surprising twist, Fennell *et al.* observed that clonal descendants of the same individual barcoded cell rose to dominance across different mice, pointing to a non-genetic, yet heritable, cell property.

Some of the fittest clones had a gene-expression signature reminiscent of leukaemic stem cells, providing a clue to how the heritable identities provide an advantage. Furthermore, the authors observed that dominant clones exhibited high expression of the gene *Slpi*, which encodes secretory leukocyte peptidase inhibitor, a protein that has an anti-inflammatory function. These clones also had lower than normal expression of certain immune-system-related genes, such as those encoding components of the major histocompatibility (MHC) system. The MHC system has a function in triggering immune defence, which suggests that clonal fitness might be related to the capacity of the cells to remain hidden from the immune system.

Notably, this gene signature was present even before the cancer cells were transplanted into mice, and it remained stable after the cancer clones had grown in the animals — even in secondary transplants into other mice — reinforcing the idea of inheritance of these characteristics (phenotype). Nevertheless, the gene-expression profiles of dominant clones were not homogeneous, suggesting that, although these profiles are heritable, their stability over the course of repeated cell divisions is less than that observed for genetic factors underlying cancer evolution.

In contrast to the heritable propagation of non-genetic phenotypes, when the authors gave chemotherapy to mice with cancer, only a subset of cancer cells adopted a transcriptional program associated with the senescent-like state, consistent with previous reports[9–12]. However, the likelihood of adopting this state differed between clones, suggesting a complex interplay between heritable pre-existing characteristics and drug tolerance actively gained after exposure to treatment.

The demonstration of non-genetic yet heritable changes in cancer cells raises the question of what mechanism propagates these changes from parent to daughter cell. The immediate candidates are changes to the nuclear complex of DNA and protein called chromatin. Such epigenetic alterations include the addition of methyl groups (methylation) to DNA and modifications to the DNA-binding histone proteins. A high level of DNA methylation can inactivate genes that help to suppress tumour formation[13]. And the removal of methyl groups from histone H3 protein (at a site called H3K4) modifies chromatin that has been altered as a result of the therapeutic use of inhibitors of EGFR, and triggers the emergence of treatment-resistant persister cells[12].

In addition, key transcription factors might enable the stable propagation of rare, treatment-resistant cellular phenotypes[8]. Data implicating other post-translational protein modifications in resistance further expand the number of potential explanations to consider[10].

These intriguing findings point to the need for a more holistic view of cancer evolution. This would help to define how genetic and non-genetic mechanisms jointly contribute to cellular phenotype, which is the substrate for selection. Attempts to meet this challenge will be greatly aided by methods such as 'multi-omic' technologies that can capture multiple layers of information at single-cell resolution — the 'atomic unit' of the evolutionary process[14]. For example, capturing genetic, epigenetic and transcriptional information from the same individual cell will allow researchers to define the relative contribution of these features to evolving populations of cancer cells.

Moreover, translating animal studies into analyses of primary human samples will require innovations in clonal tracking that do not rely on artificial cell engineering, but instead use already-existing native, heritable changes to the DNA, which can be captured at the level of single cells[15,16]. These exciting new horizons might give us an integrated perspective on cancer evolution, with insights into disease progression and relapse. This could pave the way for new therapeutic approaches to directly anticipating and addressing cancer evolution, which remains a central obstacle to achieving a cure[9–12].

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1. Fennell, K. A. *et al. Nature* **601**, 125–131 (2022).
2. Duy, C. *et al. Cancer Discov.* **11**, 1542–1561 (2021).
3. Dhimolea, E. *et al. Cancer Cell* **39**, 240–256 (2021).
4. Rehman, S. K. *et al. Cell* **184**, 226–242 (2021).
5. Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. *Science* **305**, 1622–1625 (2004).
6. Shlush, L. I. *et al. Nature* **547**, 104–108 (2017).
7. Hata, A. N. *et al. Nature Med.* **22**, 262–269 (2016).
8. Shaffer, S. M. *et al. Cell* **182**, 947–959 (2020).
9. Mikubo, M., Inoue, Y., Liu, G. & Tsao, M.-S. *J. Thorac. Oncol.* **16**, 1798–1809 (2021).
10. Berger, A. J. *et al. Nature Cancer* **2**, 1055–1070 (2021).
11. Bell, C. C. *et al. Nature Commun.* **10**, 2723 (2019).
12. Sharma, S. V. *et al. Cell* **141**, 69–80 (2010).
13. Pan, H. *et al. Cancer Discov.* **11**, 2266–2281 (2021).
14. Nam, A. S., Chaligne, R. & Landau, D. A. *Nature Rev. Genet.* **22**, 3–18 (2021).
15. Chaligne, R. *et al. Nature Genet.* **53**, 1469–1479 (2021).
16. Ludwig, L. S. *et al. Cell* **176**, 1325–1339 (2019).

