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Molecular aspects of DNA repair

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Report of a workshop held at Noordwijkerhout (The Netherlands)
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Approximately, 120 participants convened recently at the Conference Center in Noordwijkerhout, The Netherlands, to discuss aspects of the molecular biology of DNA repair. The workshop comprised a series of selected review lectures, plenary talks and working poster sessions. The organization of the latter was novel in that it was specifically designed to constitute the focus of much of the informal discussion at the workshop. Three topically distinct groups of posters were open for simultaneous viewing. Poster viewing was followed by open discussions of posters, coordinated by designated chairpersons. Summaries of each discussion were subsequently presented to the full body of conferees in workshop summary sessions, which also included brief oral presentations of selected posters deemed to be of special interest to the audience at large.

The central themes around which much of the workshop was organized included molecular cloning and gene transfer of DNA-repair genes, the isolation and phenotypic characterization of DNA-repair mutants, the repair of well defined lesions in DNA, and the biological roles of repair genes and their products. A considerable emphasis was placed on molecular approaches to DNA repair in eukaryotes, particularly higher organisms. However, continuing progress and new developments in prokaryotic systems were well represented. The following paragraphs are organized much along these topical lines and represent capsule summaries of information presented in formal talks, informal discussions and posters. Our goal has not been to be totally comprehensive, but rather to present new areas of research and to emphasize the current state of the art of the molecular biology of selected DNA-repair mechanisms.

I. Mammalian DNA-repair mutants

There is an appropriately extensive and growing interest in the isolation and characterization of laboratory-derived and naturally occurring mammalian cell lines defective in cellular responses to DNA damage. The phenotypic characterization of such mutants readily provides much fundamental information, e.g., the spectrum of sensitivity to

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DNA-damaging agents. From such information alone clues can sometimes be gleaned as to the general category of repair defect in the mutant in question. However, it is clearly important that all mutants be well characterized genetically and that their phenotype be subjected to detailed biological and biochemical analysis whenever feasible.

In theory, the availability of a complete set of mutants should provide the genetic framework that could allow the ultimate identification of all catalytic, structural and regulatory components required for DNA repair in mammalian cells. But it is clear that we have a long way to go to achieve this goal. For example, the genetic complexity evident from the human disease xeroderma pigmentosum (XP) is not yet matched by the available characterized rodent mutant cell lines. Additionally, for reasons that are not clear, most eukaryotic mutants in nucleotide excision repair reflect defects in early postulated steps in this process (e.g., DNA incision). No mutants have been identified that are defective exclusively in later steps (e.g., repair synthesis).

(a) Mutants and their characterization

Zdzienicka, Tran and Simons (Leiden), presented evidence for the isolation of 13 new mutants from mutagenized (with ENU) V79 and CHO cells using a replica-plating technique. The frequency with which these mutants arose from V79 cells is substantially higher than that reported for CHO or L5178Y cells, suggesting the presence of many hemizygous loci in these cells. One of the mutants (VH1), has been studied in some detail [Zdzienicka, van der Schans, van Zeeland, Westerveld and Simons (Leiden, Rijswijk, Rotterdam)]. Complementation analysis indicates that VH1 belongs to complementation group 1 (based on the classification of Thompson). However, it shows significant differences from the CHO cell line UV5, a previously established member of this complementation group. UV5 shows essentially no unscheduled DNA synthesis (UDS) or DNA incision following exposure to UV radiation. However, VH1 is quite competent for UDS and can incise DNA, albeit at a slower rate, despite the fact that it is highly sensitive to killing by UV radiation.

Stefanini, Mondello, Tessera and Nuzzo (Pavia), described 3 new UV-sensitive CHO mutants. Two fall into genetic complementation group 2; the third complements the phenotype of the first 2, but does not complement representatives of any of the other CHO complementation groups (currently designated 1–6). This puzzling result is unexplained and led to a general discussion of problems associated with phenotypic complementation in somatic cell hybrids. The following salient points emerged from this discussion.

(1) Depending on the nature of the mutation, widely differing phenotypes may appear within a single genetic complementation group. This possibly reflects allele-specific differences in cell lines. However, in general, for different members of a given complementation group one can reasonably expect some common denominators in the phenotypic spectrum.

(2) Phenotypic complementation is frequently incomplete, i.e., function is rarely restored to wild-type levels.

(3) Complementation can be inconsistent, e.g., cell line A may complement the phenotype of line B, but not of B′, from the same complementation group as B.

(4) Some features of anomalous complementation may be explained if mutants carry and express multiple mutations in the same repair pathway. In cells in which hemizygosity is extensive this situation could occur with a reasonable frequency, despite the diploid nature of mammalian cells.

(5) The phenomenon of intragenic complementation can result in the erroneous assignment of more genetic loci than actually exist for a particular repair pathway.

The important question arose whether there is a need for further DNA damage-sensitive mammalian cell mutants, given the many cell lines already available. The consensus opinion was that based on the large number of genetic complementation groups in XP and the large number of mutant loci identified for excision repair in yeast, it is likely that in rodent cells there are other loci yet to be identified by mutational studies. Hence, it is important to continue a systematic search for new mutants defective or deficient in nucleotide excision repair. Should we concentrate on isolating new mutants from a standard cell line for ease

of genetic analysis? Probably not. Different rodent cell lines are likely to be hemizygous at different loci and thus their use will increase the probability of finding new mutants. Additionally, new mutants might be more efficient for gene transfer. The utility of isolating laboratory-derived human repair-defective mutants to determine whether the spectrum of loci identified parallels the XP complementation groups, was stressed.

Robson, Davies, Harris and Hickson (Newcastle-upon-Tyne), described 14 CHO mutants hypersensitive to a range of DNA-damaging agents and which apparently fall into 7 genetic complementation groups. Darroudi and Natarajan (Leiden), have characterized the X-ray-sensitive CHO mutants xrs5 and xrs6 (both belonging to the same genetic complementation group) in terms of chromosome aberrations and sister-chromatid exchanges (SCEs). They found a good correlation between the extent of defectiveness in the repair of double-strand breaks and the level of chromosomal aberrations. Ferro (Leiden), introduced X-irradiated *Drosophila melanogaster* sperm (which do not carry out DNA repair) into oocytes of repair-defective females and was able to examine the fate of DNA damage in germ line cells. In general, it appears that mutants defective in repair of damage in somatic cells show the same defects in the germ line.

(b) Mutants as recipients for gene transfer

In evaluating the suitability of mutant mammalian cell lines for gene transfer by DNA transfection, several factors must be assessed.

(1) The efficiency of DNA uptake, i.e., the absolute frequency of *transfection*.

(2) The frequency of *stable* integration of transfecting sequences.

(3) The maximum size of DNA fragments that can be stably integrated.

(4) The copy number of stably integrated sequences.

There were two reports on the apparent limitations of a number of human cell lines for gene transfer experiments. Green, Dean, Mayne and Lehmann (Sussex), have shown that when DNA bearing two different dominant selectable markers was used to transfect human cell lines, initial transfection occurred at a reasonable frequency. However, if selection was maintained for only one of the markers, the other was readily lost. A second study on transfection of human and hamster cells was presented by Westerveld, Odijk and Hoeijmakers (Rotterdam), who showed that while transfection occurred in all cell lines examined, much less DNA per cell was integrated in human, BHK or V79 cells, than in CHO cells. These difficulties with human cells notwithstanding, a cell line derived from XP complementation group D has been fused to HeLa cells and a derivative (HD2) has been isolated with enhanced efficiency for stable gene transfer (Arrand, Squires, Bone and Johnson, Cambridge).

(c) Human repair-defective diseases

A novel inherited human radiosensitivity syndrome (Nijmegen breakage syndrome) has recently been recognized and in many aspects resembles the disease ataxia telangiectasia (AT) (Jaspers, Baan and Bootsma, Rotterdam). The syndrome is characterized by chromosomal instability, immune deficiency and radio-resistant DNA synthesis. However, patients do not present with ataxia telangiectasia. Two genetic complementation groups distinct from those defined for AT have been identified.

Transformation of diploid cells from repair-deficient human diseases into immortalized cell lines is a crucial requirement for gene-transfer experiments. In an attempt to improve the efficiency of immortalization of diploid cells, Klein, Pastink and van der Eb (Leiden), co-transfected normal and XP fibroblasts with the SV40 early region, together with various oncogenes. The inclusion of certain oncogenes resulted in a lower transformation frequency than with SV40 DNA alone, whereas no significant increase in the frequency of immortalization was observed under these conditions. Transfection with SV40 *ori*$^-$ DNA resulted in immortalization of XP-C and XP-G lines. Mayne (Sussex) suggested that the plasmid pSV3gpt may be generally useful for immortalization of human cells. Permanent lines of AT and Cockayne's syndrome (CS) cells have been obtained using this plasmid.

