Genome-Scale Approaches to Investigate Oxidative DNA Damage

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Summary **In the trend of biological science after the completion of the human genome project, appreciation of an organism as a system rather than the sum of many molecular functions is necessary. On the investigation of DNA damage and repair, therefore, the orientation toward systematic and comprehensive genome-scale approaches is rapidly growing. The immunoprecipitation-based technique combined with high-density microarrays is one of the promising methods to provide access to such novel research strategies. We propose this sort of research area as oxygenomics.**

Key Words**: DNA immunoprecipitation, 8-oxoguanine, acrolein-modified adenine, chromosome territory, oxygenomics**

Introduction

In the current context of life science, integrative analyses, such as diverse 'omics' analyses, have been actively conducted with an aim to comprehend organisms as a whole. A rapid paradigm shift from the molecule to the system has been taking place over the whole area of biological science.

The fact that a draft sequence of the whole human genome was published in 2001 [*1*, *2*] has not simply meant that all of the sequence information of DNA, including human genes, was acquired. That was a revolutionary achievement in biological science, which enabled the information of the whole human genome to be handled as finite data in a computer. Consequently, genomics has forced biologists to reform their fundamental attitude when developing strategies for new studies of life science. Hereafter, we need to elucidate functions of the genome and systematic and comprehensive genome-wide approaches are becoming more required. Before this transition took place, biological research generally focused on one gene or one pathway at a time, but in the current circumstances, we need to appreciate an organism as a system in a genome-scale, taking the genome database into account. Based on this trend, in this review, we present an overview of the systematic and comprehensive genome-scale approaches to investigate the dynamics of DNA damage and repair with a primary focus on newly developed experimental technologies.

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Genome-Scale Views of DNA Damage

Little information has been acquired about the distribution of DNA damage over the whole genome. The information of the genome, unlike the transcriptome or proteome, essentially does not change in the life of an organism and shows high similarity between individuals in a species. Indeed, cells regard the maintenance of the stability of genome information as a mission of the highest priority. Because it threatens genomic integrity, DNA damage induced by various causative factors must be processed immediately. Viewing this process on a genomic scale raises a new important question. It is the problem of how each kind of DNA damage is distributed throughout a genome. For example, when studying the pathogenesis of cancer, information on the frequency of DNA damage at each genomic site is extremely valuable. At present, however, we can find very few studies that analyzed the frequency of generation, accumulation or repair of DNA damage across every genomic region.

It is important to map various kinds of damage in different types of cells under various physiological and pathological conditions. The term genomic damage includes many sorts of chemical changes of DNA molecules (e.g. oxidative modification of bases, formation of bulky DNA-adducts, single or double strand breaks, etc.). Different types of damage are produced through different processes and have different influences on the cell. Therefore, each type of damage is of particular importance in biology and medicine. In addition, it is thought that distribution of these forms of DNA damage may vary depending on the cell type and on the conditions under which the cells are placed.

A powerful experimental method to deal with this problem is based on immunoprecipitation technique. The basic idea of the method is that, after fragmentation of genomic DNA extracted from a specimen, we can isolate only the fragments bearing a marker of specific DNA damage by applying the principle of immunoprecipitation. There are two variations of immunoprecipitation for this purpose that differ in the sort of antigen to which the adopted antibody binds. The first one, DNA immunoprecipitation (DnaIP), uses antibodies that recognize DNA damage itself (Fig. 1). We recently analyzed the distributions of 8-oxoguanine and acrolein-modified adenine residues (acrolein-dA) on the whole genome by cloning and mapping the fragments obtained through DnaIP [*3*]. In another application, the genomic fragments adducted with benzo(a)pyrene diol epoxides collected in the same way were cloned [*4*]. The other immunoprecipitation-based method is the example of chromatin immunoprecipitation, which uses antibodies against the proteins associated with DNA damage and repair. Armed with these immunopreciptation-based methods, one can obtain the sample for mapping the sites at which particular genomic damage is generated or processed.

