

Chapter 6

Unclassified Chromosome Abnormalities and Genome Behavior in Interphase



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Abstract The discovery and characterization of abnormal chromosomes have been an important tradition for cytogenetics. In the past 70 years, extensive efforts have been made to illustrate the molecular mechanisms of various chromosomal abnormalities and to apply them for clinical diagnosis and monitoring treatment responses. As a result, clinical cytogenetic analyses represent an essential component of laboratory medicine. However, efforts in both basic research and clinical implications have been focused on recurrent or clonal types of abnormalities, and the majority of non-clonal chromosome/nuclear aberrations remain unclassified and lack their deserved attention. In recent years, these stochastic genome-level alterations have become an important topic due to the emergence of the genome theory, in which chromosomal/nuclear variations play the ultimately important role both in somatic and organismal evolution. In this chapter, following a brief review of these studies on unclassified chromosomal/nuclear abnormalities, both the rationale and significance of studying these structures will be presented. Specifically, the dynamic relationship between normal and “abnormal” chromosomal structures, and among diverse types of “abnormal variations,” will be discussed through the lens of genome-mediated somatic evolution. This discussion will not only enforce the importance of new genomic concepts, such as system inheritance, fuzzy inheritance, and emergent cellular behavior based on interaction among lower-level agents, but can also shine light on many current puzzling issues, such as missing

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heritability and the challenge of clinical prediction based on gene mutation profiles. Together, genome-based genomic information will play an important role in future cytogenetics and cytogenomics.

Historical Perspective

Following the establishment of the correct number of human chromosomes (Tjio and Levan 1956), abnormal chromosomes were soon linked to diseases such as Down syndrome and *chronic myelocytic leukemia* or CML (Lejeune et al. 1959; Nowell and Hungerford 1960). In particular, with the introduction of various chromosomal banding methods to identify individual chromosomes (Caspersson et al. 1970), medical cytogenetics entered a new era marked by the successful identification of many known types of chromosomal abnormalities (both structural and numerical) and their linkage with an array of human diseases. Such chromosome identification capability was further strengthened due to the development of FISH technology (Langer et al. 1981; Lichter et al. 1990; Heng et al. 1991, 1992, 1997), especially once SKY (spectral karyotyping) and multiple color FISH became popular, as these techniques can rapidly and precisely identify individual chromosomes/chromosomal regions both for mitotic and meiotic chromosomes (Speicher et al. 1996; Schröck et al. 1996; Heng et al. 2003; Ye et al. 2006). In recent years, different cytogenomic methods have also been applied to chromosomal analyses including various array and sequencing platforms (Dong et al. 2018).

Despite these technical advances, however, most of these identified chromosomal abnormalities fall in the category of recurrent or clonal types (clonal chromosome aberrations or CCAs) as they are commonly shared within patient populations. Furthermore, it is relatively easy to identify these signatures by classical cytogenetic/cytogenomic methods. According to clinical cytogenetic guidelines, “current cytogenetics defines CCAs as a given chromosome aberration which can be detected at least twice within 20 to 40 randomly examined mitotic figures. Based on this definition, the frequency of CCA needs to be higher than 5–10% in an examined cell population. In literature, however, when a CCA is reported, researchers often refer to aberrations with frequencies that are over 30%.” (Heng et al. 2006a, b, 2016a).

Obviously, a large amount of “non-clonal chromosome aberrations” or NCCAs are not reported in the literature. Even though most NCCAs have a frequency of less than 10% among examined mitotic figures, the total number of them in their diverse types is enormous, given the fact that NCCAs can be detected from any individual, regardless of whether or not they are a patient. Unfortunately, however, these overwhelmingly numerous NCCAs were considered as insignificant “noise” and were largely ignored in the name of pattern identification (Mitelman 2000; Heng et al. 2006a, 2016a, b; Ye et al. 2018a).

Not surprisingly, at different fronts of genomic research, so-called genomic noise is overwhelming as well, as reflected by CNV and gene mutation profiles in patients,

as well as in normal individuals (Iafrate et al. 2004; Heng 2007a, 2015, 2017a, 2019; Liehr 2016). In fact, these unexpected findings have started to challenge gene mutation theory (Heng et al. 2011a, b; Heng 2009). To illustrate this point, in this chapter, we will mainly use cytogenetic examples.

Our interests in NCCAs, including the initial descriptions of various abnormal chromosomes and nuclei, started in the early 1980s. With the discovery of free chromatin, sister unit fibers, partially or uncompleted-packing-mitotic figures or UPMs (later termed as Defective Mitotic Figures or DMFs), various nuclear fragments, and massively newly rejoined chromosomes (Heng and Chen 1985, 1988a), it was confirmed that these structures are real (rather than non-chromatin artifacts) (Heng and Shi 1997). Even though they were initially linked to drug treatments, these were clearly chromosome-related structures, which represented opportunities to study the high-order structure of the chromosome, and could be useful for monitoring different stages of the cell cycle.

Several research projects have promoted the realization of their importance, including the development of high-resolution fiber FISH and the characterization of genome chaos during cancer evolution (Heng et al. 1992, 1997). For more details, please see Heng and Shi (1997), Heng et al. (2013a, b), and Heng (2015, 2019). A number of representative examples are listed in Table 6.1.

