Chapter 5
Differentiating Chromosome Fragmentation and Premature Chromosome Condensation

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Abstract The chromosome has long been viewed as a structure that ensures faithful segregation of the genetic materials to daughter cells. However, it is now apparent that the chromosome plays a central role in defining the genetic network through the genome context. One often-confused phenomenon bridging studies of interphase chromatin and mitotic chromosomes is chromosome pulverization, which has been inappropriately linked to premature chromosome condensation (PCC) and more recently confused with chromosome fragmentation (C-Frag), a major form of mitotic cell death. Recently there has been increased interest in genome alteration-mediated somatic cell evolution and its clinical implications, although a number of publications have continued to confuse these terminologies/concepts.

To alleviate confusion in this field we review both C-Frag and PCC. Discussion of C-Frag includes its morphological and mechanistic characterization, its relationship to genomic instability, and its utility. Discussion of PCC pertains to its mechanisms, definition, historical perspectives, and its application in basic research and clinical settings. C-Frag and PCC are then directly compared and contrasted to fully differentiate these two phenomena. Chromosome pulverization, chromosome shattering, and mitotic catastrophe are compared in relationship to both C-Frag and PCC. To avoid future confusion we suggest avoidance of the ambiguous term chromosome pulverization in

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favor of the more specific term C-Frag or PCC. Finally, future implications and perspectives of both C-Frag and PCC are discussed.

Introduction

It is well established that the chromosome plays a key role in packaging the genes to ensure faithful division of genetic material during mitosis (Heng et al. 2004). However, the chromosome serves more than this obvious purpose (Heng et al. 2011a). Recently, it has been realized that the order of genes along the chromosome and within the genome represent a new type of genetic information called system inheritance. The genome provides this new information through the genome topology, which is an important component of the genome context (all sequences of a given species plus genomic topology), which in turn defines the genetic network (Heng et al. 2009, 2011a, b; Heng 2013). Thus, the most important functions of the chromosomes are (1) defining the genetic network for all types of somatic cells, and (2) ensuring the maintenance of system inheritance (especially through the germline) by preserving the karyotype (including the order of genes along the chromosome, as well as the chromosomal compositions within a cell). As the result, alterations in chromosomal number or structure lead to extensive changes in gene expression, modifying the networks in which those genes function (Stevens et al. 2013a, b). Maintenance of chromosome makeup (both structurally and numerically) of a cell is thus of great importance, and alteration of this makeup results in multiple diseases, especially cancer, by providing evolutionary potential. Therefore, there is an urgent need to increase understanding of the chromosome and the effects of its alteration (Heng et al. 2009, 2010a, 2013a; Heng 2007, 2009; Stevens et al. 2011a; Gorelick and Heng 2011).

Premature chromosome condensation (PCC) is a phenomenon whereby chromatin condensation is induced inappropriately during interphase. The discovery of PCC has led to increased knowledge of the basic chromosome structure, the state of the chromosomes during the different stages of interphase, and identification of factors involved in the cell cycle (Bezrookove et al. 2003; Johnson and Rao 1970). One key feature of PCC is the resultant chromosomal morphology (Potu et al. 1977). Induction of PCC during S-phase results in the condensation of partially replicated chromosomes, which appear as fragmented clumps of chromosomes. Interestingly, similar morphology, often described as chromosome pulverization or shattering, has been observed following exposure to a variety of agents, including viral infection, pesticides, caffeine, and ultraviolet light, and in blood diseases (Knuutila et al. 1981; Alam and Kasatiya 1976; Cremer et al. 1980; Norrby et al. 1966; Kato and Sandberg 1968). Despite the difference of pulverization from PCC in many of these cases, and warnings of restraint in calling PCC chromosome pulverization, the use of the term pulverization has remained (Stevens et al. 2007, 2010, 2011a, b; Sandberg 1978).

More recently, a new form of mitotic cell death called chromosome fragmentation (C-Frag) has been identified (Heng et al. 2004, 2013b; Stevens et al. 2007, 2011a, 2013b; Ye et al. 2007). During chromosome fragmentation, condensed mitotic chromosomes are progressively degraded, leading to cell death. C-Frag is induced to

eliminate cells subjected to broad-ranging stress, including both external stress such as drug treatments or viral infections and internal stress including genome instability (Stevens et al. 2011a). Interestingly, incomplete chromosome fragmentation leads to fragmented pieces of chromosomes being rejoined to form highly complex chromosomal rearrangements known as genome chaos and a subtype of genome chaos, chromothripsis (Heng et al. 2006, 2009, 2011a, b; Kloosterman et al. 2011; Liu et al. 2013; Stephens et al. 2011). Thus, C-Frag can act as a double-edged sword, on one hand serving to retain genomic integrity by removing stressed and altered cells and on the other hand changing the genome and perpetuating somatic evolution.

Because of the similarities between C-Frag and PCC, these two phenomena are frequently confused. In particular, such confusion is evidenced in many recent studies seeking to understand the mechanism of genome chaos (Heng et al. 2010b, 2011a; Liu et al. 2013; Micronuclear chromosome pulverization may underlie chromothripsis 2012; Crasta et al. 2012). This review seeks to quell that confusion. To do so, C-Frag and PCC are discussed, specifically in regard to similarities and differences in morphology, mechanisms, and outcomes. The basic research and clinical utilities of both phenomena are also discussed. Similarities of the two to other confusing phenomena such as mitotic catastrophe, chromosome shattering, and chromosome pulverization are briefly addressed. Finally, future avenues of research and implications of these studies are described to provide a broad view and underscore the importance of both C-Frag and PCC.

