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THE REPLICATION OF PHAGE LAMBDA DNA

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The DNA extracted from a λ phage particle is a linear duplex with about 46,500 nucleotide pairs. The linear duplex molecule has " cohesive ends ", formed by mutually complementary sequences of twelve unpaired bases at the 5′ ends of each single strand.

On infection of a sensitive cell, the λ DNA rapidly forms hydrogen bonded circles, which are sealed to covalent circles by the host's DNA ligase. During the early part of the latent period λ DNA replicates in a monomeric circular form. This replication is semi-conservative, bidirectional, and occurs from a fixed origin. The initiation of λ DNA replication requires two phage-coded proteins, at least one host function, and transcription at the origin.

Later in infection the synthesis of closed circular molecules stops and a new, fast-sedimenting form of λ DNA appears. Mature linear monomers arise from the fast-sedimenting intermediate by the action of a phage-coded nuclease, the *ter* protein.

BIDIRECTIONAL REPLICATION OF THE *ESCHERICHIA COLI* CHROSOMOME

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The chromosome of *E. coli* is a single closed circular molecule of DNA which replicates sequentially from a fixed origin. Although early evidence was interpreted as showing that replication takes place in a single direction around this circle, more recent work shows that replication proceeds, in fact, in both directions. The first evidence, genetic in nature, was the analysis (by transduction and hybridisation) of the frequencies of genes at different positions on the chromosome. More recently, however, the early replicated DNA has been isolated and shown to be replicating bidirectionally both by biochemical analysis and direct autoradiographic visualisation.

THE ENZYMATIC BASIS OF CHROMOSOME REPLICATION IN BACTERIA

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There appear to be three distinct species of DNA polymerising enzymes in *E. coli* and in *B. subtilis*. The classical enzyme, DNA polymerase I, is probably dispensable, whereas one of the others, DNA polymerase III plays an essential role in replication in both organisms. All three species require a 3′-OH primer terminus and polymerise only in the 5′ to 3′ direction. These properties raise two important problems *vis à vis* semi-conservative DNA replication: (1) how are rounds of replication initiated on

closed circular double stranded DNA molecules? (2) how are both daughter strands synthesised concurrently at a replication fork?

Work in several laboratories indicates that RNA polymerase acts, at least in some cases, to produce primer termini for DNA strand initiation, and points to the existence of some other, at least partially distinct, mechanism of initiation. This latter mechanism appears also to be implicated at the replication fork in initiating the discontinuous " backwards " synthesis of the daughter DNA strand whose polarity runs 3′ to 5′ with respect to the overall direction of fork movement.

DNA REPLICATION IN EUKARYOTIC ORGANISMS

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In several mammalian, avian and amphibian cells it has been established that the replication of chromosomal DNA occurs in tandemly-arranged segments, and that within each segment replication proceeds bidirectionally from an initiation point or origin. The evidence for these conclusions will be examined.

Triturus contains about 10 times more DNA per nucleus than does *Xenopus*. When replication in somatic cells (tissue cultures) of these two organisms is compared, the initiation point intervals in *Triturus* prove to be a lot further apart than those of *Xenopus*, and the rate of progress of each replication fork in *Triturus* is between 2 and 3 times faster than the rate in *Xenopus*. The significance of these differences will be discussed.

Concentrating attention on *Triturus*, it will be shown that in this organism the length intervals between initiation points vary according to the duration of the S-phase of the cells whose chromosomes are replicating. In embryonic cells, with a short S-phase, the intervals are much shorter than those of somatic cells in culture, with a long S-phase; while in spermatocytes, with an extremely long S-phase, the intervals are much longer than those of somatic cells. The problem of how initiation points for replication become reduced in number, as meiosis approaches is examined.

CHROMOSOMAL PROTEINS ASSOCIATED WITH TRANSCRIPTION IN EUKARYOTIC ORGANISMS

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The problem of assignment of function to chromosomal proteins is very much in a state of flux at present. Data bearing on this problem have been obtained employing chromatin prepared from two sources—developing sea urchin embryos and newt oocytes.

It is possible to isolate from sea urchin embryos, a chromatin complex which retains stage specific template properties for RNA polymerase. During the period of development from blastula to pluteus a major shift in the pattern of transcription may be observed. Analysis of the chromatin proteins of the embryos has resolved 11 histones and some 30 non-histones. Changes occur in these protein populations as a function of embryonic development.

