

Cell fate transitions and the replication timing decision point

David M. Gilbert

Department of Biological Science, Florida State University, Tallahassee, FL 32306

Recent findings suggest that large-scale remodeling of three dimensional (3D) chromatin architecture occurs during a brief period in early G1 phase termed the replication timing decision point (TDP). In this speculative article, I suggest that the TDP may represent an as yet unappreciated window of opportunity for extracellular cues to influence 3D architecture during stem cell fate decisions. I also describe several testable predictions of this hypothesis.

All eukaryotic organisms replicate their DNA in a defined temporal order that is highly conserved between related species (Hiratani and Gilbert, 2010; Ryba et al., 2010; Yaffe et al., 2010). However, neither the mechanism orchestrating this program nor its biological significance is known. What we do know is that this replication timing program is developmentally regulated and is closely related to the 3D organization of chromatin in the nucleus. Early- and late-replicating chromosomal domains are segregated into spatially distinct compartments of the nucleus with late-replicating, silent genes generally closer to the nuclear periphery than early-replicating, active genes (Gilbert and Gasser, 2006). During mouse and human development, 400–800-kb segments of chromosomes exhibit replication timing switches that accompany changes in subnuclear position and, in some cases, transcriptional competence of genes within those segments (Hiratani et al., 2008, 2009, 2010; Hiratani and Gilbert, 2010; Pope et al., 2010; Ryba et al., 2010). Moreover, genome-wide experiments have identified a nearly precise alignment between the frequency of chromatin interactions and replication timing (Hiratani et al., 2010). Perhaps the most compelling link between replication timing and chromatin organization is illustrated by experiments introducing nuclei isolated from cells at different times after mitosis into a cell-free replication system. These studies have shown that a temporally specific replication program is established at a discrete time point after mitosis, designated the TDP, which coincides with the repositioning and anchorage of chromosome domains after mitosis (Dimitrova and Gilbert, 1999; Chubb et al., 2002; Walter et al., 2003; Lu et al., 2010). In this speculative article, I tie together developmental and cell cycle features of replication time and spatial organization and propose that the TDP could represent an important

time window to establish aspects of higher order chromosome organization that influence the commitment of stem cells to developmental fates.

Chromosome architecture and cell fate transitions

Stem cells in adult animals have two primary regulatory decisions. First, they must remain quiescent throughout the lifetime of an organism until they are recruited for regeneration, when they must respond to the appropriate cues and reenter the cell cycle. Second, once they enter the cell cycle, they must properly balance self-renewal with commitment to differentiation to maintain tissue homeostasis. Excess self-renewal leads to over proliferation (tumor formation), whereas excess commitment leads to depletion of stem cell reserves and a reduction in cell number (degeneration). Embryonic stem cells (ESCs) do not exit the cell cycle and are only concerned with the second decision. As stem cells commit to differentiation pathways during embryogenesis, lineage options become increasingly restricted, and cells can respond in very different ways to the same signaling cues even though all cells have a full complement of genetic information. This change in cellular competence can be mediated by changes in the intracellular environment (Grimm and Gurdon, 2002), changes in transcriptional feedback loops, or epigenetic/chromatin changes such as histone composition or DNA methylation (Steinbach et al., 1997; Kundu and Peterson, 2009; Kaufman and Rando, 2010). No single mechanism is involved in all cell fate transitions, and there are likely to be additional as yet-undiscovered mechanisms.

How important is large-scale chromatin architecture to these stem cell functions? Although not as well established as the roles of transcription factors (Egli et al., 2008) and histone modifications (Spivakov and Fisher, 2007), recent observations link 3D organization of chromatin to gene expression. It is now well accepted that the nucleus is structurally and functionally compartmentalized to favor transcription in specific subnuclear neighborhoods (Lancôt et al., 2007; Lieberman-Aiden et al., 2009; Hakim et al., 2010; Laster and Kosak, 2010). For example, the periphery of the nucleus (Luo et al., 2009; Peric-Hupkes et al., 2010) and the nucleolus (Németh et al., 2010) are

© 2010 Gilbert This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons license [Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>].

