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Examination of Replication Dynamics in Fragile Sites Through Molecular Combing

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EXAMINATION OF REPLICATION DYNAMICS IN FRAGILE SITES THROUGH MOLECULAR COMBING

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ABSTRACT

Chromosomal fragile sites are specific loci that exhibit instability visible as gaps and breaks on the chromosome following inhibition of DNA synthesis and are generally categorized into two main classes: rare fragile sites (RFSs) and common fragile sites (CFSs). Under standard conditions, CFSs are typically stable but are prone to breakage in cells subjected to replication stress. In recent years, their role in the generation of gross chromosome rearrangements has become increasingly evident, and fragile sites have now connected to chromosome instability in cancer cells. The connection between CFSs and cancer thus highlights the importance of the regulation of DNA replication to prevent cancer development. The study of fragile sites in the yeast model organism has provided insight into the mechanisms that lead to breakage and genome instability. Through the process of molecular combing, replication dynamics can be observed at fragile sites to further understand the consequence of replication stress on DNA damage.

LITERATURE REVIEW

Vast improvements in biomedical research have increased our understanding of life's many diseases and disorders. Developments in antibiotics and experimental medicines alone have statistically decimated communicable diseases (16), yet as humankind slowly discovers the solution to one problem, another emerges. Cancer has become one of society's greatest burdens and is now responsible for one in eight deaths worldwide (16). It is a genetic

disease, caused by the ill effects of genomic damage in the DNA of otherwise non-cancerous cells, and originates in most of the cell types and organs of the human body (16). The cells themselves are characterized by relatively unrestrained proliferation that can invade beyond normal tissue boundaries and metastasize to distant organs (16).

Though the term encompasses a large pool of diseases, all cancers are thought to share a common pathogenesis (16). Analogous to Darwinian evolution, cancer development is based on two constituent processes: (1) the continuous genetic variation in individual cells by more-or-less random mutation, and (2) natural selection acting on the resultant phenotypic diversity (16). Selection may have the ability to weed out many of the deleterious mutations or foster cells carrying alteration that can emerge as invisible and benign cell growth (16). However, occasionally cancer growth emerges when DNA replication of cells incurs genetic damage and allows an advantageous mutation to proliferate autonomously, invade cells, and metastasize (16).

I. DNA Replication

Replication of the eukaryotic genome is a difficult task, as cells must coordinate chromosome replication with chromatin remodeling, DNA recombination, DNA repair, and transcription cell cycle progression (12). This process is initiated in multiple steps along the chromosome by origins of replication (1). Studies have shown that replicating origins are first licensed during the G1 phase of the cell cycle through the stepwise assembly of pre-replication complexes (pre-RC) (1)(2). Cells are then initiated into the S phase of mitosis, where DNA replication occurs. Replication forks and origins are controlled by specific mechanisms to ensure they are activated once, and only once, per cell cycle (1) (2). Despite its many reparative functions in the face of mutations and mistakes, DNA replication can be a genotoxic process (12).

Even though the process should be as reliable as possible in order to minimize mutations, DNA replication in some regions of the genome appears to raise specific problems (10). At these regions, DNA replication forks frequently slow down or even stall

when encountering obstacles in their way, such as repetitive sequences or secondary structures (12). Most of the time, the stalled forks can easily resume synthesis after the block; however, these regions of the chromosome lead to fork collapse and accumulate abnormal DNA intermediates such as long stretches of single stranded DNA (ssDNA) or DNA breaks or gaps (12). Breaks and additional structures can prevent fork restart and cause cell lethality or genome deletions that can lead to instability (12).

Genetic instability and deletions in cancer genomes occasionally occur over chromosomal fragile sites, where they are thought to reflect an increased local rate of DNA breakage (3). Fragile sites are regions of the chromosome where inhibition of DNA replication can lead to gaps and breaks, and several fragile sites are located at or near tumor suppressor genes (6). Mutations in such genes that encode the proteins that control the cell cycle are extremely common in cancer cells and lead to no detection of incorrect growth and to the abortion of replication in the instance of damaged DNA (3). Thus it is thought that some tumor suppressor genes become deactivated due to chromosome breakage at fragile sites (16).

