Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. In epidemiological studies, it has been shown that people with elevated frequencies of CA in their peripheral blood lymphocytes have a significantly elevated risk of developing cancer [2–6]. Many types of cancers are associated with specific types of CA which are etiologic for the cancer in question [7]. Dose-response curves (DRC) for CA are different depending on the inducing agent, the cell cycle stage exposed and the type of CA analyzed. From DRC for CA induced by ionization of low linear energy transfer (LET) first interpretations of the origin of CA in a mechanistic sense were deduced [8]. DRC for dicentric chromosomes and reciprocal translocations induced by radiation in G0 lymphocytes in vivo and...
in vitro are similar and are therefore used in biological dosimetry [9–12]. The frequency of inborn CA in human population is quite high and it is of utmost importance to understand how CA originate and how they are transmitted to progenies [13,14]. Testing agents for their ability to induce CA has a firm place in screening strategies for mutagenic/carcinogenic agents [15,16]. CA are a small fraction of a huge amount of changes in chromosomal DNA and reflect an enormous plasticity of the genome which has far reaching consequences for evolution [17].

In the present study, the current status of the mechanisms of induction of CA, their identification and classification as well as their distribution among the genome is summarized and discussed.

2. DNA damage and repair lead to chromosomal aberrations

With the exception of polytene chromosomes, eukaryotic chromosomes are unicentric. They contain one continuous DNA molecule in the presynthetic phase of the cell cycle which is replicated during the S-phase. These DNA molecules are extremely long when compared to metaphase chromosomes, or to the respective fibrillar structures in interphase chromatin [18,19]. According to calculations made by DuPraw [18], human chromosome 1 contains a DNA molecule of 7.5 cm length which in interphase is packed in a fibrillar structure of 1350 μm. In metaphase, chromosome 1 is about 10 μm long. These packaging problems are mainly solved by proteins of various types [20,21]. Due to their enormous dimensions, DNA molecules in chromosomes are permanent targets of chemical or physical damage of diverse origin. One microscopically visible result of such damage are CA.

CA are induced by agents that damage chromosomal DNA [22–24]. Experimental analyses have shown that DNA double strand breaks (DSB) are the principal lesions in the process of CA formation [25–27]. DSB arise spontaneously at quite significant frequencies through a variety of cellular processes, and can be directly induced by ionizing radiation, certain antibiotics, or endonucleases [27,28]. Sources of spontaneously induced DSB are DNA replication and DNA excision repair by accumulating single strand breaks (for reviews see [29,30]), transposition [31], VDJ-recombination [32–34], antibody class switching [35], mitotic recombination [36–38] and oxidative damage [39].

UV-radiation and the majority of chemical mutagens are not able to induce DSB directly but lead to other lesions in chromosomal DNA which during repair, or DNA synthesis, may give rise to DSB and eventually to CA.

If left unrepaired, DSB may lead to broken chromosomes. In view of the enormous amount of DNA in a chromosome and its very complex protein-associated packing, it is likely that many isolated DSB will be so splinted with proteins that they probably never appear as breaks (severances) in metaphase chromosomes. Unrepaired DSB but also CA (that arise by misrepair of DSB, see below) may be cell lethal at interphase or mitosis, respectively. If repaired improperly, DSB may lead to mutations, chromosome rearrangements, and oncogenic transformation.

In eukaryotic cells, DSB are repaired by at least three different mechanisms: (i) homologous recombination repair (HRR), a highly accurate process that usually precisely restores the original sequence at the break; (ii) single-strand annealing (SSA), which leads to the formation of mainly interstitial deletions; (iii) nonhomologous DNA end joining (NHEJ) which joins two broken ends directly and usually generates small scale alterations (base pair substitutions, insertions and deletions) at the break site. Although NHEJ is considered the major pathway of DSB repair in vertebrate cells, evidence has emerged recently that vertebrate cells are also quite proficient at HRR [40–44]. This is consistent with the increasing number of vertebrate genes (e.g. XRCC2 and XRCC3) homologous to the members of the \textit{Saccaromyces cerevisiae} Rad52 gene group responsible for HRR in yeast. These results are in contrast to the current dogma that only yeast but not vertebrate cells are capable of repairing DSB efficiently by HRR and change our picture of DSB repair in vertebrate cells [45–49].