Correspondence to David M. Gilbert: gilbert@bio.fsu.edu

less-favorable locations for transcription of developmentally regulated genes. In addition, the 3D organization of chromatin can change dramatically as stem cells undergo lineage commitment (Meshorer and Misteli, 2006), and this can move specific genes into different compartments (Hiratani et al., 2010). Importantly, once established, structural constraints limit chromatin mobility throughout the remainder of the cell cycle (Chubb et al., 2002; Walter et al., 2003) so that large-scale 3D architecture is considerably less plastic than many other features of chromatin structure and could provide a scaffold to preserve a memory of cellular lineage. Thus, 3D genome architecture has attracted a great deal of interest to those interested in understanding stem cells and the reprogramming of cellular fates.

Replication timing reflects chromosome architecture

Replication timing has been indirectly linked to chromosome architecture since the 1960s, when it was discovered that the inactive X chromosome in female mammals takes on a compact structure localized to the periphery of the nucleus, known as the Barr body, which is coincident with a switch to late replication (Hiratani and Gilbert, 2010). As discussed, DNA replication takes place in spatially distinct compartments of the nucleus at different times during S phase, and it is now generally appreciated that facultative heterochromatin, including silent gene families (genes that lack poised polymerase II) and imprinted gene loci, are late replicating and generally localized closer to the nuclear periphery or nucleoli. Recently, a compelling genome-wide alignment was found between replication timing and the density of chromatin interactions within the mammalian nucleus, and spatial models of how chromatin folds within the nucleus based upon chromatin interaction maps resemble independently derived models of how chromatin is organized into spatially and temporally discrete replication domains (Ryba et al., 2010). These data provide strong support for the hypothesis that replication domains are self-interacting structural and functional units of chromosomes.

Implicit in this finding is that genome-wide replication timing profiles provide a clear and measurable property with which to evaluate higher order chromosome structure and function and can rapidly identify regions of chromosomes that undergo remodeling of these architectural features during development or in specific diseases. For example, a comprehensive survey of 10 stages of mouse embryogenesis revealed changes across half of the mouse genome mediated in units of 400–800 kb, including a set of down-regulated genes that switch from early to late replicating during loss of pluripotency (Hiratani et al., 2010). These regions remain late replicating throughout the rest of development and are difficult to reprogram back to an embryonic like state; e.g., they fail to reestablish ESC-specific replication timing and transcription in partially reprogrammed induced pluripotent stem cells (iPSCs). It follows that these regions undergo higher-order remodeling events that are difficult to reverse. In fact, these same changes were coincident with chromatin compaction and movement of these regions toward the nuclear periphery (Hiratani et al., 2010).

What regulates these events? This remains a mystery, but we do know that replication timing is reestablished in each cell cycle by events that take place while chromatin is reorganized after mitosis (Dimitrova and Gilbert, 1999; Lu et al., 2010). When nuclei from cells staged at different times in the cell cycle are introduced into a cell-free replication initiation system, chromatin in early G1 or G2 phase nuclei replicates in a random temporal order, whereas chromatin from cells in mid- to late-G1 phase replicates in the cell type characteristic temporal sequence (Lu et al., 2010). The interpretation of these results is that determinants of replication timing are established during a short 1-h interval of early G1 (TDP) and are then lost during replication. Importantly, before the TDP, early- and late-replicating chromatin are mobile and irregularly positioned but become anchored and organized in 3D space within the same short time interval as the TDP (Dimitrova and Gilbert, 1999; Chubb et al., 2002; Walter et al., 2003; Lu et al., 2010). This 3D organization is then retained throughout the remainder of interphase. These studies have suggested a model in which chromatin regulators are concentrated into spatially separated subnuclear compartments by the anchorage and folding of chromosomal domains at the TDP (Gilbert, 2001). In this view, subnuclear compartmentalization is required to establish some type of chromatin mark that is then erased during replication and requires a round of mitosis to reestablish. One possibility is that the factors regulating replication timing set thresholds for the accessibility of S phase-promoting factors to replication origins or replicon clusters (Gilbert, 2001; Thomson et al., 2010).

