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Recombination and gene conversion

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Recombination is an important aspect of DNA metabolism. It leads to rearrangements of DNA sequences within genomes. Such genome rearrangements seem to be ubiquitous, since they play a role in evolution, human health and biotechnology. In medicine one important aspect of recombination is its role as one possible step in the multistep process of carcinogenesis. Since recombination may occur as a cellular response to DNA damage, the protection of cells from recombination-inducing agents, so-called recombinagens, should eliminate possible deleterious effects resulting from damage-induced DNA recombination. During the last few years, the awareness of the importance of recombination phenomena has substantially increased and the development of assay systems detecting recombinagens has progressed. The need for considering recombinagenic effects as a safety aspect of chemicals has gained ground in the field of genetic toxicology. This paper summarizes present knowledge concerning the occurrence, inducibility, detection and toxicological interpretation of DNA recombination.

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Terminology

Recombination is the reassortment of a series of nucleotides along a nucleic acid molecule, usually of double stranded DNA, in exceptional cases also of RNA (see below). DNA recombination results from an interaction between two chromosomes or DNA double helices and may be recognized as a change in DNA sequence and/or changed allelic alignments.

Reciprocal recombination is a symmetrical exchange between two DNA double helices. Basically three events have to take place: (i) all four single strands have to be broken (this is the equivalent to two double strand breaks), (ii) an X-type reunion of the broken ends of the duplexes has to occur, and (iii) the topology of the crossing over configuration (Holliday structure) has to be solved. The three events are net results of fairly complex, interrelated enzymatic activities. In eukaryotic chromosomes, a single reciprocal exchange event will exchange the total DNA sequence distal to the point of exchange. This can involve from a few telomeric sequences up to a whole chromosome arm (for yeast it has been shown that recombination can occur within the centromeric region) (Symington and Peter, 1988). Somatic reciprocal recombination in eukaryotes was discovered in 1936 in *Drosophila melanogaster* (Stern, 1936).

Gene conversion is a nonreciprocal transfer of genetic information. The phenomenon has been discovered in fungi as an 'unusual 3:5 segregation' in cases where normally a 4:4 segregation is observed. Overall, three events have to occur: (i) a segment of one DNA duplex has to be copied, (ii) the homologous segment from the other DNA duplex has to be deleted and (iii) the deleted sequence has to be replaced by the copy of the first segment. The three steps occur through interrelated enzymatic processes. The size of the DNA segments involved varies with the different organisms: in mammalian cells the conversion tract length was found to be fewer than 350 base pairs (bp) (Liskay and Stacheleck, 1986). In the yeast *Saccharomyces cerevisiae* tract lengths over 10 kilobases (kb) have been observed both in meiosis and in mitosis (Subramani and Seaton, 1988).

Homologous recombination occurs between two DNA duplexes with the same or nearly the same base sequences. The homology between the strands which are involved in the exchange process is interpreted in the 'double-strand break repair model' proposed by Szostak et al. (1983). The influences of the length of the homology on the efficiency of the initial synapsis and subsequent strand migration were studied in several systems (Liskay et al., 1987). In monkey cells the frequency of the intramolecular, extrachromosomal excision of SV40 sequences from plasmids decreased linearly from 5243 to 214 bp, dropped nine-fold between 213 and 163 bp, and then fell linearly down to 14 bp of homology (Rubnitz and Subramani, 1984). For intermolecular, extrachromosomal gene conversion between plasmids in human EJ bladder cells, greater than 330 bp homology was necessary for optimal recombination and low levels were observed with as little as 25 bp of homology (Ayares et al., 1986). The 200 bp homology needed in mammalian cells is large compared to the homology requirements in other systems such as 50 bp for bacteriophage T4 (Singer et al., 1982) and 20–74 bp for *Escherichia coli* (Watt et al., 1985). The large minimal recognition length may represent one mechanism to reduce recombination between the highly reiterated sequences distributed throughout the mammalian and human genomes.

The term *general recombination* has been coined by Low and Porter (1978) to characterize the recA dependent homologous recombination in *Escherichia coli*. More than 30 genes are known to influence these recombination processes of which the RecBCD pathway is best understood (Porter, 1988; Mahajan, 1988). Interestingly, proteins with recA-like activity have been isolated from Chinese hamster ovary cells and from a human cell line (Kenne, 1988; Kenne and Ljungquist, 1987).

