methyltransferase enzymes from methanogenic archaea encodes Pyl. They went on to show[5] that the biological machinery associated with Pyl's synthesis and incorporation into proteins is encoded by the *pylTSBCD* cluster of genes, which can be thought of as a genetic-code expansion cassette — a gene cluster that, when transferred into an organism, enables that organism's ribosomes to recognize UAG and translate it into Pyl. The ribosome therefore 'reads through' this stop codon and catalyses a reaction between the Pyl–tRNA and the preceding peptidyl–tRNA to insert Pyl into a protein. The protein PylS was found to be the Pyl–tRNA synthetase[6], whereas PylT was identified[7] as the Pyl–tRNA. The remaining *pylBCD* genes in the cassette were therefore expected to encode the enzymatic pathway for Pyl biosynthesis.

It has been proposed that Pyl derives from lysine and some other cellular compound — possibly one of the amino acids D-ornithine[8], D-glutamate[9], D-isoleucine[8] or D-proline[5,8]. But Krzycki and colleagues[4] have now demonstrated that the protein products of the *pylBCD* genes catalyse the synthesis of Pyl from two lysines using the pathway shown in Figure 1.

The authors began by genetically engineering a common laboratory strain of the bacterium *Escherichia coli* to include the *pylTSBCD* expansion cassette from the archaeon *Methanosarcina acetivorans* and the methyltransferase gene *mtmB1* from another archaeon, *Methanosarcina barkeri*. The mRNA sequence of mtmB1 contains the UAG stop codon that specifies Pyl. Krzycki *et al.* then provided the engineered *E. coli* with lysine in which all six carbon atoms and both nitrogen atoms were isotopically labelled, and later purified the methyltransferase produced by the organism.

To decipher the biosynthetic pathway for Pyl, the authors used mass spectrometry to accurately measure the masses of peptide fragments (produced *in situ* in the mass spectrometer) of the purified methyltransferase. By comparison with a similar analysis of methyltransferase purified from engineered *E. coli* grown in unlabelled lysine, they identified a single labelled Pyl-containing peptide fragment. Further mass spectrometry experiments unambiguously revealed that all 12 carbon atoms in the Pyl residue and all three of its nitrogen atoms were isotopically labelled. Because lysine contains six carbons and two nitrogens, the results conclusively demonstrated that two molecules of lysine combine to produce Pyl, and that one of the lysines eliminates a nitrogen atom during the PylBCD-catalysed biosynthetic pathway (Fig. 1). In other words, no precursor other than lysine is used in the biosynthesis of Pyl.

These results are surprising in light of a report[8] that D-ornithine stimulates UAG read-through in an *E. coli* strain similarly engineered to contain the *pylTSBCD* expansion cassette — a finding that suggests that D-ornithine is a precursor of Pyl. To investigate the apparent disparity, Krzycki and co-workers[4] performed mass spectrometric analysis of the methyltransferase obtained from engineered *E. coli* cultures grown in a medium supplemented with both unlabelled D-ornithine and labelled lysine. They discovered that some of this methyltransferase contained labelled Pyl, as before. However, part of the protein contained desmethylpyrrolysine, an amino acid in which the methyl group of Pyl has been replaced by a hydrogen atom. Desmethylpyrrolysine can be made from one lysine and one D-ornithine, suggesting that D-ornithine was charged onto Pyl-tRNA by the Pyl-tRNA synthetase and thereby misincorporated into the methyltransferase. This implies that the Pyl biosynthetic cassette could be used to incorporate useful modified amino-acid residues into proteins — something that is of interest to many research laboratories.