HRR and SSA involve the members of the Rad52 gene group and strictly require regions of extensive sequence homology. NHEJ depends on the products of the genes XRCC4-7 and can dispense with sequence homology (for a more detailed overview of the DSB repair processes and the proteins involved refer to some recent reviews [28,46–48,50]). The essential requirement of HRR for sequence homology
is reflected by the fact that it occurs preferentially between sister chromatids in cells undergoing mitotic cell cycles or between homologous chromosomes in cells undergoing meiotic cell cycles where it may lead to gene conversion. In addition, HRR can also occur between homologous DNA sequences in different chromosomes (ectopic HRR) which may lead to exchange type CA such as dicentrics and translocations (Fig. 1, upper panel). HRR is usually initiated by one single DSB to generate both correct intrachromosomal repair products, and incorrect exchange type CA (the two DNA ends of a single DSB interact with the two strands of a homologous unbroken DNA duplex). DSB between two direct repeat sequences can be repaired by SSA which leads to the deletion of one repeat unit and the intervening sequence (Fig. 1, lower panel). NHEJ rejoins DSB in the absence of extended sequence homology and is, therefore, a universally applicable pathway for the repair of DSB occurring within chromosome regions without sequence homology. SSA and NHEJ need a single DSB to generate intrachromosomal repair products (the two

Fig. 1. Examples for the possible formation of CA by different mechanisms.
DNA ends of a single DSB interact with each other. In contrast to the restitution generated by HRR, the repair products usually contain small scale changes NHEJ and deletions SSA. To generate exchange type CA, both NHEJ and SSA require at least two initial DSB (the four ends of two DSB interact cross-wise with each other). The repair pathways mentioned before compete actively for the repair of a DSB. The regulation of these pathways (what circumstances determine which pathway is used) is still enigmatic, but appears to occur at different levels during development and cell cycle progression [51]. Likewise, it is not known as to what extent a given DSB repair process contributes to the formation of a certain type of CA.

It is important to note that, in principle, each of the mechanisms shown in Fig. 1 is able to form any possible exchange type CA. It is also important to note that the number of initial DSB required to induce an exchange type CA depends on the mechanism involved. A single DSB is sufficient for homologous recombination (HR) while at least two initial DSB are required for NHEJ and SSA. The schemes and interpretations shown in Fig. 1 are confined to CA formed with a maximum of two DSB. More than two DSB can be involved in the formation of CA by the DSB-repair mechanisms described, and it is imaginable that these different mechanisms can participate in the formation of complex aberrations (see Fig. 2 and Fig. 3e and f).

HR, the most accurate process, depends on the Rad52 and Rad51 proteins and occurs usually between extended regions of sequence homology located on sister chromatids in cells undergoing mitotic cell cycles or on homologous chromatids in cells undergoing meiotic cell cycles. If, however, HR occurs within regions of sequence homology (e.g. repeats, pseudogenes) located on different chromosomes (ectopic HR), exchange type CA like reciprocal translocations (Fig. 1 (1)) or dicentrics and the corresponding fused acentric fragments (Fig. 1 (2)) can arise. In the examples given, a single initial DSB is created within a repeat sequence (red box) on the green chromosome. The subsequent steps are shown in the double-strand-break repair (DSBR) model on the left side (DNA duplexes represented by two lines with arrowheads pointing in 3'-direction; centromeres by vertical bars). The resulting DSB-ends are 5'-3' exonucleolytically resected to produce long 3'-single-stranded tails that invade the blue chromosome at a site of a homologous DNA sequence (red hatched box). The 3'-ends of the invading strands serve as primers for semi-conservative repair synthesis (black dotted arrows) of several kilobases length 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 Holliday junctions that are endonucleolytically resolved to yield (as shown in Fig. 1 (1)) reciprocal crossover or non-crossover products (not shown). Depending on the original orientation of the sequence repeat (arrows above red boxes) in the blue and green chromosomes, reciprocal translocations or dicentrics can arise. NHEJ requires two DSB to yield an exchange type CA but is independent of sequence homology. In the non-reciprocal example shown in Fig. 1 (3), the green centromere-containing fragment fuses with the blue centromere-containing fragment to yield a dicentric while the two acentric fragments do not fuse so that breaks arise. For this, the original DSB-ends have to be enzymatically modified (fill-in DNA synthesis, exonucleolytic trimming) to form a ligatable structure. These steps are enhanced by the Ku70/Ku80 heterodimer complex. It is important to note that the reciprocal event (dicentric plus fused acentric fragment) or the formation of a reciprocal translocation is possible as well. In Fig. 1 (4), a single DSB occurs between two direct repeat sequences on the green chromosome and is processed by the Rad52- and homology-dependent SSA mechanism: extensive 5'-3' exonucleolytic resection of the DSB-ends yields long 3'-tails that anneal at regions of sequence homology. Unpaired ends are nucleolytically removed and gaps filled. The resulting intrachromosomal deletion comprises one copy of the repeat and the intervening sequence. The formation of exchange-type CA by SSA (not shown) would require two initial DSB (as shown for NHEJ) which must occur within or near regions of sequence homology on two chromosomes.