II. Fragile Sites

Chromosomal fragile sites are specific loci that preferentially exhibit chromosome instability, visible as gaps and breaks on the chromosome following partial inhibition of DNA replication (6). The study of human fragile sites has led to identification of diseases such as fragile X syndrome and trinucleotide repeat expansions (6). Fragile sites are generally categorized into two main classes: rare fragile sites (RFSs) and common fragile sites (CFSs) (6). Rare fragile sites (RFS) are uncommon in the human genome because they result from mutation, and they segregate in a Mendelian manner (6). RFSs are the result of expansions in tri- and dinucleotide repeats that allow for the formation of secondary structures, leading to fragility during replication (6). In the clinical context, some rare fragile sites are linked with conditions causing mental retardation, such as Fragile X syndrome (FRAXA) and Jacobsen syndrome (FRA11B) (6).

In contrast, common fragile sites (CFSs) are present in all individuals and are a component of normal chromosome structure (6). Under standard conditions, CFSs are typically stable, but they are prone to breakage in cells subjected to replication stress, particularly under low levels of DNA polymerase alpha, a critical enzyme of DNA replication (6). In cultured cells, they are most efficiently induced in breakage by low doses of aphidicolin (APH) treatment, an inhibitor of DNA polymerase alpha (7). Following such treatment, they become hotspots for sister chromatid exchange, and they give rise to a high frequency of translocations and deletions (6).

Common Fragile Sites	Associated Genes
FRA2G	*IGRP,RDHL,LRP*
FRA4F	*GRID2*
FRA6E	*PARKIN, MAR3K,LPA*
FRA6F	*REV3l, DIF13, FKHRL*
FRA7E	*LEP*
FRA7G	*CAV1,CAV2,TESTIN,MET*
FRA7H	*Not Identified*
FRA7I	*PIP*
FRA7K	*IMMP2L*
FRA9E	*PAPPA,ROD1,KLF4*
FRAXB	*STS*

Table 1: Human common fragile sites that have been cloned and their related genes.

Sixteen CFSs have been cloned and characterized at the molecular level (6). Most lie within or near known genes, and the two most frequently broken fragile sites in lymphoblasts, FRA3B and FRA16D, lie within tumor suppressor genes. FRA3B is centrally located within the *FHIT* gene, while FRA16D lies within the *WWOX* genes (6).

In recent years, their role in the generation of gross chromosomal rearrangements has become increasingly evident, and defects in DNA replication, or in the replication checkpoint greatly increase chromosome instability in cancer cells (7)(10). The connection between CFSs and cancer thus highlights the importance of the regulation of DNA replication to prevent cancer

development (7). A direct involvement of CFSs in cancer has not been yet established; however, a significant association between fragile sites and chromosome aberrations found in tumor cells has been demonstrated (7). Presently, a large consensus agrees that majority fragile site instability results from cells entering mitosis before completion of their replication (11), yet the mechanisms responsible for the delayed replication are still debated (11).

III. Mechanisms of Common Fragile Site Instability

The identification of the molecular mechanisms responsible for instability at fragile sites represents a major challenge (10). Characterizing these breakage and repair mechanisms may allow for a better understanding of the causes of genetic mutations that contribute to cancer.

