Cells

Were They Bacteria?

April 19, 1969

REPORTS of experiments supporting the notion that mitochondria may have evolved from endosymbiotic bacteria continue to appear. Last December, Smith and Marcker (*J. Mol. Biol.*, **38**, 241; 1968) described a particularly compelling experiment. N-formyl-methionine *t*RNA (*t*RNA$^{met}_{F}$), the *t*RNA required to initiate protein synthesis in bacteria, has not been identified in eukaryotes, except in algae such as *Euglena gracilis*, where it occurs in chloroplasts, and in yeast. But, contrary to expectation, there is less *t*RNA$^{met}_{F}$ in yeast cells than in bacteria; if this species of *t*RNA was required for the initiation of yeast cytoplasmic protein synthesis yeast cells should contain more of it than do the smaller *E. coli* cells. A possible explanation for this discrepancy was that the *t*RNA$^{met}_{F}$ in yeast was confined to the mitochondria, and Smith and Marcker convincingly showed that this is the case. Initiation of protein synthesis in yeast mitochondria is similar to that in bacteria; on the other hand, they found no trace of *t*RNA$^{met}_{F}$ in yeast cytoplasm.

Küntzel, working with another fungus, *Neurospora crassa* (*Nature*, **222**, 142; 1969), reports that the proteins from both the large and small subunits of *Neurospora* cytoplasmic and mitochondrial ribosomes are almost

totally unrelated, as judged by their behaviour on carboxymethyl cellulose columns.

But, in spite of this difference in the species of proteins in the two classes of ribosomes, it seems that the mitochondrial ribosome proteins are actually synthesized in the cytoplasm on cytoplasmic ribosomes. The evidence for this comes from experiments with inhibitors of bacterial and mitochondrial protein synthesis which have no inhibitory effect on cytoplasmic protein synthesis. These inhibitors do not stop the synthesis of the proteins characteristic of mitochondrial ribosomes, which must therefore be made in the cytoplasm and then migrate to the mitochondria either before or, less likely, after they have associated with mitochondrial ribosome RNA. This immediately raises the question of whether the genes for mitochondrial ribosome protein are in the nucleus or in the mitochondrial DNA. Estimates of the total amount of genetic information in mitochondrial DNA make it extremely unlikely, if not impossible, that mitochondrial DNA specifies the proteins of mitochondrial ribosomes. There just is not enough DNA to code the necessary information. The RNA component of mitochondrial ribosomes, on the other hand, is apparently specified by the mitochondrial DNA.

Küntzel has also recently characterized the subunits of the two classes of ribosome (*J. Mol. Biol.*, **40**, 315; 1969). The intact cytoplasmic and mitochondrial ribosomes sediment at 77*S* and 73*S* respectively. The large subunits can also be differentiated in the ultracentrifuge, where they sediment at 60*S* and 50*S* respectively. The small subunits, however, have the same sedimentation coefficient, 37*S*. But this is a coincidence; it does not, of course, mean that the two small subunits are structurally or functionally identical. In fact, as Küntzel has shown, they contain quite

different proteins and, as Reflein *et al.* reported in 1967, their RNA differs in sequence and base composition.

Two new species of RNA, apparently associated with mitochondria, have been detected in HeLa cells by Vesco and Penman (*Proc. US Nat. Acad. Sci.*, **62**, 218; 1969). The RNAs sediment at about 21*S* and 12*S* and have several unusual characteristics. They are unmethylated, have a slow rate of turnover and their synthesis is at least five times more resistant to ultraviolet light than the synthesis of nuclear RNA. The function of 21*S* and 12*S* RNA, which are not found associated with polysomes and therefore probably are not involved in protein synthesis, remains obscure.

The list of mitochondrial enzymes which are specified by nuclear rather than mitochondrial DNA also continues to grow. Longo and Scandalios (*Proc. US Nat. Acad. Sci.*, **62**, 104; 1969) have recently reported that genes for malic dehydrogenase isozymes of mitochondria of maize are inherited in a classical Mendelian fashion, which must mean that they are in the nucleus, not the cytoplasm.

