in addition to arthropods. This finding may explain why some antibiotics are effective against filariasis and may allow the development of new antibiotics for the treatment of filarial diseases.

A wake-up call

Recent weeks have heralded the most significant step forward in vector-borne parasitology with the publication of the genome sequences of the malaria parasite[7] (*Plasmodium falciparum*) and its carrier the mosquito[8] (*Anopheles gambiae*). This is in stark contrast to the handful of known tsetse fly genes and the apparent stalling of the *T. brucei* genome project. There is a danger that parasitologists will be seduced into researching exclusively malaria. This would be detrimental, as comparative genetic information from arthropod bloodsucking vectors and eukaryotic parasites, which have distinct life cycles, would yield significant added value. The sequencing of *W. glossinidia* marks the start of an effort to compile the genetic information of the players in the African sleeping disease cycle. The genome sequences of the tsetse fly and *T. brucei* are now required to develop a comprehensive understanding of the *T. brucei* life cycle that will serve as the basis to design an effective intervention strategy against the deadly African sleeping disease. □

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Replication timing and metazoan evolution

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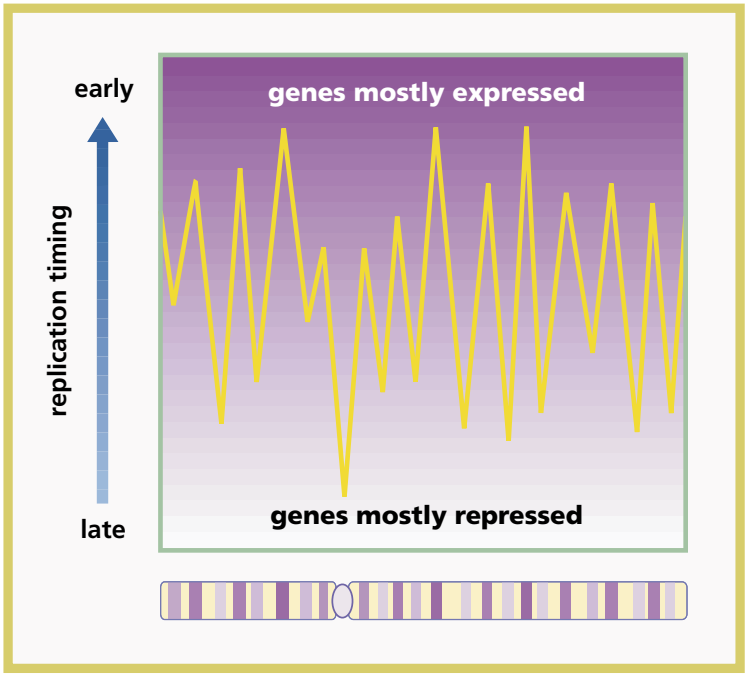
The long-presumed relationship between transcriptional activity of genes and their replication early in S-phase was challenged when a whole-genome analysis of replication timing in budding yeast found no such relationship. A new study reports the first genome-wide comparison of replication timing and gene expression in a multicellular organism, revealing a strong correlation between the two. This difference may reflect levels of nuclear organization that are important in the context of tissue-specific gene regulation.



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A link between replication and expression. Replication timing analysis along the length of a chromosome reveals domains that replicate at characteristic times. Early-replicating genes have a higher probability of being expressed, whereas late-replicating genes are less likely to be expressed. The molecular basis of this probabilistic relationship and the extent to which it has a role in the developmental regulation of gene expression are important questions for future research.

For several decades, a correlation between gene activity and replication in the first half of S-phase has been appreciated, but its molecular basis remains a mystery[1]. What has been desperately needed is a more complete sampling of genes to determine whether the apparent relationship between replication timing and gene expression could withstand a test of statistical significance. The advent of DNA array technology made this possible. Although the evaluation of replication timing for individual genes is a formidable task, the effort in these experiments is expended in synchronizing cells and isolating labeled replication intermediates. Once these replication intermediates are isolated, a profile of replication timing of the entire genome with a DNA array does not require much more effort than evaluating individual genes one at a time by traditional methods. The first whole-genome analysis of replication timing, performed in budding yeast[2], could find no correlation between gene expression and replication time, underscoring the need to perform similar analyses in a multicellular organism. On page 438 of this issue, Dirk Schübeler and colleagues[3] report such an analysis, using an established *Drosophila melanogaster* cell line.

Annotating replication timing

D. melanogaster is an ideal model organism in which to begin such a study. DNA arrays containing nearly all *D. melanogaster* genes are available, the genome is nearly 20 times smaller than in mammals, and cultured cell lines exist in which to carry out replication studies that require large populations of homotypic cells. Such cell lines do not exist for simper model organisms such as *Caenorhabditis elegans*.

