

Atl-1 protects *S.pombe* against the toxic effects of O^6 -alkylguanine in DNA

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O^6 -alkylguanine-DNA alkyltransferases protect cells and organisms against the toxic and mutagenic effects of O^6 -alkylguanine residues generated in DNA by carcinogenic and chemotherapeutic alkylating agents. They operate by alkylation damage reversal in a mechanism in which the alkyl group is transferred to a cysteine residue in the active site peptide sequence PCHRI/V. *In silico* analysis has identified a family of alkyltransferase-like proteins with extensive amino acid sequence similarity, but with tryptophan in place of cysteine in the putative active site. The *S.pombe* protein (Atl-1) has been overexpressed and affinity purified. Atl-1 binds to short oligonucleotides containing O^6 -methyl, benzyl, 4-bromophenyl or hydroxyethyl-guanine but does not remove the alkyl group or base and does not cleave the oligonucleotide in the region of the lesion.

To examine if ATL plays a role in the biological effects of alkylating agents, the gene was insertionally inactivated in *S.pombe*. The resulting deletant had normal growth rate and morphology. Incubation of crude extracts of wild type but not Atl-1 deleted *S.pombe* with [³²P]-labelled oligonucleotides results in a band shift on PAGE. The deletant was substantially more susceptible to the toxic effects of alkylating agents including N-methyl-N'-nitro-N-nitrosoguanidine, N-methyl- N-ethyl- and N-propyl-N-nitrosourea and methyl- ethyl- and n-propyl-methanesulphonate, but not to other types of DNA damaging agents. ATL therefore plays a key role in protecting cells against the growth inhibitory effects of these agents. Crossing of the Atl-1 deletant with deletants in other DNA repair pathways indicates that Atl-1 may be part of a recognized repair pathway.

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