ESSAY

Chromatin Challenges during DNA Replication: A Systems Representation

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In a recent review, A. Groth and coworkers presented a comprehensive account of nucleosome disassembly in front of a DNA replication fork, assembly behind the replication fork, and the copying of epigenetic information onto the replicated chromatin. Understanding those processes however would be enhanced by a comprehensive graphical depiction analogous to a circuit diagram. Accordingly, we have constructed a molecular interaction map (MIM) that preserves in essentially complete detail the processes described by Groth *et al.* The MIM organizes and elucidates the information presented by Groth *et al.* on the complexities of chromatin replication, thereby providing a tool for system-level comprehension of the effects of genetic mutations, altered gene expression, and pharmacologic intervention.

Bioregulatory systems often are composed of a network of molecular interactions that is so complex as to challenge comprehensive description in linear text. A graphical description akin to a circuit diagram can then be of great value. In the current work, we assembled a molecular interaction map (MIM; Kohn, 1999, 2006a,b) based on information from an excellent recent review of molecular events during chromatin replication (Groth *et al.*, 2007), and we discuss how the MIM provides a fresh integrated and detailed (albeit incomplete) view of the system. In addition, we point out some of the areas that remain to be covered to complete the picture.

During DNA replication, nucleosomes are disassembled ahead of the replication fork and reassembled, together with newly synthesized histones, on the daughter DNA strands. At the same time, the epigenetic information that is encoded as chromatin modifications is copied from the parental chromatin onto the daughter chromatin strands. These chromatin modifications (such as DNA methylation, histone methylation, and histone acetylation) are part of the complex system that influences gene expression patterns and cell differentiation state. Replication provides an opportunity for chromatin modifications to be copied faithfully or to be altered as cells change their differentiation state.

In their recent review, Groth *et al.* (2007) discussed the molecular events that bring about disassembly of nucleosomes ahead of the replication fork, assembly of nucleosomes on the daughter DNA strands, and copying of chromatin modifications. They described those events in a clear and detailed manner, thereby enabling us to assemble the relevant information in the form of a molecular interaction map (Kohn *et al.*, 2006a,b) that shows graphically how chromatin replication operates and how chromatin modification

state is preserved. The MIM retains and integrates essentially all of the molecular details presented in their review, thus providing the equivalent of a circuit diagram in electronics. The MIM provides an essential tool for troubleshooting the effects of genetic mutations, altered gene expression, or specific inhibitory drugs.

It is important to recognize that the picture presented here is confined to the molecular interactions discussed by Groth *et al.* and is necessarily incomplete. A more complete picture of how epigenetic information is preserved during replication would have to consider the copying from parental to daughter chromatin of other histone modifications, such as phosphorylation, ubiquitinylation, glycosylation, sumoylation, and ADP-ribosylation, as well as the copying of histone variant patterns.

Figure 1 defines the symbols used in the MIM (Figure 2). Appendix 1 summarizes the relevant MIM notation rules. Appendix 2 explains each interaction shown in the MIM and provides other relevant information, as well as references cited by Groth *et al.* To help the reader find any molecular species or interaction on the MIM, Appendix 3 provides a Location Table giving coordinates in the manner of a road-map. We have also prepared an electronic version (eMIM) in which the annotations and locations can be navigated automatically and links to other databases are provided (<http://discover.nci.nih.gov/mim/index.jsp>).

The bracketed numbers in the following description are the identification numbers of the interactions, as shown in the MIM and in Appendix 2.

DISASSEMBLY OF PRE-EXISTING NUCLEOSOMES

The molecules that actively disassemble nucleosomes act on the nucleosomes immediately ahead of the replication fork and therefore must be located close to those nucleosomes. (In addition to this active process, but not shown explicitly in Figure 2, is the destabilization and displacement of nucleosomes ahead of the replication fork that

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Figure 1. MIM symbols used in Figure 2 are adapted from Kohn *et al.* (2006b). The DNA helix unwinding and DNA polymerization symbols are new.

may be due simply to the progression of the replication machinery). The MIM shows that FACT, a key protein in the process, is linked to the replication fork through its binding to MCM [14, 15], the helicase complex that unwinds the DNA at the replication fork. At that location, where the helix is unwinding, FACT-bound proteins could have access to nucleosomes located immediately in front of the replication fork.

