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ABSTRACT

Characterization of the N-Methyl-N'-Nitro-N-Nitrosoguanidine Induced Response in Vibrio Cholerae.

Alkylating agents, of which methylating agents appear to be quite widespread in the environment, are efficient mutagenic and cytotoxic agents. Cells have the ability to acquire increased resistance to the deleterious effects of alkylating agents during exposure to sublethal concentrations of methylating or ethylating agents. This phenomenon has been termed the "adaptive response". Organisms across the evolutionary scale, ranging from bacteria to humans possess such a defence mechanism to protect themselves against the damage inflicted by alkylating agents. In *Escherichia coli* induction of the "adaptive response" involves the coordinate expression of at least four genes viz. *ada, alkA, alkB* and *aidB*. The repair enzymes encoded by these, in addition to the constitutively expressed methyltransferase and glycosylase encoded by *ogt* and *tag* genes respectively protect the organism against the alkylation damage.

Vibrio cholerae is known to exist freely in nature and hence has chances to get exposed to the naturally present alkylating agents. How it copes with the alkylation damage and whether it has an adaptive response is not yet known. This thesis presents the results of such a study.

Wild type *V. cholerae* cells when adapted by a stepwise treatment with sub-lethal concentrations of MNNG, acquired resistance to the killing and mutagenic effects of subsequent challenges with higher concentrations of the same. The fold increase in survival of the adapted cells was, however, only about 2 fold when compared to the control cells. Mutation experiments showed that MNNG pretreated cells resisted further mutagensis by the same agent. Studies on the reactivation patterns of alkylated phage indicated the presence of an *alkA* like gene in *V.cholerae*. Experiments done with a *rec* mutant of the *V. cholerae* strain proved that the observed effects were independent of the SOS response.

The physiological data was corroborated by direct biochemical evidence for the presence in *V. cholerae* of O^6-methylguanine-DNA methyltransferase, the central protein of the adaptive response. In *E. coli*, both the O^6-methylguanine- and the

methylphosphotriester methyltransferase activites reside on the same molecule, while in *V. cholerae* they do not. Also a counterpart of the constitutively expressed Ogt protein of *E. coli* was found to be missing in this organism. Immunoassay of the Western blotted proteins showed the absence of any antigenic similarity between the Ada proteins of *E. coli* and *V. cholerae*. DNA hybridization studies demonstrated a low level sequence homology between the *ada* genes of the two organisms. Titration of the *alkA*-specific mRNA supported by the glycosylase enzyme assay results showed that the *alkA* gene is co-induced alongwith the *ada* gene in *V. cholerae* under conditions of adaptive response.

The *alkA* like gene of *V. cholerae* was cloned using the functional complementation technique. Northern blotting experiments revealed the gene to be 1.1-kbp in size. Sequence analysis of the cloned gene indicated a limited amount of shared homology with the glycosylase gene of *E. coli*.

The overall scenario of the induced adaptive response in *V. cholerae* thus appears to be similar to those present in the well studied examples of *E. coli* and *Bacillus subtilis*, although the extent of inducibility was much lower. Even though *V. cholerae* is taxonomically closer to *E. coli*, the two transferase activities present in the organism viz. O^6-methylguanine- and methylphosphotriester methyltransferase, reside, unlike in *E. coli*, on two separate molecules as is found in *B. subtilis*.