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REVIEW

Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations

Petra Pfeiffer, Wolfgang Goedecke[1] and Günter Obe[1,2]

Institut für Zellbiologie, Universitätsklinikum Essen, Virchowstraβe 173, D-45122 Essen and [1]Institut für Genetik, Universität GH Essen, Universitätsstraβe 5, D-45117 Essen, Germany

DNA double-strand breaks (DSB) are considered to be critical primary lesions in the formation of chromosomal aberrations. DSB may be induced by exogenous agents, such as ionizing radiation, but also occur spontaneously during cellular processes at quite significant frequencies. To repair this potentially lethal damage, eukaryotic cells have evolved a variety of repair pathways related to homologous and illegitimate recombination, also called non-homologous DNA end joining, which may induce small scale mutations and chromosomal aberrations. In this paper we review the major cellular sources of spontaneous DSB and the different homologous and illegitimate recombination repair pathways, with particular focus on their potential to induce chromosomal aberrations.

Introduction

Chromosomes consist mainly of DNA, histones and non-histone proteins and are organized during interphase in domains. In most cells chromosomes are visible only during the process of cell division (Cremer *et al*., 1996; Savage, 1996; Bickmore and Craig, 1997; Kreth *et al*., 1998; Wolffe, 1998). After extensive research it became clear that, with few exceptions, G_0/G_1 chromosomes contain one DNA molecule which is replicated in the S phase of the mitotic cell cycle to yield two sister chromatids, each of which consists of one single DNA molecule (the uninemic structure of chromosomes). In meiosis, only one S phase takes place in the pre-meiotic cell cycle, followed by two meiotic divisions that separate the homologues and the sister chromatids, respectively.

Sometimes, alterations in the normal chromosome structure, so-called chromosomal aberrations (CA), are observed. CA can be classified as intra- and inter-chromosomal aberrations. The first class comprises aberrations within a single chromosome, such as terminal and interstitial deletions and inversions; the second class comprises rearrangements between two or more chromosomes, such as translocations and dicentrics. In general, CA are analysed in mitosis at a metaphase-like stage that is induced by colchicine (Levan, 1938). Fusion of mitotic cells with interphase cells induces premature chromosome condensation (PCC) in the latter which permits the analysis of chromosomes at stages in which they are normally not visible (Rao *et al*., 1982; Cornforth, 1998). Staining of chromosomes with Giemsa allows the analysis of different types of CA, such as polycentric chromosomes, ring chromosomes, chromatid interchanges and fragments. Other CA types, such as reciprocal translocations and inversions, are normally not recognizable with Giemsa staining but can be visualized by fluorescence *in situ* hybridisation (FISH) (Lucas *et al*., 1989, 1992, 1997; Gray *et al*., 1994; Natarajan *et al*., 1994, 1996a,b; Gebhart *et al*., 1996; Boei *et al*., 1998; Chudoba *et al*., 1999; Johannes *et al*., 1999; Knehr *et al*., 1999; Wojcik *et al*., 1999). Studies on radiation-induced CA in G_0/G_1 cells using the FISH methodology revealed that chromosomal rearrangements may be complex, involving three or more breaks in two or more chromosomes (Brown and Kovacs, 1993; Lucas and Sachs, 1993; Savage and Simpson, 1994a,b; Simpson and Savage, 1994, 1995a,b; Griffin *et al*., 1995, 1996; Tucker *et al*., 1995; Savage, 1996, 1997; Savage and Tucker, 1996; Edwards and Savage, 1999; Johannes *et al*., 1999; Simpson *et al*., 1999). Break points on chromosomes can be localized by Giemsa banding or with the help of special FISH techniques (Sumner, 1990; Holmquist, 1992; Folle and Obe, 1995, 1996; Bickmore and Craig, 1997; Folle *et al*., 1997; Martinez-López *et al*., 1998; Johannes *et al*., 1999).

Sister chromatid exchanges (SCE) can be detected in chromosomes differentially substituted with [^{3}H]thymidine (Taylor, 1958), 5-bromodeoxyuridine (BUdR) (Latt, 1981) or biotin-dUTP (Bruckmann *et al*., 1999a,b). There are excellent reviews and books on SCE to which the interested reader is referred (Kato, 1977; Wolff, 1977, 1982; Latt, 1981; Sandberg, 1982; Tice and Hollaender, 1984; Morris, 1991).

In 'normal' somatic cells, the frequency of spontaneously occurring CA is rather low with ~1 dicentric/1000 human lymphocytes. Exposure of cells to clastogens (i.e. agents that create structural alterations in DNA), however, can increase the frequency of CA formation by several orders of magnitude (Ishihara and Sasaki, 1983; IAEA, 1986; Ishidate *et al*., 1998; Kirkland, 1998). While certain types of CA are lethal, others may lead to oncogenic transformation, e.g. by inactivation of a tumor suppressor gene (Weinberg, 1988) or activation of an oncogene by generating novel fusion proteins capable of initiating carcinogenesis (Pierotti *et al*., 1992). Indeed, elevated frequencies of CA are often correlated with an elevated risk of cancer (Hagmar *et al*., 1998) and certain human neoplasias are associated with defined CA (Mitelman *et al*., 1997). Therefore, CA are considered a hallmark of all tumour cells and the ongoing formation of CA in these cells reflects genomic instability associated with cancer progression (Cheng and Loeb, 1997; Cho and Hedrick, 1997; Tlsty, 1997).

In spite of decades of research, the molecular mechanisms of CA formation are still not entirely understood. Principally there are two classical and one more recent theory which try to explain how CA are generated: (i) the 'breakage and reunion theory' (Sax, 1941; Lea, 1946); (ii) the 'exchange theory' (Revell, 1963); (iii) the 'molecular theory' (Chadwick and

[2]To whom correspondence should be addressed. Tel: +49 201 183 3388; Fax: +49 201 183 2866; Email: guenter.obe@uni-essen.de

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Leenhouts, 1981, 1998). Both the breakage and reunion theory and the exchange theory are non-specific with respect to the molecular target in which a 'primary break' is formed and were mainly developed from the interpretation of dose–effect relationships for different types of CA induced by ionizing radiation (IR). The breakage and reunion theory proposes breaks in the chromosome axis which may: (i) be rejoined to the original structure (restitution); (ii) lead to exchange-type aberrations by rejoining of different breaks; (iii) appear as chromosome breaks if not rejoined at all. Savage (1998) characterizes the breakage and reunion theory as 'no break—no exchange'. The exchange theory assumes the formation of 'unstable lesions'. If two such lesions come into close contact they may initiate an exchange mechanism leading to: (i) exchange-type aberrations, when complete; (ii) open breaks, when incomplete. Therefore, the exchange theory was characterized by Savage (1998) as 'no exchange—no break'. The molecular theory suggests that exchange-type aberrations may result from a single DNA double-strand break (DSB) by recombinational repair which may be characterized as 'one DSB—one exchange' (Chadwick and Leenhouts, personal communication). New insights into the mechanisms of how CA may be formed can be gained from complex chromosomal rearrangements seen with FISH following exposure of G_0/G_1 cells to ionizing radiation. Edwards and Savage (1999) state that some of these configurations cannot be explained with Revell's theory but can with Sax's theory. In his excellent review 'Insight into sites', Savage (1996) comes to the conclusion that complex exchanges are difficult to explain by random movement of broken ends and their eventual mutual reunion. Savage states 'The possibility exists that the classical theory is wrong, and that exchanges can arise from just *one* DSB interacting with proximal undamaged chromatin by a process of reciprocal recombination' (by 'classical theory' Savage refers to the theory of Sax).

