

Replication timing and transcriptional control: beyond cause and effect

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In general, transcriptionally active euchromatin replicates during the first half of S phase, whereas silent heterochromatin replicates during the second half. Moreover, changes in replication timing accompany key stages of development. Although there is not a strict correlation between replication timing and transcription *per se*, recent results reveal a strong relationship between heritably repressed chromatin and late replication that is conserved in all eukaryotes. A long-standing question is whether replication timing dictates the structure of chromatin or *vice versa*. Mounting evidence supports a model in which replication timing is both cause and consequence of chromatin structure by providing a means to inherit chromatin states that, in turn, regulate replication timing in the subsequent cell cycle. Moreover, new findings relating aberrations in replication timing to defects in centromere function, chromosome cohesion and genome instability suggest that the role of replication timing extends beyond its relationship to transcription. Novel systems in both yeasts and mammals are finally beginning to reveal some of the determinants that regulate replication timing, which should pave the way for a long-anticipated molecular dissection of this complex liaison.

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Abbreviations

LCR locus control region
TDP timing decision point

Introduction

Experiments conducted in the early 1960s showed that genetically inactive heterochromatin, contained in Giemsa dark chromosome bands or ‘G bands’, replicates late during S phase, whereas most transcription takes place in Giemsa light or ‘R bands’, which replicate early during S phase (for references see [1]). Results from modern molecular approaches have been consistent with this conclusion: out of the few dozen genes examined, nearly all transcriptionally active genes replicate early in S phase, and more than half of the developmentally regulated genes replicate late when they are not expressed [2–4].

Comprehensive studies of large DNA segments are consistent with the idea that several adjacent replication origins orchestrate the coordinate replication of large ‘replication domains’, whose boundaries coincide with the boundaries of R and G bands [5–7]. Some studies have

identified developmentally regulated switches for replication timing that encompass hundreds of kilobases [8,9,10]. In all such cases, a switch from late to early replication precedes or coincides with transcriptional activation of genes within the affected domain. Hence, it is often presumed that early replication is a necessary (albeit not sufficient) condition for transcription.

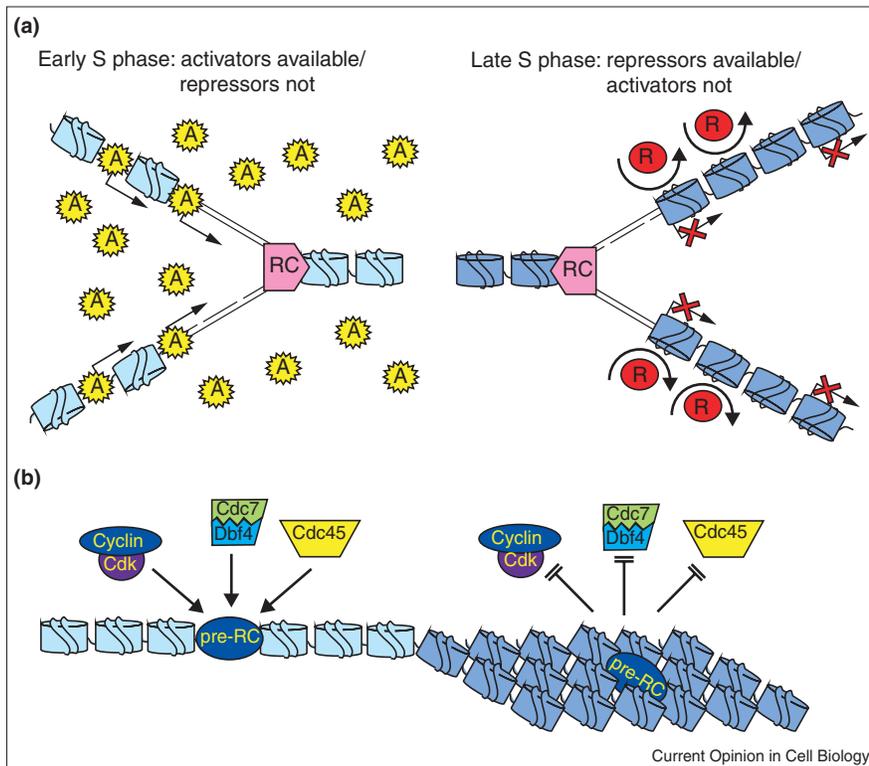
Two reciprocal but not mutually exclusive working models have been proposed to describe this relationship (Figure 1). In the first model (Figure 1a), transcriptional potential is established by synthesizing DNA at times when specific proteins are available for assembly into chromatin. For example, early replicating DNA would have a competitive advantage for binding limiting concentrations of transcriptional activators [11], whereas proteins that facilitate the assembly of heterochromatin would be available only late during S phase [12]. An alternative model (Figure 1b) proposes that the structure of heterochromatin delays the initiation of replication [13,14,15], perhaps by restricting access of essential replication proteins to chromatin. In this review, I summarize recent evidence in the context of each of these models.

Globin gene regulation

The mammalian *globin* gene loci are amongst the most well studied with respect to the role of replication timing in gene expression. In non-erythroid cells the *β-globin* gene cluster is embedded in a 200–300 kb stretch of hypoacetylated, DNaseI-resistant, late-replicating chromatin; but in erythroblasts that can be induced to express *β-globin*, roughly 1 Mb surrounding the *β-globin* gene comprises early-replicating, DNaseI-sensitive, acetylated chromatin [9,16]. Much attention has focused on the role of the locus control region (LCR) — a cluster of five DNaseI-hypersensitive sites that span a region 50–75 kb upstream of the *β-globin* genes. Naturally occurring deletions that include the LCR render the entire globin locus insensitive to DNaseI, transcriptionally inactive and late replicating [16].

