Minireview

DNA replication: telling time with microarrays

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Abstract

A long-standing hypothesis about eukaryotic DNA replication is that the late-replicating regions are transcriptionally inert and that repressing transcription delays replication initiation. But do contrasting results from yeast and a recent study in *Drosophila* imply that replication timing and transcriptional activity are differentially regulated in yeast and higher eukaryotes?

Replication timing and transcriptional activity in a metazoan

Eukaryotic DNA replication begins at multiple origins on each chromosome, with successive origins firing in a reproducible temporal sequence. The mechanism by which certain regions of the genome are reproducibly designated as earlier- or later-replicating is not well understood, but cytological observation of replicating metazoan chromosomes suggested that transcriptionally silent regions of the genome replicate late in S phase (for a review see [1]). This finding led to the hypothesis that transcription and replication timing are functionally linked and that a closed chromatin conformation that is refractory to transcription also delays replication.

The hypothesis of a connection between transcriptional activity and replication timing was bolstered by molecular analysis of budding yeast (*Saccharomyces cerevisiae*). As in higher eukaryotes, yeast DNA near or within transcriptionally repressed heterochromatin, such as that located at telomeres, replicates late in S phase [2], because of delayed initiation at origins close to telomeres [3]. It is thought that late replication initiation at such origins may be imposed by the telomeric chromatin conformation, because moving a normally early-replicating origin near to a telomere delays its activation [4]. In addition, Sir3p, a protein involved in mediating transcriptional silencing, has been shown to be important for the activation of telomeric late origins at the appropriate time within S phase [5]. The relationship between transcription and replication timing in yeast is not absolute, however, given that one of the most well-studied late-replicating regions encompasses several transcriptionally active genes [6-8]. Furthermore, a genome-wide survey of replication timing in yeast failed to identify a direct correlation between transcriptional inactivity and late replication [9].

The apparent disagreement between the yeast molecular data and the hypothesis from metazoans regarding control over replication timing and transcription raised an important question: while all eukaryotes seem to share a common mechanism for designating late-replicating DNA within heterochromatic regions, do higher eukaryotes differ fundamentally in the mechanisms used to regulate replication timing outside heterochromatin? To address this question directly, Schübeler and colleagues [10] set out to determine whether there is a relationship between replication timing and transcriptional activity in a model higher eukaryote by analyzing the expression and replication time of thousands of sequences across the euchromatic complement of the *Drosophila melanogaster* genome. To determine when a particular sequence replicates, they isolated newly replicated DNA from cultured embryonic *Drosophila* (Kc) cells in either early or late S phase. The two DNA fractions were amplified and differentially color-labeled before being mixed and hybridized to a microarray of *Drosophila* euchromatic

sequences. The representation of each sequence in the early- and late-replicating fractions allowed estimation of the relative time at which the sequences replicate during S phase. In addition, the authors isolated RNA from the Kc cells in order to determine whether the sequences on the microarray are transcriptionally active in logarithmically growing cells.

Armed with both replication-timing data and expression data for 5,077 sequences across the *Drosophila* genome, the authors [10] showed that sequences replicating earlier in S phase have a significantly greater probability of being expressed than do later-replicating regions ($p = 10^{-44}$). Unlike yeast cells, therefore, *Drosophila* Kc cells do demonstrate a clear, but not absolute, correlation between the transcriptional activity of a sequence and the time at which it replicates. It remains to be seen whether such a relationship is maintained in cells of more advanced developmental stages, or in Kc cells cultured under different conditions than those used by Schübeler *et al*. [10].

The expanding utility of microarrays

Although several groups have previously performed microarray-based analyses of replication in *Escherichia coli* and *S. cerevisiae* [9,11-13], the study by Schübeler and colleagues [10] is the first such analysis of replication in a higher eukaryote. The relatively small size of the *E. coli* and yeast genomes facilitated the production of manageable high-resolution arrays (4,115 probe regions over 4.6 Mb and up to 12,158 probe regions over 12 Mb, respectively) that, in the case of yeast, allowed for the localization of virtually every replication origin [9,12,13]. Similar use of microarrays to identify origin locations in *Drosophila* would represent a major advance in the study of metazoan replication, because few origins have been defined thus far in higher eukaryotes. In contrast to the *E. coli* and yeast arrays, however, the *Drosophila* arrays used by Schübeler *et al*. [10] consisted of 5,221 probe regions across the approximately 120 Mb euchromatic portion of the genome, and included many gaps of at least 100 kb. Although this level of resolution allowed for the definitive detection of a correlation between transcriptional activity and replication timing in *Drosophila* embryonic cells, arrays of higher resolution will be necessary to identify replication origin locations, even though overall origin size and spacing in *Drosophila* may be greater than in yeast.