Secondary Structure Hypothesis

Computational analysis performed on a subset of fragile site sequences indicated that CFSs containing frequent AT-rich islands, without any repeat motifs such as expanded trinucleotide or mini-satellite repeats, may be responsible for the formation of

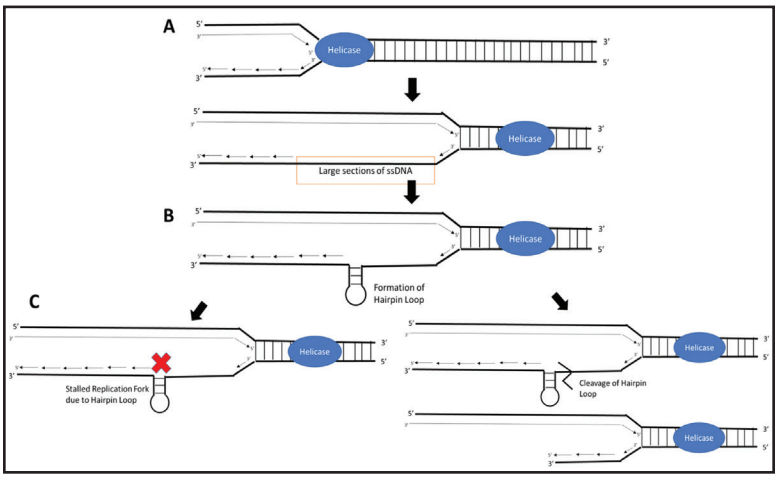

Figure 1. Formation of secondary structure. A) Low levels of DNA polymerase contributes to slow replication, leading to long stretches of ssDNA. B) Long ssDNA eventually self-pairs and forms hairpin loops. C) Hairpin loops either become barriers that halt replication or result in a cleavage that creates DNA breakage.

secondary structures (7). This idea has long been a hypothesis for explaining why CFSs are prone to breaking under conditions of replication stress (7). With this hypothesis, structures formed by these sequences stall replication forks, which may lead to DNA breaks at collapsed replication forks and chromosomal rearrangements (10). What occurs first is that low levels of DNA polymerase alpha contribute to a slow replication rate, resulting in a lagging polymerase complex (10). As a result, replication polymerase pausing may occur, and long single-stranded DNA (ssDNA) regions are produced at stalled forks (7). As DNA in this single-stranded area bends back on itself, a region of sequence can pair to create secondary structures, such as hairpin loops (10). Thus, DNA breakage occurs either directly as a result of cleavage at the site of the secondary structure or as a result that leads to a stalling of the replication fork, eventually blocking the firing of new replication origins and preventing entry into mitosis and promoting repair **(Fig. 1.)** (7)(10).

It should be noted that recent genome-wide analyses of CFSs sequences have provided contrasting results regarding the presence of flexible AT-rich regions within these sites (10). Indeed, some reports claim that CFSs are highly enriched in flexible AT-rich regions, while others fail to identify specific accumulation of such sequences in the sites (7)(10). On one side, several analyses have shown that DNA sequences within or adjacent to deletion breakpoints contain AT-rich motifs, suggesting that these regions are prone to breakage (10). On the other side, chromosomes with deletions that remove AT-rich sequences in FRA16D or FRA3B still continue to break at the corresponding fragile site (10).

Origin Paucity Hypothesis

Apart from the possibility that CFSs form secondary structures that may impair replication fork progression, findings also support a role for replication origin density in determining the fragility of CFSs (2)(7). Letessier and colleagues state that due to the scarcity of origins of replications in fragile sites, the hypothesis of paucity of origins is a causative agent in why CFSs break. It predicts that fragility is due to two reasons: there are simply fewer origins prelicensed to be activated, and all available

origins in the fragile site regions are already activated under normal growth conditions, so no additional ones can be activated during times of replication stress (14).

The activity of origins on human chromosomes greatly differs between cell types, and human CFS instability is correlated with origin paucity (11). For instance, the human common fragile sites FRA3B and FRA16D are lacking in origins in lymphoblast cells and are frequently broken in this cell type (6). Yet in fibroblast cells, these same fragile sites are not origin-poor and account for only ~5% of all fragile site breaks (11). Nonetheless, the fact that both fragile site regions still break at a detectable frequency in fibroblasts indicates that mechanisms other than origin density are also likely to contribute to their instability under replication stress. Thus, the described origin paucity model only partially explains fragile site instability (11)(14).

