development of liver cancers. So TNF-α, produced by surrounding stromal cells, activated an NF-κB-dependent pathway that inhibited cell death during the period when precancerous hepatocytes developed into cancers.

In the model described by Greten *et al.*[6], selective deletion of IKK-β — a key intermediary of NF-κB — in cells of the intestinal lining did not decrease intestinal inflammation, but did reduce the subsequent development of intestinal tumours. As in the liver-cancer model, preventing NF-κB activation led to the death of more of the intestinal cells that would otherwise have given rise to cancer. However, Greten *et al.* went one stage further by generating mice in which IKK-β was selectively deleted in inflammatory cells that infiltrate pre-malignant and malignant tumours. In these mice, the levels of messenger RNAs encoding several pro-inflammatory cytokines decreased, as did subsequent tumour development.

Cancers develop in phases. First there is thought to be an initiating event — genetic damage that renders an epithelial cell (such as a hepatocyte or a cell lining the intestine) capable of forming a cancer. Initiated precancerous cells then multiply during a promotion phase, when further genetic damage is incurred. And, in the case of inflammation-induced cancer, non-genetic stimuli also encourage the survival and proliferation of initiated cells.

The data obtained in these two cancer models[5,6] suggest that the NF-κB pathway does not affect initiation but has dual actions in tumour promotion: first by preventing the death of cells with malignant potential, and second by stimulating the production of pro-inflammatory cytokines in inflammatory cells in the tumour mass. The pro-inflammatory cytokines then signal to initiated or otherwise 'damaged' cells to promote their survival and proliferation (Fig. 1). This model is consistent with other studies[7–9] and with observed correlations between the numbers of inflammatory cells, levels of cytokines and tumour severity and prognosis in both mice and humans[4].

However, in some human and mouse cancers, the transformed cells themselves can also contribute to the overall levels of soluble pro-inflammatory cytokines. For example, TNF-α produced by skin cells (keratinocytes) is a tumour promoter in experimental skin cancer, where it produces a cascade of cytokines and of enzymes that degrade the extracellular matrix during the early stages of tumour promotion[7]. (The transcription factor implicated here, however, is AP-1 rather than NF-κB[10].) Human keratinocytes also produce TNF-α in response to ultraviolet irradiation[11]. Other studies show that TNF-α produced by hepatocytes (rather than by neighbouring inflammatory cells) acts as a hepatocyte growth factor, and that mice lacking TNF receptors are resistant to chemically induced liver carcinogenesis[12]. Moreover, as Pikarsky *et al.* and Greten *et al.* state, activation of NF-κB occurs in many malignant cells as a result of genetic mutation rather than in response to signals from surrounding cells. So pro-inflammatory cytokines contribute to tumour promotion not only by signalling from tumour-associated inflammatory cells to precancerous cells, but also through production in the precancerous cells themselves, especially at early stages.

Should future anticancer strategies focus on regulating NF-κB activation or the availability of TNF-α? It is important to note that all organs are endowed with unique cell-death pathways, as well as with damage-response pathways that typically involve short-term activation of innate immune cells. In the skin, for example, keratinocytes die through 'terminal differentiation', and inhibiting NF-κB in initiated keratinocytes actually promotes one type of cancer by reducing terminal differentiation[13]. Conversely, blocking TNF-α attenuates chemically induced skin-tumour formation[14]. The idea of therapeutically regulating TNF-α, however, must also be thoroughly investigated, as this cytokine, too, has opposing activities that depend on the cell type and environment[15]. Phase I clinical trials of TNF-α antagonists are currently under way in patients with advanced cancer, and might help researchers to understand these issues[14,15]. The new results[5,6] also suggest that any anti-inflammatory therapy would be most effective during the early stages of cancer development.

Tissues have a complicated architecture, and their cells respond to damage and die through inflammation-dependent and -independent mechanisms. So it is no wonder that inflammatory mediators such as NF-κB and TNF-α have many different effects, depending on the context in which they are called into play. The studies by Pikarsky *et al.*[5] and Greten *et al.*[6] articulate these complexities, and show that, in devising anticancer strategies, we must consider the context in which tumour cells thrive, as well as the cells themselves. ■

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Global change

Carbon conundrum on the tundra

Wendy M. Loya and Paul Grogan

Vast amounts of carbon are locked into soils at northern high latitudes. The vexed question of how these ecosystems will respond to global warming is addressed by a long-term experiment in the Arctic.

