

Chromosome aberrations: Plants to human and Feulgen to FISH

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Chromosome aberrations and their impact on human health have been recognized for a long time. In the 1950s, in India, studies on induced chromosome aberrations in plants were initiated by Swaminathan and his students. I trace here the impact of these initial studies on further developments in this field. The studies which were started in plants have been extended to mammals (including human) and the simple squash and solid staining have been improved by molecular cytogenetic techniques, thus enabling accurate identification and quantification of different types of chromosome aberrations. These studies have also thrown light on the mechanisms of chromosome aberration formation, especially following exposure to ionizing radiation.

Keywords: Chromosome aberrations, radiation, feulgen, FISH.

It is more than twelve decades since the existence of chromosomes and their division during cell proliferation were demonstrated. Since then, advances in our knowledge with regard to the fine structure of chromosomes, their replication during cell division, the localization of genes in chromosomes, transcription and expression of genes at chromosomal level have been considerable. At the turn of the last century, Hugo de Vries described mutations in the plant *Oenothera* which later turned out to be due to mal-segregation of chromosomes. Spontaneously occurring chromosome structural aberrations were studied intensively in plants using pachytene analysis during male meiosis¹ and salivary gland chromosomes of *Drosophila*². Thus, most of the fundamentals in chromosome cytology were laid out even before mammalian cytology came into being. With the introduction of hypotonic shock to spread the chromosomes in mammalian cells by Hsu³ and the technique to culture human lymphocytes using phytohaemagglutinin as a mitogen⁴ it became easy to study mammalian chromosomes. Tjio and Levan⁵ reported that the diploid number of chromosomes in man was 46. The importance of both structural and numerical chromosome changes to human health was slowly recognized. Several syndromes and diseases such as Down syndrome (trisomy 21), Edward syndrome (trisomy 18), Patau syndrome (trisomy 13), Turner syndrome (XO) and Klinefelter syndrome (trisomy X) were found to be

associated with chromosomal alterations. The spontaneous frequency of structural and numerical chromosomal alterations in humans is in the order of 6 in 1000 newborns. Chromosome analyses of spontaneous abortuses indicate that about 50% of all spontaneous abortions are chromosomally abnormal. Many types of human cancer are associated with specific and non-specific chromosomal aberrations. Several human recessive diseases such as ataxia telangiectasia, Fanconi anemia, Bloom syndrome are associated with elevated frequencies of chromosomal aberrations. Populations exposed to ionizing radiation (atom bomb survivors) and chemical mutagens (e.g., workers occupationally exposed to vinyl chloride or consuming arsenic contaminated water) show increased frequencies of chromosome aberrations in their lymphocytes as well as increased frequencies of cancer. All these observations point to the importance of studying the origin and significance of chromosome aberrations.

In the middle of 1950s, M. S. Swaminathan joined the Botany Division as Assistant Cytogeneticist and progressed to be the Senior Cytogeneticist and Head of the department. One of the many lines of research he initiated during the period was chemical and radiation-induced mutations in crop plants, such as barley, wheat, cotton, *Brassica*, etc. One aspect of mutagenesis is the induction of gross chromosomal aberrations which can be detected in somatic cells in root meristems and gametic cells such as pollen mother cells in plants. He with his students initiated several studies on the mechanisms of induction of chromosome aberrations following mutagenic treatment. I had the privilege of being his first doctoral student and in this review, an attempt is made to survey the earlier studies in plant cells and how far these concepts developed during that period have led to the understanding of formation of chromosomal aberrations in mammalian cells.

Detection of chromosomal aberrations

The ease and accuracy of detecting and quantifying chromosomal aberrations have largely depended on the progress in the development of cytological techniques over the years. Most of the important concepts on the spontaneous and induced chromosomal aberrations came from the study of plant tissues, such as root meristems (e.g., *Vicia faba*), pollen mother cells and pollen grains (e.g., *Tradescantia*) which were amenable for simple smear and squash techniques using stains such as aceto-carmine, aceto-orcein

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and Feulgen. Low number and large-sized chromosomes made these plant species ideal for chromosome studies. Already in 1930s, Karl Sax⁶ had established the basic concepts on radiation-induced chromosomal aberrations in his 'breakage first' hypothesis, implying that the initial radiation-induced event is a chromosome break and this can remain open (break), rejoin with another break (rejoin to produce a dicentric or translocation) or reconstitute. It should be appreciated that these basic ideas were generated at a time when neither chromosome structure nor DNA structure was well understood. After the World War II, the discovery of chemical mutagens came to light^{7,8} and it was soon reported that mustard gas induced chromosome abnormalities in *Tradescantia* microspores⁹.