Transgenic mice carrying human *β-globin* constructs containing the LCR show efficient, position-independent levels of gene expression, although the percentage of cells expressing the gene is still subject to variegating position effects [17]. Recently, several LCR-containing transgenes have been shown to replicate early in erythroid tissues and late in non-erythroid tissues derived from these transgenic mice, regardless of the normal replication time of the integration site [9]. For one of these transgenes, flanking sequences seemed to come under the replication-timing control of the insert. Furthermore, derivative transgenes

Figure 1



Two models that relate replication timing and transcriptional control. **(a)** In this model, replication timing is controlled actively to replicate chromosome domains at times when critical regulatory factors are available. For example, limiting amounts of transcriptional activating factors (A, yellow stars) could be sequestered by early-replicating DNA [11]. Alternatively, the availability of repressors (R) (e.g. chromatin modifiers, red circles) might be restricted to late S phase [12**]. Euchromatin is denoted as light blue nucleosomes, heterochromatin as dark blue nucleosomes, active and inactive promoters as arrows. **(b)** This model proposes that chromatin structure, by restricting the access of replication factors, determines the time at which origins in that domain will fire [13,14]. Shown is a pre-replication complex (pre-RC) assembled at a replication origin and either accessible or inaccessible to proteins that regulate initiation of replication during S phase (reviewed in [13,29]). These models are not mutually exclusive. For example, chromatin modifications could initiate changes in replication timing, whereas replication timing might maintain chromatin structure by ensuring that epigenetic states of chromatin are propagated.

with deletions in the LCR did not show proper replication-timing control. These experiments suggest that *cis*-acting elements within the LCR are sufficient to influence the replication timing of large chromosomal regions; however, they do not distinguish whether the LCR exerts a direct influence over origin firing or affects replication timing indirectly through global changes in chromatin structure.

Intriguingly, targeted deletions of the LCR at the native locus, either in knockout mice or in human chromosomes transferred into murine erythroleukemia cells, do not affect chromatin structure or replication timing, although they do substantially reduce transcription of the β -globin genes [16**,18*]. At present, the simplest way to reconcile these knockout studies with the transgenic mouse experiments is to hypothesize the existence of redundant elements upstream of the LCR that ensure proper replication timing when the LCR is deleted. These redundant elements would presumably be contained within the larger Hispanic deletion that disrupts replication control. This hypothesis will soon be put to the test through the targeted deletion of additional upstream sequences.

An important consideration is that hemoglobin consists of equal quantities of both α - and β -globin. Therefore, the genes encoding these two polypeptides must be regulated coordinately and expressed to similar levels, yet the α -globin locus is early replicating and embedded in accessible chromatin in all tissues [3,19*]. Thus, an erythroid-specific

pattern of gene expression can be achieved without marked changes in chromatin structure. Cells may be forced to adapt different strategies depending on the chromosomal context in which a gene is located. The α -globin gene is in a GC-rich region containing many housekeeping genes, whereas the β -globin gene is in an AT-rich region flanked by olfactory receptor genes and imprinted genes.

Allelic asynchrony

Homologous alleles usually replicate synchronously; however, monoallelically expressed genes such as imprinted genes [20], genes encoding olfactory receptors [9**], and the female X chromosome (reviewed in [21,22]) replicate asynchronously in mammals, with the expressed allele replicating earlier than the silent allele. Asynchrony is established early in development. With imprinted genes, asynchronous replication is erased in the germ line before meiosis and reset in late gametogenesis, remaining asynchronous throughout pre-implantation development [20]. In contrast, asynchronous replication of the X chromosome is reversed during gametogenesis and then re-established during the implantation stage of embryonic development, coincident with transcriptional inactivation and heterochromatinization of the late-replicating chromosome [21,22].

Recently, the immunoglobulin and T-cell receptor genes have been shown to replicate asynchronously [23**]. Asynchronous replication is erased in the morula and blastula, and (similar to X-chromosome inactivation) reset

at the time of implantation by a mechanism that chooses the late-replicating allele at random. Notably, it is almost always the early-replicating allele that is initially selected to undergo rearrangement, suggesting that replication timing is an early developmental marker for allelic exclusion in the immune system.

Inactivation of the X chromosome is currently the only available system in which the order of events surrounding a switch in replication timing can be observed in cell populations. Female embryonic stem cells can be stimulated to differentiate in culture. This results in the coating of one X chromosome with *Xist* RNA, a non-coding transcript only expressed from the inactive X. Subsequently — perhaps within a single cell cycle — the *Xist*-coated X chromosome is rendered transcriptionally silent and late replicating [24], coincident with hypoacetylation and K9 methylation of histone H3 [25•]. If *Xist* RNA is induced experimentally before the signal for differentiation, however, transcriptional silencing occurs but is reversible upon downregulation of *Xist* RNA [26••]. In this system, irreversible (*Xist*-independent) inactivation follows differentiation and is coincident with late replication. All of these events take place before the methylation of CpG islands on the inactive X chromosome [24], which, owing to its late onset, cannot be involved in initiating late replication. DNA methylation does seem to contribute to maintaining late replication, as mutations in the *dnmt3b* DNA methyltransferase gene [27••], or treatment of somatic cells with the de-methylating agent 5-azacytidine [28], result in early replication of the inactive X chromosome in a percentage of cells.