(d) Transfer of microbial DNA-repair genes to mammalian repair-defective lines

Attempts have been made to correct various

TABLE 1

STRATEGIES FOR THE ISOLATION OF MAMMALIAN DNA-REPAIR GENES

1. Gene transfer

Cosmid or genomic DNA (homologous or heterologous) ± dominant marker (ligated or not) → First transfection → selection for: (1) dom. marker (2) rep. proficiency → rep. proficient primary transformant → DNA ± new dom. marker copies → second transfection → selection for: (1) linked or cotransf. dom. marker (2) rep. proficiency → rep. proficient secondary transformant → cloning in: cosmid library → screening for: → species-specific repeats or linked dom. marker → gene ← Isolation of transfected sequences → analysis by: (1) transfection to mutant (2) probing independent transformants

2. Chromosome transfer

Individual chromosomes (tagged with dom. marker) in hybrid panel → microcell fusion with mutant cells → selection for: (1) dom. marker (2) rep. proficiency → rep. proficient chrom. recipient → further micro-cell fusion → deletion mapping → identification of transferred chromosome → GENE CLONING ← (see 6)

3. cDNA cloning and transfection

mRNA (rep. proficient cell) → microneedle injection → mutant cells → transient phenotypic correction → enriched mRNA (size-fractionated) → cDNA synthesis and insertion in mammalian expression vector → transfection (stable transformation) → 'rescue' of correcting cDNA → subdivide cDNA pools, repeat → micro-injection using DNA, or RNA synthesized in vitro from pooled clones (transient correction)

TABLE 1 (continued)

4. Retroviral inactivation

retrovirus —infection→ wild-type cells (hemi- or heterozygous for gene of interest) —selection for repair deficiency→ mutant → cloning of retrovirus flanking sequences (part of disrupted gene) → clone repair-proficient gene from wild-type cell

5. Evolutionary walking

Cloning of mammalian repair genes based on nucleotide sequence homology with cloned repair genes of lower species (yeast) or based on amino-acid sequence homology using antibodies against repair proteins of lower species.

6. (Sub)Chromosomal localization — chromosomal walking

search for:
(a) LINKAGE with localized RFLP (restriction fragment length polymorphism) in families with repair deficiency syndrome or
(b) PATIENTS with cytologically detectable DELETION

Cloning of sequences closer to the gene by CHROMOSOME WALKING (using 'Jumping' or 'Linking' libraries).

(Search for patients with deletions or rearrangements detectable by molecular techniques).

Test genomic clones for presence of genes and for complementation of defect.

7. Protein → gene

Protein purification → Partial amino-acid sequence
↓
Synthesize corresponding oligonucleotide
↓
Screen genomic or cDNA library
or → Elicit antibodies
↓
Immunoscreen bacterial cDNA expression library (λgt 11)

human repair-defective cells by introducing repair genes cloned from other organisms. The XP-D line HD2 referred to above is partially complemented in terms of UV resistance, DNA incision and UDS by introduction of the T4 *denV* gene which encodes endonuclease V (Arrand et al., Cambridge). Introduction of the yeast *RAD1* and *RAD3* genes also has a slight effect on the ability of these cells to incise UV-irradiated DNA. Transfection of the *denV* gene into XP-A or CS cells had no effect on UV sensitivity (Mayne and Jones, Sussex).

Fox, Brennand and Margison (Manchester), transfected CHO cells with the *E. coli ada* gene in the mammalian expression vector pZIPneoSV. The *ada* gene was introduced in two forms; either encoding the alkylguanine transferase (ATase) function alone, or encoding both the ATase and the alkylphosphotriester transferase (APase) functions. Transfected cells selected for $G418^R$ were screened for alkyl transferase activity. Cells containing both APase and ATase functions were more protected from killing and mutagenesis by alkylating agents than cells containing only the ATase function. Similar experiments were carried out by Hall, Kataoka and Karran (ICRF). The *ada* gene conferred resistance to killing and mutagenesis by MNNG in CHO cells transfected with both the ATase and APase activities. The APase function alone did not confer protection. It seems that both transferase activities may be required for optimal protection against the effects of alkylation. However, interpretation of these experiments is complicated by the fact that CHO cells

are relatively resistant to killing by alkylation despite the absence of endogenous alkyltransferase activity.

II. Strategies for cloning mammalian DNA-repair genes

The experimental approach most frequently used for the molecular cloning of human DNA-repair genes involves correction of the repair defect in repair-deficient cells by transfection with genomic DNA, followed by recovery of the correcting DNA sequences. (Table 1). This strategy has met with spectacular success using UV-sensitive CHO mutants as recipients. Results using immortalized human cell lines that are UV or X-ray sensitive have been less satisfying. A number of presentations focused on this so-called classical approach to gene cloning. Mayne and Jones (Sussex), introduced normal human DNA ligated to pSV2neo into their immortalized CS cells. G418^R resistant clones were then treated with UV light and one was isolated that shows normal UV resistance and normal recovery of RNA synthesis following irradiation. However, these phenotypes are retained only if the transfectant is repeatedly exposed to UV radiation.

Diatloff-Zito, Papadopoulo and Moustacchi (Curie Institute, Paris), have used a multi-step protocol involving repeated transfections with UV-irradiated DNA in an attempt to complement diploid Fanconi's anemia cells. In the initial transfection a co-selectable marker was used. Transfectants were selected for the marker and then screened for resistance to mitomycin C. Mitomycin C^R transfectants have been isolated by this strategy and will presumably be used for secondary transfection in future experiments.

Successful transfection of human XP-A cells by DNA-mediated transfection with mouse DNA was reported by Tanaka (Osaka). Total genomic mouse DNA was cotransfected with pSV2gpt and cells resistant to mycophenolic acid (MPA) were identified. These were then screened for enhanced UV resistance. In initial experiments, 2 UV^R clones were identified from a total of 1.6×10^5 MPA^R colonies screened. These transformants were shown to contain mouse sequences. DNA from one of these cells was used together with pSV2gpt for secondary transfection. A single secondary transfectant was isolated following the screening of $\sim 3 \times 10^5$ colonies. This transfectant contained fewer mouse sequences and was used to construct a cosmid library. From this library a cosmid containing a non-repetitive mouse insert was identified. The cosmid hybridizes to identical fragments in both primary transfectants. However, it does not complement the UV sensitivity of XP-A cells. The cosmid is currently being used to identify other cosmids with flanking mouse sequences, in the hope of generating a cosmid which is functional for phenotypic complementation.

Similar experiments using human or mouse DNA have been carried out by Blum and Herrlich (Karlsruhe). No UV^R transfectants were identified following transfection with human DNA. The transfection of mouse DNA resulted in the isolation of a single UV^R colony. Secondary transfection with DNA from these cells has not been successful. A phage λ genomic library of the primary transformant has been constructed for use in further studies.

A number of presentations highlighted alternative strategies for the identification and possible cloning of human DNA-repair genes. Schultz, Saxon, Glover and Friedberg (Stanford and Ann Arbor) established a pool of human–mouse hybrids containing randomly tagged (with pSV2neo) human chromosomes from the aneuploid repair-proficient human cell line VA13. These chromosomes were introduced into XP-A and XP-F immortalized cells by microcell fusion and selected for UV^R. In this way XP cells containing different complementing single human chromosomes were isolated. The individual complementing chromosomes were identified and maintained in human–mouse hybrids bearing a single human chromosome. Passage of these hybrids in culture has facilitated the isolation of colonies with non-complementing chromosomal derivatives which should be useful for enrichment (by a subtractive strategy) for the region of the complementing chromosome containing the gene of interest.

Retroviral vectors provide a means for identification of genes of interest by insertional mutagenesis (Brown, Mee and Wilkie, Beatson Institute, Glasgow). The vectors (based on M-MuLV) integrate at random at many sites in host ge-

TABLE 2

DOMINANT MARKER SYSTEMS

Gene	Selecting agent	Range of concentrations required for different cell types	Remarks
XGPT or *gpt* (xanthine guanine phosphoribosyltransferase)	Mycophenolic acid	Narrow	Problem with cells showing metabolic cooperation
AGPT or *neo* (aminoglycoside 3′-phosphotransferase)	Neomycin, G418	Wide 40–1000 μg/ml	
Hygromycin phosphotransferase	Hygromycin B	Wide	
Histidinol dehydrogenase	Histidinol	Moderate	Probably also metabolic cooperation
DHFR (dihydrofolate reductase)	Methotrexate	Critical	Methotrexate resistance results from amplification of DHFR gene

nomes, thus providing the opportunity for inactivation of genes. Preliminary experiments have examined inactivation of the TK and HPRT genes in rodent cells. Results to date indicate integration of only a single virus per cell, stability of the integrated viral genome and a high frequency of mutant induction.

The amino acid sequence homology between the human *ERCC1* and the yeast *RAD10* genes raises the intriguing possibility of exploiting weak nucleotide sequence homologies by evolutionary walking. Koken, Yasui, Prakash, Hoeijmakers and Bootsma (Rotterdam, Rochester and Leiden) showed that both the *RAD10* and *ERCC1* genes hybridize to the same region of the Drosophila genome. The goal is to use Drosophila and possibly other lower eukaryotes as "intermediates" to walk from yeast to mammalian cells. Specific hybridization of *RAD1* to Drosophila DNA has been demonstrated.

