division cycles of precancerous cells — but not equally rapid divisions in normal tissues — can elicit the DDR. The authors argue[1,2] that the trigger involves DNA replication 'stress': the idea is that the replication machinery performs differently when activated by aberrant, rather than physiological, stimuli. In support of this, the authors offer evidence for abnormalities in replication when proliferation is driven by oncogenic stimuli *in vitro* or in tissues. Provocatively, for example, both groups find that allelic imbalances (signifying chromosomal translocations or deletions) in the genomes of incipient cancer cells occur frequently at 'fragile sites', believed to resist easy copying by the replication machinery.

Replication 'stress' conceived in this way is somewhat nebulous. A hard look at what could distinguish aberrant from physiological stimulation of DNA replication is now needed. Much thinking about the initiation of DNA replication is dominated by a simple 'on–off' concept, in which the replication machinery is loaded onto DNA when key enzymes (cyclin–CDK complexes) are 'off', and then activated when these enzymes are turned 'on'. Yet evidence is emerging[10] for more complex regulatory circuits. Here, the nature and intensity of growth-promoting stimuli are integrated by different levels of activity of cyclin–CDK complexes and of another crucial enzyme, the anaphase-promoting complex, to affect the assembly and operation of the replication machinery. Oncogenic stimuli could perturb these circuits, leading to aberrant replication[11,12].

But how aberrant replication might trigger a DDR is far from clear. Cancer cell cycles could generate excessive amounts of normal intermediates, such as single-stranded DNA, spawn abnormal structures, such as double-strand breaks, or even lower the threshold for DDR activation[13]. Identifying the triggering events will be vital to understanding what distinguishes normal from cancer cell cycles.

Alternative scenarios are also possible. In dividing cells, replication is frequently blocked[14] by problems such as oxidative changes to DNA bases. If such problems are unresolved, stalled replication creates abnormal DNA intermediates that trigger the DDR. So, could another difference between normal and cancer cell cycles relate to metabolic changes that augment DNA base lesions? For instance, overexpression of the oncogene *Myc* leads to the production of reactive oxygen species[15]. Bartkova *et al.*[1] find that antioxidants have little effect on oncogene-induced DDRs *in vitro*. But, given the high oxygen tensions under which tissue culture is performed relative to *in vivo* conditions, it would be premature to discard this possibility altogether.

Whatever its underlying mechanism, replication 'stress' as a trigger for DDR activation calls attention to the network of tumour-suppressor proteins that monitors genome integrity during DNA replication[16]. Besides ATM, ATR, Chk2 and p53, which enforce cell-cycle checkpoints during the DDR, the network includes Fanconi anaemia proteins and the breast-cancer-susceptibility proteins BRCA1 and BRCA2, which are more directly involved in processing replication-blocking lesions[17]. The proposals discussed here suggest that oncogenic stimuli will generate selective pressure for this network to be suppressed during carcinogenesis. Conversely, the proposals could also help to explain why inherited mutations that affect network components, and thereby potentially lower the barrier to uncontrolled division, predispose people to cancer. What we know about the involvement of these tumour suppressors in cancer is not fully consistent with the predictions, however, hinting that further nuances are yet to be discovered.

An interesting twist also reported in this issue is that BRCA2-deficient cells (which cannot deal with stalled replication[18]) can be killed by overloading them with replication-blocking DNA damage, using inhibitors of DNA repair[19,20] (pages 913, 917). Along similar lines, it has been suggested that DDR inhibitors might provide a means to sensitize cancers to therapeutic radiation. The work of Bartkova, Gourgolis and colleagues[1,2] suggests that there could be a long-term price to pay in either situation — in non-malignant cells — if these interventions also overburden, or stifle, the tumour-suppressor network that senses and stops cancer cell cycles. ■

Ashok R. Venkitaraman is in the Cancer Research UK Department of Oncology and the Medical Research Council Cancer Cell Unit, University of Cambridge, Hills Road, Cambridge CB2 2XZ, UK.
e-mail: arv22@cam.ac.uk

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Climate change

Water cycle shifts gear

Thomas F. Stocker and Christoph C. Raible

Various studies indicate that the hydrological cycle is speeding up at high northern latitudes. The resulting increase in freshwater flow into the Arctic Ocean is predicted to have long-range effects.