Nucleic Acids and Interferon

June 7, 1969

THE discovery of a new type of double-stranded RNA (reported in this *Nature*) has two important implications. First, the new RNA may play a part in the induction of interferon production—interferon is the protein, discovered at the National Institute for Medical Research, Mill Hill, by the late Dr Alec Isaacs, which has the property of inhibiting the replication of a wide range of DNA and RNA viruses in animal cells. Second, it may mean that virologists will have to

modify their ideas about the way in which DNA viruses replicate in mammalian cells.

Viral infection can induce infected cells to produce interferon which is a defence against the virus. Both DNA and RNA viruses share this property, but all attempts to track down the active principle introduced by the virus have led to the conclusion that the inducer is double-stranded RNA. Neither single nor double-stranded DNA nor single-stranded RNA have much capacity for interferon induction. For the past two years, this result has puzzled virologists because, although DNA viruses induce interferon, there has been neither reason to assume nor evidence to suggest that DNA viruses manufacture double-stranded RNA during their replicative cycle.

Colby and Duesberg, however, now report that the DNA vaccinia virus does make a viral specific double-stranded RNA during growth in cultured chick cells. They infected chick cells with vaccinia and simultaneously fed either radioactively labelled uridine (to pick out newly synthesized RNA) or labelled thymidine (which indicated newly synthesized DNA) and then extracted and fractionated the nucleic acids of the infected cells. About 3 per cent of the RNA labelled by uridine turned out to be resistant to digestion by ribonucleases. This property is characteristic of double-stranded RNA. This interpretation is borne out by the chromatographic properties of this fraction, its resistance to digestion by deoxyribonucleases, its density as measured by density gradient centrifugation and by the fact that heating to 100° C, which destroys the double-stranded structure of DNA, makes the nucleic acid sensitive to RNase.

Hybridization experiments with vaccinia virus DNA prove that the double-stranded RNA is specified by the vaccinia genome, and it seemed that at low concentrations this RNA can induce the production of interferon and, moreover, that the ability to do so is lost when it is converted to single-stranded RNA by heating to 100° C. All these experiments indicate that a DNA virus can synthesize a double-stranded RNA (or at least an RNA which after extraction from the cells by a technique which removes all protein is double-stranded) which induces interferon. It is important to add this proviso because Colby and Duesberg's experiments do not prove that the RNA is double-stranded in the cell and work on RNA bacteriophages has shown that single-stranded RNA may be converted to a double-stranded form during extraction.

Saying that, of course, in no way minimizes the work of Colby and Duesberg, which seems to prove that DNA viruses induce interferon by way of an RNA molecule. It also raises an interesting question about DNA virus replication; if, as seems likely, it turns out that the RNA they have isolated is in fact double-stranded *in vivo*, what is its function in viral multiplication ? There is no obvious reason why replication of a DNA virus should involve the production of a double-stranded RNA. But another observation made by Colby and Duesberg, which confirms experiments published last year by Montagnier, is that even uninfected animal cells contain a small proportion of RNA which is double-stranded after extraction. All this suggests that double-stranded RNAs are more widely distributed than has previously been realized. So far, there is not much to suggest what they may do.

Hybrid Cell Genetics

July 12, 1969

EVER since the discovery of the technique of cell hybridization, the production at will of hybrid cells by inducing somatic cells of the same or different species to fuse in pairs has promised to be a most useful technique for mapping somatic chromosomes, for example, assigning a gene to a particular chromosome in the human set. Now Professor Henry Harris and two American colleagues, Professors E. Engel and B. J. McGee of Vanderbilt University, have shown that somatic cell hybrids undergo recombination and segregation which is the formal equivalent to the recombination and segregation that occurs at meiosis. This experiment (see page 152 of this issue) is something somatic geneticists have been waiting for; it should give added stimulus to the cytogeneticists who are trying to map human chromosomes using hybrid cells.

Professor Harris and his colleagues used two mutant strains of a line of mouse cells which have been maintained in tissue culture for thousands of generations. Each mutant strain suffers from an enzymatic defect, which means it cannot synthesize an enzyme, and is therefore dependent on an external supply of metabolites essential for growth. Hybrid cells produced by fusing these two mutant strains in pairs are, of course, capable of multiplying in the absence of an external supply of the two metabolites because each partner in the hybrid contributes the enzyme that the other lacks. But the hybrids with double the chromosome complements of the parental normal diploid cells are unstable. In particular, they tend to shed some of

their extra chromosomes when they are cultured for long periods.

