RÉSUMÉ

Horse scents

STALLIONS competing for mates judge the form of a rival male not only by listening carefully to its whinnies but by sniffing its faeces as well (D.I. Rubenstein and M.A. Hack *Evol. Ecol.* **6,** 254–260; 1992). Whinnies indicate dominance status, from the horse's mouth, as it were, irrespective of whether the two horses are familiar with each other or not. Faecal smell, though, reveals the identity and home turf of a particular horse, which can then be associated with the outcome of previous contests. Redundant signalling in one species is unusual, but may be a belt-and-braces solution for animals with large and overlapping ranges. The consequent saving on wasteful physical combat echoes Churchill's dictum that "to jaw-jaw is better than to war-war".

Under pressure

HYDROGEN is a singly valent element, as is sodium, so it stands to reason that when transformed into a solid — by pressure — it should become metallic. H. K. Mao and R. J. Hemley claimed three years ago to have achieved this remarkable state of matter. But because the pressures involved are so high, false signals from the apparatus may have confused the issue. Mao, Hemley and M. Hanfland now reply to a charge that the small quantity of powdered ruby (aluminium oxide), included in their cell to calibrate the pressure, becomes reduced by the hydrogen to create traces of aluminium metal. The authors have repeated their experiments with different proportions of ruby, and find that the indicators of metallization (such as increased optical reflectance) are unchanged (*Phys. Rev.* B**45**, 8108–8111; 1992). These are experiments at the extremes of techniques, however, and many remain to be convinced that metallic hydrogen has truly been found.

Tackling TB

TUBERCULOSIS is back with a vengeance in places such as New York City, where the disease is reaching epidemic proportions. But as A. M. R. Chowdhury *et al.* point out in *The Lancet* (**339**, 1181–1182; 1992), in the developing nations it has never been away. In Bangladesh, estimates of the percentage of people testing positive for the TB bacillus have remained at a stubborn 0.5 for over a quarter of a century, in part because TB control has been urban-based — most Bangladeshis live in rural areas and so three-quarters of patients fail to complete their course of treatment. Chowdhury *et al.* describe the results of taking treatment measures to the people who need them, in the form of a community-based programme. Completion rate was an impressive 60 per cent, and the cost per case ($108) comparatively low. Plans are being laid to expand the programme.

DNA REPLICATION

Eukaryotic initiation rites

Joachim J. Li and Bruce M. Alberts

INITIATION of chromosomal DNA replication, which defines the transition from the G1 to the S phase of the cell cycle, is a pivotal event in the life of a eukaryotic cell. It occurs with precise timing and is carefully coordinated with other events in the cell cycle. Moreover, S phase is normally an all-or-nothing event: the initiation process leads to replication of all of the DNA once and only once, followed by division of the cell in M phase. Attempts to understand how these controls are achieved have been limited by our ignorance of the initiation machinery in eukaryotic cells. Partly for this reason, identification of this machinery has become a holy grail which has attracted researchers ever since 1979, when the first eukaryotic replication origins were discovered in the yeast *Saccharomyces cerevisiae*[1].

As reported by Bell and Stillman on page 128 of this issue[2], the long and arduous search now seems to have paid off with the purification of a large multiprotein complex. The complex binds to the yeast replication origin and appears to wrap the DNA around itself. Also reported in this issue[3] is supporting evidence from Diffley and Cocker which suggests that this complex actually binds to the origin *in vivo* (see page 169).

From studies on bacterial[4] and viral[5,6] systems it was predicted that initiation of chromosomal DNA replication in eukaryotes would involve an initiator protein that binds to the origin of replication, a *cis*-acting DNA sequence that specifies the site of initiation. For reasons that are still unclear, defining such origin sequences has proven to be a daunting task in most eukaryotes. Only in *S. cerevisiae* have the replication origins been sufficiently characterized to encourage a search for an initiator protein based on its predicted origin-specific DNA binding.

Yeast origins[1] are some 100 base pairs in length and have two essential elements, termed A and B. The A element is primarily composed of an 11 base-pair consensus sequence, the only sequence common to all origins from *S. cerevisiae*. Although normally a required part of every yeast chromosomal origin, the B element can be replaced by several copies of the A element when origin function is tested on plasmids. These properties of the A element recommend it as the most likely binding site for an initiator protein in yeast.

Despite evidence suggesting that a protein actually occupies this site *in vivo*[7], the search for such a protein has frustrated researchers for many years. Several groups have recently discovered an activity that binds to one of the single strands of the A region[8–10]. Its function is not clear, however, especially because purified preparations of this DNA-binding protein exhibit only weak sequence specificity[8]. At any rate, if the protein participates in replication, its action would presumably require the prior conversion of the closed duplex origin into an open single-stranded form. Hence researchers have maintained faith in the existence of an initiator protein that binds to the duplex A element as a first step in the initiation of yeast DNA replication.

This faith has now been affirmed by the work of Bell and Stillman. They have identified and purified a DNA-binding activity that recognizes the A element as a double-stranded sequence. This activity is associated with a tight complex of six proteins called the origin recognition complex (ORC). Identifying this activity was a remarkable feat, in that it could not be detected until several steps into the purification. In addition, ORC could not be seen by gel retardation assays, and its footprint was only observable in the presence of ATP.

