Cell, Vol. 104, 329–332, February 9, 2001, Copyright ©2001 by Cell Press

Spontaneous DNA Damage, Genome Instability, and Cancer—When DNA Replication Escapes Control

Minireview

Primo Schär*
Institute of Medical Radiobiology
University of Zürich and Paul Scherrer Institute
CH-8008 Zürich
Switzerland

Human cancer can be viewed as a disease of underlying genetic instability. Most, if not all human tumors display some form of genomic instability, including subtle DNA sequence alterations, gross chromosomal rearrangements, aneuploidy, and gene amplifications. These alterations have the potential to affect the function of growth-regulating genes that are associated with the malignant transformation of cells. Therefore, to understand the early events in tumor development, we need to explore the origin of the genetic alterations that are typically found in human tumors.

The basis of genomic instability is unfaithful transmission of genetic information from a cell to its daughters. This arises from failure of cellular functions that ensure the accuracy of DNA transactions such as DNA replication, DNA damage repair, or mitotic chromosome distribution. Specific functional defects can be associated with a characteristic pattern of genomic instability. For example, inactivation of functions that increase the fidelity of DNA replication or eliminate mutagenic DNA lesions enhances the rate of subtle DNA sequence alterations. This is illustrated by the phenotypes of postreplicative mismatch repair (MMR) or nucleotide excision repair (NER) defects. Malfunction of MMR causes an increase in spontaneous mutation rate, microsatellite DNA instability, and a strong, heritable predisposition to cancer (reviewed in Jiricny, 1998). Similarly, NER failure, such as in Xeroderma pigmentosum and related genetic disorders, results in increased mutation rates induced by UV and an increased risk of cancer (reviewed in de Boer and Hoeijmakers, 2000). These specific genetic defects in DNA surveillance illustrate that increasing the rate of a particular form of genomic instability can contribute to the development of a tumor. However, the underlying causes of the more dramatic gross chromosomal aberrations that predominate in human cancers are less clear. An important question is whether the same principle applies, i.e., can connections be established between defined genetic defects that alter the rate of chromosomal aberrations and accelerated development of tumors? Perhaps the most convincing argument to date in favor of a genetic instability hypothesis is the karyotypic heterogeneity of most solid tumors (Mitelman et al., 1994), suggesting the persistent generation of novel genetic variants during tumor progression at an increased rate. The paper by Myung et al. (2001) in this issue of *Cell* provides evidence for a role of replication-associated DNA damage signaling in suppression of spontaneous chromosomal aberrations in *Saccharomyces cerevisiae*. The objective of this article is to evaluate these data and their possible implications for human cancer.

Routes to Chromosomal Aberration

If we accept that an initial mutational event early in the history of a tumor increases the rate of chromosomal instability, the question arises of what the possible genetic culprits might be. The wide heterogeneity of gross chromosomal aberrations associated with human tumors (Mitelman et al., 1994) suggests that a variety of different cellular processes and, hence, a great number of genes might be affected. For simplicity, we can assign two major categories of mechanistically distinct events: those that simply affect chromosome numbers, and those that alter chromosome structure. Chromosome number instabilities are found in most human malignancies and likely reflect malfunction of the mitotic chromosome segregation apparatus (Lengauer et al., 1998). However, changes in chromosome structure are equally frequent and point to irregularities in DNA metabolic processes rather than in chromosome distribution. Since these chromosomal aberrations usually involve breakage and rejoining of DNA segments, the underlying cause seems to be related to either the generation or the repair of DNA strand breaks. Studies in different models have established that treatment of cells with agents that induce DNA double-strand breaks (DSB's) leads to recombinational repair and can give rise to chromosomal rearrangements (reviewed in Friedberg et al., 1995). Enhanced mitotic recombination also results from metabolic accumulation of DNA strand interruptions during lagging strand DNA synthesis in yeast and human cells with a defective DNA ligase I (Lindahl and Barnes, 1992). Similarly, defects in replication associated RecQ-like DNA helicases such as the Sgs1p of yeast or the homologous Bloom's helicase (BLM) of human cells cause increased mitotic recombination and chromosomal instability (Frei and Gasser, 2000). These examples support the principle that irregularities during DNA replication can generate substrates for recombination and give rise to gross chromosomal aberrations. In tumor cells, which usually evolve in the absence of external sources of DNA damage, an enhanced rate of chromosomal instability could thus be accounted for by either increased formation of DNA strand breaks due to an endogenous DNA metabolic defect or enhanced irregular repair of strand breaks that arise during DNA synthesis or excision repair.