The reason for this tendency of hybrids to segregate from the tetraploid hybrid chromosome complement towards the diploid complement of the parents is obscure. The most likely explanation seems to be that the diploid complement has evolved as the best adapted for any cell, and redundant genetic material which offers no selective advantage is therefore eliminated. But, whatever the reason, the process of segregation can be exploited for somatic cell genetics.

Engel, McGee and Harris, for example, have shown that when hybrids of the two mutant mouse cell strains are cultured for a year or more, the proportion of segregants with less than the tetraploid chromosome set of the initial hybrids increases and eventually segregant cells with chromosome complements virtually identical to one of the parental cells emerge. What is more, these segregants are able to grow without supplies of either of the metabolites required by the parental cells. In other words, the chromosome sets of the hybrids have not only segregated back to that of a parent but there has also been recombination between the two sets of chromosomes in the hybrid, eliminating the enzymatic defects carried on the chromosomes of the parents. It still remains to be seen precisely how this recombination takes place, but there are two obvious alternatives. Either the complete defective chromosome of one parental set is replaced by its normal counterpart from the other, or the recombination may involve some exchange between chromosomes of only that part of the DNA which specifies the particular defective enzyme.

This sort of experimental approach can easily be adapted for mapping human chromosomes by, for example, fusing defective human cells with normal human or animal cells and correlating the loss of a particular chromosome with the loss of a defect or, conversely, the retention of a particular chromosome with the persistence of a defect of metabolism. These alternative states can be selected for by either adding or omitting the metabolites concerned from the culture.

Things that go Bump in the Cell

August 16, 1969

THE notion has been current for some time that the polysomes attached to the endoplasmic reticulum of mammalian cells manufacture proteins for export, whereas those in the cytoplasm make only intracellular proteins. There is now some experimental support for this idea, and indeed a further example has just appeared (Takagi, Tanaka and Ogata, *J. Biochem.* (Tokyo), **65**, 651; 1969). These workers reported earlier that only the microsomes of rat liver could be shown to synthesize serum albumin in a cell-free system. They now show that this also holds *in vivo*. They administered a pulse-label of ^{14}C-amino-acids, and followed the appearance of radioactivity in the ribosomal fraction, released by treatment with EDTA. A steep maximum occurred at one to two minutes. Extraction of the nascent protein chains, followed by assay with an antiserum to rat albumin, showed that the growing albumin chains were confined to the microsomal fraction. (Related results, it may be noted, were described by Redman and by Hallinan *et al.*).

It is only the extracellular proteins that are subject

to extremes of environmental conditions, especially *p*H, and it is presumably for this reason that disulphide bonds occur in these, rather than intracellular enzymes Now the disulphide exchange enzyme, which catalyses the correct pairing of cysteines in an open polypeptide chain, seems to be present in all mammalian tissues, and resides in the endoplasmic reticulum. This is evidently where it can do most good, if it is here alone that the disulphide proteins, which are to be carried out of the cell, are synthesized. The relation between the activity of this enzyme and the concentration of polysomes in the membrane is the subject of an arresting paper by Williams and Rabin (*FEBS Letters*, **4**, 103; 1969). The disulphide exchange activity in the microsomes can be measured in terms of the regain of enzyme activity of oxidized ribonuclease. From microsome fractions of three tissues from the rat, it was established that the specific activity diminishes in each case in proportion to increasing polysome concentration. Williams and Rabin then examined the effect on this system of the steroid-like carcinogen, aflatoxin B_1, which is known to act at membranes, and also to inhibit protein synthesis.

It indeed caused the disulphide exchange activity to rise, and finally to approach a value corresponding to the availability of all the enzyme molecules. Moreover, a given aflatoxin treatment reduced the ribosome concentration in the membrane by precisely the extent expected to produce the observed activity. It is evident, therefore, that aflatoxin induces specifically the release of polysomes from the membrane. The same kind of stimulation of enzyme activity could be engendered by stripping away the ribosomes with EDTA. But whereas in this case the exposure of the enzyme could be reversed by adding new polysomes in a sufficient concentration of magnesium, the effect of aflatoxin was irreversible.