Germinal vesicle nuclei were isolated from newt oocytes by microdissection. Centrifugal fractionation of these nuclei gives rise to a nucleoplasm supernatant and a chromatin pellet which may be seen to consist of lampbrush chromosomes, nucleoli, nucleoplasmic granules and nuclear membranes. Disulphide bond crosslinks appear to contribute to the morphological integrity of isolated lampbrush chromosomes and nucleoli. The non-histone proteins from germinal vesicle chromatin exhibit an interesting level of simplicity: they consist predominantly of two major polypeptide

components. We may be close to assigning a function to one of these non-histone species.

A TWO-STRANDED MODEL OF CHROMOSOME ORGANISATION

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Current debate on the possible structure of chromosomes usually involves only two basic possibilities: that a chromosome is either single-stranded or multi-stranded. If more than one strand were to be present it is assumed that each strand would be genetically identical. I have recently proposed a third possibility: a two-stranded model of chromosome organisation in which the two strands are very different in both physical size and genetic content. A relatively short linear strand of contiguous main gene loci has an associated array of chromomeres containing the bulk of a chromosome's DNA. Chromomere DNA is joined by single-stranded DNA overlaps to produce a long molecule which is, in fact, a chromomere polymer. Chromomere DNA forms lampbrush loops. Its genetic role is debatable, one of several possibilities being that it contains multiple repeats of identical genetic information.

A POSSIBLE GENETIC APPROACH TO THE STRUCTURE OF EUKARYOTIC CHROMOSOMES

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Recent work, especially that of Judd and his colleagues, suggests that each band in the giant salivary chromosomes of *Drosophila* often corresponds to one complementation group. However, so far, not a single well-documented amino acid change in a protein has been shown for any mutant in this organism. A possible scheme for combining fine-scale genetic mapping with amino acid sequence data is proposed.

COLCHICINE RESISTANT MUTANTS IN *CHLAMYDOMONAS REINHARDI*

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As a part of a study of the genetic control of cell division, mutants which are resistant to various inhibitors of cell division are being isolated and characterised. For example, the isolation of colchicine resistant strains is quite easy in *Chlamydomonas* and it is hoped that this approach may provide a selective technique for obtaining mutants with abnormal spindle proteins. These mutations have pleiotropic effects on growth rate and cell form and also confer cross resistance to another anti-mitotic agent, vinblastine. It will be argued that the mutant phenotypes are consistent with a change in microtubular structures. This point is being examined more directly by a study of ^{3}H colchicine binding to cell extracts.

A TEMPERATURE SENSITIVE DNA POLYMERASE MUTANT OF *USTILAGO MAYDIS*

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Among several mutants of *U. maydis* blocked in DNA synthesis at the restrictive temperature of 32° (Unrau and Holliday, *Genet. Res. Camb.*, *15*, 157, 1970), one has been shown to be defective in DNA polymerase activity. Crude cell free extracts of this strain, designated *pol* 1–1, grown at the restrictive temperature contain 10–25%

of the DNA polymerase activity of those of the wild type. Analysis of two meiotic tetrads from a cross between *pol* 1–1 and a non-mutant strain has established that the temperature sensitive phenotype and reduced polymerase activity segregate together with the expected 2 : 2 ratio in each tetrad. Partial purification of the polymerase activity from *pol* 1–1 grown at the permissive temperature of 22° has been carried out. This activity is thermolabile whilst that in extracts from a wild type strain purified in a similar manner is stable. This provides strong evidence that the *pol* 1 gene is the structural gene for the enzyme.

Pol 1–1 grown in liquid culture at 32° forms filamentous cells which are mainly uninucleate, with only limited loss of viability. The strain is only slightly sensitive to ionising radiation, UV light and nitrosoguanidine at 22° in comparison to wild type. When cells are held at 32° for several hours before UV irradiation, they do not exhibit increased sensitivity. It is probably, therefore, not defective in a major repair pathway.

Further purification and characterisation of the altered DNA polymerase is in progress.

THE EFFECT OF CYCLOHEXIMIDE ON REPLICATION OF THE NUCLEAR GENOME IN THE SIMPLE EUKARYOTE *SACCHAROMYCES CEREVISIAE*

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It has been known for some years that cycloheximide (CHI) affects the synthesis of nuclear DNA in eukaryotes. Recently, detailed investigation in *Physarum* (Muldoon *et al.*, *Biochim. biophys. Acta*, *247*, 310, 1971) revealed that the genome in this highly evolved eukaryote is organised, operationally, in the form of a relatively small number of separate " replicons "; initiation of replication of each " replicon " is sensitive to CHI, whilst chain elongation, once started, is resistant to the drug.