Tissue Dependent Hypothesis

Recent mapping of CFSs in different cell types by conventional and molecular cytogenetic approaches confirmed that their instability is tissue dependent (10). These results imply that sequence alone cannot account for CFS instability and raise further questions of whether any chromosome region can be fragile in one or another type of tissue (10). Le Tallec and colleagues have examined a wide variety of fragile sites in lymphocytes, fibroblasts, breast and colon epithelial cells, and erythroid cells (10). Interestingly, comparison of these CFSs has revealed that many of these loci are unstable in several tissues, although their level of fragility could vary from one cell type to the other (10). Together, these data suggest that finite numbers of loci constitute the pool of CFSs and that only a limited subset of these loci is fragile in a given cell or tissue type (10).

Gene Size Hypothesis

Many CFSs co-map with very large genes, ranging from 600 kb to more than 2 Mb (10). The extensive mapping performed recently in different human tissue and different species shows that between 80 and 100% of human CFSs, depending on the cell type, and 100% of those found in mouse embryonic fibroblast, are within genes over 300 kb long (10). These genes are at least 15 times larger than the median length of human genes, which

is approximately 20 kb, and account for approximately 3% of human genes (10). Notably, CFSs mapped in chicken DT40 cells also correlate with large genes (10). The most fragile region in DT40 cells overlaps the large FAM190A and GRID2 genes and is therefore orthologous to human FRA4F and murine FRA6C1 (10). These results suggest that the conservation of CFSs in vertebrates is linked to the conservation of large genes and, conversely, that chromosome regions containing large genes constitute the pool of potential CFSs for all cell types (10).

The human genome contains approximately 700 such genes, sometimes organized in clusters (10). Strikingly, according to Le Tallec and colleagues, reanalysis of the data provided by two reports that catalogued focal deletions in cancers and cancer cell lines has shown that large genes host 51.4% of recurrent deletions and that many of these genes are associated with CFSs visible in one or the other tissues in which the sites have now been mapped (10). These results lead to the conclusion that approximately half of the recurrent focal deletions found in human cancers originate from CFSs unstable in the cell types from which the cancers derive (10).

Transcription Machinery Hypothesis

Recently, it has been proposed that the transcription process may also contribute to the fragility of CFSs (7). As previously mentioned, a number of CFSs have been mapped to the coding regions of large human genes, and it has been well established that transcription of such genes requires a long time to be completed, so that transcription and replication may occur simultaneously (7)(10). Transcription machinery and replication forks may collide and cause genome stability (7). In a collision, DNA polymerase inhibits the elongating RNA polymerase and stable R-loops are created at the site of blockage, thereby contributing to breakage at long CFS-associated genes (7). It is important to note, however, that this mechanism cannot justify the fragility of all the CFSs, as only about half of them are associated with large genes (7).

Although much progress has been made in understanding the underlying causes of common fragile site instability, it must be stated that none of the mechanisms discussed above are mutually

exclusive, and a clear link between replication process and DNA breakage at these loci has not been identified (6)(7). A key role seems to be played by the ability of cells to stabilize stalled forks and to assure their safe recovery (7). Otherwise, stalled forks could disrupt replication fork progression, possibly resulting in the formation of large DNA "unreplicated" regions, which could pose a serious threat to genome stability (7). More detailed information on how cells defend themselves against this threat may come from a better elucidation of mechanisms by which proteins stabilize and/or recover stalled forks, avoiding degeneration into chromosomal instability (7).

IV. Methods to Study Fragile Site Instability

Yeast

CFSs have been highly conserved throughout mammalian evolution, where orthologs of human CFSs have been found in organisms such as primates, cats, dogs, mice, horses, and cows (6). This evolutionary conservation also extends to lower eukaryotes, most notably *Saccharomyces cerevisiae*, or yeast, which allows a more in-depth study of chromosomal breakage (6).