Together, these data imply that expression of *Xist* RNA and transcriptional silencing mark the inactive X chromosome for a switch in replication timing, whereas a complex interplay between several epigenetic factors maintains late replication. They do not yet distinguish, however, whether the initial assembly of a heritable heterochromatic state is a cause or consequence of changes in replication control.

Determinants of replication timing

Ultimately, understanding the role of replication timing in transcriptional control (or any other cellular process) will require knowledge of what programs replication origins to fire at particular times during S phase. This has been a particularly challenging problem in metazoa, because of difficulties in defining the sequence elements that comprise replication origins and the lack of convenient assays for replication [29].

There are only two mammalian loci for which both replication-timing switches and origin localization have been studied. At the mouse immunoglobulin IgH locus, new origins are activated in pre-B cells when the promoter region of this locus becomes transcriptionally active and switches from late to early replicating ([10]; C Shildkraut, personal communication). In contrast, at the human β -globin locus, replication initiates at the same site whether

the locus is early or late replicating [16••], showing that the elements that control timing are separate from those that specify origin sites at this locus.

Neither of these labor-intensive studies [10,16••] has determined, however, whether the primary switch is at the level of replication or the level of transcription. Globally, domains of histone H4 deacetylation [30] and histone H3 Lys9 methylation [31•] coincide with domains of late-replicating chromatin, and inhibitors of histone deacetylases can disrupt replication timing [32]. Although this suggests that the structure of heterochromatin is required to maintain patterns of late replication, it does not identify the event that establishes these patterns.

Budding yeast provide a much simpler model system in which to address the determinants of replication timing. Well-defined, genetically tractable replication origins initiate at defined times during S phase. When removed from their native chromosomal context, most of these origins will replicate early unless they are positioned either near a telomere or next to specific segments of DNA derived from late-replicating regions (reviewed in [13]). For a zone extending about 10 kb from the telomere, mutations in the gene encoding the silent chromatin factor Sir3p, which is enriched at telomeres, results in both firing of a silent replication origin [14] and increased gene expression [33].

Such experiments provide direct evidence that the structure of transcriptionally silent chromatin can delay initiation at some origins, presumably by restricting access of replication initiation factors to chromatin (Figure 1b). But other origins seem to have a strong propensity to remain late replicating. For example, origins at the silent mating-type locus (*HML*) still replicate somewhat later than other origins when removed from their chromosomal context, and this intrinsic late-initiating program appears to be controlled by *cis*-acting elements that are, as yet, inseparable from the elements required for initiation [34•,35].

At many budding yeast loci, transcriptional activity is not incompatible with late replication. A stretch of late-replicating DNA on chromosome XIV contains several actively transcribed genes [36], and mutations that relieve transcriptional silencing at the *HML* locus do not advance the replication timing of origins within this locus [35,37]. Moreover, a pioneering whole-genome analysis of replication timing has concluded that there is no correlation between levels of gene expression and replication timing for 137 ribosomal protein genes (accounting for about 50% of RNA polymerase II expression), small nucleolar RNA and tRNA genes [38••].

At first glance, this result may appear to reveal a fundamental difference between yeast and higher eukaryotes. However, the number of mammalian genes analysed for replication timing is a small fraction of the genome, and

even within this small sampling there exist examples of late-replicating expressed genes [3,39,40]. These results can be more inclusively interpreted by proposing that it is only the heritably repressed (heterochromatic) chromosome domains that correlate strictly with late replication, which is true in all eukaryotic systems studied to date. Telomeres and silent mating-type loci may be the only genuine heterochromatic loci in budding yeast. Studies of replication timing in fission yeast, which has become an excellent model system for studying heterochromatin [41•], have only just begun [42•] and should be instrumental in probing the liaison between late replication and heterochromatin.

Replication timing and subnuclear position

Several recent studies suggest that replication timing is re-established in each cell cycle by modifications of chromatin that take place as sequences are re-positioned after mitosis (reviewed in [13,43]). In mammalian cells, the events that establish a replication program can be teased apart by incubating nuclei from synchronized cells in extracts from replication-competent *Xenopus* eggs [44,45••]. These studies have defined a discrete window of time in early G1 phase during which the replication-timing program is established — the ‘timing decision point’ (TDP). Before this point, chromosomal domains are replicated in a random order.

The TDP takes place before the specification of replication origin sites and certain manipulations of nuclei can disrupt origin specification without disturbing the order in which chromosome domains are replicated [44]. This implies that replication timing is established at the level of chromosome domains and is independent of which sites are selected to function as replication origins. Intriguingly, the TDP takes place simultaneously with the repositioning of sequences in the nucleus after mitosis [44,45••,46]. In fact, the Chinese hamster *β-globin* locus acquires the replication-timing program of peripheral heterochromatin coincidentally with its association with the nuclear periphery at the TDP [45••].

These experiments suggest a model in which chromatin regulators disperse during mitosis and are then re-concentrated into subnuclear compartments by a clustering of related chromosomal domains [13,44,45••]. Chromatin coming into contact with these compartments would be modified in *trans* by the locally high concentrations of these regulators, setting thresholds for replication timing, as suggested in Figure 1b. The determining event is probably not histone modifications, as patterns of histone acetylation and methylation are largely maintained during mitosis [30,31•], but it could be the association of other chromatin regulators such as chromodomain proteins [13].