One limitation of Krzycki and colleagues' study[4] is that the pylBCD-encoded proteins were not purified and used to demonstrate their proposed activities. But on the basis of the similarity of the amino-acid sequences of PylB, PylC and PylD to other proteins whose functions are known, the biosynthetic pathway proposed by the authors is reasonable and chemically feasible. The door is now open for enzymologists to study the Pyl biosynthetic pathway in detail. A prime target for investigation is the PylB-catalysed lysine mutase reaction, in which an aminoethyl group ($CH_2CH_2NH_2$) shifts from one part of the molecule to another (Fig. 1a). This is particularly interesting because the amino-acid sequence of PylB suggests that it is a member of the radical *S*-adenosylmethionine protein family[10], which is not currently known to catalyse this reaction. More broadly, these findings will help us to better understand the relationship between the evolution of the genetic code and of amino-acid biosynthetic pathways. ■

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CLIMATE

Cold winters from warm oceans

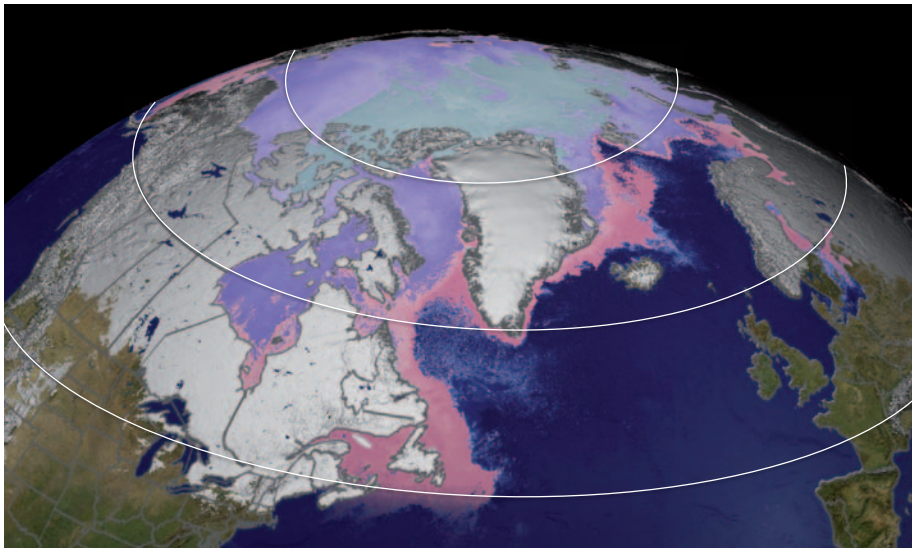
Winters are colder in northeastern North America and Asia than in other regions at the same latitude. Previous explanations may be incomplete, having overlooked the radiation of atmospheric wave energy. SEE LETTER P.621

WILLIAM R. BOOS

Barcelona and London both lie north of New York, yet winters in these two European cities are warmer than winters in the northeastern United States (Fig. 1). It would seem easy to attribute this difference simply to the fact that Europe lies downwind of the warm North Atlantic Ocean. But Kaspi and Schneider (page 621 of this issue)[1] put forward the counter-intuitive argument that the northeastern parts of North America, and also of Asia, are instead cooled in winter by heat released from oceans just off their coasts.

No exotic thermodynamics is needed to create continental cold from oceanic warmth. Instead, Kaspi and Schneider show that heat emitted by a warm swathe of mid-latitude ocean can set up a pattern of north–south flow that draws cold Arctic air into regions immediately upstream of the heat source. Waters just east of Asia and North America do indeed emit large amounts of heat into the atmosphere during winter, when prevailing eastward winds draw cold continental air over the warm waters of the Gulf Stream and the Kuroshio, Asia's analogous continental boundary current.

Two main processes were previously thought to create the wintertime thermal contrast across the Atlantic, the first of which is the warming of Europe by heat drawn out of the ocean by prevailing eastward winds[2]. The North Atlantic itself is kept warm in winter by storage of the previous summer's heat and by ocean currents that transport heat from lower latitudes. There has been some excitement in



NASA/GODDARD SPACE FLIGHT CENTER SCIENTIFIC VISUALIZATION STUDIO; G. RIGGS (NASA/SSAI)

Figure 1 | Winter temperature asymmetry. This image of snow cover over North America and Europe was taken on 23 March 2003 by NASA's Terra satellite using the MODIS instrument. Approximate latitudes at 30° N, 45° N, 60° N and 75° N are marked. Note that northeastern North America is almost entirely snow covered between 45° N and 60° N, but that there is little snow at the same latitudes in western Europe. Also shown is sea-ice surface temperature (imaged by the MODIS instrument on NASA's Aqua satellite): pink, temperatures between 0 °C and −15 °C; purple, temperatures between −15 °C and −28 °C; white (in the deep Arctic), temperatures colder than −28 °C. Kaspi and Schneider[1] invoke the influence of Rossby-wave plumes as a partial explanation of this transatlantic asymmetry.