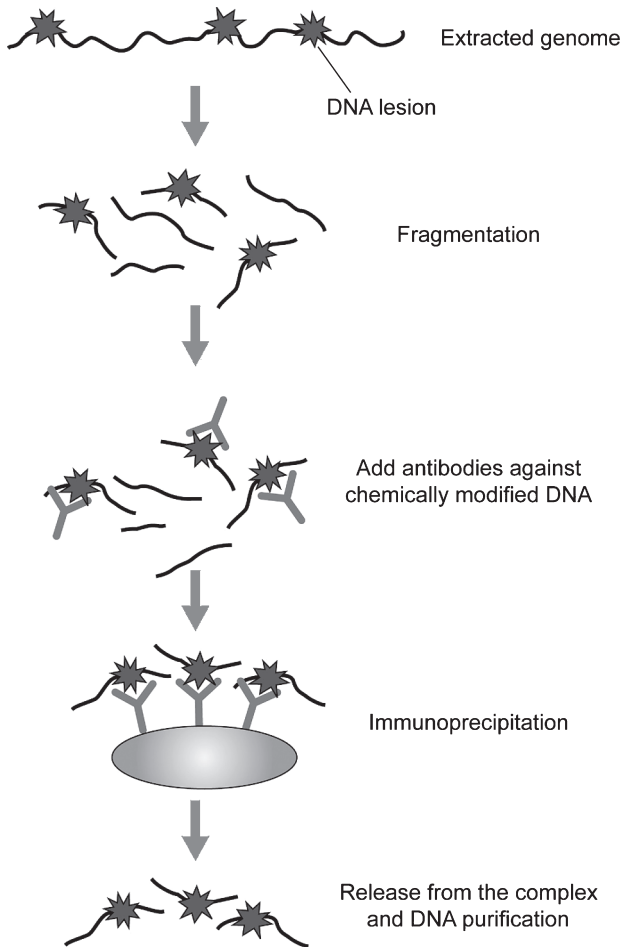

Fig. 1. Principle of DNA immunoprecipitation (DnaIP) method to analyze damaged genomic regions.

Approaches Based on Microarray Technology

DNA microarray is first on the list of the novel technologies that have made a great advance in the post-genome sequence era. The improvement in coverage and accuracy of the major genomic databases and the development of manufacturing technologies and analysis tools has supported the growth of this technology. Although it had been used to analyze transcriptomes in the early years, microarrays can now carry as many probes as can represent an entire genome on one chip, providing high-density views of genetic states. Thanks to the high capacity attained, the use of microarrays has expanded from gene expression profiling to include a variety of applications, such as array-based comparative genomic hybridization (CGH) [*5*], alternate transcripts [*6*] and microRNA [*7*] profiling, epigenomic analyses [*8*] and germline genotyping [*9*].

There have been two types of microarrays, which are made in different ways. One uses PCR products derived from genomic or cDNA clones as probes to detect targets. The prepared DNA for each probe is spotted on individual sections on the surface of a glass slide [*10*]. For the other type, an array of oligonucleotide probes is chemically synthesized on the chip itself [*11*]. In the early years, spotted microarrays were intensively used because of the cost advantage and the flexibility for customization. However, as the spotted array has a problem in specificity of hybridization, the oligonucleotide microarray, to which high-quality analyses can be applied, is generally used today.

The immunoprecipitation-based methods explained above can become more powerful tools when combined with microarray technology. The combination of the immunoprecipitation-based techniques and high-density microarrays covering a genome totally, such as a genome chip, enables us to take a snapshot of the dynamics of various genomic activities in full size.

If based on DnaIP, the DNA fragments collected using the antibody capable of catching chemically altered DNA are

applied to a genome chip, which reveals the distribution of the DNA modification throughout the genome in a single experiment. Recently, systems of array-based CGH for genomic analysis of cancer have been widely used in many laboratories. Those platforms provided by life science companies generally include the application software to analyze a vast amount of data generated from the result of the microarray experiment, which permits making full use of advanced information technologies to interpret the experimental output [*12*, *13*]. Thus far, an analysis of the methylation state of CpG islands was performed with this method [*14*]. We have not seen any publication of the example where this method was applied to DNA damage yet, but it will certainly appear pretty soon.