In view of the structure of aflatoxin, it is not surprising to find that corticosterone competes with it, and partly reverses its effect. Williams and Rabin conjecture that the attachment of polysomes to the membrane might be governed by the presence of the hormone at the polysome-binding, or some structurally related, site. Evidence for such a scheme was not lacking: when EDTA-treated microsomes were exposed to corticosterone before addition of polysomes and magnesium, the uptake of polysomes—and consequent suppression of disulphide exchange activity—greatly exceeded the normal level. This was not so when cortisol, which does not compete with aflatoxin, was substituted for corticosterone. Moreover, "smooth" membranes, which bear no polysomes in the cell and have little capacity to bind them, can be activated by corticosterone, so that they acquire a strong affinity for polysomes, with complete inhibition of the disulphide exchange enzyme. It seems then that the enzyme resides in the polysome binding site, which is operative only when the hormone is also present. One may hope ultimately for a bonus from this stylish piece of work, in terms of the nature of the carcinogenicity of aflatoxin.

Hybridization and Satellite DNA

January 31, 1970

SINCE 1961 the technique of nucleic acid hybridization has been progressively refined to increase its resolution and range of usefulness, but until recently it has always involved the use of extracted nucleic acids. In theory, at least, if hybridization could be performed with nucleic acids denatured *in situ* this would permit the intracellular localization of specific classes of DNA and

RNA. Last year, the rival groups of Gall, Birnstiel and Amaldi devised methods for partially realizing this potential. They all showed that isolated RNA can be made to hybridize with denatured intracellular RNA. Pardue and Gall (*Proc. US Nat. Acad. Sci.*, **64**, 600; 1969) have taken the technique one step further by hybridizing labelled DNA of *Xenopus* oocytes, from which ribosomal DNA had been removed, with denatured DNA of pachytene oocyte chromosomes and nuclei.

With the meiotic cells the labelled DNA was found bound to virtually all the chromosomes, but the experiments with the oocyte interphase nuclei prove the specificity of the hybridization. During oogenesis a cytologically distinct cap appears in the nuclei which contains the amplified ribosomal DNA, about 70 per cent of the total nuclear DNA. Labelled DNA, from which ribosomal DNA has been removed, fails to bind to the cap but binds to the rest of the chromatin. Three other lines of evidence indicate that hybridization is specific: first, cells treated with deoxyribonuclease fail to hybridize with the test DNA; second, alkali denaturation of the cell DNA is essential for binding test DNA; and third, the hybridization can discriminate DNAs of different sources. Mouse satellite DNA will not, for example, hybridize with *Xenopus* DNA, or that of much more closely related species, denatured *in situ*.

Apparently Pardue and Gall have used this new technique to solve a long standing question, the localization of the highly reiterated mouse satellite DNA fraction. The function of this satellite DNA has always been obscure, reducing investigators to suggest, for example, that it may be involved in chromosome "housekeeping", but Pardue and Gall claim that it is localized in the centromeres. It may

therefore play a role in chromosome pairing, and this may account for the curious properties of satellite DNA, not least its peculiar base sequence.

It is also clear from Smith's experiments (*J. Mol. Biol.*, **47**, 101; 1970) that chemical properties and intracellular localization are not the only distinguishing features of mouse satellite DNA. Infection of a confluent non-dividing culture of baby mouse cells with polyoma virus results in a cell population with synchronized DNA replication during which enough satellite DNA is replicated before replication of the rest of the DNA is really under way. In Smith's experiments, replication of satellite DNA was completed 11 to 17 hours after infection, while replication of the rest of the DNA began after about 16 hours. Moreover, autoradiographs showed that much of the early replicating DNA ended up in association with the nucleolus. Whether or not early replication of satellite DNA is a characteristic of polyoma infection rather than an inherent cell characteristic remains to be seen. If the first alternative proves to be the case it may have interesting implications for tumour virology, while if the second is correct why is centromeric DNA replicated before the rest of the chromosome?

Questions of Identity

April 4, 1970

THE nature of the signals which control the cell cycle and the onset of mitosis remains anyone's guess. It is not surprising that tissue culturalists attach great significance to the so-called serum factors—an apparently mixed bag of molecules in serum which have to be supplied to keep cultures of most cell lines alive and

multiplying. At present, however, ignorance of the precise chemical nature of these serum factors, which superficially at least have much in common with endocrine hormones, is more or less complete. And even when their chemistry has been deciphered the problem of how they function—how, for example, they induce the initiation of DNA synthesis or the structural changes at mitosis—may remain unanswered. Discovering the structure of the steroid hormones has not, after all, given many clues about how they act.

