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Assignment of ten DNA repair genes from *Schizosaccharomyces pombe* to chromosomal *Not*I restriction fragments

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Summary. Ten DNA repair (*rad*) genes from the fission yeast, *Schizosaccharomyces pombe* were mapped to the 17 *Not*I fragments of the three chromosomes. Nine of the genes map to chromosome I, but there is no evidence for significant clustering.

Key words: DNA repair – *Schizosaccharomyces pombe* – *rad* genes – Mapping *Not*I

Introduction

DNA repair processes play an important role in all living organisms. The complexity, intricacy and diversity of the repair pathways attest to their importance in maintaining genomic stability. This is confirmed by the existence in man of several genetic disorders, whose severe symptoms result from defects in DNA repair. It is becoming increasingly apparent that basic cellular processes are conserved throughout eukaryotes, and that this functional conservation extends to gene structure. In particular, at least four of the cloned human DNA repair genes that have been investigated have homologues in yeast. Thus the human *ERCC-1* and *ERCC-2* genes are homologous to the *S. cerevisiae RAD10* (Van Duin et al. 1986) and *RAD3* (Weber et al. 1990) genes respectively, and the recently cloned *ERCC-3* gene (Weeda et al. 1990) has homologues in both *S. cerevisiae* and *Schizosaccharomyces pombe* (unpublished results of M.H.M. Koken, G. Weeda and J.H.J. Hoeijmakers). Likewise human DNA ligase (Barnes et al. 1990) is homologous to the DNA ligases from *S. cerevisiae* (Barker et al. 1985) and *S. pombe* (Barker et al. 1987).

We have recently commenced a systematic study of DNA repair genes in the fission yeast *S. pombe*. Mutants in 23 independent complementation groups involved with UV or gamma-ray sensitivity, and by implication with DNA repair, have been identified previously (Phipps et al. 1985, Lieberman et al. 1989). We have cloned 10 DNA repair genes by their ability to complement radiation-sensitive mutants. In addition the *S. pombe* homologues of the *RAD6* gene of *S. cerevisiae* (Reynolds et al. 1990) and of the human *ERCC-3* gene (M.H.M. Koken, G. Weeda and J.H.J. Hoeijmakers, unpublished) have been cloned using hybridization techniques. The cloning and characterization of the genes will be described elsewhere.

The *S. pombe* genome is comprised of three chromosomes of length 3.5, 4.7 and 5.7 Mb. Digestion of *S. pombe* DNA with the rare-cutting restriction enzyme, *Not*I, produces 17 fragments, most of which can be readily resolved using pulsed-field gel electrophoresis (PFGE). These fragments have been ordered on the three chromosomes (Fan et al. 1988). In this report we describe the mapping of ten DNA repair genes to these fragments using PFGE and hybridization techniques.

Materials and methods

DNA preparation. Our procedure for preparing DNA in agarose plugs was modified from that of Anand (1986). *S. pombe* spheroplasts were prepared by digestion of the cell walls with NovoZym (Novo BioLabs), using the procedure of Beach et al. (1982), and resuspended in 10 mM TRIS-HCl, 1.2 M sorbitol to give a concentration of 5×10^8 cells/ml. Molten 1% agarose (InCert agarose, FMC Bioproducts) at 37° C was added and gently pipetted up and down. The suspension was transferred to plug moulds cooled to 4° C by placing on a bed of ice and the agarose allowed to set. Using a rubber teat, the solid plugs were blown directly into NDS (0.5 M EDTA, 10 mM TRIS-HCl, 1% lauroyl sarcosine, pH 9.5). The lysed cells were incubated twice in proteinase K (Sigma) overnight. Remaining proteinase K was removed by rinsing twice in 10 ml TE (10 mM TRIS-HCl, 1 mM EDTA) on ice for approximately 30 min, in an excess of NDS for 30 min and finally stored in NDS at 4° C.

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Digestion. Each plug was cut into 3 pieces. Each piece, equivalent to approximately 0.5 μg DNA, was rinsed twice in a minimum of 10 ml TE buffer containing 0.1 mM phenylmethylsulphonyl fluoride (PMSF), and once in the recommended digestion buffer but without any protein or dithiothreitol. Plugs were then transferred to 60 μl of complete digestion buffer and allowed to equilibrate at room temperature for 10 min. Digestion was with 40 units of enzyme at the recommended temperature for 4 to 6 h. In cases where a second digestion followed, plugs were equilibrated overnight in the appropriate buffer first. Digestion was halted by adding 100 μl of STOP (10 mM EDTA, 3% Ficoll, 0.5 × TAE (1 × TAE = 40 mM TRIS-acetate, 2 mM EDTA, pH 8.5) and orange-G dye) and the plugs loaded into the gel. Molecular weight markers used were lambda phage DNA ladders and chromosomal DNA from *S. cerevisiae*. To avoid any local high salt effects in the gel, they were briefly washed in TE to remove most of the storage buffer before use.