Using cell autoradiography as well as bulk DNA analyses on synchronised and randomly dividing populations of *Saccharomyces cerevisiae*, we have found that in this relatively simple eukaryote, there is a cycloheximide transition point in the cell cycle which cocurs about 10 minutes (10% of the cycle) before initiation of DNA replication. Addition of CHI prior to this point prevents DNA replication entirely; after the transition point, replication of the genome is resistant to the presence of the drug and appears to go to completion. The implications for the structure and functional organisation of the yeast chromosome will be discussed.

A PREMEIOTIC STAGE CONTROLLING ZYGOTENE CHROMOSOME PAIRING IN *TRITICUM AESTIVUM*

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and

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Genotypes of wheat, *Triticum aestivum*, deficient for chromosome 5D, show a marked sensitivity of chiasma frequency to temperature. Careful examinations of meiotic prophase show reductions of chiasma frequency to result from failure of zygotene chromosome pairing.

Experiments involving temperature changes, followed by sequential measurement of chiasma frequency, have been used to locate the time of temperature sensitivity. Comparison of timings made in this way with independent timings of meiosis show the temperature sensitive stage to occur in the premeiotic interphase,

before DNA replication. The significance of this result for a general model of chromosome pairing will be briefly discussed.

RIBOSOMAL RNA GENES AND NUCLEOLAR ORGANISERS IN HEXAPLOID WHEAT

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In several eucaryotes, ribosomal RNA genes have been shown to be localised in the nucleolar organiser regions of the chromosomes (Birnstiel, M. L., Chipcase, M. and Speirs, J. *Progress in Nucleic Acid Research and Molecular Biology* (1971), *11*, 351-389). In hexaploid wheat at least four chromosomes carry nucleolar organisers (Crosby, A. R., *Am. J. Botany* (1957), *44*, 813-822). We have carried out hybridisation studies of ^{3}H labelled ribosomal RNA, isolated from wheat roots, to DNA's purified from leaves of the hexaploid wheat variety Chinese Spring, and also from related ditelosomic stocks each of which carries deletions for a different pair of nucleolar organisers. This has enabled us to estimate the proportion of ribosomal RNA genes at two of the nucleolar organiser sites. The site carrying the most ribosomal RNA genes is the most active in RNA synthesis. All the ribosomal RNA/DNA hybrids have similar melting profiles. The expected increases in the number of ribosomal RNA genes in plants tetrasomic for the nucleolar organiser chromosomes were not found. These and other results to be presented suggest that the number of ribosomal RNA genes at a particular nucleolar organiser site may not be invariant but may be under the control of a genetic regulatory mechanism.

EFFECTS OF MITOCHONDRIAL MODIFICATIONS ON CELLULAR TOXICITY OF CHLORIMIPRAMINE IN *SACCHAROMYCES CEREVISIAE*

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Chlorimipramine is an inhibitor of yeast grown both on fermentable and non-fermentable media but there is a marked differential sensitivity of most strains on the latter medium. The toxic effect on non-fermentable substrate is believed to be due to an effect on mitochondrial respiration. Mutants have been isolated which show resistance to the drug on one or other of these media.

Anaerobic growth and induction of the petite (ρ^-) condition cause an alteration in resistance levels of cells growing on fermentable medium, that is, affect the cellular response to the drug as distinct from the antimitochondrial effect. Petites derived from wild type, sensitive strains, in general show increased drug resistance and this is also true in the case of anaerobically grown cultures of wild type strains. Spontaneous, high level resistant mutants when converted to petite generally show a reduction in cellular tolerance to the drug. When these petite strains are crossed to wild type, restoration of the ρ factor usually does not restore the original cellular tolerance of the derived diploids. Mitochondrial DNA recombination may account for this finding.

Since the change to petite and anaerobic growth are known to result in an alteration in membrane components, it may be concluded that these alterations affect drug reactivity with membranes.