Schübeler *et al.*[3] isolated nascent DNA synthesized either early or late during S-phase, labeled each preparation differentially with either a red or a green fluorochrome, and then hybridized these preparations to an array containing 5,221 unique genes separated by an average of 20 kb between probes. The relative fluorescence intensities of the different fluorochromes provided an internal control for the temporal order in which various sequences replicated, whereas similar replication times for neighboring genes provided confidence that the method was working. To analyze large blocks of heterochromatin, which contain repetitive DNA sequences that confuse hybridization analyses, the array also contained probes corresponding to certain transposable elements, some of which are localized exclusively to these blocks of repetitive DNA. As expected from cytogenetic studies, these sequences were replicated later than 98% of sequences found within euchromatin. Surprisingly, other heterochromatic regions adjacent to these repetitive blocks replicated much earlier. These regions contain unusual genes whose expression requires genetically defined components of heterochromatin. This result suggests that different types of heterochromatin in *D. melanogaster* replicate at different times. Also unexpected was the finding that some late replicating segments were localized within cytogenetically defined euchromatin, although these could be small regions of heterochromatin that escape cytogenetic detection (see figure).

These unanticipated results further begged the question: does transcriptional activity correlate with early replication? To address this question, Schübeler *et al.*[3] examined the expression of 5,077 genes by hybridizing cDNA from their cell line to the same DNA array used to annotate replication timing. When the replication timing of genes was related to their probability of being expressed, a strong correlation between early replication and transcriptional activity was found. As expected from the prior analysis of individual genes, the correlation is not complete. Nearly 20% of the earliest replicating genes are silent, and more than 20% of the latest replicating genes are expressed. However, the size of this sampling confirms beyond any reasonable doubt that this long-suspected relationship holds true genome-wide and is statistically (P value of 10^{-44}!) and biologically significant. This result turns our attention to the following questions. Why doesn't this relationship exist in yeast, and what does this dichotomy between yeast and metazoa tell us about the differences in genome organization between single-celled and multicellular organisms?

Order in the metazoan nucleus

As anyone who has worked in a small office knows, the more material you put into a small area, the more organized you have to be if you want access to things in a hurry. Each cell in a metazoan organism must maintain its entire genome while retaining the ability to work quickly with some parts and pack other parts out of the way. By contrast, the single-celled budding yeast uses virtually all of the genes in its much smaller genome and doesn't require as much long-term storage space.

Several observations suggest that yeast and metazoa organize their nuclei differently. It has been long recognized that yeast lack a nuclear lamina[4]. Also, it has been shown that chromatin is considerably more mobile in yeast nuclei than in metazoa; this difference may be due to differences in the number of anchorage sites for chromatin in yeast as opposed to metazoa[5]. Recently it was found that more than 20% of the *D. melanogaster* genome is organized into groups of 10–30 adjacent genes (averaging 100 kb) that are similarly expressed but are not otherwise functionally related[6]. Intriguingly, replication origins in metazoa are frequently organized into similarly sized clusters of origins that fire synchronously during S-phase[7]. Thus, it seems reasonable to postulate that temporally differentiating the replication of transcriptionally active and inactive portions of the genome is part of a larger process that metazoans use to maintain order in the nucleus.

The missing link

Do the results of Shübeler *et al.*[3] imply that the power of yeast genetics will not be useful for probing the relationship between transcription and replication timing? Not exactly. It is clear that several highly conserved features of chromatin structure affect both transcriptional activity and replication timing in yeast, including histone acetylation (O. Aparicio, personal communication) and the levels of silent chromatin proteins[8–10]. Similarly, inhibitors of histone deacetylation advance replication timing in mammalian cells[11] (R. Wu and D.M.G., unpublished). Thus, it is not a coincidence that telomeres and mating-type loci are arguably the only truly heterochromatic sites in the yeast genome, and these are late-replicating.

The key to finding the missing link between single-celled and multicellular organisms may require a molecular, or indeed structural[12], definition of heterochromatin and euchromatin. The genes that Shübeler *et al.*[3] examined are all localized to cytogenetically defined euchromatin. Yet, many of the transcriptionally silent genes in this cell line are likely to be developmentally regulated genes that are in chromatin configurations different from that of expressed genes. Furthermore, both the Shübeler *et al.*[3] study and studies recently carried out in fission yeast[13] suggest that there are several types of heterochromatin, only some of which replicate late. This view is supported by genetic studies in budding yeast that reveal distinct molecular differences between silent chromatin found at different loci[14]. These differences are likely to be buried deep within the language of the histone modification code[15]. Hence, the genetic dissection of the molecular basis for differences in replication timing in yeast may provide an ancestral glimpse of an epigenetic mechanism that was exploited during metazoan evolution. □

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