Discussions of global climate change tend to focus on increasing surface temperatures. By contrast, changes in the water cycle — precipitation, evaporation and river discharge — have received little attention. Yet water has profound effects on our planet's climate. The natural greenhouse effect is caused primarily by water vapour; the radiative balance at the Earth's surface is modified by snow and ice cover; the distribution of vegetation types is sensitive to the local water balance; and regional climate patterns are influenced by ocean currents.

Progress in modelling the many aspects of the water cycle is therefore essential to assess the changes that will result from rising levels of greenhouse gases in the atmosphere. A step forward has been made by Wu and co-workers[1], who, in a study published in *Geophysical Research Letters*, have investigated changes in the freshwater balance of the high northern latitudes.

Wu *et al.*[1] used a climate model that links the influences of the oceans, atmosphere and land surface on climate, and that, for example, has been used to demonstrate the contribution of rising greenhouse gases to warming during the twentieth century[2]. Four simulations modelling the climate over the past 140 years form an ensemble that shows large seasonal cycles and interannual variability. The ensemble gives a figure for mean discharge from Eurasian rivers of about 2.3 Berings (Be; see Fig. 1). This compares with an estimate of 1.9 Be based on observational measurements[3]. Given the complexity of the hydrological cycle, and the processes that need to be resolved, this discrepancy is remarkably small.

The ensemble suggests an average increase in Arctic river discharge since the mid-1930s of about 1.8 ± 0.6 mBe yr^{-1}, which compares well with the observation-based estimates of 2 ± 0.7 mBe yr^{-1} (ref. 3).