The precedent for this model derives from several elegant studies in budding yeast. The 32 telomeres in a diploid yeast cell are clustered into three to eight sites at the nuclear periphery. Sir proteins, which are required for late replication and transcriptional silencing near telomeres

[14,33], are present at low levels throughout the overall nucleus but are concentrated at telomere clusters. Mutations that disrupt telomere clustering also relieve silencing of telomeric genes [47]. Recently, it was shown that several non-telomeric late replicating origins are closer to the nuclear periphery during G1 phase but are found farther from the periphery at later points in the cell cycle, whereas early replicating origins are found throughout the nucleus at all times during the cell cycle [15••].

In fact, the late replication program of one budding yeast origin is established during early G1 phase, and *cis*-acting elements that are separable from this origin are necessary for both peripheral localization and late replication during this same window of time [15••,48]. Together, these results suggest that replication origins are modified at the nuclear periphery during G1 phase to delay their initiation time — a conclusion that provides optimism that the power of yeast genetics may allow the molecular basis of the TDP to be dissected.

The periphery is not the only heterochromatic nuclear compartment, but the extent to which other compartments can delay replication timing in *trans* is not yet clear. For example, genes that associate in *trans* with intranuclear pericentromeric heterochromatin are often (but not always) silenced [49,50,51•]. This association seems to be established by specific transcriptional repressors [52,53] and disrupted by specific transcriptional activators [51•]. Out of the few genes that associate with these pericentromeric subnuclear compartments that have been examined, however, none is heterochromatic [53] or late replicating ([51•]; A Fisher, personal communication).

In contrast, a strong correlation has been found between late replication, transcriptional inactivity and proximity to the nuclear periphery ([7,45••,54•]; C Shildkraut, personal communication). Importantly, in both yeast [15••] and mammalian cells [46,55], sequences can move away from the periphery after the TDP and retain their late replication program. Thus, studies relating subnuclear position to replication timing may be ambiguous unless carried out with cells synchronized at the TDP.

Other roles for replication timing

The incomplete correlation between replication timing and transcription may reflect other roles for replication timing. In several model systems, defects in replication timing are associated with defects in chromosome condensation, sister chromatid cohesion and genome stability [56••,57•]. Moreover, abnormal allelic asynchrony has become a useful clinical marker for predicting malignant cancers [58•,59•,60••], and defects in replication timing have been associated with chromosome instability in several inherited human diseases (for example, [27••,61,62]).

In both yeast and mammals, late replication correlates more faithfully with heterochromatin than with transcriptional

silencing, and heterochromatin has been shown recently to be required for sister chromatid cohesion and segregation [41*]. In addition, the histone deacetylase HDAC2 localizes to sites of late-, but not early-, replicating DNA [12**], suggesting a means by which late replication might maintain the hypoacetylated structure of heterochromatin (Figure 1a). If the primary role of replication timing is to maintain the structure of heterochromatin, which is neither necessary (e.g. for the α -globin gene) nor sufficient (e.g. for genes in heterochromatin [63]) to repress transcription, then the incomplete correlation between replication timing and transcriptional control would not be surprising.

Conclusions

In all eukaryotes, replication timing is linked indirectly to transcriptional control through its liaison with chromatin structure. Heterochromatin seems to be universally late replicating. The mechanism that links these two has remained elusive, and evidence exists for both of the models shown in Figure 1. Although some systems provide direct evidence that silent chromatin is responsible for late replication, this model (Figure 1b) does not offer a functional significance to replication timing. The simplest interpretation is that both models are correct. Although switches in replication timing can be initiated by changes in chromatin structure, once established that structure must be re-assembled in each cell cycle. Duplicating chromatin at specific times during the cell cycle might facilitate the re-assembly of the very domain structure that dictates, in turn, replication timing in the subsequent cell cycle.

Testing this hypothesis will require a better understanding of what regulates replication timing. This will be a formidable challenge, particularly when one considers that origins do not fire simply early or late, but continuously throughout S phase [38**,44]. New approaches, in both yeast and mammalian cells, are beginning to dissect how this program is reset in each cell cycle; and recent links to genome stability and human disease should provide added incentive for more research. Sorely needed, however, are systems for probing the molecular events that initiate replication-timing switches, such as those available for studying X-chromosome inactivation. Central to this goal is a description of when such switches occur during development.