It should be pointed out that, historically, it was highly significant when researchers could identify the linkages of these common and signature chromosomal abnormalities to various diseases, which supported the gene mutation theory of cancer and human diseases. Prior to the acceptance of the genetic basis of cancer, for example, the highly diverse chromosomal changes detected from cancer were used as evidence against the idea that cancer is caused by genetic aberrations. The identification of a specific translocation from CML and the subsequent cloning of the Bcr/Abl fusion gene have played highly significant roles in the acceptance of the gene mutation theory of cancer (Rowley 2013). Now, based on how challenging it has proven to be to identify commonly shared genetic aberrations for most cancer cases, coupled with the new realization that the majority of nonrecurrent genomic variants are of importance for somatic evolutionary potential, the new era of studying NCCAs is arriving. This transition represents an era in which it is necessary to deal with bio-complexity and uncertainty (Horne et al. 2013).

In the case of cancer research and, in particular, when studying the process of genome chaos, increased nuclear abnormalities are also linked to different types of chromosomal abnormalities and, ultimately, to CIN-mediated cancer evolution (Sheltzer et al. 2011; Siegel and Amon 2012; Zhu et al. 2012; Heng et al. 2013a, b; Heng 2015). Many interesting phenomena, including micronuclei clusters, giant nuclei, rapid nuclear fusion/fission/budding/bursting, and entosis, are now under increased investigation, leading to the realization that these abnormal nuclei can also change the chromosomal coding. In other words, genome reorganization can unify different types of chromosomal/nuclear variations under the evolutionary mechanism of genome-based selection (Heng 2015, 2019; Ye et al. 2018a, b, 2019a, b).

Table 6.1 Examples of various NCCAs reported in literature

Experiments and new concepts	Key findings	Main conclusions	Comments	References
Using drug treatment to induce elongated chromosomes in frog and human blood culture	Elevated frequencies of free chromatin, unit fibers, and DMFs were observed	They are chromatin materials rather than non-DNA contaminations. Both unit fibers and DMFs potentially represent various stages of the process of high-order structural formation. These chromosomal aberrations can be induced by drug treatment, especially within the G2 phase of the cell cycle High-resolution fiber FISH was initially developed using free chromatin and elongated chromosomes	Despite a few publications, it failed to generate follow-up studies from others due to the reasons that the mechanism of their generation is not clear, and there is no guideline to score these structures	Heng and Chen (1985), Heng et al. (1988a, 1992, 2013a, b) and Heng (2015, 2019)
Using topo II inhibitors and other reagents to induce chromosomal de-condensation or DMFs	Elevated frequencies of DMFs, massive chromosome fragments, elongated chromosomes, and newly formed joined chromosomes	Various chromosomal aberrations can be induced from various cell lines Both the compromise of the G2-M checkpoint and interference with condensation is required to induce DMFs (unpublished data)	Observed mitotic cell death, genome chaos during the 1980s. But these data were held until 2004, waiting for additional mechanistic studies	Heng et al. (1988b), Haaf and Schmid (1989), Smith et al. (2001) and Heng (unpublished observations)
Examining the baseline and inducibility of free chromatin and DMFs using normal individuals' blood culture	Free chromatin, C-Frag, aneuploidy, and translocations can be detected from hundreds of normal individuals with variable frequencies	Various aberrations can be observed from normal individuals, albeit at much lower frequencies	There likely is a base level of NCCAs for normal individuals	Heng et al. (2004a)

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Table 6.1 (continued)

Experiments and new concepts	Key findings	Main conclusions	Comments	References
Watching karyotype evolution in action using in vitro immortalization model	Massive chromosomal aberrations, including karyotype chaos, were observed during the punctuated phase of cancer macroevolution	In the punctuated discontinuous phase of genome evolution, there is no traceable clonal expansion between cellular generations, and the frequencies of NCCAs reach their peak	CCAs are often observed from the stepwise micro-evolutionary phase, while the peak of NCCAs is mapped into the macro-evolutionary phase	Heng et al. (2006a, b, c, 2011a, b) and Heng (2015, 2019)
Comparing frequencies of NCCAs from cell lines with different degrees of CIN; compare the baseline of NCCAs to induced NCCAs; compare the transcriptome profile of cell populations with different degrees of NCCAs; examine drug resistance from cell lines with variable degree of NCCAs	The frequencies of NCCAs are linked to the degree of CIN, transcriptome dynamics, cancer evolutionary potential, and drug resistance	NCCAs can be used as an index of CIN and evolutionary potential	NCCAs are not insignificant noise but valuable chromosomal variants	Stevens et al. (2013, 2014) and Heng et al. (2011a, b, 2013a, b)
Linking various chromosomal and nuclear abnormalities to cancer and other types of diseases	Linking aneuploidy to metastasis; describing entosis; giant nuclei in cancer; mosaicism in diseases	There are many diverse types of NCCAs; NCCAs are associated with an array of diseases	Most of the different types of abnormalities are linked by CIN	Ye et al. (2019a, b), Bloomfield and Duesberg (2016), Zhang et al. (2014), Iourov et al. (2008, 2010, 2019) and Horne et al. (2015)

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Table 6.1 (continued)

Experiments and new concepts	Key findings	Main conclusions	Comments	References
The establishment of the concepts of system inheritance and fuzzy inheritance	Cellular inheritance can be classified into gene-defined “parts inheritance” and genome-defined “system inheritance”	System inheritance represents a new type of coding which determines the gene interaction relationship. The order of genes and other DNA sequences within a chromosome and among different chromosomes provides the physical platform for gene interaction to work. The main function of sexual reproduction can maintain the chromosomal coding for a given species	System inheritance explains why chromosomal variations are important, and fuzzy inheritance explains why there are so many different types of the chromosomal variants	Heng (2009, 2015), Heng et al. (2009, 2011b, 2016a, b) and Ye et al. (2019a, b)
	By and large, genomic information is fuzzy rather than precise. This fuzziness is the genomic basis for heterogeneity There is a high level of dynamics in the chromatin loop domain during the normal cell cycle	Fuzzy inheritance can be observed from multiple levels (e.g., gene and epigenetic levels) of bio-informational organization	System inheritance and fuzzy inheritance explain why it is challenging to understand missing heritability based on a gene-centric view	Heng et al. (2001, 2004b)