A MUTANT OF *ASPERGILLUS NIDULANS* LACKING NADP-LINKED GLUTAMATE DEHYDROGENASE

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Amongst mutants selected for loss of ammonium repression in *Saccharomyces cerevisiae*, a mutant in which this phenotype results pleiotropically from loss of NADP-linked (" anabolic ") glutamate dehydrogenase has recently been reported (Grenson and Hou, *Biochem. Biophys. Res. Commun.*, *48*, 749, 1972). We have directly selected a mutant (designated *gdhA-1*) lacking this enzyme in *Aspergillus nidulans* and found that it is also derepressed with respect to ammonium repression at least for the syntheses of nitrate reductase and xanthine dehydrogenase. It exhibits wild-type sensitivity to the toxic ammonium analogue, methylammonium (Arst and Cove, *J. Bacteriol.*, *98*, 1284, 1969). Inducibility of nitrate reductase and xanthine dehydrogenase is unaffected by the *gdhA-1* mutation as is sensitivity to molybdate toxicity (whereas mutations affecting control of nitrate reductase can lead to altered growth responses in molybdate (Arst, MacDonald and Cove, *Molec. Gen. Genetics*, *108*, 129, 1970)). Growth properties conferred by *gdhA-1* resemble approximately those of strains of *Neurospora crassa* mutant at the probably corresponding *am-1* locus (Fincham, *J. Biol. Chem.*, *182*, 61, 1950): a growth lag on ammonium and nitrogen sources metabolised via ammonium but nearly wild-type growth rate on nitrogen sources metabolised via glutamate. The *gdhA-1* mutation does not seem to affect levels of NAD-linked (" catabolic ") glutamate dehydrogenase although it eliminates, within the limits of detection, the NADP-linked enzyme.

NADP-GLUTAMIC DEHYDROGENASE AND AMMONIUM REGULATION IN *ASPERGILLUS NIDULANS*

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In *A. nidulans*, mutants deficient in NADP-glutamic dehydrogenase (NADP-GDH) activity have been isolated. These mutants grow poorly on nitrate, urea or ammonium but have normal growth on certain amino acids as nitrogen sources. The NADP-GDH mutants are derepressed for certain ammonium repressed uptake and enzymes systems. The NADP-GDH and four other classes of ammonium derepressed mutants will be compared with respect to their NADP-GDH activity, growth responses and map position in the genome. The possible role of NADP-GDH in ammonium regulation in *A. nidulans* will be briefly discussed.

THE AMMONIUM POOL AND AMMONIUM REGULATION IN *ASPERGILLUS NIDULANS*

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In *A. nidulans* the presence of ammonium in the culture medium results in the low activity of a number of uptake and enzyme systems. This is referred to as ammonium repression although the mechanism is unknown. A simple hypothesis concerning ammonium repression is that the concentration of the intracellular ammonium pool should determine the activity of the ammonium regulated systems. A high ammonium pool should result in low activity, *i.e.* repression, and *vice versa*.

To check this hypothesis cells of the wild type and five classes of ammonium derepressed mutants were held in media at various concentrations of urea and

ammonium. The intracellular ammonium pool and the associated levels of glutamate uptake and methylammonium uptake were measured. The results show that the level of glutamate uptake or of methylammonium uptake is not determined by the concentration of the intracellular ammonium pool. It appears that extracellular ammonium is the necessary and sole condition for ammonium repression in the wild type. This finding has significance for theories of ammonium regulation and possible roles for some classes of ammonium derepressed mutants.

THE GALACTOSIDE PERMEASES OF *KLEBSIELLA AEROGENES* STRAIN V9A

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Escherichia coli possesses three galactoside permeases, the lactose permease (Lac-P), melibiose permease (Mel-P) and the methyl-galactoside permease (MG-P), with considerable specificity for both induction and uptake. Thus lactose only induces, and is only accumulated by, Lac-P; Lac-P and Mel-P are induced by and accumulate melibiose; MG-P is induced by and takes up D-fucose, but not lactose or melibiose (B. Rotman, A. K. Ganesan & R. Guzman, *J. Molec. Biol.*, *36*, 247, 1968).

In *Klebsiella* V9A, Lac-P$^-$ mutants will grow on lactose after induction by melibiose, galactose, TMG (methylthio-β-D-galactopyranoside), IPTG (isopropyl-thio-β-D-galactopyranoside) or D-fucose. Lac-P$^-$ Mel-P$^-$ double mutants can grow on both lactose and melibiose after induction with TMG, IPTG or D-fucose, but cannot accumulate lactose in the presence of IPTG. Adding a third mutation prevents growth on lactose or melibiose after induction by any of these substances, but the triple mutant is still able to grow on galactose. From this and other evidence it is concluded that the *Klebsiella* strain has three galactoside permeases, Lac-P, Mel-P and MG-P, with the following properties: MG-P is induced by TMG, IPTG and D-fucose, and accumulates lactose and melibiose. Lac-P is not induced by, but does accumulate, melibiose. Mel-P is not induced by, but does accumulate, lactose. These properties are strikingly different from those of the corresponding permeases in *E. coli*.