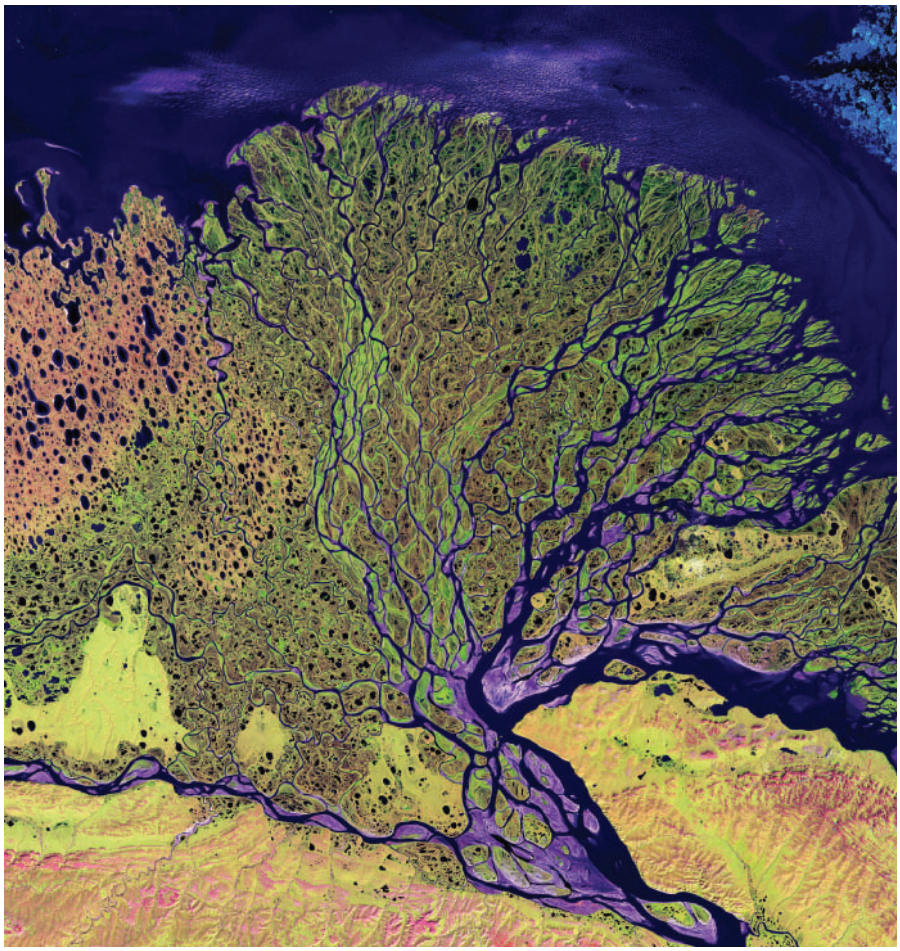

Figure 1 **The vast delta of the Lena River, Russia, which is some 250 km wide. Large rivers such as this collect water from Eurasia and northern North America, and discharge it into the Arctic seas. About half of the total volume delivered to the Arctic comes from Eurasian river systems and amounts to about 1.9 Berings; the Bering (1 Be = 10^3 km^3 yr^{-1}) is a convenient unit of water flux for large-scale hydrological studies. Like the now popular Sverdrup (1 Sv = 10^6 m^3 s^{-1}), used in oceanography, the Bering was proposed by the late Max Dunbar (personal communication). Discharge from Eurasian rivers may increase by 20–70% by the end of this century[3].**

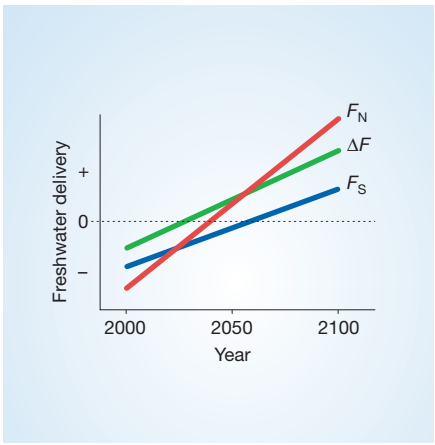

Figure 2 **Projected freshwater delivery to the ocean in the high northern (F_N) and high southern (F_S) latitudes. Delivery is projected to increase in both hemispheres, but at a faster pace in the north owing to greater warming. As simulated by the climate model of Wu *et al.*[1], this results in a transfer of fresh water between the hemispheres of $\Delta F = F_N - F_S$. Such a transfer may change the distribution of the major water masses in the world ocean, and thus affect the meridional overturning circulation.**

As in the observations, this trend was magnified by at least a factor of three during the last 30 years of the twentieth century. In the model, a comparison between simulations with and without greenhouse gases reveals that the increased discharge is clearly associated with warming induced by rising concentrations of greenhouse gases. Such trends in water balance are also detectable in the latest data from observations of the atmosphere[4], particularly in the mid-to-high latitudes of the Northern Hemisphere. The level of natural variability is still large, however, and the changes in the southern high latitudes are less clear because reliable data are not available for the pre-satellite era.

Both observations and models therefore suggest an accelerated hydrological cycle in the atmosphere, which manifests itself as increasing river discharge in the circum-Arctic regions and consequently in a freshening of the northern North Atlantic[5]. Our knowledge of decadal climate variability in the high northern latitudes is limited[6], and this variability might affect the fraction of climate change attributable to various causes, particularly with regard to the hydrological cycle. But it seems unlikely that the simulated changes would be quantitatively consistent with natural variability purely by chance.