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Experiments and new concepts	Key findings	Main conclusions	Comments	References
Link stress and stress responses to cell death and the induced emergence of outliers following genome reorganization	While induced cell death can eliminate a large portion of cells, the induced surviving cells with new genomes can escape death and become dominant	Highly diverse genome alterations generated from different molecular mechanisms, including aneuploidy, micronuclear clusters, entosis, and chaotic genomes, share the same fact: Their genome systems have altered due to the changing of the chromosomal coding	That is the reason why it is essential to study the informational and evolutionary meaning of chromosomal variations, rather than the molecular mechanisms that lead to them, as there are so many ways to achieve new systems by altering the chromosomes	Stevens et al. (2011), Heng et al. (2011a, b), Ye et al. (2018a, b, 2019a, b), Heng et al. (2016b, 2019) and Home et al. (2014)
Search for the evolutionary and informational mechanism of the highly diverse nuclear and chromosomal variations	The highly diverse abnormal nuclei and chromosomes can contribute to the formation of new genomes: a key strategy of survival			
Genome theory aims to unify multiple levels of genomic and non-genomic variants in both somatic and organismal evolution	Nearly all genomic variants are potentially useful for cellular adaptation, but as a trade-off, they can lead to diseases conditions	The genome is the basic unit for macroevolution	NCCAs (at the genome level) and other stochastic genomic and non-genomic alterations serve as evolutionary potential	Heng (2009, 2015, 2017a, b, 2019)

Examples of Unclassified Chromosome/Nuclear Abnormalities

As a freshly graduated student, one of us (HH) was very surprised and excited upon initially observing high frequencies of unknown chromosome/chromatin abnormalities and later realized that these high frequencies are observed even on chromosomal slides prepared from normal individuals without any special treatment. At that time, however, the majority of cytogeneticists dismissed these structures, and many considered them simply as contaminations or artifacts of slide-making. It was difficult to even publish these observations in mainstream cytogenetics journals.

A few years later, some of these elongated chromatin structures and chromosomes were used for the development of high-resolution fiber FISH (Heng et al. 1992, 1997). Despite this success, the biological meaning of these structures has been continuously ignored.

The third wave of studying these variants was triggered by the linkage of NCCAs and genome instability using various *in vitro* and *in vivo* cancer models, especially once the frequencies of NCCAs were linked to cancer evolutionary potentials (Heng et al. 2004a, 2006a, b, c). With the introduction of chromosomal coding and system inheritance, all of a sudden, it made the perfect sense to us why NCCAs are important and are detectable from normal and disease tissues but at different frequencies, and why there is a relationship among stress, cellular adaptation, system survival, and disease conditions. We have thus published accumulated data over the course of nearly three decades (Heng et al. 2004a, 2008, 2011a, b, 2013a, b; Heng 2019; Stevens et al. 2007, 2011, 2013). Furthermore, with the appreciation of fuzzy inheritance and emergent properties, more attention has been paid to the characterization and classification of different types of chromosomal/nuclear variants (Heng 2019; Ye et al. 2019a, b; Heng et al. 2019). Some examples of unclassified chromosome/nuclear abnormalities are listed below.

Free Chromatin

Free chromatin refers to those released chromatin materials detected from conventional cytogenetic preparation. They often display a spindle- or ropelike shape, and there is no apparent nuclear envelope. The generation of free chromatin can be achieved by various drug treatment and manipulating release conditions. For example, using a special high-PH buffer, an extremely long linear structure can be released (Heng et al. 1992; Heng and Tsui 1994; Heng 2000). Despite that elevated frequencies of free chromatin can be observed in some pathological conditions, even under routine slide-making conditions, the biological significance is still unclear. Potential causes might include the instability of the nuclear envelope and cell cycle checkpoints (Fig. 6.1).

Defective Mitotic Figures or DMFs

DMFs refer to partially condensed mitotic figures in which condensed chromosomes or chromosomal regions and uncondensed chromatin fibers coexist. There are three types of DMFs according to their morphological features, and the common

Fig. 6.1 (continued) comparison between interphase nuclei and various free chromatin generated from protocols releasing free chromatin (Heng et al. 1992). Interphase nuclei (**b** and **c**) and free chromatin (**d–i**) were prepared from a human-hamster hybrid cell line 4AF/106/KO15, which contains an altered human chromosome 7. (**b**, **d**, **f** and **h**) FISH detection results. The yellow signals represent a human chromosome (the FISH probe used is total human DNA). (**c**, **e**, **g** and **i**) Corresponding DAPI staining. From **d** to **h**, there is an increased degree of stretching. (Reused from Heng et al. 2013a)