FACT binds and removes H2A:H2B dimers from nucleosomes [6], leading to nucleosome disruption. H2A:H2B dimers can exchange continually, whereas the nucleosomal core histones, H3:H4, are more stable (Kimura and Cook, 2001). As long as H2A:H2B dimers remain bound to (H3:H4)₂ tetramers on DNA [5], the nucleosome remains intact; when FACT removes the H2A:H2B dimers, the nucleosome is destabilized, and H3:H4 as dimers or tetramers can translocate to nucleosomes assembling behind the replication fork.

The FACT-bound H2A:H2B dimers [6] provide a pool of H2A:H2B for later use in assembly of nucleosomes behind the replication fork (details of the H3:H4 translocation mechanism are unknown).

ASSEMBLY OF NEW NUCLEOSOMES

Transfer of histones from destabilized nucleosomes to newly synthesized DNA may be both by transfer of intact (H3:H4)₂ tetramers [8] and by transfer of H3:H4 dimers [10] (Groth *et al.*, 2007). H3:H4 dimers from disassembled nucleosomes may transfer randomly to the leading and lagging DNA strands and then combine with newly synthesized H3:H4 dimers [11] to form new tetramers. This is an attractive mechanism whereby the histone modification state could be copied from the old to the newly synthesized H3:H4 dimers (Groth *et al.*, 2007), analogous to the way DNA methylation is copied from pre-existing DNA to daughter DNA (see below). (For simplicity, the MIM show H3:H4 binding to the leading strand only.) To complete the assembly of new nucleosomes, two H2A:H2B dimers then add to the (H3:H4)₂ tetramers [12, 13]. The MIM suggests that FACT binds

H2A:H2B (pre-existing and/or newly synthesized) [6] in competition with (H3:H4)₂ tetramers; consequently, there may be a pool of FACT-bound H2A:H2B that would make H2A:H2B dimers available wherever (H3:H4)₂ tetramers may occur.

To see this situation in more detail, note that there are three branches in the binding line that connects H2A:H2B dimers with (H3:H4)₂ tetramers: 1) binding to a pre-existing (H3:H4)₂ tetramer ahead of the replication fork (this branch normally tends to go in the direction of dissociation) [5]; 2) binding to a pre-existing tetramer behind the fork (on a semiconservatively replicated DNA strand) [12]; and 3) binding to a tetramer consisting of a pre-existing H3:H4 dimer and a newly synthesized H3:H4 dimer [13]. The MIM suggests that FACT could, in principle, facilitate H2A:H2B transfer in either direction across the replication fork. Normally, the accumulation of newly replicated DNA would pull the transfer from ahead of the fork to behind it. When a replication fork has stalled, however, histone transfers may go in either direction, with potentially long-term effects on the local histone modification state (see below).

COPYING THE DNA METHYLATION STATE ONTO NEWLY SYNTHESIZED DNA

DNA methylation at cytosines in CpG sequences is part of the mechanism that serves to silence genes that are not to be expressed in particular functional states of the cell. The pattern of gene silencing is usually passed on from mother to daughter cell during DNA replication by copying both the DNA methylation pattern and the histone modification pattern (i.e., epigenetic information) from parental to replicated chromatin.

The DNA methyltransferase DNMT1 has multiple binding capabilities that allow it to play a central role in this process. Its DNA methyltransferase function is accomplished through binding to hemimethylated CpG sites, whereupon the enzyme methylates the cytosine on the complementary (newly replicated) DNA strand [27, 28]. (The enzyme reaction symbol [27] implies the existence of an intermediate in which enzyme is bound to substrate.) The efficiency of this process is enhanced by the binding of DNMT1 to PCNA [29]. The binding to PCNA places the enzyme behind the replication fork near sites of DNA synthesis [20], where a pre-existing methylated DNA strand would be paired with a newly synthesized unmethylated strand. This arrangement allows DNA methylation pattern to be copied at the precise time and place of DNA replication. We shall see that an analogous arrangement allows the histone modification pattern to be copied.

