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Origin of DNA replication at the human lamin B2 locus

OBR or ABR?

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Unlike in prokaryotes and simple eukaryotes such as *Saccharomyces cerevisiae*, the origins of DNA replication (oris) in mammalian cells are not dictated by a specific nucleotide sequence.[1] Nevertheless, recent studies show that mammalian replicons are organized in groups of site-specific but flexible replication oris, for which chromatin context plays an important role.[2] Despite that our knowledge on mammalian oris has been substantially expanded in recent years, mainly through genome-wide analysis, the mechanism of replication initiation at individual oris is still poorly understood.[1,3] For example, two opposing models of replication initiation-sites have been extensively debated over the past two decades without a clear conclusion.[4,5] The OBR (origin of bidirectional replication) model suggests that DNA replication initiates from a defined initiation site, from which replication progresses in both directions.[5,6] In stark contrast, a defused/delocalized initiation model suggests that replication initiates from any sites within a broad replication zone.[4,7] The prototype of the OBR model in mammalian cells is the ori located at the human lamin B2 locus, which was determined by ligation-mediated (LM) PCR-based replication initiation point (RIP) mapping.[6] In their paper, Abdurashidova et al.[6] concluded that the replication initiation at the lamin B2 locus starts at nucleotide (nt) positions 3933 and 3930 on the "upper" and "lower" strands, respectively, thus defining the mammalian OBR model (**Fig. 1**). Contrarily to the OBR model, data from 2D replicon mapping demonstrated that replication initiates from almost any sites within the entire 55 kb intergenic region of the CHO DHFR locus,[4,7] although data from another laboratory suggested an OBR exists in this locus.[5] When discussing these two replication models, it is important to note that OBR at the human lamin B2 locus has yet to be reproduced by other laboratories, and RIP mapping at nucleotide resolution has never been carried out on the ori at the CHO DHFR locus.

Using a relatively simple one-way PCR-based RIP mapping method,[8] we have recently found that the initiation of DNA replication occurs within a 1,143-bp DNA segment at the human DBF4 promoter locus.[9] Interestingly, three distinct DNA segments were found within this ori: the 449-nt-long replication initiation zone I on the sense strand (i.e., the DBF4 transcription orientation) spans from nt-235 to nt-683 relative to the Dbf4 ATG translational start-site; the 331-bp initiation zone II on the antisense strand spans from nt+130 to nt+130 (i.e., downstream of the ATG); and the 365-bp space between these two zones does not contain any detectable initiation site.[9] We found that replication initially starts anywhere from initiation zone I and progresses unidirectionally in the sense orientation. Equally defused replication initiation from zone II seems occur only when replication emanated from zone I has passed through zone II. Replication from initiation zone II then progresses unidirectionally in the antisense direction. (It should be noted that the overall replication from the DBF4 ori is still bidirectional). We termed this mode of replication initiation ABR (asymmetric bidirectional replication).[9] The ABR model suggests that replication initiation does not require a specific nucleotide sequence. However, two separate zones of replication initiation are utilized in a given replicon. Similarly, by genome-wide analysis, Cayrou et al.[2] recently demonstrated that most oris associated with transcriptional promoters contain bimodal replication sites that are separated by ~1 kb. Although these authors did not carry out a RIP assay, the bimodal replication model is strikingly similar to that of ABR. Furthermore, Lubelsky et al.[10] unequivocally showed recently that there is no OBR at the CHO DHFR ori locus. All of these data are consistent with the notion that there may not be a precise OBR in mammalian cells. The ori at the human lamin B2 locus is the only known exception to this rule. To confirm this, therefore, we re-examined the RIP pattern at the human lamin B2 locus using one-way PCR-based RIP mapping.[8]

We did find that replication initiates at nt3930 on the lower strand (i.e., sense strand) as reported previously by Abdurashidova et al.[6] Interestingly, however, we found that the synthesis of leading strand also starts from nt3730 and nt3763, suggesting there is a short initiation zone (**Fig. 1**). Most surprisingly, we did not find a RIP at nt3933 on the upper strand (i.e., anti-sense strand), which is contradictory to the previous report by Abdurashidova