At the Indian Agricultural Research Institute (New Delhi), studies on chromosome aberrations were carried out with crop plants, namely barley (*Hordeum vulgare*), different species of wheat (*Triticum monococcum*, *T. durum* and *T. aestivum* and their relatives) in the fifties. Nitrogen mustard, a bifunctional alkylating agent which was extensively used for chemotherapy of tumours at that time, was the first chemical tested for its chromosome breaking ability in wheat (*T. dicoccum*, *T. aestivum*) maize (*Zea mays*), pea (*Pisum sativum*) and broad bean (*Vicia faba*)¹⁰. Feulgen squashes of root tips and anthers were used. With this technique only gross chromosomal aberrations, such as fragments could be scored in mitotic cells and exchanges, deletions and duplications could be scored in meiotic cells.

With advancement in techniques, mammalian cells could be cultured and well spread chromosome preparations could be made with ease. However, individual chromosomes in human or mouse could not be identified easily except by length and the position of the centromere. A breakthrough came in late 1960. We had reported several types of localized chromosome breakage following high LET irradiation of diploid wheat seeds¹¹. In further studies with alkylating agents and base analogues, non-random induction of chromosome breaks along the chromosomes of *Vicia faba* was observed and this was interpreted as to the differential base composition (i.e. AT-rich and GC-rich) of these regions¹². The GC-rich regions of *Vicia faba* were found to be heterochromatic in nature and late replicating, the alkylating agents specifically bind to these regions. When mitotic preparations of *Vicia faba* root tips were treated with alkylating agents which were attached to fluorochromes (quinicrine mustard), the heterochromatic regions could be identified as specific fluorescent spots. This observation originally made at Stockholm by an Indian visiting scientist, Ulhas Wagh formed the basis for the chromosome banding techniques¹³. Strangely, Wagh happens to be one of the last authors in that paper!. This was followed by the development of several banding techniques, such as G banding, R banding, etc. to identify individual chromosomes, especially in human and other mammalian species. Chromosome banding allowed detection of specific chromosomal translocations in different human cancers,

such as chronic myelogenous leukemia, Burkitt's lymphoma, and in several chromosomal disorders in newborns in humans. Karyotypic analysis using banding techniques enabled one to trace the relationships between different species of animals and plants.

One of the areas of research initiated by Swaminathan was the origin of different crop plants, such as *Triticum*, *Brassica*, *Solanum*, etc. by analysis of chromosome morphology and pairing behaviour during meiosis in interspecies crosses^{14,15}. Though it was recognized that the hexaploid *Triticum aestivum* comprised three diploid genomes (AA, BB, DD), the contributor of the B genome was attributed to two different species, namely, *Agropyron triticum* and *Aegilops speltoides*. In spite of the fact that plant chromosomes were not amenable to good banding, the resolution was good enough to trace the origin of B genome of the hexaploid wheat conclusively as *Aegilops speltoides*¹⁶.

Radiation-induced chromosome aberrations in plant cells

Due to limited resolution of the cytological techniques available in the 1950s, the types of chromosome aberrations that could be scored were limited to simple breaks, exchanges and anaphase bridges in mitosis and reciprocal translocations, duplications, deficiencies and inversions in meiosis¹⁷. However, by employing a species such as diploid barley, detailed analysis of the types of aberrations such as dicentrics and rings became feasible. Some of the basic concepts on the organization of the interphase nucleus and formation of radiation-induced chromosome exchange aberrations were developed¹⁸⁻²¹. For example, the importance of how different chromosomes and their arms are organized in the formation of exchange aberrations (dicentrics, centric rings and reciprocal translocations) was recognized and a model was proposed¹⁹. According to this model, exchanges are formed at nuclear sites, where two or more chromosome strands lie in close proximity. Centric rings (asymmetrical exchanges between the two arms of a chromosome) are formed at sites where only two chromosome strands come together, whereas inter-chromosomal exchanges can occur at sites where two or more strands come close together, allowing the possibility of more interactions. Dose response kinetics following low and high LET radiations fit this model. This would also predict that after high LET irradiation, more complex exchanges would be formed, though such complexes could not be recognized at that time with the techniques available (see the section on 'Radiation induced complex exchange aberrations' below). Similarly, if the chromosomes are separated, for example the nucleolus separating the satellited chromosomes, interchanges between these chromosomes will be restricted¹⁸. Similar observations were made later in mammalian cells, i.e. exchanges between the two X chromosomes (which are separated in the interphase due to the inactivation of