COPYING HISTONE MODIFICATIONS DURING THE ASSEMBLY OF REPLICATED CHROMATIN

The MIM suggests that all of the proteins implicated in chromatin modification behind the replication fork may be recruited to the replication sites by binding to proliferating cell nuclear antigen (PCNA) directly or through a sequence of no more than two intermediaries: CAF-1, DNMT1, and histone deacetylase (HDAC) bind PCNA directly [19, 29, 43]. Asf-1, MBD1, and HP1 may bind PCNA via CAF-1 [17, 35, 39] (but requires experimental verification). The histone H3 methylases G9a and EZH2 both bind DNMT1 [30, 31], and the histone H3 methylases SETDB1 and Suv39h bind MBD1 and HP1, respectively [36, 41]. Moreover, the binding of HP1 to MBD1 [40] and the binding of MBD1 to methyl-CpG [34]

Figure 2. Molecular interaction map (MIM) of the network that mediates replication of chromatin and its epigenetic modification state, as described by Groth *et al.* (2007). Each interaction is identified by a number that corresponds to the numbering used in the text and in the Annotations (Appendix 2). The Annotations describe the interactions and give supporting references as cited in Groth *et al.* (2007). To assist in finding molecular species or interactions on the MIM, a table of coordinates is provided in Appendix 3. The MIM is heuristic, as defined in Kohn *et al.* (2006a). That means that bindings and/or modifications of a molecular species may exist concurrently (see Figure 1). An interactive electronic version of this MIM can be found at <http://discover.nci.nih.gov/mim/index.jsp>. (A small filled circle ["node"] on an interaction line represents the molecular species resulting from that interaction; an isolated node represents another copy of the molecular species that is at the other end of the line [Kohn *et al.*, 2006b]. The boxed "2" on the interaction line indicates that two copies of H3:H4 are involved in the binding. The MIM notation rules are summarized in Appendix 1.)¹

would further solidify the integration of all these proteins (at least 11 different proteins) within a complex.

The two methylases that bind to DNMT1 act on different H3 sites: G9a methylates lysine-9 [32], and EZH2 methylates lysine-27 [33], thereby perhaps coordinating the methylation of the two sites. The remaining two methylases (SETDB1 and Suv39h) also act on H3 lysine-9, making three different methylases that act on the same site. The reason for this duplication is unclear; perhaps their actions are invoked in structurally different types of chromatin (e.g., G9a in euchromatin, SETDB1 in euchromatin or heterochromatin, and Suv39h in heterochromatin).

Although there is no clear evidence of the role of HDACs binding to PCNA, it is tempting to speculate that it is dedicated to deacetylation of histones incorporated into nucleosomes formed behind the replication fork.

Methylase HP1 binds stably to lysine-9 sites on histone H3 [38a], suggesting the possibility that HP1 is recruited to such sites on pre-existing H3, putting it in position to act on newly synthesized H3 on the same newly formed nucleosome. The interlocked binding network may localize other components of the methylation system through HP1 to those newly formed nucleosomes that require H3 methylation.

The system of interlocked components of the chromatin modification system can link also to hemi-methylated CpG sites on DNA, mediated via MBD1 [34] and possibly also via DNMT1 (if this enzyme binds stably to its substrate). The interlocked system of chromatin modification molecules, as already mentioned, could be envisioned as a molecular cluster localized to replication sites through binding to PCNA. (Such a cluster however could not be mediated by a single PCNA trimer alone, because the trimer probably has only three binding sites.) When new nucleosomes bearing a methylated H3 appear, all of the players may become bound together at the site of newly synthesized/assembled chro-

¹ References relating to each interaction shown in Figure 2 are cited in Appendix 2 and in the annotations associated with the eMIM at <http://discover.nci.nih.gov/mim>.

matin, allowing prompt, efficient, and coordinated copying of the epigenetic information.

