shells made from silica — preserved in open-ocean sediments from the northeastern North Pacific (Fig. 1). The authors measured the ratios of stable oxygen isotopes in the diatoms. These ratios reflect past changes in the temperature and isotopic composition of sea water, which, in turn, vary with changes in global sea level and local salinity. The same principle was used in earlier studies[8] of planktic foraminifera, but diatoms can thrive in colder and less saline environments[9] than can many planktic foraminiferal species. Maier and colleagues' measurements reveal that large and abrupt intrusions of low-salinity waters occurred at their study site, coinciding with the timing of some Heinrich events. The authors interpreted these intrusions as evidence of meltwater originating from the Cordilleran Ice Sheet.

The researchers went on to carry out a series of computational climate-modelling experiments, of a type known as hosing experiments. Such simulations are used to study anomalies in global climate and ocean circulation that arise in response to abrupt climate change, and involve artificially introducing fresh water into the oceans at high latitudes, typically routed to the North Atlantic[5]. Maier *et al*. extended the hosing approach in two ways. First, they used a climate model that represents isotopic tracers, which thus enabled a more direct comparison of the simulations with their measurements. And second, they performed two sets of simulations, one in which only the North Atlantic was hosed, and the other in which both the North Atlantic and North Pacific were hosed.

The authors found that the simulation in which freshwater input was confined to the North Atlantic did not indicate that low-salinity waters entered the North Pacific Ocean, contradicting the findings from their diatom measurements. Despite this difference, the simulation did reproduce the ocean–atmosphere dynamics thought to have occurred across the Pacific in response to perturbations in the North Atlantic[5]. It also revealed poleward routing of warm, subtropical ocean waters due to shifts in tropical rainfall. Maier *et al*. therefore propose that the rerouted warm waters might have been responsible for the melting of parts of the Cordilleran Ice Sheet, adding fresh water to the North Pacific — a scenario that they could model by hosing the North Pacific as well as the North Atlantic.

Indeed, when the authors simulated this scenario, it provided a better match to the diatom observations. Moreover, the simulation suggests that salinity changed only negligibly at depth in the North Pacific. This might explain why no change in salinity was recorded in the isotopic study of foraminifera — it is thought that these organisms do not dwell at the topmost part of the ocean in this region. However, the diatom data indicate that freshwater influxes to the North Pacific did not occur during every Heinrich event. This could be because all Heinrich events are not created equally[1,4]. Sure enough, when Maier and colleagues performed additional simulations of North Atlantic perturbations involving exceptionally cool background temperatures, they found that the conditions produced were not conducive to melting of the Cordilleran Ice Sheet.

The new study is a major advance in our understanding of freshwater events in the North Pacific, but questions remain owing to the limitations of the time resolution of the sediments in the core that was analysed, and because the low abundance of diatoms in some sedimentary layers prevented the authors from carrying out their analysis for the corresponding periods of geological time. For example, the lack of evidence of freshwater pulses in proxies of the North Pacific surface ocean during some Heinrich events is puzzling, given the presence of glacial detritus. Furthermore, we still do not know how stable the Cordilleran Ice Sheet would be in response to shifts in Pacific climate that are unrelated to Heinrich events.

Importantly, further research is required to determine whether Cordilleran-meltwater events influenced circulation in the Pacific, or even in the Atlantic. More broadly, a more-refined understanding of Cordilleran-meltwater pulses and the associated effects on regional temperature and precipitation will benefit our theories of abrupt climate change. ■

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DNA DAMAGE

Breaking the replication speed limit

Inhibitors of PARP proteins are used in cancer treatment. It emerges that PARP inhibitors exert their effect by accelerating DNA replication to a speed at which DNA damage occurs. SEE LETTER P.279

ANNABEL QUINET & ALESSANDRO VINDIGNI

The two strands of DNA's double helix unwind to be copied, with a structure called a replication fork forming at the point of separation. The speed at which the replication fork progresses along DNA — and so the speed of replication — must be controlled to guarantee faithful duplication of the genome. On page 279, Maya-Mendoza *et al.*[1] define a molecular network involved in the regulation of replication-fork speed. Changes to this network can cause that speed to increase above a safe threshold, causing DNA damage and genomic instability.