On page 440 of this issue[1], Mack and colleagues describe how they dug deep into the carbon balance of an arctic tundra ecosystem, and came up with some surprising results. Their research reveals that tundra plants and soils respond in opposing ways to long-term nutrient fertilization. In their experiment, intended to simulate increased nutrient availability under warmer temperatures, plants grew better and stored more carbon, but valuable soil carbon was lost. In the context of global warming, the main implication of their findings is that the losses of deep-soil carbon that they observe could mean even greater increases in carbon dioxide concentrations in the atmosphere.

The carbon balance of terrestrial ecosystems is the difference between carbon storage in plants and soils, and carbon losses resulting from decomposition of these tissues. In the cold, wet climate of high latitudes, decomposition proceeds slowly, and carbon accumulates in thick layers of organic matter on top of mineral soils. This net carbon storage in tundra and boreal forests accounts for an estimated one-third of the global soil-carbon pool[2], and is equivalent to two-thirds of the carbon presently found in the atmosphere. Hence the worry that changes in climate resulting in a warmer, drier environment might alter this balance through accelerated decomposition of soil organic matter. This could result in net carbon losses, positive feedback into increases in atmospheric concentrations of CO_2, and accentuated climate change. On the

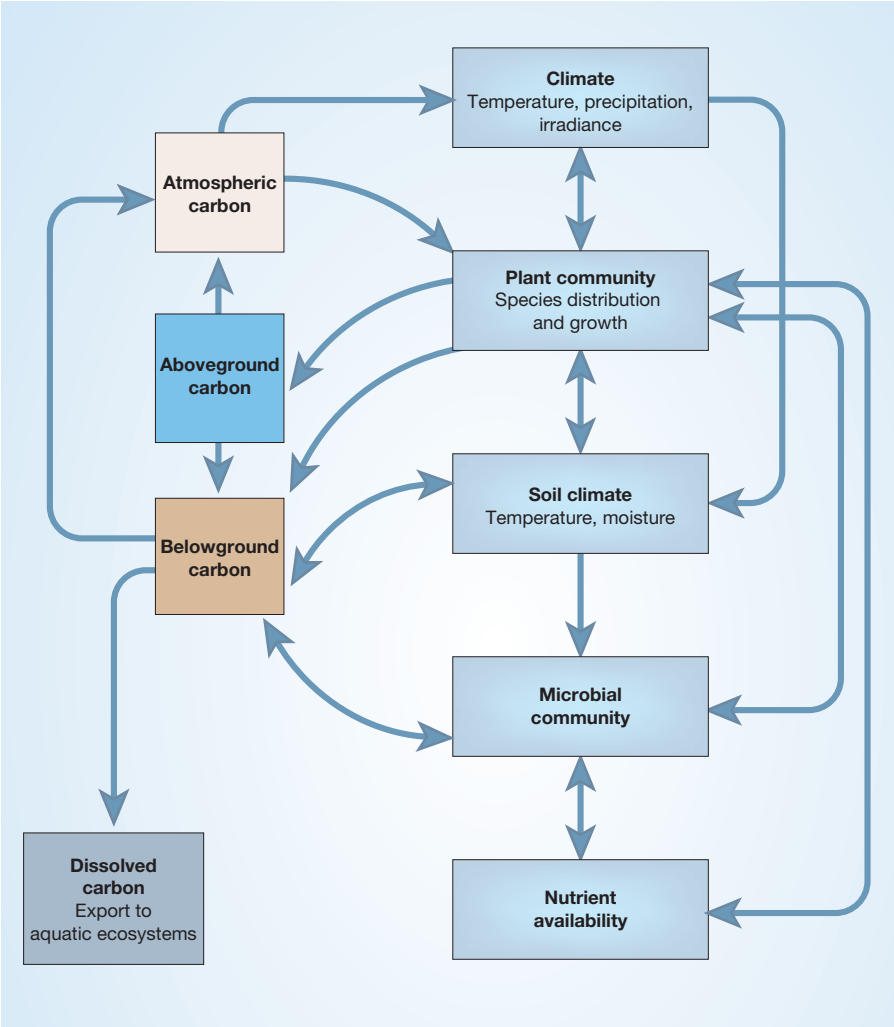

Figure 1 **Carbon connections in high-latitude terrestrial ecosystems. On the left are the main pools in which carbon is stored; on the right are the factors that determine the exchange of carbon between those pools. The interactions tested experimentally by Mack *et al.*[1] in their fertilization experiments are those between nutrient availability and the plant community, and the consequences for carbon storage above and below ground.**

other hand, warming-induced decomposition could also release nutrients that stimulate plant growth, potentially allowing plants to store more carbon and offset losses of soil carbon.