CONTROL OF REPLICATION TIMING

DNA replication at particular genomic sites, known as replication origins, is initiated through the joint action of many proteins, including the MCM complex, which is thought to be the helicase that unwinds DNA ahead of the replication fork. Initiation at replication origins in DNA is controlled in part by a kinase, Hsk1/Cdc7 and its regulatory subunit Dpf1/Dbf4 (for a MIM of the regulation of replication origins; see Aladjem *et al.*, 2004 or <http://discover.nci.nih.gov>). Hsk1/Cdc7 phosphorylates the p150 subunit of CAF-1 [22], an evolutionarily conserved three-subunit protein that exists in complex with newly synthesized H3.1:H4 dimers and deposits them in the construction of newly replicated chromatin. (CAF-1 does not associate with H3.3, whose incorporation into chromatin is independent of DNA replication and which is considered to be a replacement histone, as opposed to the major histone H3.1; Groth *et al.*, 2007). CAF-1 functions in concert with Asf-1, which can bind to H3:H4 dimers directly [25]. A complex containing Asf-1, CAF-1, and newly synthesized H3:H4 may localize to replication sites behind the replication fork by way of direct binding between CAF-1 and PCNA [19]. The binding of the complex to PCNA however is potentially blocked by the ability of CAF-1 to form a homodimer [23]. Phosphorylation of the p150 subunit by Cdc7:Dfb4 [22] stabilizes p150 in a monomeric conformation and allows p150 binding to PCNA. This scenario is supported by the findings that 1) H3:H4 binding to CAF-1 and deposition onto chromatin are promptly blocked by inhibitors of DNA replication and 2) newly synthesized H3:H4 may not lead to the direct formation of tetramer (references cited by Groth *et al.*, 2007). The mechanism described here may be how the timing of deposition of newly synthesized H3:H4 on DNA is coordinated with the timing of DNA replication. Because histone modifying enzymes (methylases, HATs, and HDACs) are also recruited to chromatin via PCNA, histone modifications concomitant with the deposition of new nucleosomes ensure the perpetuation of epigenetic marks on newly synthesized chromatin.

LINKS TO OTHER MIMs

Each molecular species is generally represented only once in a MIM, in contrast to other types of network diagrams. Hence, MIMs tend to be smaller than other equally detailed representations of biological networks. Nonetheless, they can become too large for easy navigation and comprehension. We counter this tendency by abbreviating parts of networks in a given MIM to include only the pertinent interactions and linking the abbreviated subnetwork to MIMs that shows further details. In Figure 2, we used that strategy for MCM, PCNA, and Hsk1:Dpf1, each of which is shown enclosed in a dashed green box and assigned an identification number that links to a more detailed MIM.

PCNA recruits many proteins to sites of DNA replication. Figure 2 shows only those involved in nucleosome formation and chromatin modification. The abbreviated PCNA box [48] however links to a MIM that shows the association of PCNA with other proteins involved in DNA replication, DNA repair, and cell cycle control (<http://discover.nci.nih.gov/mim/kohnk/fig6b.html>; Kohn, 1999). Links to other MIMs can be accessed at <http://discover.nci.nih.gov/mim/index.jsp>.

WHAT DO WE GAIN FROM THE MIM?

The MIM in Figure 2 displays a network of 52 interactions, involving 27 primary molecular species (not counting multimolecular species derived from binding and modification interactions), all of which function together in the process of chromatin replication. The MIM is a system diagram showing all of the interactions and their known molecular consequences. We need such a system diagram, because many or all of the interactions may have to be considered in evaluating functional hypotheses or experimental inferences.

The value of this systematic MIM representation is illustrated by its depiction of the role of PCNA. It has been noted that PCNA may be a hub for the assembly, close behind the replication fork, of a set of proteins that function in chromatin replication and modification (Groth *et al.*, 2007). The MIM for the first time displays the intricacies of that assembly, showing how each component relates to the others. Examination of the MIM suggests which of those proteins can assemble, with or without PCNA, at DNA sites of CpG methylation and/or sites of histone H3 lysine-9 methylation. The interactions that may be required to coordinate particular chromatin replication functions also can be read from the MIM. Another interesting possibility suggested by the MIM was that CAF-1 might promote dissociation and stabilization H2A:H2B dimers from nucleosomes on either side of the replication fork, thereby perhaps allowing nucleosome transfer in reverse at stalled replication forks with consequent loss of epigenetic information.

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APPENDIX 1

MIM NOTATION RULES

These rules are adapted from Kohn *et al.* (2006a). A complete description with examples is provided in Kohn *et al.* (2006b).

1. A named molecular species usually appears in only one place on a map.
2. Interactions between molecular species are shown by different types of connecting lines (Figure 1).
3. Interaction lines can change direction, but not by more than 90° at a corner; this restriction prevents ambiguities at branch points.
4. When lines cross, it is as if they do not touch.
5. Color is optional and does not affect symbol definitions. We use red for inhibitions and other negative actions, green for stimulatory or catalytic actions, blue for covalent modifications, and purple for replication, transcription, and translation.
6. A small filled circle ("node") on an interaction line indicates the consequence or product of the interaction. Thus the consequence of binding between two molecules is formation of a dimer, which is represented by a node on the binding interaction line. The consequence of a modification (e.g., phosphorylation) is production of the modified (e.g., phosphorylated) molecule; the phosphorylated product is represented by a node placed on the modification line.
7. Multiple nodes on an interaction line represent exactly the same molecular species. A node should not be

placed at a line crossing or at a corner where a line changes direction.

