

have more plasticity in its origin selection¹⁴². Data from these newer mapping approaches show that 7 replication origins can be kilobase- sized zones in metazoans and were reported to fire stochastically with no sequence specificity^{82, 109, 158}. Consistently, metazoan ORC was shown to bind DNA with no apparent sequence specificity in vitro^{159–161}. Despite the lack of sequence specificity, G-rich sequences which have the potential to form G-quadruplex secondary structure have been found to be associated with sites of efficient¹ in metazoan species, it has been more difficult to locate replication origins and independent studies with different methods do not show high concordance of origin features and locations^{90, 151}, likely due to the large genome sizes, the existence of large replication initiation zones, analysis of different cell types, movement of Mcm2–7 double hexamers and technical limitations. Interestingly, bioinformatic analysis of some other fungi, such as *Neurospora crassa* and *Aspergillus nidulans*, suggest that AT-hooks at their Orc4 N-terminus might be used to specify replication origin location at AT-rich sequences in their genomes (N. Zali and B. Stillman, unpublished). One possibility is that ORC from *S. cerevisiae* has lysine-rich sequence in an intrinsically disordered region (IDR) of the Orc1 subunit that binds to a minor groove in the origin DNA and this amino acid sequence is conserved in other eukaryotes and has been shown in ORC from human cells to bind DNA, possibly a short sequence the ORC1 IDR^{139, 143}. Following the release of the first draft of the human genome, techniques such as quantitative mapping of RNA-capped short nascent strands (SNS) and 2-dimensional gel electrophoresis were employed to map replication origins^{84, 153–157}. They are .organized into spatially and temporally regulated replication timing domains⁶