479 The Presence of Origins of Replication in a Bovine Satellite DNA. R.H. Lussier*, J.E. Palardy* and L.H. Matsumoto, Department of Biology, Rhode Island College, Providence, RI.

Bovine satellite I DNA is a tandemly repeated DNA. A 1400 base pair clone of this repeat was used to demonstrate the presence of origins of replication in satellite DNA from cultured Madin-Darby bovine kidney cells. Since bovine cells initiate the replication of satellite I DNA at 1.5 hours into S-phase, we examined the DNA from synchronized cells 15 minutes before and after this time point for origins of replication. Cells were synchronized by isoleucine and glutamine deprivation followed by hydroxyurea arrest at the G1/S border. Cells were synchronously released into S phase by removal of hydroxyurea. Total DNA from cells at times around 1.5 hours into S phase were treated with S1 nuclease. Newly initiated regions of DNA replication, eye forms, contain single-stranded regions sensitive to S1 nuclease. The entire digest was sedimented in a 1.40 gm/ml CsCl-ethidium bromide gradient for 4 hours at 40 krpm. The end labeled (by polynucleotide kinase and γ-^{32}P-ATP) Hinf I fragments of pBR322 of approximately 75 to 400 bp do not enter the CsCl-ethidium bromide gradient. In parallel tubes, fragments generated from S1 nuclease treated DNA that did not enter the gradient were collected by centrifugation. These newly initiated fragments of DNA were denatured and covalently bound to DBM paper. Three subfragments of the cloned 1400 bp repeat were ^{32}P-end labeled and used as probes in a dot hybridization procedure. The results of the hybridization show that at 1.5 hours into S phase one probe hybridized extensively, a second hybridized moderately and a third had almost no hybridization. When these experiments were repeated with a different set of labeled subfragments, the results corroborated the initial observation that replication of bovine satellite I DNA is initiated in an origin of replication localized in a specific part of the tandem repeat. (Supported by NIH Grant GM31964).

480 Extrachromosomal ribosomal DNA molecules in pea roots produced by cells that differentiate from G2 phase. E. Kraszewska*, C.A. Bjerknes*, S.S. Lamm*, and J. Van't Hof, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

The root-tip meristem is a complex tissue composed of two general cell types: those that proliferate and those beginning to differentiate. The two types are not mutually exclusive because the progenitors of those that differentiate are the dividing cells. Differentiation occurs either before chromosomal DNA replication, when the cells are in the G1 phase, or after, when they are in the G2 phase. Autoradiography and cytophotometry of carbohydrate-starved cultured roots showed that certain cells during their final S phase replicated 70 - 80 % of their genome, stopped temporarily in late S phase, and while replicating the remaining 20 - 30 % of their DNA, produced extrachromosomal DNA molecules (exDNA) of replicon size (54 - 73 kb). Digestion of exDNA with the restriction enzyme BamH I produced fragments similar in size to those of mitochondrial but dissimilar to those of plastid DNA. Restriction enzymes inhibited by methylated nucleotide sequences (Hpa II and Msp I) ,however, digested organellar DNAs but failed to cleave exDNA indicating that exDNA was highly methylated and of nuclear origin. Finally, DNA/RNA hybridization experiments demonstrated that exDNA contained ribosomal genes. Cells that produce exDNA eventually differentiate to form epidermal and peri-stelar tissues and it is proposed that the extrachromosomal molecules are involved in the process of differentiation. These experiments are the first to show that certain cells of higher plants, like those of some lower eukaryotes, have extrachromosomal ribosomal DNA molecules.

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481 Amplification of Thymidylate Synthase Genes in FdUrd-Resistant Mouse Fibroblasts. C.-H. Jenh*, C. Rossana* and L. F. Johnson, Dept. of Biochemistry, The Ohio State University, Columbus, Ohio.

We have previously isolated a FdUrd-resistant mouse fibroblast cell line, LU3-7, that overproduces thymidylate synthase (TS) and the mRNA for the enzyme 50-fold as compared to the parental 3T6 cell line. We have also cloned cDNA corresponding to mouse TS mRNA into pBR322. In the present study, we have used the cloned cDNA as a hybridization probe in Southern blot analysis of DNA from the parental and overproducing cell lines. These analyses showed that the TS gene is amplified in LU3-7 cells about 50-100 fold as compared to the parental cells. The same size restriction fragments were detected by the probe in 3T6 and LU3-7 cells, suggesting that major rearrangements have not occurred in the vicinity of the TS gene during the amplification process. However not all of the restriction fragments were amplified, suggesting that there may be multiple genes or pseudogenes for the enzyme. Assuming that only a single structural gene was amplified, the size of the TS gene is at least 15 kb in length and contains at least 3 introns. When LU3-7 cells were grown in the absence of selective pressure, the level of TS overproduction and the number of copies of the TS gene decreased. Restriction site polymorphisms were detected when comparing TS restriction fragments in DNA isolated from various types of mouse cells. The mouse cDNA also cross-hybridized with hamster and human TS gene sequences. TS cDNA has been partially sequenced using the technique of Maxam and Gilbert. The nucleotide sequence and the predicted amino acid sequence show considerable homology to bacterial TS sequences in the region of the active site of the enzyme.

482 THYMIDINE STRESS AND NASCENT REPLICON MATURATION J.B. Schvartzman and D.B. Krimer*. Institute of Cell Biology (CSIC). Velázquez 144, Madrid-6, SPAIN.

In higher eukaryotes, the growing of nascent DNA molecules occurs in two clearly distinguished phases: before and after the achievement of replicon size. At the beginning, the growth rate of nascent molecules depends on the rate of synthesis and ligation of Okazaki pieces. Later on, as replicon size is achieved, this growth rate is a function of the timing of ligation of neighbouring replicons and replicon-clusters. The maturation of early, middle and late-S replicating replicons is known to occur synchronously during late S and G2. However, the reason for this delayed maturation of nascent replicons is not clearly understood. We have previously shown that in cultured cells of Pisum sativum synchronized by a protracted treatment with FdUrd, the rates of DNA synthesis, cell progression through S and nascent DNA maturation, are determined by the concentration of exogenously supplied thymidine (Thd). Our current objetive is to examine whether these effects are a consequence of Thd starvation, or the reflection of a natural occurring process. For this purpose, seedlings of Pisum sativum were continuously labeled with 100 uCi/ml tritiated Thd with specific activities ranging from 1 to 100 Ci/mmol, and the size of the nascent molecules were determined after 30, 60, 120 and 240 min using alkaline sucrose gradients. Alternatively, the seedlings were labeled with 100 uCi/ml tritiated Thd (spec. act. 100 Ci/mmol), and chased for 30, 90 and 210 min with different Thd concentrations. The results obtained showed that in asynchronously growing untreated cells, high concentrations of exogenously supplied Thd accelerated nascent replicon maturation without affecting the rate of DNA synthesis. This observation strengthens the hypothesis suggesting that the endogenous pool size of dTTP is one of the factors controlling the timing of nascent replicon maturation.