In the present review we try to reconcile the classical breakage and reunion theory and the exchange theory with the molecular theory and our present understanding of the molecular mechanisms of DSB repair and genetic recombination.

The initial lesion

The use of a variety of chemical and physical DNA-damaging agents that dramatically increase the formation of CA first gave hints as to the nature of the critical lesion that causes CA. Early investigators developed the concept of frank 'chromosome breaks' as the initial lesion in CA formation (Lea, 1946). The broken ends of these breaks are free to interact either directly with each other (restitution) or to unite with the ends produced by a second break when the two occur in close spatial and temporal proximity. The current dogma is that chromosome breaks are produced by DSB. Considering the uninemic structure of mammalian chromosomes, a chromosome or chromatid break must involve the equivalent of at least one DSB.

Artificially induced DSB

In general it can be noted that agents which induce DNA strand breaks directly (e.g. IR and endonucleases) lead to CA in the cell cycle stage in which the lesion was induced: CA formation is independent of S phase. In contrast, most chemicals result in DNA damage other than strand breaks and give rise to CA only when the cell passes through S phase: CA formation is dependent on S phase. In the following paragraphs we focus exclusively on agents that induce DSB directly.

Ionizing radiation

The agent most frequently used for generating DSB, and consequently CA, is ionizing radiation (Cornforth, 1998). Sparsely ionizing radiations, such as X- and γ-rays, deposit their energy in cellular structures through discrete ionization events that are essentially randomly distributed in space. Unlike chemical agents, whose damaging potential is strongly dependent on diffusion processes and thus may be affected by subcellular structures, IR is typically highly penetrating: the physics and subsequent chemistry associated with photon absorption and the ionization events that occur along fast electron tracks are complete within a few microseconds ('on/off' character of damage delivery).

IR causes a wide spectrum of chemically different types of lesions in DNA of which the so-called locally multiply damaged sites (LMDS) are assumed to be biologically most important (Ward, 1988, 1990). LMDS may consist of single-strand breaks (SSB) on opposite strands which, if located close to each other, may give rise to DSB. Other LMDS containing chemically modified base and sugar moieties (for a review see Friedberg *et al*., 1995) have the potential to cause DSB following strand cleavage by cellular base damage-specific enzymes. Thus, DSB induced by IR may arise as a direct consequence of one or more ionizing events or indirectly as a consequence of repair processes that eliminate closely spaced base or sugar damage on opposite strands. Due to the presence of complex chemical alterations in the DNA, DSB generated by IR are particularly challenging to repair because they first have to be converted into 'clean' structures containing 5′-phosphate and 3′-hydroxyl groups which are accepted substrates of cellular enzymes.

Endonucleases

Although most studies to investigate the formation of CA have been performed with IR, the first direct evidence that DSB are indeed the initial lesions in the process of CA formation came from experiments with a single-strand-specific endonuclease from *Neurospora crassa*. The enzyme alone did not induce CA, but elevated frequencies of CA were observed when it was applied to cells that had been pre-treated with X-rays and thus contained SSB in their chromosomal DNA (Natarajan and Obe, 1978; Natarajan *et al*., 1980; Obe *et al*., 1982; Nowak and Obe, 1984).

Restriction endonucleases (RE) have also proven an important tool in the exploration of the biological effects of DSB in living cells because they generate, unlike IR, no other lesions but DSB (Bryant and Liu, 1994; Thacker, 1994). The fact that RE treatment increases the frequency of CA has furthermore supported the idea that DSB are a primary lesion in the process of CA formation (Natarajan and Obe, 1984; Obe *et al*., 1992; Yates and Morgan, 1993). As with all other DSB-inducing agents, it was shown that the type of CA formed depends on the stage of the cell cycle in which the cells were treated with RE (Obe and Winkel, 1985; Obe *et al*., 1993). (i) Chromosome-type aberrations occur if DSB are induced in G_1 chromosomes. The resulting aberrations are replicated during S phase so that both sister chromatids carry the same aberration at the same site. (ii) In contrast, induction of DSB in G_2 usually affects only one sister chromatid, thus giving rise to chromatid-type aberrations. (iii) RE treatment in S phase generates both types of CA, with the chromosome-type resulting from DSB in

yet unreplicated DNA and the chromatid-type from DSB in replicated DNA.

An important feature of RE-induced DSB is that they are well defined with respect to structure (5′- or 3′-overhangs or blunt ends) and location within a given DNA sequence. This has not only facilitated the analysis of mutational spectra caused by DSB in defined chromosomal loci but also permitted, by comparison of the initial DSB ends and the resulting product, deduction of the mechanisms underlying DSB-induced mutagenesis (Winegar *et al*., 1992; King *et al*., 1993; Phillips and Morgan, 1994; Pfeiffer, 1998). Although simpler in structure than IR-induced DSB (always having 'clean' ends with 5′-phosphate and 3′-hydroxyl groups), RE-induced DSB cause the same cytotoxic effects, such as increased levels of cell killing (Bryant, 1985; Costa *et al*., 1993; Obe *et al*., 1995; Winckler *et al*., 1988) and oncogenic transformation (Bryant and Ritches, 1989). This and the fact that the mutational spectra caused by IR and RE are very similar and range from microscopically visible CA to small scale mutations, as measured in non-essential selectable genes like the *APRT* or *HPRT* gene, has provided further evidence for the notion that DSB are critical lesions in CA formation (Obe *et al*., 1986; Miles and Meuth, 1989; Phillips and Morgan, 1994; Costa and Thacker, 1996; Cornforth, 1998).

Cellular (spontaneous) sources of DSB

Since in most laboratories IR or other agents are used to induce DSB artificially, the fact that DSB also occur spontaneously at quite significant frequencies is often neglected. In fact, spontaneous DSB can arise at any stage of the cell cycle: in cells in a non-dividing or resting state, during DNA replication and during the subsequent spatial resolution of the sister chromatids. In the following paragraphs we have tried to briefly summarize the main causes of spontaneous DSB.

Topoisomerases

During the cell cycle, spontaneous DSB may arise by topoisomerase (Topo)-mediated DNA cleavage. Topoisomerases are ubiquitous enzymes that change the superhelical state of the DNA (eukaryotic enzymes can relax both positive and negative supercoils), which is essential for DNA replication, recombination, chromosome segregation and transcription. While Topo I generates a reversible SSB, Topo II generates a reversible DSB and is required specifically during mitosis and meiosis for the separation of sister chromatids. Both enzymes are involved in genome stabilization and can promote illegitimate recombination, which in turn may lead to the formation of CA (for a review see Wang, 1996).

Replication

One of the most prevalent sources of DSB is DNA replication, which takes place in every dividing cell. Although the process itself is remarkably accurate, human cells are estimated to suffer ~10 DSB/cell cycle, as estimated by the incidence of spontaneous SCE (for a review see Haber, 1999a). This high frequency may result from the fact that any SSB in the parental strand, e.g. at a Topo I site, can be converted into a DSB upon arrest of the replication fork at this lesion (Roth and Wilson, 1988). Furthermore, defects in the maturation of Okazaki fragments may lead to the accumulation of DSB (Lieber, 1997; Tishkoff *et al*., 1997). DSB occurring during replication are acted upon by mechanisms of homologous recombination and vertebrate cells lacking the Rad51 strand transfer protein (see below) exhibit many broken chromosomes and are inviable, probably because they cannot repair these lesions (for a review see Haber, 1999a).