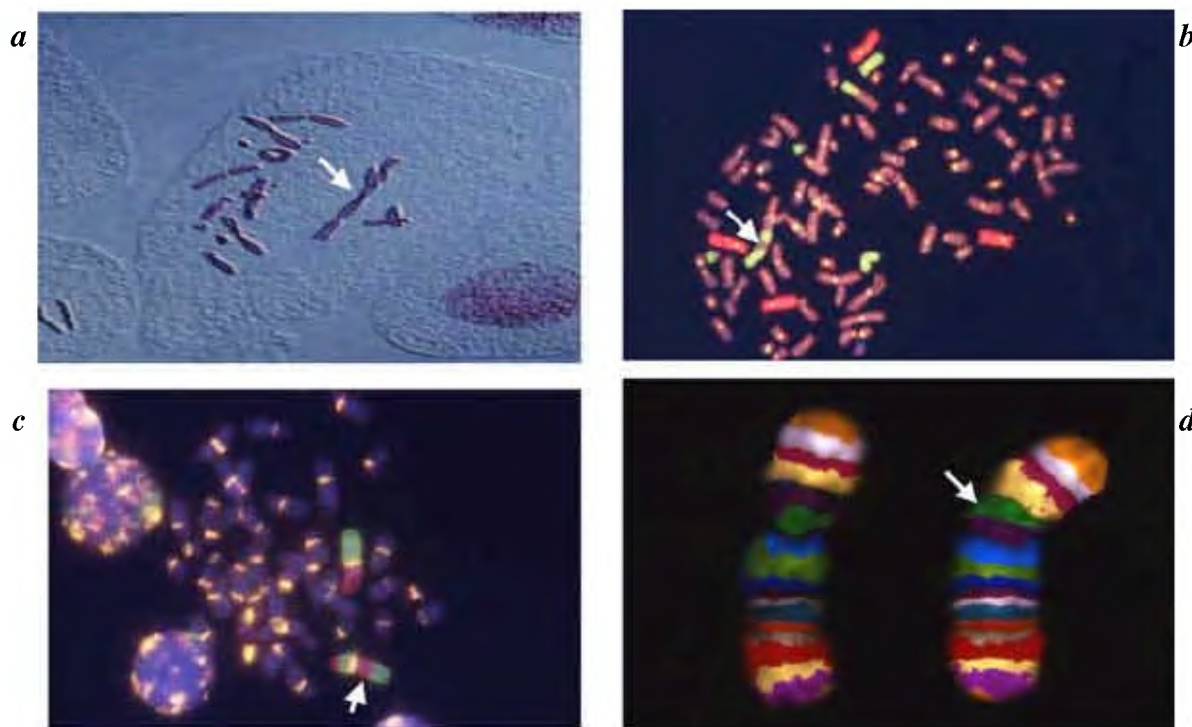


Figure 1. *a*, X-rays induced dicentric (arrow) and fragment in *Vicia faba* – Feulgen staining. *b*, Two colour FISH in X-irradiated human lymphocyte following two colour FISH, showing interstitial translocation (arrow) and another simple translocation; *c*, X-rays induced pericentric inversion (arrow) in chromosome #1 of a human lymphocyte following FISH employing arm-specific DNA libraries; *d*, X-rays induced paracentric inversion (arrow) in chromosome 5 of a human lymphocyte following m-band FISH.

one of X chromosomes forming a chromocenter) in Chinese hamster and *Microtus agrestis* embryonic cells^{22,23}.

Chromosome aberrations in mammalian cells

The basic principles laid out for the formation of ionizing radiation-induced chromosome aberrations⁶ were found to be true to mammalian cells as well. These include: the primary lesion induced is a chromosome break which can reconstitute, or remain open (detected as a break in metaphase) or rejoin with another break if present close in time and space to give rise to dicentric, translocations and intra-changes. The dose response curve following low LET (Linear Energy Transfer) radiation (e.g., X-rays, γ rays) for induction of exchanges is linear-quadratic, whereas, for high LET radiation (e.g., neutrons, α particles) it is linear. Though at the time when the principles were laid down, the structure of chromosomes was not known, with the discovery of DNA structure by Watson and Crick and the demonstration that chromosomes replicate similar to a double-stranded DNA molecule²⁴ and inhibitors of DNA synthesis such as fluorodeoxyuridine induces chromosome breaks, it became clear that DNA is the main target for induction of chromosome aberrations both following radiations and chemical mutagens²⁵. DNA double strand break (DSB) was proposed to be the important lesion leading to chromosome aberrations²⁶. First direct evidence for the involvement of DSBs in the formation of chromosome aberrations came from studies involving combina-

tion treatment of X-rays and *Neurospora* endonuclease (an enzyme that converts single strand regions into double strand breaks)²⁷ and restriction endonucleases (which induce exclusively DSBs)^{28,29}.