So far, the number of loci for which replication timing changes have been identified is a tiny representation of the genome. Moreover, tissue-specific differences in Giemsa R and G banding patterns have not been observed, raising the question of how frequently changes in timing of replication occur during development. Whole-genome analyses of replication timing, such as those carried out recently in budding yeast, need to be performed in different cell lineages before we can evaluate the significance of replication timing switches during the course of development. At least this approach is one that is within our technological grasp.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Stambrook PJ, Flickinger RA: **Changes in chromosomal DNA replication patterns in developing frog embryos.** *J Exp Zool* 1970, **174**:101-113.
 2. Taljanidisz J, Popowski J, Sarkar N: **Temporal order of gene replication in Chinese hamster ovary cells.** *Mol Cell Biol* 1989, **9**:2881-2889.
 3. Hatton KS, Dhar V, Brown EH, Iqbal MA, Stuart S, Didamo VT, Schildkraut CL: **Replication program of active and inactive multigene families in mammalian cells.** *Mol Cell Biol* 1988, **8**:2149-2158.
 4. Goldman MA, Holmquist GP, Gray MC, Caston LA, Nag A: **Replication timing of genes and middle repetitive sequences.** *Science* 1984, **224**:686-692.
 5. Tenzen T, Yamagata T, Fukagawa T, Sugaya K, Ando A, Inoko H, Gojobori T, Fujiyama A, Okumura K, Ikemura T: **Precise switching of DNA replication timing in the GC content transition area in the human major histocompatibility complex.** *Mol Cell Biol* 1997, **17**:4043-4050.
 6. Strehl S, LaSalle JM, Lalande M: **High-resolution analysis of DNA replication domain organization across an R/G-band boundary.** *Mol Cell Biol* 1997, **17**:6157-6166.
 7. Sadoni N, Langer S, Fauth C, Bernardi G, Cremer T, Turner BM, Zink D: **Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments.** *J Cell Biol* 1999, **146**:1211-1226.
 8. Selig S, Okumura K, Ward DC, Cedar H: **Delineation of DNA replication time zones by fluorescence *in situ* hybridization.** *EMBO J* 1992, **11**:1217-1225.
 9. Simon I, Tenzen T, Mostoslavsky R, Fibach E, Lande L, Milot E, Gribnau J, Grosveld F, Fraser P, Cedar H: **Developmental regulation of DNA replication timing at the human β globin locus.** *EMBO J* 2001, **20**:6150-6157.
 10. Ermakova OV, Nguyen LH, Little RD, Chevillard C, Riblet R, Ashouian N, Birshtein BK, Schildkraut CL: **Evidence that a single replication fork proceeds from early to late replicating domains in the IgH locus in a non-B cell line.** *Mol Cell* 1999, **3**:321-330.
 11. Gilbert DM: **Temporal order of replication of *Xenopus laevis* 5S ribosomal RNA genes in somatic cells.** *Proc Natl Acad Sci USA* 1986, **83**:2924-2928.
 12. Rountree MR, Bachman KE, Baylin SB: **DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci.** *Nat Genet* 2000, **25**:269-277.

Mouse transgenes that contain the locus control region (LCR) of the human β -globin gene and that are integrated at different chromosomal sites replicate late in most cell types derived from the transgenic mice, but replicate early in erythroid cells. Additional evidence suggests that specific deletion mutations in the LCR abolish the late – but not the early – replicating state, whereas other deletions abolish both early and late control of replication timing. These results imply that the LCR contains dominant *cis*-acting elements that can regulate the time at which mammalian replication origins fire.

DNA methyltransferase 1 (DNMT1) localizes to sites of nascent DNA replication and contains a polybromodomain for recruiting chromatin modifiers such as histone deacetylases. Using transient transfection with epitope-tagged proteins, the authors show that the amino terminus of DNMT1 can recruit the histone deacetylase HDAC2 to replication sites during late but not early S phase. They propose a model whereby late replication maintains heterochromatin through HDAC2 deacetylation of histones during chromatin assembly.