Meiosis

Meiosis is the best paradigm for a cellular process in which DSB are formed by a special system to initiate highly efficient homologous recombination (HR) between chromatids of the maternal and paternal homologues in the germ cells to achieve increased genetic variability (Sun *et al*., 1989). In yeast, a Topo II-like enzyme, Spo11 endonuclease, creates DSB during prophase of meiosis I. Although meiosis-specific DSB have not yet been confirmed physically in other organisms than yeast, the recent findings of proteins from various species sharing sequence homology with the yeast Spo11 protein suggest a common mechanism for the initiation of meiotic HR (Keeney *et al*., 1997, 1999; McKim and Hayashi-Hagihara, 1998; Romanienko and Camerini-Otero, 1999).

V(D)J recombination

The process which produces the enormous repertoire of antigen-binding proteins, T cell receptors and immunoglobulins during the development of lymphoid cells is another remarkable example of a programmed DNA rearrangement with the purpose of generating diversity. To this end, germline V, J and, in some cases, D segments are fused in a site-specific recombinational joining process to give rise to a functional antigen-binding molecule (Grawunder *et al*., 1998). The V(D)J recombination process is initiated by a DSB induced by the Rag1/Rag2 transposase and is completed by the general DSB repair system which depends, as discussed below in more detail, on DNA-dependent protein kinase (Gellert, 1992; Jackson and Jeggo, 1995; Roth and Craig, 1998).

Other recombination mechanisms

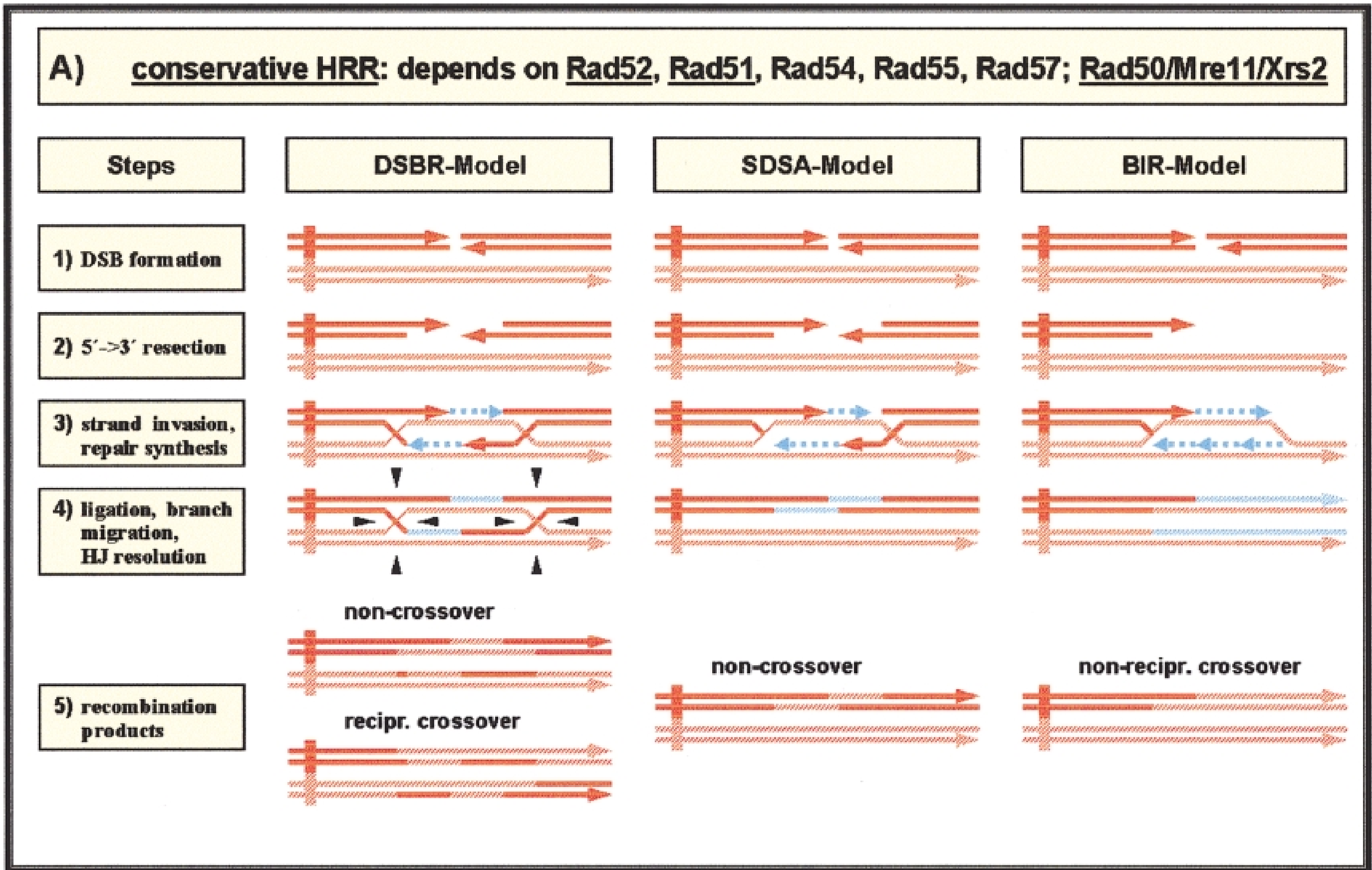
Another well-studied example involving the spontaneous induction of DSB is the mating type switch in yeast, which is initiated by HO endonuclease (Haber, 1992). Furthermore, the excision and re-insertion of transposable elements (transposons and retrotransposons) are processes that involve a DSB intermediate and may contribute to the restructuring of chromosomes. Although best studied in bacteria, yeast, *Drosophila* and maize, it is known that transposable elements are also active in humans and may contribute to CA (Finnegan, 1994; Lim and Simmons, 1994; Erickson and Lewis, 1995; Hall and Collis, 1995; Britten, 1997; Labrador and Corces, 1997). Since neither mating type switching nor transposition will be discussed here in further detail the interested reader is referred to the reviews cited in this paragraph.