Chromosome Fragmentation

C-Frag Is a Major Type of Mitotic Cell Death

C-Frag is a major form of mitotic cell death that occurs directly during mitosis, both in vivo and in vitro, and results in the progressive degradation of condensed, mitotic chromosomes (Figs. 5.1 and 5.2) (Stevens et al. 2007). C-Frag has been previously observed but was often referred to as PCC (Stevens et al. 2007, 2010). The realization that C-Frag resulted from the degradation of mitotic chromosomes resulted in the conclusion that C-Frag and PCC are distinct mechanisms (Stevens et al. 2007, 2010, 2011a). In addition to the distinctive morphology of C-Frag, degraded chromosomes are mitotic, as evidenced by phosphorylation of histone H3 at Ser10, whereas viability is lost during the degradation of chromosomes, as well as when they are exclusively induced from the window of mitosis (Stevens et al. 2007).

C-Frag differs from apoptosis in both morphology and mechanism. Morphologically, on cytogenetic slides apoptotic cells appear as clusters of small round regions of condensed DNA (Fig. 5.3) (Stevens et al. 2007). Mechanistically, C-Frag is not affected by overexpression of Bcl-2 or caspase inhibition, and fragmented chromosomes have been shown to not react to TUNEL staining, although double-strand breaks are detectable during C-Frag by γ-H2AX (Stevens et al. 2007). Apoptosis, on the other hand, is inhibited by caspase inhibition and Bcl-2 overexpression, and degraded DNA from apoptotic cells exhibit positive TUNEL staining. Interestingly although C-Frag

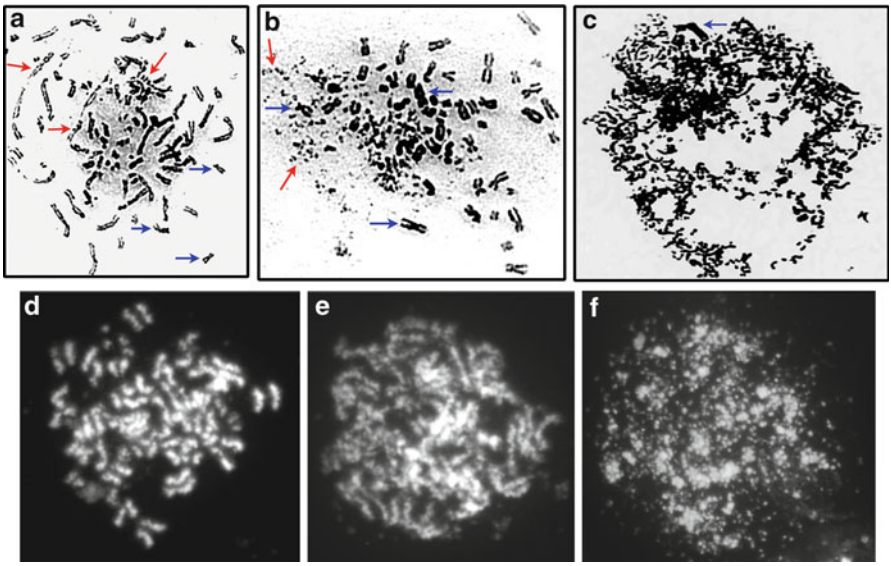

Fig. 5.1 Examples of various stages of chromosome fragmentation (C-Frag). Giemsa (**a**–**c**) and DAPI (**d**–**f**) images of early- (**a**, **d**), mid- (**b**, **e**), and late-stage (**c**, **f**) C-Frag. In early-stage C-Frag, a limited number of chromosomes begin to degrade, as evidenced by broken chromosomes (*red arrows*), while most remain unphased (*blue arrows*). As C-Frag continues, more chromosomes show signs of degradation and chromosome morphology begins to be lost. At late stages of C-Frag, nearly all chromosomes are degraded, although one or more chromosomes may still be intact (*blue arrow*) (**a**–**c** Adapted from Stevens et al. 2007)

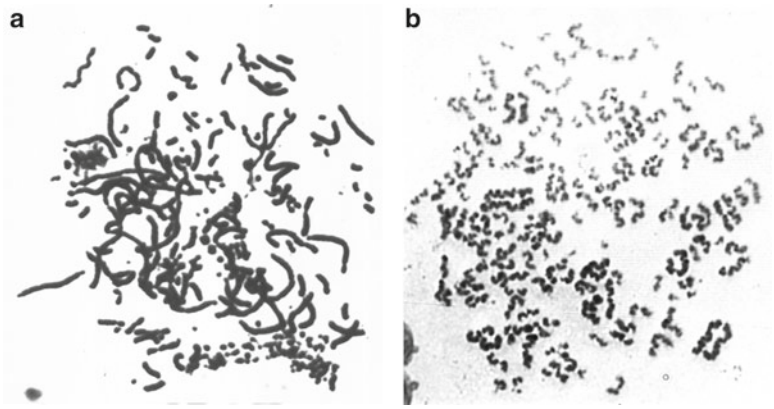

Fig. 5.2 C-Frag occurring at later stages of mitosis and earlier stages of mitosis. The morphology of C-Frag is also dependent on the stage of mitosis in which it occurs. C-Frag occurring early in mitosis (pre-metaphase) results in chromosomes that are not highly condensed and in which separation of sister chromatids is not detectable (**a**). Occurrence of C-Frag at or beyond metaphase results in further condensation of chromosomes, and separation of sister chromatids is evident (**b**). Occurrence of C-Frag in response to various drug treatments more commonly results in chromosome degradation at or after metaphase, especially if the treatment includes inhibitors of microtubule dynamics