Site-specific recombination represents events occurring at highly preferred sites. A classical example of a double-site-specific recombination (with defined recombination sites on both DNA duplexes taking part in the exchange) is the integration of the circularized bacteriophage lambda genome into the bacterial genome and its subsequent excision requiring highly preferred attachment sites on both participating DNA molecules. In other cases, a special site is required on only one of the participating DNA molecules (integration of bacteriophage Mu and of transposable elements (transposons)) (Kleckner, 1981). Some site-specific recombination systems require the interacting DNA sequences to have a specific relative orientation. A model accounting for the site orientation specificities (polarity) has been proposed by Gellert and Nash (1987).

The class of *illegitimate recombination* events requires little or no DNA homology. Basically a cut and join reaction must operate. They do not appear to require the proteins promoting general or site-specific recombination. In bacterial and fungal systems illegitimate recombination normally occurs at a lower frequency than either general or site-specific recombination (Brunier et al., 1988). Illegitimate recombination has been observed in prokaryotes as well as in lower and higher eukaryotes, including man (for references see Brunier et al., 1988). In contrast to bacteria and lower eukaryotes, illegitimate recombination dominates in mammalian somatic cells. In mammalian cells only 1 proper targeted homologous recombination occurs for every 100–1000 nonspecific integrations of incoming DNA (Subramani and Seaton, 1988). This fits with the observation of a high frequency (15–20%) of generation of insertional mutations (including nonlethal ones)

in transgenic mice (Covarrubias et al., 1987). This observation suggests that the integration of vector DNA into transcriptional units must occur frequently.

The terms *mitotic recombination* and *somatic recombination* are often used as synonyms, but the term 'mitotic recombination' is preferred, because in addition to somatic cells, it also includes the mitotically dividing premeiotic cells of the germ line in which mitotic recombination occurs as well (e.g., Darras and Francke, 1987).

Four general features distinguish *meiotic recombination* from mitotic recombination (Giroux, 1988). (i) Meiotic recombination is regulated at the whole genome level as well as at the level of the individual chromosomes: at least one recombinational event (crossing over) occurs between each pair of parental homologs (bivalents) and the recombinant chromatids subsequently segregate from each other at the first meiotic division. In mitosis, recombinant homologous chromatids segregate independently of each other in the next cell division. (ii) Meiotic recombination occurs at the four-strand stage, whereas mitotic recombination occurs at the two-strand and four-strand stage. (iii) All parental homologs exhibit substantial physical interaction, homologous pairing. (iv) A unique chromosome-associated structure, the synaptonemal complex, is elaborated in meiotic prophase nuclei and forms the axis of the paired homologs during synapsis. Synapsis is not observed between homologous chromosomes in mitotic nuclei (dipteran insects appear to be exceptional in this respect showing somatic pairing in prophase chromosomes and polytenic chromosomes).

Recombination may result in *loss of heterozygosity*. In cells that are heterozygous for two alleles of a marker gene on the two homologous chromosomes, reciprocal recombination in the chromosome segment between the marker gene and the centromere or gene conversion in the region of the marker gene will lead to loss of heterozygosity. In proliferating tissue, a clone (in the case of gene conversion) or two clones (in the case of reciprocal recombination) of cells homozygous for a marker allele will develop. If the marker allele results in an easily recognizable phenotype, groups of phenotypically altered cells (a *somatic single spot*) representing the clone which lost the heterozygosity may be detected. If, on a particular chromosome arm, two transheterozygous markers are present, both clones resulting from a reciprocal recombination event between the centromere and the most proximal marker will be phenotypically detectable. This will form a *twin spot* with adjacent cell groups exhibiting each one of the marker alleles. A twin spot may also be observed with two complementing alleles at the same locus if both homozygous cell clones exhibit different phenotypic characteristics and are both distinguishable from the surrounding heterozygous, phenotypically wild-type, tissue. Twin spots have been observed, e.g., in *Drosophila melanogaster* (Becker, 1976; Würgler and Vogel, 1986), *Musca domestica* (Nöthiger and Dübendorfer, 1971), the mouse (Fahrig and Neuhäuser-Klaus, 1985), and in man (Festa et al., 1979).