Two presentations addressed the use of cDNA's to correct repair-defective cells with selectable

TABLE 3

VECTOR SYSTEMS FOR GENERATING STABLE TRANSFORMANTS

1. Integration vectors: Integration occurs by recombination.
2. Extrachromosomal vectors
   BPV (based on Bovine Papilloma virus), limited host range (only mouse and rat cell lines) reference: e.g. Law et al. (1983).
   EBNA (based on Epstein–Barr virus; stable; wide host range and high copy number, probably not so suitable for rodent cells) reference: e.g. Yates et al. (1985).

References (for Tables 2 and 3)

F.C. Garapin, F. Horodniceanu, P. Kourilsky and A.-C. Garapin (1981) A new dominant hybrid selective marker for higher eukaryotic cells, J. Mol. Biol., 150, 1–14.

M.-F. Law, J.C. Byrne and P.M. Howley (1983) A stable bovine papillomavirus hybrid plasmid that expresses a dominant selective trait, Mol. Cell. Biol., 3, 2110–2115.

R.C. Mulligan and P. Berg (1981) Selection for animal cells that express the *Escherichia coli* gene coding for xanthine–guanine phosphoribosyltransferase, Proc. Natl. Acad. Sci. (U.S.A.), 78, 2072–2076.

K. O'Hare, C. Benoist and R. Breathnach (1981) Transformation of mouse fibroblasts to methotrexate resistance by a recombinant plasmid expressing a prokaryotic dihydrofolate reductase, Proc. Natl. Acad. Sci. (U.S.A.), 78, 1527–1531.

J.L. Yates, N. Warren and B. Sugden (1985) Stable replication of plasmids derived from Epstein–Barr virus in various mammalian cells, Nature (London), 313, 812–815.

TABLE 4

POTENTIAL PITFALLS IN THE CLONING OF MAMMALIAN DNA REPAIR GENES, AND HOW TO AVOID THEM

1. *Suitability of recipient cell*
   (a) Is it really a mutant? (Hypermethylation and viral transformation, for example, may cause altered phenotypes).
   (b) Is the defect well characterised biochemically — and known to be in a repair pathway rather than in some ancillary process or in regulation?
   (c) Is there a reliable way of selecting resistant variants of the mutant?
   (d) Does it have a low reversion frequency?
   (e) Does it take up DNA at a reasonable frequency and amount per cell?
2. *Nature of DNA to be used for genomic transfection to the mutant*
   (a) If homologous DNA is used, it must first be "tagged" by random ligation with a marker sequence or gene to permit ultimate rescue.
   (b) If heterologous, check first that it can correct the defect of the recipient cell.
   (c) If the DNA is ligated in vitro to a marker, it will necessarily be digested, either with an infrequently cleaving enzyme such as *SalI* and *ClaI*, or with a more frequent cutter (e.g. *MboI*) to yield fragments with an average size of 100–40 kb. Inevitably this will disrupt a fraction of the copies of the gene of interest, depending on the gene size in relation to the average size of the digested DNA. The advantage is that a random distribution of marker gene copies over the transferred genomic DNA is ensured, increasing the chance that a dominant marker copy is situated close to the repair gene, facilitating linked secondary transformation and retrieval of the gene of interest.
   (d) If the DNA is prepared as random fragments, not ligated to the dominant marker, there is a greater chance of finding the complete gene. On the other hand the likelihood that a dominant marker copy will be located close to the repair gene is lower than in (c), because of the larger size of the genomic DNA and the fact that the cell is likely to concentrate most dominant marker copies in tandem head-tail arrays. This in turn reduces the chance of linked secondary transfection and cloning of the gene via a nearby dominant marker.
3. *Selection of repair-proficient transformants*
   (a) Reconstruction experiments comparing survival of wild-type and mutant cells under given selection conditions will help in design of the selection; however, in reality, transfectants may not behave like wild-type cells.
   (b) Choice of selecting agent; a physical selecting agent (e.g. UV light) has to be applied at intervals, rather than continuously; in the case of chemical agents, stability, uptake, metabolism and excretion are important considerations.
   (c) Selection regime; low doses of selecting agent over a long period are more likely to induce revertants than high doses over a short period; the latter is therefore preferable.
   (d) There is a choice as to the interval between transfection and starting the selection process. The optimum is established by trial and error!
4. *The possibility that a repair-proficient primary 'transformant' is in fact a revertant*

   The use of dominant marker genes considerably reduces the chance that a repair-proficient transformant is in fact a revertant, because the selection for uptake of the dominant marker gene reduces the number of cells transfected to the fraction that has integrated exogenous DNA (usually less than 1%).

   Two rigorous tests can be carried out to exclude the chance of dealing with revertants:

   (1) One can try to perform a linked secondary transfection (i.e. linked transfer of integrated dominant marker and repair gene using the DNA of a primary transformant). Because of the limited number of dominant marker gene copies (usually less than 200) in the genome of the primary transformant the number of secondary transformants containing a dominant marker gene is small. The chance of the secondary transformant also being a revertant is very low.

   (2) When heterologous genomic DNA is used for the primary transformation, one can perform secondary transfection adding new dominant marker copies to the DNA of the primary transformant. Subsequently, one should examine whether a repair-proficient secondary 'transformant' contains part of the heterologous DNA of the primary transformant. The presence of heterologous DNA in the secondary transformant by accident is extremely unlikely.
5. *The secondary transformants may contain no heterologous DNA detectable by Southern blots*
   (a) This does not necessarily mean that heterologous DNA is absent. The amount of DNA and/or the occurrence of species specific repeats in the heterologous DNA might be below the level of detection by Southern blot analysis. It may be possible to detect it in a library where the DNA fragment is relatively enriched.
   (b) If the transfected DNA was linked to the dominant marker, it will still be possible to retrieve it.

phenotypes for complementation. Belt, de Wit, Backendorf, Hoeijmakers, Bootsma and van de Putte (Rotterdam and Leiden) have size fractionated poly(A)$^+$ RNA and tested fractions for phenotypic complementation of XP-A cells following microinjection. A class of mRNA ~ 1.4 kb in size is active for complementation and has been used as a template for constructing cDNA. It is proposed to clone the cDNA into an EB viral-based expression vector which transfects human cells with high efficiency. Zoller, Angel, Herrlich and Rahmsdorf (Karlsruhe) are using a similar strategy with mRNA shown to complement XP-A and CS cells.

Table 1 presents a summary of various cloning strategies for the isolation of human DNA repair genes. Tables 2 and 3 list currently available vectors and dominant selectable markers for DNA transfection, and Table 4 is offered in the interests of sharing accumulated experiences about experimental pitfalls for potential investigators venturing into these treacherous waters.

III. Cloned DNA-repair genes

This section overlaps closely with that just presented and describes studies on repair genes which have recently been cloned, or which have been identified in primary or later generation transfectants and the isolation of which can be reasonably expected in the near future. Smulson, Bhatia, Giri, Pommier, Cherney, Dritschilo, Chen and Alkhatib (Georgetown and NIH) have cloned the gene for human poly(ADP-ribose) polymerase, implicated in the rejoining of DNA-strand breaks. Polyclonal antibodies to the polymerase were used to screen a λ expression library of cDNA from a human hepatoma. Using hybrid-selected and hybrid-arrested translation, a positive clone was shown to specify a 116 kDa polypeptide which reacts specifically with the polyclonal antibodies. The cDNA (1–1.5 kb) was used to screen a second library with larger inserts. A second insert of 3–4 kb was thus identified which, following transfection into cells, gave a pronounced increase in poly(ADP-ribose) polymerase activity and an enhancement in the rejoining of DNA strand breaks induced by γ rays. However, there is no enhanced resistance of these cells to γ-irradiation (Dritschilo, Cherney, Bhatia, Thraves, Giri, Alkhatib and Smulson).

S. Prakash (Rochester) described properties of some of the cloned *RAD* genes required for nucleotide excision repair in the yeast *S. cerevisiae*. There are indications that the products of the *RAD7* and *RAD23* genes overlap, since double mutants are more UV-sensitive than single mutants. The *RAD7* gene has a very hydrophilic 5′ end, deletion of which reduces the ability of the cloned gene to complement the UV-sensitivity of *rad7 Δ* mutants. However, this plasmid has no complementing activity in a *rad7 Δ rad23 Δ* double mutant. Mutagenesis of the cloned *RAD3* gene converted Lys^{48} to Met. This amino acid substitution results in a complete defect in the excision of pyrimidine dimers. However, when coupled with the *cdc9* DNA ligase *ts* mutation, the *rad3 cdc9* double mutant shows the presence of strand breaks at the restrictive temperature after UV-irradiation. These results suggest that the *RAD3* gene may be required both for incision and post-incision events during excision repair. Rad3 protein has been purified to near physical homogeneity from a yeast strain carrying an overexpression plasmid. The purified protein has a DNA-dependent ATPase activity which can be inhibited with antisera to Rad3 protein.