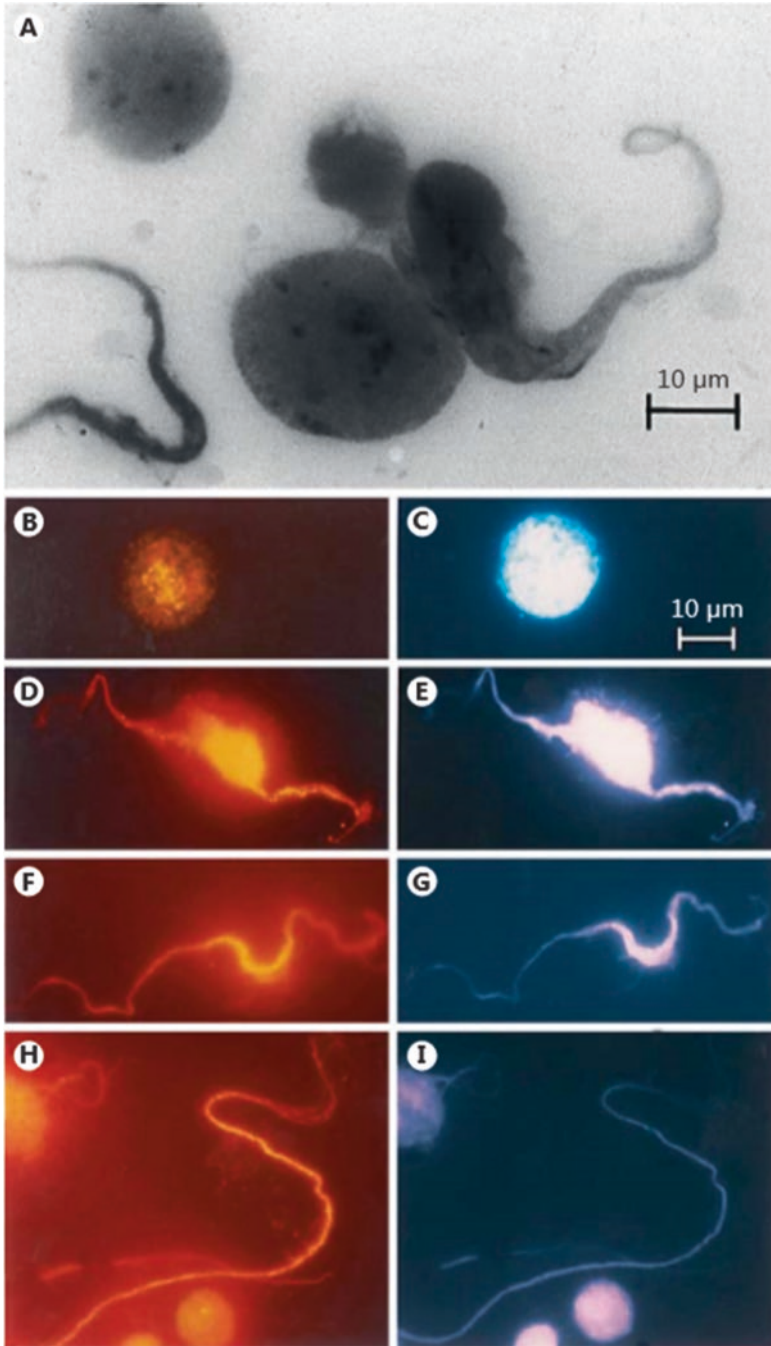


Fig. 6.1 Examples of free chromatin. (a) An example of the typical morphology of free chromatin (spindle and rope shapes) and three interphase nuclei detected from routine chromosome preparations without any treatment (reverse DAPI staining image). (b–i) FISH signals and morphological

feature is the mixed degree of condensation. Elevated DMFs can be obtained by using topo II inhibitor, especially in cells with a G2-M checkpoint deficiency (Heng et al. unpublished observation). Using DMF as a case study, it was realized that even the same types of chromosomal abnormalities can be linked to different errors from different phases of the cell cycle. For example, DMFs can be generated from interfering with different stages of the cell cycle, such as directly interfering with condensation in the G2 phase or indirectly interfering with DNA replication in S phase (Heng and Chen 1985; Heng et al. 1988a; Haaf and Schmid 1989; Smith et al. 2001). Even without drug treatment, the baseline of DMFs is elevated for many cancer patients, as well as in other illness conditions such as GWI and CFS (Liu et al. 2018; Heng et al. unpublished data) (Fig. 6.2).

Chromosome Fragmentations or C-Frag

C-Frags refer to the phenomenon of fragmented chromosome or nuclei. Often, different proportions of chromosomal fragments and chromosomes coexist. C-Frags represent a form of mitotic cell death (Heng et al. 2004a; Stevens et al. 2007). There are different subtypes of C-Frags based on the time fragmentation occurs (in an earlier or later stage of metaphase) and/or the degree of fragmentation (the proportion of chromosome vs. fragments). Further studies are needed to investigate if interphase nuclei can be fragmented as well. Importantly, various types of stresses (genomic and environmental alike) have been linked to the induction of C-Frags (Stevens et al. 2011; Stevens and Heng 2013), revealing the general link between various molecular pathways or mechanisms to the same end product, mitotic death. Such a connection is of importance for unifying highly diverse molecular mechanism and diverse chromosomal variations. Studies of C-Frags also help us to understand the mechanism of genome chaos (Heng et al. 2006c; Liu et al. 2014; Heng 2015, 2019). Furthermore, nuclear fragmentations are also observed (Ye et al. unpublished observations) (Fig. 6.3).

Unit Fibers

Unit fibers describe various treatment-generated (chromosomal isolation or drug treatment to interfere with condensation) substructures of metaphase chromosomes (Bak et al. 1979; Heng et al. 1988b). Unit fibers display a constant diameter of approximately 0.4 μm , which have been observed from cells of different species, including frog and human. The detection of unit fibers strongly suggested that there might be an intermediate structure between metaphase and interphase chromatin fiber. The further characterization of both unit fibers and DMFs will illustrate how the last step of chromosome packaging is achieved (Heng et al. 2013a, b; Heng 2019).