Ubiquity of recombination phenomena

Recombination in RNA viruses, was only detected in 1982 in the foot and mouth disease virus (King et al., 1982). Subsequently another member of the picornavirus family, poliovirus, and more recently the mouse hepatitis virus, a coronavirus, were also shown to recombine. Since replication of these viruses takes place in the cytoplasm, without a DNA intermediate, exchange of genetic information has to occur by a sequence rearrangement between RNA molecules (review: King, 1987). The importance of RNA recombination is not merely academic. These days, most cases of poliomyelitis in Western countries are caused by neurovirulent revertants of the attenuated vaccine virus; according to Kew and Notlay (1984), reversion is frequently associated with genetic recombination between the three poliovirus serotypes of which the vaccine is usually composed.

In the case of retroviruses, recombination used to optimize the maintenance of viability and the amount of increase in variation in virus populations, occurs during minus or plus strand DNA synthesis (Temin, 1991).

Recombination may also lead to changes of host ranges or virulence, as in the case of the

influenza virus where recombination between two virus types leads to the appearance of the extremely virulent 'Hong Kong type'.

In bacteria the transfer of genetic information between individual microorganisms occurs in nature. There are three modes of gene transfer: transformation (uptake of naked DNA molecules by competent bacterial cells), transduction (DNA is moved between bacteria by bacteriophages; specialized transduction involves specific segments of bacterial genomes; generalized transduction transfers any piece of a bacterial genome) and conjugation (DNA transfer between two cells in direct contact). With all these modes of gene transfer, donor DNA without an origin of replication must recombine with a replicon (typically the bacterial chromosome) in the recipient cell in order to be stably inherited and expressed. Recombination in this case includes all facets of the introduction and stabilization of new genetic information in the recipient cell.

Pathogenic microorganisms have developed recombinational strategies to escape the host antibody response. This is especially important for extracellular pathogens that cycle between the vertebrate bloodstream and hematophagous arthropoda: the longer the parasite survives in the bloodstream, the greater the probability of transmission through an arthropod to another animal.

Antigenic variation is achieved by genetic changes in a small fraction of the multiplying cells. Multiphasic antigenic variation has been described in, e.g., *Trypanosoma brucei*, *Neisseria gonorrhoeae*, and *Borellia hermsii* (for references see Plasterk et al., 1985). The cyanobacterium Anabena undergoes, in the absence of a nitrogen source, two specific DNA rearrangements through site-specific recombination between short repeated sequences to activate two nitrogen-fixation genes (nif) during heterocyte differentiation.

In the ciliated protozoa, such as Oxytricha, Tetrahymena, and Paramecium, genomic reorganization during differentiation of the somatic macronucleus is a general characteristic of the phylum (reviewed in Blackburn, 1987). Large numbers of rejoinings and deletions of internally eliminated sequences occur frequently throughout the genome of the developing macronucleus.

In yeast (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*), trypanosomes, and certain bacteria (*Neisseria gonorrhoeae*, Borellia spp.) nonreciprocal recombination is used for mating type switching (review: Strathern, 1988). In the best studied case of *Saccharomyces cerevisiae*, an early step in switching is a double strand break induced specifically by the HO endonuclease at the active mating type locus MAT. Then in α cells sequences from the silent HMR locus and in a cells from the silent HML locus located near MAT on chromosome III replace the actual MAT sequences. A similar conversion mechanism has been postulated to occur in the activation of immunoglobulin light chain genes in chickens (Thompson and Neiman, 1987).

In genetic studies with heterozygous diploid fungi (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, *Aspergillus nidulans*, *Ustilago mayadis*, etc.), spontaneous mitotic recombination is observed. As discussed in detail by Hastings (1988), important substrates (but not the only ones) for mitotic gene conversion and reciprocal recombination are double strand cuts and gaps as well as double-stranded discontinuities formed subsequently to DNA damage. Evidence is consistent with the view that mitotic recombination is repair. Interestingly recombination leading to excision of sequences has also been observed in the mitochondrial genome of yeast (de Zamaroczy et al., 1983).

In heterozygous plants of *Glycine max* and Nicotiana, environmental mutagens can induce spots resulting from mitotic recombination (Vig, 1982).

