pathogen interaction and of the evolution of tuberculous disease.

Many questions about the intracellular lifestyle of M. *tuberculosis* remain to be answered. Which bacterial gene products are involved in the remodeling of the phagosome? How are the intravacuolar bacteria presented to T cells to elicit cell-mediated immunity? What are the implications of preventing vacuolar acidification for the immune response to M. *tuberculosis* infection? If we can answer these questions, we will not only have a better understanding of microbial persistence and normal intracellular trafficking, but we may also have a key to preventing one of the most insidious causes of human morbidity and mortality.

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DNA Replication Origins in Animal Cells: A Question of Context?

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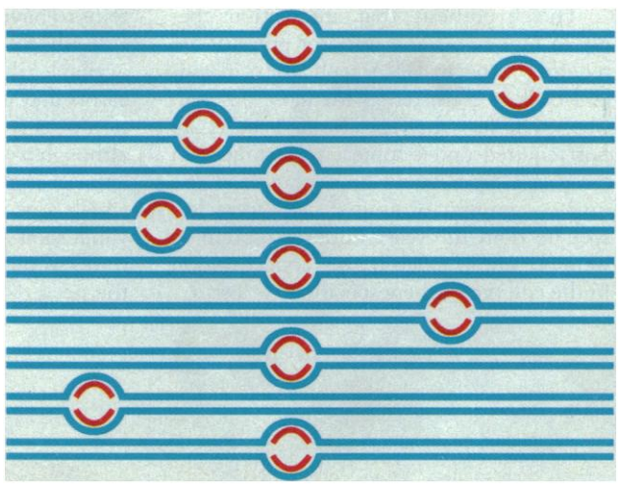
The process of DNA replication, which occurs during each cell division, starts at "origins," places in DNA molecules where the synthesis of a new DNA strand begins. In simpler genomes such as prokaryotes and the budding yeast *Saccharomyces cerevisiae*, replication origins require well-defined sequences called "replicators" (*1–5*) and their interaction with a specific initiator protein complex (*2*, *6*). How DNA duplicates itself in more complex metazoan (animal) cells has not been as clear. The field is still pondering whether the replication origins of animal cells correspond to specific nucleotide sequences and, if they do, what those sequences are and how they function.

Until recently, replicators [cis-acting sequences essential for origin function that are identified genetically] had been demonstrated for just two animal cell origins of replication. In one case several short (several hundred base pairs), partially redundant, cis-acting sequences are needed for DNA synthesis at an origin used for amplification of chorion genes in the third chromosome of the fruit fly, *Drosophila melanogaster* (*7*). In the second case, a replicator responsible for an origin of replication downstream of the dihydrofolate reductase (DHFR) gene in CHO (Chinese hamster ovary) cells has been localized within 16 kilobase pairs (*8*). Now a third example of an animal cell replicator has been provided in a recently published study of DNA replication in the human β-globin gene region (*9*). A replication origin normally located just upstream of the β-globin gene is removed by a naturally occurring deletion of this locus.

These three examples convincingly demonstrate the existence of replicators in animal cells. It is likely that most other animal origins will also prove to be associated with replicators. This view is reinforced by the results of experiments in which initiation sites (the sequences where DNA synthesis starts, which are defined by physical methods) were mapped by measuring the time during S phase when a DNA segment replicates or by measuring the average direction of replication fork movement through a DNA segment. In most cases, initiation sites seemed to occur at discrete locations (*8–20*). And, in the three cases where a replicator has been defined (*8*, *9*, *13*, *14*), these initiation sites occur in the same region as the replicator.

However, animal replicators may be less specific or may function differently than those of prokaryotes and budding yeast. For example, in some cases animal cells can initiate replication without replicators or specific initiation sites. DNA molecules injected into *Xenopus* eggs or incubated in egg extracts (*21–23*) replicate once per cell cycle without detectable sequence dependence. Initiation can take place at multiple sites, perhaps randomly, during *Xenopus* embryonic development (*24*), during *Drosophila* embryogenesis (*25*), and in repeated histone genes in cultured (postembryonic) *Drosophila* cells (*26*). In cultured human cells, most genomic sequences that are greater than 10 kilobase pairs can support autonomous plasmid replication (*27*), and replication within these plasmids initiates at multiple, perhaps random, sites (*28*).

