were clearly potential targets of HBx. Sure enough, HBx expression was soon found to be associated with activation of both c-*src* and *fyn* genes, which encode the Src and fyn nonreceptor tyrosine kinases, respectively (*9*).

As they could detect no direct interaction between HBx and either of these Src kinases, or the carboxyl-terminal Src kinase (Csk), Schneider and co-workers turned their attention to other upstream regulators of the Src family. This led them to the kinase Pyk2, a cytosolic kinase that is activated by transient calcium ion ($Ca^{2+}$) fluxes and is known to activate Src kinases. In the new work, these investigators make a compelling case for involvement of this $Ca^{2+}$-dependent Src kinase signaling pathway in at least some of HBx's activities (*1*). First, HBx production leads to activation of Pyk2 through addition of phosphate groups (phosphorylation). Dominant-negative mutations in Pyk2 block HBx induction of AP-1 activity, and also produce a substantial decline in fyn kinase activity. In addition, expression of dominant-negative mutant Pyk2 in cells replicating wild-type HBV has exactly the same effect as seen in X-null mutant cells: reduced viral DNA synthesis in the presence of normal levels of viral RNA. These findings are consistent with the notion that HBx acts upstream of Pyk2, perhaps on a pathway that leads to $Ca^{2+}$ release. Consistent with this notion are two further lines of evidence. First, molecules that chelate intracellular $Ca^{2+}$ also block the HBx induction of Pyk2 activation. Second, the impaired replication of HBx-deficient HBV mutants can be partially overcome by agents ($Ca^{2+}$ ionophores and thapsigargin) that increase the concentration of cytosolic $Ca^{2+}$.

A signaling pathway that depends on $Ca^{2+}$ fluxes is an attractive biochemical locus for HBx action because it affects so many cellular processes, including transcription, translation, cell cycle control, and apoptosis. Many of these cellular processes have been suggested to be affected by HBx. The outstanding questions now are (i) how does HBx influence the release of $Ca^{2+}$ and from which organelles (see the figure), and (ii) are the effects of $Ca^{2+}$ release limited to those caused by Pyk2 activation, or does HBx activate other $Ca^{2+}$-dependent signaling events? If the latter is true, as seems likely, it is conceivable that many of the other phenotypic effects of ORF X expression that have been described may be attributable to this activity. Time will tell.

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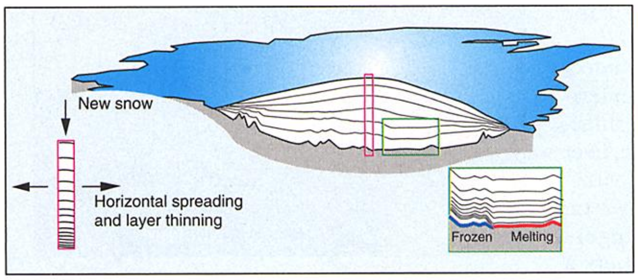
**PERSPECTIVES: GLACIOLOGY**

# How Ice Sheets Flow

Christina L. Hulbe



**Peering into an ice sheet.** Internal layers thin with depth (and age) because of ice flow. Where basal melting occurs, ice is lost at the bottom of the ice sheet and layers experience relatively less thinning than they would over a frozen bed.

Visitors to alpine glaciers routinely marvel at the torrents of melt water rushing from under the ice. As early as the late 18th century, alpine geologists had surmised the importance of this liquid water to glacier flow. In his epic *Voyages in the Alps* (*1*), Horace-Bénédict de Saussure observed "Almost all glaciers [...] of any appreciable size, have beneath them, even in winter, streams of water that flow between the ice and the bed that supports it. One therefore understands that these ice masses, driven by the loss of contact with the bed on which they rest, freed by the loss of contact with the bed on which they rest, freed by water from contact that ice could make with that same bed, sometimes even lifted by this water, must little-by-little, slide and descend following the valley slope or ridge they cover."

It is perhaps less obvious that basal melt water is also important to the much larger, and considerably colder, polar ice sheets. Yet the effects of water beneath ice sheets can be profound, as exemplified by the unexpectedly fast-flowing networks of ice streams embedded within the West Antarctic Ice Sheet (*2*) and by the flotillas of icebergs cast into the North Atlantic by surges of the Laurentide Ice Sheet, which covered much of North America during the Pleistocene glacial cycles (spanning the last 2 million years) (*3*).

On page 2338 of this issue, Fahnestock *et al.* (*4*) report a striking example of the power of basal melting in an unexpected place: the northeast sector of the Greenland Ice Sheet. They use an innovative combination of remote observation and theory. The technical aim of their work is narrow: to deduce surface snow accumulation and basal melting rates from the geometry of ice layers within the ice sheet, measured remotely by airborne radar. But the mark they hit is much broader.

Snow falling on the ice sheet surface forms layers that move downward and densify to become glacier ice as new snow falls above them. The downward-moving layers thin as a result of horizontal spreading of the flowing ice (see the figure). The process is easy to imagine for a simple scenario in which the ice sheet is frozen to its bed and is close to a steady-state mass balance. In that case, the rate of new snow accumulation is matched by the spreading rate and the ice layers thin with depth (and thus age) in a predictable way. But when Fahnestock *et al.* (*4*) applied a commonly used model of that process to a set of well-dated layers in the Greenland Ice Sheet, the model failed in some interesting locations.

