foraminifera, do not indicate such a lead (*11*), even though they were measured in a Santa Barbara Basin core also studied by Herbert *et al.*

Alkenones are mainly produced by coccolithophorids, microalgae that follow a distinct bloom pattern (*12*). The alkenones may therefore record the temperatures at which those blooms occurred and not the mean pattern of surface water temperature change. Strong support, however, for the inferences from the alkenone data comes from a continental record: a calcite vein from a submerged fissure called Devils Hole in Nevada (*13*). The lead in the independently dated Devils Hole oxygen isotope record over oceanic isotope records was first interpreted as posing a fundamental challenge to the Milankovitch theory, which argues that changes in orbital configuration drive ice age cycles (*1*). The problem was that the early onset of deglaciation in the Devils Hole record appeared to be in conflict with the orbital time scale of marine records of continental glaciation (*13*). But new temperature records from the tropical Pacific (*4*) and the more proximal California Current (*6*) suggest that Devils Hole variability is not a record of glaciation but rather a response to the temperature of Pacific source waters, which warmed early in the deglacial cycle, thus accounting for the timing mismatch. Although this eliminates the chronological mismatch with orbital theory, the early temperature response itself remains to be explained beyond the high-latitude Northern Hemisphere ice sheet forcing that Milankovitch envisioned (*1*).

If the large lead of California Current temperature is correct, it requires an explanation that is separate from the global response. Herbert *et al.* (*6*) hypothesize that the California Current collapsed during glacial maxima and that the invasion of warm gyre waters from the south produced the temperature lead. They further argue that there is a regional pattern to this effect, with the largest lead characterizing sites in the southern part of the California Current—where warm gyre waters can readily invade—and a smaller or absent lead north and south of this point.

The authors suggest that the presence of the Laurentide ice sheet—the massive ice sheet that covered much of North America—perturbed the northwesterly wind field along the California margin in a way unfavorable to the propagation of the California Current. This scenario might also explain some of the difference between the alkenone record and other temperature proxies (*11*). A seasonal invasion of warm gyre waters might favor coccolithophorid blooms, thereby leaving a particularly strong imprint of warmer temperatures in the alkenone record. Regardless of the exact causes, the timing of temperature change in the California Current and other regions challenges the paleoclimate community to understand what appears to be a globally asynchronous climate response during ice age terminations.

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14. I am grateful for discussions with A. Mix, J. Kennett, M. Bender, M. Kienast, E. Bard, and J. McManus.

Published online 7 June 2001;
10.1126/science.1061078.
Include this information when citing this paper.

PERSPECTIVES: DEVELOPMENT

The Message Is in the Translation

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Messenger RNAs (mRNAs) in the developing embryo must be translated into proteins at the right time and in the correct place to ensure that these proteins direct tissue formation appropriately. Assembly of multiprotein complexes on mRNAs ensures that translation is switched off until the time is right and the transcripts have reached the correct location within the embryo. Recent work on *cyclin B* mRNA (which encodes a cell cycle factor) and on other mRNAs suggests that the timing and location of mRNA translation may also control progression of cells through the cell cycle. Like protein degradation, control of mRNA translation is proving to be an elegant way for cells to modulate when and where proteins carry out their duties.

The accumulation and destruction of the crucial cell cycle factor cyclin B is essential for progression of the cell through the final stages of division (mitosis). Cyclin B binds to Cdk1 (Cdc2) protein kinase, forming activated M-phase promoting factor (MPF), and, although it is synthesized throughout the cell cycle, cyclin B begins to accumulate only during interphase. Both MPF activation and the onset of mitosis are triggered when cyclin B reaches a critical amount and the Cdk1 subunit is appropriately modified by both phosphorylation and dephosphorylation (the addition and removal of phosphate groups). Exit from mitosis requires destruction of cyclin B by an elaborate protein machine known as the anaphase-promoting complex (*1*). Although it is axiomatic that regulated protein destruction is the driving force behind cell cycle progression, recent evidence suggests that at least in early embryos, regulating the production of cyclin B is also essential for cell division. New work indicates that control of *cyclin B* mRNA translation takes place in different parts of the embryo and within different regions of rapidly dividing embryonic cells. Regulation of *cyclin B* mRNA translation appears to be part of a wider translational control network that is critical for pattern formation (the establishing of body structures in the appropriate region of the embryo) during early development of the fruit fly *Drosophila melanogaster*.