In most cases, at least as long as the number of initial breaks remains small, DSB are likely to be repaired correctly or lead to small scale DNA alterations in the range of a few base pairs or kilobases which can be analyzed only by restriction mapping or sequencing. In some cases, DSB may lead to large scale alterations visible as CA under the light microscope. Therefore, CA are not special phenomena resulting from specific cellular activities, but are just the microscopically
Fig. 2. Schematic representation of five chromosomes with the same CA seen with Giemsa-stain (a), after painting with different single paints (b, d, f), with a dual-paint (c) and with mFISH painting (e). Classification of patterns using S&S and PAINT nomenclature are given for each subset.
Fig. 3.
visible part of a wide spectrum of products generated by different DSB repair mechanisms. Microscopical analyses of CA depend on staining of chromosomes. Especially, the methodology of fluorescence in situ hybridisation (FISH) uncovered unex-pected complexities of CA and this will be discussed in Section 3.

3. The impact of FISH-painting on the scoring and interpretation of chromosome-type aberrations

For many decades, studies of CA, and the theories derived from them, have been based primarily upon solid-stained microscopical preparations. Only asymmetrical forms of CA which give rise to acentric fragments [52] are readily visible in solid-stained preparations, and it seems that the dicentric shown in Fig. 2a is directly related to the acentric fragment, and has arisen from a simple, reciprocal, pairwise exchange following irradiation in G1 (a cyclical ex-change of order 2, or “c2” [53,54]. Solid-stained chromosome-type aberrations almost always lead to this conclusion, so that classification, interpretation, and foundational quantitative theory are all based upon the assumption of an almost exclusive domi-nance of c2 exchanges. Studies of chromatid-type aberrations indicate that many exchanges are “complex”, involving multiple breaks and/or chromosomes [52]. Such events were deemed to be rare for chromosome-type changes, and few are seen even with the application of chromosome-band ing techniques. However, in retrospect, early work from Drosophila [55–57] and from clinical findings [13,14,58] should have made us think more carefully.

When fluorescence in situ hybridization methods using chromosome-specific probes (“FISH-painting”) [59–64] began to be applied to induced chromosome-type aberrations, the picture changed and the older aberration scoring systems [52] proved to be inadequate. The positional arrangement of postulated initial breaks within chromosomes can be used to classify potential complex exchange configurations into fam-ilies by the CAB system (CAB: number of chromo-somes, number of arms, number of breaks) [65,66]. Random rejoining interactions between the break-ends produce many different exchange configurations from each family, each of which can generate a number of different single-paint patterns. Classification of these patterns forms the basis for the S&S nomenclature system that can be used for scoring [65,67–69].

The alternative scoring nomenclature system, “PAINT” [69,70] treats each painted element in iso-lation, assigning the rejoining events to one of five categories, and also provides a very versatile scheme for descriptive mapping of each element. Descriptions of patterns by both systems are used in Fig. 2. In the case of complex configurations (defined as three or more breaks in two or more chromosomes),
a variety of different single-paint patterns can arise from the same configuration, depending upon the position occupied by the painted participant. And because there are a limited number of possible patterns, different configurations can generate the same single-paint pattern [65,68,70].