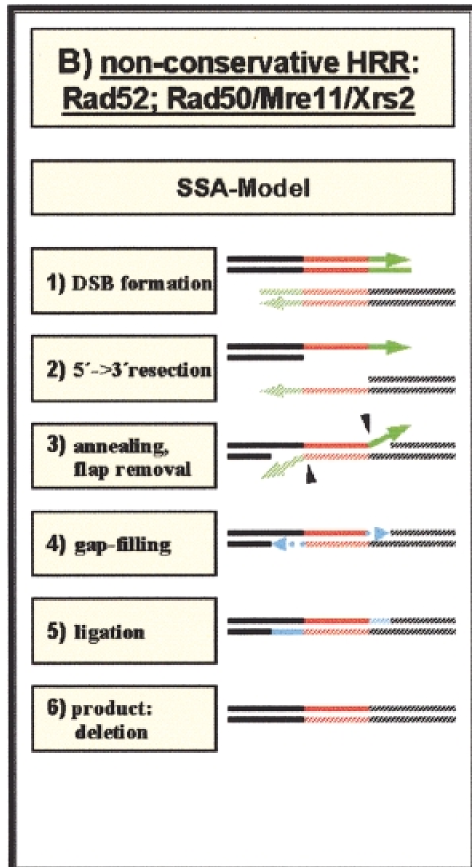
Fragile sites

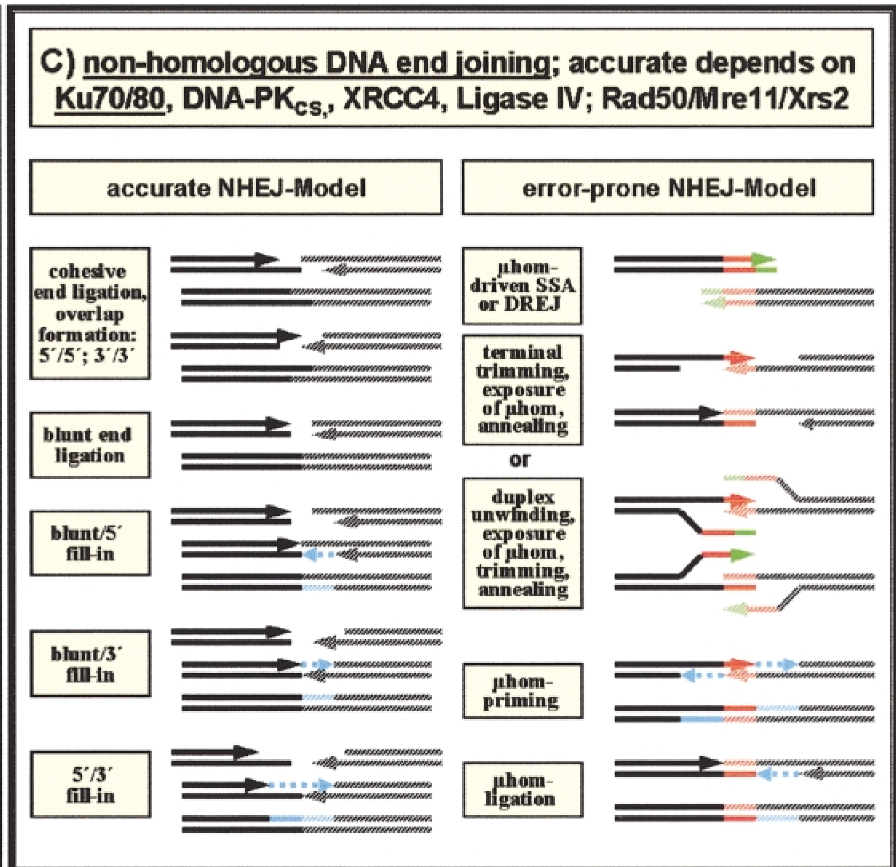
Extended micro- and minisatellite sequences may also be regarded as a potential source of DSB in the mammalian genome. These sequences undergo a dynamic process of expansion and deletion which is often associated with genetic diseases (Sutherland *et al*., 1998). Although the precise mechanism of micro- and minisatellite instability is not yet entirely understood, DSB have been shown to occur as intermediates of this process in yeast (Debrauwere *et al*., 1999). In this context, it is interesting to note that the formation of DSB is meiosis specific, since it is known that germline transmittance can vary between males and females for such instabilities (Jeffreys *et al*., 1998).

DSB as a result of excision repair

Excision repair processes that eliminate mismatches and base damage from DNA may also be regarded as a potential source


A) conservative HRR: depends on Rad52, Rad51, Rad54, Rad55, Rad57; Rad50/Mre11/Xrs2
Steps
DSBR-Model
SDSA-Model
BIR-Model
1) DSB formation
2) 5′->3′ resection
3) strand invasion, repair synthesis
4) ligation, branch migration, HJ resolution
5) recombination products
non-crossover
recipr. crossover
non-crossover
non-recipr. crossover


B) non-conservative HRR: Rad52; Rad50/Mre11/Xrs2
SSA-Model
1) DSB formation
2) 5′->3′resection
3) annealing, flap removal
4) gap-filling
5) ligation
6) product: deletion


C) non-homologous DNA end joining; accurate depends on Ku70/80, DNA-PKCS, XRCC4, Ligase IV; Rad50/Mre11/Xrs2
accurate NHEJ-Model
error-prone NHEJ-Model
cohesive end ligation, overlap formation: 5′/5′; 3′/3′
blunt end ligation
blunt/5′ fill-in
blunt/3′ fill-in
5′/3′ fill-in
µhom-driven SSA or DREJ
terminal trimming, exposure of µhom, annealing
or
duplex unwinding, exposure of µhom, trimming, annealing
µhom-priming
µhom-ligation

of DSB (Doutriaux *et al*., 1986). In this context, it is important to consider the frequencies at which certain DNA lesions occur spontaneously in the cell (for a review see Friedberg *et al*., 1995). One of the most important sources of spontaneous damage to DNA is attack by reactive oxygen species (ROS) generated in various redox reactions of aerobic metabolism, which causes about 20 000 oxidative lesions (e.g. 8-oxo-guanine) daily in the DNA of each cell (many of the lesions induced by ROS also occur after irradiation with IR). Spontaneous hydrolysis of the β-glycosidic bond between a purine (depurination) or, less frequently, a pyrimidine (depyrimidination) and its deoxyribose leads to the formation of ~10 000 apurinic/apyrimidinic (AP) sites/cell/day and deamination of cytosine to uracil and 5-methylcytosine to thymine occurs at a frequency of ~100/cell/day. Such base damage is acted upon by the base excision repair system and removed by glycosylases which are specific for certain types of base damage (e.g. hOGG1 removes 8-oxoguanine from G:C pairs; uracil glycosylase removes uracil from DNA; thymine glycosylase removes thymine from G:T mismatches; Croteau and Bohr, 1997; Cunningham, 1997; Wilson,D.M. and Thompson, 1997). If located close together (<10 bp apart) on opposite strands, simultaneous excision of such modified bases can lead to the formation of DSB.

All the examples given above show that DSB, the supposed initial lesion in the formation of CA, are not as rare as one might expect but may occur spontaneously (deliberately or accidentally) by several cellular processes. The different repair pathways that may act on this dangerous type of lesion are reviewed in the following paragraphs.

Mechanisms of DSB repair

Sequencing of the break points of chromosomal exchanges was most helpful in identifying the nature of the processes underlying CA formation (for a review see Cornforth, 1998). Today it is generally accepted that mechanisms involved in the repair of DSB and genetic recombination are mainly responsible for formation of CA. As discussed in the next paragraph, the most convincing support for this notion is probably derived from the analysis of so-called chromosome instability syndromes, rare human autosomally inherited disorders which are associated with dramatically increased frequencies of CA.

Human chromosome instability syndromes

Human chromosome instability syndromes include Nijmegen breakage syndrome (NBS), Fanconi anemia (FA), Bloom syndrome (BS), Werner syndrome (WS) and ataxia telangiectasia (AT) (Bay *et al*., 1996; Lavin *et al*., 1999). Apart from serious physiological defects, cancer proneness and premature ageing (WS), patients suffering from these diseases exhibit highly elevated frequencies of spontaneous CA at the cellular level. In NBS, the NBS1 protein (or nibrin), a component of the Rad50/Mre11 protein complex which is involved in HR and non-homologous DNA end joining (NHEJ), is mutated (Carney *et al*., 1998; Varon *et al*., 1998). In FA, at least eight different complementation groups (FA A–H) have been identified of which two genes (*FA-A* and *FA-C*) have been cloned (Joenje *et al*., 1997). The hypersensitivity at the cellular level to DNA crosslinking agents indicates a potential role of the corresponding gene products in HR (Thompson, 1996). The genes defective in BS (*BLM*) and WS (*WRN*) have been identified to be RecQ-like helicases implicated in the control of HR (for a review see Wu,L. *et al*., 1999). In AT, the ATM kinase is defective, which is involved in cell cycle regulation and appears to play a role in activation of the cellular responses to DNA damage (Meyn, 1997; for a review see Lavin *et al*., 1999).