Insects, in particular *Drosophila melanogaster*, have played a key role in the development of the concept of mitotic recombination. Mitotic recombination was demonstrated as early as 1936 by Stern in his classical twin spot experiments where the somatic events became visible as adjacent color and bristle differences in a heterozygous animal with otherwise wild-type phenotype. Sobels (1956, 1957) for the first time demonstrated the induction of recombination by a chemical agent. Mitotic recombination was also observed in the housefly *Musca domestica* (Nöthiger and Dübendorfer, 1971). Developmentally controlled genomic rearrangements occur in Drosophila fol-

licle cells which show chorion gene amplification. The observation of three different karyotypes in the ovarian nurse cells of individual *Anopheles gambiense* is additional evidence for mitotic recombination in insects in vivo (Hunt, 1987).

Important biological phenomena depending on genetically programmed mitotic recombination occur in the mammalian immune system. Functionally immunoglobulin genes in B-cells are the result of mitotic rearrangements of coding elements (review: Engler and Storb, 1988). Also the phenomenon of immunoglobulin class switching to produce, often as a result of antigen stimulation, differentiated lymphocytes, occurs through recombination between tandem repetitive sequences in the heavy chain gene (Iwasoto et al., 1990). Matsuoka et al. (1990) have characterized circular DNA in mouse splenocytes which represent the excised DNA sequences (these represent just one class of circular DNAs observed in various mammalian cells). In contrast to mammals, in the chicken the immunoglobulin light chain repertoire is generated by a high frequency of segmental gene conversion between a unique rearranged functional variable (V) gene and a pool of V pseudogenes (Reynaud et al., 1987).

Gene rearrangements are also involved in the formation of the T-cell receptor gene complex. In this case the γ-chain genes encoded by immunoglobulin-like gene segments rearrange during T-cell development and some template-independent sequences (1–12 base pairs) are inserted enzymatically into the γ-chain genes (Quertermous et al., 1986).

Recombination events appear to play an important role in two widely occurring phenomena in eukaryotic chromosomes: (i) the conservation and homogenization of repeated DNA sequences and (ii) in the maintenance of telomeres. It is likely that sequence homogeneity is maintained by gene conversion because of its 5–10-fold predominance (in yeast as well as in mammalian cells) and of the drawback of reciprocal recombination in the form of unequal crossovers. Such conversion events have been invoked for the sequence homogeneity in many types of repeated eukaryotic sequences such as rDNA, histone genes, α, β, γ, δ globin genes, murine serum amyloid A genes, bovine vasopressin and oxytocin genes, rodent cytochrome P450 genes and immunoglobulin genes (references in Subramani and Seaton, 1988).

Every time a linear chromosome is replicated it is in danger of being whittled away from the ends, because polynucleotide synthesis can proceed in only one direction, from 5′ to 3′; this leads to a problem in copying the 3′ ends of both strands of a linear chromosome. A common solution to this problem is to have simple repeated sequences at the ends, the telomeres and a specialized system for generating additional repeats compensating for loss in replication. Whereas in Tetrahymena a telomerase (a riboprotein representing a DNA polymerase carrying its own template) builds up new telomeres (Greider and Blackburn, 1989), in other organelles (e.g., Tetrahymena mitochondria) and other organisms such as yeast and higher eukaryotes, recombination seems to be responsible for maintaining the average telomere length (review: Traverse and Pardue, 1988; Szostak, 1989; Brown et al., 1990).

Recombination is also involved when mobile genetic elements or viruses integrate into chromosomal DNA or when these sequences get excised. In some cases cellular enzymes are involved, in other cases element-specific enzymes, e.g., transposases encoded by the mobile element, are involved. Well characterized examples are insertion sequences and transposons in bacteria, the 2μ plasmids in yeast, the Ac/Ds system in *Zea mays*, mobile elements like P, copia, etc., in Drosophila and the animal retroviruses known to be widespread in all vertebrates. The fact that the integration of foreign DNA into mammalian cells can result in revertable mutations has been demonstrated for the dilute and the hairless locus in the mouse (Copeland et al., 1983; Favor et al., 1987; Stoye et al., 1988).