Pulsed-field gel electrophoresis (PFGE). PFGE was carried out using the Waltzer apparatus (Southern et al. 1987). Standard conditions were electrophoresis in 1.5% agarose gels for 22 h at 20° C at a voltage of 150 V (5.8 V/cm), and a pulse time of either 30, 45 or 60 s. With the Waltzer system, the gel rotates 110° between each pulse, the direction of the electric field remaining constant. The buffer used was 0.5 × TAE. DNA was transferred onto nylon filters (Hybond N^+, Amersham International) and hybridized with ^{32}P-labelled probes using standard procedures (Sambrook et al. 1989). Inserts were isolated from vector sequences by gel purification in all cases.

Results and discussion

We have recently cloned the *S. pombe rad2, rad4, rad8, rad9, rad11, rad13, rad15* and *rad17* genes by complementation of the defects in the corresponding mutants. *Rhp6*$^+$ is the *S. pombe* homologue of the *S. cerevisiae RAD6* gene (Reynolds et al. 1990) and *ERCC-3*^{sp} is the *S. pombe* homologue of the human *ERCC-3* gene. The latter was isolated by low stringency hybridization of an *S. pombe* gene bank with fragments of *ERCC-3* cDNA (unpublished work of M.H.M. Koken, G. Weeda and J.H.J. Hoeijmakers).

S. pombe DNA immobilized in agarose was digested with *Not*I and in some instances also with *Sfi*I, and electrophoresed using the Waltzer PFGE system (Southern et al. 1987). The DNA was transferred to nylon filters and successively hybridized with fragments of each of the cloned genes. Figure 1 reproduces the *Not*I restriction map of *S. pombe* as reported by Fan et al. (1988). The *Not*I fragments are indicated, together with the assignments to them of the various DNA repair genes. We have been able to define further the location of *rad15* by digestion with *Sfi*I. The 500 kb *Not*I fragment J is split by *Sfi*I into a 185 kb distal fragment and a 315 kb proximal fragment. *rad15* hybridizes to the former. The

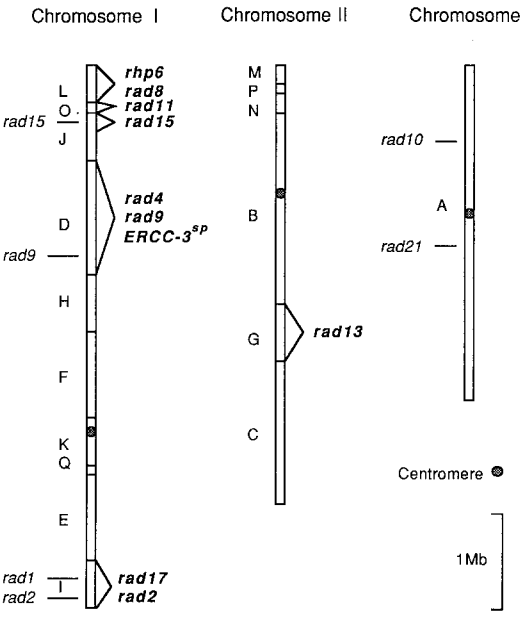

Fig. 1. Assignment of DNA repair genes to *Not*I fragments. Genetic locations are indicated to the left of the chromosomes, locations determined by restriction mapping to the right

rad1 gene was cloned and mapped by Sunnerhagen et al. (1990). Also shown in Fig. 1 are the approximate locations of *rad* genes which have previously been mapped genetically (Munz et al. 1989).

The distribution of the ten genes that have been successfully mapped is markedly non-random, in that nine of them are on chromosome I. Furthermore seven of the genes map to the top third of chromosome I. This region of the chromosome, however, covers 2.3 Mb of DNA, so this cannot be construed as evidence for close clustering of repair genes. Nevertheless it is of interest to determine whether this regional clustering has any evolutionary significance. In *S. cerevisiae,* DNA repair genes appear to be scattered through the chromosomes (reviewed in Hoeijmakers and Bootsma, 1990). Cloned human DNA repair genes also map to different chromosomes, although four of these genes (*ERCC-1, ERCC-2, XRCC-1,* DNA ligase I) are located on chromosome 19 (Mohrenweiser et al. 1989, Barnes et al. 1990, Hoeijmakers and Bootsma 1990), and, of these, the *ERCC-1* and *ERCC-2* genes are found on the same 250 kb fragment (Mohrenweiser et al. 1989).

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