The model failed because the downward-moving layers do not thin as much as they ought to thin. The implication, which the authors demonstrate quite elegantly, is that the bottom of the ice sheet is melting, in some places at rates of up to 20 cm/year. The largest inferred melting rates exceed what can be explained by typical heat flow in old (and hence relatively cold) cratonic crust. The basal melting anomaly is large enough in both magnitude and spatial distribution to suggest that a feature similar to North America's Yellowstone caldera lies beneath the ice sheet. Such calderas are caused by the collapse of massive magma chambers, indicating substantial geophysical activity. The area where large basal melting begins lies upstream of a heretofore unexplained

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channel of fast-flowing ice embedded within the northeast sector of the ice sheet (*5*). Basal melt water, lubricating the interface between the ice and its bed, must be the cause of the enhanced flow.

With the exception of his descriptions of the physics, de Saussure's theories about subglacial water were arguably quite advanced. Water submerges the bed, thereby diminishing the basal drag that resists the gravitational driving stress. It also saturates and weakens subglacial tills, and under pressure, basal water can cause the overlying ice to float (*6–8*). Recently, another attribute of water has been added to the list: Melt water, flowing down the glacier from its source, may redistribute energy within the glacier system. The effectiveness of this redistribution is still under investigation (*9*), but it may be critically important to one of Earth's three remaining great ice masses, the West Antarctic Ice Sheet.

The temperature at the bottom of an ice sheet depends on the air temperature at the ice surface, the rate at which snow accumulates, the thickness of the ice, the geothermal gradient in the rock beneath the ice, and variations arising from horizontal motion of the ice. In general, the thinner the ice, the more likely it is that the geothermal gradient is accommodated by diffusion of heat through the ice and that the ice is frozen to its bed. But in West Antarctica, the fast-flowing ice streams are both thin and melted at the bed. And an increasing range of evidence suggests that this has been the case since at least the Last Glacial Maximum about 20,000 years ago (*10*, *11*). Weak, water-saturated marine sediments beneath the ice sheet are the cause of the fast flow, but this subglacial till would consolidate without sufficient water (*7*, *12*). The apparent contradiction of thin yet fast-flowing ice can only be resolved by recognizing the importance of melt water and its distribution at the base of the ice sheet. Melt-water thermodynamics is thus of great concern to glaciologists trying to predict future ice sheet retreat rates and corresponding changes in global sea level.

The notion of thin, fast-flowing West Antarctic ice streams throughout the Pleistocene (*10*, *11*) has implications beyond Antarctica. Many of the Northern Hemisphere ice sheets of recent glaciations were marine-based as well, and reconstructions of their thickness histories rely in part on fundamental assumptions about basal energy balance that may need reevaluation. Fahnestock *et al.*'s discovery of substantial basal melting in an unexpected place reminds us that basal melt water matters and that it may be important in places we never suspected.

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PERSPECTIVES: DNA REPLICATION

# Genomic Views of Genome Duplication

**Bruce Stillman**

Genomewide views of biological processes offer new perspectives on the organization of chromosomes and the replication of their DNA. In a pair of recent *Science* papers (see page 2357 of this issue), two groups report different, but complementary, global approaches for mapping the distribution of origins of DNA replication (*oris*) throughout the genome of the budding yeast *Saccharomyces cerevisiae* (*1*, *2*). DNA replication simultaneously proceeds along discrete sections of the chromosomes, and the *oris* are the DNA sequences where replication begins. Both techniques not only reveal the position of many yeast genome *oris*, but also provide a wealth of information about the way in which the genome is duplicated. The results also hint that the proteins that initiate DNA replication may be important in nonreplication events as well.

Enhanced online at www.sciencemag.org/cgi/content/full/294/5550/2301

*S. cerevisiae* was the first eukaryote to have its genome sequenced. Scientists have probed the sophisticated genome of this well-researched eukaryote to learn how whole chromosomes are replicated. The 16 chromosomes of the budding yeast contain multiple *oris*. Two decades ago, small pieces of yeast DNA called autonomously replicating sequences (ARSs), when inserted into bacterial plasmids, were found to promote the replication of these circular DNAs during each S phase of the cell cycle (*3*). Later, many, but not all, of these ARSs were found to correspond to *oris* in yeast chromosomes.

The detailed structures of individual ARSs and *oris* were determined a decade ago. Simultaneously came the discovery of the origin recognition complex (ORC), a six-subunit protein complex that binds to *oris* and coordinates the assembly of a pre-replication complex (pre-RC) at each *ori* (*3*). The pre-RC contains, among other components, the six different, but related, mini-chromosome maintenance (MCM) proteins (*3*) (see the top figure on the next page). We have learned much about how DNA is replicated in the budding yeast (*3*), including the distribution of *oris* in two of its chromosomes (*4*).

Brewer and Fangman and their colleagues teamed up with Davis and his group (*1*) to undertake the first genome-wide analysis of *oris*. With high-density DNA microarrays, they determined exactly when each region of the yeast genome replicated (at a resolution of about 10 kilobase pairs) (*1*). To do this, the investigators modified one of the most elegant biology experiments ever undertaken—the famous Meselson and Stahl experiment that proved semiconservative DNA replication (that is, each DNA strand is a template for the new strand being synthesized). Unfortunately, the yeast lacks the enzyme thymidine kinase and hence its DNA cannot be labeled with the modified nucleotide bromodeoxyuridine during replication. So, instead, the investigators prelabeled budding yeast DNA with the isotopes $^{15}$N and $^{13}$C (the resulting DNA strands being called heavy heavy, HH). Yeast cells arrested in late G$_1$ phase of the cell cycle were synchronously released from this block in the presence of two other isotopes, $^{14}$N and $^{12}$C (light medium). At various time points during S phase, DNA was isolated—both DNA that replicated semiconservatively (called heavy light, HL) and DNA that remained unreplicated (HH). By calculating

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