about the galaxies that must have driven reionization[7].

Two major challenges for detecting the 21-cm signal involve foregrounds and calibration. The cosmic signal is dwarfed by radio emission from the Milky Way, as well as by terrestrial radio emission. Despite the favourable location of the EDGES experiment in the Australian outback, transmission from local radio and TV stations causes the loss of isolated regions of the spectrum. In addition, Galactic radio emission from energetic electrons spiralling in magnetic fields forms a spectrally smooth foreground that is one-thousand times brighter than the 21-cm signal. This smooth Galactic foreground can be fitted with a simple polynomial, and so removed, leaving the cosmic signal in the residuals. Unfortunately, this procedure removes much of the signal, potentially throwing the baby out with the bath water.

Another important limitation of the current experimental set-up is the absence of a method for calibrating the frequency response of the radio antenna. This necessitates fitting a combination of the foregrounds and the antenna's response. Given these limitations, it is impressive that the authors[2] are able to achieve residuals at the level of tens of millikelvin, comparable to the expected signal, and to place weak constraints on the duration of reionization.

Bowman and Rogers' technique allows them to rule out only models in which reionization occurs most abruptly — corresponding to a redshift interval of less than 0.1. As yet, the technique has had little effect on most models of the reionization epoch and the first galaxies. Figure 1 shows the expected evolution of the Universe as traced by emission or absorption of the 21-cm spectral line[8]. There is an initial absorption regime where the hydrogen gas is cooling through its cosmic expansion, and the excitation temperature of the 21-cm transition, which characterizes the relative populations of its two energy levels, is held equal to the gas temperature by collisions between hydrogen atoms. This absorption dies away as the gas gets diluted. Then the first stars form and emit UV photons that again set the excitation temperature of the 21-cm transition equal to the gas temperature, reinvigorating a second absorption trough. As these stars die, some of them produce black holes whose X-ray emission is expected to heat the gas to above the temperature of the cosmic microwave background, pushing the 21-cm signal into emission.

The authors[2] focus their efforts on this final phase, in which the signal is seen in emission and the progressive ionization of the diffuse hydrogen gas cuts off the signal, indicating the end of reionization. The same technique could ultimately be applied to detecting earlier periods for which our picture of the astrophysics is highly uncertain.

In the meantime, considerable time and money is being dedicated to the construction of low-frequency radio interferometers such as MWA, LOFAR and PAPER, which will target spatial fluctuations in the 21-cm signal (Fig. 1a). The EDGES experiment represents a cheaper method for measuring only the sky-averaged, broad-brush features in the evolution of the signal. Despite its limitations, it opens the possibility of an alternative experimental avenue that should be pursued in parallel to the more ambitious interferometers. Bowman and Rogers[2] have taken the first step on this journey, which will hopefully lead to new insights about the first stars and galaxies and the reionization epoch. ■

Jonathan Pritchard *and* **Abraham Loeb**
are in the Institute for Theory and Computation, Harvard University, Cambridge, Massachusetts 02138, USA.
e-mails: jpritchard@cfa.harvard.edu; aloeb@cfa.harvard.edu

1. Loeb, A. *How Did the First Stars and Galaxies Form?* (Princeton Univ. Press, 2010).
2. Bowman, J. D. & Rogers, A. E. E. *Nature* **468,** 796–798 (2010).
3. Furlanetto, S. R., Oh, S. P. & Briggs, F. H. *Phys. Rep.* **433,** 181–301 (2006).
4. Shaver, P. A., Windhorst, R. A., Madau, P. & de Bruyn, A. G. *Astron. Astrophys.* **345,** 380–390 (1999).
5. Becker, R. H. *et al. Astron. J.* **122,** 2850–2857 (2001).
6. Komatsu, E. *et al. Astrophys. J. Suppl. Ser.* **180,** 330–376 (2009).
7. Robertson, B. E., Ellis, R. S., Dunlop, J. S., McLure, R. J. & Stark, D. P. *Nature* **468,** 49–55 (2010).
8. Pritchard, J. R. & Loeb, A. *Phys. Rev. D* **82,** 023006 (2010).
9. Santos, M. G. *et al. Astrophys. J.* **689,** 1–16 (2008).

