DNA replication: the unbearable lightness of origins

Now that the genomes of various metazoans have been sequenced, one of the biggest challenges in biology is to understand their functional organization. Programmed alterations of gene expression during development and cell differentiation frequently involve long-range modifications in the organization of chromosomal loci. These modifications include nuclear localization, genetic locus conformation (for example, looping), replication timing, chromatin organization, DNA methylation and many others. These features seem to be cell-type- or differentiation-stage-specific and are frequently inherited during cell division. However, their relationship to the regulation of gene expression remains largely unknown. Two studies published in this issue of *EMBO reports* now show that, in cells of vertebrate animals, changes in gene expression are also accompanied by modifications in the activity of DNA origins of replication (Dazy *et al*, 2006; Gregoire *et al*, 2006). These studies raise the possibility that origin regulation is part of the mechanism that integrates changes in gene expression with cell proliferation in various developmental contexts.

Until recently, DNA origins of replication were seen as stable entities of eukaryotic chromosomes with specific origins required to replicate particular parts of the genome—known as replicons. This vision has evolved slowly to accommodate an increasing body of evidence indicating that gene loci frequently contain several origins that are used at relatively low frequencies (initiation zones; reviewed by Gilbert, 2004). This means that a specific genomic region can be replicated by different origins in different cells, or in different alleles—a phenomenon that could explain the asynchronous replication of many gene loci in mammalian nuclei (Norio *et al*, 2005). The two new studies support this vision by showing that both the chicken β -globin and the mouse HoxB loci contain several active origins. In addition, they help to bring into focus another aspect of origin plasticity in metazoans. Previous studies have shown that changes in origin activity can take place concomitantly with global modifications in cell-cycle duration and organization—for example, during early embryonic development in *Drosophila* and *Xenopus* (Hyrien *et al*, 1995; Sasaki *et al*, 1999)—or with endoreduplication (Lunyak *et al*, 2002). By contrast, evidence of specific origin regulation during less marked developmental transitions was limited to a few cases (Maric *et al*, 2003; Norio & Schildkraut, 2004; Norio *et al*, 2005). The two studies in this issue show that origin regulation is a common event.

Hence, far from being a static component of the genome, origins of replication can be activated or silenced as part of the normal programme of cell differentiation.

Dazy and colleagues examined the chicken β -globin locus and its surrounding sequences—by measuring the level of enrichment of RNA-capped short nascent DNA strands—to determine how terminal differentiation of primary erythroid progenitors into erythrocytes affects origin activity. Before the induction of terminal differentiation—when the locus is transcriptionally active but the level of transcription is low—they found that nascent DNA strands are highly enriched at four sites. This indicates the presence of at least four separate origins of replication. In addition, the amount of nascent DNA strands remains higher than the background level across most of the locus, indicating the possible presence of a single large initiation zone. During the induction of terminal differentiation—when transcription is significantly upregulated—the amount of nascent DNA increases at various sites. These increases can be interpreted either as an extension of the initiation zone to include origins that were previously silent, or as an increase in the activity of pre-existing origins. Interestingly, the largest increases occur in the proximity of the locus control region (5'HS1-4). This situation is reminiscent of the increase in origin activity previously detected near the 3'-regulatory region of the mouse immunoglobulin heavy-chain locus, during the transcriptional upregulation that accompanies the late stages of B-cell development (Norio *et al*, 2005). However, Dazy and colleagues show that changes in origin activity are not strictly linked to modifications in transcription as origin activation takes place also in the proximity of the folate receptor gene, which is transcribed at a constant low level before and after induction of terminal differentiation.

These conclusions are strengthened further by the study from Gregoire and colleagues (this issue), who have examined the changes in origin activity that occur in the HoxB locus during *in vitro* differentiation of the mouse embryonic carcinoma cell line P19. In these cells, retinoic acid induces the transcription of nine HoxB genes, activating them in a collinear manner over several days. Modifications in origin activity were also measured in this study by changes in the enrichment of nascent DNA. In undifferentiated P19 cells, when the HoxB genes are transcriptionally silent, the authors found that the locus is occupied by a large initiation zone spanning more than 100 kilobases. By contrast, when transcription is activated by retinoic acid treatment, most of the origins become silent. Only one origin of replication seems to be unaffected by cell differentiation. This origin is located at the 3'-end of the locus and overlaps with an enhancer element that mediates the early expression pattern of *Hoxb1*. However, the presence of other unaffected origins cannot be ruled out as nascent DNA strands were quantified at relatively few locations across the HoxB locus. Nevertheless, these results clearly support a model in which origin activity is developmentally regulated but not strictly dependent on transcription.