The notion that replicators may function differently in animal cells than in yeast and prokaryotes is reinforced by experiments analyzing origins of replication with two-dimensional (2D) gel electrophoretic mapping techniques. These results show that the initiation sites of animal replication origins are distributed over broad regions (initiation zones) of several kilobase pairs or more (*7*, *24*, *26*, *29–32*). When origins have been studied both by 2D gel mapping and by other techniques, the 2D gel results consistently imply that initiation events take place over a broader region than suggested by the other techniques. For the DHFR origin, both replication timing and determination of average fork direction suggest that initiation sites occur within a few kilobase pairs (*8*, *10*, *13*, *14*). However, results from 2D gels suggest that initiation can occur at multiple sites, perhaps anywhere, within an initia-



Events in an animal cell initiation zone. Each pair of parallel blue lines are the parental strands of a DNA molecule in a different cell. The red lines are the newly synthesized daughter strands at initiation sites. [Adapted from (*6*)]

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tion zone of 55 kilobase pairs surrounding the replicator and initiation sites localized by the other techniques (*29, 30*). Similarly, 2D gel analysis of the chorion gene in *Drosophila* suggested that initiation events could take place at multiple sites throughout a region of several kilobase pairs, which includes the more circumscribed replicator described above (*7*).

The apparent disagreement between the 2D gel techniques and other techniques concerning the distribution of initiation sites can be explained by the model (*6*) shown in the figure. This model suggests that an initiation zone consists of multiple potential initiation sites, some of which (such as the central initiation site in the figure) are used more frequently than others. Measurements of the average direction of replication fork movement or of replication timing would detect the frequently used initiation sites but would be insensitive to the others. The 2D gel techniques are more sensitive to individual initiation events and would thus better define the breadth of the initiation zone.

This model is supported by several observations. In yeast cells, closely spaced simple origins can generate 2D gel patterns resembling the signals from animal cell initiation zones (*33*). The initiation zones at the *Drosophila* chorion gene amplification origin and at an amplification origin in the fly *Sciara coprophila* consist of major initiation sites flanked by multiple dispersed minor initiation sites (*7, 32*). The 2D gel signals from the DHFR initiation zone suggest that most initiation events occur near the primary initiation site detected by other techniques, with fewer initiation events elsewhere in the initiation zone (*30*). Although there are some remaining discrepancies in quantitation between different results at each of these animal origins, further study is likely to resolve these discrepancies.

The hypothesis that interactions between replicators and initiator proteins are required to begin DNA replication in animal cells is consistent with most observations. The fact that specific replicators are not required under some circumstances in some cell types could be explained if the initiator protein of animal cells were less specific than the yeast initiator protein or were present at much higher concentration in the cell types where specificity is lost.

However, the known features of animal replication origins can also be explained by an alternative hypothesis—any animal DNA sequence has the potential to serve as an origin but most sequences in postembryonic cells are prevented from becoming origins by aspects of nuclear and chromatin structure that we do not yet understand. The essential cis-acting sequences that specify origin locations in animal cells may do so by setting up chromatin and nuclear structures that repress origin activity at some sequences and favor it at others (*6*). Several recent experiments favor this second hypothesis and indicate that chromatin structure is crucial for the formation of initiation zones. For example, the boundaries of both the DHFR initiation zone (*30*) and the human ribosomal DNA initiation zone (*31*) coincide with boundaries between transcribed and nontranscribed sequences. Within the ribosomal DNA of *Xenopus* early embryos, replication initiates at apparently random sites, but these sites are spaced at surprisingly regular intervals corresponding approximately to the size of the ribosomal repeat unit, suggesting that some repeating aspect of chromatin structure may establish initiation zone boundaries (*24*). When naked DNA is incubated in *Xenopus* egg extracts, a DNA molecule containing the primary initiation site of the DHFR initiation zone is replicated no more efficiently than control DNA segments from outside the initiation zone or from phage λ (*34*). However, if intact CHO cell nuclei are incubated in the same extracts, then most initiation events downstream of the DHFR gene occur at the primary initiation site (*34*). Proteins bound to CHO nuclear DNA may be able to suppress initiation from some potential initiation sites while retaining it at others. Proteins that control chromatin and nuclear structure seem more likely than specific initiator proteins to have such global effects.

Only recently have techniques become available that can characterize the replicators and initiation sites of DNA replication origins in animal cells. Consequently, although we do not yet know enough about animal replication origins to determine which aspects of these two hypotheses are valid, tools are available that will permit us to do so in the near future. Most urgent is the need to elucidate the important motifs within the replicators of several animal origins. This should permit identification of the proteins that bind to replicators and determination of whether they function directly as initiator proteins or as proteins that establish a structural context permissive for initiation (or possibly both).

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