8. An isolated node (a node that is not on a line) is an abbreviation that represents another copy of the same molecular species that is defined at the other end of the line pointing to the node (to avoid ambiguity, only one arrow should point to an isolated node).

9. Molecular interactions are of two types: reactions and contingencies, as listed in Figure 1. Reactions operate on molecular species; contingencies operate on reactions or on other contingencies.

APPENDIX 2

ANNOTATIONS

The bracketed numbers refer to the interactions as marked on the MIM and used in the text. References are as cited by Groth *et al.* (2007).

[1–5] The nucleosome core particle consists of two H3:H4 dimers [1] bound together to form an (H3:H4)₂ tetramer [2] that is bound to DNA [3]. Two H2A:H2B dimers [4] bind individually to each H3:H4 dimer in a DNA-bound (H3:H4)₂ tetramer, thus forming a complete nucleosome. Interaction [5] shows the binding of H2A:H2B dimers to DNA-bound (H3:H4)₂ tetramers; the boxed “2” on the interaction line indicates that two H2A:H2B dimers bind per (H3:H4)₂ tetramer.

[6] FACT (“facilitates chromatin transcription”) binds H2A:H2B dimers, thereby dislodging them from the nucleosomes. Because FACT is bound via MCM ahead of the replication fork (see interactions [13, 14] below), the presumption is that FACT here is acting on pre-existing nucleosomes ahead of the replication fork.

[8–11] The remaining (H3:H4)₂ tetramers [2] may be transferred intact, retaining their posttranscriptional modifications and may bind randomly to either daughter DNA strand [8] behind the replication fork. (The mechanism of transfer of H3:H4 is unknown.) Recent evidence however suggests that newly synthesized H3:H4 [9] are deposited onto newly synthesized DNA as dimers, not tetramers (Polo and Almouzni, 2006). H3:H4 dimers from dissociated nucleosomes may be transferred randomly to the daughter strands [10] and may then become associated with newly synthesized H3:H4 dimers [11] from a chaperone-bound pool ([17], described below); this mechanism would help explain how posttranscriptional information is copied to newly synthesized core histones (Groth *et al.*, 2007).

[12, 13] Two H2A:H2B dimers (possibly released from FACT binding) then add to the (H3:H4)₂ tetramers on daughter strand, whether the tetramers were transferred intact [12] or assembled from a pre-existing and a newly synthesized H3:H4 dimer [13]. (FACT also mediates H2A:H2B dimer transfer during transcription, thereby facilitating RNA polymerase progression [Belotserkovskaya and Reinberg, 2004].)

[14–16] FACT binds to the MCM helicase complex [14] (Gambus *et al.*, 2006; Tan *et al.*, 2006). MCM binds and unwinds DNA ahead of DNA polymerase during replication [15, 16] (Takahashi *et al.*, 2005). (The arrow marked “H” represents the helix unwinding process.) Thus FACT may coordinate H2A:H2B histone transfer and DNA unwinding during replication.

[17–18] CAF-1 (chromatin-assembly factor, an evolutionarily conserved 3-subunit protein) binds to a complex of newly synthesized H3:H4 and Asf-1 ([17] and see [25a]

below). The newly synthesized H3:H4 dimers can then be deposited from this chaperone complex onto newly synthesized DNA [11, 18]. A newly synthesized H3:H4 dimer may be first to bind newly synthesized DNA, then being joined by a transferred H3:H4 dimer (not shown), or these bindings may occur in the reverse order (as indicated in the MIM: [10] followed by [11]). The predeposition complex specifically contains H3.1:H4 (Tagami *et al.*, 2004). Hydroxyurea blocks replication fork progression and consequently may prevent CAF-1-dependent histone deposition. Asf1 can then buffer the excess cytosolic newly synthesized H3:H4. (The order of binding of CAF-1 and Asf1 to newly synthesized H3:H4 shown in the MIM is hypothetical.)