Formation of the anterior–posterior axis in the *Drosophila* embryo is specified by the localized expression of several mRNAs inherited by the egg at the time of fertilization. One of these mRNAs encodes Hunchback (Hb), a transcription factor that is required for segmentation in the anterior thoracic region of the embryo. Although *hb* mRNA is uniformly distributed, it is translationally repressed in the posterior region through the combined action of

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two RNA binding proteins, Pumilio (Pum) and Nanos (Nos) (*2*). Mutations in either *pum* or *nos* lead to synthesis of Hb in posterior cells of the fly embryo, which suppresses formation of abdominal structures in this region. Pum is a uniformly distributed, sequence-specific protein that interacts with the nanos-response element, a 32-nucleotide sequence present in the 3′ untranslated region (UTR) of *hb* mRNA. Nos has relatively little selectivity and is expressed in a posterior-to-anterior gradient. The combined action of asymmetrically localized Nos and sequence-specific binding by Pum thus generates a posterior-to-anterior gradient of Hb mRNA translational repression, which leads to a complementary anterior-to-posterior gradient of Hb protein expression.

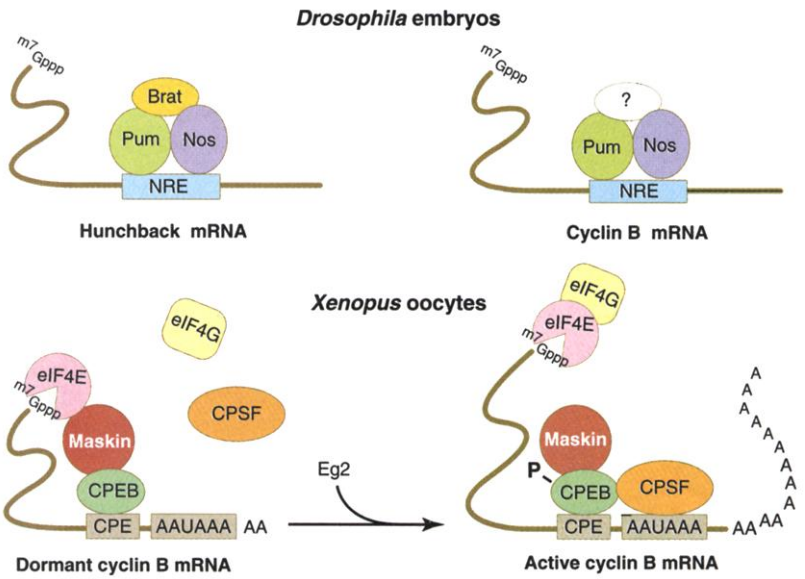
The *nos* and *pum* mutations also induce proliferation of migrating pole cells, germ cell progenitors that do not usually divide at this stage (*3*). These pole cell divisions are stimulated by the inappropriate translation of *cyclin B* mRNA, which is normally repressed because these cells remain in G_2 during migration and do not divide. Sequences similar to nanos-response elements in the 3′ UTR of *cyclin B* mRNA (*4*) are required for Pum and Nos to repress the translation of *cyclin B* mRNA (*3*). Thus, the Nos and Pum proteins acting through nanos-response elements repress translation of both *hb* and *cyclin B* mRNAs contributing to embryonic patterning and the control of cell division (see the figure).

Pum and Nos inhibit *cyclin B* mRNA translation and hence the proliferation of pole cells, but have no such effect on the proliferation of somatic cells in the posterior region of the embryo. This suggests that there must be a pole cell–specific third factor that combines with Pum and Nos to inhibit *cyclin B* mRNA translation, although no such factor has been identified so far. However, a protein that appears to act with Nos and Pum to repress *hb* mRNA translation has been isolated. Using a four-hybrid screening assay, Sonoda and Wharton (*5*) have identified Brain tumor protein (Brat), a factor that efficiently interacts with Pum and Nos only when these proteins are assembled on the nanos-response element of *hb* mRNA. Strikingly, Brat can discriminate between *cyclin B* and *hb* mRNAs, and does not bind to a ternary complex composed of the *cyclin B* nanos-response element, Nos, and Pum (see the figure). The Brat protein, like Pum, is located throughout the embryo, and mutations in the *brat* gene lead to ectopic expression of Hb protein in the posterior of the fly embryo. Consistent with biochemical and four-hybrid screening data, *brat* mutations do not affect *cyclin* mRNA expression or the proliferation of pole cells. However, *brat* mutations do lead to hyperproliferation of larval brain cells, although this appears to be independent of Nos and Pum. Pole cells may contain a translational corepressor of *cyclin B* mRNA that is functionally related to Brat (see the figure). Similarly, functional homologs of Nos and Pum may act with Brat to control translation and cell proliferation during later stages of development.