Next, Wu *et al.*[1] ran their simulations to the end of the twenty-first century, assuming the increased levels of greenhouse gases predicted by two standard emissions scenarios[7]. In these simulations, the acceleration of freshwater delivery to the Arctic, which started in the last three decades of the twentieth century, continues, and by about 2020 the discharge has risen above the upper end of the simulated range of variability. These results reinforce predictions that fundamental environmental changes will occur in the high latitudes of the Northern Hemisphere[8]. The warming will also melt the permafrost and change the seasonal snow cover, both of which will increase the warming and further accelerate the water cycle.

Wu and colleagues' model predicts that freshwater delivery to the high latitudes will increase in both hemispheres. But the rate of change is larger in the Northern Hemisphere, which will warm more rapidly (Fig. 2). This results in a net transfer of fresh water from the Southern to the Northern Hemisphere. Such a transfer, if it occurs for long enough, could change the properties of the principal water masses in the world's oceans and thus alter the balance of deep-water formation in the far north and far south[9]. Deep-water formation at high latitudes is the process by which warm water, flowing from low latitudes, cools, becomes denser and sinks. The return flow at depth completes the 'meridional overturning circulation' — a major mechanism in oceanic circulation.

As more fresh water is delivered to the Arctic, salinity will be further reduced in areas where deep-water formation takes place, so reducing water density. This might produce a decrease in the meridional overturning circulation in the North Atlantic. Indeed, various models[7] predict such a reduction, and it is confirmed by the latest climate models, which have a much higher resolution[10].

The range of possible effects is still wide. But it is clear that further and more rapid warming will increase the vulnerability of this circulation system[11], possibly leading to a permanent circulation change in the North Atlantic[12]. Potentially irreversible changes in the climate system — such as a shutdown of the meridional overturning circulation, or complete melting of the Greenland ice sheet[13] — are now on the radar screens of climate modellers. Such risks will be assessed

in the forthcoming Fourth Assessment Report of the Intergovernmental Panel on Climate Change, due in early 2007.

The evidence for such dramatic changes is still ambiguous, but distributed modelling[14] and coordinated analyses[15] will allow us to improve our estimates of the probability of such events. Results such as those of Wu *et al.*[1] help us on our way. They testify to the ability of today's models to provide a detailed picture of the environmental changes that continued anthropogenic warming of the planet will cause. ■

Thomas F. Stocker and Christoph C. Raible are at the Division of Climate and Environmental Physics, University of Bern, Sidlerstrasse 5, CH-3012 Bern, Switzerland.
e-mail: stocker@climate.unibe.ch

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Evolution

Warm-hearted crocs

Adam P. Summers

Our ideas about how crocodiles evolved have just taken a battering. It seems that these cold-blooded creatures, with their limited capacity for prolonged activity, might have had active, warm-blooded ancestors.

A crocodile, quietly submerged, waiting to ambush its next meal, is a testament to the benefits of allowing the environment to determine body temperature. Cool water keeps the metabolic rate low, allowing astonishing periods between breaths that can range into hours. Writing in *Physiological and Biochemical Zoology*, Seymour and colleagues[1] propose that inside these armoured predators beats a heart that harks back to ancestors that were terrestrial, active and — most surprisingly — 'warm-blooded'.

Warm-blooded, or 'endothermic', vertebrates (birds and mammals) have high resting metabolic rates, enabling them to maintain a constant body temperature that is usually above that of their environment. Endotherms use more oxygen and need more fuel than cold-blooded 'ectothermic' vertebrates, such as fish, lizards and frogs, with their lower and more variable body temperatures. But, as a consequence, endotherms have gained a capacity for prolonged strenuous activity that can't be matched by ectotherms[2].

Birds and mammals have independently evolved a four-chambered heart divided into two sides, so that the oxygenated blood coming from the lungs is separated from the deoxygenated blood arriving from the rest of the body (Fig. 1a). The three-chambered heart of most reptiles and amphibians is more flexible, allowing controlled mixing of pulmonary and systemic blood (Fig. 1b). This is especially important in diving, where shunting blood from the lungs accelerates the transition to a lower metabolic rate, increasing the time between breaths[3].