In the context of chromosome instability, the defect which predisposes individuals to familial breast cancer should also be mentioned. Homozygous loss of either of the two breast cancer genes (*BRCA1* and *BRCA2*) leads to drastically increased chromosomal instability. Both gene products have been shown to be related to HR and NHEJ (Zhang *et al.*, 1998). While BRCA1 interacts with the Rad50/Mre11 complex (Zhong *et al.*, 1999) and is suggested to control homology-directed DNA repair (Moynahan *et al*., 1999), BRCA2 interacts with Rad51 (Scully *et al*., 1997), a RecA-like strand transfer protein which participates in HR. In total, the evidence derived from these genetic defects suggests that the repair of DSB by HR and NHEJ pathways plays a crucial role in the formation of CA.

Some general remarks on DSB repair

All excision repair processes (mismatch repair, base excision repair and nucleotide excision repair) that eliminate base or sugar damage from DNA rely on the presence of an undamaged strand opposite the lesion which is used as a template to restore the original sequence in the damaged strand. In the case of a DSB, however, both strands are damaged so that no template is available for repair synthesis, implying that the sequence information has to be restored by different means. To this end, several different repair processes have evolved which can be formally subdivided into two groups: (i) homology-dependent and (ii) homology-independent mechanisms. The former are related to HR (also called homologous recombination repair, HRR) and thus essentially require extensive regions of sequence homology (usually several hundred base

Fig. 1. Schematic presentation of the different models of conservative HRR (**A**), non-conservative HRR (**B**) and NHEJ (**C**). In all models, DNA duplexes are shown as two lines with arrowheads pointing in the 3′-direction; continuous and hatched lines distinguish between the strands that serve as templates for DNA synthesis (blue dotted arrows) to indicate which sequence is copied (blue continuous or hatched lines). Regions of homology are shown in red, regions of non-homology in black. For all pathways the most important factors promoting the corresponding reactions are listed at the top of each panel.
(A) Conservative HRR occurs normally between extensive regions of sequence homology as present in the homologue or sister chromatid (small vertical rectangles represent the centromeres). Endonucleolytic resolution of Holliday junctions (HJ) in DSBR is marked by pairs of black horizontal and vertical arrowheads (resolution of both HJ in the horizontal direction yields non-crossover products, resolution of one HJ in the horizontal and the other in the vertical direction yields crossover products). (B) In non-conservative HRR, represented by the SSA model, two DSB ends interact directly at homologous repeat units. Note that one repeat unit and the intervening sequence which forms unpaired flap ends (green) is lost by nucleolytic trimming (oblique black arrowheads).
(C) In NHEJ, two pathways, one 'accurate' and one 'error-prone', are distinguished. The 'accurate' pathway is considered to be dependent on Ku, while the factors promoting the 'error-prone' pathway are still unknown. Note that 'accurate' NHEJ will induce small scale mutations unless the ends are not directly ligatable, as seen by the blue fill-in stretches in the blunt/5′, blunt/3′ and 5′/3′ terminus configurations. The main feature of 'error-prone' NHEJ is the formation of deletions at regions of microhomology (μhom), marked in red (see text for further details).

pairs; Figure 1A and B), while the latter are related to illegitimate recombination, also referred to as NHEJ, which can dispense with sequence homology (but often involves microhomology patches of 1–10 bp) and simply paste two broken ends together (Figure 1C).

Homology-dependent mechanisms (HRR)

Most of our knowledge about homology-dependent pathways originates from decades of basic research in bacteria, phages and yeast, where HRR is most efficient. In vertebrate cells the low frequency of accurate gene targeting events and the high frequency of end fusions of transfected DNA led to the idea that NHEJ is much more efficient than HRR: the opposite of what is found in yeast (Roth and Wilson, 1988). However, recent evidence indicates that when DSB are created within chromosomes (as opposed to transfection of DNA fragments) vertebrate cells are also quite proficient at HRR (Liang *et al.*, 1998). Genetic studies in yeast have shown that HRR is strictly dependent on Rad52 and Rad51 (see below). The recent identification of homologues of these genes in mammalian cells indicates that HRR is a highly conserved process which may play a much more important role in the repair of DSB in vertebrate cells than previously anticipated. Since we do not intend to here describe in detail all factors involved in HRR, the interested reader is referred to some excellent reviews that focus on the historical background as well as the genetic and biochemical characterization of the proteins involved (Shinohara and Ogawa, 1995; Kanaar *et al.*, 1998; Haber, 1999b; Pâques and Haber, 1999; Thacker, 1999a).

Homology-dependent pathways are subdivided into conservative (Figure 1A) and non-conservative processes (Figure 1B). The former group is characterized by accurate repair of the DSB achieved by copying the sequence information of the sister chromatid, yielding two intact copies (= conservative), and comprises three HRR pathways: DSB repair (DSBR), synthesis-dependent strand annealing (SDSA) and break-induced replication (BIR). Non-conservative HRR is represented by the single-strand annealing (SSA) process in which two direct repeats interact with each other so that one repeat copy and the intervening sequence is lost (= non-conservative). Since the mechanistic models for conservative HRR (Figure 1A) have been tested and verified in most cases only for the situation in *Saccharomyces cerevisiae* the reader should bear in mind that although there is good evidence that these mechanisms also exist in mammalian cells, it remains to be proven that the details of the processes are similar.

Conservative HRR mechanisms (Figure 1A)

The best paradigm for conservative HRR, also termed gene conversion, is found in meiosis, because, as compared with mitotic HRR, the levels of meiotic HRR are highly elevated. This is mainly due to the induction of meiosis-specific DSB by Spo11 endonuclease (see above), which greatly facilitates analysis of the recombination events. The fact that the frequency of HRR in yeast is directly related to the appearance of DSB led to the conclusion that most, if not all, recombination events are induced by DSB (Wu,T.C. and Lichten, 1994; Baudat and Nicolas, 1997). This is an important finding to mention in the context of earlier HR models that suggested that HR can be initiated by an SSB (Meselson and Radding, 1975; see below).

The basic feature of conservative HRR is accurate reconstitution of the broken chromosome by copying information from the homologue or sister chromosome to restore the original sequence at the break. In the case of gene conversion, HRR takes place between two different alleles of the same gene so that the sequence of the broken allele (recipient) is converted to the sequence of the donor allele (DSBR and SDSA). Sometimes, gene conversion may affect not only a single gene (short track) but may comprise several contiguous genes (long track), including the entire distal part of a chromosome (BIR).

In yeast, about eight proteins are directly involved in the homologous exchange mechanism. They are classified into two families participating in strand transfer (Rad51p, Rad52p, Rad54p, Rad55p and Rad57p) and nucleolytic strand resection of DSB ends (Rad50p, Mre11p and Xrs2p), respectively. In addition, proteins involved in stabilizing DNA single strands, DNA synthesis (repair synthesis and replication) and removal of non-homologous ends play essential roles (Chen and Kolodner, 1999). In mammalian cells it appears that several different homologues of the recombination proteins exist (e.g. seven Rad51 homologues have been identified to date) whose exact function is still unclear (for a review see Kanaar *et al.*, 1998; Thacker, 1999b). The functional homologue of yeast Xrs2 protein, a member of the Rad50/Mre11 nuclease complex, appears to be the human NBS1 protein, which is defective in Nijmegen breakage syndrome (for a review see Featherstone and Jackson, 1998). Furthermore, the proteins encoded by the breast cancer tumor suppressor genes *BRCA1* and *BRCA2* interact with Rad51 and the Rad50/Mre11 complex and may play a role in the regulation of these processes (Scully *et al.*, 1997; Zhang *et al.*, 1998; Moynahan *et al.*, 1999; Zhong *et al.*, 1999). These findings underscore the notion that HRR processes play an important role in the repair of DSB in mammalian cells. In the following paragraphs the major models of the conservative HRR pathways (Figure 1A) are briefly described.