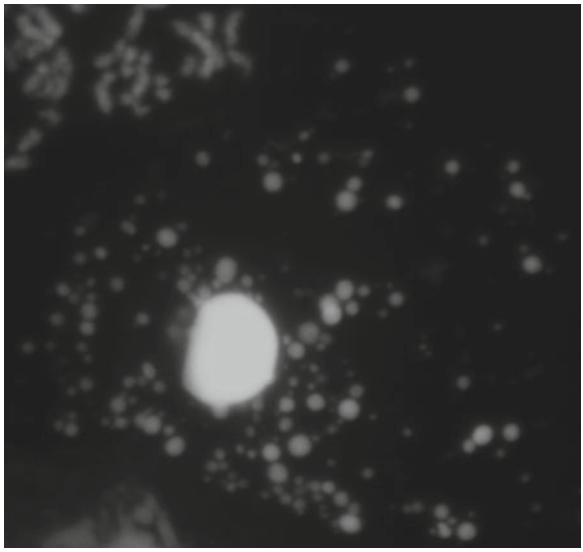

Fig. 5.3 Example of an apoptotic cell. Although apoptosis can result in condensation of fragmented DNA, this condensation differs in morphology from chromatin condensation. Apoptotic DNA fragments aggregate into small, condensed, circular clusters of DNA that differ drastically from condensed chromosomes

differs from apoptosis, poly ADP ribose polymerase (PARP) degradation does take place during fragmentation, indicating that C-Frag may be a programmed cell death where DNA repair by PARP is undesirable during the process (Stevens et al. 2011a).

C-Frag also differs in from mitotic catastrophe (MC), an apparently different form of mitotic cell death (Stevens et al. 2011a). Although the Committee on Cell Death Nomenclature has advised against the term mitotic catastrophe, studies reporting death by mitotic catastrophe are pervasive (Kroemer et al. 2005). Multiple reports have described MC as a cell death that occurs following an abnormal or abortive mitosis (Castedo et al. 2004a; Chan et al. 1999; Roninson et al. 2001). In some cases MC is linked to caspase activation (Castedo et al. 2004a). Morphologically, MC results in multilobulated nuclei and micronuclei that form during the death process (Castedo et al. 2004b). MC morphology is therefore easily distinguishable from C-Frag (Stevens et al. 2011a). Although C-Frag appears to be distinct from other forms of cell death, including apoptosis and MC, it is related to these other deaths in that it works in concert with them to ensure that abnormal and damaged cells are effectively eliminated (Stevens et al. 2011a).

Morphological Characterization of C-Frag

Broadly, C-Frag appears as three distinct morphological groupings: early C-Frag, where limited fragmentation has taken place on a limited number of chromosomes; late C-Frag, where nearly all chromosomes are degraded and most chromosome morphology is lost; and intermediate C-Frag, where extensive chromosomal degradation may have occurred but chromosome structure is largely still apparent

(Fig. 5.1) (Stevens et al. 2007). C-Frag can further be classified based on when it occurs during mitosis. C-Frag occurring during or following metaphase or after extended mitotic arrest results in small, tightly condensed chromosomes that are degraded during the process. C-Frag that occurs before metaphase results in degraded chromosomes that tend to be longer, not overly condensed, and have sister chromatids which have not separated (Fig. 5.2).

C-Frag Occurs as a Response to Stress

Mechanistically, C-Frag occurs as a general response to stress (Stevens et al. 2011a). C-Frag was originally identified in cells treated with genotoxic drugs; however, C-Frag is not caused directly by genotoxicity by a specific drug/reagent. C-Frag has been shown to occur in response to a number of treatments that can broadly be summed as stresses to the cellular system. Initially, C-Frag was described as chromosome pulverization resulting from measles infection, but it has subsequently also been shown to occur in knockouts of ATR, ATM, and p53 (Nichols and Levan 1965; Brown and Baltimore 2000; Fukasawa et al. 1997). Furthermore, C-Frag can be induced by endoplasmic reticulum stressors such as A31187, DTT, and thapsigargin. In fact, simply increasing the temperature at which cells are cultured increases the frequency of C-Frag. Finally, stress induced by the effects of genomic instability results in C-Frag. Cell lines with high levels of genomic instability also show increased levels of spontaneous C-Frag (Stevens et al. 2011a).

Further support for the mechanistic link between stress and C-Frag is evidenced by centrosome amplification (Stevens et al. 2011a). Centrosome amplification also occurs during times of stress. HSP90 is a core component of the centrosome, and inhibition of its function with 17-DMAG increases C-Frag (Stevens et al. 2011a). Taken together, C-Frag can be described as a mitotic cell death that occurs when mitotic cells are exposed to stress in general or which occurs when cells that have encountered lethal levels of stress are able to bypass cell-cycle checkpoints and enter mitosis (Fig. 5.4) (Stevens et al. 2011a).