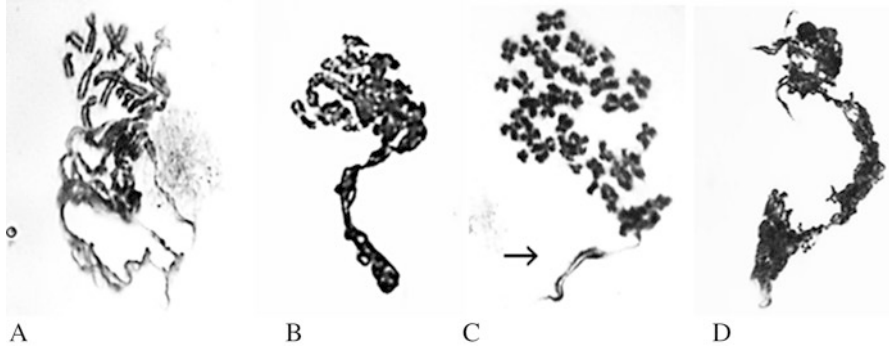


Fig. 6.2 Examples of DMFs detected from Gulf War illness patients. (a–c), Type 1 DMFs with the typical polarizing shape, in which the condensed chromosomes group at one end, and the uncondensed chromatin extends out in the opposite direction (Giemsa staining). In (c), an arrow indicates a less condensed chromosome. (d) Type 2 DMF with more a random distribution of de-condensed chromosomes. In this image, there is a mixture of DMFs and sticky chromosomes. (Reused from Liu et al. 2018)

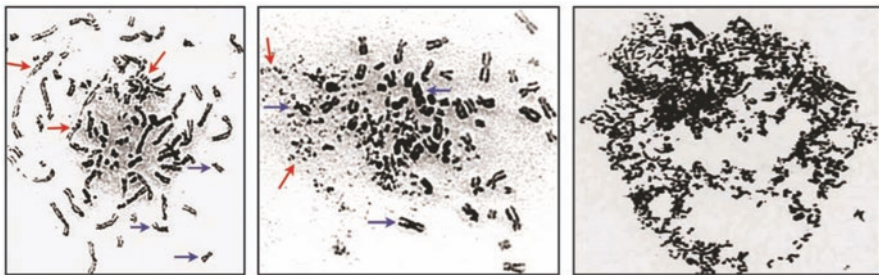


Fig. 6.3 Morphological features of chromosome fragmentation. Chromosomes undergoing fragmentation display many breaks and often seem frayed. Giemsa staining shows that chromosome fragmentation is a progressive process, with early stages showing few fragmented chromosomes (left, chromosome fragmentation (red arrows); intact chromosomes (blue arrows)), mid stage with approximately half of the chromosomes fragmented (middle), and late stage with nearly all chromosomes except for one at the top showing degradation (right). (Reused from Stevens et al. 2007)

Sticky Chromosomes

Sticky chromosomes have traditionally been described in plant chromosome research, and less attention has been paid to these structures in human chromosome studies. Sticky chromosomes can be induced by various drugs, and they are frequently observed from studies of plant hybrids. Sticky chromosomes are often observed from samples displaying high frequencies of DMFs (Heng et al. 2013b). Recently, sticky chromosomes were also detected from GWI patients (Liu et al. 2018). Sticky chromosomes can be linked to aneuploidy and translocation as well.

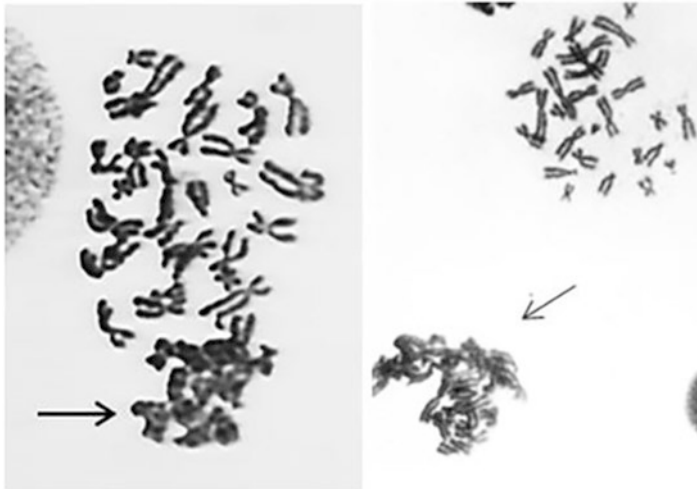


Fig. 6.4 Images of sticky chromosomes. Left: A portion of the mitotic figure displays sticky chromosomes, where multiple sticky chromosomes form a cluster (as indicated by the arrows). Right: A comparison between nonsticky chromosomes (top right) and sticky chromosomes (indicated by an arrow): This image is different from left image, as the sticky chromosome cluster likely belongs to a different mitotic figure. (Reused from Liu et al. 2018)

We also found that cells displaying high levels of sticky chromosomes might be more frequently involved in exchanging DNA among cells, an example of fuzzy inheritance. For more information, see Heng (2019) (Fig. 6.4).

Micronuclei Clusters

Unlike classical micronuclei (the small nuclei that result from chromosomes or chromosomal fragments getting separated from the daughter nucleus during cell division), the term micronuclear cluster refers to a group of various sizes of nuclei, often burst dividing from a single cell (Heng et al. 2013a, b; Heng 2019; Ye et al. 2019a). Micronuclei clusters can also be derived from giant nuclei which contain hundreds of chromosomes (Heng et al. 2013a, b, 2016a, b; Liu et al. 2014; Zhang et al. 2014; Chen et al. 2018). In a recent case study of the relationship between micronuclei and genome chaos, a general model was proposed that illustrates the mechanism of how micronuclei can promote the formation of new genome systems by reorganizing the chromosomal coding (Ye et al. 2019b) (Fig. 6.5).

