REGULATION OF S-PHASE LENGTH IN MAMMALIAN CELLS

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Mammalian cells have many more potential replication origins than they activate in each S phase, some origins being more efficient than others. We have used molecular combing to study the replication dynamics along the AMPD2 domain in Chinese hamster cells. We found that oriGNAI3, which is nested within a large matrix attachment region, is the most efficient replication origin of the locus when cells are grown under conditions allowing fast progression of replication forks. However, slowing down replication speed triggers the recruitment of otherwise silent origins, so that cells adjust origin density to the rate of fork progression and complete S phase in a timely fashion. We have shown that this compensatory process does not depend on the intra-S checkpoint and occurs almost instantaneously in response to speed variations. Surprisingly, when shifted back to a growth medium supporting a high replication speed, even though the number of active origins decreases immediately, the cells have to go through a complete cell cycle before the hierarchy of origins, ie the prominence of oriGNAI3, is restored. Interestingly, we have observed a strict correlation between replication speed during a given S-phase and the size of chromatin loops in the next G1-phase. These data suggest a new level of origin programming, in which the replication speed determines the spacing of nuclear matrix-attached sequences and, in turn, controls the density of initiation sites. Origin hierarchy would thus reflect their relative affinity for the nuclear matrix.

Key words: Replication origins, fork speed, S-phase regulation, matrix attachment