Although it is unclear how Nos and Pum regulate translational repression during *Drosophila* development, the *Xenopus* (frog) embryo may hold some clues. The *Xenopus* homolog of Pum is known to interact with cytoplasmic polyadenylation element binding protein (CPEB), a factor that promotes translation by extending the polyadenylate [poly(A)] tail of mRNAs (*6*). In addition, because *Drosophila nos* and *pum* mutations increase the polyadenylation of *hb* mRNA (*7*), the inhibitory activity of these proteins might involve modulation of poly(A) tail length. Consistent with this speculation, regulated changes in polyadenylation and deadenylation are known to be important for translational control during early development in amphibians and in mammalian neurons (*8*). How changes in poly(A) tail length control translation of mRNAs in *Drosophila* is not known, but the biochemistry of this process during early development of *Xenopus* embryos may provide important clues. Frog oocytes are arrested at the end of first meiotic prophase in G_2 of the cell cycle. These large cells contain many translationally dormant mRNAs (including *cyclin B* mRNA) that have relatively short poly(A) tails. When the oocytes are induced to reenter meiosis (that is, M phase), these short poly(A) tails are lengthened and translation ensues.

In the activated *Xenopus* egg, elongation of the poly(A) tail depends on two cis elements in the 3′ UTR of *cyclin B* mRNA: the short uridine-rich cytoplasmic polyadenylation element (CPE), and the hexanucleotide AAUAAA (see the figure). The CPE is bound by the sequence-specific RNA binding protein CPEB, whereas the AAUAAA is bound by a group of proteins collectively known as cleavage and polyadenylation specificity factor (CPSF). Polyadenylation is initiated by CPEB phosphorylation (*9*), which causes it to bind and recruit CPSF into an active cytoplasmic polyadenylation complex (*10*). Translational regulation by polyadenylation appears to involve the protein Maskin. Before poly(A) tail addition and translational activation, this protein interacts with CPEB and the cap binding factor eIF4E (*11*). The association of Maskin with eIF4E inhibits translation by preventing the interaction of eIF4E with eIF4G, the factor that helps to position the 40*S* ribosomal subunit on the 5′ end of the mRNA. At a time that is coincident with polyadenylation, Maskin dissociates from eIF4E, allowing eIF4G binding and initiation of mRNA translation.

Whereas translational repression and activation of *cyclin B* mRNA probably occur throughout the frog oocyte, the situa-



The alchemy of mRNA translation. Control of mRNA translation in the *Drosophila* embryo and the *Xenopus* oocyte. **(Top)** In the *Drosophila* embryo, a complex among Pum, Nos, *hb* mRNA, and the nanos-response element (NRE) is recognized by Brat, an inhibitor of mRNA translation. In the same cells, a complex among Pum, Nos, and *cyclin B* mRNA is also assembled on the nanos-response element, but is not recognized by Brat. Instead, it might interact with another unidentified inhibitory factor. **(Bottom)** In the *Xenopus* oocyte, the CPEB-Maskin-eIF4E trimer prevents the translation of *cyclin B* mRNA. The phosphorylation of CPEB by Eg2 stimulates the recruitment of CPSF, resulting in the addition of a poly(A) tail to the *cyclin B* mRNA. This event may be responsible for the dissociation of Maskin from eIF4E, the assembly of the eIF4E-eIF4G initiation complex, and translation of *cyclin B* mRNA.

tion in the embryo appears to be very different. Here, CPEB and Maskin are mostly confined to animal-pole blastomeres, the cells that give rise to ectodermal structures such as the skin and nervous system. Within these blastomeres, CPEB and Maskin and *cyclin B* mRNA are associated with mitotic spindles and centrosomes (*12*), the microtubule machinery that separates chromosome pairs during the final stages of cell division. The abrogation of CPEB or Maskin activity by microinjection of antibodies or dominant-negative mutant proteins inhibits cell division and induces multipolar spindle assembly and accumulation of excess centrosomes. Importantly, injection of a mutated form of CPEB that is unable to associate with microtubules has little effect on cyclin B synthesis but causes *cyclin B* mRNA to detach from the spindles. This leads to a dramatic decrease in cyclin B protein accumulation at the spindles and the blocking of cell division. Disruption of spindle-associated translation of *cyclin B* mRNA thus appears to block normal progression through the cell cycle. This unexpected observation suggests that controlling the location of cyclin B production, in addition to regulating the time and place of cyclin B destruction, may be essential for cells to progress through the cell cycle.