Replication forks that encounter damage in the genome sometimes temporarily stop, allowing DNA repair to occur before replication continues. Proteins of the poly(ADP) ribose polymerase (PARP) family, particularly PARP1, assist in the repair of breaks in single strands of DNA through a process called PARylation[2], in which the proteins synthesize chains of ADP-ribose molecules that attract repair proteins to the damaged DNA. PARP inhibitors — drugs that block the PARylation activity of PARP proteins — are showing promise as therapeutics to treat various cancer types[3]. Previous models have proposed that, by preventing PARP activity, PARP inhibitors cause replication forks to stall for abnormally long periods, and eventually to collapse, when they encounter DNA damage[4]. This leads to accumulation of DNA damage owing to improper replication and death of the treated cells[4].

Maya-Mendoza *et al.* challenge the idea that PARP inhibitors perturb the ability of replication forks to progress. The authors found that treating proliferating human cells with the PARP inhibitor olaparib *in vitro* led to aberrant acceleration of fork speed. They provide evidence that, if fork speed increases above a threshold speed of 40% faster than normal, there is insufficient time for the forks to recognize damaged DNA in need of repair. This leads to accumulation of DNA damage and reduced cell viability. Supporting this idea, the authors found that violation of the threshold speed led to the activation of proteins involved in a DNA-damage response, although the mechanism by which this occurs needs to be further investigated.

To uncover the pathway by which PARP

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inhibition speeds up replication forks, Maya-Mendoza *et al.* investigated the protein p21, which can inhibit DNA replication[5]. Expression of the *p21* gene is controlled by PARP1 (ref. 6). Moreover, the protein p53, which is a central player in maintenance of genome integrity, activates expression of *p21* (ref. 7) and is itself activated by PARylation[8]. The authors found that loss of *p21* led to an increase in replication-fork speed similar to the acceleration caused by PARP inhibitors. Loss of *p21* in addition to PARP inhibition increased fork speed more than either manipulation in isolation. Combining these observations, the authors propose the existence of a fork-speed regulatory network that has two interacting arms — the p53–p21 axis and PARylation. Each arm acts to keep fork speed below the threshold, with inhibition of either p21 or PARylation throwing the network out of balance and so increasing fork speed (Fig. 1). Several steps of this regulatory pathway will require further investigation. For example, exactly how the arms interact to properly control replication-fork speed is a key question to address.

PARP inhibitors are used to treat tumours that have deficiencies in a pathway called homologous repair that repairs double-stranded DNA breaks. These defects make the tumour cells particularly susceptible to PARP inhibitors. To explain, the single-stranded DNA breaks that accumulate owing to PARP inhibitors are converted to double-stranded breaks when the damaged strand is replicated. In normal cells, the breaks can then be repaired through homologous repair, but when this pathway is defective, the inability to repair these defects leads to cell death. Most notably, breast, ovarian and prostate cancers caused by mutations in the genes *BRCA1* and *BRCA2* are susceptible to PARP inhibition[9,10].

Maya-Mendoza *et al.* found that PARP inhibition accelerates fork speed above the threshold in *BRCA1*-deficient cells. On the basis of these results, the authors suggest that the susceptibility of tumours harbouring *BRCA* mutations to PARP inhibitors might not be due to increased stalling and collapse of replication forks, as originally believed, but instead to aberrant acceleration that compromises the ability of forks to detect and repair DNA damage.

In summary, Maya-Mendoza *et al.* have provided a fresh view of why PARP inhibitors are toxic to cancer cells, and have outlined a previously unknown network that controls replication-fork speed. Their work has the potential to revolutionize current models of how cells cope with DNA damage — but it also raises several questions.

For example, do other PARP proteins help to control fork speed? The authors report that PARylation levels were not affected by PARP1 depletion. This observation implies that other members of the PARP family are involved in controlling replication-fork speed.