Therefore, when only one, or a few chromosomes are painted, false inferences can be drawn about the degree of complexity (or otherwise) of the underlying exchange. Conversely, it is seldom possible from a single-paint pattern, to assign the CAB family of origin. Solid-staining in Fig. 2a shows a simple two-break (c2) asymmetrical interchange, “dicentric + acentric fragment”.

If with a single-chromosome FISH protocol, one of the dicentric participants is painted (Fig. 2b), the pattern (S&S 2A) is precisely that expected for a c2 dicentric, and, taken alone, confirms our inference. Multi-color protocols have been developed, two to three homologue pairs being painted in distinctive colors, often with the addition of a pan-centromeric probe. These protocols increase the number of aberrations recovered and can enhance the accuracy of interpretation [64,71–82]. Fig. 2c shows a result from a dual-paint protocol, and now, by chance, a symmetrical interchange between two of the other chromosomes (a “reciprocal translocation”, S&S 2B) is detected suggesting again a simple c2 exchange. The majority of symmetrical events are not seen with solid staining, yet these are the types that will be transmitted to future cell generations, and are therefore most likely to be of genetical significance. It is this efficiency to detect symmetrical, transmitted events that is making FISH so useful in the fields of long-term retrospective dosimetry [83–95], and of clinical and cancer cytogenetics [96–99].

If the initial single-paint had colored the other participant in the dicentric (Fig. 2d) we find that its terminal segment has gone to another centric chromosome, and not to the fragment. Formally, such an event requires the interaction of at least three breaks (a c3 exchange), so this signal (S&S 2G) indicates that the underlying exchange configuration is “complex”. The 2A pattern of Fig. 2b has given us a false signal; it is a pseudosimple pattern [66,82]. In critical quantitative work concerning aberration mechanistics, it is necessary to correct for such pseudosimple patterns [69,100].

Anomalous single-paint patterns of many kinds are relatively common at radiation doses used in cytogenetic experiments, increasing in number and variety as dose or LET rises [66,76,100–111]. Because such anomalous patterns are not seen with solid staining, it is obvious that for many years we have been under-estimating the cytogenetic damage that radiation causes, and of course this will have had an effect upon our theoretical ideas about aberration formation.

These data show that the underlying exchange configuration is “simple” or “complex”, not the pattern. The pattern is only a signal that alerts us (or fails to alert us) to the status of the exchange from which it was generated.

Relatively recently, it has become possible with the aid of differential fluorochrome mixes and sophisticated computer software to paint (pseudocolor) distinctively all the chromosomes in the karyotype [112–117]. So, for the first time, we can actually view the exchange configuration which is generating the single-paint patterns. This removes much (but not all) of the ambiguity of interpretation which arises from single- and multi-paint protocols. It has, however, introduced a number of other problems, not least the classification of actual configurations, which we have not faced before [53].

Fig. 2e shows a “multiplex-FISH” (m-FISH) picture of our chromosome group, and it can be seen at once that the situation is even more complicated than deduced from previous paintings. Using S&S nomenclature the configuration comprises a group of four single-paint patterns, 2A/2B/2G/3T. Simplistically, this suggests that it originates from the interaction of five breaks in four chromosomes, probably complex family CAB 4/5/5. Of the 544 possible rejoining interactions (restitution excluded) amongst the 10 break-ends, just 24 will give this collection of patterns. However, 12 of these involve all 10 ends in a single exchange cycle (an irreducible c5). The other 12 can be resolved, visually, into two cycles, a c2 and a c3. Such reducible complex patterns are termed “sequential exchange complexes” [53,82,118]. Whilst these constitute genuine random possibilities from a c5 exchange, they could also have arisen from two space-separated, independent exchanges, but there is no way to decide which is the actual origin. The relevance of the distinction lies in the fact that in the irreducible c5 situation, the red/yellow 2B is a pseudosimple,
but in the c2 + c3 case, it is a truly simple (c2) reciprocal translocation. These formation-mode problems are an inherent feature of all configurations derived from complex-generating families $\geq C/((A/4))$.

Interestingly, had the single-paint used happened to correspond to the yellow chromosome (Fig. 2f) the S&S 3T pattern would have signaled that a minimum of five breaks was involved in the exchange, and that the underlying configuration belongs to either CAB family 3/5/5, 4/5/5, or even higher. Comparison of panels (b), (d) and (f) emphasizes the limitations and care needed in interpreting patterns when only a few chromosomes are painted [68].