13. Gilbert DM: **Nuclear position leaves its mark on replication timing.** *J Cell Biol* 2001, **152**:F11-F16.
14. Stevenson JB, Gottschling DE: **Telomeric chromatin modulates replication timing near chromosome ends.** *Genes Dev* 1999, **13**:146-151.
15. Heun P, Laroche T, Raghuraman MK, Gasser SM: **The positioning and dynamics of origins of replication in the budding yeast nucleus.** *J Cell Biol* 2001, **152**:385-400.
- Using fluorescence *in situ* hybridization coupled with targeting green fluorescent protein to specific chromosomal sites, the authors show that late replicating origins tend to localize close to the periphery of the nucleus specifically during G1 phase, whereas early replicating origins are localized more randomly. These results suggest that replication origins are modified at the nuclear periphery during G1 phase to delay their initiation time (see also the accompanying commentary by Gilbert (2001) [13]).
16. Cimbora DM, Schubeler D, Reik A, Hamilton J, Francastel C, Epner EM, Groudine M: **Long-distance control of origin choice and replication timing in the human β -globin locus are independent of the locus control region.** *Mol Cell Biol* 2000, **20**:5581-5591.
- Human chromosome 11 and derivatives containing deletions in the β -globin upstream region were introduced into murine erythroleukemia cells. In this system, the wild-type β -globin locus is in an open chromatin conformation and replicates early, whereas a naturally occurring deletion (Hispanic thalassemia) that includes the locus control region (LCR) and additional upstream sequences renders the whole β -globin locus late replicating and insensitive to DNaseI, with replication initiating from a different origin. The authors show that a targeted deletion of the LCR reduces transcription of the β -globin gene but does not effect replication timing or the choice of replication origins. Presumably, sequences contained within the additional upstream sequences of the Hispanic thalassemia deletion ensure early replication of the β -globin locus when the LCR is deleted.
17. Alami R, Greally JM, Tanimoto K, Hwang S, Feng YQ, Engel JD, Fiering S, Bouhassira EE: **β -globin YAC transgenes exhibit uniform expression levels but position effect variegation in mice.** *Hum Mol Genet* 2000, **9**:631-636.
18. Schubeler D, Groudine M, Bender MA: **The murine β -globin locus control region regulates the rate of transcription but not the hyperacetylation of histones at the active genes.** *Proc Natl Acad Sci USA* 2001, **98**:11432-11437.
- Mice carrying a targeted deletion of the locus control region (LCR) of murine β -globin (but kept alive with a human transgene) show normal developmental regulation of globin genes (albeit expressed at very low levels) and formation of chromatin that is sensitive to DNaseI. Although replication timing is not examined in these knockout mice, the results on DNase I sensitivity imply that replication timing of the murine β -globin locus is regulated by elements outside the LCR, as seems to be the case in humans (Cimbora *et al.* [2000] [16**]).
19. Brown KE, Amoils S, Horn JM, Buckle VJ, Higgs DR, Merckenschlager M, Fisher AG: **Expression of α - and β -globin genes occurs within different nuclear domains in haemopoietic cells.** *Nat Cell Biol* 2001, **3**:602-606.
- The subnuclear location of α - and β -globin loci is compared in primary human lymphoblasts and erythroblasts. In lymphoblasts, where both genes are silent, β - but not α -globin is positioned next to centromeric heterochromatin. These data and those of other laboratories (references therein) show that these two coordinately expressed genes are regulated very differently with respect to replication timing, subnuclear position and chromatin structure.
20. Simon I, Tenzen T, Reubinoff BE, Hillman D, McCarrey JR, Cedar H: **Asynchronous replication of imprinted genes is established in the gametes and maintained during development.** *Nature* 1999, **401**:929-932.
21. Avner P, Heard E: **X-chromosome inactivation: counting, choice and initiation.** *Nat Rev Genet* 2001, **2**:59-67.
22. Maxfield Boumil R, Lee JT: **Forty years of decoding the silence in X-chromosome inactivation.** *Hum Mol Genet* 2001, **10**:2225-2232.
23. Mostoslavsky R, Singh N, Tenzen T, Goldmit M, Gabay C, Elizur S, Qi P, Reubinoff BE, Chess A, Cedar H *et al.*: **Asynchronous replication and allelic exclusion in the immune system.** *Nature* 2001, **414**:221-225.
- Asynchronous replication of B- and T-cell receptor loci is erased in the morula and blastula and then re-established around implantation, similar to X-chromosome inactivation. In B cells, the earlier replicating copy of the κ locus is almost always the rearranged copy. Using allele-specific markers, the authors show that the pattern of replication is established randomly and maintained clonally, which suggests that replication timing serves as a basis for initially choosing one allele to undergo rearrangement.