C-Frag and Genomic Instability

C-Frag has direct implication for genomic instability. Cell-cycle checkpoint function is often abrogated in cells with genomic instability such as cancer cells. Loss of cell-cycle checkpoint function is common in cancer. Thus, mitotic cell death is the major type of cell death that occurs during cancer therapy, and C-Frag is a major form of mitotic cell death. Normally, damaged or highly stressed cells are arrested at cell-cycle checkpoints where the damage can then be fixed, or signals for apoptosis are given. In cells with genome change, however, gene networks are altered and their function changes, resulting in the ability to escape checkpoints;

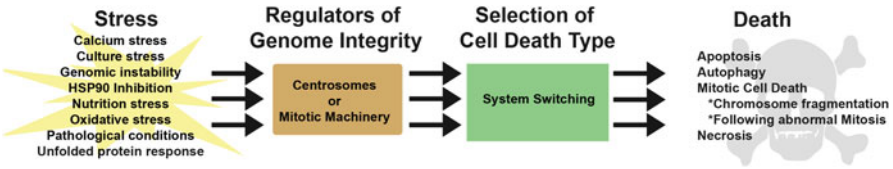

Fig. 5.4 Relationship between cellular stress and death. Cells subjected to levels of stress respond in multiple ways. Sublethal doses of stress in most cases result in adaptation of the cell without disruption to the genome system. Lethal doses of stress, however, affect individual cells differentially. The type of cell death that is induced is dependent on a number of factors including the integrity of the affected cell genome, the current state of the cell (such as the point in the cell cycle where the cell resides during the stress encounter or the intactness of cell-cycle checkpoints), the availability of cell death-inducing networks, and the degree and type of stress that is encountered (Adapted from Stevens et al. 2011a)

therefore, mitotic cell deaths, especially C-Frag, are the last line of defense to eliminate cells with altered genomes.

In the case of genomic instability, C-Frag can be a double-edged sword. Although C-Frag does eliminate cells with altered genomes, C-Frag can induce further genome change. C-Frag-related genomic change occurs in three ways. First, C-Frag can eliminate single (or multiple) chromosomes without inducing cell death, resulting in aneuploidy (Stevens et al. 2007). This chromosome elimination is more common when treatment dosages are sublethal. Interestingly, in yeast it has been shown that genome duplication followed by the loss of chromosomes is a major mechanism in the development of aneuploidy. C-Frag is one way to lose chromosomes and create aneuploidy. Second, in certain cases the process of C-Frag can stop, leaving large regions of chromosomes partially digested. Repair mechanisms such as nonhomologous end-joining can repair these partially digested chromosomes, resulting in highly rearranged chromosomes that are indicative of genome chaos. Interestingly, this could provide a mechanism for chromothripsis, one subtype of genome chaos where portions of one or more chromosomes are inserted into another chromosome, resulting in a highly rearranged chromosome with repeating segments of other chromosomes (Stephens et al. 2011; Liu et al. 2013). Third, a fraction of C-Frag cases directly contribute to genome chaos (Liu et al. 2013). Following induction of C-Frag, chromosome repair mechanisms including nonhomologous end-joining reattach chromosomal fragments to form new chimeric chromosomes (Fig. 5.5). Cells with genome chaos persist typically only for a few weeks until a stable genome is selected, but in some cases genome chaos can persist (Stephens et al. 2011; Liu et al. 2013).

Utility of C-Frag

The implications and utility of C-Frag are wide ranging. First, C-Frag functions in concert with other forms of cell death to eliminate diseased, stressed, or altered

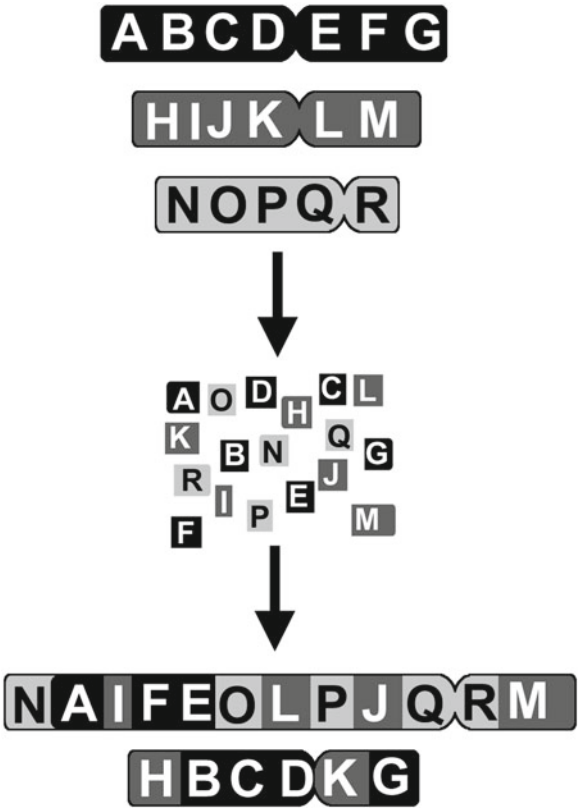

Fig. 5.5 Incomplete C-Frag can lead to multiple types of genome chaos. Following exposure to stress, normal chromosomes (*top*) undergo C-Frag (*middle*). Activation of various repair mechanisms such as nonhomologous end-joining results in rejoining of the fragments in a manner that reshuffles the genome (represented by the changing of the lettered regions of the genome and the chromosome number), resulting in genome chaos (*bottom*) (Adapted from Heng et al. 2011b)

cells (Stevens et al. 2011a). In combination with measures of other types of cell death, C-Frag is useful in measuring the amount of cell death occurring in a given sample. C-Frag is especially useful in measuring cell death in cancer samples because mitotic cell deaths are the main types of cell death that occur during cancer treatment and C-Frag is a major form of mitotic cell death. Therefore, C-Frag has obvious utility in evaluating the efficacy of new chemotherapeutic drugs. Second, C-Frag is a type of nonclonal chromosome aberration (NCCA) and is useful as an indicator of genome stability (Stevens et al. 2011a). Spontaneous C-Frag increases with genome instability. Thus, the presence of C-Frag occurring spontaneously at increased frequencies indicates increased genome instability. Incomplete C-Frag can lead to further induction of genomic instability through the development of genome chaos or aneuploidy. Therefore, C-Frag also gives a window into the probability of genome change, which in turn can be used to monitor the evolutionary potential for targeted cells (Stevens et al. 2007, 2011a, b, 2013b).

