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GENETICS AND BIOCHEMISTRY OF DNA REPAIR IN NEUROSPORA CRASSA.

Progress Report

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Genetics and Biochemistry of DNA Repair in Eukaryotic Microorganism, Neurospora crassa.

by

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1. ABSTRACT

Most organisms possess enzymes that can repair DNA damaged by various radiation and radiomimetic chemicals. There are at least three key enzymes involved in this process, these are DNA polymerase, deoxyribonuclease, and ligase. The specific role of these key enzymes in DNA repair in eukaryotes has not yet been elucidated due to the lack of a biochemical genetic approach. The aim of the present study is to characterize and compare DNA polymerases and deoxyribonucleases from the wild type and mutant strains of eukaryotic organisms such as Neurospora crassa. Mutants of Neurospora, presumably defective for deoxyribonuclease and DNA polymerase, have been recently isolated in my laboratory. These mutants are being biochemically characterized and compared with the wild type enzyme to establish which enzymes control a particular function in DNA repair and other aspects of DNA biosynthesis. After biochemical characterization, these mutants will be also examined for their ability to repair damage caused by exposure to radiation, radiomimetic drugs and carcinogens. Also, a number of Neurospora mutants which are known to be sensitive to UV radiation or to MMS (methyl methane sulfonate) or to histidine or defective in recombination are being characterized for DNA polymerases and nucleases to elucidate the biochemical basis of these defects.

These studies will provide a detailed knowledge of the different aspects of DNA repair and the role of the key enzymes there in. The results of present studies involving a eukaryote can be meaningfully generalized for similar studies in humans. Efficient DNA repair in man is necessary for the maintenance of normal growth and for exposure to the sunlight and to various radiomimetic chemicals present in our environment. Due to the introduction of various chemicals in our environment, there is increased risk of damage to our own genetic material (including that of our crop and cattle) and the outcome of the proposed study may provide a meaningful insight into these problems.

PROGRESS REPORT

Following is a summary of work accomplished during the current grant period.

I. Isolation and partial characterization of DNA polymerases from Neurospora crassa. (El-Assouli and Mishra).

DNA polymerases from the wildtype and one repair deficient and a recombination deficient strain of Neurospora has been characterized.

We have for the first time described conditions for the assay of Neurospora DNA polymerase and have recently described our method for the isolation and characterizatión of Neurosproa DNA polymerase (7). We prepare cell free extract from actively growing culture and remove nucleic acid by precipitation with polyethylene glycol as described previously (See ref. 18). The supernatant obtained after centrifugation is extensively dialyzed and then used as source of the enzyme. The DNA polymerase activity was dependent on the amount of enzyme preparation added to the complete reaction mixture and also on the period of incubation. Activated primer template was most effective; native DNA and denatured DNA showed little or no activity when used as primer template in a complete reaction mixture (El-Assouli and Mishra, 1977) (see ref. 7).

The DNA polymerases from different Neurospora strains have been analyzed by DEAE cellulose chromatography and then each peak of activity has been characterized with respect to its physio-chemical properties ([illegible] S values, Molecular weight, templat specificity, elution on ion exchange chromatography, treat-ability, inactivation by SH blocking agent and ability to use artificial Nucleic acid polymers as Primer Templat

The wild type strain of Neurospora was found to have multiple molecular form of DNA polymerases. The young growing cultures there are two molecular form of DNA polymerases however towards the end of log growth phase two more (high molecular wt) forms of DNA polymerases appear. The appearance of the late form of DNA polymerases in old culture is concommitant with the appearance of high deoxyribonuclease activity. In view of this fact it is suggested that the late appearing forms of DNA polymerases may have role in DNA repair where as the early form is involved in DNA replication. We plan to examine the inducibility of the late appearing forms of DNA polymerase following exposure to UV and X-ray. It is assumed that if the late appearing forms are involved in DNA repair then, the level of these enzymes will be increased following exposure to UV or X-ray.

Neurospora DNA polymerases seem to have some properties similar to that of mammalian DNA polymerases particularly the presence of the mammalian β-polymerase like activity in Neurospora crassa which is resistant to SH-blocking reagents (El-Assouli 1979, El-Assouli and Mishra 1979).

The late appearing DNA polymerases are the major abundant forms of enzymes in Neurospora conidia. Besides the cytoplasmic form of DNA polymerases, a DNA polymerase characteristic of Mitochondria has also been characterized during present investigation by El-Assouli and Mishra (1979).

DNA polymerase after DEAE cellulose chromatography was compared from the wild type and mutant strains. The uvs-1 (sensitive to UV light) and rec-2 (recombination deficient) mutants were found to show similar profile of DNA polymerases as the wild types, the mutant and wild type DNA polymerases were comparable in their specific activity and heat lability and other physico-chemical properties. These data suggest that at least these two mutants examined did not show any defect in DNA polymerases. However a large number of other mutants remains to be examined to elucidate the possible role of DNA polymerases in Neurosproa DNA repair.

II. Sensitivity of Neurospora repair deficient mutants towards Histidine. (DeLange and Mishra)

Recently Newmeyer and others (1979) as well as DeLange (1979) have shown that UV sensitive mutants of Neurospora are also sensitive to Histidine (200-500 μg/(ml) added to growth medium. Thus sensitivity to a histidine seems to be an additional criterion of Neurospora DNA repair mutants. This idea is strengthened by our recent observation that nuclease deficient mutants of Neurospora (isolated in my laboratory as well as by Ishikawa et al) were found to grow equally well as the wildtype strain in the presence of histidine. Thus unlike the UV radiation sensitive mutants of Neurospora, the deoxyribonuclease deficient mutants are not resistant to histidine. These data may be interpreted to mean that the deoxyribonuclease mutants are deficient in DNase which is not involved in DNA repair. It is quite possible that these DNase mutants are defective for enzyme involved in DNA salvage pathways. Thus it seems that this method can be used to delineate the role of nucleases in DNA repair and DNA salvage pathways. This project is being continued by Dr. Delange and is discussed in detail later in this proposal.

III. Restriction endonuclease activity in Neurospora. (Mishra and Forsthoefel).

This year, we have been successful in detecting an EcoR1 like restriction endonuclease activity in a preparation of Neurospora membrane. The Neurospora enzyme makes an EcoR1 like cleavage in covalently closed circular DNA of plasmid pBR322. The Neurospora enzyme makes similar cleavage in SV40 and ØX174 DNA the plasmid (pBR322) DNA is routinely prepared in our laboratory where as SV40 and ØX174 was a gift from Drs. Daniel Nathans and Clyde Hutchison. The number of restriction endonucleases have been described from bacteria and its role in DNA recombination have been reviewed (see Roberts 1976) but this discovery by us is the first report of restriction endonuclease from Eukaryotes. This enzymes is being further characterized in my laboratory.

IV. Isolation and characterization of DNase mutants of Neurospora. (Forsthoefel).

Earlier Ishikawa et al. have reported the isolation of two DNase mutants of Neurospora called nuc-1 and nuc-2; these were selected by their inability to grow on medium containing DNA as the only phosphate source. A number of similar DNase mutants have been isolated in my laboratory. These map at two different sites on linkage group II and are non allelic to nuc-1 and nuc-2. All these DNase mutants were found to grow in presence of histidine added to the growth medium at concentration which inhibits the growth of Neurospora mutants sensitive to ultra violet. These data suggest that the DNase missing from the nuclease mutants of Neurospora is not involved in DNA repair. These DNase mutants are being compared with the wildtype strains for the profit of DNase obtained after DEAE-chromatography.