24. Keohane AM, O'Neill L P, Belyaev ND, Lavender JS, Turner BM: **X-inactivation and histone H4 acetylation in embryonic stem cells.** *Dev Biol* 1996, **180**:618-630.
25. Heard E, Rougeulle C, Arnaud D, Avner P, Allis CD, Spector DL: **Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation.** *Cell* 2001, **107**:727-738.
- Modifications in histone H3 are shown to constitute an early change in chromatin during X-chromosome inactivation in differentiating embryonic stem cells. They occur immediately after *Xist* RNA coating and before either histone H4 deacetylation or transcriptional inactivation of several genes. Relating histone H3 modifications to the onset of late replication will be important for studies of both X-chromosome inactivation and autosomal heterochromatin (see Cowell *et al.* [2002] [31*]).
26. Wutz A, Jaenisch R: **A shift from reversible to irreversible X inactivation is triggered during ES cell differentiation.** *Mol Cell* 2000, **5**:695-705.
- Xist* RNA is induced experimentally from several independent integration sites in both the X chromosome and autosomes of undifferentiated embryonic stem cells. This results in *Xist* RNA coating and gene repression of the *cis* chromosome. Without inducing differentiation, X chromosomes remain early replicating, and transcriptional repression is reversible upon removal of *Xist*. After differentiation, however, the *Xist*-marked chromosome becomes late replicating and the repression of gene expression becomes independent of *Xist*. These results suggest that an epigenetic change elicited by *Xist* RNA can mark a chromosome for inactivation but that the heritable propagation of that epigenetic change requires additional events that are associated with changes in replication timing.
27. Hansen RS, Stoger R, Wijmenga C, Stanek AM, Canfield TK, Luo P, Matarazzo MR, D'Esposito M, Feil R, Gimelli G *et al.*: **Escape from gene silencing in ICF syndrome: evidence for advanced replication time as a major determinant.** *Hum Mol Genet* 2000, **9**:2575-2587.
- The ICF (immunodeficiency, centromeric heterochromatin, facial anomalies) syndrome is associated with hypomethylation of satellite regions, which results from mutations in the *DNMT3B* DNA methyltransferase gene. This report finds that regions of hypomethylation are associated with advanced replication time and nuclease hypersensitivity. Some X chromosomes are hypomethylated but still silenced; escape from silencing is only seen with early replicating X chromosomes, which suggests that replication timing is a major determinant that maintains gene silencing.
28. Hansen RS, Canfield TK, Fjeld AD, Gartler SM: **Role of late replication timing in the silencing of X-linked genes.** *Hum Mol Genet* 1996, **5**:1345-1353.
29. Gilbert DM: **Making sense of eukaryotic DNA replication origins.** *Science* 2001, **294**:96-100.
30. Belyaev ND, Keohane AM, Turner BM: **Histone H4 acetylation and replication timing in Chinese hamster chromosomes.** *Exp Cell Res* 1996, **225**:277-285.
31. Cowell IG, Aucott R, Mahadevaiah S, Burgoyne PA, Huskisson N, Bongiorno S, Prantera G, Fanti L, Pimpinelli S, Wu R *et al.*: **Heterochromatin, HP1 and methylation at lysine 9 of histone H3 in animals.** *Chromosoma* 2002, DOI 10.1007/s00412-002-0182-8.
- Methylated Lys9 of histone H3 is shown to localize to G-banding patterns of autosomes, as well as the inactive mammalian X chromosome. Chromatin containing this modification replicates late in S phase but the modification is stable throughout the cell cycle, including mitosis. Since methylation of histone H3 on Lys9 therefore precedes the timing decision point, this modification is not sufficient to delay replication but may serve as a memory for the re-establishment of late replicating domains.
32. Bickmore WA, Carothers AD: **Factors affecting the timing and imprinting of replication on a mammalian chromosome.** *J Cell Sci* 1995, **108**:2801-2809.
33. Wyrick JJ, Holstege FC, Jennings EG, Causton HC, Shore D, Grunstein M, Lander ES, Young RA: **Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast.** *Nature* 1999, **402**:418-421.
34. Sharma K, Weinberger M, Huberman JA: **Roles for internal and flanking sequences in regulating the activity of mating-type-silencer-associated replication origins in *Saccharomyces cerevisiae*.** *Genetics* 2001, **159**:35-45.
- This study evaluates the sequence requirements for replication of two origins that are inactive at their native silent mating-type locus but that function as origins in plasmids. Most evidence suggests that silent origins are simply very late replicating origins that can be activated with sufficient time under the appropriate conditions (Vujcic *et al.* (1999) [35]). These origins are particularly interesting for discussing replication timing because one of them has been shown (Bousset and Diffley [1998] [64]) to replicate late even when present on a plasmid – unlike most other late replicating origins. This report shows that both flanking and internal sequences contribute to the activity and (probably) the late replication program of these origins.

