3011 A Unique Nuclear Protein Which Appears in Response to Lead Intoxication. K.R. Shelton and P.M. Egle,* Department of Biochemistry, Virginia Commonwealth University, Richmond, Virginia 23298.

A characteristic feature of lead intoxication is the appearance of inclusion bodies in the nuclei of kidney proximal tubular cells. It has been proposed that these inclusion bodies, which are enriched in lead, immobilize lead and thereby protect nuclear functions. In the present study a unique protein has been identified in nuclear inclusion bodies from rats. The protein has a molecular weight of 32,000 and an isoelectric point of 6.3. It has been further characterized by ^{125}I-tryptic peptide mapping. The protein has not been detected in liver or kidney from normal animals and is at least 200-fold more abundant in lead-treated rats. Identification of a nuclear inclusion body protein which is either sequestered with great selectivity or induced by the administration of lead increases the possibility that the inclusion bodies represent a protective response and, further, provides an opportunity for characterizing this response. (Supported by USPHS Grant # ES 02377).

3012 Extrachromosomal DNA of pea (Pisum sativum) root-tip replicates by strand displacement. D.B. Krimer* and J. Van't Hof, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

Extrachromosomal DNA isolated from pea-root meristems was analyzed by electron microscopy. Two different categories of extrachromosomal DNA molecules were observed: (i) double-stranded linear molecules, heterogeneous in size, with a modal length between 10-15 µm, and (ii) free single-stranded forms with a mean length value of 3.8 µm. 74% of the double-stranded molecules had single-stranded branches that were slightly shorter than the free single-stranded population. The characteristics of structure and size of these branched molecules indicate that they are replicative forms (RF) producing free single-stranded molecules (SF) by means of a strand displacement mechanism. The branches on the double-stranded molecules were shorter and more heterogeneous in length than free SF DNA since a portion of their length remained attached to the RF DNA and the length awaiting displacement would differ depending on how close the process was to completion. The SF molecules themselves, apparently, are not capable of further replication, since single-stranded replicative forms were not observed. Also, double-stranded DNA comparable in size to SF molecules were infrequent in the preparations. These data indicate that after being excised out of the chromosomes, the extrachromosomal DNA is capable of autonomous replication which occurs via a strand displacement mechanism, resulting in the release of SF molecules. If the displaced SF DNA were identical to that of its replacement on the duplex RF DNA, then a kind of amplification would result. On the other hand, if the displaced strands have modified bases while their replacement do not, the information on the duplex RF DNA could be altered. In either case, a change in genetic information would occur that may be useful during cell differentiation.

3013 Histone-like proteins and chromosomal structure in certain Archaebacteria. D.G. Searcy and G.R. Green, Zoology Department, University of Massachusetts, Amherst, Ma.

Small basic DNA-binding proteins have been described in many organisms, including eukaryotes and prokaryotes. However, differences exist between eukaryotic histones and bacterial proteins, such as the protein HU in E. coli. There is a second group of prokaryotes: the Archaebacteria. In several ways their chromosomal proteins are remarkably histone-like.

We have studied two thermophilic Archaebacteria. The first of these, Thermoplasma acidophilum, has a small basic protein (MW=9934) bound to its DNA. The protein can be dissociated by salt concentrations that are 10 times physiological. It protects the DNA from nuclease digestion, and with the DNA it forms nucleosome-like particles. Each particle consists of a core of 4 molecules of the protein plus a loop of 40 bp DNA. Within this small loop the duplex structure of the DNA is highly deformed. Nevertheless, the DNA is markedly stabilized against thermal denaturation. Fluorescence measurements suggest that tyrosinyl residues from the protein core are intercalated into the DNA during particle formation.

The second thermophilic Archaebacterium we have examined is Sulfolobus acidocaldarius. This organism has two abundant chromosomal proteins having molecular weights about 14,500 and 36,000. The proteins appear to exist in a 2:1 complex, which then binds to the DNA, protecting it against nuclease digestion and thermally stabilizing it.

When high-salt extracts of Sulfolobus were analyzed by chromatography on DNA-cellulose, a different protein bound most tightly (eluting about 1 M NaCl). It was not a chromosomal protein, and the two legitimate chromosomal proteins did not bind tightly, apparently because they failed to re-form their native complex. These observations have serious implications for several previous studies upon "histone-like" proteins, where often they have been identified entirely by their affinity for DNA-cellulose.

3014 DNA Methylation in the Indian Muntjac H. Vasilikaki - Baker* and Y. Nishioka, Department of Biology and Centre for Human Genetics, McGill University, Montreal, Canada.

We have studied methylation in the muntjac genome using anti-5-met-C antibody (a gift from Dr. B.F. Erlanger, Columbia University) and restriction endonucleases. Following denaturation of chromosomal DNA by photo-oxidation, the major antibody binding sites were visualized by immunofluorescence. Above background, the large metacentric autosomes showed a bright band of fluorescence at the tip as well as a less intense fluorescent band proximal to the first one approximately one third the distance from the centromere. In addition, a brightly fluorescing centromere was observed on the acrocentric chromosome whose homologue is attached to the X chromosome. Finally, a region of fluorescence was visible in the "neck" which fuses the X chromosome to the autosomal acrocentric. In order to estimate the extent of DNA methylation, total genomic muntjac DNA was cleaved with either HpaII or MSPI, and the resulting digestion patterns were compared to those of mouse DNA cleaved in the same manner. In the case of mouse DNA, a significant difference was observed between the two enzyme patterns generated, while very little difference was detected with muntjac DNA, suggesting that muntjac DNA does not contain many C*CGG sequences. An investigation is in progress to further characterize the muntjac genome.