

REVIEW ARTICLE

CHROMOSOMAL ABERRATIONS INDUCED WITH ENDONUCLEASES*

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ABSTRACT

Experiments with *Neurospora crassa* endonuclease, pancreatic DNase I and bacterial restriction endonucleases indicate that DNA double-strand breaks are ultimate lesions for the induction of chromosomal aberrations. Cellular uptake of such enzymes can be mediated by different methods which are discussed here.

Eukaryotic chromosomes are complex structures containing mainly DNA, basic proteins (histones) and nonhistone proteins (van Holde, 1989). It is generally agreed that before DNA-replication (G1-phase of the cell cycle) chromosomes contain one continuous DNA molecule, which in the largest human chromosome is 7.3 cm long, while in metaphase the respective chromosome is only 10 μ long (DuPraw, 1970). After completion of DNA synthesis (S-phase of the cell cycle), each chromosome contains two identical chromatids with one DNA molecule in each of them. The DNA molecules in chromosomes are complexed with proteins which makes them supercoiled. The structure of the chromosome can be changed spontaneously or by mutagenic agents, which can

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induce chromosomal aberrations in a S-phase independent or S-phase dependent manner. S-phase independent chromosome breaking agents induce aberrations in the stage of the cell cycle in which the cells are exposed. Treatment in a presynthetic phase of the cell cycle (G1 or G0 phase) leads to chromosome type aberrations, such as polycentric chromosomes or ring chromosomes, treatment during the S-phase leads to both chromosome- and chromatid-type aberrations, and treatment after the S-phase (G2-phase) leads to chromatid-type aberrations such as chromatid interchanges and various types of chromatid intrachanges. S-phase dependent chromosome breaking agents lead to changes in the DNA which during DNA synthesis are transformed to chromatid aberrations, i.e., treatment in the G1- or S-phase leads to chromatid type aberrations, treatment in the G2 phase does not lead to aberrations in the ensuing metaphase, but leads to chromatid type aberrations in the next metaphase, when the chromosomes with "S-dependent" lesions went through a S-phase. This pattern of S-dependent and S-independent activities gives a first clue as to the origin of chromosomal aberrations. S-independent chromosome breaking agents such as ionizing radiation, bleomycin and endonucleases are able to induce DNA double strand-breaks (DSB) directly. S-phase dependent agents induce chemical lesions of different types which during the S-phase may lead to DSB. The finding that restriction endonucleases (RE) induce chromosome aberrations in a S-phase independent manner supports the idea that DSB are ultimate lesions for the production of chromosomal aberrations.

We discuss here the importance of endonucleases for the understanding of the origin of chromosomal aberrations, using the chromosome breaking activities of *Neurospora crassa* endonuclease (NE), pancreatic deoxyribonuclease (DNaseI) and restriction endonucleases (RE) as examples.

Neurospora crassa endonuclease (NE)

NE is a single-strand specific endonuclease which recognizes single-strand gaps in DNA and cuts the intact strand opposite to the gap. When CHO cells or human peripheral lymphocytes are irradiated in the G2-phase of the cell cycle and posttreated with NE, many more chromatid type aberrations are induced than without NE. The explanation for this finding is that X-ray induced DNA single-strand breaks (SSB), which when induced in G2 do not lead to chromosomal aberrations, are transformed to DSB by NE and by this give rise to chromatid type aberrations (Natarajan and Obe, 1978; Obe *et al.*, 1982; Nowak and Obe, 1984). A transformation of SSB to DSB has been shown in neutral sucrose gradients following X-irradiation of CHO cells in G2 and posttreatment with NE (Natarajan *et al.*, 1980). This system not only works with X-rays but also with other agents which lead to SSB in the G2-phase of the cell cycle, such as bleomycin, the alkylating agents dimethyl sulfate, ethyl metanesulfonate, methyl-nitrosourea,

methyl-nitro-nitrosoguanidine, methyl methanesulfonate, hydrazine sulfate and caffeine. No influence of NE posttreatment on the frequencies of chromosomal aberrations in the G₂-phase of the cell cycle was found with the polyfunctional alkylating agents trenimon, diepoxybutane, diethyl stilbestrol, ultraviolet light and neutrons (Natarajan and Obe, 1978; Nowak and Obe, 1984; Nowak *et al.*, 1984). These results confirm the data obtained with X-rays and bleomycin, namely, agents which are able to induce SSB in DNA of G₂ chromosomes induce higher frequencies of chromatid type aberrations when the cells are posttreated with NE. Therefore this system can be used as a cytological test to detect SSB induced by various agents in the G₂-phase of the cell cycle (Nowak *et al.*, 1984 (Figure 1).