The system that applies the products of chromatin immunoprecipitation (ChIP) to the microarray (= DNA chip) is already named "ChIP-chip" or "ChIP-on-chip" [*15–17*]. In short, chromatin immunoprecipitation is the method to isolate, using specific antibodies, chromatin fragments that are bound by a particular nuclear protein or associated with a particular histone-modification signature [*18*, *19*] (Fig. 2). The ChIP-chip method has the potential to disclose the patterns of distribution on the genome of many kinds of DNA-binding proteins or histone-modifications in a single assay. The ChIP-chip technique is rapidly spreading as a tool to analyze the molecular phenomena involved in the control of various functions of the genome, such as transcriptional regulation or DNA replication and repair [*20–23*]. Also regarding the analysis of DNA damage, genome-scale assays with the ChIP-chip technique have been carried out by utilizing antibodies against the associated proteins that are recruited to the damaged site in the genome or the histone that received chemical modification. Employing an antibody against phosphorylated H2AX, a histone variant that aggregates at the DNA ends generated by double strand breaks, the distribution of the damage was analyzed in senescent human cells [*24*].

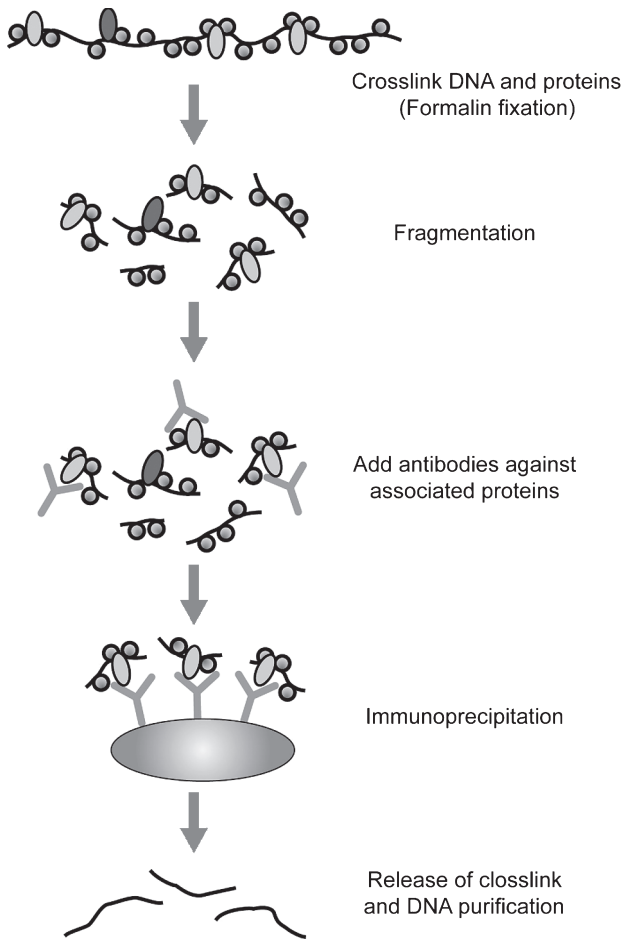

Fig. 2. Principle of chromatin immunoprecipitation (ChIP) method.

Approaches Based on Cytological Methodology

The achievements of the genome projects of major model organisms have, no doubt, defined a new paradigm in 21st century biology. Knowledge of the genome sequence has certainly expedited the search for genes responsible for specific medical disorders, simplified the search for homologues of genes among the species sequenced and allowed us to predict likely gene units that used to be unknown. Sequence information alone, however, is not always sufficient to predict how frequently a gene is transcribed, how the frequency varies depending on the cell type and what function the gene product might perform, because the extremely complex gene expression pathways found in mammalian cells are regulated at multiple different levels. While DNA-binding proteins and their interactions with the initiation complex drive transcription, it is now clear that the efficiency and the selectivity of this process are strongly influenced by higher nuclear organization [*25*]. Spatial organization of genomes also influences DNA replication and repair in mammalian cells [*26*, *27*]. Thus, the study of the spatial architecture within the eukaryotic nucleus is of greater importance in the post-genome sequence era.

Recently, technologies to visualize chromosomes and other nuclear structures, especially during interphase, under a microscope are growing rapidly. This owes mainly to the development of the fluorescence imaging technique and the improvement of the quality of genome databases. Chromosomes in mitosis or meiosis and during interphase can be visualized by the application of fluorescence *in situ* hybridization (FISH) [*28*, *29*], whereas the spatial localization of the proteins involved in transcription, replication or repair and the modification of histones can be detected using fluorescence immunocytochemistry [*30*]. Further, experi-