VIROLOGY

One protein, many functions

The Lassa virus nucleoprotein coats the viral genome to make a template for RNA synthesis. A study shows that it also binds the 'cap' structure of cellular messenger RNAs and directs immune evasion using a novel mechanism. SEE ARTICLE P.779

FÉLIX A. REY

Lassa fever is a dreadful human haemorrhagic disease caused by the Lassa virus, a member of the Arenaviridae family[1]. The disease is prevalent in West Africa, causing 5,000 deaths each year and infecting hundreds of thousands more[2]. Arenaviruses are distributed worldwide and cause persistent infection in rodents, in which they generally don't cause disease. Humans become infected by exposure to material contaminated by infected mice, for example when the animals infiltrate food stores. The Lassa virus genome is a negative-sense, single-stranded RNA (nsRNA) molecule, and is coated by a nucleoprotein to form a nucleocapsid — a complex in which multiple copies of the nucleoprotein wrap around the genomic RNA, each one contacting a fixed number of nucleotides. In this issue (page 779), Qi *et al.*[3] report the crystal structure of the Lassa virus nucleoprotein, and reveal that it has a striking array of activities.

The nucleocapsids of nsRNA viruses serve as templates for the virus's polymerase enzyme (also known as the large or L protein), which replicates the genome to make new infectious particles. Qi and colleagues' crystal structure[3] shows that the Lassa virus nucleoprotein is made of two domains — an amino-terminal domain and a carboxy-terminal domain — with a positively charged groove in between, where the genomic RNA is expected to bind. This organization has been observed in all nsRNA viruses for which the nucleoprotein structure is known.

Before replication, the polymerase transcribes the genome into messenger RNA molecules to be translated into the viral proteins. Efficient translation of mRNAs by cellular ribosomes occurs if the mRNAs have a 'cap' structure at the 5′ end of the molecule. But arenaviruses, along with a subset of nsRNA viruses (those that have segmented genomes; Fig. 1, overleaf), cannot themselves cap mRNAs. They therefore steal caps from cellular mRNAs and transfer them to nascent viral transcripts, in a process known as cap snatching. Arenaviruses do this by cleaving off the 5′ end of cellular mRNAs using an 'endonuclease' activity that resides in the amino-terminal domain of the L protein[4,5], and then transferring the mRNA fragment to nascent transcripts.

Qi and colleagues' structure of the Lassa virus nucleoprotein shows that its amino-terminal domain has a cap-binding site, which holds the 5′ end of cellular mRNAs in place while the L protein cleaves off the rest. This additional function of the arenavirus nucleoprotein has not been observed in counterparts of the protein from any other virus family. The authors[3] show that when key residues in the cap-binding site are mutated, transcription is impaired.

Furthermore, the structure shows that the carboxy-terminal domain of the Lassa virus's nucleoprotein is folded in the same way as cellular 3′–5′ exonucleases — the enzymes that remove nucleotides one at a time from the 3′ end of RNA or DNA molecules, often completely degrading the nucleic-acid molecules in the process. Indeed, one of the closest structural homologues of the nucleoprotein's carboxy-terminal domain is the human DNA 3′–5′ exonuclease enzyme TREX1. What are the implications of this?

The detection of foreign nucleic acids to induce production of type I interferon (IFN) proteins is central to the innate antiviral defence of cells; misregulation of this system causes autoimmune problems. TREX1 is necessary for the degradation of single-stranded DNA derived from endogenous retroelements[6] (which constitute 90% of the approximately three million transposable elements in the human genome). Such single-stranded DNA accumulates in TREX1-deficient cells, inducing the IFN response and causing autoimmune disease. Qi *et al*. identified the amino acids of the 3′–5′ exonuclease active site of the Lassa virus nucleoprotein by superposition of their crystal structure[3] on that[7] of TREX1. Remarkably, these amino acids correspond to residues that were recently shown to have a critical role in the IFN-counteracting activity of the nucleoprotein of the lymphocytic choriomeningitis virus[6], the best-studied arenavirus. This suggests that the 3′–5′ exonuclease activity is the way by which arenavirus nucleoproteins inhibit IFN induction.