Recombination in mammalian somatic cells

The lack of cytological demonstration of somatic chromosome pairing in mammalian cells and several unsuccessful efforts to detect recombination between cellular genes at the functional level in cultured mammalian cells (for references see Wasmuth and Hall, 1984) led for a long time to the common belief that, in contrast to sister

chromatid exchanges (SCE), mitotic recombination between homologous chromosomes does not occur in human somatic cells with the very special exception of cells from patients with Bloom's syndrome (Kuhn, 1974; Therman and Kuhn, 1976). Whereas spontaneous recombination between homologous chromosomes, resulting in so-called chiasmata, is usually extremely rare in human leukocytes, a frequency of about 2% was observed in leukocytes of Bloom's syndrome patients. In addition, in a child with Bloom's syndrome who developed acute lymphocytic leukemia (ALL) the skin on one leg had adjacent areas of increased and decreased pigmentation similar to the 'twin spots' seen in Drosophila (Festa et al., 1979). This twin spot is the phenotypic manifestation that reciprocal mitotic recombination had taken place in vivo and resulted in the loss of heterozygosity for two genes involved in the control of skin pigmentation. This added confidence to earlier rare claims that mitotic crossing over had been observed in man and in a number of primates (Therman and Kuhn, 1976). There exist a few recent reports on the cytogenetic detection of symmetrical exchanges after exposure to genotoxic agents, e.g., cigarette smoke in heavy smokers (Obe and Herha, 1978; Littlefield and Joiner, 1986).

The whole issue of recombination in somatic mammalian cells got a boost when modern DNA technologies were applied to mammalian cells. It was primarily the interest in using homologous recombination for targeted integration of DNA sequences in mammalian cells with the potential use for gene therapy that stimulated research. With foreign DNA injected into nuclei, the most spectacular observation was the high frequency of extrachromosomal recombination events relative to chromosomal recombination (Subramani and Seaton, 1988). These frequencies differ by several orders of magnitude. One clue to this puzzling observation is that extrachromosomal recombination occurs at this high frequency only for about 90 min after injection. This suggests that naked DNA may be a better substrate for recombination than chromosomal DNA.

With respect to mechanisms and with respect to the expectation that, as in other organisms, double strand breaks may be a substrate for recombination, the observation is important that the frequency of recombination between plasmids is increased, on average 10-fold, if one of the plasmids is linearized. These observations prompted the investigation of cell extracts from mammalian cells. It was found that mammalian cell extracts can initiate and catalyze enzymatic homologous recombination (Kucherlapati et al., 1985). In addition, a recA-like activity, similar to *E. coli* RecA protein, has been isolated from a Chinese hamster ovary cell line and from human cells (Kenne, 1988). The activity mediated homologous pairing and the formation of D-loop structures in the DNA in the presence of ATP and Mn^{2+}.

It is an important fact that extrachromosomal recombination events in mammalian cells are nonconservative (loss of some sequences), whereas there exist indications that chromosomal events are conservative. The fact of the nonconservative nature of extrachromosomal events suggests that perhaps these processes are catalyzed by some machinery whose primary role might involve repair of damaged DNA. In contrast, chromosomal recombination appears to occur in a conservative manner, conserving the complete DNA sequences (Subramani and Seaton, 1988).

From a toxicological point of view it is important (i) that recombination events are involved in the generation of chromosome rearrangements and of spontaneous gene mutations in mammalian cells, (ii) that often highly repetitive sequences are involved and (iii) that in cultured mammalian cells as well as in mammalian cells in vivo chemically induced recombination events do occur. Reports concerning chromosome inversions (Malissen et al., 1986) and reciprocal translocation events involved in Burkitt's lymphoma (Moulding et al., 1985) as well as the observation of a twin spot in a Bloom's syndrome patient (Festa et al., 1979) show that intra- and interchromosomal recombination occurs in humans in vivo. In tumor cell lines loss of heterozygosity is an important phenomenon (Okamoto et al., 1988). As has been shown, e.g., by Cavenee et al. (1983) for retinoblastoma, a substantial fraction of these losses of heterozygosity are the consequences of homologous recombination events. Modern DNA techniques are intensively