THE *ACU-6* GENE AND PEP CARBOXYKINASE IN *NEUROSPORA CRASSA*

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Seven classes of acetate non-utilising (*acu*) mutants are known in *Neurospora crassa*. Mutants at the *acu6* locus are unable to derepress phosphoenolpyruvate (PEP) carboxykinase following transfer of mycelium from sucrose to acetate medium (Flavell & Fincham, *J. Bacteriol.*, *95*, 1056 and 1063, 1968).

An *acu-6* revertant, which shows temperature-sensitive growth on acetate but not sucrose medium, has been isolated. The temperature-sensitive property appears to map in the *acu-6* gene and strains carrying this mutation possess abnormally thermolabile PEP carboxykinase thus supporting the suggestion that this gene is the structural gene for PEP carboxykinase.

Some pairs of *acu-6* mutants exhibit interallelic-complementation. At least one of these strains possesses a PEP carboxykinase-like protein, as judged by polyacrylamide gel electrophoresis. Heterokaryons between complementing pairs of *acu-6* mutants contain very low levels of PEP carboxykinase activity (about 5% of the wild type level). This activity is abnormally thermolabile. It is suggested that interallelic-complementation at the *acu-6* locus is mediated by hybrid enzyme formation.

AUTOSOMAL LINKAGE ANALYSIS

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The results of an analysis for genetic linkage detection, involving 24 autosomal markers (ABO, Rh, MNSs, Lu, P, Ke, Fy, Jk, Diego, Se, Le, Gm, Inv, PTC, Hp, Tf, AK, 6PGD, PGM1, AP, Gc, El, E2 and Yeast factor) will be presented. The 900 two-generation families studied were ascertained from Morton's data (*Cold Spring Harb. Symp. quant. Biol., 29*, 69-79, 1964) on a north-eastern Brazilian population. The calculations were performed by a multi-allelic generalisation of the lod score method, considering sexual difference in the recombination fraction, given at values of 0·10 and 0·30. The computer analyses were based on the manipulation of W arrays or double vectors, whose description and use will be discussed. (J. H. Edwards, A marker algebra, *Clinical Genetics*, in press)

FISHER'S THEORY OF SELECTION FOR THE SEX-RATIO

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Bodmer and Edwards (*Ann. Human Genetics, 24*, 239, 1960) examined Fisher's theory mathematically. Using their model a re-analysis will be made including a demonstration of some convergence properties of the system. These convergence properties highlight the limitations of Fisher's theory, and indicate the necessity of considering additional factors. Some of the possible factors of importance will be indicated.

UNIVALENT SEX CHROMOSOMES AND MEIOTIC ARREST IN MAN

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A significant correlation has been found between a high frequency of cells with univalent sex chromosomes at diakinesis/metaphase I in human male meiosis and low numbers of cells in metaphase II. The results, obtained from meiotic studies in a series of men attending a subfertility clinic, indicate breakdown of maturation in germ cells with unpaired X and Y chromosomes.

GONADAL SIZES IN EMBRYOS OF SEX REVERSED MICE

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Gonadal volumes were measured in litters of mouse embryos, which were segregating for the *Sex reversed* (*Sxr*) factor (Cattanach *et al.*, *Cytogenetics, 10*, 318, 1971). It was found that in embryos aged 15 and 16 days, the volumes of testes were very much greater than those of ovaries. The mean testicular volumes of XX mice carrying the *Sxr* factor were only slightly below those of XY males. It is concluded that the *Sxr* factor increases the growth rate of the developing gonads, which differentiate into testes.

It is possible that the ultimate small size and lack of spermatogenesis of the testes in *Sxr*, XX adult mice may be foreshadowed by a gonadal growth rate in the embryo which is slightly below that of normal XY mice. A similar situation may occur in Klinefelter's syndrome in man.

THE "BACKCROSS" IN SOMATIC CELL GENETICS

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Since Mendel, the backcross technique is a most efficient tool in genetic analysis *via* sexual reproduction. Its application in genetic analysis *via* somatic cell hybridisation also promises to be fruitful. A progress report will be presented on somatic crosses of the following type: mouse fibroblasts 3T3 TK^- x human lymphocytes, isolate hybrid clones and from these isolate TK^- segregants, backcross the latter to human Lesch Nyhan fibroblasts ($HGPRT^-$) and isolate backcross clones. The clones arising from the backcross still show loss of human chromosomes. However, differently from the original cross, which gives almost exclusively clones with 2 S mouse, the chromosomes of the TK^- segregant are not found duplicated in the backcross hybrid clones. Clearly, the backcross technique will have wider applications at the intraspecific level for the assignment of recessives.