Replication timing is regulated in 400–800-kb units or subdomains. In fact, larger coordinately replicating domains are found to change replication timing in smaller, 400–800-kb increments during differentiation (Hiratani et al., 2008; Ryba et al., 2010). Moreover, the regions between adjacent early- and late-replicating domains traverse spatially segregated compartments of the nucleus and are suppressed for origin activity so that a single unidirectional replication fork duplicates the intervening DNA, often over the course of several hours (Ryba et al., 2010). Interestingly, a histone modification signature exists at the early-replicating boundaries of these intervening timing transition regions (Ryba et al., 2010), and it is known that at least some of these same chromatin marks are erased at the replication fork and reestablished after the following mitosis (Jansen et al., 2007; Aoto et al., 2008; Scharf et al., 2009). However, there are some aspects of higher order chromatin architecture that are clearly not necessary to maintain replication timing once chromatin compartments are established. Chromatin within nuclei isolated from quiescent cells, which show measurable changes in global chromatin architecture including massive decondensation of selected regions, retained replication timing determinants (RTDs; Lu et al., 2010).

Hypothesis: the TDP as a window of opportunity

The TDP may be defined by the establishment of a replication timing program, but the close linkage between replication timing and chromosome architecture implies that important organizational events that determine the folding of chromatin, its degree