In summary, these two studies show that transcription activity does not strictly correlate with origin activity. Depending on the gene locus in question, active transcription is associated with active origins (β -globin), silent origins (HoxB), or both (folate receptor). Studies of replication timing have previously established a correlation between late replication and transcriptional silencing for specific genes (Goldman *et al*, 1984; Hatton *et al*, 1988; Kitsberg *et al*, 1993; Perry *et al*, 2004) and at the genomic level (Macalpine *et al*, 2004; Schubeler *et al*, 2002). These studies have depicted a relatively simple image of genome organization in higher eukaryotes in which gene-rich regions replicate mostly in early S phase, gene-poor and heterochromatic regions replicate mostly in late S phase, and only a relatively small number of tissue-specific genes can change their timing of replication during cell differentiation—these are mostly located in A+T-rich portions of the genome (Hiratani *et al*, 2004). If changes in origin activity and replication timing are linked, this would suggest that origin regulation is not a widespread event in metazoan cells. However, we have shown recently that replication timing has limited predictive value as an indicator of replication origin activity (Norio *et al*, 2005). In addition, the changes in origin usage described in this issue take place in portions of the genome that replicate in early S phase even when the β -globin and HoxB loci are transcriptionally silent (Fisher & Méchali, 2003; M.-N. Prioleau, personal communication). Therefore it seems possible that the real extent of origin regulation in metazoan cells has been overlooked.

Previous studies have also shown that metazoan origins are inhibited within some actively transcribed portions of the genome (Haase *et al*, 1994; Mesner & Hamlin, 2005; Saha *et al*, 2004; Sasaki *et al*, 2006). In principle, the passage of an RNA polymerase could interfere with DNA replication initiation by destabilizing pre-replication complexes, or inhibiting their assembly and their activation—either directly or indirectly by inducing chromatin modifications. The new studies by Dazy *et al* (2006) and Gregoire *et al* (2006) seem to argue against these explanations by showing that origin activation can take place in actively transcribed genes and that origin silencing is not limited to transcribed regions. However, this is not necessarily the case. Transcription might occur only on part of the alleles, and initiation of DNA replication at each origin could be infrequent. Therefore, initiation events could be restricted to alleles that are not actively transcribed. Moreover, transcription can occur at different times during the cell cycle. Transcription that is limited to specific portions of the cell cycle might not interfere with origin activity. Hence, additional studies are required to clarify the effect that transcription might have on origin activity.

Dazy *et al* and Gregoire *et al* also tackle the thorny issue of the role of histone acetylation in origin selection (reviewed by Zhou *et al*, 2005). Previous reports have shown that histone acetylases are important modulators of origin activity in eukaryotes and could be involved in origin selection as well as in regulating the frequency and timing of origin activation (Aggarwal & Calvi, 2004; Danis *et al*, 2004; Iizuka *et al*, 2006; Kemp *et al*, 2005; Pappas *et al*, 2004; Pasero *et al*, 2002; Vogelauer *et al*, 2002). However, the two new studies do not find any clear causal link between histone acetylation and replication origin activity during normal cell proliferation and differentiation. In the chicken β -globin locus, origins of replication are associated with either histone acetylation or deacetylation both before and after induction of terminal differentiation. In addition, in

the mouse HoxB locus, histone acetylation correlates with origin silencing rather than origin activation. These results also make it unlikely that histone acetylation in the proximity of origins could have a role in regulating their timing of activation. For example, across the β -globin locus, origins with a different histone acetylation signature are present within relatively short distances. If their timings of activation were different, only the early origins would have the time to fire whereas the others would be passively replicated. Clearly the origins of the β -globin locus fire during the same portion of the S phase. Therefore, although histone acetylation could affect origin activity in specific cases, this role does not seem to be generally required for origin selection or replication timing specification. After these studies, one important question remains: What controls origin activity? Other chromatin modifications? Specific transcription factors? Changes in the structural organization of gene loci? Now that some of the usual suspects have been eliminated, the search for the culprit continues.

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