Though the study of CFSs in their natural context within human cells allows for relevant research in comparison to using model organisms, research in S. cerevisiae allows for CFSs regions to be examined out of context, fundamentally separating structural and context-based mechanisms of CFS breaks (2)(6). Yeast fragile sites, in contrast to human fragile sites, are primarily sequence oriented and do not appear to vary in origin usage (6)(13). Yeast as an experimental model, due to their small genome size, also promote the discovery of stalled replication forks by facilitating the collection of larger data sets. For example, according to the National Center for Biotechnology Information (NCBI), the human genome is 3,300 Mb long. If we were to locate human fragile site FRA3B, which is 1 Mb, in relation to the human genome, there is only a 0.033% chance of finding the sequence. In contrast, the smaller 12.4 Mb length of the yeast genome yields a higher percentage of 8.06% in the location of the fragile site. Therefore, it is relatively easier to locate the hard sought needle in a smaller haystack, in comparison to a larger one. Yeast are also

excellent models to study replications fork collapses that lead to breakage, using the powerful method of molecular combing.

Molecular Combing

Understanding the mechanisms involved in the initiation and regulation of DNA replication requires the identification and characterization of origins of replication (4)(5). Classical techniques employed to identify origins of replication include competitive PCR and 2D-gel electrophoresis, which rely on identifying newly synthesized DNA fragments and three-dimensional structures, respectively (4). Molecular combing represents a powerful, more direct method to study replication dynamics at the level of single molecules (12). Using this approach, DNA fibers are stretched and aligned on a glass surface by the force exerted by a receding air/water interface (12). DNA is consistently stretched in a uniform manner, and an array with thousands of DNA molecules is generated (9). Furthermore, because all molecules are identically stretched, reliable measurements of the replication units and of their size distributions can be readily obtained (4).

Analysis of the replication signals on a whole genome basis, issued either from single or sequential labeling, provides useful parameters for dynamic replication studies (4). For instance, because the rate of DNA synthesis correlates with fork densities and distributions, the spatial and temporal organization of DNA replication can be directly deduced on a genome-wide basis (4). Moreover, measurements made on an appropriate set of replication data can reveal the frequency of origin activation during the S phase, with firing events mapped in time (4)(13). Together, these analyses might, in turn, reveal correlation between the different parameters governing DNA replication in a variety of genetic backgrounds (4). In this manner, a comprehensive understanding of the dynamics of genome duplication is feasible, making quantitative studies possible and allowing thoughtful planning of such studies (4)(9).

According to Herrick et al., 1999, the method of DNA combing was first developed during attempts to specifically anchor individual DNA molecules to a solid surface in order to map genetic alterations in the human genome (12). The simple principle behind this method involves the physico-chemical binding of the molecules by one or both of their extremities to a silanized glass coverslip,

and the uniform alignment and homogenous extension of all attached molecules (8). DNA combing has been used to study DNA replication by the direct labeling of replicating DNA sequences using halogenated thymidine analogs, such as iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU), allowing origins of replication to be directly visualized and mapped on a genome wide basis (1)(8)(12). IdU and CldU are incorporated into a yeast artificial chromosome during S phase of the cell cycle and base pair with adenine during their respective 10-minute pulses (6). A later 90-minute pulse of excess thymidine with nocodazole ensures completion of replication and prevents entry into another cell cycle **(Fig. 3)**(6).

Letessier and colleagues used combing to elucidate the fragility of FRA3B, where interestingly, even though they found fork symmetry to be greatly increased after aphidicolin treatment of cells, the stalling showed no difference between the locus and bulk genome regardless of the growth condition, concluding that the replication dynamics along FRA3B are not localized to the *FHIT* gene (11).

Combing studies of replication in S. cerevisiae are more complicated, as they are unable to incorporate the thymidine analogs into their DNA because they lack the nucleotide salvage pathway that enables the uptake of extracellular thymidine or its analogs (1). To overcome this limitation, *S. cerevisiae* strains are engineered to incorporate IdU and CldU (1). Ectopic incorporation of the *Herpes simplex* virus thymidine kinase (HSV-TK) and the human equilibrative nucleoside transporter 1 (hENT1) have been shown to improve thymidine uptake and incorporation (1)(12).