With solid staining (e.g., Feulgen or Giemsa), asymmetrical chromosome exchanges such as dicentric and rings can be detected easily (Figure 1 *a*), whereas symmetrical exchanges such as translocations and interstitial deletions cannot be detected. Chromosome banding techniques can be employed for analysis of such aberrations as has been done in the case of different human cancers, where analysis of few cells can reveal the pattern. In case of studies on induced chromosome aberrations, large number of cells, each carrying a different type of aberration, have to be scored. Chromosome banding techniques are too time consuming and labour intensive for such studies. Fluorescence *in situ* hybridization (FISH) introduced in late 1980s has proved to be very useful for analysis of chromosome aberrations³⁰.

FISH technique involves hybridization of DNA probes to denatured metaphase chromosome preparations followed by direct or immunological staining procedures. Whole chromosome-specific DNA libraries could be generated by fluorescent sorting of chromosomes based on their length and differential base composition (AT or GC-rich) isolation followed by amplification of DNA. Such libraries have been generated for human³¹, mouse³² and Chinese hamster^{33,34}. Whole chromosome, arm-specific and region-specific DNA probes were also generated by micro-dissection from mitotic or meiotic chromosome preparations

of human³⁵, mouse³⁶ and Chinese hamster³⁷, followed by amplification by polymerase chain reaction. Combination of these types of probes with those specific for centromeres and telomeres allows accurate detection of all types of chromosome aberrations. Thus, some of the early findings on plant cells by us at the Indian Agricultural Research Institute could be confirmed and extended with greater details.

Radiation-induced chromosome fragments

Though chromosome fragments are usually pooled into one class, namely acentric fragments, the use of telomere and centromere-specific probes allows the identification of different types of fragments, namely compound fragments (with telomeric signals at both ends) which accompany dicentric and centric ring, terminal fragment (with telomeric signal at one end only) and interstitial fragments (appearing as double minutes without any telomeric signals). Contrary to expectations, true terminal fragments are induced at a very low frequency (about 8% at 3 Gy of X-rays) in human lymphocytes, whereas interstitial fragments are high, about 25% (ref. 38). It should be pointed out that interstitial deletions are common types of aberrations encountered in many human tumours.

Radiation-induced translocations

FISH technique allows easy identification of both chromosomal interchanges and intrachanges. With whole chromosome paints combined with centromeric probing one can discern between dicentrics and translocations. It soon became clear that in addition to symmetrical or reciprocal translocations, other types such as terminal and interstitial translocations are induced by ionizing radiation (Figure 1 b)³⁹. When all types of translocations are taken into account, more translocations (T) were found to occur more than dicentrics (D) in human lymphocytes (a D:T ratio of about 1.7) following X-rays. The D:T ratio appears to be dependent on the karyotype of the species under study, namely, mouse carrying 40 acrocentric chromosomes, has a ratio of 1:1 (ref. 40) and Chinese hamster, similar to human, carrying metacentric, sub-metacentric and acrocentric chromosomes, has a ratio of about 1.5 (ref. 41). In plant chromosomes, such an analysis is difficult because of the fact that plant chromosomes are rich in repetitive sequences, compared to mammalian chromosomes, which makes it difficult to paint individual chromosomes⁴². Radiation-induced balanced translocations, however, can be detected during meiosis by the characteristic formation of quadrivalents and multivalents in plants and mammals, though individual chromosomes involved in the translocations cannot be discerned in plants easily, unless specific marker chromosomes (e.g., satellite chromosomes) are involved.

