THU-S25-16

ApaA and GMP stimulate the pol III holoenzyme activity in the presence of the protein factors from E. coli K-12, Y. Kobayashi and K. Kuratomi, Dept. of Biochem., Tokyo Med. Coll., Tokyo, Japan.

We have tested the stimulatory effect of F1 and F2 proteins on the DNA polymerase III holoenzyme activity (pol III holo) in the presence of diadenosine 5',5'-ppp,p'4'-tetraphosphate (ApaA) or cGMP, or of both compounds. When activated salmon sperm DNA was used as a template-primer, the combined factors, F1 + F2, stimulated the activity of pol III holo several fold in the presence of both cGMP and ApaA. On the other hand, F1 + F2 enhanced the enzyme activity in the presence of either cGMP or ApaA, where p(dT)10-poly(dA) was used as another template-primer. The addition of ATP and spermidine to the assay systems was a prerequisite for the appearance of the above stimulatory effects. The results of binding assays showed that the presence of pol III holo and template-primer increased the amount of [3H]cGMP or [3H]ApaA bound to F1 + F2 fraction.

THU-S25-18

Endogenous nuclear peroxidase levels in regenerating rat liver.

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The activity of a nuclear NADH-peroxidase was followed in purified nuclei from rat liver following partial hepatectomy. Nuclear and post-100,000g superenatant fractions were isolated in 250mM sucrose, 2mM MgCl2, 50mM Tris-HCl (pH 7.4). Fifteen to twenty-fold increases in specific activity of this nuclear enzyme were observed as early as 3 hours post hepatectomy, returning to control levels after 90 hours. Using activated DNA as template-primer, utilisation of N-thymidine triphosphate was used to follow DNA polymerase levels post hepatectomy. Close temporal agreement with the nuclear peroxidase activities could be seen. Putative roles for this endogenous enzyme are discussed.

THU-S25-20

Restriction mapping of two filamentous phages of Xanthomonas oryzae.

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Several plaque type mutants of bacteriophage Xf have been found in this lab. A small plaque mutant was isolated on a rifampicin-resistant strain of X. oryzae. RFI DNA of the two phases is cleaved once into linear RFIII DNA by EcoRI, HindIII, KpnI, SalI and XmnI. Both genomes have two specific recognition sites for BamHI, 3 sites for HindIII, 4 sites for PstI and 5 sites for Ball. However, SacI has only one recognition site on the Xf genome but two sites on Xf genome but two sites on that of the mutant. At least 5 restriction endonucleases PstI, HpaI, SmaI, XbaI and XhoI failed to cut RF DNA of the two phases. The mutant has been estimated to contain 1 extra kilobasepair of DNA. Attempts have been made to determine the possible relationships of the two phases and the function and origin of the extra DNA in the mutant.

POSTERS RELATED TO S25

THU-S25-17

Repair enzymes involved in N4-hydroxyxanthine-induced mutagenesis.

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Mutagenesis induced by OH Cyd was tested in E. coli mutants impaired in different genetic traits: dam [DNA-adenine methylation enzyme], mutS, mutL, mutR or urac [mismatch repair system]. The results point to moderate involvement of these genetic traits in OH Cyd mutagenesis, except for the dam mutant which is hypermutated by OH-Cyd. Comparison of mutation rates of T4rII phages at 30°C and 43°C suggest that OH-Cyd residues in DNA are sensitive to the T4 DNA polymerase proof-reading system. The reason for the high mutagenic specificity of OH-Cyd[AP-AP transition] will be discussed in the light of the above results.

THU-S25-19

Enhancement by 1-methyladeninomamide of DNA synthesis and cell proliferation in the cultured rat liver cells.

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Sinceorton's proposal (1958) a certain correlation has been suggested between cell proliferation and NAD metabolism, especially poly ADP-ribosylation of nuclear proteins. Here we report that 1-methyladeninomamide (1-MNA), an intermediate derived from nicotinamide and SAM, stimulates the proliferation of rat liver cells (RLC) in culture. At concentrations higher than 20 μM 1-MNA enhanced DNA synthesis with concomitant decrease in the cellular NAD. DNA replicating activity measured on the isolated nuclei were unaffected by 1-MNA, whereas, as reported earlier, it was strongly inhibited by NAD. These results indicate that in RLC cells 1-MNA enhances DNA synthesis by lowering the cellular NAD concentration.

THU-S25-21

Distribution and levels of DNA polymerase in regenerating rat liver cells.

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Male Wistar rats were sacrificed at intervals following partial hepatectomy and nuclear and cytoplasmic fractions prepared in 0.25M sucrose; 2mM MgCl2, 50mM Tris-HCl (pH 7.4) with either 4mM MgCl2 or 4mM CaCl2. Cytoplasmic polymerase α(NEM sensitive) was significantly increased at 18 h, reaching a five-fold maximum at 45 h after hepatectomy. Nuclear activity, however, showed three distinct peaks at 3, 27 and 45 h. The peak at three hours coincided with a slight, but not significant, drop in activity in the cytoplasmic fraction. Extraction in CaCl2, resulted in a drop in activity in both fractions and did not significantly alter the distribution of enzyme between the fractions. It is concluded that the nuclei from hepatectomised rats maintain their synchrony for at least two cell cycles and that this is not reflected in the cytoplasmic levels of polymerase α.