the popular press over the idea that Europe might be plunged into a deep freeze if northward ocean heat transport were to weaken as the planet warms[3], but simulations of such an ocean circulation decline suggest that it would merely counteract part of the warming that Europe would otherwise experience because of an increase in greenhouse-gas emissions[4]. The issue has been further muddled by conflation of the density-driven ocean circulation that transports heat, which might be altered by redistribution of ocean salinity in a changed climate, with the Gulf Stream, a wind-driven current that owes its existence to the less mutable principle of the conservation of angular momentum on a rotating planet[5].

The other accepted cause of Europe's relative winter warmth, and one that seems less challenged by Kaspi and Schneider's result[1], is the steady pattern of wind set up by mountains. As eastward mid-latitude winds impinge on the Rocky Mountains, the downstream flow is perturbed to blow southward over the northeastern United States and northward over Europe, drawing cold and warm air, respectively, into those regions[2,6]. Mountains perturb the flow by creating a particular type of wave that exists in planetary oceans and atmospheres, called a Rossby wave, which also happens to be central to the east-coast cooling mechanism proposed by Kaspi and Schneider.

Waves in general exist because some force accelerates a disturbed medium back towards its equilibrium position, with gravity providing the restoring force for the familiar example of ripples on the surface of a pond. In Earth's Northern Hemisphere, the Coriolis force accelerates moving masses to the right, and this force can produce waves that typically span thousands of kilometres and involve almost entirely horizontal motions[7]. These are Rossby waves, and they are peculiar in that their crests propagate only westward relative to the flow in which they are embedded. When Rossby waves are triggered by eastward winds hitting mountains, some waves travel against the flow fast enough to be stationary relative to Earth's surface. The north–south flow set up by these stationary waves creates temperature contrasts downstream of mountains by transporting air from distant latitudes.

Kaspi and Schneider[1] show that Rossby waves can also induce southward winds upstream of a mid-latitude heat source by a mechanism quite distinct from that involving flow over mountains. Rossby waves are dispersive, meaning that different frequencies travel at different speeds, and as waves of different frequencies pass through each other, they create envelopes of wave energy. Rossby-wave energy emitted by an atmospheric heat source can propagate upstream faster than the mid-latitude eastward flow, producing horizontal circulations that spread out to the west of the wave source in elongated plumes[7]. Although such Rossby-wave plumes had been known to exist in both the atmosphere and the ocean, it seems that, until now, no one had looked for them in this mid-latitude atmospheric context.

Using an idealized numerical model of an atmosphere over an entirely water-covered planet, Kaspi and Schneider[1] studied the response to a mid-latitude heat source that

had a spatial scale similar to that of the Gulf Stream or the Kuroshio. In their model, a cold region formed upstream of the heating owing to the southward transport of air from more polar regions, and the width of this cold region increased with the rotation rate of their model planet in a manner consistent with the existing theory of Rossby-wave plumes. Kaspi and Schneider argue that upstream cooling by these plumes would explain why the coldest regions of eastern Asia and North America have similar horizontal extents despite large differences in the topography and size of the respective continents.

Many questions remain, one of which is whether signatures of this upstream cooling mechanism can be found in observations and in numerical models with more realistic representations of continents and oceans. It is also unclear how much of the temperature contrast across the Pacific and Atlantic can be explained by Rossby-wave plumes. The authors estimate that these plumes might account for about half, a fraction not inconsistent with a previous finding[2] that the north–south flow patterns set up by mountains account for almost half of these temperature contrasts.

As to whether northeastern North America might warm if oceanic northward heat transport wanes with global warming, that seems unlikely. Simulations using climate models suggest that poleward ocean heat transport warms nearly all longitudes of the extratropical Northern Hemisphere[2,4], and those models should include the Rossby-wave-plume mechanism, even though it may have gone unrecognized. Perhaps even when ocean heat transport is reduced, cold and dry continental air still extracts abundant thermal energy from the warmer ocean just off eastern coasts. Or perhaps the dynamics are relatively insensitive to climate change because the Gulf Stream and Kuroshio are wind-driven.