Premature Chromosome Condensation (PCC)

Definition and Historical Perspectives

Premature chromosome condensation (PCC) is a process whereby interphase chromatin is induced to condense into chromosomes abnormally (Johnson and Rao 1970; Sperling and Rao 1974a; Rao 1982). This condensation should not be confused with apoptotic condensation where degraded interphase DNA condenses (Figs. 5.3 and 5.6) (Martelli et al. 1997). During PCC, mitosis-promoting factor (MPF), which is composed of cyclin b and CDK1 (cdc2p34), is activated, moves from the cytoplasm to the nucleus, stimulating the events of mitosis, including breakdown of the nuclear envelope and condensation of chromatin (Nurse et al. 1976; Masui 2001; Wasserman and Masui 1976). The morphology of PCC differs depending on the stage of interphase in which PCC is induced. Induction of PCC during G_1 or G_2 results in condensation of complete chromosomes (Fig. 5.6) (Johnson and Rao 1970; Potu et al. 1977; Hanks et al. 1983). In the case of G_1 PCC, the chromatin has not been replicated, so the

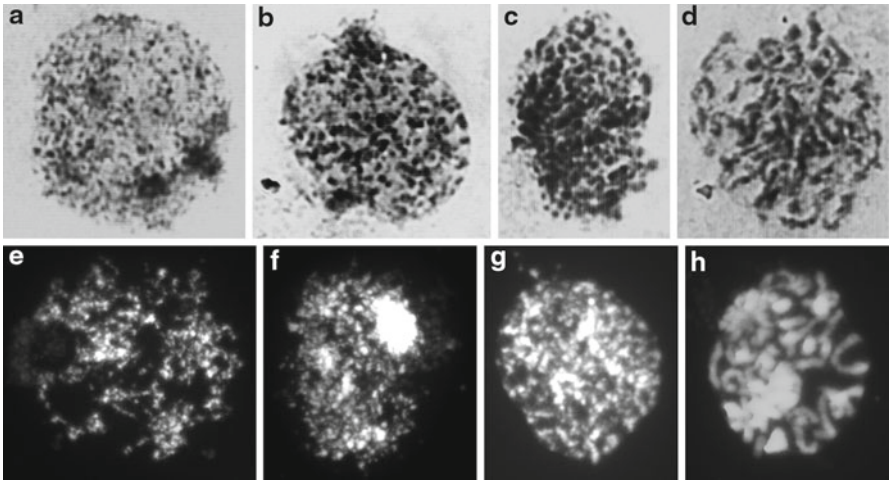

Fig. 5.6 Examples of various stages of premature chromosome condensation (PCC): Giemsa (**a**–**d**) and DAPI (**e**–**h**) staining. Treatment of cells with the phosphatase inhibitor calyculin A induces PCC in cells where cyclin b and CDK1 are present, primarily S-phase and G_2. The morphology of the resultant PCC figure depends on the stage of the cell cycle in which PCC is induced. During S-phase PCCs take on a "pulverized" appearance (**a**–**c**, **e**–**g**). PCCs in early S-phase cells result in condensation of small regions of the chromosomes (**a**, **e**). The visibly condensed regions have replicated whereas the unreplicated regions remain less tightly condensed. As S-phase proceeds, larger portions of the genome are replicated and chromosome morphology becomes more apparent (**b**, **c**; **f**, **g**). Notice the uniformity in size of the condensed regions within each cell. G_2 PCC causes condensation of fully replicated chromosomes, resulting in long chromosomes with little chromatid separation (**d**, **h**)

chromosomes lack sister chromatids. G_2 PCC chromosomes appear similar to early prophase chromosomes: they tend to be long, skinny, and not very condensed. Induction of PCC during S-phase results in the most unique chromosome morphology of the three stages of interphase. S-phase chromatin has not been fully replicated, and PCC induced during S-phase results in figures where replicated regions of chromosomes have normal condensed chromosome morphology while intervening unreplicated regions appear to lack chromatin (Fig. 5.6). This appearance can lead to the false conclusion that there are breaks between the replicated regions.

PCC was first described in 1970 when it was found that fusion of mitotic and interphase cells by the Sendai virus would cause condensation of the chromatin from the interphase cell (Johnson and Rao 1970). This finding demonstrated that mitotic cells could induce condensation of interphase chromatin but did not identify the reason for this. Subsequent work showed that MPF was the factor that initiated the PCC process (Masui and Markert 1971). MPF was soon after identified as a heterodimer of cyclin b and cdc2 (CDK1) (Lee and Nurse 1987).

Other methods to induce PCC have also been developed (Miura and Blakely 2011). Soon after the discovery that inactive Sendai virus could induce fusion-based PCC, protocols were developed that used ethylene glycol to induce fusion, eliminating the need for viral production (Lau et al. 1977). A number of chemicals have also been discovered to be capable of inducing PCC. For example, caffeine in combination with inhibitors of DNA synthesis such as hydroxy urea has been shown to induce PCC (Rybaczek and Kowalewicz-Kulbat 2011). The maturation of MPF requires phosphorylation of CDK1, which occurs spontaneously without dephosphorylation by protein phosphatase 2C (PP2C). Okadaic acid was found to dephosphorylate PP2C, inactivating it and inducing PCC (Ghosh et al. 1992). Subsequently calyculin A, a molecule with increased affinity for PP2C over other protein phosphatases, was isolated and found to potently induce PCC (Miura and Blakely 2011).