35. Vujcic M, Miller CA, Kowalski D: **Activation of silent replication origins at autonomously replicating sequence elements near the HML locus in budding yeast.** *Mol Cell Biol* 1999, **19**:6098-6109.
36. Friedman KL, Diller JD, Ferguson BM, Nyland SV, Brewer BJ, Fangman WL: **Multiple determinants controlling activation of yeast replication origins late in S phase.** *Genes Dev* 1996, **10**:1595-1607
37. Ehrenhofer-Murray AE, Kamakaka RT, Rine J: **A role for the replication proteins PCNA, RF-C, polymerase epsilon and Cdc45 in transcriptional silencing in *Saccharomyces cerevisiae*.** *Genetics* 1999, **153**: 1171-1182.
38. Raghuraman MK, Winzeler EA, Collingwood D, Hunt S, Wodicka L, Conway A, Lockhart DJ, Davis RW, Brewer BJ, Fangman WL: **Replication dynamics of the yeast genome.** *Science* 2001, **294**:115-121.
Microarray technology is used to determine the replication times of thousands of sites across the budding yeast genome. This study reveals a wealth of information about origin positions and replication fork movement, but also suggests that replication timing is not correlated with transcription *per se* throughout the yeast genome as a whole.
39. Gartler SM, Goldstein L, Tyler-Freer SE, Hansen RS: **The timing of XIST replication: dominance of the domain.** *Hum Mol Genet* 1999, **8**:1085-1089.
40. Gilbert DM, Cohen SN: **Position effects on the timing of replication of chromosomally integrated simian virus 40 molecules in Chinese hamster cells.** *Mol Cell Biol* 1990, **10**:4345-4355.
41. Bernard P, Maure JF, Partridge JF, Genier S, Javerzat JP, Allshire RC: **Requirement of heterochromatin for cohesion at centromeres.** *Science* 2001, **294**:2539-2542.
See annotation Kim and Huberman (2001) [42**].
42. Kim SM, Huberman JA: **Regulation of replication timing in fission yeast.** *EMBO J* 2001, **20**:6115-6126.
These two reports [41*,42**] illustrate the potential of *Schizosaccharomyces pombe* to reveal important insight into the regulation and function of replication timing. Fission yeast heterochromatin contains Swi6 – a structural and functional homologue of HP1 – that is found in metazoan heterochromatin. By studying fission yeast heterochromatin, Bernard *et al.* [41*] show that centromeric heterochromatin recruits cohesin and is required for proper sister chromatid–centromere cohesion and chromosome segregation. The study by Kim and Huberman [42**] represents the first characterization of replication timing in *S. pombe*, demonstrating that origins of replication in fission yeast, as in all other eukaryotic organisms, are initiated in a temporal sequence.
43. Cimbora DM, Groudine M: **The control of mammalian DNA replication: a brief history of space and timing.** *Cell* 2001, **104**:643-646.
44. Dimitrova DS, Gilbert DM: **The spatial position and replication timing of chromosomal domains are both established in early G1-phase.** *Mol Cell* 1999, **4**:983-993.
45. Li F, Chen J, Izumi M, Butler MC, Keezer SM, Gilbert DM: **The replication timing program of the Chinese hamster β -globin locus is established coincident with its repositioning near peripheral heterochromatin in early G1 phase.** *J Cell Biol* 2001, **154**:283-292.
The transcriptionally inactive β -globin locus in Chinese hamster ovary cells becomes localized to the nuclear periphery between one and two hours after mitosis and then replicates late during S phase – within the same time window as peripheral heterochromatin. Using a system in which G1 phase nuclei are stimulated to enter S phase by introduction into *Xenopus* egg extracts, the authors show that the β -globin locus acquires a late replication program between one and two hours after mitosis. This defines a timing decision point for the β -globin locus and suggests that this locus acquires the replication timing program of peripheral heterochromatin on association with the periphery.
46. Bridger JM, Boyle S, Kill IR, Bickmore WA: **Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts.** *Curr Biol* 2000, **10**:149-152.
47. Cockell M, Gasser SM: **Nuclear compartments and gene regulation.** *Curr Opin Genet Dev* 1999, **9**:199-205.
48. Raghuraman M, Brewer B, Fangman W: **Cell cycle-dependent establishment of a late replication program.** *Science* 1997, **276**:806-809.
49. Dernburg AF, Broman KW, Fung JC, Marshall WF, Phillips J, Agard DA, Sedat JW: **Perturbation of nuclear architecture by long-distance chromosome interactions.** *Cell* 1996, **85**:745-759.
50. Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG: **Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division.** *Mol Cell* 1999, **3**:207-217.
51. Francastel C, Magis W, Groudine M: **Nuclear relocation of a transactivator subunit precedes target gene activation.** *Proc Natl Acad Sci USA* 2001, **98**:12120-12125.
This report shows that, on experimental induction of globin gene expression in murine erythroleukemia cells, the β -globin locus moves away from centromeres. The significance to my discussion is that the β -globin locus is known to be both sensitive to DNase I and early replicating in this same cell line before activation of the globin gene (Hatton *et al.* [1988] [3]). Thus, localization of β -globin to centromeres is not sufficient to render it late replicating.
52. Trinh LA, Ferrini R, Cobb BS, Weinmann AS, Hahn K, Ernst P, Garraway IP, Merkenschlager M, Smale ST: **Down-regulation of TDT transcription in CD4⁺CD8⁺ thymocytes by Ikaros proteins in direct competition with an Ets activator.** *Genes Dev* 2001, **15**:1817-1832.
53. Sabbattini P, Lundgren M, Georgiou A, Chow C, Warnes G, Dillon N: **Binding of Ikaros to the $\lambda 5$ promoter silences transcription through a mechanism that does not require heterochromatin formation.** *EMBO J* 2001, **20**:2812-2822.
54. Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA: **The spatial organization of human chromosomes within the nuclei of normal and emerlin-mutant cells.** *Hum Mol Genet* 2001, **10**:211-219.
A global evaluation of the subnuclear positions of human chromosomes reveals that gene-rich chromosomes are more internal, whereas gene-poor chromosomes are located toward the nuclear periphery.
55. Li G, Sudlow G, Belmont AS: **Interphase cell cycle dynamics of a late-replicating, heterochromatic homogeneously staining region: precise choreography of condensation/decondensation and nuclear positioning.** *J Cell Biol* 1998, **140**:975-989.
56. Loupart M, Krause S, Heck MS: **Aberrant replication timing induces defective chromosome condensation in *Drosophila* ORC2 mutants.** *Curr Biol* 2000, **10**:1547-1556.
See annotation Pflumm and Botchan (2001) [57*].
57. Pflumm MF, Botchan MR: **Orc mutants arrest in metaphase with abnormally condensed chromosomes.** *Development* 2001, **128**:1697-1707.
This paper, together with Loupart *et al.* (2000) [56**], shows that mutations in *Drosophila* origin recognition complex subunits result in death at late larval stages, with defects in replication timing and chromosome condensation [56**], as well as in sister chromatid cohesion [57*]. Together, these papers show that there is a link between replication timing and chromosome condensation/segregation.
58. Amiel A, Elis A, Sherker S, Gaber E, Manor Y, Fejgin MD: **The influence of cytogenetic aberrations on gene replication in chronic lymphocytic leukemia patients.** *Cancer Genet Cytogenet* 2001, **125**:81-86.
See annotation Smith *et al.* (2001) [60**].
59. Sun Y, Wyatt RT, Bigley A, Krontiris TG: **Expression and replication timing patterns of wild type and translocated BCL2 genes.** *Genomics* 2001, **73**:161-170.
See annotation Smith *et al.* (2001) [60**].
60. Smith L, Plug A, Thayer M: **Delayed replication timing leads to delayed mitotic chromosome condensation and chromosomal instability of chromosome translocations.** *Proc Natl Acad Sci USA* 2001, **98**:13300-13305.
These three papers (Amiel *et al.* (2001) [58*], Sun *et al.* (2001) [59*] and this one [60**]) show that aberrations in replication timing are associated with many different types of human cancers. In addition, these authors show that certain chromosomes carrying translocations have replication timing defects that are accompanied by delays in mitotic chromosome condensation and an increased frequency of chromosomal instability.
61. Barbosa AC, Otto PA, Vianna-Morgante AM: **Replication timing of homologous α -satellite DNA in Roberts syndrome.** *Chromosome Res* 2000, **8**:645-650.
62. Hellman A, Rahat A, Scherer SW, Darvasi A, Tsui LC, Kerem B: **Replication delay along FRA7H, a common fragile site on human chromosome 7, leads to chromosomal instability.** *Mol Cell Biol* 2000, **20**:4420-4427.
63. Clegg NJ, Honda BM, Whitehead IP, Grigliatti TA, Wakimoto B, Brock HW, Lloyd VK, Sinclair DA: **Suppressors of position-effect variegation in *Drosophila melanogaster* affect expression of the heterochromatin gene light in the absence of a chromosome rearrangement.** *Genome* 1998, **41**:495-503.
64. Bousset K and Diffley JF: **The Cdc7 protein kinase is required for origin firing during S phase.** *Genes Dev* 1998, **12**:480-490.