[19–21] CAF-1 binds to PCNA [19] and is thereby directed to sites of DNA replication [20, 21] (Taddei *et al.*, 1999; Sporbett *et al.*, 2002). (The arrow marked “P” represents replication by DNA polymerase on the leading strand; replication on the lagging strand is not shown.) CAF1:PCNA remains bound to newly synthesized DNA for 20 min (corresponding to the replication of 20–40 kb of DNA), thereby providing a “window of opportunity” for modification of newly synthesized chromatin (Groth *et al.*, 2007).

[22] Binding of CAF-1 to PCNA depends on phosphorylation of the CAF-1 large subunit (human p150), catalyzed by the essential kinase Hsk1/Cdc7:Dpf1/Dbf4 (Gerard *et al.*, 2006). This kinase is enclosed in a dashed green box to indicate that its interactions are shown in more detail in another MIM (Aladjem *et al.*, 2004; see eMIM).

[23–24] CAF-1 p150 contains a dimerization domain [23] (Moggs *et al.*, 2000; Gerard *et al.*, 2006). Dimerization competes with PCNA for binding to CAF-1 monomer [19]. The Cdc:Dbf4-catalyzed phosphorylation of CAF-1 p150 inhibits the dimerization [24] (i.e., stabilizes p150 in a monomer configuration) and thereby enhances the binding of CAF-1 p150 monomer to PCNA (Gerard *et al.*, 2006).

[25a–c] Asf1 (“anti-silencing function 1”) is a highly conserve chaperone that binds soluble H3:H4 [25a]. Asf1 may work together with CAF-1 to assemble nucleosomes during replication (Tyler *et al.*, 1999; Mello *et al.*, 2002; Groth *et al.*, 2005). Asf1 binding to H3:H4 dimer prevents the dimers from coming together to form tetramers [25b and c] (English *et al.*, 2005, 2006; Mousson *et al.*, 2005). One might anticipate (hypothetically) that, in the absence Asf1, newly synthesized unmodified (H3:H4)₂ tetramers could add onto newly replicated DNA and cause loss of histone modification information.

[26] H3.3 is not associated with CAF-1, and is incorporated into chromatin as a “replacement histone” in a replication-independent manner (Groth *et al.*, 2007).

[27–29] DNMT1, a maintenance DNA methyltransferase that acts on hemimethylated CpG sites to establish symmetrical C-methylations [27, 28], binds PCNA [29] and is thereby recruited to DNA replication forks (Leonhardt *et al.*, 1992; Chuang *et al.*, 1997). Although CAF-1 acts to maintain nucleosomal density throughout the replicated genome, DNMT1 acts primarily on epigenetically silenced DNA and functions to maintain the CpG and H3 methylations that confer silencing (Groth *et al.*, 2007). Although DNMT1 is an essential methylase, the binding of DNMT1 to PCNA is not required for maintenance of DNA methylation in human cells (Spada *et al.*, 2007).

[30–33] DNMT1 binds histone H3 methyltransferases G9a [30] and EZH2 [31], thereby perhaps recruiting them via PCNA to newly replicated DNA, where they could methylate H3 on newly formed chromatin. G9a methylates H3 at K9 [32] (Esteve *et al.*, 2006), whereas EZH2 (a polycomb

protein) methylates H3 at K27 [33] (Vire *et al.*, 2006). These DNMT1–methyltransferase complexes may constitute binary memory modules that coordinate the maintenance of DNA and H3 methylations during replication of epigenetically silenced DNA (Esteve *et al.*, 2006).

[34–37] MBD1 binds methylated CpG in DNA [34]; it also binds CAF1 [35] and SETDB1 [36], an H3K9 methyltransferase [37] (Sarraf and Stancheva, 2004). The CAF1:MBD1:SETDB1 complex maintains H3K9 methylation and stable silencing at certain genes in replicating cells (Groth *et al.*, 2007). SETDB1 that is bound via MBD1 to methylated CpG in front of the replication fork may translocate to methylated CpG sites behind the replication fork, where SETDB1 would be in position to methylate newly deposited H3 (Sarraf and Stancheva, 2004).