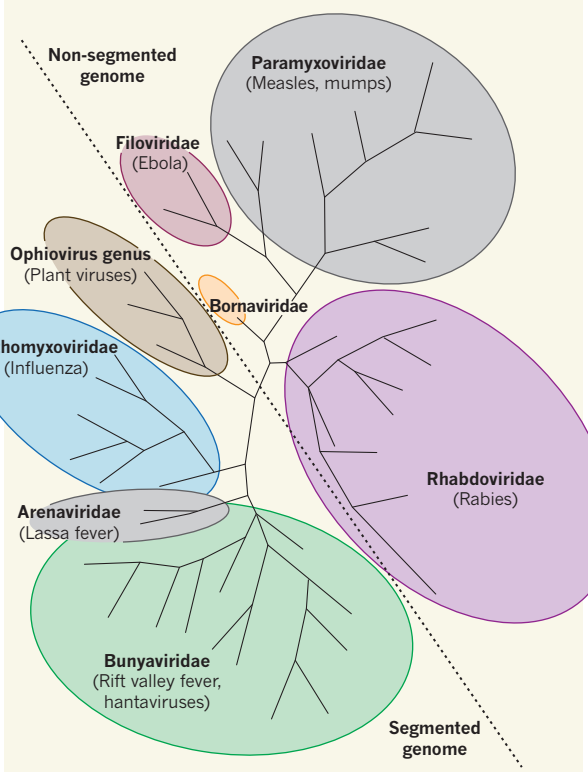

Figure 1 | Phylogeny of nsRNA viruses. This unrooted phylogenetic tree of the known nsRNA viruses (adapted from ref. 20) is overlaid with coloured ellipses representing the various virus families. The ophioviruses constitute a genus rather than a family. Representative human pathogens/diseases in each of the families are indicated in parentheses. The diagonal dashed line separates the nsRNA families that have a single RNA genomic molecule (non-segmented genomes) from those that have several genomic segments. The arenaviruses have two genomic segments, bunyaviruses have three, ophioviruses three or four (depending on the virus) and orthomyxoviruses six to eight. Qi *et al*.[3] report the crystal structure of the nucleoprotein from the Lassa virus (a member of the Arenaviridae family). The structure reveals an unexpected biological function of the nucleoprotein, and casts fresh light on the evolutionary history of nsRNA viruses.

The authors verified[3] that the Lassa virus nucleoprotein does indeed degrade short RNA molecules similar to those generated as by-products during replication and transcription of the virus. They also showed that the wild-type nucleoprotein inhibits IFN production in virus-infected cells, whereas mutants devoid of exonuclease activity do not, even though they still undergo replication and transcription.

Why are so many unrelated activities concentrated in a single protein? The answer is probably related to the extreme compactness of arenavirus genomes, which code for only four proteins — fewer than in any of the other nsRNA virus families, and fewer than in any other human pathogenic virus. Of these four proteins, two of them (the nucleoprotein and the L protein) are present in all nsRNA viruses, and form the replicative foundation of these viruses. The structure of the Lassa virus nucleoprotein thus also provides further insight into the evolutionary history of nsRNA viruses, as described below.

The amino-acid sequence of the arenavirus L protein has the signature of RNA-dependent RNA polymerases (RdRps, enzymes that catalyse the replication of RNA from an RNA template). L proteins are found in all nsRNA viruses, with the exception of those of the Orthomyxoviridae family, in which the polymerase is split into three smaller polypeptides (PA, PB1, PB2) and functions as a heterotrimer containing these three proteins[8] — PA has the cap-snatching endonuclease site[9], PB1 acts as the catalytic RdRp[10] and PB2 has the cap-binding site[11]. Qi *et al*.[3] have now shown that, in arenaviruses, the cap-binding site resides in the nucleoprotein. The Bunyaviridae family of nsRNA viruses, meanwhile, have endonuclease activity in the amino-terminal domain of the L protein[12], but their cap-binding site has not yet been identified. Thus, the three families of nsRNA viruses that have segmented genomes and that have been studied in detail (Bunyaviridae, Orthomyxoviridae and Arenaviridae) share a cap-snatching strategy for genome transcription. This is not the case in the non-segmented viruses, in which the L protein has a capping activity.