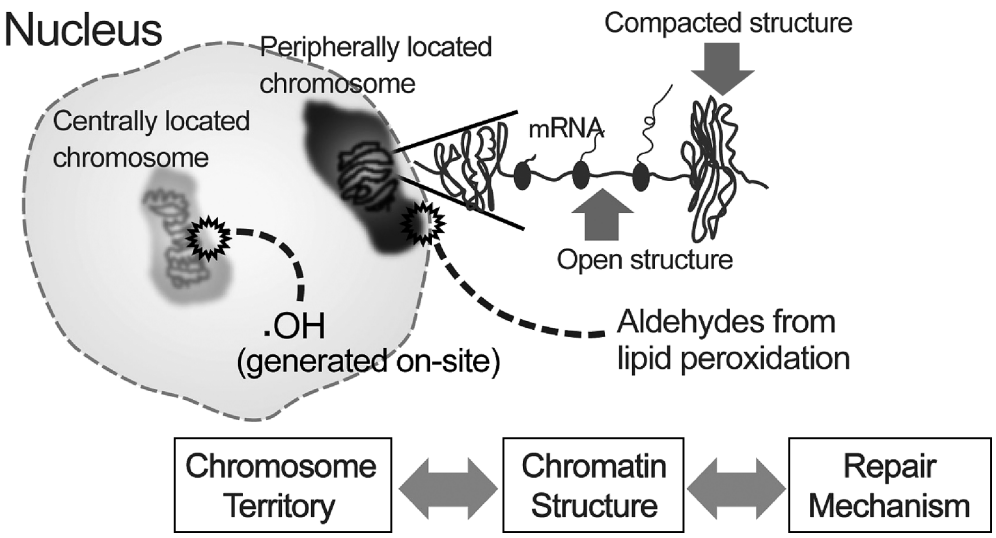

Fig. 3. Frequency of DNA damage is determined by distinct mechanisms in the cell.

mental systems to monitor the motion of these nuclear components in a living cell along the time axis are now being developed, making use of fluorescently conjugated proteins microinjected into the cell or constructs fused with a fluorescent protein transfected into the cell [*31*].

There have also been recent studies that analyzed the distribution of the marker relevant to DNA damage using traditional cytogenetical methods with metaphase spreads. Ohno *et al.* [*32*] detected 8-oxoguanine residues on metaphase chromosomes of human lymphocytes by applying fluorescent immunostaining. On the other hand, Surrallés *et al.* [*33*] combined FISH on metaphase spreads with the immunoprecipitation-based isolation procedure. In order to elucidate the site-specificity of nucleotide excision repair (NER), they labeled the genomic sites where unscheduled DNA synthesis had occurred after UV irradiation with 5-bromodeoxyuridines (BrdUrd) in human fibroblasts deficient in NER, collected the fragments containing BrdUrd-labeled repair patches after DNA extraction and hybridized the fluorescent DNA probes prepared from the collected fragments to the metaphase chromosomes.

Recently, the concept of chromosome territory (CT) has been established [*34*, *35*]. This concept indicates that each chromosome occupies a spatially limited volume in the nucleus, even at interphase. The arrangement of CTs appears to be different among different types of cells [*36*]. It is possible that the genome areas susceptible to oxidative damage may differ depending on the spatial organization of the genome in each cell. Accordingly, we decided to explore the relationship between the distribution of the oxidative DNA damage on the genome we detected and the arrangement of CTs in biologically identical cells. Interestingly, we found that a chromosome on which 8-oxoguanines were detected very frequently concentrated at the center of the nucleus, whereas a chromosome with a high incidence of acrolein-dA was located toward the nuclear envelope in the result of chromosome painting, i.e. FISH with the probe representing a whole chromosome, against interphase cells [*3*]. This may be explained by the fact that acrolein, an aldehyde, comes from the cytoplasm or directly from the nuclear membrane (Fig. 3). We propose this sort of research area as “oxygenomics”.

Concluding Remarks

This is the post-genome sequence era, given the completion of genome projects of humans, mice, rats and other species. The sequence information in public databases is a great advantage for researchers of oxidative DNA damage who are aiming for genome-scale approaches. Furthermore, current advances in microarray technologies for high-resolution analyses and visualization technologies for *in situ* detection promise to provide them with clear and detailed views of genome dynamics involved in DNA damage and repair.

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Abbreviations

BrdU, 5-bromodeoxyuridines; ChIP, chromatin immunoprecipitation; CGH, comparative genome hybridization; CT, chromosome territory; DnaIP, DNA immunoprecipitation; FISH, fluorescence *in situ* hybridization; NER, nucleotide excision repair; UV, ultraviolet.

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