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Molecular cloning of cellular unintegrated forms of mouse mammary tumor virus (MMTV)

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Circular unintegrated MMTV DNA was extracted from the soluble nuclear fraction of MMTV-infected rat hepatoma cells HTC-M1. The linear 9–10 kb molecules produced by restriction digestion at the unique EcoRI site were isolated by preparative agarose gel electrophoresis and cloned in λgt*WES*. 7 independent clones were identified with MMTV cDNA (0.025% of total) which contained MMTV DNA of homogeneous length: about 10 kb (2) or 9 kb (5). All 5 9-kb clones had identical restriction maps, consistent with that of 9-kb unintegrated DNA in HTC-M1 cells, which contains 1 copy of the terminal repeat. The 2 10-kb clones had different maps, possibly due to rearrangements. In preliminary infectivity studies with cloned DNA, the transfected cells displayed new MMTV-containing bands in Southern blot analyses, possibly from new integrated MMTV sequences. Experiments to test their organization and possible expression are in progress.

Maternal effects of repair-deficient mutants on mutation fixation in *Drosophila melanogaster*

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The experiments are concerned with the role of different repair-deficient mutants of *Drosophila* in chemically induced mutagenesis in germ cells. Premutational lesions induced in mature sperm are processed only after insemination, by the repair systems present in the oocyte cytoplasm. Differences in repair capacity of the oocyte lead to maternal effects on mutation frequencies. We studied these effects of 2 repair mutants on sex-linked recessive lethals induced by monofunctional alkylating agents. The agents EMS, MMS, ENU, and MNU differ with respect to their reaction kinetics and site specificity in alkylating nucleic acids. With all compounds the mutation rates were lower when oocytes of the postreplication-repair-deficient mutant *(mei-41^{D5})* were used instead of wildtype ones. In contrast the mutation frequencies were increased when using excision-repair-deficient oocytes *(mei-9^{L1})*. Supported by Swiss National Science Foundation, project No. 3.156-0.77.

Cauliflower mosaic virus DNA passaged through bacteria remains infectious for plants

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The genome of cauliflower mosaic virus (CaMV) consists of a 8024 basepair long double-stranded DNA with 3 discontinuities[1,2], 2 in one and 1 in the other strand. The virus is being studied for 2 main reasons: a) The genomic organisation could serve as a model for the genomic organisation of plant DNA. b) The viral DNA might be developed into a suitable vector for genetic manipulation of plant DNA. Cloning of the DNA, infectivity assays of native and cloned DNA as well as in vitro mutagenesis studies are essential for the characterization of the viral DNA, its functions and its potential usefulness.

CaMV DNA was cloned in the *Sal*I site of bacteriophage λgt*WES* and in the *Sal*I, *Pst*I and *Bam*HI sites of bacterial vector pBR322. Infectivity of DNA of viral origin restricted with an enzyme cleaved once was compared to infectivity of cloned DNA restricted in the cloning sites. Both native and cloned DNA was infectious: In a typical experiment 9 out of 10 plants, each treated with 2 µg DNA, showed the typical mosaic and leaf wrinkling symptoms 20 days (in some cases as early as 10 days) after inoculation. Virus production occurred in all cases. Virus was isolated from the infected leaves and its DNA compared: linearized native and cloned DNA had given rise to particles containing circular DNA with all 3 discontinuities.

These experiments show that the infected plant cells possess all the enzymes necessary to ligate virus DNA and to produce the discontinuities. Thus the basis is established for experiments on in vitro mutagenesis of the virus and for its potential use as a vector for genetic engineering of plants.

1 T. Hohn, B. Hohn, A. Lesot and G. Lebeurier, Restriction map of original and cloned cauliflower mosaic virus DNA. Gene *11*, 1 (1980).

2 A. Franck, H. Guilley, G. Jonard, K. Richards and L. Hirth, Nucleotide sequence of cauliflower mosaic virus DNA. Cell, 1980, in press.

Evaluation of sister chromatid exchange in aplastic anemia

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The rate of sister chromatid exchanges (SCE) in chromosomes of cells that have been cultured for 2 replication cycles in the presence of bromodeoxyuridine (BUdR) is considered to be a sensitive indicator for lesions at the DNA-level. The formation of SCE has been postulated to be associated with the action of DNA repair enzymes, the exact biochemical mechanisms, however, are still unknown. In order to obtain insight into the etiology of idiopathic aplastic anemia (AA), the frequency of SCE was determined in lymphocytes of 7 juvenile patients with a severe form of AA, in lymphocytes of two children with transitory anemia as well as of 6 control individuals of the same age. The mean numbers of SCE per metaphase were 9.3 (range:

6.9–11.3) in the control group, 11.1 (range: 10.7–11.4) in the children with transitory anemia, but 15.3 (ranges: 11.1–21.84) in the patients with AA. The slight, but consistent (with 1 exception) excess of spontaneous SCE in AA indicates DNA alteration, possibly as a result of an abnormality of DNA repair as has been suggested on the grounds of other methods of approach (Morely et al., Lancet *2*, 9 (1978)).

The application of genetic methods to biotechnology

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Specifically blocked mutants were used to examine the reasons that *Pencillium chrysogenum* and *Cephalosporium acremonium* require different sulfur sources for optimal production of their respective β-lactam antibiotics. Penicillin producing strains of *P. chrysogenum* can obtain sulfur for antibiotic synthesis very efficiently by the reduction pathway from inorganic sulfate. In contrast, *C. acremonium* obtains it from methionine by reverse transsulfuration. It was found that the cleavage of cystathionine acts as an 'inducer' of the transfer of the cysteine moiety into the β-lactam antibiotics in both fungi. The formation of cystathionine from inorganic sulfide can occur by 2 alternative routes. The alternative pathway via homocysteine is the main route for optimal β-lactam synthesis in *P. chrysogenum.* This is not the case in *C. acremonium,* where direct anabolic synthesis of cysteine is operative. A specific block in the final step of the anabolic cysteine synthesis facilitates the conversion of sulfide to cystathionine by the remaining alternative pathway in *C. acremonium.* The respective mutants were prepared in *C. acremonium.* In contrast to the parent strain they could use either methionine or sulfate with equal efficiency. These mutants of *C. acremonium* resembled *P. chrysogenum* in their ability to utilize inorganic sulfate for antibiotic synthesis.

Oocyte types isolated from adult human ovary and their chromosomes

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Single oocytes were isolated by mechanical disruption of human ovarian tissue which had been removed during operations for various clinical reasons. 320 oocytes of the following types could be distinguished by direct observation in an inverted microscope and analized cytogenetically: 203 normal oocytes containing a nucleus in the dictyate stage; 26 already matured oocytes displaying metaphase-I and II-chromosomes; 2 vacuolized oocytes with scattered metaphase-II-chromosomes, 10 fragmented oocytes containing nuclear fragments of different sizes and finally 34 'ghost' oocytes in which no chromatin could be found by cytogenetic techniques.

As former investigations about degeneration in the juvenile mouse ovary (v. Weymarn et al., Anat. Embryol., 1980, in Press) indicated, a connexion seems to exist between the different degeneration types, resulting in a degeneration sequence: Thus, normal oocytes mature in situ up to metaphase-II, become vacuolized and then fragmented, while the atretic and 'ghost' oocytes can be direct degeneration products of normal and mature oocytes as well as of vacuolized and fragmented ones.