Thompson (Lawrence Livermore Laboratories), reported the molecular cloning of two human genes which complement CHO mutant cell lines. One of these genes (designated *ERCC2*), was isolated by DNA-mediated transfection of the mutant cell line UV5, a member of CHO genetic complementation group 1. A cosmid identified in a library constructed from secondary transfectants fully complements all the phenotypes of the UV5 mutant and has no effect on the phenotype of mutants from other genetic complementation groups. A second gene (designated *XRCC1*) which complements the cell line EM9 was isolated by a similar strategy. This gene (in a cosmid isolated from a library constructed from tertiary transfectants) complements defective strand break rejoining and increased levels of SCEs in the EM9 cell line.

van Duin, van den Tol, Odijk, de Wit, Westerveld, Koken and Hoeijmakers (Rotterdam), described further characterization of the human

DNA repair gene *ERCC1*. Using this gene as a probe against XP and Fanconi's anemia cell lines, no major deletions or rearrangements of the gene were detected in these cells. Transfection of XP cells (exclusive of complementation groups B and I) has not revealed phenotypic complementation. Low constitutive transcription of the *ERCC1* gene is observed in a variety of cells and tissues and Ercc1 protein is not detected following overexpression in *E. coli* or CHO cells. However, expression of an *ERCC1–lacZ* fusion protein is observed and the fusion protein will be used to raise antisera against Ercc1 protein.

The UV-sensitive CHO cell lines 27-1 (complementation group 3) and UV-61 (complementation group 6) are the subjects of attempts by Weeda, van Ham, Westerveld, de Wit, Odijk, Hoeijmakers and van der Eb (Rotterdam and Leiden), to clone further human *ERCC* genes. Genomic DNA from HeLa cells was co-transfected with pSV3gpt into these cell lines. With UV-61 a number of primary and one secondary UV^R transfectants have been isolated, but no human sequences have been detected in the latter. Work with the 27-1 cell line has progressed further. DNA from a UV^R secondary transfectant has been used to construct a cosmid library. However, thus far, none of the single cosmids which contain human and plasmid sequences complement the phenotype of 27-1 cells.

A parallel study by MacInnes, Chen, Tesmer, Reynolds, Okinaka, Nickols and Strniste (Los Alamos), addressed the complementation of UV 135 cells (CHO genetic complementation group 5) and xrs6 cells (X-ray-sensitive CHO genetic complementation group 2). The latter cells were transfected with human genomic DNA ligated to pSV2gpt, selected for gpt^+ and screened for X-ray resistance. Primary transfectants containing relatively little human DNA have been used to generate a cosmid library. A cosmid library has also been constructed from a tertiary UV-135 transfectant. As yet, no single cosmid complements either cell line.

As indicated previously, the cell line HD2, derived from a fusion of XP-D and HeLa cells, has proven to be a particularly good recipient for transfection (Arrand et al., Cambridge). Following transfection of HD2 with hamster DNA ligated to a cosmid arm containing the *neo* gene, $G418^R$ UV^R colonies were isolated. Cosmid rescue of the *neo* gene and flanking sequences and their evaluation for complementation is in progress.

Another cell line which can be stably transfected at high efficiency is the SV40-transformed Muntjac line SVM. This cell line is UV- and MNU-sensitive and shows evidence suggestive of a defect in post-replication repair. Excision repair is normal, but there is a marked increase in the level of SCEs induced by UV or MNU. SVM cells were cotransfected with mouse genomic DNA and pSV2neo. One transfectant shows increased resistance to UV and MNU, improved levels of maturation of high molecular weight DNA on a damaged template, but no correction of the increased SCEs. Similar features were observed in a secondary transfectant. These observations raise the possibility that there are two mutations in the SVM line, only one of which is corrected by transfection. Alternatively, the possibility was considered that the presence of the SV40 genome may exert epigenetic effects which partly mimic the phenotype of these cells.

A defect in post-replication repair is also observed in the CHO line UV-1 which is sensitive to UV, MNNG, MMS and mitomycin C (Hentosh, Neft and Waldren, Eleanor Roosevelt Institute, Denver). Transfection with human DNA restored resistance to all of these agents except mitomycin C. A cosmid library has been constructed from one of the transfectants.

A common feature that characterizes many of the transfection experiments reported is the observation of partial correction of a particular phenotype, and/or correction of some, but not all the phenotypes of the recipient mutant cell line. It is a moot point whether these results are to be interpreted as a sign of failure to identify the appropriate gene, or as a clue to the intricacies of regulation and expression of genetically and biochemically complex repair systems.

Further evidence of possible complex regulatory influences following DNA transfection of cells comes from studies by Kaina, van Zeeland and Thielmann (Heidelberg and Leiden). A CHO cell line devoid of detectable alkyl transferase activity was used as a recipient for transfection. These investigators linked human genomic DNA to a *gpt*-containing vector which was alkylated in

vitro, in order to facilitate selection for sequences which repaired the alkylation damage and hence restored the integrity of the *gpt* marker. Primary and secondary transfections produced cells with elevated resistance to alkylation, but the resistant cells contained no detectable transferase activity. Alkylation resistance correlated with reduced tumorigenicity, increased serum dependence and reduced growth rate in the transfected cells. These features may be aspects of some common regulatory process which is affected by the transfection.

The *E. coli* formamide pyrimidine glycosylase (*fpg*) gene which encodes the FaPy-DNA glycosylase has been cloned by Boiteux, O'Conner and Laval (Villejuif). Since there are no known mutants for this gene, they selected transformants containing multiple copies of the gene, as evidenced by overexpression of the enzyme in crude extracts. A subclone containing a 1.8 kb insert was isolated and shown to encode FaPy-DNA glycosylase. Characterization of the cloned gene is in progress.

IV. Analysis of mutations in mammalian cells

Current technology permits mutations in mammalian cells to be analyzed in at least 4 different types of systems: (a) Endogenous mammalian genes (e.g. *hprt, aprt*). (b) Bacterial genes integrated into the mammalian genome. (c) Genes in extrachromosomally replicating shuttle vectors. (d) Mammalian viral genes.

1. Effects of DNA methylation

Using the *tk* gene integrated into human cells, Giphart-Gassler, den Dulk, Vloemans and Tasseron (Leiden), found that in all tk^- derivatives of their cell lines, the tk^- phenotype could be attributed to methylation. The methylation sites were restricted to the promoter sequence in tk^- derivatives of one line, but were more extensive in derivatives of a second line. Gebara, Harcourt, Steingrimsdotter, Arlett and Lehmann (Sussex) carried out similar studies with the *gpt* gene integrated into the genome of human fibroblasts. The gpt^- phenotype can result from a wide variety of alterations including deletions, or, in many cases, methylation. This raised the general question as to whether mutations in endogenous mammalian genes could also in some cases be attributed to gene inactivation by methylation.

Glickman, Grosovsky and Drobetsky (Toronto) reported that 200 spontaneous and induced mutations in the hamster *aprt* gene could be traced to direct alterations in the base sequence of the *aprt* gene. Vrieling, Simons and van Zeeland (Leiden) and Thacker (Oxford) have both attempted to reactivate hamster $hprt^-$ mutants with 5-azacytidine (an analogue which results in hypomethylation of cytosine in DNA). No restoration of the $hprt^+$ phenotype was obtained. There is thus no evidence that mutations in the *aprt* or *hprt* genes result from inactivation by methylation. Nonetheless, the possibility remains that such a mechanism may occur in genes which are normally subject to methylation control.

2. New systems for mutational analysis

Several new systems were described for the analysis of mutations. Vrieling, Simons and Van Zeeland (Leiden) have used amplification in vitro as a method for sequencing mutations in the *hprt* gene. Starting with 1 μg of poly A^+ RNA, three overlapping fragments of the *hprt* coding region were amplified using reverse transcriptase followed by the DNA polymerase chain reaction, which were then cloned and sequenced. Pastink, Vreeken, Schalet and Eeken (Leiden) studied the 40-kb *white* locus of Drosophila and were able to locate small deletions or insertions in the gene by heteroduplex mapping. The regions around the deletions could then be sequenced. Gentil, Teoule and Sarasin (Villejuif and Grenoble) described a system whereby oligonucleotides containing specific mismatches could be inserted into the intron of the large T antigen gene of SV40. Certain alterations of these mismatches generate a new *Bgl*II site in the SV40 DNA and the sensitivity or resistance to this restriction enzyme was used to screen and select mutants without any selective pressure on the viral cycle.

3. Results of mutational analyses

The analysis by Glickman et al. involving cloning and sequencing of 34 UV-induced mutations in the *aprt* gene generated results similar to those obtained with microbial systems and with mammalian shuttle vectors. Nearly all the mutations

were targeted to dipyrimidine sites, and 50% were G · C → A · T transitions which probably resulted from pyrimidine(6–4) photoproducts. Several transversions and double mutations were also found.

None of 29 ENU-induced *hprt* mutations in hamster cells (Vrieling et al.) contained any detectable deletions or rearrangement, although *hprt* mRNA levels were reduced or absent in 30% of the mutants.

X-Ray-induced mutations at the *white* locus of Drosophila (Pastink et al.) could be identified mainly as large deletions or rearrangements, but in 4 cases where gross alterations could not be seen, small deletions were identified by heteroduplex mapping and in each case were in regions flanked by short repeats, one copy of which was preserved in the deletion mutants.

In the SV40 intron system (Gentil, Teoule and Sarasin) G.U. mismatches were introduced at various sites within the potential *Bgl*II cutting sites. The results showed that G · U could be converted into a G · C base-pair or into a T · A base-pair, but not into A · T or C · G base-pairs. The mechanism(s) of these conversions are unclear.