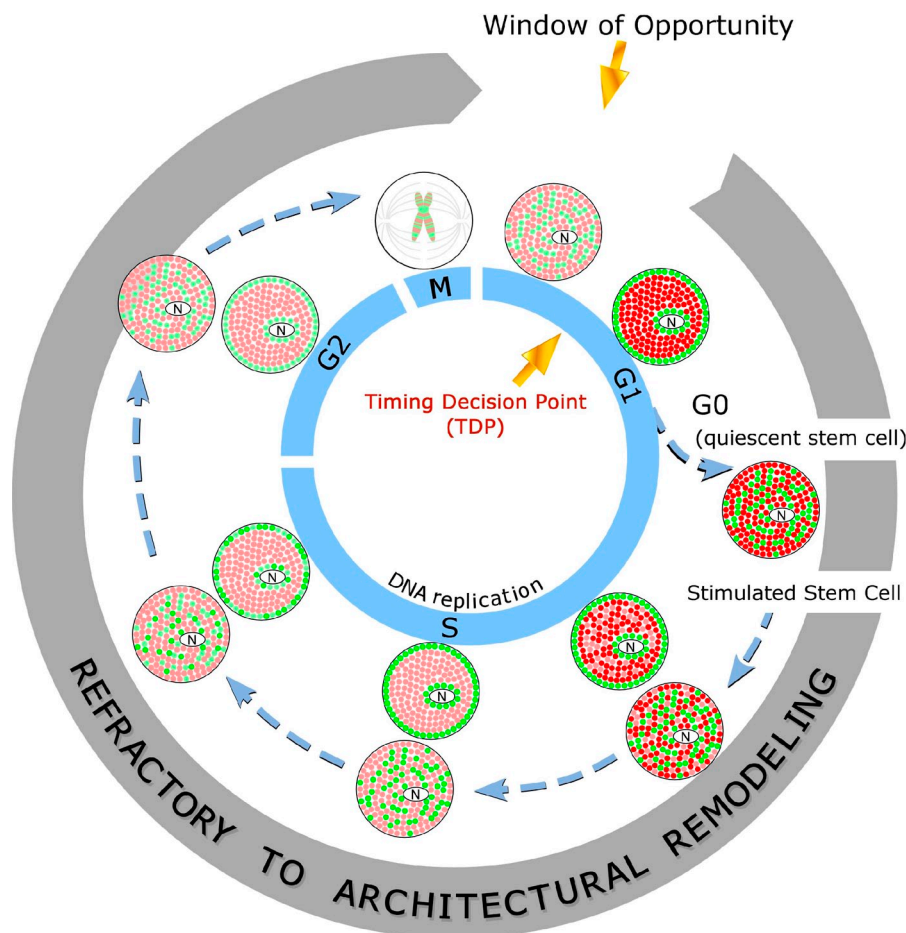


Figure 1. The TDP as a novel window for cellular reprogramming. In this model, early- and late-replicating chromatin domains are labeled as red or green, respectively, with light colors representing lack of RTDs and bright colors representing the presence of RTDs. Because replication timing and spatial organization of chromatin are established simultaneously during early G1 phase at the TDP (Dimitrova and Gilbert, 1999) and because there is a strong genome-wide correlation between 3D chromosome architecture and replication timing (Ryba et al., 2010), it is hypothesized that spatial reorganization at the TDP drives the assembly of RTDs, potentially by creating subnuclear compartments that set thresholds for initiation of replication (Gilbert, 2001). These RTDs are maintained until the time of replication in S phase. During replication, the potential RTDs are modified or removed at the replication fork, which is indicated by early-replicating domains changing to light colors first, followed by late-replicating domains. In G2 phase, there are no RTDs on chromatin, but the general spatial organization is maintained until being disrupted during mitosis. If cells withdraw from the cell cycle and enter quiescence, aspects of the spatial organization of chromatin change but RTDs remain intact, and upon return to the cell cycle, replication proceeds in the normal temporal order despite spatial disruption. As developed in the text, dismantling and reassembling higher-order chromosome architecture may provide a window of opportunity to reprogram 3D architecture and replication timing to influence cellular identity in response to extracellular cues during differentiation. Once established, replication timing influences the type of chromatin assembled (Lande-Diner et al., 2009), invoking a domain-wide change in chromatin structure that in turn may serve to reinforce maintenance of the new 3D architecture at each TDP. N, nucleolus. This figure is modified from Lu et al. (2010).

of compaction, and the potential interactions between genomic segments are also established at this time. In considering causality, it makes little sense that replication timing would be the driving force behind chromatin architecture because this would mean that a chromatin architecture would be established for a replication program that might not be played out if the cells subsequently exit the cell cycle. Moreover, it is not obvious why the genome would need a cell type-specific temporal sequence to carry out the housekeeping function of genome replication. More sensibly, the 3D architecture is a result of cell type-specific factors present during passage through the TDP, and the replication timing program is a reflection of that architecture. However, that does not imply that replication timing does not influence architecture because different types of chromatin are assembled at different times during S phase (Lande-Diner et al., 2009), so changes in replication timing could rapidly and simultaneously influence the structure of chromatin across large segments of chromosomes, which could profoundly affect the structure assembled in the next cell cycle.

I would like to propose that the TDP may represent an as of yet unappreciated window of opportunity for extracellular cues to reprogram chromatin interactions and subnuclear position

during cell fate transitions (Fig. 1). Once established, these features of chromatin architecture may not be changeable until the next TDP. Consistent with this hypothesis, experiments in which ectopically inserted LacO sequences were artificially targeted to the nuclear periphery with LacI fusions to inner nuclear envelope proteins demonstrated that the targeting protein is not sufficient to reposition chromatin; repositioning requires mitosis. Repositioning silenced some genes in the vicinity of the LacO, but the targeting was probabilistic; not all cells managed to reposition the LacO regions (Finlan et al., 2008; Kumaran and Spector, 2008; Reddy et al., 2008). Moreover, many transcriptional regulators dissociate from chromatin during mitosis and reassociate thereafter, and it is reasonable to presume that the stoichiometry and binding affinities of transcription factors present at the TDP would have a profound influence on the resulting chromatin architecture (Egli et al., 2008). As chromatin decondenses, it likely experiences a series of stochastic inter- and intra-chromosomal interactions of variable stabilities, facilitated by the high mobility of chromatin at this time (Chubb et al., 2002; Walter et al., 2003). This would create the opportunity to selectively reinforce or discourage certain interactions to reinstate or alter chromatin folding or positioning, which

has been proposed to maintain active and repressed states of gene expression (Fraser and Bickmore, 2007; Deng and Blobel, 2010). This model implies that the architecture assembled at the TDP affects the time and thus type of chromatin assembly during the upcoming S phase, which in turn will influence the probability of reassembling the same chromatin architecture in the next cell cycle. This could create a self-reinforcing feedback loop that would tend toward maintaining a 3D architecture characteristic of the previous cell cycle but that would be vulnerable to change at each TDP.