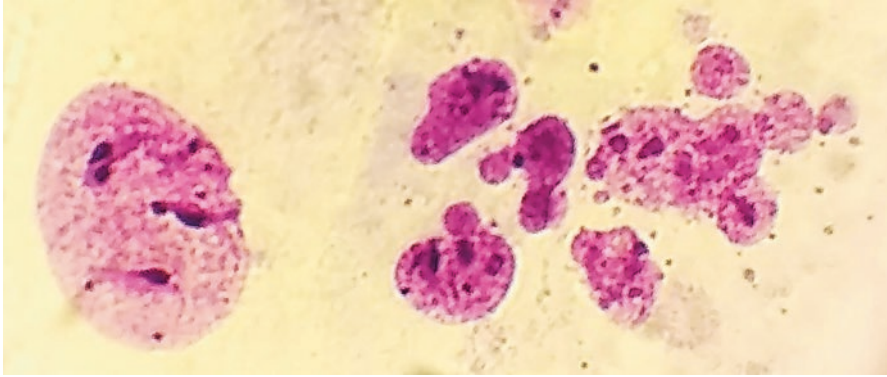


Fig. 6.5 Morphological comparison between normal interphase nucleus and micronuclei cluster. A normal nucleus is displayed at the left corner. A micronuclei cluster is located at middle to right. There are more than ten individual nuclei with different sizes (micronuclei were stained by Giemsa)

Fusion/Fission/Budding/Bursting/Entosis

Nuclei can exhibit many bizarre ways of dividing or rejoining, including cell-to-cell fusion, fission, budding, bursting, and entosis (cannibalism or emperipolesis) (Erenpreisa et al. 2005; Walen 2005; Heng 2013). On the surface, there are many differences (in regard to both morphology and mechanisms) among these many different types. Fundamentally, however, they all share the key features of altering the system inheritance or chromosomal coding and a high degree of uncertainty. Evolutionarily speaking, they all represent a stress response for cellular adaptation or survival. Despite the massive cell death involved, some outliers will have the chance to become the dominating population or serve as essential transitional populations for a new stable population to be possible. For example, entosis is a way of changing the genome through polyploidy, and polyploidy is linked to aneuploidy, translocations, and genome chaos; fusion/fission cycles are associated with genome chaos and can produce cells with altered genomes.

Chaotic Genome

This category includes many drastically altered chromosomes and nuclei (Heng et al. 2004a, 2008, 2013a, b; Liu et al. 2014; Heng 2015, 2019). For example, in addition to giant nuclei, an entire genome can form one single giant chromosome. There are chromatid rings and many other forms of alterations, most of which have yet to be named. In general, almost any form of abnormality can be detected.

It should be pointed out that chaotic genomes were initially described by cytogenetic analyses and later confirmed by sequencing. Furthermore, chromothripsis belongs to one subtype of genome chaos (Heng 2007c; Liu et al. 2011; Stephens

et al. 2011; Heng et al. 2006a, b, c, 2008, 2011a; Setlur and Lee 2012; Righolt and Mai 2012; Forment et al. 2012; Crasta et al. 2012; Baca et al. 2013; Horne and Heng 2014; Liu et al. 2014).

The main reason that detections of chromothripsis have been more frequently reported than other types of genome chaos by current sequencing analysis is that these locally limited alterations can be favored by evolutionary selection and are easily detectable in clonal populations (Liu 2011; Heng et al. 2013a, b; Liu et al. 2014; Heng 2015, 2017a, b, 2019). In fact, due to the limitations of DNA sequencing (which is unable to detect cell subpopulations below 10–15%), only clonal chaotic genomes can be detected (single-cell sequencing can solve this problem, but a large number of cells are needed). In contrast, cytogenetic method is so far the most effective and economic one, as it is comprised of single-cell-based populational analysis.

By tracing the process of genome chaos using an *in vitro* model, it becomes clear that different types of chromosomal/nuclear abnormalities are linked by the degree of CIN, the phase of evolution, and the level of system stress and stress response. For example, cells with giant nuclei can be generated by the genome chaos process, and giant cells can be linked to micronuclei clusters and more complicated translocations. To make the situation more complicated, some transitional structures can trigger further stress responses even though these will not be survived at the end of the chaotic process. As a conclusion, it is possible that in the future, we will need to monitor evolutionary mechanisms rather than specific types of chromosomal abnormalities as they are constantly changing.

Nevertheless, before we achieve the future goal of using quantitative general biomarkers (rather than using one specific type of abnormalities alone), further characterization and classification of types of abnormalities are needed, as many of them involve different names, and some confusion about them exists as well. For example, despite their similar morphological features, C-Frag differs from PCC (premature chromosome condensation), both from a morphological and mechanistic point of view (for more details, please see Stevens and Heng [2013]). Similarly, many terms are overlapping, such as chromosome pulverization, shattering, and mitotic catastrophe. These can all be termed as forms of C-Frag, a means of mitotic cell death. More generally, they are unified by genome chaos. Clearly, one important concept is the heterogeneity of cell death (Stevens et al. 2013). Drastically altered chromosomal morphological features do not mean the elimination of the system but the emergence of a new system, albeit at very low frequencies (Fig. 6.6).