V. Use of plasmids to study repair in mammalian cells

The use of plasmids to study DNA repair in mammalian cells has advantages and shortcomings. Plasmids present a clearly defined system in which the results of damage introduced into a specific gene can be analyzed. Sometimes, however, the results do not reflect the situation occurring in chromosomal DNA. Both aspects were exemplified by Knox and Roberts (Surrey). On the one hand, pSV2gpt is inactivated much more readily by difunctional than by monofunctional agents. On the other hand, the inactivation dose-response of the plasmid after treatment with cisplatin was the same following transfection into two isogenic cell lines which differed widely in terms of sensitivity to cisplatin. It is likely that if the mechanism of toxicity of DNA-damaging agents is mediated by the production of a rare highly toxic lesion (such as a cross-link), this mechanism will not be applicable to plasmid inactivation because of the small size of the DNA target.

Debenham, Jones and Thacker (Oxford) described a system for measuring the fidelity of rejoining of breaks produced in plasmid DNA by digestion with various restriction enzymes. An ataxia telangiectasia (AT) cell line appears to be deficient in accurate rejoining of these breaks, as does one of three different X-ray sensitive V79 hamster mutants.

Plasmids can also be used to study recombination in mammalian cells following transfection. This recombination occurs at very high frequency (~ 10%) and its implications are not clear. An improved system for measuring recombination following integration generates much more acceptable frequencies (10^{-3}–10^{-7}) (Lydall and Roberts, Surrey). This frequency was not increased by treatment of cells with cisplatin.

Kraemer (NIH) reviewed work from his laboratory in which shuttle vectors were used to study either repair or mutagenesis. Plasmids containing the *cat* gene express chloramphenicol acetyltransferase (CAT) when transfected into mammalian cells. Prior UV-irradiation of the plasmid inactivates CAT expression. This inactivation occurs at 10–15-fold lower UV fluences if the plasmid is transfected into XP-A or XP-D cells as compared to normal cells. If the UV-irradiated plasmid is first pretreated with photoreactivating enzyme to remove pyrimidine dimers but leave other photoproducts intact, the D_0 of the plasmid when transfected into XP cells is increased ~ 3-fold, suggesting that pyrimidine dimers are responsible for a part but not all of the inactivation.

The plasmid pZ189 replicates rapidly in human cells and contains the *supF* gene which can be used as a target for mutagenesis studies. Following replication, the plasmid can be rapidly recovered from human cells and used to transform suitable host bacteria in which mutations in the *supF* gene can be easily identified and sequenced. The majority of UV-induced mutations following transfection into either normal or XP cells were transitions, principally G · C → A · T. All types of transversions were found in normal cells, but to a much lower extent in XP cells. There were some marked hotspots. Treatment with photoreactivating en-

zyme removed 90% of the mutations produced in XP cells, but the hotspots persisted even after photoreactivation. Mutations are therefore thought to occur principally, but not exclusively at TC and CC pyrimidine sites. However, there was no correlation between the frequency of mutation at any particular site and the frequency with which lesions were produced at these sites.

VI. Detection of repair and consequences of defined lesions in DNA

1. Preferential repair of active genes

Smith (Stanford) reviewed evidence from the Hanawalt group (chiefly Bohr, Madhani, Mellon and Okumoto) indicating that pyrimidine dimers are preferentially removed from transcriptionally active genes. Rodent cells are relatively inefficient in carrying out excision repair of pyrimidine dimers, even though rodent and human cells are equally tolerant of the toxic effects of UV. Effective repair of the DHFR and HMB-CoA reductase genes has been demonstrated in CHO cells. It seems to be most efficient in the 5′ end of the DHFR gene and is reduced in the flanking sequences. Similar differences in repair between the active *c-abl* and inactive *c-mos* genes have been reported for mouse cells. In human cells overall excision repair is much more efficient than in rodent cells, but occurs more rapidly in amplified DHFR genes. Mullenders reported confirmation of this more rapid repair in the adenosine deaminase gene. CS cells show normal levels of excision repair from bulk DNA but fail to effect recovery of RNA synthesis following UV-irradiation. Preliminary data obtained by Mellon (presented by Smith) and by Mayne and Mullenders suggest that CS cells may be specifically deficient in this rapid excision repair of active genes, as postulated several years ago by Mayne and Lehmann.

Mullenders also presented indirect evidence for preferential repair in active genes. Active genes are thought to be located at attachment sites of DNA to the nuclear matrix, and repair synthesis at early times following UV-irradiation was shown to be preferentially located at these attachment sites. Data suggesting repair in matrix-associated DNA in XP-C cells (Mullenders) seems to conflict with the lack of repair of the DHFR gene in XP-C cells published by Bohr et al. Since in XP-C cells only part of the matrix-associated DNA is efficiently repaired, a likely explanation could be that the *dhfr* gene resides in that part of the matrix-associated DNA that lacks efficient repair in XP-C cells.

2. Use of defined lesions in DNA

Essigmann (MIT) described work in which a particular type of lesion was synthesized chemically and then inserted at a single precise location in a plasmid. The plasmid could then be transfected into bacterial or mammalian recipients and the biological consequences examined. Insertion of an O^6-methylguanine residue into single sites in plasmid molecules produced exclusively $G \cdot C \rightarrow A \cdot T$ transitions at the site of the lesion in both bacterial and mammalian cells. The mutation frequency was greatly enhanced in recipient cells which did not contain an active O^6-methylguanine DNA-methyltransferase. The system also revealed interesting strand-specific biases in the production of mutations.

Sage, Boyer and Moustacchi (Paris) determined the sites of formation of photoadducts of various psoralen derivatives reacted with purified *lacI* DNA fragments, using exonuclease blockage and sequencing techniques. A number of derivatives, including 8-MOP and 5-MOP exhibited preference for TpA sequences.

Vos, Islas and Hanawalt (Stanford) compared introduction and removal of psoralen interstrand crosslinks and crosslinkable monoadducts in different genes in human cells. DNA restriction fragments were subjected to Southern analysis after denaturation–renaturation to determine crosslinking frequency. Similarly treated randomly sheared DNA was separated by centrifugation in CsCl gradients at pH 10.8, slot-blotted and probed for various sequences. Crosslinkable monoadducts were determined from changes in the crosslink frequency brought about by further UVA irradiation of the DNA just prior to this analysis. The first method showed that removal of crosslinks from the *dhfr* gene was more extensive than removal of the monoadducts; however, the rates were significantly less than those for pyrimidine dimers. Removal of monoadducts was less effi-

cient from the non-transcribed ribosomal spacer DNA. The second method showed that removal of monoadducts and DNA crosslinks was in the order DHFR > bulk > ribosomal spacer.

Dosanjh and Essigmann (MIT) studied the distribution of aflatoxin adducts in DNA by examining the lengths of the products of a specific cleavage reaction. With pure DNA, all guanine residues were sites of addition, with GpGpG and GpC having higher than average frequencies of adduction. However, when rat liver was perfused with aflatoxin B_1, it appeared that nearly all the liver DNA had been adducted in two specific sites within the *c-k-ras* proto-oncogene.

Weinfeld, Liuzzi and Paterson (Edmonton) presented a post-labelling method for quantitation of pyrimidine dimers, in which dimers containing trinucleotide diphosphates are generated by incubation of UV-irradiated DNA with snake venom phosphodiesterase and alkaline phosphatase. These products of digestion are substrates for phosphorylation by polynucleotide kinase, while the products of native DNA are not. This method is reported to be useful at the femtomole level; its extension may allow specific detection of 6–4 photoproducts and AP sites.

Roza, Vermeulen, Eker, Hoeijmakers and Lohman (Rijswijk and Rotterdam), used a monoclonal antibody that recognizes T^T dimers to detect lesions in single cells irradiated with doses as little as 1 J/m^2. They could detect the activity of injected photoreactivating enzyme, but found no evidence for an endogenous PHR system. At present it is not clear if the antibody is specific only for T^T dimers, but it shows no reaction with the oligonucleotide dGTTG containing a 6–4 photoproduct.

Mullaart, Lohman and Vijg (Rijswijk) used the monoclonal antibody described above to show that rat keratinocytes in vivo remove more dimers than either keratinocytes in vitro or cultured fibroblasts. Together with recent results concerning repair of genes in rodent cells, these observations suggest that a system for repair of more inaccessible regions of the rodent genome is characteristically lost during cell culture. Mullaart et al. also presented evidence that the high level of UDS observed at early times in rat fibroblasts (compared to human cells) has no obvious trivial explanation. They proposed that in these cells some excision of nucleotides 5′ to sites of damage is followed by resynthesis up to the damage and ligation, leading to repair synthesis without concomitant removal of lesions. This model clearly needs further examination.

Walker and Schrader (Ontario) studied excision repair in quiescent and con-A stimulated human lymphocytes following exposure to UV or DMS. A 2–4-fold increase in repair rate was noted in stimulated cells, and repair patch sizes also appeared to increase. At 2 days after stimulation, the repair patches were 2–3-fold larger than in quiescent cells. However, it is possible that these changes reflect an artifact associated with the effects of HU, included to suppress normal DNA replication.