DNA Labeling

With different strains and different experiments, the process of DNA labeling and combing varies. In a typical analysis, yeast cells are first arrested in G1 phase of the cell cycle with α-factor pheromone and later released synchronously into S phase (6)(12). A pulse of IdU is added just before the cells are released from G1 arrest, and the cells are allowed to grow for ten minutes (6). The cells are then switched to a medium containing CldU and allowed to grow for another ten minutes (6). They are subsequently switched to a 90-minute pulse of excess regular thymidine and nocodazole, which allows the completion of regular synthesis and

prevents the cells from entering another cycle of mitosis (12).

DNA is then purified in an agarose plug to limit shearing, or tearing, and placed into a solution of YOYO-1, zymolyase, and proteinase (6). YOYO-1 is a florescent dye that binds genomic DNA and allows for measurement of DNA length, while zymolyase breaks down the cell wall and proteinase consumes residual proteins in order to isolate the DNA (6). The DNA solution is combed onto a coverslip through the combing machine. DNA fibers are attached to the coverslip by their ends, and then the machine creates an upward pulling motion of 300 µm a minute (6). DNA fibers are denatured with NaOH, and after neutralization, IdU and CldU are detected with monoclonal antibodies and visualized with fluorescent secondary antibodies (6). Fluorescence in situ hybridization is also used to detect the fragile site sequence within the yeast, using the Genomic Morse Code (GMC) strategy (4)(9)(11)(14). As the last step, DNA fibers are visualized using an epifluorescence microscope that is coupled to a CCD camera. A visual representation of molecular combing can be found in **Figure 2.**

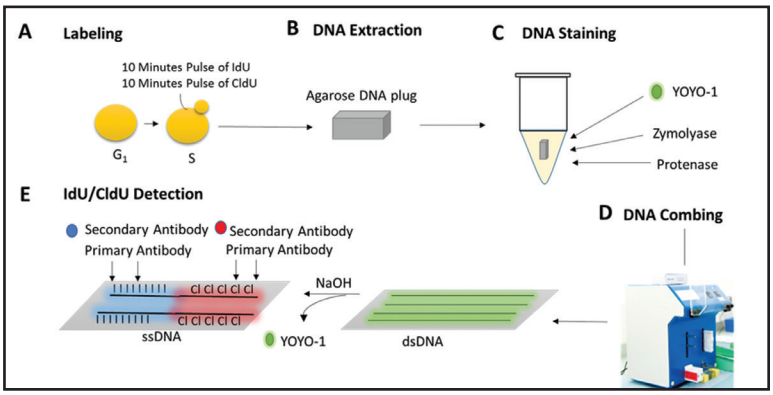

Figure 2. Molecular combing analysis of DNA replication in yeast. (A) Cells are released synchronously into S phase from an α-factor arrest (G1) in the presence of IdU and CldU to label newly-synthesized DNA in their respective 10-minute pulses. (B) Cells are harvested and are embedded into agarose DNA plugs to protect chromosomal DNA from mechanical shearing during the extraction procedure. (C) DNA staining occurs when a plug is put into solution with YOYO-1. Zymolyase breaks down cells wall while proteinase consumes protein to isolate DNA. (D) DNA solution is combed on slides by a combing machine into single strands of DNA. (E) Incorporated IdU and CldU is detected by immunofluorescence using a combination of primary and secondary antibodies and through FISH probes that can determine locations of stalled forks in the fragile site sequence (6).