The Evolutionary Mechanism of Stochastic Chromosome/ Nuclear Alterations

Prior to recent evolutionary mechanism-focused research, most chromosomal/nuclear abnormalities are studied by different investigators within the premise of studying specific molecular mechanisms. For example, aneuploidy has mainly been

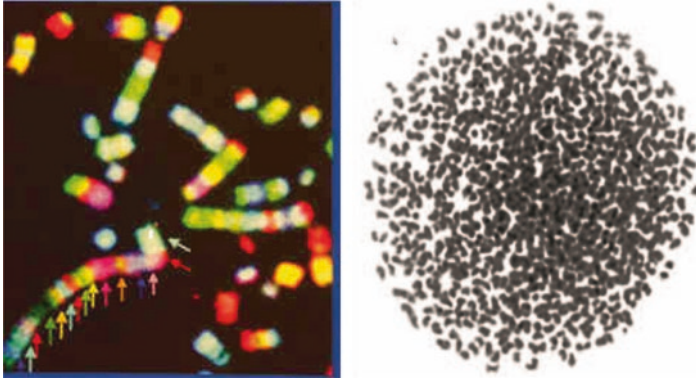


Fig. 6.6 Examples of structural and numerical chaotic genomes. Despite that there are many subtypes of chaotic genomes, structural chaotic genomes commonly involve multiple translocations (as in the SKY image, in which the chromosomes in the left corner are formed by at least 15 large chromosome fragments, some of which are indicated by arrows with different colors) (left image). On the other hand, numerical chaotic genomes can contain hundreds of chromosomes, as exemplified by the right image, in which the genome contains over 700 human chromosomes or $> 15n$ of DNA content. Two images are reused from Heng (2013) and Liu et al. (2014)

linked to the chromosome segregation mechanism. With various large scale -omics studies, however, many different specific molecular mechanisms have been linked to aneuploidy, which makes aneuploidy research much more complicated. This situation calls for a new strategy of studying the general evolutionary mechanisms of aneuploidy which can unify diverse molecular mechanisms (Ye et al. 2018a, b). Obviously, such a strategy should be used for studying all types of chromosomal/nuclear abnormalities (Heng 2015, 2019).

The General Causative Factor of Genome Alterations

Even though many different molecular mechanisms can be linked to a given type of abnormality (e.g., over a dozen different treatments/mechanisms can be linked to C-Frag) (Stevens et al. 2011), the general causative factors can be described as internal genomic stochasticity and stress response-mediated cellular adaptation, in addition to bio-errors produced under dynamic environmental conditions. It is important to point out that even the process of cell death can eliminate many unwanted cells (to reduce the average population size); under many circumstances, the process itself can trigger further system changes with unexpected consequences (such as the creation and/or favoring of some outliers which provide resistance). The long-term consequences, for better or worse, depend on the multiple levels of the systems and the fate of evolutionary selection.

The Evolutionary Mechanism of Genome Alterations

- (a) Promoting genomic variants at the somatic cell level: solving the conflicts of constraint (germline) and dynamics (somatic)

In working to solve the conflict between species' genomic stability and the genomic dynamism necessary for adaptation (the two faces of the coin that are essential for evolution), it was realized that genome integrity is maintained by the stability of the genomic landscape of the germline (which is ensured by the function of the sex) (Heng 2007b; Gorelick and Heng 2011; Heng 2015, 2019). The genomic dynamics of the somatic cell, on the other hand, are achieved by the fuzzy inheritance of somatic cells and environmental interaction (which is promoted by the needs of cellular adaptation within changing environments). Therefore, as long as the germline's karyotype coding is preserved, somatic alterations can be pushed to very high levels. As the trade-off for the benefit of cellular adaptation, there are many disease conditions caused by the increased variants generated (Heng et al. 2016a, b; Heng 2017b).

Interestingly, the concept of system inheritance, combined with the separation of germline constraint and somatic dynamics, can also explain part of the missing heritability (Heng 2010, 2019). The gene-centric concept will not be able to identify the missing heritability. Unfortunately, current major efforts are still within the genome centric framework, although they are making greater use of computational models.

- (b) Genome reorganization and evolutionary potential

With so many different types of unclassified chromosomal abnormalities, and even due to the presence of just one given type, there are high degrees of morphological heterogeneity, which makes it rather challenging to understand the main function of these abnormalities. As different types of chromosomal abnormalities can be linked to many different molecular mechanisms, molecular mechanistic understanding as a whole becomes less certain. As a result, even though increased molecular knowledge is available, much of this knowledge can only explain limited cases. Examples can be found in aneuploidy and micronuclei research (Ye et al. 2018b, 2019a). As a result, the underlying common principles that can unify all of these chromosomal and nuclear variants are lacking, and the incidence of clinical prediction based on individual molecular mechanisms is low.

Clearly, a correct approach is to go above the individual molecular mechanisms (as there are so many) to search for an evolutionary and informational mechanism, which is applicable to all chromosomal abnormalities.

One holistic understanding is that regardless of their morphological and mechanistic differences, all of these NCCAs are simply chromosomal or nuclear variants with altered chromosomal codes. In other words, their informational meaning and evolutionary mechanism is the same: the creation of a new information package with evolutionary potential.

A general model has been proposed when discussing the mechanism of how genome chaos leads to a new system by reorganizing the chromosomes (Heng et al.

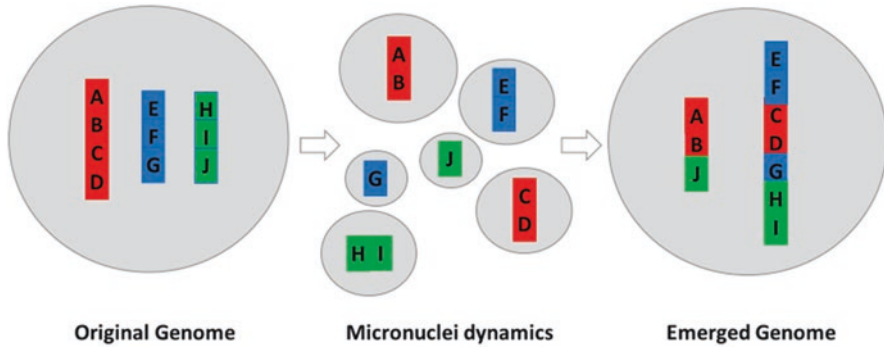


Fig. 6.7 The diagram of how micronuclei create a new genome by reorganizing karyotype coding. When under a high level of stress (either internal or environmental), the cluster of micronuclei is formed, which can lead to death, proportional survival (partial population survival without altering the genome), the formation of an emergent genome through a fusion/fission cycle, or simply the combination of micronuclei with other nuclei, resulting in a new cell with an emergent genome (defined by altered chromosomal coding). (Reused from Ye et al. 2019a)