FISH-painting has thrown up a further anomaly not encountered in solid-staining studies, namely the “one-way” exchange (also called “non-reciprocal translocations”, “incomplete exchanges”, “terminal translocations” [70]). These patterns are “incomplete” in the sense that certain expected segments appear to be missing. With single-paint protocols, there are six frequently recurring patterns ($I \rightarrow VI$ [100]) plus a few rarer types. At reasonable radiation dose levels, one-way exchanges constitute typically $\sim 20–30\%$ of total exchange patterns, and currently, this frequency appears to be independent of dose or radiation quality.

Initially, it was thought that these represented exchanges where some segments had failed to join up (i.e. “incomplete” in the accepted cytogenetic definition [52]), but careful work using telomere probes has shown that the majority arise from complete exchanges where a terminal segment is so small that it does not register as a distinct visible signal [119–128]. As inferred from earlier studies [52] incompleteness is rare for chromosome-type aberrations (2–6%). This is in sharp contrast to chromatid-type exchanges where incompleteness of exchanges is between 30 and 50%.

The frequency and degree of chromosome involvement in complex exchanges has surprised everyone, and raised a host of problems regarding mechanisms of formation. How and where within the nucleus do so many chromosome regions and lesions come together? The situation is exacerbated by the findings that the nucleus has a very compartmentalized architecture, chromosome arms occupying very localized "domains" [52,129–137]. So chromosome “painting” has not only become a useful tool, but has given fresh impetus to re-examine the foundations of our thinking about the origin of CA. This section shows that if we paint DNA molecules participating in CA with different colors we may get different types of CA.

FISH studies discussed so far cannot be used to analyze paracentric inversions, transpositions within the same chromosome, longitudinal orientation of inserted segments and homologue/homologue events. G-banding would allow to do this to a certain extent, but the analysis is difficult and needs great experience. The methodology of m-BAND in which single chromosomes are covered with pseudocolored transverse bands is well suited to recognize such CA types [138,139]. Fig. 3a–f shows examples of different m-banded human chromosomes and of chromosome 5 involved in complex aberrations induced with the restriction endonuclease Alu.

Not only frequencies and types of CA but also inter- and intrachromosomal distributions of breakpoints have been intensively studied. This aspect is discussed in Section 4.

4. Intra- and interchromosomal distribution of breakpoints

The intrachromosomal distribution of CA breakpoints in plant and animal (including human) cells is not always random [140–156]. Results of intrachromosomal localization of “breakpoints” are difficult to interpret.

Firstly, we are only able to observe and score a selection of the original number, the residue that has been “fixed” in visible aberrations. It is by no means certain that these represent an unbiased sample with respect to positioning. As a constant reminder of this fact, it has been proposed that aberration derived breakpoints be termed “selected observed residual breakpoints” (SORB) [157].

Secondly, metaphase chromosomes are highly compacted and coiled structures (Section 1) and the condensation process, which is often differential along the chromosome, may alter the observed position relative to that in the extended interphase chromatin, where the aberration was originally formed.

Thirdly, with respect to chromosome-type (but not chromatid-type) aberrations observed in G-banded chromosomes, there is an inherent pattern recognition artifact (the “three-band uncertainty”) which biases placement of the SORB to a pale band [158]. This,
coupled with the variable efficiency with which different kinds of aberrations can be detected within different banded regions [159] introduces further bias into SORB location.

Fourthly, primary lesions are handled by different repair pathways which rarely give rise to CA and by this influence the location of SORB.

The discussion shows that SORB are at best a far-away echo of the location of primary lesions.