The double-strand break repair (DSBR) model

First proposed by Resnick (1976) and Resnick and Martin (1976) and later elaborated by Szostak *et al.* (1983), the DSBR model comprises the following steps: after DSB induction (e.g. by Spo11p in meiosis) the ends are $5'\rightarrow3'$ exonucleolytically resected to produce long $3'$ single-stranded tails that invade the homologous chromosome (meiosis) or sister chromatid (mitosis) at a site of sequence homology. The mechanism of resection is not yet clear but genetic evidence from yeast indicates involvement of the Rad50/Mre11/Xrs2 complex (for a review see Haber, 1998) although *in vitro* Mre11 has been shown to possess only $3'\rightarrow5'$ activity (Paull and Gellert, 1998; Trujillo *et al.*, 1998). The key step of strand invasion and exchange is carried out within a filament of Rad51 protein (Nishinaka *et al.*, 1998). The $3'$-ends of the invading strands serve as primers for semi-conservative repair synthesis (mostly short tracks of a few kilobases) so that one newly synthesized strand is present in each of the donor and recipient. The resulting joint molecule contains a heteroduplex region bordered by two branched structures, so-called Holliday junctions (HJ) (Holliday, 1964), which are not fixed in space but may branch migrate to enlarge the heteroduplex region. Endonucleolytic resolution of the HJ yields either crossover or non-crossover products (also called splice or patch recombinants, respectively).

The synthesis-dependent strand-annealing (SDSA) model

Since most mitotic gene conversions in yeast are not associated with a crossover, a second group of gene conversion models

emerged (Nasmyth, 1982; Hastings, 1988; Thaler and Stahl, 1988) whose designation SDSA was derived from a similar process occurring in *Drosophila* (Nassif *et al*., 1994). The basic feature of these models (for a review see Pâques and Haber, 1999), of which only the simplest is outlined in Figure 1A, is that the newly synthesized DNA strands (short tracks) are displaced from the template and returned to the broken molecule, allowing the newly synthesized strands to anneal to each other. Unlike in the DSBR model, where repair synthesis is semi-conservative, repair synthesis in the SDSA model is conservative (all newly synthesized sequences are on the same molecule).

The break-induced replication (BIR) model

As mentioned above, gene conversion usually involves relatively short conversion tracks. However, sometimes very long conversion tracks are observed. The central feature of this so-called BIR pathway is that only one DSB end invades the homologue or sister chromatid and initiates both leading and lagging strand synthesis in a true replication fork (Mosig, 1987; Kogoma, 1996, 1997; Chen and Kolodner, 1999). Once BIR starts, it can either proceed to the chromosome end or be converted into gap repair (DSBR) if the second end of the DSB becomes involved. BIR may be a biologically important pathway for the repair of chromosome ends: a chromosome that has lost a telomere has a single DSB end because the distal, acentric fragment is lost so that no second end can participate in a DSBR or SDSA event (for a review see Pâques and Haber, 1999).

The Meselson–Radding model

This model (not shown), originally developed by Meselson and Radding (1975) (Wagner and Radman, 1975) to explain meiotic gene conversion events in fungi, is mentioned here too to complete the picture of HRR. It is similar to the DSBR model with the major difference that the HR process is initiated by an SSB instead of a DSB. The 3′-end of the SSB induced in one DNA molecule is used as a primer for displacement synthesis. The displaced 5′ single-strand pairs with the complementary sequence in the homologue or sister chromatid and induces an SSB in the latter which initiates a reciprocal strand exchange to create a joint molecule with a single HJ that can branch migrate. As in the DSBR model, resolution of the HJ yields either crossovers or non-crossovers. We found it important to mention this model here because it may be one explanation as to how CA are induced by agents that do not induce DSB directly.

Non-conservative HRR (Figure 1B)

The single-strand annealing (SSA) model

If a DSB occurs between two flanking homologous regions (repeats), repair of the broken chromosome is very efficient and results in a deletion embracing one copy of the repeat and the intervening sequence. The underlying SSA mechanism, first observed in mammalian cells (Lin *et al*., 1984) and characterized in detail in *Xenopus* oocytes (Maryon and Carroll, 1991a,b; Carroll *et al*., 1986), is initiated by extensive 5′→3′ resection of the DSB ends (possibly also mediated by the Rad50/Mre11/Xrs2 nuclease complex) until substantial regions of homology (~400 bp) flanking the break are exposed on long single-stranded 3′-tails which subsequently undergo strand annealing. In yeast, this process does not require Rad51 but is dependent on Rad52, which is consistent with its role as a DNA end-binding and strand-annealing protein (van Dyck *et al*., 1999). The association of Rad52 proteins with each other may help to bring the ends together to begin the search for homology (Haber, 1999b).

Homology-independent mechanisms (Figure 1C)

As opposed to the situation in yeast, where HRR pathways predominate in the repair of DSB, homology-independent (illegitimate) recombination mechanisms, also termed NHEJ, which are able to rejoin DSB ends directly, appear to prevail in mammalian cells. The independence of NHEJ of sequence homology does not necessarily mean that homology is never involved. On the contrary, whenever short regions of sequence homology, so-called micro-homology patches in the range of 1–10 bp, are available they will most probably be used.

NHEJ was observed first during transfection experiments in mammalian cells (Pellicer *et al*., 1980; Perucho *et al*., 1980) and later also reported for yeast (Orr-Weaver and Szostak, 1983). In the following years, the mechanisms of NHEJ were studied in detail with the help of RE-cleaved plasmid or SV40 DNA in mammlian cells (Wilson,J.H. *et al*., 1982; Roth *et al*., 1985; Roth and Wilson, 1986), extracts from *Xenopus laevis* eggs (Pfeiffer and Vielmetter, 1988; Thode *et al*., 1990) and yeast (Goedecke *et al*., 1994; Kramer *et al*., 1994; Mézard and Nicolas, 1994). The simplest type of NHEJ is the ligation of compatible ends. However, NHEJ is also able to rejoin non-complementary ends irrespective of their sequence and structure. This has implications for the mutagenic potential of this DSB repair pathway: (i) the original sequence is only restored if the DSB generates two complementary or blunt ends that can be precisely religated; (ii) if, however, two non-matching ends (for instance after irradiation) arise they first have to be transformed into a ligatable structure by enzymatic modification which often causes base pair substitutions, insertions and/or deletions (for a review see Pfeiffer, 1998). Although this will usually lead to small scale mutations at the resulting repair site (junction), the consequences of NHEJ appear to be tolerable in multicellular organisms because the chance that small alterations at break points affect a critical region within an expressed essential gene is low due to the favourable ratio of non-coding to coding DNA (only a few per cent of the total genomic DNA in a mammalian cell has a coding function), and even in the case of such an unlikely event the intact allele may compensate for the defective allele in diploid cells.

Analysis of hamster cell lines that are hypersensitive to IR and defective in DSB repair and V(D)J recombination led to the identification of four complementation groups involved in NHEJ (for reviews see Jeggo, 1990, 1998; Zdzienicka, 1999). The corresponding genes (*XRCC4–XRCC7*) encode the XRCC4 protein, an essential co-factor of DNA ligase IV, and the three components of the DNA-dependent protein kinase (DNA-PK) represented by the 70 (*XRCC6*) and 86 kDa (*XRCC5*) subunits of the heterodimeric DNA end-binding Ku complex (Ku70/80) and the catalytic subunit of protein kinase (DNA-PK_{CS}, *XRCC7*) (Jackson and Jeggo, 1995; Lieber *et al*., 1997; for a review see Featherstone and Jackson, 1999). In addition to these components, the Rad50/Mre11/Xrs2 nuclease complex (see HRR mechanisms) also appears to be involved in NHEJ, as indicated by the recently discovered direct interaction of Mre11 with Ku70 in rodent cells (Goedecke *et al*., 1999).