Given that this window of time is small (the first hours after mitosis) and that remodeling chromatin architecture is probabilistic, this model provides an explanation for the inefficiency of cellular reprogramming in iPSC technology, the ability of cell division to improve reprogramming efficiency, and the ability of cell synchronization to improve animal cloning (Egli et al., 2008). In addition, the finding that quiescent cells retain RTDs could help to explain how an adult stem cell, emerging from quiescence after stimulation by specific differentiation factors, can ensure self-renewal and maintenance of the stem cell population before committing to differentiation. Such a stem cell would retain RTDs regardless of the stimuli and assemble chromatin in the temporal order dictated by the prior TDP. This would preserve a memory of the stem cell identity until mitosis, when cell division could renew the stem cell. If the emerging cell was to commit before cell division, it would abrogate the possibility of self-renewal.

Another interesting nuance of this model relates to the fact that the length of G1 phase is very short in mouse ESCs (Panning and Gilbert, 2005). In addition, chromatin is very dispersed relative to differentiated cells (Hiratani et al., 2010), and there are a larger number of smaller early- and late-replicating domains (Hiratani et al., 2008). It is possible that under these conditions, replication initiates so rapidly after mitosis that there is little time to reorganize the nucleus, potentially contributing to the plasticity of pluripotent cells (Hiratani et al., 2008). The length of G1 phase in human ESCs is still a matter of debate (White and Dalton, 2005; Neganova and Lako, 2008) but is likely to be similar to mouse ESCs and should be resolved once the pluripotent states of human ESCs grown under different conditions are clarified (Hanna et al., 2010). Indeed, lengthening G1 phase appears to be central to at least some differentiation pathways (Zhang et al., 2009; Borghese et al., 2010).

Testable predictions

A hypothesis is only as useful as the testable predictions that it imparts. This model makes at least two such predictions. First, in cycling stem cells, if one stimulates differentiation, individual cells that are exiting mitosis should differentiate more rapidly than cells at other times during the cell cycle. Second, adult stem cells emerging from quiescence should require mitosis to initiate the process of differentiation, and this window should close again at the following TDP. Practically speaking, this second prediction is similar to the first, but the implications are that quiescent cells are not poised or vulnerable to certain differentiation signals despite their altered chromatin organization. Moreover, this second prediction contradicts as yet unsubstantiated

claims that nuclei from quiescent cells are more efficient substrates for animal cloning (Kasinathan et al., 2001).

Sophisticated live cell imaging systems that can track individual cells throughout multiple cell cycles, coupled with fluorescent beacons as indicators of cell fate transitions, should allow these predictions to be tested. If the TDP represents a critical time window for reprogramming, then the window should not close until several hours after nuclear reassembly and the resumption of transcription, when the completion of chromatin repositioning is observed. If, instead, mitosis is a more critical time period (e.g., through the eviction of transcription factors [Egli et al., 2008] or binding of factors specifically in mitosis [John and Workman, 1998; Zaidi et al., 2010]), the window should close considerably earlier. Similarly, these same experiments would also be able to identify whether another phase of the cell cycle, such as S phase or G2 phase (during which chromatin mobility is restrained), is a more critical window of plasticity or whether cells are equally vulnerable to reprogramming regardless of their position in the cell cycle, either of which would also rule out the proposed hypothesis. Of course, the markers chosen to assess differentiation will be critical because many genes are likely to be regulated by mechanisms that are independent of 3D architecture. In fact, we have found that transcription from CpG-rich promoters is independent of replication timing changes, implying that regulation of many genes is independent of higher-order chromatin folding (Hiratani et al., 2008; Hiratani et al., 2010).

In conclusion, spatial compartmentalization of chromatin is a cell cycle-regulated feature of chromosome organization that may dictate the time and place of chromatin assembly in the upcoming S phase, which would implicate the TDP as an important regulatory decision point for cellular identity. Therefore, more thought, discussion, and experimentation into the role that events occurring at the TDP might play in maintaining stable cell identities during development and disease are needed.