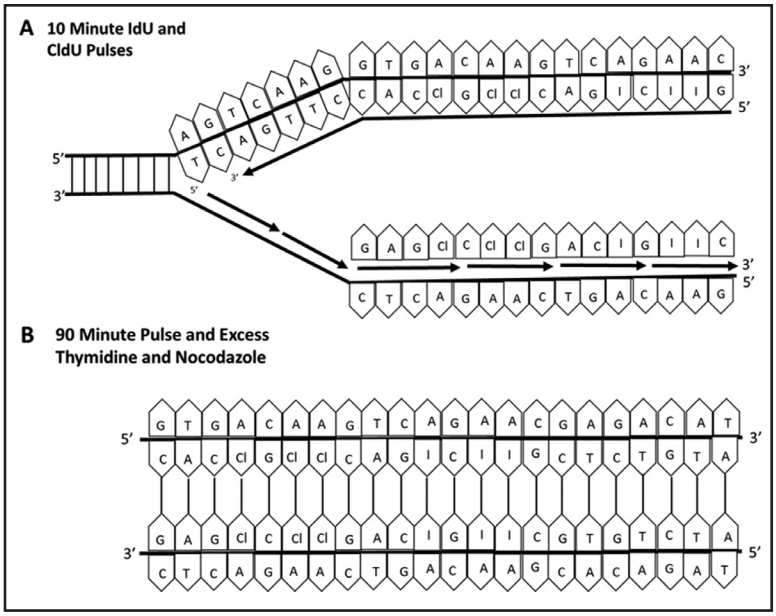

Figure 3. Incorporation of IdU and CldU into yeast DNA."I" and "Cl" designate IdU and CldU. The lagging and leading are not drawn to scale. (A) Halogenated thymidine analogs act as regular thymidine, so nucleoside linkages occur between adenine and the analogs. (B) Chase of regular thymidine ensures completion of synthesis.

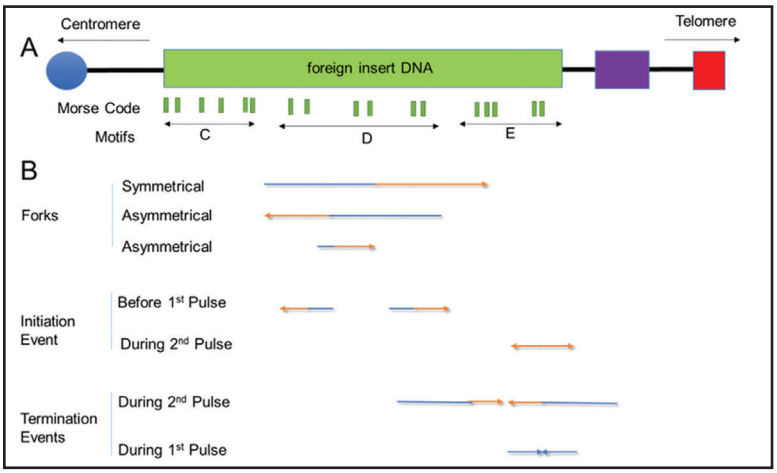

Figure 4. Combing analysis of replication along a yeast chromosome. (A) Morse code used for FISH probes (green bars) organized in 3 motifs (C to E) that identify the locations of stalled forks in the fragile site sequence. (B) Examples of DNA fibers displaying different replication tracts (newly synthesized DNA labelled with IdU then CldU, respectively revealed in blue and red). Arrowheads indicate the direction of fork progression.

Analysis of Combed DNA Fibers

Replication tracks are examined to determine replication fork speed, fork stalling, and frequency of initiation and termination events along a fragile site (6). Replication tracts will be identified with different fluorescent colors, corresponding to the thymidine analogs: IdU (blue) and CldU (red) **(Fig. 4.)**. Identification of stalled replication forks are sites in which replication patterns are asymmetrical, presenting unequal IdU and CldU tracks (13)(14). Location of the stalled forks within fragile sites is determined by FISH probes (green bars) organized in three motifs (C to E) that identify the fragile site region **(Fig. 4.)**.