One possibility, of course, is that at least some of the control mechanisms regulating mitosis involve localized changes in the intracellular ionic environment. It has been suggested often that the condensation of chromosomes during prophase, and perhaps the polymerization of the mitotic spindle, is controlled in this way. Certainly manipulation of the tonicity and ionic composition of a cell's environment can induce several morphological transformations which closely mimic those occurring during mitosis. Robbins, Pederson and Klein (*J. Cell. Biol.*, **44**, 400; 1970), for example, have reported a detailed analysis of the changes brought about by exposing HeLa cells to hypertonic saline solutions. In 1·6x isotonic saline the HeLa chromosomes condense preferentially at the nuclear envelope and the nucleolus, the nuclear envelope becomes ruffled, the nucleolus loses its fibrillar component, polysomes break down and macromolecular synthesis is markedly impaired. All these changes are, of course, seen during mitotic prophase. In 2·8x isotonic saline the process goes a stage further with the nuclear envelope selectively dispersing. These morphological transformations are rapidly reversible and during recovery from osmotic shock the HeLa nucleus looks very similar to a telophase nucleus. Robbins *et al.* have not, however, been able to induce

the polymerization of a mitotic spindle, the lysosomes and Golgi complex do not go through the structural changes characteristic of mitosis, and the pattern of inhibition of macromolecular synthesis induced by hypertonicity is not the same as that during mitosis. There is therefore no question of being able to induce mitosis at will by altering the tonicity of a cell's environment, but the morphological similarities between mitotic cells and cells exposed to a hypertonic environment are most striking, even accepting the differences. It is therefore hard to resist the suggestion that localized ionic changes play at least some part in controlling mitosis and that experiments of the sort Robbins *et al.* report are more than an interesting gimmick.

The problems of coming to grips with the control mechanisms of the cell cycle remain, however, but cell biologists have Caspersson and his colleagues to thank for an important technical development (*Exp. Cell Res.*, **58**, 128, 141; 1970) which potentially makes possible the identification of individual interphase chromosomes and the differentiation of active and inactive chromatin. Caspersson's group reported that certain fluorescent acridine derivatives, in particular quinacrine mustard, bind specifically to the *M* chromosome of *V. faba*. In this issue of *Nature* Pearson *et al.* (page 78) and George (page 80) report adaptations of this technique for the identification of the human *Y* chromosome in interphase cells. The importance of a rapid method for determining the sex chromosome composition of human cells and distinguishing *XY* from *XYY* cells needs no emphasizing. No doubt this is the first of many exploitations and developments of the Caspersson group's discovery.

The Last Word Perhaps

May 2, 1970

LAST October a report by Dr Eugene Bell of Massachusetts Institute of Technology which was published and publicized in *Nature* (**224**, 326; 1969) caused something of a stir among cell and molecular biologists. Bell claimed he had identified a new class of DNA, informational DNA, residing in particles called I-somes in the cytoplasm of embryonic chick muscle cells. He went on to throw down his gauntlet by speculating that it is this informational DNA, not messenger RNA, which acts as the intermediary carrying genetic information from the nucleus to the cytoplasm. Bell envisaged that I-DNA was only transcribed and its mRNA translated after it had reached the cytoplasm, and his observation that I-DNA is associated in some way with polysomes seemed to support this idea. These speculations had obvious attractions; for one thing, no one has shown convincingly how mRNA migrates from the nucleus to the cytoplasm; none of the nuclear RNA fractions identified so far can be assigned with conviction as mRNA precursors; and the I-DNA theory could easily account for specific gene amplification. Furthermore, Bond *et al.* (*Science*, **165**, 705: 1969) had, a few weeks previously, reported the discovery of similar cytoplasmic DNA fractions in rat liver.

Bell's iconoclastic ideas would clearly not remain unchallenged for long; could his experiments be repeated or could evidence for I-DNA be found in other cell types? Fromson and Nemer (*Science*, **168**, 266; 1970) seem to have been first off the mark and their results, obtained with a different cell type, sea-urchin embryo cells, throw doubt on the whole I-DNA story. It looks as though I-DNA and I-somes are

artefacts resulting from damage to cell nuclei during the cell disruption procedures used by Bell. Cytoplasmic I-DNA is, they say, nothing more than contaminating nuclear DNA.