Our own analyses showed that in G-banded Chinese hamster ovary (CHO) cells about 75% of SORB in chromosome and chromatid aberrations induced by the restriction endonucleases AluI and BamHI as well as of chromatid type aberrations induced by DNaseI occurred in Giemsa-light bands. Breakpoint clusters produced by these endonucleases showed co-localization in specific G-light bands of CHO chromosomes, the heterochromatic long arm of the X chromosome was nearly devoid of breakpoints [160–162]. Exposure of metaphase spreads to these enzymes led to G-band patterns after Giemsa staining, but no endonuclease digestion was evident in the heterochromatin of the X chromosome [162]. It is possible that SORB positions reflect a better access of the enzymes to nuclease sensitive sites in G-light bands which are preferentially digested by the enzymes even in fixed metaphase preparations. SORB induced by neutrons and /H9253-rays in CHO chromosomes were also not randomly distributed, more than 60% occurred in G-light bands and major breakpoint clusters co-localized with those produced by endonucleases [163]. Following exposure to ionizing radiation energy deposition per unit volume should be random in the nucleus. However, it can be expected that less DSB are induced in highly condensed as compared to loose chromatin, because the DNA in the former will be more protected from radiation-induced radicals. This could be one explanation for the higher density of radiation-induced SORB in G-light bands, as compared to G-dark bands.

Of the various properties relating chromatin structure and chromosome organization to the distribution of SORB the visually most striking is the close correspondence with sites of hyperacetylation observed in CHO chromosomes [164].

There is an intimate relationship between histone acetylation status of chromatin and its transcriptional activity [165,166]. Acetylation of histones by histone acetyltransferases (HATs) is in dynamic equilibrium with deacetylation, mediated by histone deacetylases (HDACs) [167] and many protein modifiers of transcription have been shown to possess either HAT or HDAC components (for a review see [168]). Several DNase I hypersensitivity sites in the genome show co-localization with CIIP sequences, also contain HDACs [169–172].

Transcriptionally active chromatin has a more accessible structure than transcriptionally inactive chromatin, as indicated by its increased sensitivity to nuclease digestion [162,173]. Current ideas on chromatin remodeling during transcriptional activation envisage conversion of the highly ordered and compact 30 nm fiber, thought to be formed from a solenoidal arrangement of six to seven nucleosomes per helical turn and including histone H1 linkers [174], to an open ‘beads-on-a-string’ 10 nm fiber, lacking H1, that allows progression of RNA polymerase during transcription.

During interphase, transcribed and potentially transcribable (poised) chromatin (henceforth collectively referred to as active chromatin) have both a more open conformation and a higher level of histone acetylation than transcriptionally silent or ‘heterochromatic’ chromatin. When antibodies recognizing acetylated isoforms of histone H4 were used to investigate the nuclear distribution of acetylated chromatin by immunofluorescence in situ [175,176], it was found, perhaps surprisingly, that hyperacetylated H4 was present in transcriptionally inactive metaphase chromosomes. The distribution of acetylation is not uniform, but reflects almost exactly the distribution of actively transcribed DNA during interphase [176–178]. The production of G-band patterns in metaphase chromosomes exposed to endonucleases also shows that characteristics of interphase chromatin can be traced to metaphase chromosomes [162].

Principally, the same problems discussed with respect to the intrachromosomal distribution of SORB, pertain to their interchromosomal distribution. It has been shown repeatedly that the interchromosomal distributions of SORB induced by low LET radiation in presynthetic human lymphocytes in vitro are not random [179–181]. One reason for deviations from randomness may be non-random arrangements of chromosomes in the interphase nucleus [129,134,182,183].
The interchromosomal distribution of SORB of chromatin-type aberrations induced by low LET radiation in S-phase CHO cells showed interesting deviations from randomness. In the majority of chromosomes, the distribution of SORB was as expected according to chromosome length for simple breaks or for relative corrected lengths in exchanges calculated according to Savage and Papworth [157,184]. Chromosomes 2 and 8 were more often involved in exchanges than expected from randomness, and Z3 showed more chromatid breaks and interchanges [185].

Probably, chromosomes have different phenotypes with respect to their characteristics to form radiation-induced CA. Chromosomes 2 and 8 may have an “exchange” phenotype, chromosome Z3 a “break” and “exchange” phenotype [185].

In conclusion, it can be stated that deviations from random intra- and interchromosomal distributions of SORB reflect the complex interplay of primary lesions in chromosomal DNA, structure and intranuclear arrangement of chromosomes at the time of exposure to a chromosome damaging agent and different types of DSB repair activities. Telomeres, interstitial telomere repeat like sequences and subtelomeric regions seem to play important roles in the formation of CA and this will be discussed in Section 5.