Radiation-induced interchanges and intrachanges

While exchanges between chromosomes can be detected by whole chromosome painting, intrachanges (exchanges within a chromosome) cannot be discerned. Three types of intrachanges are recognized namely, pericentric inversions (exchange between two arms of a chromosome), paracentric inversions (exchange within one arm of a chromosome) and acentric rings (appearing as 'minutes'). Employing arm-specific DNA libraries it is easy to detect pericentric inversions, when each arm is painted with different colours (Figure 1 c). It has been found that the frequencies of radiation-induced pericentric inversions are about 7 to 8 times higher than chromosome interchanges, when calculated on the basis of the target size⁴³. This increase is attributed to the so-called 'proximity effect', implying close proximity of the two arms of a chromosome in the interphase nucleus, promotes exchanges between the arms⁴⁴. Similar effects due to proximity of heterochromatic regions forming chromocenters in the interphase nucleus in plant cells have been described in *Vicia faba* and *Secale cereale* following chemical and radiation treatments^{12,45}. For identification of paracentric inversions (Figure 1 d), region-specific DNA libraries are needed and they have been generated for human chromosomes⁴⁶ and Chinese hamster chromosomes³⁷. Paracentric inversions and interstitial deletions have been found to persist for a long time in radiation workers following exposure to high LET radiation and has been proposed as a fingerprint for past exposures⁴⁷.

Heterogeneity of induction of radiation-induced chromosome aberrations

Our earlier studies with plant chromosomes have demonstrated that some chromosomes and some regions of chromosomes are more prone for aberration formation than others following radiation^{11,18,45}. The distribution of break points among the chromosomes in a genome depends on the initial DNA damage (DSBs) and its subsequent repair or mis-repair. In a detailed study utilizing Chinese hamster cells, it was shown that initial damage is not randomly distributed among the chromosomes, light G bands (transcriptionally active regions) were more affected in comparison to dark bands (condensed regions). It was also shown that the repair of breaks was very fast in light bands in comparison to dark bands⁴⁸. Deletion break points and exchange break points were also found to be differentially distributed among the chromosomes⁴⁹. With the generation of chromosome-specific DNA libraries, it was possible to study the question of heterogeneity in greater detail. It was found that both high and low LET radiations induce preferentially more aberrations in Chinese hamster chromosome #8 in comparison to others^{40,50}. In Chinese hamster chromosomes, telomeric repeat sequences are distributed both at terminal ends of all the chromosomes and near the centromeres of most of the chromosomes. It

was found that chromosome #8 is rich in interstitial telomeric sequences which tend to aggregate with other chromosomes in interphase nucleus, thus forming 'chromocenter'-like structures, which allow increasing interaction between chromosomes to form exchange aberrations⁵¹. The long arm of X chromosome can be divided into two regions, namely q1 and q2, with an intervening fragile site. Since q2 region has common DNA sequences with the Y chromosome, the q1 and q2 regions can be differently painted, thus allowing the possibility to quantify the frequencies of aberrations induced in these two regions. It was found that q2 region is more involved in aberrations than q1 region, indicating the role of the DNA sequences in bestowing heterogeneity to radiation response³⁷. There are several studies indicating the existence of heterogeneity among the human chromosomes for response to ionizing radiation^{52,53}, but there appears to be inter-individual variability which makes generalization difficult. Thus, there are several factors, such as interphase organization, transcriptional activity of different chromosome regions, repetitive sequences, interstitial telomeric sequences which contribute towards the observed heterogeneity to radiation response at the chromosomal level⁵⁴.

Radiation-induced complex exchange aberrations

Even with single colour painting, it was observed very early that in addition to simple exchange aberrations (dicentric and reciprocal translocations), complex aberrations (involving more than two chromosomes and three breaks) are induced³⁸. However, discrimination between simple and complex exchanges based on single colour can be misleading. With multi-colour FISH involving combination of different fluorochromosomes, one can paint all the human chromosomes in different colours and using a sophisticated image analysis system, these can be recognized and exchanges between chromosomes identified. There are three systems available at present, namely, m-FISH⁵⁵, spectral karyotyping-SKY⁵⁶ and COBRA⁵⁷. Analysis of complex exchanges using SKY revealed cyclic non-reciprocal interaction, illustrating that the breaks induced in several chromosomes are able to form exchanges among each other leading to complexity⁵⁸.

Conclusions

The improvements in making cytological preparations and staining techniques over the last fifty years have helped us to gain insights into the mechanisms involved in the formation of chromosome aberrations following treatment with ionizing radiation. The basic concepts, based on the observations made in plant cells, developed during the forties and sixties still hold true. Molecular cytogenetic techniques which became available in the last two decades along with the sophisticated image analysis systems have thrown new light in resolving various steps involved,

from the initial DNA damage, its repair or mis-repair and the formation of aberrations at chromosomal level.

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