Irregular repair of a DNA strand break can be defined as the events that occur when its allocation to the appropriate repair pathway fails. Allocation to the proper repair system is not as trivial as it might seem, as the choice depends on where in the genome and in which physiological context strand breaks occur; i.e., a particular DNA repair pathway may be appropriate ("regular") in one situation, but inappropriate ("irregular") in another. For example, when a DSB arises at the site of a stalled replication fork during DNA synthesis, the S phase cell recognizes and reacts to the problem. Homologous recombination may be utilized to repair the DSB

*E-mail: schaer@imr.unizh.ch

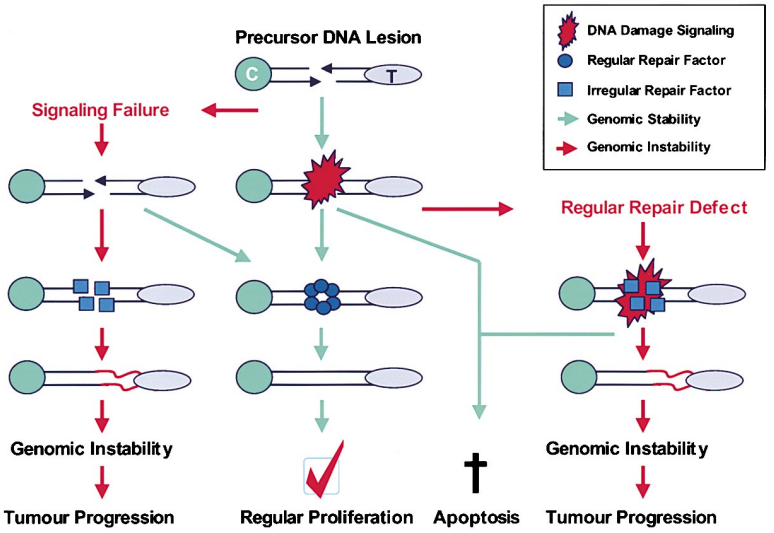

Figure 1. Options for Mutagenic and Nonmutagenic Repair of an S Phase DNA Lesion in the Presence or Absence of Regular DNA Damage Signaling and Repair Pathways

Depicted is an example of a DNA DSB arising during DNA replication. Damage signaling either provides for coordinated repair by an appropriate repair pathway (i.e., sister chromatid recombination) that restores the intact chromosome or subjects the cell to programmed cell death (center). Both events preserve genomic stability. If the regular repair system is nonfunctional (right side), "irregular" pathways may gain access to the damage and perform mutagenic repair (i.e., telomere addition, translocations, and end-to-end ligation), or damage signaling induces apoptosis. If damage signaling fails (left), coordinated repair of the DSB is disturbed and the option for regular repair may be lost as the cell progresses in the cell cycle. Irregular repair events then give rise to genomic instability.

using the sequence information from the unbroken sister chromatid (Paulovich et al., 1997; Johnson and Jasin, 2000). If sister chromatid recombination is nonfunctional because of a mutation inactivating homologous recombination, the cell can try to repair the damage through alternative pathways (i.e., nonhomologous endjoining or telomere addition) which, in this particular physiological context, are inappropriate and more error prone. Similarly, if the DSB arises unnoticed because of a failure in damage signaling, the cell passes on to later stages of the cell cycle where the option of repair by sister chromatid recombination may be lost. However, if the cell is to survive, the DSB must be repaired. Homologous recombination between non-sister chromatids or ectopic regions would risk loss of heterozygosity or the formation of chromosomal translocations. Nonhomologous end joining would increase the chance of chromosomal end-to-end ligation or of losing DNA sequence information, and addition of a new telomere to the broken chromatid would lead to the loss of the fragment distal to the DSB. This is just one possible scenario to illustrate that "irregular repair" of a DSB can occur not only when the appropriate pathway is nonfunctional, but also when the cellular DNA damage response is faulty and the correct order of DNA repair and cell cycle progression is disturbed (discussed in Paulovich et al., 1997). The underlying principle of the "irregular repair" idea is depicted in Figure 1, and leads to the argument that a failure in DNA damage signaling could be as harmful as a specific DNA repair defect, because it allows mutagenic repair of DNA damage while lowering the rate of apoptotic cell death. Consistent with this, most human tumors not only display chromosomal instability but also miss some checkpoints (reviewed in Hartwell and Kastan, 1994).