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Particle physics

Mirror symmetry validated for antiprotons

Ralf Lehnert

A comparison of the charge-to-mass ratio for the proton and the antiproton has been performed with record-breaking precision — the best such test yet for a mirror-like symmetry that relates matter and antimatter. **See p.53**

Merging the theory of relativity and the microscopic world of quantum physics into a single framework has yielded some profound insights into the nature of the Universe. Among them is the concept of antiparticles, the elusive twins of ordinary particles such as the electron, the proton and the neutron. But determining the properties of these particle twins is a difficult task, because they don't mix well — to the extent that, on contact, a particle–antiparticle pair annihilates, leaving only a flash of intense light. However, the theories of relativity and quantum physics also imply that a mirror-like symmetry exists, called CPT invariance, that ensures that a particle and its antiparticle have the same mass, but opposite charge. On page 53, Borchert *et al.*[1] report the most stringent test of this aspect of mirror symmetry performed so far, by studying the motion of single negatively charged hydrogen ions and antiprotons in an apparatus known as a Penning trap.

The challenges posed by antimatter experiments are often circumvented by establishing physical laws using matter and then extrapolating them to antimatter by assuming CPT invariance. This approach has been extraordinarily successful, and will indisputably continue to hold sway in fundamental-physics research. This fact alone highlights the necessity for improved CPT tests.

But there is also a more intriguing side to mirror symmetry. Certain observations are difficult, although not impossible, to reconcile

with our current CPT-invariant laws. One such observation is the imbalance of matter and antimatter in the Universe, despite the prevailing view that the Big Bang created equal quantities of both. There are also conceptual issues associated with our existing laws that could be resolved by allowing minuscule departures from mirror symmetry. These exciting developments have imparted particular urgency to the need for improved CPT tests[2]. Observations of departures from mirror symmetry, however small, would have a huge impact, requiring serious revisions to relativity, quantum physics or both.

In preparation for the authors' measurement of the charge-to-mass ratio of antiprotons, these antiparticles were created at CERN, the European particle-physics laboratory near Geneva, Switzerland. Borchert *et al.* stored the antiparticles in a Penning trap, a device designed to suspend charged particles or antiparticles inside a vacuum with carefully chosen magnetic and electric fields, ready to be deployed for experiments. Such a delicate storage method prevents the antiprotons from making contact with ordinary matter, which would lead to their immediate disintegration.

The key idea behind the measurement is based on the circular trajectory of a charged particle when it moves in a constant magnetic field, such as that in a device known as a cyclotron: the rotational frequency of this motion is proportional to the antiproton's charge-to-mass ratio. For the actual measurement, Borchert *et al.* extracted a single antiproton from storage and shuttled it to a region of the trap that had judiciously tuned electromagnetic fields. This region was equipped with ultrasensitive detectors capable of picking up the motion of the single antiproton by detecting its electromagnetic signature in a circuit. The authors obtained the cyclotron frequency from this motion.

In principle, comparing this antiproton measurement with the corresponding measurement for the proton provides a test of mirror symmetry. However, the proton's opposite electric charge requires an inversion of the electric trapping field, an adjustment that introduces large systematic errors. So, instead, Borchert *et al.* used a negatively charged hydrogen ion. The effects of introducing two electrons to a proton to produce this ion are well understood, so the authors were able to correct for these effects in their data analysis. The final result was the ratio of the cyclotron frequencies of the antiproton and the hydrogen ion, minimizing uncertainties due to possible irregularities in the magnetic field. Borchert and colleagues measured the magnitude of this ratio to be one, with a fractional uncertainty of 16 parts per trillion. This value is precise enough to be considered confirmation of mirror symmetry, and it improves the precision of the previous best test[3] by a factor of 4.3.