[38–42] HP1 binds H3K9me3 in heterochromatin or at stably silenced genes [38a], where H3K9me3 marks gene repression (Maison and Almouzni, 2004; Feldman *et al.*, 2006). HP1 binds CAF1 p150 directly [39] (Murzina *et al.*, 1999). HP1 binds MBD1 [40] (Fujita *et al.*, 2003) and H3K9 methyltransferase Suv39h [41, 42] (Aagaard *et al.*, 1999). Dnmt1 can bind HP1 directly [40a], thereby helping to maintain DNA methylation status at pericentric heterochromatin, as well as gene silencing (Fuks *et al.*, 2000; Lehnertz *et al.*, 2003; Esteve *et al.*, 2006; Smallwood *et al.*, 2007). MBD1 (via HP1) binds to a pre-existing methylated nucleosomal H3; Suv39h and SETDB1, in complex with MBD1 and/or HP1, could then methylate the corresponding site in a newly synthesized H3 molecule in the newly constituted nucleosome. These actions are said to be *in trans* (defined in Figure 1), indicated by the gapped stimulation symbols [38b and c].

[43–45] Histone deacetylases (HDAC) bind PCNA [43] and are thereby recruited to DNA replication sites [20] (Milutinovic *et al.*, 2002), where they deacetylate lysines 4 and 12 of histone H4 [44]. HDAC may also bind to DNMT1 [45] (Fuks *et al.*, 2000; Rountree *et al.*, 2000).

[46] Newly synthesized H3:H4 dimers become acetylated at K5 and K12 of H4 (Loyola *et al.*, 2006). These acetylations of H4 are introduced by the two-subunit acetyltransferase HAT1:RbAp46 and may be required for repair of stalled replication forks (Barman *et al.*, 2006). The H4K5/K12 acetylations are removed 20–60 min after replication (Taddei *et al.*, 1999).

[47] The MCM complex is a helicase that plays a critical role in DNA replication. A MIM showing the interactions of this complex can be viewed at <http://discover.nci.nih.gov/mim/view.jsp?MIM=replication&selection=map&subMenu=replication>.

[48] PCNA serves as a platform for other proteins involved in DNA repair and cell cycle checkpoint control. A MIM showing other interactions of PCNA can be viewed at http://discover.nci.nih.gov/mim/kohnk/fig_6b.html (Kohn, 1999).

[49] Hsk1/Cdc7:Dpf1/Dbf4 is a kinase complex that phosphorylates CAF-1 p150 [22]. It also phosphorylates MCM proteins and other members of the replication pre-initiation complex (not shown). A MIM showing the interactions of this kinase can be viewed at <http://discover.nci.nih.gov/mim/view.jsp?MIM=replication&selection=map&subMenu=replication>.

APPENDIX 3

LOCATION TABLE

Appendix Table A1. shows the MIM coordinates for molecular species and interactions.

Table A1. Location table (MIM coordinates)

Molecular species							
Asf-1	A2	H3	C6	Hsk1/Cdc7	A3		
CAF-1	A2	H3 (new)	C2	MBD1	E2		
DNMT1	E1	H3.3	A6	MCM	C5		
Dpf1/Dbf4	A3	H4	D6	Me-CpG	E2		
EZH2	F1	H4 (new)	D2	PCNA	C4		
FACT	A5	HAT1	C5	polymerase	E4		
B9a	F1	HDAC	B4	repln fork	E5		
H2A	B7	helicase	E5	SETDB1	F2		
H2B	B7	HP1	E3	Suv3			
Interactions							
[1]	D6	[11]	D3	[21]	E5	[29]	D3
[2]	C6	[12]	C4	[22]	A2	[30]	E1
[3]	E6	[13]	C4	[23]	B2	[31]	E1
[4]	B7	[14]	B5	[24]	B2	[32]	F2
[5]	C6	[15]	D5	[25a]	B1	[33]	F2
[6]	B6	[16]	E5	[25b]	C2	[34]	E2
[7]	N	[17]	A1	[25c]	C2	[35]	D3
[8]	D4	[18]	C1	[26]	C6	[36]	E3
[9]	C2	[19]	B3	[27]	E2	[37]	F4
[10]	D4	[20]	C4	[28]	E2	[38a]	F4
[38b]	F6	[41]	E3	[45]	B3	[49]	A4
[38c]	F6	[42]	F4	[46]	D5		
[39]	D3	[43]	B4	[47]	C5		
[40]	E3	[44]	B4	[48]	B4		

N, unassigned.

^a Brackets are as shown in the text and numbering is as in the MIM.

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