Spontaneous DNA Damage, Failing Checkpoints, and Chromosomal Instability

The data presented by Myung et al. (2001 [this issue of *Cell*]) argue for a role of an S phase DNA damage checkpoint in signaling of spontaneous DNA damage and suppression of chromosomal aberrations in *Saccharomyces cerevisiae*. The paper shows that mutations in *RFC5*, *MEC1*, *DDC2*, *DUN1*, and *PDS1* increase the rate of chromosomal aberrations by more than two orders of magnitude, while mutations in *MEC3*, *RAD53*, and *CHK1* cause intermediate effects. DNA damage checkpoints controlled by *RAD9*, *RAD17*, and *RAD24* appear to contribute only marginally to chromosomal stability in this assay, and mitotic checkpoint deficient *bub3* and *mad3* mutants did not show any effect at all.

RFC5 encodes a subunit of the replication factor C that functions as a PCNA loader during DNA replication and has also been implicated in signaling of replication associated DNA damage. *MEC1* encodes a member of the subfamily of phospho-inositide kinase type protein kinases (PI(3)K) that also includes the human *ataxia telangiectasia mutated* (ATM) and ATR proteins. It acts as a signal transducer in response to DNA damage and phosphorylates a number of downstream signaling factors such as Ddc2, Rad53p, and Chk1p. Therefore, the common chromosomal instability phenotype of *rfc5-1*, *mec1*, *ddc2*, and *dun1* mutants suggests that key components of an S phase DNA damage signaling pathway constitute a system for suppression of spontaneous chromosomal aberrations. However, there are some uncertainties associated with this interpretation of the genetic data. For example, Rfc5p is primarily a replication protein. It is therefore unclear whether the increase in chromosomal aberrations in the *rfc5-1* mutant is due to a failure in DNA damage sensing or to a defect in DNA replication that generates DNA lesions. The same applies to other factors discussed in this context, including Pol2p and Dpb11p, which are both essential subunits of DNA polymerase ϵ. Nevertheless, since the chromosomal aberration rates in *rfc5-1 mec1* and *rfc5-1 dun1* double mutants are not significantly different from those of either of the single mutants, the conclusion that the *rfc5-1* defect feeds into a DNA damage signaling pathway that involves Mec1p and Dun1p seems valid. The observation that *mec1* and *dun1* mutants are more severely affect in chromosomal stability than the *rad53* mutant is incompatible with the current view that Mec1p signals through Rad53p to Dun1p. However, the current set of genetic data is too limited to provide strong sup-

port for alternative models for functional interactions in S phase checkpoint signaling.

The role of Pds1p is also not entirely clear. Pds1p was first identified as an anaphase inhibitor that responds to DNA damage or malformation of mitotic spindles, but has also been associated with a Mec1p-independent late S phase checkpoint (Clarke et al., 1999). Mutation of *PDS1* causes a chromosomal destabilization phenotype comparable to that of *rfc5* or *mec1* mutants. The dramatic increase of chromosomal aberrations in the *pds1 mec1* double mutant indicates that Mec1p and Pds1p may function in separate but partially redundant pathways for DNA damage signaling. However, the detailed architecture of the relevant signaling pathways involved remains to be resolved.

The genetic assay used in this study selects for a specific class of chromosomal aberration events: for deletions of nonessential telomeric regions located on chromosomes V and VII (Chen and Kolodner, 1999). It may therefore bias an assignment of characteristic patterns of genomic rearrangements to specific DNA damage signaling defects. Nevertheless, in this particular system, the predominant events caused by mutations in *RFC5*, *MEC1*, and *DUN1* were deletions associated with simple addition of new telomeres. In *tel1* mutants, however, which showed wild-type levels of chromosomal instability, telomere additions were totally suppressed. *TEL1* encodes a Mec1p-related protein and thus belongs to the family of ATM-related protein kinases. Its primary role is the control of telomere lengths, but it has also been implicated in DNA damage signaling through phosphorylation of checkpoint factors such as Rad9p and Rad53p. The finding that the loss of Tel1p affects the ability to add new telomeres to broken chromosomes, but does not increase the rate of chromosomal aberrations suggests a function of this kinase in telomere regulation, rather than in suppression of chromosomal rearrangements through DNA damage signaling. However, this seems to be true only as long as the related Mec1p kinase is active. Inactivation of Mec1p in a *tel1* mutant increased the rate of chromosomal rearrangements synergistically, suggesting that the two related protein kinases can partially substitute for each other in signaling of spontaneous DNA damage in S phase.