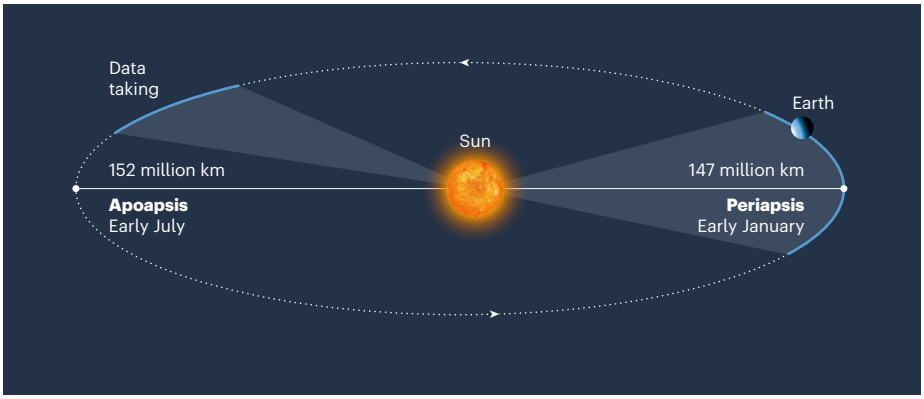

Figure 1 | Testing the effects of the Sun's gravitational field on antimatter. Variations in the position of Earth in relation to the Sun — ranging from periapsis (the closest point in its orbit) to apoapsis (the farthest point) — cause small changes in the Sun's gravitational potential on Earth over a timescale of months. Borchert *et al.*[1] collected data over several months to assess how these changes affected the properties of the antiproton relative to its the antimatter twin, the proton. (Adapted from Fig. 3 in ref. 1.)

This measurement also places severe constraints on deviations from Albert Einstein's special theory of relativity, one of the pillars on which mirror symmetry rests, when interpreted within a framework known as the standard-model extension. This framework predicts the observable effects of hypothetical departures from Einstein's ideas in both general relativity, the theory that governs gravity, and the standard model of particle physics, the theory that describes how fundamental particles interact with each other. The standard-model extension contains coefficients describing physics that is dependent on the orientation of the laboratory, but this dependence is incompatible with the principle of relativity. The fact that Borchert *et al.* measured a constant cyclotron-frequency ratio as the orientation of the Penning-trap apparatus changed with Earth's rotation implies that the measurement was in agreement with relativity — an observation that provides four times the precision of previous limits for ten of the coefficients of the standard-model extension.

The authors also used the Penning trap to study the question of whether antimatter obeys the weak equivalence principle. This principle states that all bodies in the same gravitational field experience the same acceleration, regardless of their properties. Its origin is often attributed to Galileo Galilei, who is said to have dropped unequal masses from the top of the Leaning Tower of Pisa in Italy. To test whether the antiproton has an anomalous coupling to the gravitational field, in violation of the weak equivalence principle, the team used cyclotron-frequency data collected over extended periods spanning months. On such timescales, the distance between Earth and the Sun varies enough to induce changes in the Sun's gravitational potential at the location of the Penning trap (Fig. 1). This made it possible to estimate constraints on how much the measured cyclotron-frequency ratio varied with the gravitational potential — the world's first such analysis.

The next step will be to design a portable trap for antiprotons, which would allow cyclotron-frequency measurements to be performed in a low-noise laboratory without the disturbances present at the antiproton facility at CERN[4]. Other developments might take inspiration from quantum computing. A closely related mirror-symmetry test exists that involves the anomaly frequency — the difference between the cyclotron frequency and the precession frequency of the particle's spin angular momentum in a magnetic field. A comparison of the anomaly frequencies of the proton and antiproton in a Penning trap could benefit from laser-based techniques that were originally developed for manipulating ions in quantum-computing experiments[5]. Finally, theoretical insights into mirror-symmetry violations in the context of gravity, reported last year[6], have the potential to provide much more sophisticated interpretations of tests of CPT invariance. With these developments on the horizon, the future for mirror-symmetry studies involving antiprotons looks bright indeed.

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1. Borchert, M. J. *et al. Nature* **601**, 53–57 (2022).
2. Colladay, D. & Kostelecký, V. A. *Phys. Rev. D* **58**, 116002 (1998).
3. Ulmer, S. *et al. Nature* **524**, 196–199 (2015).
4. Smorra, C. *et al. Tech. Design Rep. BASE-STEP* CERN-SPSC-2021-007, SPSC-TDR-007 (2021).
5. Nitzschke, D. *et al. Adv. Quant. Tech.* **3**, 1900133 (2020).
6. Kostelecký, V. A. & Li, Z. *Phys. Rev. D* **104**, 044054 (2021).

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