Kaspi and Schneider's work[1] provides fresh insight into processes that create a notable asymmetry in Earth's climate. It also raises numerous issues regarding how east-coast Rossby-wave plumes might be involved in the seasonal cycle, interannual variability, and climate change over centennial and longer timescales. ■

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DEVELOPMENTAL BIOLOGY

A hair-raising tale

Signals from the external microenvironment or 'niche' determine the fate of stem cells. In the hair follicle, stem cells themselves seem to act as a niche for the adjoining muscle cells that cause goosebumps.

BRUCE A. MORGAN

When you are angry, cold or scared, your hairs stand up. This response is mediated by the arrector pili muscle, which runs from the bulge — the permanent portion of the hair follicle — to the skin surface. The muscle's contraction shifts the angle of the hair relative to the skin. Piloerection, or goosebumps, makes the hair a better insulator, and alters its appearance to send a message, gruff or bluff. Writing in *Cell*, Fujiwara *et al.*[1] describe how the stem cells of the bulge act as a niche for these smooth muscle cells, coordinating their differentiation and ensuring their correct localization with high reproducibility. Although piloerection still seems to occur even when the muscle cells are not attached in precisely the right position, these data provide valuable insight into stem-cell biology.

The hair follicle has become a focus of research, in part because, in adults, its lower portion undergoes cycles of degeneration and regeneration. The keratinocytes that regenerate the follicle and hair shaft are derived from a stem-cell population housed in the bulge (Fig. 1).

Early observations suggested a simple model in which label-retaining cells (LRCs) — the most quiescent population of stem cells in the follicular epithelium — lie at the base of a stem-cell/progenitor-cell hierarchy[2]. At the outset of the regeneration phase, LRCs were thought to undergo asymmetrical division, generating transient amplifying cells — cells with less proliferative and developmental potential that ultimately form the differentiated cell types of the lower follicle and hair shaft. The LRCs reside in the bulge region that is adjacent to the attachment site of the arrector pili muscle (APM), and the APM was speculated to serve as a niche to maintain follicular stem cells.

Direct analysis of keratinocyte dynamics in the follicle has revealed a more complicated story that further emphasizes the potential importance of a niche. For instance, the most quiescent LRCs have a more restricted function in follicular regeneration (perhaps serving as a 'reserve' stem-cell pool), whereas other keratinocyte precursors in the permanent follicle have more active roles in the cyclical regeneration process[3].

Moreover, asymmetrical division does not seem to be the mechanism that generates a fate-restricted progenitor that is distinct from the stem cell. Instead, position within the follicle seems to have a more essential role in the retention of stem-cell character. The cells that actively maintain the follicle are arranged both in a stem-cell zone — which encompasses the LRCs and is characterized by the expression of the marker protein CD34 — and in the secondary germ, which lies below it (Fig. 1).

The stem-cell zone is a component of the permanent follicle, whereas the secondary germ is a transient structure incorporated into the regenerating lower follicle. Notably, there is substantial flux of keratinocytes out of and into the stem-cell zone, not only in response to injury, but also with each cycle of regeneration and degeneration[3–5]. Despite all this movement, zones of gene expression and correlated biological properties are maintained. In this context, local cues might be expected to play an essential part in sustaining this follicular architecture.

Fujiwara *et al.*[1] set out to test the function of proteins produced by stem cells and selectively deposited in the basement membrane of the bulge region. They chose nephronectin, a protein that keratinocyte stem cells deposit in the extracellular matrix of the bulge. They found that nephronectin guides the attachment of APM cells to the bulge by binding with high affinity to the α8β1 integrin receptor on the surface of these cells.

The authors report that mice lacking either nephronectin or the α8 integrin chain in their skin still form the APM in most of their follicles. But when the gene encoding nephronectin is mutated, the APM most frequently attaches slightly higher than normal on the follicle. Similarly, although in α8-deficient mice the APM usually finds its normal attachment site, it attaches higher up on the follicle more frequently than in normal animals. APM attachment close to its normal site in both mutants may be explained by the presence above the bulge of EGFL6 — another ligand that binds to the α8β1 integrin receptor, albeit