used to analyze the molecular nature of chromosome rearrangements and gene mutations (reviewed in Mohrenweiser and Jones, 1990). In many cases the mutations causing genetic diseases are the result of more complex changes than just base pair substitutions. They may be insertions, deletions or rearrangements of DNA sequences. Repetitive elements appear to have a role in the occurrence of many of these changes, examples are given in Table 1. Several thalassemias are the result of deletions occurring in the region of the Kpn (L1) repetitive elements in the β globin gene complex (Gilman, 1987). Alu sequences have been identified at the 5′ and 3′ boundaries of a deletion in the α-chain of the β-hexosaminase A gene (Myerowitz and Hogikyan, 1987). An exon-Alu recombinational event is proposed to account for a 5-kb deletion in the low-density lipoprotein (LDL) receptor gene (Lehrman et al. 1986), while Alu-Alu mediated recombinational events have been identified in another deletion (Lehrman et al., 1985) and in a duplication of seven exons (Lehrman et al., 1987) at this locus. Many other deletions and rearrangements at the LDL receptor gene locus also appear to involve the Alu repeats (Langlois et al., 1988).

Unequal crossing over and gene conversion events can cause alterations in gene structure and genetic disease. Deletions involving the human somatomammotropin gene (Simon et al., 1986) and the steroid 21-hydroxylase genes (Higashi et al., 1988) are suggested to have arisen through a mechanism involving unequal crossing over between members of the gene family. Gene conversion, as illustrated by the case involving recombination between the steroid 21-hydroxylase pseudogene and the functional gene, is also a mechanism for generation of nonfunctional genes (Amor et al., 1988).

Mutations in somatic cells were studied at the HPRT locus. Precursors to T-lymphocytes appear to have a greater likelihood of producing major deletions or rearrangements of the HPRT locus, perhaps due to the action of recombinases that are associated with T-cell antigen receptor gene maturation in the thymus (Alt et al., 1987; Marrack and Kappler, 1987).

The observation that recombination in cultured human cells can be increased by chemical treatment dates back to the studies of Therman and Kuhn (1976) when they showed that mitomycin C is capable of enhancing the recombination frequency in Bloom's syndrome cells. Only recently such studies were continued with other cell types (Kenne and Ljungquist, 1984, 1987; Kenne, 1988). A chemically induced, dose-dependent increase in homologous recombination between duplicated herpes simplex thymidine kinase genes in mouse L cells was observed. In Chinese hamster ovary cells containing duplicated truncated *neo* genes stably integrated in the genome a dose-dependent increase was found within a 400-bp region after treatment with methyl methanesulfonate (MMS), but not with nitrogen mustard (NH2). Studies with human cell lines

TABLE 1

EXAMPLES OF HUMAN MUTATIONS ASSOCIATED WITH INSERTIONS, DELETIONS OR DUPLICATION IN CODING REGIONS OF GENES

Gene	Size	Event	Reference
Alu sequences			
LDL receptor	5 kb deletion	Alu-Alu recomb. (dominant)	Lehrman et al., 1985
LDL receptor	5 kb	exon-Alu recomb.	Lehrman et al., 1986
LDL receptor	14 kb	duplication involving Alu repeats in introns 1 and 8	Lehrman et al., 1987
Hexosamidase A	7.6 kb	Alu-Alu recomb.	Myerowitz and Hogikyan, 1987
δ, β Globin		deletion begins in Alu repeat	Ottolenghi and Gigglioni, 1982
KpnI (L1 sequences)			
α Globin	12.6 kb	KpnI (L1) sequences, deletion	Gilman, 1987
$\gamma\delta$, β Globin	80 kb	deletion + insertion of *Kpn*I sequences	Mager, 1985

that differ in their nucleotide excision repair capacity indicated that unexcised DNA lesions, rather than excision repair per se, stimulate intrachromosomal homologous recombination (Bhattacharyya et al., 1990).