5. Subtelomeric segments and telomeres

The incubation of chromosome preparations in a buffered salt solution at high temperature followed by Giemsa staining produces darkly stained subtelomeric segments (T-bands) with the rest of the chromosome lightly stained [186]. Electron microscopic analyses showed that T-banded sections of human chromosomes are intricate fibrous structures [187]. T-bands are a subset of GC-rich R-bands highly resistant to heat denaturation and to digestion with the restriction endonuclease MseI [188].

Scanning microphotometer systems and application software were used to generate computer graphic images allowing to obtain quantitative data on the structure of nuclei and chromosomes at the light microscopic level [189,190]. We used this system to study T-banded chromosome segments and detected differential distributions of high-density chromatin in the chromatids of these regions [191,192].

In some chromosomes high density areas in sister chromatids were arranged like sister chromatid exchanges and these were called t-SCE (Fig. 3b) [193]. The density patterns and the minute t-SCE may result from characteristic DNA-protein complexes in T-band areas of the chromosomes [194]. These findings fit to the idea that subtelomeric regions of chromosomes seem to be hot spots for the formation of symmetrical exchanges (SCE) which are not visible in the normal light microscope [195].

Cryptic aberrations in subtelomeric chromosomal regions have been shown to be associated with several human congenital abnormalities [196–199].

Since eukaryotic chromosomes are linear, they have two ends with special structures called telomeres which guarantee DNA synthesis at the ends of chromosomal DNA [200–205] and seem to play an important role in the formation of CA. The mammalian telomere is composed of a TG-rich double stranded region with a long 3’-extension of (T2AG)n repeats. According to proposed models, the 3’ tail displaces parts of the double stranded TG-rich sequence and forms a so-called t-loop. Telomeres are stabilized by several telomere-specific proteins including telomere repeat binding factors TRF1 and TRF2 [206,207].

Disruption of this structure, for example by over-expression of a dominant negative TRF2 protein, leads to DNA damage and to end-to-end fusions of chromosomes [208,209].

In contrast to these t-loop-guarded telomere ends, ends created by DSB are recombinogenic and are therefore used as substrates for DSB repair mechanisms (see Section 1). In addition, when DSB occur at interstitial telomeric repeat arrays, they can be stabilized by de novo synthesis of telomeric repeats [210–213]. It is supposed that the formation of new telomeric sequences protects DSB from nucleolytic degradation in a similar way to that which natural telomeres do [214]. The rather common “one-way exchanges” in which telomeric regions are involved are discussed in Section 3.

Apart from telomere-specific proteins, DNA repair proteins are also associated with telomeres, and these provide the basis for a further mechanism, by which telomeres may interfere with DSB repair activities. In yeast, DNA repair proteins stored in telomeres are translocated to sites of DSB due to DNA damage [215,216]. In mammalian cells, localization of DNA
repair proteins on telomeres has been shown as well and it appears that the intact telomere structure is required to maintain chromosomal integrity by DSB repair [217].

Thus, apart from guaranteeing DNA synthesis at the ends of chromosomal DNA, telomeres may have a dual role in (i) providing a stable chromosome end and (ii) saving the integrity of damaged chromosomal DNA by supplying stored DNA repair proteins.

In addition to the telomeric repeats at the ends of the chromosomes, rodent cells contain large interstitial telomere repeat-like sequences which have been shown to be involved in chromosomal rearrangements [218–222]. Except for chromosomes 1 and 2, Chinese hamster chromosomes have interstitial telomeric blocks near the centromeric regions (Fig. 3g). These regions tend to associate together in the interphase nucleus forming a chromocenter like organization. The presence of repetitive sequences and the proximity of such regions from different chromosomes promote exchange aberration formation following treatment with ionizing radiation, short wave UV and restriction endonucleases [223–226]. In addition, these types of aberrations occur spontaneously during culture, leading to amplification of telomeric repeats as well as to the origin of marker chromosomes [226]. It has also been demonstrated that the telomerase activity is upregulated both in vitro (Chinese hamster cells, human tumor cell lines) [227,228] and in vivo [201]. This effect should have consequences for repair of DNA damage as well as for formation of aberrations in these regions.

6. Concluding remarks

In conclusion it can be said that CA are microscopically visible changes in the single DNA molecules of chromosomes and chromatids and therefore can be regarded as “a kind of visible chemistry” in the sense of Darlington’s statement given at the beginning of this review.

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