The phylogenetic diagram shown in Figure 1 is based on the conserved RdRp modules from all nsRNA viruses, and shows how the different families cluster according to whether or not they have segmented genomes. Structural data show that the nucleoproteins from all non-segmented nsRNA viruses have evolutionarily conserved folds, but this isn't the case for the segmented ones. So, although the nucleoprotein structures of the bunyaviruses[13] and the orthomyxoviruses[14] both contain two domains (an amino-terminal and a carboxy-terminal domain, as seen for the Lassa virus nucleoprotein[3]), the individual folds of the domains are unrelated. By contrast, the amino- and carboxy-terminal domains of the nucleoproteins of the Bornaviridae[15], the Rhabdoviridae[16,17] and the Paramyxoviridae[18] (all of which have non-segmented genomes) have a conserved three-dimensional fold, suggesting a common ancestry, despite the absence of any detectable similarity in their amino-acid sequences.

Qi and colleagues' crystal structure[3] of an arenavirus nucleoprotein illuminates the protein's roles in the virus's cycle, while adding to our understanding of the evolutionary history of nsRNA viruses. It also highlights the fact that each family of nsRNA viruses seems to have developed different immune-defence strategies and shows that the arenaviruses' mechanism of immune evasion is a novel one. Considering that fatal infections by pathogenic arenaviruses — and chiefly by the Lassa virus[19] — are characterized by a generalized immune suppression, these new results have major implications for finding new ways to combat these diseases. ■

Félix A. Rey *is in the Structural Virology Unit, Department of Virology, Institut Pasteur, 25 rue du Dr Roux, Paris 75015, France.*
e-mail: rey@pasteur.fr

1. Buchmeier, M. J., De la Torre, J. C. & Peters, C. J. in *Fields Virology* Vol. 2 (eds Knipe, D. M. & Howley, P. M.) 1791–1828 (Lippincott Williams & Wilkins, 2007).
2. Ogbu, O., Ajuluchukwu, E. & Uneke, C. J. *J. Vector Borne Dis.* **44,** 1–11 (2007).
3. Qi, X. *et al. Nature* **468,** 779–783 (2010).
4. Lelke, M., Brunotte, L., Busch, C. & Günther, S. *J. Virol.* **84,** 1934–1944 (2010).
5. Morin, B. *et al. PLoS Pathog.* **6,** 1–11 (2010).
6. Stetson, D. B., Ko, J. S., Heidmann, T. & Medzhitov, R. *Cell* **134,** 587–598 (2008).

7. de Silva, U. *et al. J. Biol. Chem.* **282,** 10537–10543 (2007).
8. Ruigrok, R. W., Crepin, T., Hart, D. J. & Cusack, S. *Curr. Opin. Struct. Biol.* **20,** 104–113 (2010).
9. Dias, A. *et al. Nature* **458,** 914–918 (2009).
10. Poch, O., Sauvaget, I., Delarue, M. & Tordo, N. *EMBO J.* **8,** 3867–3874 (1989).
11. Guilligay, D. *et al. Nature Struct. Mol. Biol.* **15,** 500–506 (2008).
12. Reguera, J., Weber, F. & Cusack, S. *PLoS Pathog.* **6,** e1001101 (2010).
13. Raymond, D. D., Piper, M. E., Gerrard, S. R. & Smith, J. L. *Proc. Natl Acad. Sci. USA* **107,** 11769–11774 (2010).
14. Ye, Q., Krug, R. M. & Tao, Y. J. *Nature* **444,** 1078–1082 (2006).
15. Rudolph, M. G. *et al. Structure* **11,** 1219–1226 (2003).
16. Albertini, A. A. *et al. Science* **313,** 360–363 (2006).
17. Green, T. J., Zhang, X., Wertz, G. W. & Luo, M. *Science* **313,** 357–360 (2006).
18. Tawar, R. G. *et al. Science* **326,** 1279–1283 (2009).
19. Baize, S. *et al. J. Virol.* **83,** 5890–5903 (2009).
20. Fauquet, C. *et al. Virus Taxonomy* (Elsevier, 2005).