What does the study tell us about DNA damage signaling? The source of DNA damage must be endogenous since the cells were not exposed to DNA-damaging conditions. Also, since the major signaling pathway includes the putative S phase–specific DNA damage sensor Rfc5p, the precursor lesion is probably a product of DNA replication. This is most likely to be DNA ends that arise as a consequence of stalled or collapsed DNA replication forks (Haber, 1999). Regarding the relevant signaling endpoint, I think the study provides some interesting clues. First, RFC5, MEC1, and DUN1 appear to belong to the same pathway for suppression of chromosomal aberrations. Unlike Rfc5p and Mec1p, Dun1p is not known to be required for DNA damage–dependent S phase arrest. Thus, the cell cycle arrest subroutine of the checkpoint may not be a primary contributor to chromosomal stability as measured by this assay. Instead, DNA damage signaling might be required for the regulation of DNA repair activities to coordinate individual DNA repair pathways. This might involve not only Dun1p-, Rad53p-, and Chk1p-mediated processes but also a direct regulation of the Mre11p-Rad50p-Xrs2 complex. This heterotrimer, which has DNA binding and nuclease activity, has been implicated in various DNA transactions, including DSB processing and recombinational repair, telomere silencing and maintenance, and DNA damage signaling (Haber, 1998). This multifunctionality makes the Mre11 complex a potential mediator at the interfaces between DNA damage sensing and signaling, and/or signaling and repair. The data from a previous study (Chen and Kolodner, 1999) show that the Mre11 complex, but neither homologous recombination nor nonhomologous end-joining, is necessary for chromosome stability. Thus, the role of the Mre11 complex in this context could be more closely associated with transmission of DNA damage signals than with a direct engagement in DSB repair processes. This is an attractive possibility that warrants further investigation.

Checkpoint Failure, Chromosomal Instability, and Cancer

To gain insight into the complex network of signal transduction pathways that monitor the state of DNA and provide for genetic stability, we are forced to use simple models that allow for a genetic and molecular dissection of general principles and individual functions. The example discussed above illustrates an approach with the yeast model that suggests a connection between an S phase checkpoint defect and a specific form of chromosomal instability. This is consistent with previous studies in yeast that have associated other cell cycle defects with increased mitotic recombination and chromosome nondisjunction (reviewed in Paulovich et al., 1997). What is the broader significance of these observations with regard to the more complex situation in human cells? Is there evidence for a causal relationship between checkpoint failure, genomic instability, and cancer? Studies of human cancer predisposition syndromes and mouse knockout models have revealed several connections between defects in DNA damage checkpoint genes and tumorigenesis. Well documented examples are the *ataxia telangiectasia mutated* (*ATM*) gene, *BRCA1* and *BRCA2*, the *NBS1* gene, the *BLM* and *WRN* genes mutated in patients with Bloom's and Werner's syndrome, respectively, and, of course, p53. In all of these cases, inactivating mutations cause defects in DNA damage signaling, give rise to some form of chromosomal instability, and increase the risk of cancer. *ATM*, for instance, is mutated in Ataxia telangiectasia patients (AT) that suffer from an increased incidence of leukemia and lymphoma. Cells from AT patients are hypersensitive to DSB inducing agents and show increased chromosomal aberration as well as a failure to induce a DNA damage response. The ATM-mediated DNA damage response consists of phosphorylation of key components of cell cycle checkpoints including p53, BRCA1, CHK2, and NBS1 (reviewed in Rotman and Shiloh, 1999). The related human protein kinase ATR interacts functionally with BRCA1 in a manner that suggests parallel action of ATM and ATR to enforce cell cycle checkpoints in response to distinct forms of DNA damage (Tibbetts et al., 2000). In mice, the loss of ATR is lethal. Homozygous $Atr^{-/-}$ embryos die early in development as a consequence of extensive apoptosis and the

cells from such embryos exhibit high levels of chromosomal aberrations (Brown and Baltimore, 2000).