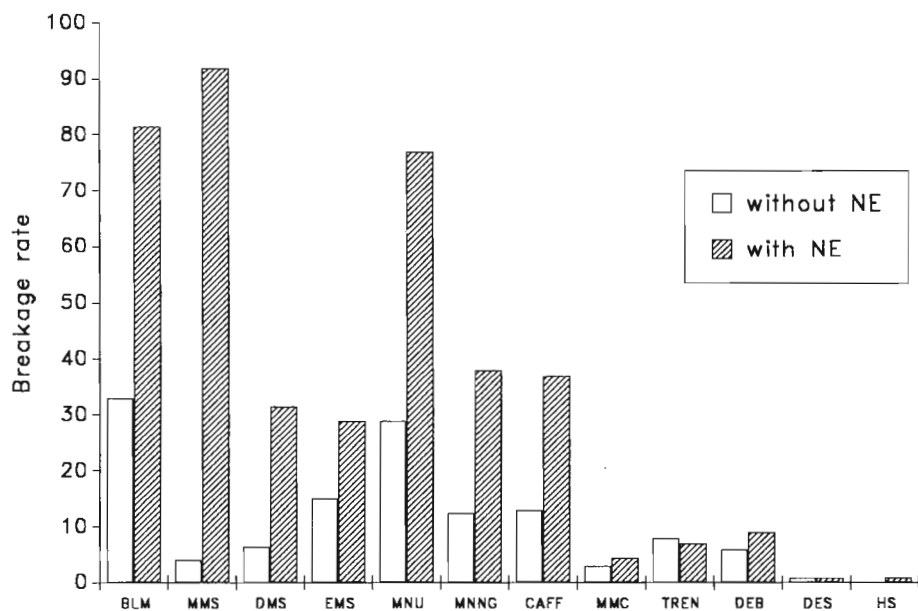


Figure 1 - Breakage rate obtained following treatment of G₂-phase cells with various chemical mutagens and posttreatment with *Neurospora crassa* endonuclease. The breakage rate was calculated by associating break events to aberrations as follows: Chromatid and isochromatid/chromosome breaks - 1; exchange type aberrations such as chromatid interchanges - 2. The breakage rate gives the number of break events per 100 metaphases. BLM - bleomycin; MMS - methyl methanesulfonate; DMS - dimethyl sulfate; EMS - ethyl methanesulfonate; MNU - methyl-nitrosourea; MNNG - methyl-nitrosoguanidine; CAFF - caffeine; MMC - mitomycin; TREN - trenimon; DEB - diepoxybutane; DES - diethyl stilbestrol; HS - hydrazine sulfate. The breakage rates induced by MMC, TREN, DES, HS and DEB are not influenced by posttreatment with NE. (Data from Nowak *et al.*, 1984).

DNase I

Pancreatic deoxyribonuclease (DNase I) binds to the minor groove of DNA and produces SSB with 3'-OH and 5'-phosphate ends (Suck *et al.*, 1988). In chromatin, DNase I induces SSB in the opposite polynucleotide strands and this leads to DSB with various base overhangs (van Holde, 1989; Lewin, 1990). DNase I leads to chromosomal aberrations in CHO cells in a S-independent manner. Polycentric chromosomes induced by DNase I in the G1-phase of the cell cycle are linearly correlated with the dose of the enzyme (Folle *et al.*, 1991).

Class II restriction endonucleases

Class II restriction endonucleases (RE) bind to the major groove of DNA at specific palindromic sequences and produce DSB with 3'-OH and 5'-phosphate ends (McClarín *et al.*, 1986; Kessler, 1987). Depending on the RE the recognition sequence can be 4 to 8 base pairs long and the DSB produced can have blunt or overlapping (sticky) ends. With different methods it has been shown that RE induce DSB in the chromatin of exposed cells (Bryant, 1984; Natarajan *et al.*, 1985; Costa and Bryant, 1990, 1991; Morgan *et al.*, 1990; Chung *et al.*, 1991). RE induce chromosomal aberrations in a S-independent manner (Obe and Winkel, 1985) whose frequencies are linearly correlated with dose (Bryant, 1984; Winegar *et al.*, 1989; Johannes and Obe, 1991). These results clearly support the idea that DSB are the ultimate lesion for the production of chromosomal aberrations (Figure 2).