I thank M.W. Davidson and A. Robins for helpful comments and K. Womble for help with the figure.

Work in the Gilbert laboratory is supported by the National Institute of General Medical Sciences (grants GM083337 and GM085354).

Submitted: 21 July 2010

Accepted: 3 November 2010

References

- Aoto, T., N. Saitoh, Y. Sakamoto, S. Watanabe, and M. Nakao. 2008. Polycomb group protein-associated chromatin is reproduced in post-mitotic G1 phase and is required for S phase progression. *J. Biol. Chem.* 283:18905–18915. doi:10.1074/jbc.M709322200
- Borghese, L., D. Dolezalova, T. Opitz, S. Haupt, A. Leinhaas, B. Steinfarz, P. Koch, F. Edenhofer, A. Hampl, and O. Brüstle. 2010. Inhibition of notch signaling in human embryonic stem cell-derived neural stem cells delays G1/S phase transition and accelerates neuronal differentiation in vitro and in vivo. *Stem Cells*. 28:955–964. doi:10.1002/stem.408
- Chubb, J.R., S. Boyle, P. Perry, and W.A. Bickmore. 2002. Chromatin motion is constrained by association with nuclear compartments in human cells. *Curr. Biol.* 12:439–445. doi:10.1016/S0960-9822(02)00695-4
- Deng, W., and G.A. Blobel. 2010. Do chromatin loops provide epigenetic gene expression states? *Curr. Opin. Genet. Dev.* 20:548–554. doi:10.1016/j.gde.2010.06.007
- Dimitrova, D.S., and D.M. Gilbert. 1999. The spatial position and replication timing of chromosomal domains are both established in early G1 phase. *Mol. Cell.* 4:983–993. doi:10.1016/S1097-2765(00)80227-0