With the exception of DNA-PK_{CS}, homologues of all com-

ponents of the mammalian NHEJ machinery have also been identified in yeast (for a review see Critchlow and Jackson, 1998). However, efficient NHEJ is only detectable in yeast with the Rad52-dependent HRR pathway disabled. When such strains, furthermore, carry mutations in Ku70/80, ligase IV and/or Rad50/Mre11/Xrs2, NHEJ efficiency drops drastically to a residual error-prone activity that creates deletions which indicate that the Ku-dependent NHEJ pathway is dominant (Boulton and Jackson, 1996; Critchlow and Jackson, 1998). Consistent with this, increased frequencies of imprecise end joining were also observed in the Ku80-deficient xrs6 hamster cell line (Liang and Jasin, 1996; Feldmann *et al*., 2000) and in partially purified protein fractions from calf thymus (Mason *et al*., 1996) and *Xenopus* eggs (Göttlich *et al*., 1998). Together these findings indicate the existence of at least two different pathways of NHEJ, one that is dependent on Ku and joins DNA ends accurately and one that is independent of Ku and forms deletions whose break points display patches of micro-homology. In the following, the models of how these two pathways may act are briefly explained.

'Accurate' non-homologous end joining (NHEJ)

It should be noted that although termed 'accurate', this NHEJ pathway can only restore the original sequence correctly when two compatible ends which are directly ligatable are provided. As soon as the ends are not compatible, small scale mutations will be created at the junctions (Figure 1C, left).

The mechanisms of 'accurate' NHEJ have been most extensively studied in *Xenopus* egg extracts (Pfeiffer and Vielmetter, 1988). In this *in vitro* system, the major NHEJ pathway was shown to work with high fidelity because it not only precisely religates cohesive overhangs or blunt ends to restore the original restriction site but also tends to preserve the sequences of interacting non-complementary DNA ends by generating two major types of products (fill-in and overlap). The type of product formed depends on the structure of the ends being joined: fill-in junctions arise typically during the joining of abutting ends (blunt/5′-overhang; blunt/3′-overhang; 5′-overhang/3′-overhang) while overlap junctions are formed between two overhangs of the same polarity (5′/5′ and 3′/3′). In the fill-in pathway, the sequences of participating 5′- and/or 3′-overhangs are fully preserved by fill-in DNA synthesis in a process in which the ends are transiently held together by non-covalent interactions while the 3′-hydroxyl group of the 5′-overhang or blunt end is used as a primer to direct repair synthesis of the 3′-overhang (Thode *et al*., 1990). In the overlap pathway, overhangs form, by pairing of single fortuitously complementary bases, incompletely matched overlaps whose structure determines the patterns of subsequent repair reactions (Pfeiffer *et al*., 1994a,b). Based on these data, it was suggested that an alignment factor may function to maintain the two ends in alignment to facilitate their biochemical reconfiguration into a ligatable structure (Thode *et al*., 1990). Similar joining events have been observed in mammalian cells *in vivo* and *in vitro*, which indicates that the mechanisms found in the *Xenopus* system also apply for mammalian systems (Bøe *et al*., 1995; Daza *et al*., 1996; Roth and Wilson, 1986). Recent evidence suggests that the highly abundant Ku70/80 heterodimer, which is able to bind to a great variety of DNA ends, may function as the proposed alignment factor by protecting DNA ends from degradation and thus enhancing the accuracy of NHEJ (Liang and Jasin, 1996; Critchlow and Jackson, 1998; Feldmann *et al*., 2000).

'Error-prone' NHEJ

This pathway is characterized by two features: (i) it is independent of Ku70/80 (DNA-PK), in fact, it is detectable only when Ku70/80 is not functional (Boulton and Jackson, 1996; Critchlow and Jackson, 1998; Feldmann *et al*., 2000); (ii) it creates deletions whose break points are flanked by micro-homologies. The latter feature indicates a mechanism in which micro-homology patches located close to the DSB ends are exposed on single strands by exonucleolytic resection and/or helicase-mediated duplex unwinding and is reminiscent of the SSA pathway discussed above with the difference that SSA requires extensive regions of homology (Figure 1C, right). For this reason, error-prone NHEJ has also been designated direct-repeat end joining (DREJ) (Thacker *et al*., 1992; Mason *et al*., 1996; Thacker, 1999c), micro-homology-based NHEJ (Lehman *et al*., 1994), modified SSA (Nicolás and Young, 1994; Nicolás *et al*., 1995) and micro-homology-driven SSA (Göttlich *et al*., 1998).

All models assume a patch of micro-homology to be located at the tip of at least one of the interacting single strands. Occurring at the tip of a 3′ single strand, it could act as a primer for DNA fill-in synthesis (micro-homology priming); exposed at the tip of a 5′ or 3′ single strand it could adjoin the recessed strand of the partner terminus and be fixed by ligation (micro-homology ligation). In both cases, one strand would be quickly stabilized in the intermediate, thus facilitating enzymatic processing of the second strand (Göttlich *et al*., 1998). Although the factors involved in the error-prone NHEJ pathway are still unknown, the similarity between this pathway and SSA could indicate that some of the factors involved in SSA possibly also participate in the error-prone NHEJ pathway.

DSB repair and CA formation

Regarding the several pathways described, it becomes clear that only the conservative HRR mechanisms will most likely restore the original sequence at the break site. All other mechanisms (SSA and 'accurate' and 'error-prone' NHEJ) have a high mutagenic potential: SSA as well as 'error-prone' NHEJ will obligatorily produce more or less extended deletions and even 'accurate' NHEJ will produce point mutations at repair sites, such as base pair substitutions, small insertions and/or deletions, if the ends are not compatible and thus cannot be directly religated (for a review see Pfeiffer, 1998).

Due to their dependence on extensive sequence homology, HRR pathways in mitosis are expected to occur mainly in late S phase and G_2, when the chromosomes have replicated, so that two identical copies are available, which is consistent with the high frequencies of spontaneous SCE (~5/cell in human lymphocytes; the exact value is not known because of the effect of BUdR on SCE frequencies). In contrast, SSA and NHEJ should not be restricted to these stages of the cell cycle but are expected to act mainly during G_1. How these different mechanisms are regulated during the cell cycle is not yet clear but will be important for an understanding of the formation of CA.

Although conservative HRR mechanisms are supposed to be mostly accurate they can also be mutagenic because of their potential to generate gene conversions which may result in loss of heterozygosity (LOH), when the broken wild-type allele is replaced by a non-functional mutant allele (e.g. inactivation of a tumour suppressor gene). In this context, the BIR pathway is particularly important because the conversion

tracks can comprise a whole chromosome arm. BIR is the mechanism thought to account for the recombination-dependent maintenance of telomeres in cells in which telomerase, the enzyme that normally adds the short telomeric repeat sequences at the ends of chromosomes, is not active (for a review see Pâques and Haber, 1999). Another source of small scale mutations occurring during repair synthesis in HRR may be mis-incorporation of nucleotides or slippage at micro- and minisatellite sequences or sites of small tandem or inverted repeats leading to base pair substitutions, insertions or deletions (repeat expansions and contractions), respectively (Rippley, 1982; Streisinger and Owen, 1985; Pâques *et al*., 1998).

