PERSPECTIVES: DNA REPLICATION

Choosing a Place to Begin

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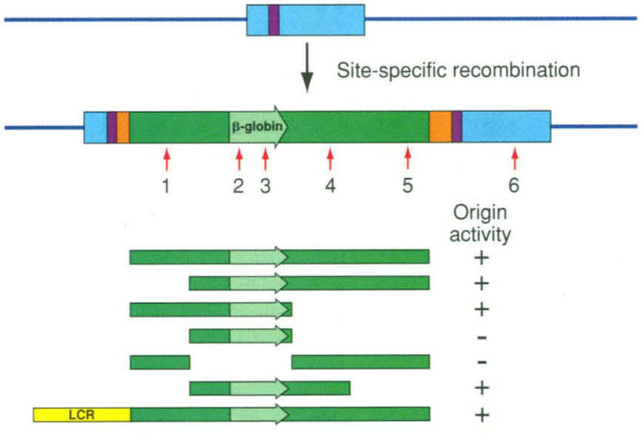
Before it can divide, a cell must first duplicate its own DNA, creating a copy of its genome for each daughter. In viruses, bacteria, and yeasts, DNA replication begins at sites in the genome called origins. Specific nucleotide sequences (or replicators) at each origin are required for initiation of DNA replication. Now in this issue of *Science*, Aladjem *et al.* (*1*) show that specific sequences are also necessary for initiation at origins near the human β-globin gene and are thus likely to be necessary for all replication origins in animal cells, as in viruses, bacteria, and yeasts.

Aladjem *et al.* (*1*) used a genetic approach to characterize replication origins in animal chromosomes. Previous studies, which relied entirely on biochemical methods for identification of initiation sites, could not determine which nucleotide sequences are important. As a result, the field has been clouded by uncertainty. The advance by Aladjem *et al.* (*1*) dissipates the clouds by showing that replicators *are* essential and, in combination with improved biochemical methods (*2*), should now allow rapid progress in understanding the mechanism and regulation of the origin of replication in animal cells.

To ensure that all sequences that they evaluated for origin function would be tested in the same environment, Aladjem *et al.* constructed a monkey cell line with a recombinase target site integrated at a single position in one of its chromosomes (see the figure). They also constructed plasmids containing the previously mapped (*3*, *4*) human β-globin initiation region (green box in figure) or deletion derivatives of the initiation region plus a recombinase target site. The β-globin initiation region (about 8 kb) includes the complete β-globin gene plus noncoding flanking sequences. Simultaneous introduction into target cells of a plasmid and recombinase led to integration of the β-globin initiation region or one of its derivatives at the same chromosomal position in each recombinant cell line (*1*).

To test for origin activity, Aladjem *et al.* used polymerase chain reaction primers specific for human β-globin DNA sequences to measure the abundance and sizes of nascent strands at several positions (red arrows in the figure). They found short nascent strands in the initiation region but none in the flanking DNA (position 6). Nascent strand sizes were consistent with the existence of origins at positions 2 and 4, with possible additional origins near positions 1 and 5. In an interesting twist, the activities of all origins in the initiation region appear to be coordinately regulated. Deletion of either the single segment containing the β-globin gene or both segments flanking the gene eliminated origin activity throughout the β-globin initiation region (*1*).



In animals, too. Sequence-dependent DNA replication has been demonstrated in animal cells by Aladjem *et al.* (*1*), who inserted the intact human β-globin initiation region (green box) and various deletion derivatives of the initiation region (green boxes at bottom of figure) or the initiation region plus LCR (yellow box) into a constant location in a monkey cell chromosome by using site-specific recombination at the recombinase target site (purple box). They then tested origin activity for DNA replication by measuring the abundance of short nascent strands at the six different positions indicated by red arrows. Dark blue line, monkey chromosome sequences; blue boxes, sequences introduced along with the recombinase target site; orange boxes, sequences introduced along with the initiation region; light green arrows, the β-globin gene. Only distances within the initiation region are to scale.

When the human β-globin initiation region is in its normal location in a human chromosome, the capacity for and timing of replication during S phase appear to be regulated by the locus control region (LCR) about 50 kb upstream of the initiation region (*4*, *5*). The LCR also regulates β-globin gene transcription. To test whether the LCR affects initiation efficiency or timing when the β-globin initiation region is inserted at the target site in monkey cells, Aladjem *et al.* examined the effect of inserting a miniaturized version of the normal LCR (see the figure) adjacent to the initiation region. With or without the LCR, replication initiated efficiently in early S phase (*1*). This lack of LCR effect on initiation would be expected if the target site for initiation region integration into the monkey chromosome were constitutively permissive for early replication.

What types of proteins are likely to interact with the replicator sequences? Obvious candidates are the animal cell homologs of proteins originally identified in budding yeast as essential for origin function, including the six subunits of the origin recognition complex (ORC) (*6*). Budding yeast uses specific origins under all conditions, and the sequences essential for those origins usually occupy less than 150 base pairs. The most important component of a budding yeast origin is a binding site for the ORC. The other sequences within budding yeast origins may bind accessory proteins or may directly facilitate DNA unwinding (*7*).

