Review of: "Determination of human DNA replication origin position and efficiency reveals principles of initiation zone organisation"

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The existence of specific DNA replication origins in mammalian genome remains a matter of debate. While many specific origins have been reported, other studies suggest that replication initiates in broad zones. In the past decade, several genome-wide replication origin mapping studies using different methods to isolate DNA from replication origins (e.g., short nascent strands, replication bubbles, Okazaki fragments, nascent DNA with incorporated nucleotide analogs, etc.) followed by mass sequencing have identified a large number (from some 20,000 to over 175,000) of origins. However, although the identified origins by different methods largely overlap, a consensus has not been reached as to whether replication origins are spatially stochastic or localized at defined sites.

A big improvement of the method in this manuscript over the previous genome-wide origin mapping studies is that it allows measurement of the efficacy of replication origins, in addition to determining origin locations, by taking the advantage of the semiconservative nature of DNA replication to separate replicated from unreplicated DNA. This allows the authors to classify the identified origins into high, medium and low efficiency origins, and make several potentially important correlations of the origins with DNA sequence and chromatin structure features. Nonetheless, this study still did not resolve the issue about the sequence/structure specificity of mammalian replication origins.

There are several caveats in the manuscript that need to be addressed.

1. The method in this study was modified from that in reference 23. In reference 23, nascent DNA in isolated nuclei was labeled with a nucleotide analog for 15 min after release from mimosine, but the labeling time was 3 hrs under otherwise similar conditions in the current study. It says in reference 23 that DNA synthesis was about 200 bp/min in the in vitro replication with the isolated nuclei. Very long DNA could be synthesized in 3 hrs, which might result in calling multiple origins as one, or at least a reduction of the resolution. Although the authors speculated that the processivity of replication was low in the assay, so that they were still able to map origins with a relatively high resolution of several hundred bp, it did not seem necessary to label nascent DNA for such a long time. No explanation was given for this. A shorter labeling time may improve the resolution (say, to 100-200 bp) and may even change the conclusions.
2. As stated above, a big advance of this study was the measurement of the efficiency, as well as the

location, of origins. However, it would be much better if the efficiency of some identified origins in the three efficiency categories are measured and confirmed by an independent method.

A few minor mistakes and improper statements are found. The legends for some figures do not have enough information.