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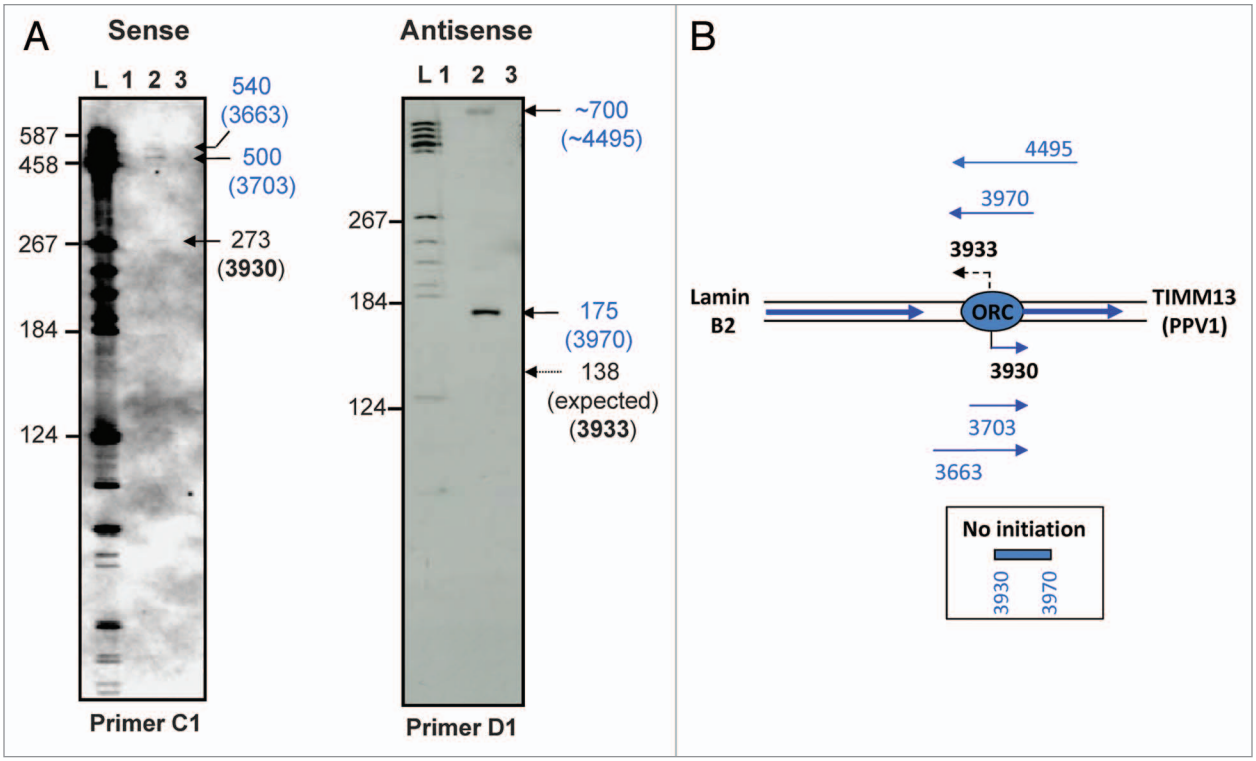

Figure 1. RIP map at the lamin B2 locus. (A) RIP mapping was performed exactly as described previously.[8,9] Size markers (in base) are shown left of the gel picture. Numbers correspond to those of the file humanlaminbb (GeneBank accession number M94363) and Abdurashidova et al.[6] Arrows show RIP positions, and the positions written in black are reported by Abdurashidova et al.,[6] and those written in blue are identified by this work. It should be noted that part of the gel on the left in panel (A) was included in our previous publication as supplementary to validate the one-way PCR-based RIP-mapping technique.[9] L, 1, 2, 3 are DNA size ladder (marker), no template control, 4 h post-mitosis in the presence of emetine, and mitosis (0 h) plus emetine, respectively. Primers used are the same as previously described:[6,12] 5'-TCG CAT CAC GTG ACG AAG AGT CAG C-3' (C1) and 5'-GTC ACA GCA CAA CCT GCA AAA ACG G-3' (D1). (B) A schematic RIP map at the human lamin B2 *ori* locus. A potential ORC binding site and the directions of lamin B2 and TIMM13 transcription are shown (thick blue arrows). The numbers and thin arrows in blue are leading strand RIPs detected by this work. The OBR positions nt3930 and nt3933 (in bold) were reported previously.[6] We could detect a RIP at nt3930, but not at 3933 (broken arrow line). A 40-bp non-replication initiation space is shown as a bar.

et al.[6] Instead, RIPs were found at nt3970 and nt4495 (**Fig. 1**). Our data, therefore, suggest that a 40-bp non-initiation space exists between two short "initiation zones" (i.e., nt 3663-nt 3930 and nt3970-nt4495) (**Fig. 1B**). Taken together, our data does not support the existence of a precise OBR at the human lamin B2 locus. In fact, the initiation mode in this locus is rather similar to the ABR model found at the human DBF4 ori locus,[9] although initiation and non-initiation zones at the lamin B2 locus are much smaller than those in the DBF4 locus. It is unclear why our data are not exactly the same as that of Abdurashidova et al.[6] It is, however, possible that a small artifact (e.g., a single-strand break or inaccurate ligation) happened at an early stage of the LM-PCR-based RIP mapping can lead to a false positive, as this technique is very complex and needs many different steps.

Bidirectional replication requires that two separate sets of large replication complexes simultaneously bind to one specific initiation site, which can be very challenging. Furthermore, this can also be error-prone, as deletions at the ori region can occur at high frequency due to crowed replication machineries in a small area. The ABR model will not have this problem, since two replication zones are separated by non-initiation space. In addition to the human lamin B2 and DBF4 loci, RIP was also mapped at the nucleotide resolution in the II/9A ori of *Sciara coprophilla*.[11] The RIP data from the II/9A locus showed a similar mode to our current RIP data obtained from the human lamin B2 locus. Furthermore, the bimodal replication initiation[2] found at most oris located at promoters appear to support ABR as the prevalent mode of replication initiation.

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