SOLAR SYSTEM

Pluto is again a harbinger

New astronomical and laboratory data show that the abundances of the two dominant ices, nitrogen and methane, on the surfaces of the Solar System's two largest dwarf planets are surprisingly similar — raising fresh questions.

S. ALAN STERN

Combining state-of-the-art telescopic and ground-based laboratory data, Tegler *et al.*[1] have recently reported that the proportions of nitrogen (N_2) and methane (CH_4), the dominant surface ices on the two largest dwarf planets, Pluto and Eris, are surprisingly similar. More specifically, they found that the N_2 and CH_4 abundances on Eris are near 90% and 10%, respectively, and that those on Pluto are 97% and 3%. Intriguingly, these abundances are also similar to those on the dwarf planet and Kuiper-belt escapee Triton, which orbits Neptune.

Tegler and colleagues' results, published in the *Astrophysical Journal*, represent the first quantitative comparison of the abundances of volatile ices on the surface of any bodies beyond Neptune. They have significant implications for understanding Pluto and Eris, as well as the Kuiper belt, the disk-shaped region beyond Neptune's orbit where these two dwarf planets and other bodies reside (Fig. 1). The findings also provide reassurance that the detailed study planned for the Pluto system by NASA's New Horizons mission[2], which is now en route for a 2015 fly-by, will be of relevance to a broader suite of small planets common to the outer Solar System.

The discovery of Pluto by Clyde William Tombaugh in 1930 can be considered the technical discovery of the Kuiper belt. But the Kuiper belt's existence was firmly established only in the 1990s, with the discovery of additional bodies there[3,4]. Interestingly, a wide variety of attributes now known to be common to many large Kuiper-belt objects were first identified in studies of Pluto[4]. These include Pluto's rocky interior, its icy red surface, the presence of its satellites, its high orbital inclination and its resonant orbit with Neptune (Pluto's orbital

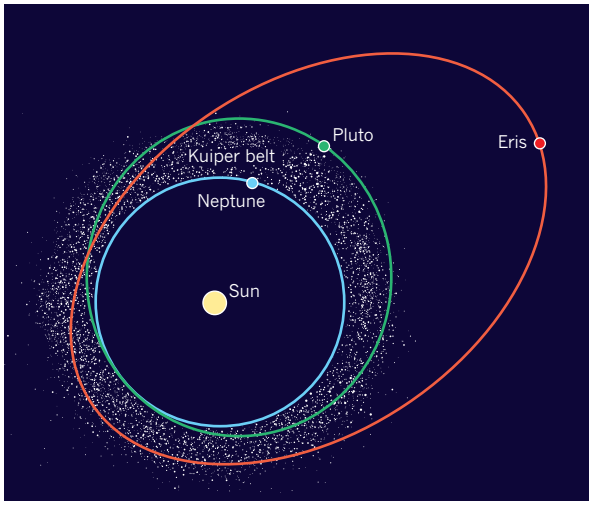

Figure 1 | Pluto, Eris and the Kuiper belt. Tegler and colleagues' demonstration[1] that Pluto and Eris have similar surface abundances of nitrogen and methane ices suggests that such abundances may be common, or at least not uncommon, among large objects in the Kuiper belt, the disk-shaped region beyond Neptune's orbit where the two dwarf planets reside. White dots represent objects in the classical Kuiper belt. Neither Centaurs (Kuiper-belt escapees) nor objects in the 'scattered belt' beyond Pluto's orbit are shown. Other large dwarf planets smaller than Pluto and Eris are also not shown.