It is unclear whether precise spatial control of cyclin mRNA translation is specific to early embryonic development, or is common to all cells. In early embryos, the cells are generally large and the production of mitotic cyclins at their site of action (the centrosome and spindle apparatus) may be particularly important for cell cycle progression. In smaller somatic cells, by contrast, there would appear to be relatively little need for local translation of *cyclin B* mRNA. However, cyclin B Cdk1 is capable of modifying an enormous number of cellular proteins, at least in vitro. Local translation of this relatively nonspecific kinase could help to generate critical substrate specificity, even in smaller somatic cells. Thus, cell cycle progression and patterning during embryonic development may be regulated by the local control of mRNA translation as well as by the temporal and local control of protein degradation.

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PERSPECTIVES: SIGNAL TRANSDUCTION

Bringing Channels Closer to the Action!

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Cells are arrayed with a large number of surface receptors that enable them to recognize and respond to neurotransmitters, hormones, odorants, and growth factors. When these extracellular ligands bind to their receptors, they activate a cascade of intracellular signals that alter effector molecules such as enzymes or ion channels, leading to the generation of physiological responses. Many plasma membrane receptors belong to the extensive G protein–coupled receptor (GPCR) family. When bound to their ligands, GPCRs become activated and interact with heterotrimeric guanine nucleotide binding proteins (G proteins), which dissociate into Gα and Gβγ subunits. These subunits then amplify and propagate signals within the cell—by regulating the production of second messenger molecules such as adenosine 3′,5′-monophosphate (cAMP)—resulting in altered activity of effector proteins such as enzymes or ion channels.

There are many GPCRs at the cell surface that activate different G proteins and modulate different downstream effector molecules. Moreover, different GPCRs expressed in the same cell can activate the same G protein and effector molecule, yet elicit completely different physiological responses. How, then, do cells manage to ensure that one signaling pathway is selectively and rapidly engaged without the activation of other pathways? The idea is emerging that cells might achieve the required specificity and rapidity by organizing macromolecular signaling complexes in the plasma membrane that contain the GPCR, its G protein, the enzyme generating the second messenger, and the effector protein (see the figure). An elegant example of how such a signaling complex might work is presented by Davare *et al.* on page 98 of this issue (*1*).

Stimulation of the β_2 adrenergic receptor (β_2AR), a GPCR, by its ligand results in activation of a signaling pathway that ultimately increases the activity of the L-type class C calcium channel, $Ca_v1.2$. An increase in $Ca_v1.2$ channel activity results in altered contraction of heart muscle and modulation of nerve impulses in brain neurons. By immunoprecipitating β_2AR from rat hippocampal neurons, Davare and colleagues (*1*) provide evidence that β_2AR is associated with the central pore-forming α_{1C} subunit of $Ca_v1.2$. This association appears to be specific for β_2AR because the α_{1C} subunit was not detected in immune complexes containing other neuronal GPCRs. The authors pinpoint the carboxyl terminus of β_2AR as the site where this receptor interacts with the $Ca_v1.2$ α_{1C} subunit. They also show that β_2AR colocalizes with the $Ca_v1.2$ channel at postsynaptic sites (including the dendritic spines) of excitatory neurons.

Although these results indicate an association between the β_2AR and the $Ca_v1.2$ calcium channel, a much more elaborate complex presumably exists. Stimulation of β_2AR results in activation of cAMP–dependent protein kinase A (PKA), phosphorylation of the α_{1C} subunit, and increased activity of the $Ca_v1.2$ channel (*1*, *2*). Attenuation of channel activity depends on specific phosphatases that dephosphorylate the α_{1C} subunit. Both PKA and the phosphatase PP2A directly associate with the α_{1C} subunit and modulate its activity (*2*). Davare *et al.* now demonstrate that the α and βγ G protein subunits as well as adenylyl cyclase (the enzyme that catalyzes cAMP production) also associate with the α_{1C} subunit. Thus, β_2AR and the $Ca_v1.2$ calcium channel presumably assemble into a macromolecular complex that includes the G protein subunits, adenylyl cyclase, PKA, and the counterbalancing phosphatase PP2A (see the figure). But how does this concoction of proteins propagate signals within the cell?

The investigators address this question by recording the activity of the $Ca_v1.2$ calcium channel in rat hippocampal neurons before and after activation of β_2AR with albuterol. (Albuterol is a selective β_2AR agonist that mimics epinephrine, the natural

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