthesis initiation factor, and *CDC*60 [12], the gene for a leucyl-tRNA synthetase (both genes are required for progress through START A in the yeast cell cycle which also requires elevated levels of the cAMP-PK activity). Other genes implicated in this pathway include those for isoleucyl-tRNA synthetase and methionyl-tRNA synthetase [13]. We suggest that the germinating spores of *A. nidulans* amino acid auxotrophs are similarly defective in the RAS/cAMP pathway.

The close similarity between many of the proteins involved in the RAS/cAMP pathway of the lower eukaryotes and mammalian cells suggests a similar function. Although the results described here were obtained with a filamentous fungus, there is some justification in speculating on their relevance to higher eukaryotes. If the hypersensitivity of *Aspergillus* spores to stress and DNA damage is indeed due to a defective RAS/cAMP pathway, then it is tempting to suggest a role for the pathway in the transformation process in mammalian cells. A defective RAS/cAMP pathway caused by mutation in one of the component genes or a gene in an essential biosynthetic pathway (e.g. amino acid metabolism or cell cycle progression) might act to suppress the normal DNA repair pathways in the cell, and thereby increase the mutation rate. This is consistent with the generally accepted hypothesis that transition from normal cell to malignant tumour cell is a multistage process involving sequential mutations in a small number of genes (for review see [14]). A defect in the RAS/cAMP pathway might represent the proposed 'initial mutator phenotype' [15] which increases the probability of a cell accumulating further mutations. These would occur with increased liklihood in a cell if its normal DNA repair capacity was suppressed, particularly if also exposed to a mutagen.

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