A muscular ridge in the three-chambered heart can completely separate oxygenated from deoxygenated blood, so mere separation of blood flow does not explain why a four-chambered heart is so beneficial that it evolved twice[4]. But in the four-chambered heart, the division of blood flow also allows the pressure to vary between the pulmonary and systemic circulatory systems. The side that sends blood to the lungs is much weaker than the other side, because high pressures of blood in the lungs could cause fluid to leak across the respiratory membranes. The stronger side of the heart sends blood to the systemic arteries, where the higher pressure works against higher resistance resulting from muscle contraction forces, multiple capillary beds and the extensive filtration across the kidneys. The systemic blood pressures of modern endotherms are three times those of ectotherms.

The crocodilian heart is an interesting mosaic: it is four chambered but it also has a shunting system, although a quite different one from that of other reptiles (Fig. 1c). Seymour *et al.*[1] propose that the ancestors of modern alligators and crocodiles were endothermic and required a four-chambered heart for pressure separation, and that it has since re-evolved a shunting system. The crocodilian ancestors from the Triassic (248 million to 206 million years ago), such as the long-legged *Terrestrisuchus* and the bipedal *Saltoposuchus*, were rather small (less than 2 metres long), completely terrestrial animals that are thought to have had an active lifestyle. They would also have had a diaphragmaticus, a muscle that inflates the lungs by pulling backwards on the liver[5]. This is analogous to the push that our diaphragm muscle exerts on the liver for the same purpose, and it allows higher ventilation rates than contemporary crocodiles require.

In the Jurassic (206 million to 144 million years ago), larger, fully aquatic crocodilian forms developed, and presumably adopted the same sit-and-wait predatory strategy that characterizes modern crocs. Because heat loss in water can be many times higher than that in air, and because larger animals have higher metabolic rates, there would have been considerable selective pressure

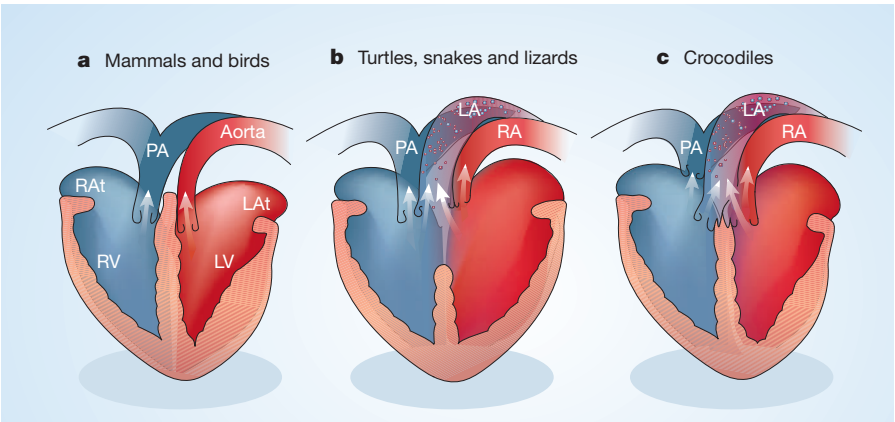

Figure 1 **Pump rooms.** a, **The four-chambered heart of mammals and birds separates oxygenated (red) from deoxygenated (blue) blood, and applies different pressures to the blood that goes to the lungs through the pulmonary artery (PA) and the blood that is pumped through the aorta to the rest of the body.** b, **The three-chambered heart of most reptiles allows oxygenated and deoxygenated blood to mix and does not permit the large pressure difference between the pulmonary and aortic systems.** c, **Modern crocodiles have a four-chambered heart, but can 'shunt' blood between the two systems through the foramen of Panizza, and are hypothesized[1] to have ancestors that were able to maintain large blood-pressure differences. RAt, right atrium; LAt, left atrium, RV, right ventricle; LV, left ventricle; RA, right aorta; LA, left aorta.**