Van der Schans, Baan, Groenendijk and Lohman (Rijswijk) used fluorometric quantitation of DNA combined with alkaline elution to show that human lymphocytes removed alkaline-labile sites generated after exposure to X-rays or ethylating agents faster than did granulocytes. White blood cells from mice appeared to remove alkaline-labile sites from X-rays to a greater extent when incubation was in vivo, but the initial rates of removal in vitro and in vivo appeared similar. Immunochemical detection was also used to show differential formation of AAF adducts in rat liver and spleen, and the formation of the deacetylated adduct in cultured cells.

Waters (Swansea) compared the repair of adducts of 4NQO and 3-Me-4NQO in cells of XP complementation groups A, C, D and G. Only XP-D and XP-G cells showed enhanced sensitivity to killing by 3Me-4NQO (which causes only C8 adducts in guanine), and repair of these adducts appeared insensitive to aphidicolin. With 4NQO (which also makes N2 guanine and the N6 adenine adducts), all but XP-C cells showed enhanced sensitivity, and repair was sensitive to aphidicolin. This suggests that repair of the C8 adduct does not require the XP-A or XP-C gene product, and does not require DNA polymerase α. Repair of the other adducts appears to require all but the XP-C gene product, and does utilize DNA polymerase α. Direct measurement of radiolabelled adducts supported the conclusions concerning XP-A and XP-D.

Thomale, Huh and Rajewsky (Essen) investigated the effects of O^6EtG in two rat fibroblast cell lines, one deficient in removing O^6EtG and the other proficient. There was no significant repair of O^4-EtT in both cell lines, as judged by immuno-slot-blotting using highly specific monoclonal antibodies. Following exposure to ENU the frequency of transformation was 20 and 50 times higher when measured as the capacity to form foci or growth in soft agar, respectively. Cells from agar colonies derived from repair-proficient lines showed no tumor formation in syngeneic Fischer rats, while those from repair-deficient lines did.

Nivard, Vogel and van Zeeland (Leiden) determined the frequency of *N*7-alkylguanine, O^6-alkylguanine, and 3-alkyladenine in DNA after injection with ^{3}H-labelled alkylating agents (ENU, EMS, MMS) in excision repair-proficient and repair-defective male (*mei-9* and *mus* (2)201) *Drosophila melanogaster*. The initial role of O^6- to *N*7-alkylguanine was very similar to that in DNA from mammalian cells. Adduct removal was observed, with more efficient removal of 3-alkyladenine in repair-proficient cells. In wild-type cells the frequency of recessive lethal mutations correlates with the frequency of O^6-alkylguanine. Repair-deficient strains are hypermutable when treated with alkylating agents which preferentially alkylate N-atoms in DNA (MMS, EMS), whereas they were not hypermutable with those which preferentially alkylate O-atoms (ENU).

Trgovčević, Brčić-Kostić and Salaj-Šmic (Zagreb), showed that *uvrA recA* cells can complete a cycle of replication if irradiated with UV light below a certain critical dose (0.07 J/m^2). This critical dose was increased by photoreactivation conditions.

VII. The purification and characterization of proteins for DNA repair

1. Prokaryotes

The enzymology and molecular mechanisms of nucleotide excision repair, repair of alkylation damage, enzymatic photoreactivation and mismatch repair in *E. coli* and phage T4 were addressed, with particular emphasis on the following parameters: (a) the basis for substrate specificity by repair enzymes; (b) mutual allostery between repair enzymes, DNA and associated cofactors; (c) regulation of levels of gene products; (d) structure–function relationships; (e) new repair enzymes; (f) relationships between repair in vivo and in vitro.

West (ICRF), reviewed the molecular mechanism of post-replication gap-filling by genetic recombination as currently understood in *E. coli*. A nuclease has recently been purified from extracts of *S. cerevisiae* which cleaves synthetic Holliday junctions at or close to the site of the crossover.

The diversity of repair-enzyme specificities was addressed thermodynamically to distinguish the affinities of the *E. coli* UvrA protein and DNA photolyase with damaged and undamaged sites. These were measured either through nuclease protection experiments at equilibrium, by gel retardation, or by filter-binding assays. The absolute affinity constants derived from such measurements, however, appear to be less meaningful than the relative difference between the binding to damaged versus the undamaged sites, so that a significant *discrimination factor* could be assigned to an enzyme.

The *E. coli* DNA photolyase system was described by Husain, Sancar and Sancar (North Carolina), whose determinations led to a discrimination factor of approximately 10^5 in the enzyme. Mazur and Grossman (Johns Hopkins) reported a discrimination factor approximating only 10^3 for the DNA binding of the UvrA protein as a dimer in the absence of ATP. This discrimination factor is biologically inadequate for the distinction between damaged and undamaged regions in DNA. Hence, it would appear that other factors have to be involved to increase the discrimination of the Uvr system to the level of the photolyase repair system.

The significance of these thermodynamic considerations reflects a concern, for example, of the consequences of introduction of foreign genes into a receptive host under conditions in which the concentrations of gene products is not under normal control and which may lead to variations in a cellular response to a uniquely synthesized gene product. Therefore, the investigator must be aware of the potential enzymology under cellular conditions which either approach or exceed the K_m for an enzyme.

Events occurring at K_m levels were described by Oh and Grossman (Johns Hopkins). The UvrA protein monomer is converted into a dimeric species in the presence of ATP. This dimeric form of UvrA when interacting with supercoiled DNA, leads to localized topological unwinding of the DNA by 10 degrees per UvrA dimer in the presence of ATP-γ-S, a poorly hydrolyzable ATP analog. The unwinding is further increased when UvrB is included in the system.

The effect of UvrB protein on UvrA was previously described by both Seeberg and by Sancar who showed that the presence of UvrB protein can influence the ATPase associated with the UvrA protein. Similar effects were observed by Sancar and his colleagues when examining the size of a UvrA nuclease-protected region of a defined DNA fragment containing a single psoralen monoadduct. When UvrB protein was added along with UvrA protein, the protected region decreased from 35–40 base pairs to approximately 20 base pairs. This implies that either the addition of UvrB protein significantly changes the conformation of both the DNA and UvrA protein, or its presence can induce the release of one of the UvrA monomers.

Oh and Grossman (Johns Hopkins) similarly found that the UvrA-associated ATPase has a rather low intrinsic turnover number, which is significantly increased in the presence of both single-stranded DNA and UvrB protein. Such a change implies that the simultaneous presence of both UvrB and UvrA proteins results in a unique catalytic profile. It has been demonstrated that the UvrAB protein complex acts as a DNA helicase, unwinding DNA in the $5' \rightarrow 3'$ direction. These experiments further suggest that the topological unwinding imposed on supercoiled DNA by UvrAB complex represents a short unwound region, which can be translocated along DNA molecules until a damaged site is reached. Support for this model is provided by the observation that the helicase activity reported by Oh and Grossman is sensitive to the presence of pyrimidine dimers. This property, unique to the UvrAB helicase, expressly allows this system to seek out a damaged site and pause until the incision–excision step is completed.

The specificity of the UvrABC system when examined under greater than K_m levels of protein is significantly reduced, allowing this protein complex to act on undamaged DNA. However, in this case, a 9 nucleotide rather than a 12–13 nucleotide fragment is incised. The biological significance of these findings is that constitutive levels of the Uvr proteins are maintained at fairly low levels in order to restrict the Uvr system to acting only on damaged DNA. Therefore, the system must be regulated to some extent in order to maintain K_m, or lower levels of these proteins.

Analysis of the sequences of the *uvrA* gene and protein (Van Houten et al.) has revealed two potential nucleotide binding sites of the Walker A-type consensus containing $G–X_4–GKT(S)–X_6–I$ and two zinc binding sites of the type $C–X_2–C–X_{5-10}–C–X_2–C$, the former sites being implicated in the ATP hydrolysing activity and the latter sequences in the DNA-binding property of the *uvrA* protein. This group has also shown experimentally that purified *uvrA* protein contains zinc in double molar ratios. Also the *uvrB* protein contains a potential nucleotide binding site which may relate to the indications from Grossman and his colleagues that the *uvrB* protein can catalyze ATP hydrolysis (see below). Studies in several laboratories on the substrate specificity of the UvrABC enzyme have shown that the enzyme acts on any type of bulky lesion. However, van Houten reported that DNA containing mismatches or extrahelical loops in one strand are not substrates.

The regulation of cellular levels of many repair proteins is well understood. Sedgwick and Lindahl (ICRF), described the post-translational control of the *ada* adaptive system in *E. coli*. This system is responsible for removal of alkylated triesters and O^6-alkylguanine residues from MNNG-damaged DNA. The adaptive system results in the transfer of the alkyl group on the phosphotriester to a cysteine residue at the *N*-terminal residue of the Ada protein. The other active cysteine residue at the carboxyl end (residue 321) is receptive to the O^6-alkyl group of guanine or the O^4-alkyl group of thymine. The scheme shown in Fig. 1 summarizes this adaptive-repair system.