PCC has also been shown to occur as a result of various genetic manipulations. Attenuation of the G_2 checkpoint by deletion of key checkpoint proteins such as ATR can sensitize cells to PCC, especially in the presence of inhibitors of DNA synthesis (Nghiem et al. 2001). Other systems have been developed where PCC is induced in syncytia by coculture of one cell line expressing a receptor such as CD4 and another expressing a ligand such as the HIV envelope protein (Castedo et al. 2002, 2004c). As further discussed next, many phenomena previously described as PCC are actually C-Frag. Such confusion is caused by similar phenotypical characteristics.

Induction of PCC by fusion or by inhibitors of PP2C does not differ mechanistically because both types of PCC are caused by the exposure of interphase chromatin to activated MPF, regardless of the origin of the MPF. Aside from fusion-based PCC resulting in two closely located mitotic figures (the original mitotic chromosomes and the PCCs), G_1 PCC is rarely detectable by drug-induced PCC. Drug-induced PCC requires the presence of cyclin b and CDK1 to have an effect, and cyclin b in most cases is not expressed until S-phase (Bezrookove et al. 2003).

Application of PCC

The major application of PCC has been to induce condensation of interphase chromatin to allow for cytogenetic analysis (Bezrookove et al. 2003; Potu et al. 1977; Cheng et al. 1993). In addition, the PCC method has been used to address many basic questions of cell biology, and it has been instrumental in identification of the proteins and complexes of the cell cycle, especially mitosis (Potu et al. 1977; Sperling and Rao 1974b). PCC has also aided in developing the current understanding of how mitosis occurs. More recently PCC has been used to uncover unique mechanisms of DNA repair and to determine bystander effects of nuclei exposed to radiation (Terzoudi et al. 2008, 2010). In addition to its use in basic research, PCC has been an important method for karyotyping tissues and cells with low mitotic indices, as mitotic figures are required for cytogenetic analysis (Bezrookove et al. 2003; Miura and Blakely 2011). PCC is commonly used to measure radiation exposure and DNA damage caused by other exposures (Miura and Blakely 2011; Balakrishnan et al. 2010). Interestingly, fusion-based PCC could be used to determine the stage of the cell cycle that is the most susceptible to various forms of DNA damage. In this method PCC is induced in lymphocytes from patients with radiation exposure to visualize chromosome breaks to ascertain radiation exposures. PCC has also been used to karyotype tumor tissue as it reduces the need for cell culture. It is important to optimize conditions of PCC induction when it is used for monitoring DNA damage, because the condition of the cells in which PCC is induced, the concentration of okadaic acid or calyculin A, and the time of treatment all affect the quality of the figures produced (Miura and Blakely 2011).

Direct Comparison of C-Frag and PCC

Differences

C-Frag and S-phase PCC figures can be difficult to differentiate, but the phenomena can be differentiated by a number of factors (Table 5.1). Fusion-based PCC is the easiest form of PCC to differentiate from C-Frag as fusion-based PCC results in figures containing a mitotic cell located closely to the nucleus undergoing PCC. Although there are instances of the two figures overlapping, this is uncommon. Visual assessment of ploidy levels of the involved nuclei can be helpful in cases of overlap. Although most cell lines have some degree of aneuploidy, nuclei from fused cells can be differentiated from aneuploidy cells by having an idea of the average chromosome count of the studied population. Differentiation of nonfusion-based PCC and C-Frag is slightly more difficult. Morphologically, C-Frag results in a higher diversity of chromosomal morphology within a given mitotic figure. C-Frag, especially in cases of intermediate-stage fragmentation, produces mitotic

Table 5.1 Diverse mechanisms of chromosome fragmentation (C-Frag)

Factors of pulverization/shattering	Species	Year	References
UV exposure	*Tradescantia paludosa*	1954	Lovelace (1954)
Exposure of male mice to methyl methanesulfonate before fertilization of female mice. Shattering seen in filial cells	Mouse	1975	Brewen et al. (1970)
Infection of lymphocytes with JM-V herpesvirus	Chicken	1976	Yoon et al. (1976)
Treatment with fungicide *N*-trichloromethylthio-phthalimide	Human	1978	Sirianni and Huang (1978)
Pulverization caused by UV light and caffeine	Chinese hamster	1980	Cremer et al. (1980)
Exposure to alpha-amanitin	Rat	1985	Magalhães and Magalhães (1985)
Doxorubicin treatment. Pulverization inhibited in drug-resistant cells	Human	1986	Tapiero et al. (1986)
Herpes simplex virus type 1 infection	Human	1986	Peat and Stanley (1986)
Hepatitis B infection. Pulverization occurs in both hepatocellular cell line derived from a tumor and in peripheral lymphocytes from the patient	Human	1986	Simon and Knowles (1986)
Herpes simplex virus (HSV) type 1 infection. Endoreduplication was noted. Also HSV infection increased the mitotic index		1986	Chenet-Monte et al. (1986)
Friend leukemia cells exposed to high levels of ADM	Mouse	1986	Patet et al. (1986)
Following incubation of cells with temperature-sensitive DNA polymerase α in S-phase at 39 °C that were then cultured in a permissive temperature	Mouse	1987	Eki et al. (1987)
Photoirradiation of G_2 or early prophase cells	CHO	1990	Fernandez et al. (1990)
Hepatitis B integration and genomic instability	Human	1993	Grabovskaya et al. (1993)
N-Methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), sodium selenite, and caffeine treatment of CHO cells	Chinese hamster	1994	Balansky et al. (1994)
Culture of a fibroblast line generated from a patient with xeroderma	Human	1995	Casati et al. (1995)
Associated with ubiquitin-activating enzyme E1 activity	Mouse	1995	Sudha et al. (1995)
Vaccination against hog cholera virus	Swine	1998	Genghini et al. (1998)
Radiation exposure	Mouse	1998	Ganasoundari et al. (1998)
Maintenance of diploid karyotype in PA-1 cells by removal of tetraploid cells	Human	1999	Gao et al. (1999)

