DNA by inactivation of an *ada* encoded O^6-methylguanine-DNA methyltransferease.

The other line of studies concerns cellular responses of *S. cerevisiae* to DNA damaging agents.We have identified several novel *S. cerevisiae* genes, the expression of which is induced by UV light or MMS. One of these genes, *DIN7*, has been cloned and its nucleotide sequence determined. It has been found that the DIN7 amino acid sequence is homologous to that of RAD2 and RAD27 of *S. cerevisiae*, RAD13 and EXO I of *S. pombe*, and human XPGC. At present, we are searching for a phenotype of a strain in which *DIN7* has been disrupted.

1. REGULATION OF THE FIDELITY OF DNA REPLICATION IN *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE*

Z. Cieśla, I. Fijałkowska, P. Jończyk, E.Śledziewska-Gójska, M.U. Fikus, M. Maliszewska-Tkaczyk, P. Mieczkowski, A. Nowicka

We have been studying mechanisms which lead to decreased fidelity of DNA replication in *Escherichia coli* exposed to DNA damaging agents such as UV light. This phenomenon is part of the inducible SOS response. We have recently concentrated on the involvement of DNA polymerase III and the products of the *umuDC* genes in inducible mutagenesis. In particular, we wished to elucidate the role of the ε-subunit of pol III, endowed with proof-reading 3'-5' exonuclease activity, in SOS mutagenesis. Our previous work showed that overproduction of epsilon counteracts the SOS mutagenic response. To explore further the nature of this antimutagenic effect of epsilon, we have constructed plasmids encoding truncated forms of epsilon which lack proof-reading activity, but retain the ability to bind to DNA. Unexpectedly, overproduction of this truncated epsilon causes a significant decrease in the frequency of UV-induced mutations. This antimutagenic effect is effectively relieved by excess UmuD,C proteins. We propose that the DNA binding property of epsilon, rather than its 3'-5' exonuclease activity, affects processing of premutagenic lesions, possibly by interference of free epsilon with interaction of the UmuC-UmuD-RecA complex with pol III holoenzyme.

Extensive previous work suggested that RecA, UmuC and UmuD' proteins help DNA polymerase III to proceed beyond a lesion in the template, resulting in generation of mutations. It has been postulated that these proteins form a complex, a "mutasome". We are trying to elucidate what kinds of specific protein-protein interactions are involved in SOS mutagenesis. To

investigate *in vivo* protein-protein interactions within the "mutasome", we have used a two-hybrid system based on restoration of the function of a yeast transcriptional activator, GAL4. We have been able to demonstrate specific interaction between UmuD/UmuD and UmuD′/UmuD′ proteins, which are the active forms of UmuD. Interaction between UmuD and UmuD′ proteins has also been shown. We also found that UmuC protein interacts with UmuD′ protein, but not with UmuD protein. The interaction between UmuD′ protein and several UmuC mutant proteins has been studied. We have been able to divide these mutant proteins into two subclasses: those in which interaction with UmuD′ is impaired and those which exhibit normal UmuC/UmuD′ interaction.

The other project concerns a fundamental question in mutagenesis, namely whether mutagenesis during DNA replication proceeds differently within the two strands on DNA (leading vs. lagging strand). We have developed a unique system that permits inversion of a selected mutational target gene inside the chromosome with respect to the origin of DNA replication, such that in one case a particular strand is replicated as leading strand, and as lagging strand in the other case. This approach is initially performed with the *lac* operon. Currently we have integrated into the *E. coli* chromosome six defined *lacZ* alleles (each in two defined orientations with regard to the replication origin) that allow rapid analysis of mutagenic specificity. Measurements of spontaneous and UV-induced mutagenesis are under investigation.

Another project is devoted to the mechanisms of repair of DNA damaged by alkylating agents. We earlier found that induction of the adaptive response to alkylating agents in *E. coli* does not influence the level of GC→AT transitions induced by MMS. We now use *lacZ* mutants of phage M13mp18 to compare the repair of DNA lesions leading to GC→AT transitions induced by MMS *in vivo* or *in vitro*. It was shown that, in contrast to *in vivo* mutagenesis, DNA modified by MMS *in vitro* is efficiently repaired in adapted *E. coli* cells and that MMS treatment of the cells blocks this repair. This result pinpoints O^6-methylguanine as a premutational lesion leading to GC→AT transitions induced by MMS, and shows that MMS treatment of the cell interferes with the repair of O^6-methyl-guanine-DNA in adapted *E. coli* cells. In agreement with this, it was shown that MMS, as well as another SN2 alkylating agent (DMS), causes depletion of *ada*-encoded O^6-methyl-guanine-DNA methyltransferase activity *in vivo*. Analysis of the repair capacity of *E. coli* cells expressing a high level of the second O^6-methylguanine-DNA methyltransferase, encoded by the gene *ogt*, cloned on the expression vector pLEX5AA,

shows that Ogt is less sensitive to *in vivo* inactivation by MMS than Ada protein.

In studies on cellular responses of *S. cerevisiae* to DNA damaging agents, we succeeded in the isolation of novel genes, the expression of which is induced by UV light and MMS. One of these genes, *DIN7*, has been characterized in detail. We have cloned *DIN7* and its nucleotide sequence has been determined. By comparison of the predicted DIN7 amino acid sequence with those in the data base, we found it homologous to that of previously reported proteins: *S. cerevisiae* RAD2, which has ssDNA endonuclease activity, and its *S. pombe* and human homologs RAD13 and XPGC. DIN7 is also homologous to RAD27 of *S. cerevisiae*, which is endowed with both DNA endonuclease and 5′-3′ exonuclease activities. Finally, it is homologous to Exonuclease I from *S. pombe*. Thus it seems likely that DIN7 is also endowed with nuclease activity. At present we are looking for a phenotype of a strain in which *DIN7* has been disrupted Interestingly, we have recently found that expression of *DIN7* is induced during meiosis.

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