Mutations in human *NBS1* underlie the AT-related Nijmegen breakage syndrome, a condition that is characterized by ionizing-irradiation sensitivity, a failure to arrest the cell cycle at G1/S in response to DNA damage, chromosomal instability, and cancer predisposition (Carney et al., 1998). The similarity of this syndrome to AT suggested that the underlying genetic defects might be related, and this was confirmed by the finding that ATM phosphorylates NBS1 in response to DNA damage (Lim et al., 2000). NBS1 is an ortholog of yeast Xrs2p and is part of the human MRE11-RAD50-NBS1 complex. The discovery of mutations in human *MRE11* causing yet another AT-like disorder (ATLD) substantiated the idea that ATM-NBS-mediated DNA damage signaling in humans involves the entire MRE11 complex, and that a failure of this pathway can result in chromosome instability and cancer.

Mutations in BRCA1 and BRCA2 predispose women to an increased risk of breast cancer and are also associated with high levels of chromosomal abnormalities. The DNA damage–induced functional interaction of ATM and ATR with BRCA1, and the coexistence in a superprotein complex of BRCA1 with ATM, the MRE11-RAD50-NBS1 complex, the mismatch repair proteins MSH2 and MSH6, and the Bloom's helicase BLM (Wang et al., 2000) suggest that the chromosomal abnormalities found in BRCA1 and BRCA2 tumors may also result from a failure of ATM/ATR dependent checkpoints.

Conclusion

These selected examples illustrate that DNA damage checkpoints form an integral part of the cellular defense against chromosome instability and may help to avoid cancer. The general principle seems established, but where do we go from here? Quite clearly, back to sorting out the details. A major limitation to genetic dissections of DNA damage signaling pathways is the pleiotropic nature of checkpoint defects and the functional ambiguity of factors acting at the interfaces between DNA damage sensing, signaling, and repair. A combination of genetics and biochemistry will be needed to unravel the general architecture of the network of interacting checkpoint pathways on the one side and to explore the rules of communication between DNA damage signaling and repair factors on the other side. Thus, there is plenty of fascinating work ahead.

Selected Reading

Brown, E.J., and Baltimore, D. (2000). Genes Dev. *14*, 397–402.

Carney, J.P., Maser, R.S., Olivares, H., Davis, E.M., Le Beau, M., Yates, J.R., 3rd, Hays, L., Morgan, W.F., and Petrini, J.H. (1998). Cell *93*, 477–486.

Chen, C., and Kolodner, R.D. (1999). Nat. Genet. *23*, 81–85.

Clarke, D.J., Segal, M., Mondesert, G., and Reed, S.I. (1999). Curr. Biol. *9*, 365–368.

De Boer, J., and Hoeijmakers, J.H. (2000). Carcinogenesis *21*, 453–460.

Frei, C., and Gasser, S.M. (2000). J. Cell Sci. *113*, 2641–2646.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995). DNA Repair and Mutagenesis. (Washington, DC: American Society for Microbiology).

Haber, J.E. (1998). Cell *95*, 583–586.

Haber, J.E. (1999). Trends Biochem. Sci. *24*, 271–275.

Hartwell, L.H., and Kastan, M.B. (1994). Science *266*, 1821–1828.

Jiricny, J. (1998). EMBO J. *17*, 6427–6436.

Johnson, R.D., and Jasin, M. (2000). EMBO J. *19*, 3398–3407.

Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1998). Nature *396*, 643–649.

Lim, D.S., Kim, S.T., Xu, B., Maser, R.S., Lin, J., Petrini, J.H., and Kastan, M.B. (2000). Nature *404*, 613–617.

Lindahl, T., and Barnes, D.E. (1992). Annu. Rev. Biochem. *61*, 251–281.

Mitelman, F., Johansson, B., and Mertens, F. (1994). Catalog of Chromosome Aberrations in Cancer. (New York: Willey-Liss).

Myung, K., Datta, A., and Kolodner, R.D. (2001). Cell *104*, this issue, 397–408.

Paulovich, A.G., Toczyski, D.P., and Hartwell, L.H. (1997). Cell *88*, 315–321.

Rotman, G., and Shiloh, Y. (1999). Oncogene *18*, 6135–6144.

Tibbetts, R.S., Cortez, D., Brumbaugh, K.M., Scully, R., Livingston, D., Elledge, S.J., and Abraham, R.T. (2000). Genes Dev. *14*, 2989–3002.

Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S.J., and Qin, J. (2000). Genes Dev. *14*, 927–939.