(continued)

Table 5.1 (continued)

2-Methoxyestradiol, an endogenous metabolite of estrogen	Human	2000	Tsutsui et al. (2000)
Following vaccination of pigs for swine fever	Swine	2002	Genghini et al. (2002)
Streptozotocin treatment	Human	2003	Bolzán and Bianchi (2003)
Viral infection in pigs	Swine	2004	Sutiaková et al. (2004)
Vitamin C treatment of lymphocytes	Human	2008	Nefíc (2008)
Shattering by UV light and caffeine exposure	Chinese hamster	2009	Hübner et al. (2009)

Source: Adapted from Stevens et al. (2010)

figures where some chromosomes are degraded to the point that chromosomal morphology is lost while some chromosomes may remain intact. In contrast, the chromosomal morphology and the number of gaps between condensed regions in S-phase PCC chromosomes is dependent on the degree of replication that has occurred. Although certain chromosomes may replicate at different times, overall replication tends to occur similarly across the genome. Thus, induction of PCC in S-phase cells results in figures with a more regular morphology than those produced by C-Frag. An exception to this rule occurs in micronucleated cells. Micronuclei can replicate at different times than does the main nucleus. When the cell completes G_2 and enters into M-phase, partially replicated micronuclei can be induced to undergo PCC, resulting in a small region of a mitotic figure showing similar morphology to C-Frag. Care should be taken during cytogenetic analysis to note the prevalence of micro- and multinucleated cells within the population as C-Frag may be over estimated in these populations. C-Frag occurring in later stages of mitosis can also be easily differentiated as PCC does not produce highly condensed chromosomes. Taken together, careful morphological inspection during cytogenetic analyses can differentiate C-Frag and PCC.

C-Frag and PCC can also be differentiated biochemically. Although morphologically S-phase PCC figures appear to have breaks interspersed in regions of condensed chromatin, these gaps are not formed by DNA breaks (Gollin et al. 1984): they are simply composed of the unreplicated regions of DNA that do not readily condense. The lack of DNA breaks in S-phase PCC has been confirmed by electron microscopy and the lack of γ-H2AX staining, which is indicative of double-strand breaks. The chromosomal pieces observed in C-Frag, on the other hand, show extensive γ-H2AX staining, although to date electron microscopy has not been performed on C-Frag. A prepulse of bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdUrd) before induction of S-phase PCC demonstrates active DNA replication during S-phase PCC whereas culturing mitosis-arrested cells in BrdUrd and subsequently inducing C-Frag by doxorubicin treatment did not reveal active DNA replication, demonstrating that C-Frag occurs during mitosis and is not related to S-phase. Furthermore, during C-Frag induction of PCC does not result in PARP degradation. Outside the nucleus, PCC and C-Frag also differ. C-Frag is associated with multiple centrosomes while

spindle formation is inhibited during PCC (Ghosh et al. 1992). Thus, C-Frag and PCC are distinguishable at both morphological and biochemical levels.

Overlap

Although PCC and C-Frag can be differentiated in most cases, there are some areas of overlap between the two phenomena. This overlap centers on the G_2 checkpoint. In cancer, the checkpoint function is often abrogated (Kastan and Bartek 2004), allowing cells with DNA damage that would normally arrest before mitosis until that damage was repaired to abnormally enter mitosis. Entry into mitosis with DNA damage then can result in the induction of C-Frag (Stevens et al. 2011a). Thus, cells that abnormally slip past the G_2 checkpoint are prematurely entering mitosis. Although C-Frag and PCC are distinct phenomena, as with many cellular phenomena there is some overlap.

Pulverization, Shattering, and Mitotic Catastrophe

Pulverization and Shattering

A number of reports of chromosome pulverization and chromosome shattering predate discovery of PCC (Lovelace 1954; Nichols and Levan 1965; Kato and Sandberg 1967). Subsequent discovery of PCC led to the shattered figures being called PCC, although there must be some doubt to PCC being the mechanism in all cases of shattering or pulverization because the continued use of the terms 'shattering' and 'pulverization'. Although some cases of pulverization are indeed PCC, such as in the case of binucleate cells in which the nuclei asynchronously replicate and in cases of cellular fusion that result in obvious PCC figures (Kato and Sandberg 1967), other cases wherein pulverization is induced by various widely ranging stresses, including viral infection, chemical exposure, radiation exposure, and genetic defects, this pulverization is most likely C-Frag (Table 5.2) (Stevens et al. 2010; Nichols and Levan 1965). Recently chromosome pulverization has been raised as a potential mechanism behind chromothripsis, a form of genome chaos where a single chromosome recombines multiple times to form a highly complex chromosome (Crasta et al. 2012). However, pulverization is synonymous with PCC, and PCC does not result in strand breaks; thus, pulverization cannot contribute to chromothripsis (Stevens et al. 2010; Micronuclear chromosome pulverization may underlie chromothripsis 2012; Gollin et al. 1984). Chromosome pulverization, or shattering, is a morphological description, whereas C-Frag and PCC are both morphological and mechanistic descriptions. Therefore, the use of chromosome pulverization/shattering should be avoided in favor of either S-phase PCC or C-Frag, depending on which process is observed. As we previously suggested, the use of C-Frag will reduce such confusion (Stevens et al. 2010).