Predicting the direction of change is complicated by factors that may act independently of one another or may interact to control the carbon balance in high-latitude ecosystems (Fig. 1). Some observations[3] suggest that the natural balance of carbon storage and carbon losses is variable — under some conditions the tundra stores carbon; at other times there is a net loss. But it is difficult to predict long-term patterns without decades of monitoring or experimental manipulations that accelerate natural processes.

Mack *et al.*[1] present insights from using both approaches. For more than two decades they have investigated the effects of adding nitrogen and phosphorus to test how nutrient availability controls the carbon balance of moist acidic tundra, a circumpolar ecosystem type. By constructing a carbon budget for both fertilized and unfertilized plots, Mack *et al.* confirm observations that increased nutrient availability increases carbon storage in plants through accelerated growth of woody shrubs[4]. They also found greater accumulation of carbon in standing dead material and leaf litter on the soil surface, as well as in roots and organic matter in the upper layers of the soil.

However, after digging a little deeper, they discovered that the lower layer of organic matter decreased in thickness, and that there was less carbon in the underlying mineral soil. Also, compared with controls, they found fewer roots growing in the deep soils in the fertilized plots, although total root growth was estimated to be the same. The increase in carbon storage in plants and at the soil surface could not offset the losses deep down, and Mack *et al.*[1] calculate that fertilization caused an overall net carbon loss of 2 kg m^{-2} within two decades, as carbon below ground decreased from about 9 kg m^{-2} in control plots to about 7 kg m^{-2} in fertilized plots.

So where did the carbon go? Although the pathways through which the carbon escaped were not measured directly, the physical reduction in organic matter clearly indicates a loss of carbon from the soil. The authors hypothesize that increased nutrient availability stimulated the activity of decomposer microorganisms, which then proceeded to consume the older, deeper organic matter. If so, these data challenge the dogma that soil microbial activity is generally limited by the availability of carbon[5], and they support research indicating that microbes in subsurface soil layers may be constrained more by nutrients than by carbon[6]. In addition, some carbon was probably leached away by melting snow or by rainfall, possibly affecting aquatic ecosystems. Microbial activity, in the form of nitrification and denitrification, and nitrate leaching, would also explain where some of the nitrogen has gone — despite inputs of 10 g m^{-2} yr^{-1}, the researchers did not find more nitrogen in the fertilized tundra.

The implications of this fertilization experiment, according to Mack *et al.*, are that predicted increases in nutrient availability associated with decomposition of organic matter under warmer temperatures could prime further losses of organic matter. If that loss is accounted for by release of CO_2 into the atmosphere, there would be an additional positive-feedback effect, with enhanced greenhouse-gas-induced warming further increasing decomposition until soils are depleted of carbon and nitrogen. The results question the hypothesis that increased nutrient availability could increase total ecosystem carbon storage through enhanced aboveground productivity[7]. And they are alarming in that the Arctic has been warming at accelerated rates during the past three decades[8], coincident with an increase in shrub expansion[9] that possibly signifies that regional carbon losses may already be occurring.

However, it is necessary to remember that this is not a direct observation of warming-induced changes. Among other methodological limitations[10], the direct addition of nutrients in the mineral forms most readily taken up by plants means that microbes are cut out of the initial decomposition process that would lead to the theoretical release of these nutrients. Thus, the natural pathways of carbon and nutrient cycling (Fig. 1) have been bypassed. How an experimentally manipulated supply of nutrients compares with a warming-induced increase is unknown. Moreover, interactions between changing climatic variables — temperature, precipitation, irradiance and higher CO_2 levels — may or may not result in the accelerated decomposition of organic matter or shifts in species composition. In experiments where air temperatures have been warmed by constructing greenhouses on the tundra,

the plant response is not as dramatic as it is with high levels of fertilization[11].

Nonetheless, the authors have demonstrated that long-term fertilization resulted in net carbon loss from the tundra ecosystem, probably as a result of increased decomposition of soil organic matter. Research to find out whether decomposition is stimulated directly by increased nutrient availability, or indirectly through changes in the plant or microbial community, will help to identify the pathways involved and to link experiments with forecasts of global climate change.