CONCLUDING REMARKS

Molecular combing is a versatile new tool with a wide range of applications (8). In conjunction with other methods, it is a particularly appealing approach to investigating factors involved specifying and determining replication dynamics along yeast fragile sites (8). Combing may also facilitate more specific investigations into the role of chromatin structure in mediating between transcription and replication, the relationships between DNA repair, recombination, and replication, and the underlying mechanisms controlling the transition to and progression of DNA replication through S-phase of the cell cycle (8)(13). A variety of experiments now underway should confirm the utility and reliability of molecular combing as a new approach to the study of DNA replication (8). However, fluorescent signal recognition and analysis of combed molecules is currently a time-consuming manual process (9). Nonetheless, this time consumption can be overcome through the development of recognition and analysis software tailored for signals on combed DNA (9). Work is currently being carried out to automate signal recognition and analysis fully, opening up the possibility for high throughput, large-scale studies that can shed greater light on the mechanisms that cause genome instability.

REFERENCES

Bianco JN, Poli J, Saksouk J, Bacal J, Silva MJ, Yoshida K, Pasero P. (2012). Analysis of DNA replication profiles in budding yeast and mammalian cells using DNA combing. Methods, 57(2), 149-157.

Bielinsky AK. (2003). Replication origins: why do we need so many?. Cell Cycle, 2(4), 306-308.

Bignell GR, Greenman CD, Davies H, Butler AP, Edkins S, Andrews JM, Stratton MR. (2010). Signatures of mutation and selection in the cancer genome. Nature, 463(7283), 893-898.

Caburet S, Conti C, Bensimon A. (2002). Combing the genome for genomic instability. TRENDS in Biotechnology, 20(8), 344-350.

Conti C, Caburet S, Schurra C, Bensimon A. (2001). Molecular combing. Current protocols in Cytometry, 8-10.

Durkin SG and Glover TW. (2007). Chromosome fragile sites. Annu Rev Genet 41, 169-192.

Franchitto A. (2013). Genome instability at common fragile sites: searching for the cause of their instability. BioMed research international, 2013.

Herrick J, Bensimon A. (1999). Single molecule analysis of DNA replication. Biochimie, 81(8), 859-871.

Lebofsky R, Bensimon A. (2003). Single DNA molecule analysis: applications of molecular combing. Briefings in functional genomics & proteomics, 1(4), 385-396.

Le Tallec B, Koundrioukoff S, Wilhelm T, Letessier A, Brison O, Debatisse, M. (2014). Updating the mechanisms of common fragile site instability: how to reconcile the different views? Cellular and Molecular Life Sciences, 71(23), 4489-4494.

Letessier A, Millot GA, Koundrioukoff S, Lachagès AM, Vogt N, Hansen RS.

Debatisse M. (2011). Cell-type-specific replication initiation programs set fragility of the FRA3B fragile site. Nature, 470(7332), 120-123.

Liberi G, Cotta–Ramusino C, Lopes M, Conti C, Bensimon A, Foiani M. (2006). Methods to study replication fork collapse in budding yeast. Methods in enzymology, 409, 442-462.

Michalet X, Ekong R, Fougerousse F, Rousseaux S, Schurra C, Hornigold N, Bensimon A. (1997). Dynamic molecular combing: stretching the whole human genome for high-resolution studies. Science, 277(5331), 1518-1523.

Ozeri-Galai E, Lebofsky R, Rahat A, Bester AC, Bensimon A, Kerem B. (2011). Failure of origin activation in response to fork stalling leads to chromosomal instability at fragile sites. Molecular cell, 43(1), 122-131.

Palumbo E, Matricardi L, Tosoni E, Bensimon A, Russo A. (2010). Replication dynamics at common fragile site FRA6E. Chromosoma, 119(6), 575-587.

Stratton MR, Campbell PJ, Futreal PA. (2009). The cancer genome. Nature, 458(7239), 719-724.

Waga S, Stillman B. (1998). The DNA replication fork in eukaryotic cells. Annual review of biochemistry, 67(1), 721-751.