Transcriptional regulation is effected by Ada protein alkylated at the *N*-terminal site. Neither partially methylated Ada protein nor unmethylated protein can efficiently regulate transcription

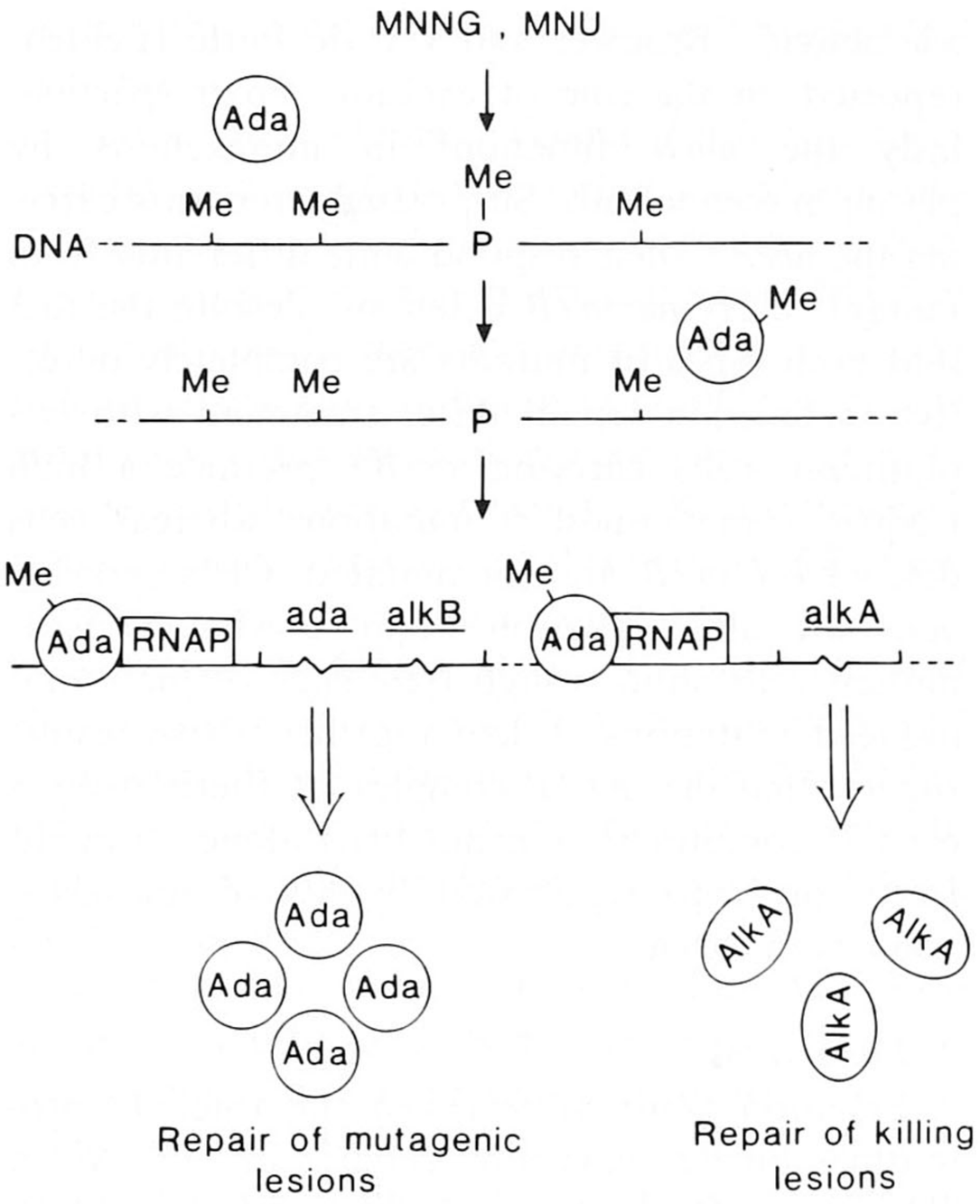

Fig. 1. Schematic model of the mechanism of induction of the adaptive response to alkylating agents. Exposure of cells to agents such as MNNG and MNU generates methyl phosphotriesters and several types of base lesions in DNA. Ada protein (20 molecules per cell) abstracts a methyl group from a phosphotriester and is converted to an efficient transcriptional activator. This covalently modified form of the Ada protein binds in the promoter regions of inducible genes and stimulates transcription by RNA polymerase (RNAP). The enhanced levels of Ada and AlkA protein result in an alkylation-resistant phenotype of the cells by the improved repair of O^6-methylation and 3-methylpurines, respectively. (Reproduced from Cell, 45 (1986) 322, with permission from Cell Press.)

of the *ada, alkB, alkA* or *alkB* genes. Control of the level of Ada protein may also be achieved through the action of an as yet undefined protease which cleaves the protein at a specific Lys–Gln residue. A homologous cleavage site has been identified by Sancar and his colleagues in the UvrB protein, suggesting that this 80 kDa protein can also be proteolyzed to generate a smaller product. Caron and Grossman have characterized some of the properties of this proteolysis product of UvrB protein (designated UvrB*; 70 kDa). The modified protein possesses some of the functional attributes of native UvrB protein. It can interact with UvrA protein however, it cannot participate in the incision or DNA helicase reactions. The function of the putative protease in *E. coli* which generates this protein (and perhaps the truncated Ada protein as well), may be to reduce levels of UvrB protein to control spurious nicking of the *E. coli* chromosome by UvrABC enzyme.

Further studies on the substrate specificity of the *E. coli* UvrABC enzyme have revealed interesting limitations on the attack of DNA containing psoralen cross-links. Seeberg (Kjeller) reported that the enzyme effectively attacks DNA with psoralen monoadducts. However in his hands no incision of cross-linked DNA is observed. This result is at variance with that reported by Sancar and his colleagues.

Franklin and Lindahl (ICRF) described a new enzyme in the base excision repair pathway in *E. coli*. This enzyme can remove 5′ terminal AP sites in duplex DNA, generating small gaps which are now a good primer-template for DNA polymerase I.

Aspects of DNA repair in vivo were addressed in experiments by Gruskin and Lloyd (Vanderbilt). The action of T4 endonuclease V and UvrABC enzyme in vivo were examined independently. These investigators showed that the T4 enzyme acts processively on UV-irradiated plasmid DNA in *uvrA*$^-$ *recA*$^-$ cells. The uvrABC enzyme also acts processively and interestingly, during repair by the latter system, the superhelical density of the DNA is preserved.

The ultimate goal of many biochemists studying DNA-repair enzymes is to correlate the structure of enzymes with their function by mutagenizing cloned repair genes and examining the properties of the products encoded. In an effort towards the realization of this goal, Recinos, Stump and Lloyd modified the T4 enzyme at residues 128–132 (Trp–Tyr–Lys–Tyr–Tyr). When Lys^{130} was changed to His^{130} or to Gly^{130}, intracellular accumulation of endonuclease V was significantly decreased for the Gly^{130} enzyme and slightly decreased for the His^{130} enzyme. However, on a molar basis, the various gene products generally yielded comparable levels of the catalytic activities tested, with $Lys^{130} > His^{130} > Gly^{130} \gg$ control *denV*$^-$ product.

Lahue, Au, Shin-San Su, Weish and Modrich (Duke) presented an update of the biochemistry of mismatch correction in *E. coli*. The products of the *mutH, mutL* and *mutS* genes have been isolated in near homogeneous, biologically active forms. The addition of purified DNA helicase II, SSB protein and DNA polymerase III holoenzyme to these three Mut proteins generates an in vitro system that mimics mismatch repair in vivo. MutS protein binds specifically to regions of DNA containing a single mismatch, with decreasing affinity for G · T, C · A, G · A and C · T mismatches. MutH protein nicks DNA at d(GATC) sites in hemimethylated DNA, with scission occurring on the hemimethylated strand. Mismatch repair in vitro also requires d(GATC) sequences. A substrate lacking hemimethylated heteroduplexes is not subject to correction.

The molecular biology of mismatch correction in *E. coli* in vivo was addressed by Dzidic, Jones, Dohet, Doutmaux and Radman (Paris) and by Radman, Jones and Wagner (Paris). The specificity of mismatch repair compensates for replication errors by repairing G · T, A · T and frameshift mismatches most efficiently, particularly in GC-rich regions. The loss of 5-methylcytosine from DNA by deamination to thymine is effectively repaired by mismatch repair which appears to be specific with respect to sequence and type of mismatch (G · T). This mode of mismatch repair generates very short patches (VSP repair) and is not methylation-directed.

Methylation-directed repair operates during recombination of phage λ, but generally does not contribute to the formation of recombinants involving close markers. VSP repair also acts during λ recombination and does produce recombinants involving close markers, because it only acts on a particular class of hyperrecombinogenic markers which form G · T mismatches in hybrid DNA and because its action does not result in extensive excision tracts.

The *dam* gene, which is involved in adenine methylation in *E. coli*, appears to be present in *S. typhimurium* (Ritchie, Podger and Hall, North Ryde, Australia). A mutation in the *dam* gene (*dam*-2) decreases the susceptibility of frameshift mutation induction by 9-aminoacridine. This was correlated with a reduced level of adenine methylase activity. Brouwer and van de Putte (Leiden) reported on the role of excision repair (particularly the *uvrB* function) in mutagenesis by platinum compounds. Surprisingly, mutants carrying the *uvrB5* allele respond quite differently from mutants carrying *uvrB* deletions, despite the fact that both types of mutants are completely defective in excision repair. After treatment with *cis*-platinum, cells carrying *uvrB5* produce a high frequency of frameshift mutations whereas cells deleted for *uvrB* are not mutated. Cells carrying *uvrA* are also mutation negative whereas *uvrC* mutant cells show a high frequency of platinum-induced mutations. Taken together, these results suggest that the *uvrAB* complex or alternatively a *uvrAB5* complex — but not *uvrA* alone — might force (or help) replication bypass of unexcised adducts in DNA.