- Egli, D., G. Birkhoff, and K. Eggan. 2008. Mediators of reprogramming: transcription factors and transitions through mitosis. *Nat. Rev. Mol. Cell Biol.* 9:505–516. doi:10.1038/nrm2439
- Finlan, L.E., D. Sproul, I. Thomson, S. Boyle, E. Kerr, P. Perry, B. Ylstra, J.R. Chubb, and W.A. Bickmore. 2008. Recruitment to the nuclear periphery can alter expression of genes in human cells. *PLoS Genet.* 4:e1000039. doi:10.1371/journal.pgen.1000039
- Fraser, P., and W. Bickmore. 2007. Nuclear organization of the genome and the potential for gene regulation. *Nature.* 447:413–417. doi:10.1038/nature05916
- Gilbert, D.M. 2001. Nuclear position leaves its mark on replication timing. *J. Cell Biol.* 152:F11–F15. doi:10.1083/jcb.152.2.F11
- Gilbert, D.M., and S.M. Gasser. 2006. Nuclear structure and DNA replication. *In* DNA Replication and Human Disease. M.L. DePamphilis, editor. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 175–196.
- Grimm, O.H., and J.B. Gurdon. 2002. Nuclear exclusion of Smad2 is a mechanism leading to loss of competence. *Nat. Cell Biol.* 4:519–522. doi:10.1038/ncb812
- Hakim, O., M.H. Sung, and G.L. Hager. 2010. 3D shortcuts to gene regulation. *Curr. Opin. Cell Biol.* 22:305–313. doi:10.1016/j.ccb.2010.04.005
- Hanna, J., A.W. Cheng, K. Saha, J. Kim, C.J. Lengner, F. Soldner, J.P. Cassidy, J. Muffat, B.W. Carey, and R. Jaenisch. 2010. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc. Natl. Acad. Sci. USA.* 107:9222–9227. doi:10.1073/pnas.1004584107
- Hiratani, I., and D.M. Gilbert. 2010. Autosomal lyonization of replication domains during early mammalian development. *In* The Cell Biology of Stem Cells. E. Meshorer and K. Plath, editors. Landes and Springer, Austin, TX. 41–58.
- Hiratani, I., T. Ryba, M. Itoh, T. Yokochi, M. Schwaiger, C.W. Chang, Y. Lyou, T.M. Townes, D. Schübeler, and D.M. Gilbert. 2008. Global reorganization of replication domains during embryonic stem cell differentiation. *PLoS Biol.* 6:e245. doi:10.1371/journal.pbio.0060245
- Hiratani, I., S. Takebayashi, J. Lu, and D.M. Gilbert. 2009. Replication timing and transcriptional control: beyond cause and effect—part II. *Curr. Opin. Genet. Dev.* 19:142–149. doi:10.1016/j.gde.2009.02.002
- Hiratani, I., T. Ryba, M. Itoh, J. Rathjen, M. Kulik, B. Papp, E. Fussner, D.P. Bazett-Jones, K. Plath, S. Dalton, et al. 2010. Genome-wide dynamics of replication timing revealed by in vitro models of mouse embryogenesis. *Genome Res.* 20:155–169. doi:10.1101/gr.099796.109
- Jansen, L.E., B.E. Black, D.R. Foltz, and D.W. Cleveland. 2007. Propagation of centromeric chromatin requires exit from mitosis. *J. Cell Biol.* 176:795–805. doi:10.1083/jcb.200701066
- John, S., and J.L. Workman. 1998. Bookmarking genes for activation in condensed mitotic chromosomes. *Bioessays.* 20:275–279. doi:10.1002/(SICI)1521-1878(199804)20:4<275::AID-BIES1>3.0.CO;2-P
- Kasinathan, P., J.G. Knott, Z. Wang, D.J. Jerry, and J.M. Robl. 2001. Production of calves from G1 fibroblasts. *Nat. Biotechnol.* 19:1176–1178. doi:10.1038/nbt1201-1176
- Kaufman, P.D., and O.J. Rando. 2010. Chromatin as a potential carrier of heritable information. *Curr. Opin. Cell Biol.* 22:284–290. doi:10.1016/j.ccb.2010.02.002
- Kumaran, R.I., and D.L. Spector. 2008. A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. *J. Cell Biol.* 180:51–65. doi:10.1083/jcb.200706060
- Kundu, S., and C.L. Peterson. 2009. Role of chromatin states in transcriptional memory. *Biochim. Biophys. Acta.* 1790:445–455.
- Lancôt, C., T. Cheutin, M. Cremer, G. Cavalli, and T. Cremer. 2007. Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. *Nat. Rev. Genet.* 8:104–115. doi:10.1038/nrg2041
- Lande-Diner, L., J. Zhang, and H. Cedar. 2009. Shifts in replication timing actively affect histone acetylation during nucleosome reassembly. *Mol. Cell.* 34:767–774. doi:10.1016/j.molcel.2009.05.027
- Laster, K., and S.T. Kosak. 2010. Genomic Pangea: coordinate gene regulation and cell-specific chromosomal topologies. *Curr. Opin. Cell Biol.* 22:314–319. doi:10.1016/j.ccb.2010.04.009