Finally, these findings add to a growing body of evidence suggesting that increases in aboveground carbon storage through woody-plant encroachment may be offset by losses of soil carbon[12,13]. Here again, we have a reminder of the need for accurate assessments of carbon storage, both above and below ground, in order to predict ecosystem response to environmental change. ■

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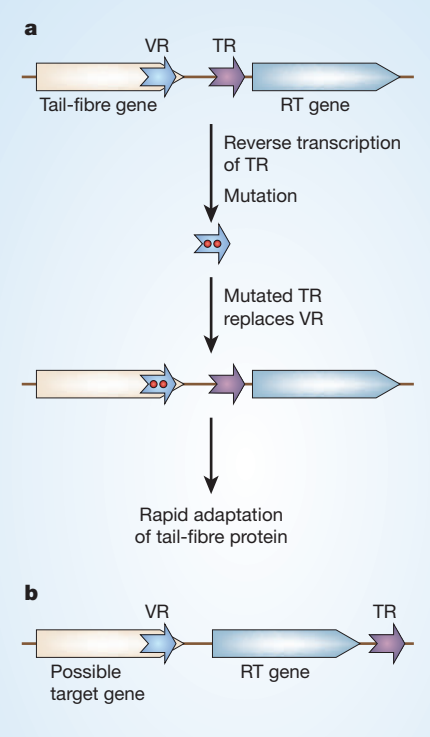

Figure 1 **Generating diversity with reverse transcriptase.** a, **Bacteriophage BPP-1, a virus that attacks bacteria of the *Bordetella* genus, contains a reverse transcriptase (RT) enzyme, which, unlike other viral RTs, is not needed for replication. Instead, its role seems to be to create vast diversity within a strictly circumscribed region of the bacteriophage genome, namely the region that encodes the tail-fibre protein, which is needed for the virus to bind to bacteria. This region includes, in addition to the tail-fibre gene, two nearly identical sequences called the variable repeat (VR) and the tandem repeat (TR). TR provides an invariant master source of sequence information. It is copied by RT, during which, as Doulatov *et al.*[1] show, mutations (dots) occur specifically at adenine residues by a process that is not yet understood. The product then replaces a segment of the VR, creating a slightly altered tail-fibre gene.** b, **Doulatov *et al.* also discovered similar VR–TR–RT cassettes in various bacteria, as well as the one shown, from *Nostoc* species (blue-green algae).**

Evolution

A is for adaptation

Jef D. Boeke

Studies of a bacterial virus have revealed an unexpected weapon that helps it to overcome its host's rapidly changing defences. A look at other organisms hints that the mechanism might be widespread.

Adapt or die. This axiom has been used so many times, in so many different contexts, that its origin is difficult to trace. But it aptly describes an intriguing mechanism exploited by viruses that infect *Bordetella* bacteria. This mechanism guarantees the viruses' survival in the face of host adaptations that would otherwise severely limit their ability to infect and multiply. On page 476 of this issue, Doulatov *et al.*[1] describe how these viruses use a form of the enzyme reverse transcriptase (RT) to generate variability very specifically in the gene encoding a protein required for binding to the bacterial surface. RTs use RNA sequences as templates to generate complementary DNA (cDNA). In this case, it is proposed that the cDNA product directs RT-induced mutations to just the right spot in the viral genome. Natural selection then acts on the mutated progeny viruses to select for those 'winners' that can infect bacteria efficiently. This work sheds new light on the pervasive presence of RT genes in many genomes, suggesting another way in which such genes can benefit the organisms in which they reside.

The *Bordetella* genus includes the causative agents of human whooping cough and canine kennel cough. These bacteria have an elaborate system for evading the immune system of the organism they infect — the nature of their surface proteins is constantly changing. This presents a formidable challenge to any virus (bacteriophage) attempting to infect *Bordetella* cells, because the first step of viral multiplication is attachment to the bacterial cell surface. Such viruses must evolve exceedingly rapidly to keep pace with a dynamic surface structure as their bacterial host undergoes its own infectious cycle. This is evolution on a very fast track indeed.

The bacteriophage BPP-1 has evolved just such a diversity-generating system, which spawns variant progeny bacteriophages possessing altered surface-binding properties at very high rates (at least 0.1% of the progeny can have significantly altered surface properties). Previous work found that this process is facilitated by interactions between the bacteriophage-encoded RT and two closely related sequences, 134 base pairs long, that lie respectively within and near the gene encoding the surface-binding protein (Fig. 1)[2]. These sequences are called the variable repeat (VR) and the tandem repeat (TR). The TR is an unchanging sequence that provides the raw material for generating variability. On the basis of limited information, it was proposed that the TR is transcribed into an as-yet-undefined RNA, which could in turn be copied by the RT in a highly error-prone manner, generating a hypervariable cDNA product. This would replace a segment of the VR in the genome, producing a swarm of viruses with altered VR sequences in the next generation. So, this VR–TR–RT 'cassette' would maintain TR in a pristine state, while generating diversity in VR.

Doulatov and colleagues[1] now provide more support for this model, and fill in some of the gaps. They show that, remarkably, the diversity-generating system mutates only adenine (A) bases in the TR: by replacing one of the three possible non-A bases in TR with A, they found that the previously invariant base is converted to a hypervariable position in VR. The 23 A residues in the TR sequence