But what sequences should be added to increase the array resolution? The microarray probes utilized by Schübeler *et al*. [10] are derived from cDNAs and expressed sequence tags (ESTs) representing fewer than half the predicted number of *Drosophila* genes [14,15]. An obvious way to increase the resolution of the array would therefore be to add probes corresponding to other previously characterized or predicted genes. It would be of particular appeal to ask if the predicted genes show the same distribution of replication timing and transcriptional activity as probes derived from cDNA and EST sequences. Furthermore, the inclusion of intergenic regions would also be key, since most *Drosophila* replication origins are likely to localize to non-coding regions [16,17].

Finally, the α-heterochromatin, which comprises roughly one-third of the *Drosophila* genome [18], was largely excluded from the arrays used by Schübeler *et al*. [10]. The repetitive nature of heterochromatin renders it difficult, at present, to analyze by microarray. There are, however, known unique gene sequences located within the heterochromatin [19] that could eventually be added to microarrays. Inclusion of these genes would provide valuable information regarding the relationship between replication timing and transcriptional activity. Since *Drosophila* heterochromatin is known to replicate late in S phase (reviewed in [20]), it would be interesting to determine whether expressed α-heterochromatic genes as a group are exceptions to the correlation between late replication and transcriptional inactivity.

How significant is the difference between yeast and *Drosophila*?

The finding that there is indeed a relationship between transcriptional activity and replication timing in *Drosophila* was surprising, given the lack of such a correlation in budding yeast. But this finding does not necessarily indicate that yeast and higher eukaryotes are inherently different in the mechanism used to regulate replication timing. Although the chromatin surrounding late origins in yeast does not always inhibit transcription, chromatin conformation clearly influences replication timing. The firing time of yeast origins can be advanced by relaxing a tight chromatin structure, or can be delayed by inducing a denser chromatin structure near origins [21,22]. In addition, there is evidence of a spatial overlap in the organization of transcriptionally silent and late-replicating regions within the nucleus. Regions containing silent genes tend to localize to the nuclear periphery in yeast and mammalian cells [23-25]. Late-replicating regions in these organisms also tend to be associated with the nuclear periphery [24,26,27]. This localization is observed in yeast even if the late-replicating region contains transcriptionally active genes [27]. Furthermore, current evidence suggests that there could be an overlap in the time within the cell cycle when the replication-timing program and transcriptional silencing are established [24,28-32].

The importance of intranuclear position

It is not known why late-replicating and transcriptionally repressed regions of the genome tend to be located at the nuclear periphery, nor is there solid evidence that factors at the periphery are necessary for the establishment of such chromosomal characteristics. In fact, telomeres can move to the nuclear periphery in yeast cells that are silencing-defective [33], and it has been reported that artificially tethering an

early origin to the periphery does not delay its replication [21]. Nevertheless, evidence is mounting that the peripheral positioning of silent and late-replicating regions may be important to both processes. For example, abolishing the attachment of yeast telomeres to the edge of the nucleus through the mutation of the telomere-binding Ku complex advances replication timing and prevents gene silencing in telomeric regions [34,35].

Two possible models have emerged to account for the potential relationship between localization at the nuclear periphery and the establishment of late replication and transcriptional silencing. One proposes that clustering away from the center of the nucleus sequesters certain regions from various transcription factors, thereby rendering them transcriptionally silent [25]. A similar model could also be suggested for the establishment of late replication. Perhaps a more persuasive model is that factors at the nuclear periphery establish a chromatin conformation [1,25] that consistently confers late replication and may also be refractory to transcription.

Such a model, in which the same mechanism creates both late-replicating and transcriptionally inactive chromatin, is attractive but the actual situation is likely to be more complicated. There is evidence that late replication and transcriptional activity can be separated functionally in human cells. Sharp and colleagues [36] describe a case in which part of human chromosome 10 is translocated to the transcriptionally silent and late-replicating X chromosome. Although several genes within the translocated portion of chromosome 10 are rendered transcriptionally silent by the spreading of X inactivation, the translocated DNA apparently does not become late-replicating, as is often the case when an autosome is translocated to the X. This observation suggests that, as in yeast, late replication and transcriptional inactivity can be separated in a higher eukaryote.

Perhaps the mechanisms regulating replication timing and transcriptional activity in yeast and higher eukaryotes can occur independently but have a certain probability of coinciding at the same chromosomal regions. If so, the probability for such coincidence would then be greater in metazoan cells than in yeast, possibly because of differences in the mechanism of transcriptional regulation or overall level of chromatin compaction. Higher eukaryotes would therefore demonstrate a stronger correlation between transcriptional activity and replication timing than yeast. Further analysis of those *Drosophila* sequences that do not show a correlation between replication timing and transcriptional activity may provide insight as to whether or not such a model will hold true.

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