2011a). This model was also applied to explain how micronuclei clusters can form different genomes (Ye et al. 2019a). (Fig. 6.7, Micronuclei cluster model of the reorganizing of the genome)

This model can be applied to explain how different chromosomal/nuclear abnormalities contribute to new genome formation, including polyploidy/aneuploidy, sticky chromosomes, giant nuclei, and entosis (Ye et al. 2019b). All of these are associated with the stress response and unstable genome status, in conjunction with system adaptation and survival. Fundamentally, they all contribute to the emergence of an end product with altered genomic coding.

(c) Heterogeneity of abnormalities caused by fuzzy inheritance and dynamic environments

Of course, fuzzy inheritance at the chromosomal level represents the basis for the heterogeneity of chromosomal abnormalities. Fuzzy coding is responsible for the potential phenotype, and it is the environment that selects the specific phenotypes. However, the selected phenotypes can easily be altered again under different selective conditions as the inherited code itself is highly flexible, and the phenotypes themselves exist within a range of potential options, a concept which differs from classical genetic frameworks (Heng 2015, 2019; Ye et al. 2018a, b). Nature has beautifully solved the key conflict of survival as a species (by not changing the entire system) and while rendering the species' bio-information flexible enough to adapt to current conditions. Clearly, the fuzzy inheritance of somatic cells, including the separation of germline and somatic cells, plays an important role.

It should be pointed out that there is emerging interest in somatic mosaicism (Yurov et al. 2007; Iourov et al. 2008, 2010, 2019; Biesecker and Spinner 2013; Heng et al. 2013a, b) and core genomes-associated multiple levels of genomic

interactions (Heng et al. 2013a, b, 2016a; Shapiro 2017, 2019; Heng 2019), which are closely related to fuzzy inheritance and genome-based evolution. These mechanisms, including minimal genomic variations in the germline, somatic alteration and mosaicism, and the host microbiome, allow diverse variants to be achieved by the same core genome interacting with other genomic and environmental factors. Under many conditions, such genome level interaction plus epigenetic changes can provide enough variations without relying on the changing of gene mutation frequencies within a population, the key mechanism of natural selection. Just passing the core genome is sufficient for passing the potential of different combinations of genomic interaction. As long as such interaction is there, there is no need to accumulate gene mutation for most traits as the environments are constantly changing back and forth.

Future Perspectives

In recent years, there have been increased reports on the significance of using various chromosomal/nuclear abnormalities in both genomic research and clinical implications (Chandrakasan et al. 2011; Heng et al. 2013a, b; Stepanenko and Kavsan 2014; Stepanenko and Dmitrenko 2015a, b; Niederwieser et al. 2016; Bloomfield and Duesberg 2016; Stepanenko and Heng 2017; Poot 2017; Rangel et al. 2017; Iourov et al. 2019; Vargas-Rondón et al. 2017; Liu et al. 2018; Heng et al. 2018; Frias et al. 2019; Ramos et al. 2018; Chin et al. 2018; Salmina et al. 2019). With an appreciation of the importance of karyotype or chromosomal coding, and of how these stochastic abnormalities can play a key role in somatic evolution, a new wave of studies will likely soon come of age. Along with some frequently discussed perspectives (Heng et al. 2016a, 2018; Heng 2013, 2015, 2019; Heng and Regan 2018; Ye et al. 2018a, 2019a, b), several issues should be addressed for further classifying and applying the knowledge of chromosomal abnormalities in clinic settings. First, the baselines of some major types of abnormalities in normal individuals and in patients are needed to be established and give reference to age, gender, and possible racial difference. Of course, for many common and complex diseases or illnesses, research is needed to examine if elevated levels of NCCAs are involved. Second, a quantitative measurement based on total chromosomal abnormalities is needed to link to different types of diseases, treatments, and overall system instability. Such studies might lead to new biomarkers based on the pattern of genome dynamics. The possibility of combining chromosomal and nuclear abnormalities together to predict system instability and evolutionary potential should also be studied. Third, the pattern of chromosomal abnormalities should be used to study the behavior of outliers within different phases of somatic evolution. The profile of outlier versus average is particularly interesting during phase transitions (Heng 2015, 2019). Fourth, another challenge is to integrate different types of variants into somatic chromosomal mosaicism (Iourov et al. 2019). Obviously, mosaicism plays an important role during the emergence of systems behavior (Heng et al. 2019).

Lastly, it should be noticed that the concept of chromosomal coding mainly applies to eukaryotes with typical chromosomes. As the chromosome represents a major innovation of our evolutionary history, the function of chromosome-based genomes drastically differs from that of prokaryotic genomes. As soon as chromosomes were formed on Earth, prokaryotes and eukaryotes have followed different games of evolution. For example, meiosis has become a main constraint for maintaining species' identities, while the breakage of chromosomal coding has become the major tool for rapid macroevolution, with increased system complexity. The chromosome-based information package has likely provided the separation of germline and somatic cells, which further increased the power of fuzzy inheritance. Of course, more research is needed to compare the evolutionary and informational mechanism of non-chromosome-based and chromosome-based genomes.

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