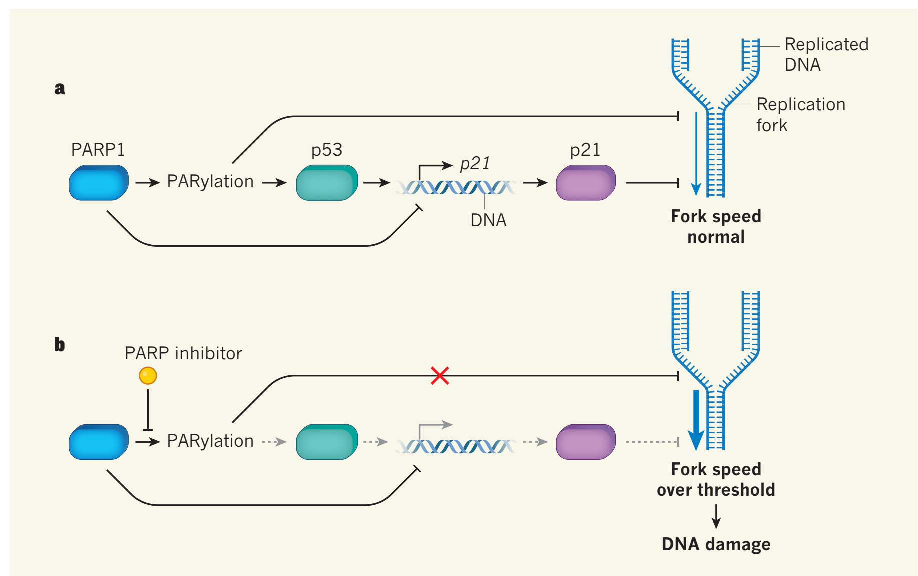

Figure 1 | A regulatory network controlling replication-fork speed. DNA undergoing replication unwinds at dynamic structures called replication forks, which move through the genome as replication progresses. Maya-Mendoza *et al.*[1] have unravelled a two-armed regulatory network that controls replication-fork speed. **a**, In one arm, the protein PARP1 limits fork speed through an enzymatic process called PARylation. In the other, the protein p53 activates the gene *p21*, which encodes a protein that also limits fork speed. The two arms are interconnected because PARylation regulates p53 activity and PARP1 inhibits *p21* expression. Together, the network limits replication-fork speed to normal levels to ensure genomic stability. **b**, Drugs called PARP inhibitors, which prevent PARylation, prevent the network from properly inhibiting replication-fork speed, although the effect of the drugs on the p53–p21 arm remains to be fully elucidated (indicated by faded, dashed arrows). Fork speed rises to more than 40% faster than normal, and crossing this threshold leads to DNA damage and genomic instability.

By what mechanism do PARP activity and p21 control replication-fork speed? PARP activity is crucial in the control of replication-fork reversal — a mechanism by which replication forks reverse their course when they face DNA breaks[11,12], enabling the damage to be dealt with. Perhaps PARP activity and p21 affect fork speed by suppressing replication-fork reversal or other mechanisms used by replication forks to cope with DNA breaks.

There are other areas of interest for future research. For example, the effect of increased fork speed on polymerase enzymes, which carry out DNA replication, should be examined to determine whether the enzymes exacerbate the situation by introducing more errors into the newly replicated genome as a consequence of increased fork speed. Whether the toxic effects of PARP inhibitors on cancer cells are mainly linked to the fact that the forks do not detect damage when the threshold speed is violated remains to be confirmed.

"Maya-Mendoza et al. have provided a fresh view of why PARP inhibitors are toxic to cancer cells."

Notably, PARP inhibitors have also been effectively used in combination with chemotherapeutic agents, which induce DNA damage by impairing the ability of replication forks to progress. It would therefore be interesting to determine whether the same mechanism underlies the effects of PARP inhibitors when used in combination with chemotherapy. Finally, Maya-Mendoza and colleagues' findings will no doubt prompt many research groups to explore whether surpassing the threshold fork speed provides a more general way to explain the molecular basis of cancer and tumour sensitivity to chemotherapy. ■

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