Using a panel of A-element point mutants, Bell and Stillman established a tight correlation between the ability of the mutant DNAs to bind ORC *in vitro* and their ability to function as plasmid origins *in vivo*. This correlation is complemented by the experiments reported by Diffley and Cocker in which they detected the signature of the ORC footprint *in vivo*. Together, the two sets of results argue strongly that an initiator protein from eukaryotic cells has finally been captured. That ORC forms a similar footprint on several different yeast origins further suggests that the same initiation machinery can work on many distinct replication origins scattered throughout a eukaryotic genome.

Additional analysis of ORC may soon provide more evidence of its role in initiation. Because of its many subunits, one might expect it either to exhibit several different biochemical activities or to be regulated in a complicated way. It would be encouraging to find that ORC exhibits biochemical activities that are associated with known bacterial[4] and viral[5,6] initiation complexes (for example duplex DNA unwinding, helicase activity and ATP hydrolysis). These activities have not been detected to date. Although this might be explained by trivial reasons, it is conceivable that the biochemical activities of a protein involved in such a highly regulated event

are maintained under tight control. Learning how to unleash them *in vitro* could therefore reveal how this protein is regulated *in vivo*. Ultimately, direct demonstration that ORC initiates yeast DNA replication will rely on the establishment of an *in vitro* system in which exogenous templates containing a yeast origin can be replicated. That is the next technical challenge to be tackled, and it should be greatly facilitated by the ability to purify large quantities of ORC.

Assuming that ORC proves to be the initiator protein, at least four ramifications arise. First, a great deal of effort in the cell-cycle field is directed towards understanding the events that lead from the activation of cyclin-dependent kinases in G1 phase to the initiation of DNA replication at the G1/S boundary. Identification of the initiator protein allows us to replace the abstract notion of a G1 to S transition with a defined molecular change, which provides an important tool for working backwards to define and order the activating events in G1. For example, genetic analysis in *S. cerevisiae* has identified a series of cell division cycle (*cdc*) genes whose actions appear to be interposed between the activation of the CDC28 kinase (the *S. cerevisiae* homologue of $p34^{cdc2}$ kinase) and the initiation of DNA replication[11–14]. How these genes promote DNA replication is unclear. We can now ask how the product of each of these genes affects the different components of the initiator protein complex. Some of the products may be involved in the synthesis, assembly, modification or activation of ORC subunits, whereas others may serve as actual subunits of the complex.

The identification of this putative initiator protein also promises to shed light on a second biological mystery: how initiation of DNA replication at each origin is limited to only one round per cell cycle (discussed in ref. 15). Explanations of how initiation occurs once and only once must ultimately converge on the actions of the initiator protein. With the protein in hand we can now test some of the models that have been proposed. For example, is the initiator protein inactivated during each initiation event, or is the freshly replicated chromosomal template altered to make it impervious to the continued action of the initiator? Moreover, once the initiation machinery has 'fired', what happens to the initiator protein as the system is reprimed for the next cell cycle? By following the fate of the protein during the replication reaction and throughout the cell cycle, we can expect to learn how the cell prepares itself for a new round of initiation and how it prevents those preparations from occurring prematurely.

A third question concerns the time within S phase at which a eukaryotic origin will initiate DNA replication. This question has been examined most rigorously in *S. cerevisiae*[16]. Although many yeast origins initiate at the onset of S phase, a few appear to delay their action until later in S phase. From recent work it seems that proximity to a telomere at the end of a chromosome is sufficient to induce a yeast origin to 'fire' late. The discovery of ORC should allow us to rephrase the problem in terms of telomeric effects on ORC binding and ORC activation.

Finally, complete characterization of ORC and each of its protein components should provide useful biochemical and immunological tools for identifying similar components of the replication apparatus in higher eukaryotes. Indeed, the discovery of homologues to ORC and analysis of their DNA-binding sites could provide a shortcut to defining replication origins in higher eukaryotes.

Work remains before we can confirm the function of the origin recognition complex, but its discovery has moved research in eukaryotic DNA replication into a promising new phase. The field is now poised to emerge from the shadows of its more developed bacterial and viral counterparts, providing us with the opportunity to solve salient problems specific to eukaryotic cells. □

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Fashioned by fire

The series of fires that swept through Yellowstone National Park in the summer of 1988 exposed the mountain slopes to the destructive effects of the intense local thunderstorms. On page 147 of this issue, G. A. Meyer *et al.* show that erosion after such fires follows a pattern that has sculpted the landform of Yellowstone over the millennia. The authors looked at the stratigraphy of alluvial fans in two creeks, and identified and dated 18 fire-related debris-flow runouts and another 17 fire-related sediment runouts from the past 3,500 years. Extended periods of drought (350–100 BC and AD 1000–1200) feature strongly in the stratigraphic record. But, as the recent experience shows, a single dry summer can leave its mark, so that rapid climate variability may equally be implicated. The picture (from August 1989), of a region above Soda Butte Creek, shows another characteristic of the fire damage — the speed with which vegetation can recolonize and restabilize the soils. **R.P.**