Table 5.2 Identifiable differences of chromosome fragmentation and premature chromosome condensation (PCC)

Chromosome fragmentation	PCC
Morphological	
Single cell involvement	If fusion induced, normal, intact mitotic cells will be in close proximity to fragmented cells
Can affect single chromosomes	Impacts entire genome regularly, except in limited multinucleated cells
Results in chromosome degradation	Unknown, may activate chromosome breakdown
Chromosome morphology lost as process progresses	Chromosome morphology dependent on position in cell cycle
Differential cut size	Differential condensation states
Mechanistic	
Occurs during mitosis	Occurs in interphase cells exposed to active MPF
Not inhibited by roscovitine	Inhibited by roscovitine
Induced by stress during mitosis	Induced by cell fusion or activation of MPF
γ-H2AX positive	γ-H2AX negative
No active DNA incorporation	Actively incorporating DNA

Source: Previously published from Stevens et al. (2010)

Mitotic Catastrophe

The term mitotic catastrophe has been applied to a number of phenomena where cell death is linked to mitosis. Various reports have shown mitotic catastrophe to occur with multiple phenotypes, which range from cell death following abnormal mitosis where apoptotic pathways are activated, to cell deaths that occur directly during mitosis which may or may not be associated with apoptosis (Chan et al. 1999; Roninson et al. 2001; Castedo et al. 2004b; Hübner et al. 2009). Despite warnings against its use because of the lack of a solid, recurrent morphological or mechanistic definition, the term mitotic catastrophe has remained pervasive in the literature (Kroemer et al. 2005). Many reports of mitotic catastrophe rely on DNA content measures to define mitotic cells without using specific markers of mitosis such as histone H3 phosphorylation at serine 10 or without cytogenetic or in-depth morphological characterization. This carelessness has led to increased confusion in the field.

C-Frag has been shown to differ from a well-described model of mitotic catastrophe. In this model, cells lacking 14-3-3σ, which plays a role in the G_2 checkpoint, have been shown to undergo a mitotic cell death following low-dose doxorubicin treatment; however, when these cells were treated no C-Frag was detectable (Stevens et al. 2007; Chan et al. 1999). Other models of mitotic catastrophe have been developed that are based on cellular fusion (Castedo et al. 2002, 2004a). In this case it is likely that a significant portion of cells are undergoing PCC. The fact that descriptions of mitotic catastrophe are wide ranging should, however, not detract from the important message that mitotic cell deaths play a major role in the reduction of tumor size following chemotherapy. It is apparent that at least two distinct types of mitotic cell death occur: C-Frag, which occurs directly during mitosis, and another form of cell death that occurs following an abnormal or failed mitosis.

Future Perspectives

A major drawback of cytogenetic analyses is the requirement for condensed chromosomes to perform most analyses. PCC has proven to be an important cytogenetic method that increases the ability to observe mitotic figures in cells or tissues which are not actively dividing, and it will continue to have multiple clinical and basic research implications. PCC has proven to be instrumental in the development of our knowledge of the cell cycle, especially the proteins and complexes that are involved in mitosis. PCC will continue to be an important tool in measuring DNA breaks associated with radiation and other hazardous exposures. Development of improved methods of inducing PCC, especially methods of inducing G_1 PCC to allow cytogenomic analysis of tissues with little or no mitotic activity, will improve the power of PCC and cytogenomics in general.

Multiple questions about C-Frag remain and should be addressed in future work. First, although C-Frag differs from apoptosis, these two types of cell death appear to complement each other in that they both eliminate unfit cells. Further work will determine potential links between these deaths and other deaths such as autophagy and necrosis, and whether other forms of cell death may be activated late in the process of C-Frag (Stevens et al. 2007). Determination of whether there is a specific order of C-Frag may reveal more of the biology behind C-Frag. Identification of whether there are specific sequence motifs that are targeted early in C-Frag, and whether portions of the genome are more resistant to degradation, would also be interesting; current genomic technologies such as next-generation sequencing put this research within grasp and could aid in identification of the proteins/systems directly responsible for fragmenting the chromosomes.

As C-Frag represents a type of NCCA, a key question is what is the relationship between C-Frag and overall frequency of the NCCAs? How to use the C-Frag to predict the outcome of cancer therapy and potential risk of induced genome chaos? Increasing the knowledge of how C-Frag is related to genome chaos and other chromosome aberrations may provide new insights into the somatic evolution leading to cancer. Finally, as C-Frag may also be involved in developmental processes, more research is needed to address its significance in both development and evolution (Fujiwara et al. 1997; Gernand et al. 2005).

Conclusion

Chromosome fragmentation and PCC are processes that are distinct both morphologically and mechanistically, although they have been confused in the past. C-Frag is a major form of mitotic cell death that is induced by various forms of stress whereas PCC is a phenomenon in which interphase chromatin is forced to abnormally condense outside mitosis. Both C-Frag and PCC are relevant for both basic research and in the medical clinic, and future studies of both phenomena will increase our understanding of the chromatin and chromosomes.

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