Fromson and Nemer's most telling piece of evidence is that the amount of I-DNA in cytoplasmic fractions depends on the cell disruption techniques used. When the cells are homogenized in a Dounce homogenizer the cytoplasmic fractions of sea-urchin embryo cells contain I-DNA and I-somes which behave as Bell described. But if the cells are disrupted by passage through a hypodermic needle, very little I-DNA is found, only 20 per cent of the amount after Dounce homogenization. Moreover, even in the preparations containing so-called I-DNA its association with polysomes is apparent rather than real. Some I-DNA co-sediments with polysomes, but rigorous buoyant density analysis shows that this DNA is not associated with the polysomes.

Bell described some I-DNA as being associated with RNA and protein in particles he named I-somes. Nemer and Fromson interpreted these as being spurious associations of fragmented nuclear DNA with the subribosomal particles containing RNA and protein which Spirin and his colleagues first described in 1964 and named "informosomes". Preparations obtained after disruption of cells by passage through hypodermic needles contain substantial yields of "informosomes" as well as polysomes and both are free of any DNA. It looks therefore as though the informational DNA theory, in spite of its attractions, has suffered a considerable reverse.

Seeking Integration Sites

July 4, 1970

ONE of the surest ways of raising doubts about the validity of an apparently important experiment is to lapse into complete silence after the first announcement. And inevitably the longer the silence the louder the sceptics can be heard. It is now two years since Weiss, Ephrussi and Scaletta (*Proc. US Nat. Acad. Sci.*, **59**, 1132; 1968) reported an ingenious but inconclusive attempt to answer two of the central questions of tumour virology. First, is the DNA of SV40 or polyoma virus integrated into the chromosomes of the host cells during transformation ? And second, is there just one specific integration site or are there several sites ?

Now, at last, Weiss (*Proc. US Nat. Acad. Sci.*, **66**, 79; 1970) has broken the two year silence. Her approach throughout has been to correlate the loss of human chromosomes from a hybrid cell, made by fusing an SV40 transformed human cell with a mouse cell, with the loss of the T antigen induced by SV40 virus. If one or more human chromosomes carry SV40 genomes the T antigen should be lost when those chromosomes are lost.

In 1968 Weiss and her two colleagues reported that the hybrid cells which had lost T antigen had invariably lost almost all the human chromosomes. This result is obviously compatible with the idea that the SV40 genome is integrated into several human chromosomes. But there are equally plausible alternative explanations. Loss of T antigen might, for example, reflect loss of capacity to synthesize it rather than loss of the SV40 genome itself. And if the chromosome containing a putative unique integration site for the virus conferred some selective advantage it might well be among the last human chromosomes to be shed from the hybrid.

Weiss's latest experiments seem to eliminate at least the first objection. A clone of cells with 5–7 human chromosomes but no T antigen was reinfected with SV40 virus and within two days 5–15 per cent of cells once again contained T antigen. Clearly these cells are capable of making T antigen if the SV40 viral genome is present. They had presumably lost the T antigen because they had lost the chromosomes carrying SV40 DNA.

It has once again proved impossible to determine precisely which chromosomes have SV40 integration sites. Weiss has shown, however, that in mixed populations of T antigen positive and negative cells the former have no obvious selective advantage. And the very complexity of the patterns of retention of human chromosomes in cells that contain or have lost T antigen makes it highly unlikely that all SV40 genomes are integrated in one chromosome. It is becoming obvious, however, that the cytological techniques Weiss exploits are unlikely ever to yield an unambiguous answer to this question. That will probably depend on the isolation of individual chromosomes and the detection of integrated viral genomes by chemical methods or autoradiography.

Although no one is precisely sure where they reside, complete SV40 virus genomes must be present in cells transformed by SV40 virus because wild type virus can be rescued from transformed cells by fusing them with permissive cells which allow the virus to replicate. Wever, Kit and Dubbs (*J. Virol.*, **5**, 578; 1970) have now determined the initial site of virus synthesis in such heterokaryons. Assaying for SV40 virus, Wever *et al.* found it first in the nucleus from the transformed cell and only later in the nucleus of the permissive cell. The first event in rescue appears therefore to be the activation of the SV40 genome in the transformed cell nucleus and not the transfer of SV40 DNA to the permissive cell nucleus.