The studies with mammalian cells in vitro together with the in vivo observations in human tumor cells and germ cell mutations as well as the in vivo chemical induction of recombination in somatic cells in the mouse indicate, taken together, that in genetic toxicology the importance of induced recombination has so far been underestimated. It appears that, due to the frequent occurrence of recombination and the increasing number of detected chemical and physical recombinagens and because other conditions such as nucleotide pool inbalances and environmental stress can increase recombination, these effects have to be taken into account in the risk assessment of genotoxic chemicals. Whereas the consequences of gene conversion, affecting a limited number of base pairs, may be comparable to the classical induction of gene mutations, the reciprocal recombination events eventually inducing homozygosity for a sizable fraction of the genome (up to a whole chromosome arm if recombination occurs within the centromere (Symington and Petes, 1988; Lieberman et al., 1988)) appear to be of special importance in contributing to the formation of genetically altered cell clones in the adult human body potentially leading to diseases such as cancer (e.g., Fearon and Vogelstein, 1990), certain types of coronary heart diseases (Mulkvag et al., 1988; Penn et al., 1986; Bridges et al., 1990), autoimmune defects and diabetes (Karsten and Kryspin-Sorensen, 1988), and aging phenomena in general (Kirkwood, 1989).

The importance of recombination and gene conversion in toxicology

In summary, the toxicological considerations are based on the following facts.

Spontaneous mitotic recombination does occur in human cells. In addition chemical and physical recombinagens that are genotoxic agents induce mitotic recombination and gene conversion in human cells.

In human populations heterozygosity for a normal and a defective allele must be quite common. It has been suggested that every one of us carries several defective genes in a heterozygous state (Muller, 1950; Vogel, 1979). The importance of loss of heterozygosity, for which mitotic recombination and gene conversion are important mechanisms, in dominantly inherited cancer susceptibilities has first been observed for retinoblastoma and Wilms' tumor (reviewed in Bishop, 1991; Marshall, 1991). Müller and Scott (1992) have identified a total of 32 clinical/genetic entities which, in the same way as retinoblastoma and Wilm's tumor, show familial clustering of neoplasms due to autosomal dominantly inherited cancer susceptibilities. This preliminary list indicates that the generation of some neoplasms in practically all organs can result from loss of heterozygosity. In addition loss of heterozygosity leading to somatic mosaicism may, in addition to carcinogenesis, also affect other biological functions, and, consequently, health (Hall, 1988), e.g., in certain cardiovascular diseases (including atherosclerosis), diabetes, aging, etc. In the case of inherited heterozygosity, even heterozygous carriers for rare autosomal recessive syndromes are common in the general population (Heim et al., 1992). For example (assuming Hardy-Weinberg equilibria), if a syndrome has a true incidence of 1/40,000, the heterozygote frequency is 1/100; for a true incidence of 1/360,000, the frequency is 1/300; and even if the incidence is $1/10^6$, the frequency is still 1/500. As discussed in detail by Heim et al. (1992) there is, for example, cumulative evidence that the heterozygote phenotypes for the autosomal recessive syndromes ataxia telangiectasia, xeroderma pigmentosum and Fanconi's anemia are different from wild type at both the cellular and clinical levels. With respect to spontaneous somatic mutations we have to stress that these occur more frequently than generally expected based on the frequencies found in germ cells. Their incidence varies from person to person due to individual genomic instabilities (Müller, 1990).

Not only somatic cells undergo mitotic recombination and gene conversion, but premeiotic, mitotically dividing cells of the germ line show these phenomena as well (Darras and Francke,

1987). In germ cells mitotic recombination may result in changes leading to hereditary diseases (e.g., Duchenne muscular dystrophy through the formation of deletions).

Human chromosomes contain structures that can act as preferred substrates for mitotic recombination (repetitive sequences, Alu sequences, fragile sites) that may also represent hot spots for recombination. Mitotic recombination in humans is under genetic control (e.g., increased frequency in Bloom's syndrome), therefore subpopulations with increased sensitivity are to be expected.

In conclusion, recombinagenic effects have to be considered as deleterious effects in humans and have to be included in risk estimates for effects from chemical and physical genotoxic agents.

Assay systems are available with organisms of different genetic complexity to screen specifically for recombinagens: bacteria (Hoffmann, 1992), yeast (Zimmermann et al., 1984; Zimmermann, 1992), Drosophila (Vogel, 1992), cultured mammalian cells (Kenne and Ljungquist, 1984; Kenne, 1988; Bhattacharyya et al., 1990) and the mouse in vivo (Fahrig and Neuhäuser-Klaus, 1985). Such studies do complement the classical mutagenicity testing of chemicals and complex mixtures and make it possible to improve the testing in safety toxicology.

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