EFFECT OF TRANSPOSON Tn*10* ON THE SURVIVAL AND MUTATION FREQUENCY OF HALOGEN LIGHT-IRRADIATED *E. coli* K-12 STRAIN AB1157

Celina Janion and Anna Wójcik
Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland

Transposons are widely used as tools for constructions of bacterial strains and as carriers for genetic material. We have found that Tn*10* transposon, when inserted into chromosome of *E. coli* strain AB1157, renders the cells more sensitive to and less mutable by halogen light irradiation. These effects are not dependent on the chromosomal locus from which Tn*10* derives or at which is inserted.

Halogen light contains a broad spectrum of UV radiation (UVA, UVB and UVC), and both its potency and specificity of mutation results from formation of pyrimidine dimers and 6-4 photoproducts in DNA. The induced mutations are UmuD, UmuC-protein dependent. We believe that the effects of Tn*10* on survival and mutation frequency in halogen light-irradiated bacteria (tested as *argE3* to Arg^+ reversion) is a result of UmuD and UmuC depletion, since: (i) the decline in survival is about the same in Tn*10*-bearing bacteria as in bacteria with deleted *umuDC*; (ii) transformation of Tn*10* bearing bacteria with plasmid overproducing UmuDC or UmuD'C leads to increase in survival and mutation frequency; and (iii) insertion of Tn*10* into chromosomal DNA has no effect on the frequency of mutations induced by EMS, a mutagen which activity is *umuDC* independent. However, transformation of bacteria deleted in *umuDC* with plasmids carrying *umuDC* or *umuD'C* leads to recovery of mutability, but not of survival of halogen light irradiated cells, indicating that the mechanisms leading to *umuDC*-dependent mutagenesis and to *umuDC*-dependent protection of survival must be different.

MECHANISMS OF MUTAGENESIS BY CYTOSINE ANALOGS

Hikoya Hayatsu[1] and Kazuo Negishi[2]
[1]Faculty of Pharmaceutical Sciences, and [2]Gene Research Center,
Okayama University, Tsushima, Okayama 700, Japan

Mutations occur by base structure changes in DNA. Relations between a certain type of such a change and its resulting mutation specificity could offer insights into mutagenesis mechanisms in general. Nucleobase analog-induced mutagenesis provides an opportunity for such an insight. We have discovered that N(4)aminocytidine, a nucleoside analog bearing an extra amino group on the N(4)amino moiety of cytidine, is a potent mutagen towards phages, bacteria, mammalian cells and fruit flies. Our studies on the mechanisms of this mutagenesis, using mainly the *E. coli* systems, have revealed that (1) N(4)aminocytosine is incorporated into DNA during the process of replication (2) during that process this analog may be incorporated as a substitute of either cytosine or thymine, thereby causing a C to T transition, and (3) this analog as a constituent of the template DNA may direct incorporation of either guanine or adenine, thereby causing a G to A transition. This ambivalent nature of N(4)aminocytosine may be derived from a tautomerism between its amino and imino forms, with the amino form basepairing with guanine and the imino form with adenine. A recent finding in our laboratory is that Ni^{2+} ion can enhance significantly the mutagenic potency of N(4)aminocytidine. This phenomenon is probably related to the fact that Ni^{2+} forms a tight complex with N(4)aminocytidine.
Overall, the structural alteration in cytosine, namely, the presence of an additional amino group at the N(4)amino moiety, results in a high mutability to cause specifically transitions.

MUTAGENIC PATHWAYS OF THE BASE ANALOG, 6-*N*-HYDROXYLAMINOPURINE, IN BACTERIA AND YEAST

Youri I.Pavlov
Universitetskaya emb. 7/9, Department of Genetics, Sankt-Petersburg State University, Sankt-Petersburg, 199034, Russia

Base analog 6-*N*-hydroxylaminopurine (HAP) is a potent mutagen in variety of prokaryotic and eukaryotic organisms. HAP mutagenesis is not affected by defects in generalized repair mechanisms - mismatch, excision, mutagenic and recombination repair, but is elevated several-fold in the DNA polymerase mutants with defective exonucleolytic proofreading in bacteria and yeast, suggesting that HAP-induced errors could be partially eliminated by editing function of DNA polymerases. To investigate this effect in more detail we designed in yeast a special system of strains which are viable despite being completely proofreading-defective, possessing defects of exonuclease sites of both replicative DNA polymerases, delta and epsilon. Antimutator mutations impairing polymerase III function in *E. coli* strongly suppressed HAP mutagenesis. In yeast, mutations affecting any of three replicative DNA polymerases, PCNA or RF-C were HAP-antimutators.
We found that mutants in the yeast *AAH1* (adenine aminohydrolase) gene increase HAP mutagenesis, suggesting that this enzyme plays a role in HAP detoxification. Another gene, *HAM1* (HAP-mutability) gene, in which mutations confer HAP hypermutability, has homologs in the wide range of organisms, from *E.coli* to *C.elegans* and man. The homologous *E. coli* gene is located in the 65-68 min region of the chromosome, while we mapped a newly obtained point mutation conferring HAP hypermutability to 18.7 min. Thus, a suprisingly large number of genes control HAP mutagenesis, raising the possibility that this base analog might pose an everyday threat to the cell.

TEMPLATE - DIRECTED BASE PAIRING OF 2-CHLORO-2'-DEOXYADENOSINE CATALYZED BY AMV REVERSE TRANSCRIPTASE

Jarosław T. Kuśmierek and Agnieszka M. Bukowska
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

2-Chloro-2'-deoxyadenosine (2ClA) is used for treatment of several lymphoid malignances. It is known that 2ClA is incorporated into DNA in cells. The aim of our study was to determine the coding properties of 2ClA located at preselected sites in oligonucleotide templates. 2ClA phosphoramidite was prepared and was used for synthesis of 25-mer templates with 2ClA located at site 21 from the 3'-end. Kinetic parameters (K_m and V_{max}) for site specific incorporation of dNTPs by AMV reverse transcriptase were determined. Frequency of incorporation (V_{max}/K_m) of dTTP opposite 2ClA is 25 - 50 fold lower than opposite A in each of four sequences studied. Substitution of chlorine in adenine strongly influences the K_m values (0.08 - 0.18 μM for A and 1.8 - 4.9 μM for 2ClA), whereas V_{max} values are similar, especially when these values for A and 2ClA are compared in the same sequence. The extension of 2ClA · T pair occurs with about 3 times lower frequency than extension of A · T pair. The frequency of insertion of dCTP, dGTP and dATP opposite 2ClA was at least several times lower than opposite A. The obtained results suggest that the presence of 2ClA in DNA template considerably slows DNA replication but does not lead to base substitution mutations.