- Lieberman-Aiden, E., N.L. van Berkum, L. Williams, M. Imakaev, T. Ragoczy, A. Telling, I. Amit, B.R. Lajoie, P.J. Sabo, M.O. Dorschner, et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science.* 326:289–293. doi:10.1126/science.1181369
- Lu, J., F. Li, C.S. Murphy, M.W. Davidson, and D.M. Gilbert. 2010. G2 phase chromatin lacks determinants of replication timing. *J. Cell Biol.* 189:967–980. doi:10.1083/jcb.201002002
- Luo, L., K.L. Gassman, L.M. Petell, C.L. Wilson, J. Bewersdorf, and L.S. Shopland. 2009. The nuclear periphery of embryonic stem cells is a transcriptionally permissive and repressive compartment. *J. Cell Sci.* 122:3729–3737. doi:10.1242/jcs.052555
- Meshorer, E., and T. Misteli. 2006. Chromatin in pluripotent embryonic stem cells and differentiation. *Nat. Rev. Mol. Cell Biol.* 7:540–546. doi:10.1038/nrm1938
- Neganova, I., and M. Lako. 2008. G1 to S phase cell cycle transition in somatic and embryonic stem cells. *J. Anat.* 213:30–44. doi:10.1111/j.1469-7580.2008.00931.x
- Németh, A., A. Conesa, J. Santoyo-Lopez, I. Medina, D. Montaner, B. Péterfia, I. Solovei, T. Cremer, J. Dopazo, and G. Längst. 2010. Initial genomics of the human nucleolus. *PLoS Genet.* 6:e1000889. doi:10.1371/journal.pgen.1000889
- Panning, M.M., and D.M. Gilbert. 2005. Spatio-temporal organization of DNA replication in murine embryonic stem, primary, and immortalized cells. *J. Cell. Biochem.* 95:74–82. doi:10.1002/jcb.20395
- Peric-Hupkes, D., W. Meuleman, L. Pagie, S.W. Bruggeman, I. Solovei, W. Brugman, S. Gräf, P. Flicek, R.M. Kerkhoven, M. van Lohuizen, et al. 2010. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell.* 38:603–613. doi:10.1016/j.molcel.2010.03.016
- Pope, B.D., I. Hiratani, and D.M. Gilbert. 2010. Domain-wide regulation of DNA replication timing during mammalian development. *Chromosome Res.* 18:127–136. doi:10.1007/s10577-009-9100-8
- Reddy, K.L., J.M. Zullo, E. Bertolino, and H. Singh. 2008. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. *Nature.* 452:243–247. doi:10.1038/nature06727
- Ryba, T., I. Hiratani, J. Lu, M. Itoh, M. Kulik, J. Zhang, T.C. Schulz, A.J. Robins, S. Dalton, and D.M. Gilbert. 2010. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res.* 20:761–770. doi:10.1101/gr.099655.109
- Scharf, A.N., T.K. Barth, and A. Imhof. 2009. Establishment of histone modifications after chromatin assembly. *Nucleic Acids Res.* 37:5032–5040. doi:10.1093/nar/gkp518
- Spivakov, M., and A.G. Fisher. 2007. Epigenetic signatures of stem-cell identity. *Nat. Rev. Genet.* 8:263–271. doi:10.1038/nrg2046
- Steinbach, O.C., A.P. Wolffe, and R.A. Rupp. 1997. Somatic linker histones cause loss of mesodermal competence in *Xenopus*. *Nature.* 389:395–399. doi:10.1038/38755
- Thomson, A.M., P.J. Gillespie, and J.J. Blow. 2010. Replication factory activation can be decoupled from the replication timing program by modulating Cdk levels. *J. Cell Biol.* 188:209–221. doi:10.1083/jcb.200911037
- Walter, J., L. Schermelleh, M. Cremer, S. Tashiro, and T. Cremer. 2003. Chromosome order in HeLa cells changes during mitosis and early G1, but is stably maintained during subsequent interphase stages. *J. Cell Biol.* 160:685–697. doi:10.1083/jcb.200211103
- White, J., and S. Dalton. 2005. Cell cycle control of embryonic stem cells. *Stem Cell Rev.* 1:131–138. doi:10.1385/SCR:1:2:131
- Yaffe, E., S. Farkash-Amar, A. Polten, Z. Yakhini, A. Tanay, and I. Simon. 2010. Comparative analysis of DNA replication timing reveals conserved large-scale chromosomal architecture. *PLoS Genet.* 6:e1001011. doi:10.1371/journal.pgen.1001011
- Zaidi, S.K., D.W. Young, M.A. Montecino, J.B. Lian, A.J. van Wijnen, J.L. Stein, and G.S. Stein. 2010. Mitotic bookmarking of genes: a novel dimension to epigenetic control. *Nat. Rev. Genet.* 11:583–589. doi:10.1038/nrg2827
- Zhang, X., I. Neganova, S. Przyborski, C. Yang, M. Cooke, S.P. Atkinson, G. Anyfantis, S. Fenyk, W.N. Keith, S.F. Hoare, et al. 2009. A role for NANOG in G1 to S transition in human embryonic stem cells through direct binding of CDK6 and CDC25A. *J. Cell Biol.* 184:67–82. doi:10.1083/jcb.200801009