With respect to the potential of the different repair pathways to create CA, it is important to consider the number of DSB necessary to initiate a particular repair event. Each DSB end is highly recombinogenic because it can invade a DNA duplex at any site of more or less extended sequence homology. This aspect is particularly important in the light of the high proportion of repetitive DNA, mini- and microsatellites and pseudogenes in mammalian genomes, a situation that does not exist in yeast to a comparable extent. Since duplex invasion initiates any type of conservative HRR and can lead to crossover events in the DSBR and BIR pathways, HRR has a high potential to induce exchange-type CA if the invasion does not occur within the appropriate site of the homologous or sister chromosome but at repeat sequences in another chromosome (ectopic recombination). Ectopic reciprocal recombination between repeated sequences located on the same or different chromosomes can create a CA, such as translocations, inversions and deletions (Lehrman *et al*., 1985, 1986; Vnencak-Jones *et al*., 1988; Liefshitz *et al*., 1995). Since HRR is initiated by a single DSB, it is important to note that *one initial DSB* can be sufficient to induce any exchange-type aberration by ectopic recombination. This mechanism might also account for the occurrence of some complex exchanges found in FISH-painted chromosomes in cells irradiated in G_0/G_1, because a single DSB end can invade non-homologous chromosomes at sites of sequence homology, a situation expected to be 'amply satisfied in the genome' (Savage, 1996).

In the context of one DSB being sufficient to initiate an HR event, we would also like to remember the Meselson-Radding model, which suggests that one SSB is sufficient to initiate an HR event (Meselson and Radding, 1975; Wagner and Radman, 1975). Although this model appears to be less likely in the light of the high prevalence of DSB in meiosis, suggesting that most if not all HRR events are initiated by DSB (Baudat and Nicolas, 1997; Wu,T.C. and Lichten, 1994), it could be useful in explaining the formation of CA during S phase by chemicals that do not induce DSB directly but SSB or base modifications which will be converted to SSB or gaps upon excision by repair enzymes.

Due to their potential to induce crossovers, and thus exchange-type CA, it appears necessary that conservative HRR pathways are tightly regulated in mammalian cells. Apart from the possibility that HRR is cell cycle regulated (it is expected to be most efficient after chromosome replication, during late S phase and G_2), another regulatory element is very likely the mismatch repair system which detects mismatches within the heteroduplexes formed between sequences that are not entirely identical but only very similar (homologous) and thus suppresses recombination (Radman, 1989, 1991; Rayssiguier *et al*., 1989, 1991).

As mentioned, SSA and NHEJ do not depend on the presence of sister chromatids and therefore are expected to occur principally throughout the whole cell cycle, especially during G_1. In both SSA and NHEJ, two DSB ends have to interact with each other directly, which means that these mechanisms require *two initial DSB* (four ends) to induce an exchange-type CA. In the presence of two DSB on different chromosomes, SSA in yeast was shown to occur at equal frequencies intrachromosomally (creating two interstitial deletions) and also interchromosomally (creating reciprocal translocations), which argues for the idea that each DSB end can search the whole genome for a partner (Haber and Leung, 1996). Therefore, SSA appears to be an important mechanism in the formation of exchange-type CA. The fact that exchange-type CA may also involve interactions between DNA sequences of little or no homology indicates that mechanisms of NHEJ also play a role in the formation of CA (Thacker, 1999c; Klugbauer *et al*., 2000).

Therefore, we may summarize that all pathways of DSB repair, both homology-dependent and homology-independent, have the potential to induce CA of the exchange type and can principally explain the origin of chromosome- as well as chromatid-type CA, depending on the cell cycle stage in which DSB are induced. Since it is not yet satisfactorily known how the different mechanisms of DSB repair are regulated during the cell cycle, it is impossible to distinguish which mechanism(s) is(are) responsible for a given type of CA.

Apart from exchange-type CA, chromosome breaks (induced in G_1) and chromatid breaks (induced in S phase or G_2) also occur spontaneously or after exposure to chromosome-breaking agents. In metaphase, breaks are visible as acentric fragments which can either be terminal or interstitial. It is conceivable that such breaks arise by DSB that have been incompletely repaired (see 'signal model' of Bryant, 1998) or not repaired at all. In PCC, chromosome breaks appear as single fragments in G_1 and as double fragments in G_2. Chromatid breaks appear as single fragments in G_2 PCC. Isochromatid breaks look like chromosome breaks and can only be differentiated from the latter by their origin in S or G_2. Double fragments can also result from repair of DSB, giving rise to polycentric chromosomes or centric ring chromosomes which can be clearly seen in FISH analyses. The exchange theory postulates that breaks result from incomplete exchanges; this would also be the case with incomplete DSB repair. It is also possible that breaks are unrepaired DSB. Irrespective of whether breaks are the result of incomplete repair or unrepaired DSB, the ends of such DSB may be protected from nucleolytic degradation by accretion of the DNA end-binding Ku heterodimer and other proteins associated with it.

When PCC is analysed in irradiated G_1 cells there are more fragments when PCC is induced shortly after as compared with later times after irradiation. This may indicate that 'early' fragments are DSB which are repaired over time and later fragments are DSB that remained unrepaired or are not repairable (Greinert *et al*., 1995, 1999; Cornforth, 1998). In similar experiments with G_0 lymphocytes using the FISH methodology it was shown that incomplete exchange-type CA are replaced by complete forms in a time span of 2 h after irradiation and it is tempting to assume that incomplete exchanges are repair intermediates (Greinert *et al*., 1995, 1999).

S phase-dependent clastogens do not lead to DSB directly and the question remains whether in these cases, also, DSB are the primary lesion in the formation of CA. In the sense of the Meselson–Radding model (see above), CA formation could

start from a single-strand break or gap induced directly or enzymatically at DNA sites damaged by S phase-dependent clastogens which initiate recombinational exchange (by inducing a second SSB in the partner DNA molecule). This is open to debate and it is imaginable that at sites damaged by S phase-dependent clastogens DSB may be directly formed enzymatically.

An important aspect in considering the mechanisms of CA formation are dose–effect relationships obtained after CA induction by DSB-inducing agents, especially IR. Taking into account the complexity of DSB repair discussed in this review and other factors, such as chromatin organization, dose–effect relationships of CA frequencies may be an average result of different processes and this may not give decisive information on the mechanisms of CA formation. In general, it can be expected that the probability of CA formation will increase with the number of initial DSB; as long as DSB are rare, the corresponding ends will either initiate an accurate mitotic HRR event using the sister chromatid in late S phase or G_2 or will be rejoined to their original partner DSB end by SSA or NHEJ. However, when large numbers of DSB are induced, as for example after IR irradiation (1 Gy produces ~40 DSB/cell), it is possible that the HRR, SSA and NHEJ mechanisms become overloaded, so that more errors in the form of CA occur by using homologous sequences in different chromosomes or rejoining originally unrelated ends, respectively.

Coming back to the three theories mentioned in the introduction, we may summarize as follows. When the terms 'breaks' in the breakage and reunion theory and 'instabilities' in the exchange theory are replaced by the molecular term 'DSB', all three theories, namely the breakage and reunion, the exchange and the molecular theory, are in accord with the DSB repair mechanisms described in this review. Therefore, we conclude that CA can arise by different types of repair mechanisms starting with a single or more DSB within a DNA duplex. The subsequent recombination mechanisms may introduce further strand breaks to initiate strand exchange. In this sense, the origin of CA may be described as 'no DSB—no aberration'.

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