2. Eukaryotes

Smerdon (Zurich) reviewed the role of chromatin during excision repair of DNA in eukaryotes. Recent evidence suggests that repair patches proceed through a nascent, unligated non-nucleosome structure, a ligated non-nucleosome structure and a ligated nucleosome structure. Repair within chromatin is not uniform. For example, after UV-irradiation it occurs preferentially at the 5′ and 3′ ends of nucleosome core particles.

Using a micro-injection system, Hoeijmakers, Vermeulen and Jaspers (Rotterdam) identified factors in crude cell extracts which transiently correct the repair defect in fibroblasts of all 9 xeroderma pigmentosum complementation groups. The correction is measured as an increase of UV-induced UDS and is complementation group-specific. A beginning has been made towards the purification of the XP-A correcting factor. The activity is sensitive to proteolytic degradation, precipitates between 25 and 40% $(NH_4)_2SO_4$, and binds to phosphocellulose, heparin sepharose and DEAE columns. The protein has a high affinity for single-stranded DNA and binds more efficiently to UV-irradiated as compared to unirradiated DNA. Using the same microinjection procedure Wunder (Paris) showed that Fanconi anemia fibroblasts can be corrected with extracts from wild-type cells. DNA synthesis rcovery after treat-

ment within 8-methoxypsoralen + UVA was used as a test.

Toney and Henner (Dana Farber Cancer Center) used a polyclonal antibody raised in rabbits against purified AP-endonuclease from bovine thymus, to screen protein extracts from different tissues of cow, mouse and human. The antibody detects several protein bands in extracts of tissues from each organism, with a major signal at 37K. Tissue distribution correlates with proliferative potential and expression of this class of AP-endonuclease is increased during S-phase.

Scovassi, Stefanini, Izzo, Lagomarsini and Bertazzoni (Pavia) described an activity gel technique allowing them to measure ADP-ribosyltransferase activity after treatment of human lymphocytes with alkylating agents or following PHA stimulation.

Pedrini, Tornaletti, Russo, Parodi, Westergaard, Palitti and Kihlman (Pavia, Aarhus, Rome and Uppsala) presented evidence that caffeine induces topoisomerase II-linked double-strand breaks in the same manner as antitumor DNA intercalators and non-intercalator epipodophyllotoxins. They showed that the drug inhibits decatenation activity of purified topoisomerase II in vitro. Evidence was presented that caffeine intercalates into DNA.

The role of DNA topoisomerase II in the production of DNA damage in cells treated with antitumor DNA intercalators and non-intercalator demethylepipodophyllotoxins was addressed by Pommier and Kohn (NIH). These agents produce protein-linked strand breaks in DNA. Pommier and Kohn have shown that DNA topoisomerase II is responsible for the nicking and is the protein bound at the termini generated by strand breakage. Treatment of cells with such drugs results in enhanced SCEs and mutagenesis related to double-strand break formation. The damaging effects appear reversible as DNA replication resumes, and double-strand breaks disappear. Cell lines resistant to these drugs have been isolated. However, topoisomerase II isolated from such mutants appears sensitive to drug action. It was suggested that intercalating drugs such as amsacrine and non-intercalating drugs such as a demethylepipodophyllotoxin etoposide (VP16) may be useful to monitor the distribution and extent of topoisomerase molecules in the chromatin structure.

Davies, Hoban, Davies, Harris and Hickson (Newcastle upon Tyne) isolated a CHO mutant hypersensitive to topoisomerase II inhibitors. More protein-associated DNA breaks are induced in the mutant than in wild-type cells with equimolar drug doses. The higher sensitivity was not due to higher uptake and the rate of repair of strand breaks was equivalent in the mutant and wild-type cell lines. Cell fusion showed that the mutation is recessive.

Eker, Hessels and Yasui (Delft and Rotterdam) compared photoreactivating enzymes from different origins. Two types of enzymes can be distinguished, depending on the type of chromophore present. Type I enzymes have reduced FAD as a chromophore, which absorbs light at 380 nm (*E. coli, S. cerevisiae*), whereas type II enzymes have two chromophores; reduced FAD and a 8-hydroxy-5-deazaflavin moiety absorbing at 440 nm (*S. griseus, A. nidulans, S. acutus, H. cutirubrum*). Protein sequence comparisons between type I and type II enzymes showed strong sequence conservation, indicative of a common ancestor for all these proteins.

L. Prakash (Rochester) reported on the *S. cerevisiae RAD6* gene product. The protein was purified from a strain transformed with a plasmid which overexpresses the cloned gene. The most striking feature of the protein is a highly acidic *C*-terminus, including a stretch of 13 consecutive Asp residues. The physical structure of the Rad6 protein was shown to be monomeric and globular, with the polyaspartate tail extending freely. Deletion of the 23 residues of the carboxy-terminus abolished sporulation, but had little, if any, effect on viability, resistance to UV or UV-induced mutagenesis. Inclusion of the first 4 residues of the polyacidic tail restored the sporulation ability. Affinity-purified Rad6-specific polyclonal antibodies were used to detect an immunoreactive protein in the fission yeast *S. pombe, D. melanogaster* and in human cells. The presumably homologous protein in *S. pombe* lacks the polyaspartic terminal tail.

Mismatch correction in human cells was reported by Brown, Zbinden, Cerutti and Jiricny (Switzerland). They could show by transfection with SV40 DNA containing a heteroduplex with a

G · T mismatch, that preferential correction of the G · T mispair occurs in favor of G in about 90% of cases. This occurs following transfection of CV-1 monkey cells as well as XP-A cells, 3 strains of BS cells and with normal human fibroblasts. Since the mismatch was at a CpG dinucleotide, the authors speculated that this correction might be directed to mismatches generated by deamination of 5-^{me}C in DNA.

Willis and Lindahl (ICRF) have investigated DNA ligase activities in extracts of various human repair-deficient syndromes. All cell lines studied except Bloom's syndrome (BS), have normal levels of both DNA ligases I and II, with a ratio of DNA ligase I: DNA ligase II of 1.6 : 1. In the BS line GM3034C the activity of DNA ligase I is reduced ~ 70% yielding a ratio of 0.5 : 1. In addition, the enzyme from these cells is more heat-labile than that from normal cells. Two additional independently derived BS cell lines also contain altered DNA ligase I activity. These data suggest that the molecular deficiency in BS is a missense mutation in the gene for DNA ligase I.

VII. Systems inducible by DNA damage

A number of presentations focused on the issue of damage-inducible functions in mammalian cells. The O^6-MeG-DNA methyltransferase activity in the rat hepatoma cell line H_4 increases up to 5 times, with a maximum 48 h after pretreatment with a single low dose of MNNG, MMS or EMS (Frosina and F. Laval, Villejuif). A similar effect was found after a single pretreatment with UV, X-rays, cis-Pt, ellipticine or bleomycin, but not with heat shock. The removal of 3-MeA and 3-MeG from the cellular DNA in pretreated cells incubated with MNU was also enhanced, but 7-MeG removal was not affected by pretreatment. 3-MeA glycosylase activity was enhanced. The repair of O^4MeT by extracts of H_4 cells does not occur at all. These enhanced activities are observed after a *single* pretreatment with agents which induce different types of DNA damage. These results therefore suggest that the repair of alkylated bases can be increased in mammalian cells by a process which is different from the adaptive response. An adaptive response in this cell line was independently observed after *repeated* treatments and was dose-dependent.

The cloning and characterization of a human DNA-damage inducible gene was reported by Kepten, Sharon and Ben-Ishai (Haifa). UV-responsive clones were isolated from a c-DNA library obtained from UV-irradiated transformed XP-C cells, by differential hybridization screening of irradiated and unirradiated cells. One of those, designated pBR-C5, was homologous to a 1.2 kb poly(A)$^+$ RNA transcript. The 1.2-kb transcript is elevated several fold after UV-irradiation and is also induced in repair-proficient human fibroblasts. It is also induced in fibroblasts treated with 4-NQO and in response to heat stock stress.

The induction of "SOS-phenomena" in normal and repair-deficient human cells after UV-treatment was presented by Abrahams, van der Kley and van der Eb (Leiden). UV pretreatment of host cells resulted in enhanced reactivation (ER) and enhanced mutagenesis (EM) of UV-irradiated herpes simplex virus. ER and EM followed similar kinetics in normal and in XP cells from complementation groups A, C and D. However, certain XP strains did not express an ER phenomenon, whereas the EM response was normal. Interestingly, these latter XP cells originated from patients that were reportedly free from cancer in sunlight-exposed skin areas. In BS and CS, the ER and EM responses were both expressed. However, the ER response in BS cells was unusually high. In AT cells the EM response was unusually low, whereas ER was normal.

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