Cellular uptake of endonucleases

Proteins cannot penetrate the cell membrane passively. Analyses with REs have shown that various methods can be used to introduce these enzymes into living cells, such as treatment of cells with RE in the presence of inactivated Sendai viruses (Bryant, 1984; Natarajan and Obe, 1984), by applying an osmolytic shock to the cells (Winegar and Preston, 1988), by applying RE included in liposomes (Mixich, 1991), by treating the cells with RE in the presence of glycerol, sorbitol or streptolysin O (Johannes and Obe, 1990; Johannes and Obe, 1991; Bryant, 1992) or by electroporation (Winegar *et al.*, 1989; Johannes and Obe, 1991). Comparative analyses have shown that the cellular uptake of Alu I in the presence of glycerol or sorbitol is energy-dependent, while the uptake *via* electroporation is independent of cellular energy (Johannes and Obe, 1991). The exact mechanism of uptake is not clear. The energy requirements indicate that in the presence of hypertonic concentrations of glycerol or sorbitol the uptake is mediated by an

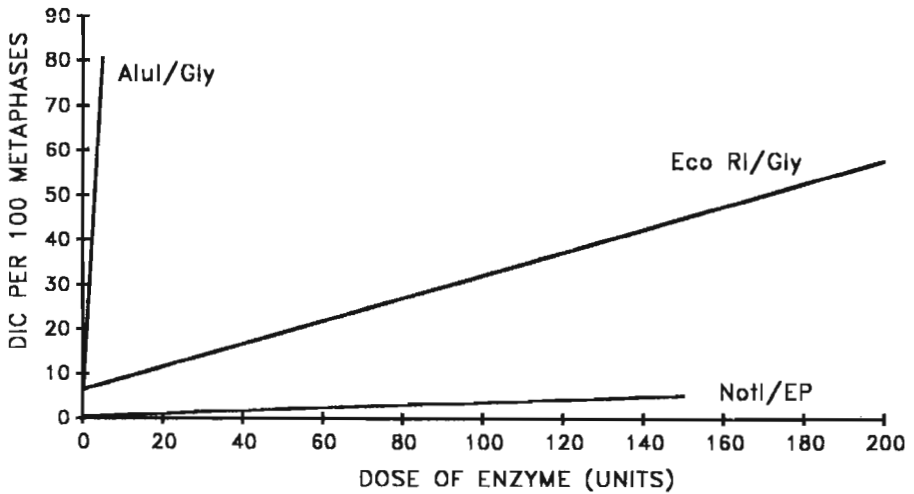


Figure 2 - Linear dose-effect relationships for dicentric chromosomes (DIC) induced in G1-phase CHO cells by AluI (recognition sequence AG/CT, blunt ends, cutting site indicated by the slash), Eco RI (recognition sequence G/AATTC, cohesive ends of 4 bases). NotI (recognition sequence GC/GGCCGC, cohesive ends of 4 bases). Gly: treatment in the presence of 2.2 M glycerol; EP: treatment *via* electroporation. DIC were calculated from all polycentric chromosomes by subtracting 1 from the number of centromeres in a polycentric and taking the result as number of dicentrics (e.g. a trivalent results in 2 dicentrics). These data indicate that the frequencies of chromosomal aberrations are dependent on the number of recognition sequences available to the enzyme. (Data from Johannes and Obe, 1991 and from Obe *et al.*, 1992).

energy-dependent endocytosis. Electroporation and streptolysin O lead to a partial breakdown of the cell membrane which may allow the enzyme to passively enter the cytoplasm. Viral preparations are acting in a similar way in that they induce a partial breakdown of the cell membrane. An osmolytic shock may lead to a breakdown of RE-containing vesicles in the cytoplasm which seems to be necessary for enzyme molecules to reach the nucleus.

DNaseI was shown to enter cells when applied *via* electroporation (Folle *et al.*, 1991) or enclosed in liposomes (Zayac-Kaye and Ts'o, 1984).

The uptake of NE was mediated by viruses (Natarajan and Obe, 1978) but it was shown that the enzyme can enter the cells in the absence of viruses (Obe and Natarajan, 1980). An explanation of this may be that the high concentration of $(\text{NH}_4)_2\text{SO}_4$ in which the NE-preparations are suspended induce a hypertonic shock and by this mediates the uptake of NE. It is not known how much enzyme enters the cells; a satisfactory dosimetry is therefore not possible. Studies concerning the cellular uptake of enzymes may help to solve this problem.

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RESUMO

Experimentos com endonuclease de *Neurospora crassa*, DNase I pancreático e endonucleases de restrição de bactérias indicam que quebras duplas na fita de DNA são lesões finais para a indução de aberrações cromossômicas, absorção pelas células de tais enzimas podem ser medidas por diferentes métodos, que são discutidos aqui.

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