The results of Aladjem *et al.* indicate that sequences distributed over several kilobases—much larger than a budding yeast origin—contribute to origin function in the β-globin initiation region, suggesting a possible requirement for extra sequences in addition to those needed for ORC binding. If so, what could be the role of the additional sequences? One possibility is suggested by the observations that, although specific origins are used in the chromosomes of adult animal cells (*8*), randomly located origins are used in small circular plasmids (*9*) and in rapidly dividing embryonic cells (*10*). When SV40-transformed Chinese hamster cells are treated with a protein kinase inhibitor in early G_1 phase, they too use random origins (*11*). These observations suggest that many sequences can potentially serve as origins in animal cells, and a protein kinase–requiring step in early G_1 is needed to confer origin specificity. If this view is correct, then some of the replicator sequences identified by the assay of Aladjem *et al.* may prove to function by giving par-

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ticular potential origins a competitive advantage over the vast excess of alternative potential origins in the same chromosome.

How could specific sequences give nearby potential origins an advantage over more distant potential origins? Several possibilities come to mind. First, certain sequences may induce a local chromatin structure that promotes origin function, perhaps by facilitating access to initiation factors. Second, certain sequences may specify localization within a portion of the nucleus that is favorable for origin function. Third, specific sequences may favor neighboring over distant potential origins by permitting earlier replication of the nearby origins. Earlier origins can suppress neighboring potential (but later) origins, because forks from the earlier origins replicate the later ones before they have a chance to initiate replication. Perhaps the reason for little origin specificity in small plasmids is that their miniature dimensions do not permit the necessary internal differences in chromatin structure, nuclear location, or replication timing.

Thus, the report by Aladjem *et al.* (*1*) provides solid evidence for specific replication origins determined by specific DNA sequences (replicators) in adult animal cells. It describes a genetic technique that should permit fine mapping of replicators. Future analyses of replicators and of the proteins that bind to them are likely to shed light on such still mysterious subjects as the mechanism of initiation of DNA replication, the roles of chromatin structure and nuclear location in initiation, and the molecular mechanisms that determine replication timing.

References and Notes

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PERSPECTIVES: GLOBAL WARMING

Global Climate Data and Models: A Reconciliation

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The global warming issue has been confounded for several years by satellite observations of a cooling trend in the troposphere (the lowest 10 to 15 km of the atmosphere) (*1–4*). Such cooling is at odds with measurements at meteorological stations (*5*, *6*), which show warming at Earth's surface, and with climate models, which yield warming at the surface and in the troposphere (*1*, *7*).

Wentz and Schabel (*8*) have now discovered an oversight in the satellite data analysis that, at least to first order, removes the inconsistency between surface and satellite observations. The modified temperature profile is also in close accord with published climate model simulations. We caution that there are remaining uncertainties in the data, the models, and the analyses that are likely to prolong the debate about temperature trends. But the apparent reconciliation, if it stands up, can potentially lead to improved understanding of how sensitive the climate system is to natural and anthropogenic forcings such as changes of solar irradiance, volcanic eruptions, and greenhouse gases.

The oversight was failure to account for decay of satellite altitude caused by atmospheric drag. The Microwave Sounding Unit (MSU) on polar-orbiting weather satellites scans across the subsatellite orbital track, making measurements at discrete angles relative to the nadir. But as the satellite falls, the measurements at a specific "look" angle (relative to the nadir) have a changing angle relative to Earth's surface. This angle change has little or no effect on temperatures measured for the stratosphere (labeled channel S) and middle troposphere (channel MT), because they are inferred from near-nadir measurements (*2*). However, the lower tropospheric temperature (channel LT) is obtained from measurements made at several look angles, and thus it must be corrected for satellite altitude change. Altitude decay is most rapid, and thus the correction is largest, near the times of peak solar activity that occurred around 1980 and 1990. Wentz and Schabel do not reanalyze the MSU data from scratch, but they calculate the mean correction for 1979 to 1995 to be +0.12°C per decade (see the figure).

The open triangle near the bottom of the figure is the global mean MSU LT temperature trend for 1979 to 1995 without correction for orbital decay. The solid triangles, connected by the solid line, are the MSU data at all three levels, LT, MT, and S, including the correction of LT calculated by Wentz and Schabel. The triangle at 1000 mbar is the surface temperature change based on surface air measurements over land and sea surface temperature measurements over ocean (*5*, *6*).

The correction of Wentz and Schabel makes the MSU temperature profile more self-consistent. Before the correction, MT was warming relative to LT, which presented an enigma. Each of the MSU measurements refers to a large layer of atmosphere, as indicated by the "weighting functions" on the right side of the figure. The broad weighting function for MT includes a contribution of about 15% from the lower stratosphere, a region that is known to have cooled rapidly in this period, on the basis of radiosonde, MSU S, and other measurements (*1–3*). The correction of Wentz and Schabel removes the enigma, as it leaves MT cooling relative to LT.

The observed temperature change is compared with our published climate model simulations in the figure. The climate model (*7*) was driven by observed SSTs and measured climate forcings added cumulatively one by one, as indicated in the figure. The climate forcings, which are imposed changes of Earth's radiation balance with space, are those measured during the era of satellite data, specifically stratospheric aerosols from volcanic eruptions, increasing greenhouse gases, changes of solar irradiance, and ozone depletion. An ensemble of five simulations, illustrated individually elsewhere (*7*), was made for each set of forcings, the individual runs differing because of small perturbations of initial atmospheric conditions and the chaotic nature of the atmosphere. Circles in the figure are the five-run means after weighting the calculated temperature profiles with the MSU weighting functions (*2*). Standard deviations among the five runs are about 0.02°C at each of the four levels shown in the figure.

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