hNCCs upregulated genes associated with translation, such as several ribosomal subunits. At the same time, hNCCs exposed to ZIKV downregulated nucleosome-associated genes, and this downregulation was accompanied by deficits in histone H3 expression and acetylation. Recent evidence suggests that ZIKV RNA can directly bind to Musashi-1 protein in CNS progenitors and that this interaction enables viral replication[14]. It would be interesting to explore whether similar ZIKV expansion processes take place in the PNS in infected humans.

Going one step further, the authors differentiated hNCCs into human peripheral neurons (hPNs) and found that ZIKV can also infect these cells *in vitro*. Transcriptional studies of infected hPNs indicated perturbations of the WNT signaling pathway. Moreover, the levels and phosphorylation of c-Jun, a JNK-pathway-related protein, was increased in hPNs. Oh *et al.*[8] suggest that this may contribute to apoptosis in these neurons.

How cell-specific is ZIKV infection in the PNS, and how does this lead to GBS? Further *in vitro* and *in vivo* studies will need to explore the specificity aspect in detail, but this study shows that muscle cells are not affected, while hNCC derivatives such as Schwann cells are. Even if ZIKV can infect neurons of the PNS, the precise mechanism of ZIKV-related GBS remains mysterious. What route would ZIKV use to reach these neurons *in vivo*, and how is the immune system reacting or cross-reacting? A recent study showed that ZIKV-infected hNCCs can release cytokines, which may affect differentiation and interactions with other cells[13]. But future *in vivo* studies will need to explore the detailed cross-talk between hPNs and the immune system, and examine how neuroinflammation occurs.

Beyond demonstrating that ZIKV can infect PNS cells, the study by Oh *et al.*[8] highlights the power of stem-cell-based approaches for asking fundamental disease questions in human cells. Moreover, and similarly to studies in cortical neural cells[15], these experiments suggest that because large quantities of hNCCs can be easily derived from human pluripotent stem cells, high-throughput screens to identify drug candidates are possible. Ultimately, the combination of *in vivo* and *in vitro* studies in multiple experimental models will be essential in developing strategies to prevent or treat GBS and other conditions in ZIKV-infected individuals.

COMPETING FINANCIAL INTERESTS
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Loopholes in the DNA contract kill neurons

Karl Herrup, Kai-Hei Tse & Hei-Man Chow

Hexanucleotide repeat expansions in *C9orf72* gene locus create double jeopardy, first by leading to DNA–RNA R-loops that spawn double-strand breaks and second by the synthesis of dipeptide repeats that hinder DNA repair. This two-pronged mechanism may explain neurodegeneration in amyotrophic lateral sclerosis and frontotemporal dementia.

Neurons must maintain genomic integrity at all costs. Any condition that increases DNA damage or decreases DNA repair threatens this integrity and predisposes the cells of the brain to neurodegeneration[1]. The situation is uniquely fraught for neurons, as they must live for decades (in humans) in a permanently postmitotic state, excluded from forms of DNA repair, such as homologous recombination, that are only active during cell division. This vulnerability is explored by Walker *et al.* in this issue of *Nature Neuroscience*[2]. These authors report that the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), a GGGGCC (G_4C_2) hexanucleotide repeat expansion in an intron of *C9orf72*, directly induces DNA damage while at the same time inhibiting DNA repair. They propose that this double whammy to the cell is the root cause of the neurodegeneration in both diseases.

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The authors began by studying the effect the number of G_4C_2 repeats on the formation of an odd structure formed by stable DNA–RNA hybrids known as R-loops. While R-loops can form naturally during transcription, they are usually quickly resolved by the senataxin helicase once the polymerase has passed. In certain GC-rich regions, however, hybridization between guanine-rich RNA and cytosine-rich DNA results in an unusually stable RNA–DNA duplex. This leaves the R-loop unresolved and the DNA sense strand excluded from the helix. As a result, the region is now susceptible to double-strand breaks and recombination. This is exactly what occurs in cells with the *C9orf72* hexanucleotide expansion: as the expansion increases in size, the number of R-loops and subsequent DNA damage also increase.

As if this weren't bad enough, the authors report that the problems caused by these repeats extend beyond the direct R-loop-induced damage. Although the hexanucleotide repeat is in an intron, the RNA transcript can and does undergo an abnormal repeat-associated non-ATG (RAN) translation. As a result, the GGGGCC hexanucleotide repeat is translated into a glycine-alanine dipeptide repeat (DPR). These DPRs are prone to forming β-sheet-rich aggregates[3] that can ensnare other proteins, such as p62. In response, cells increase their levels of p62, which sequesters and inhibits the E3 ubiquitin ligase RNF168. With RNF168 inhibited,

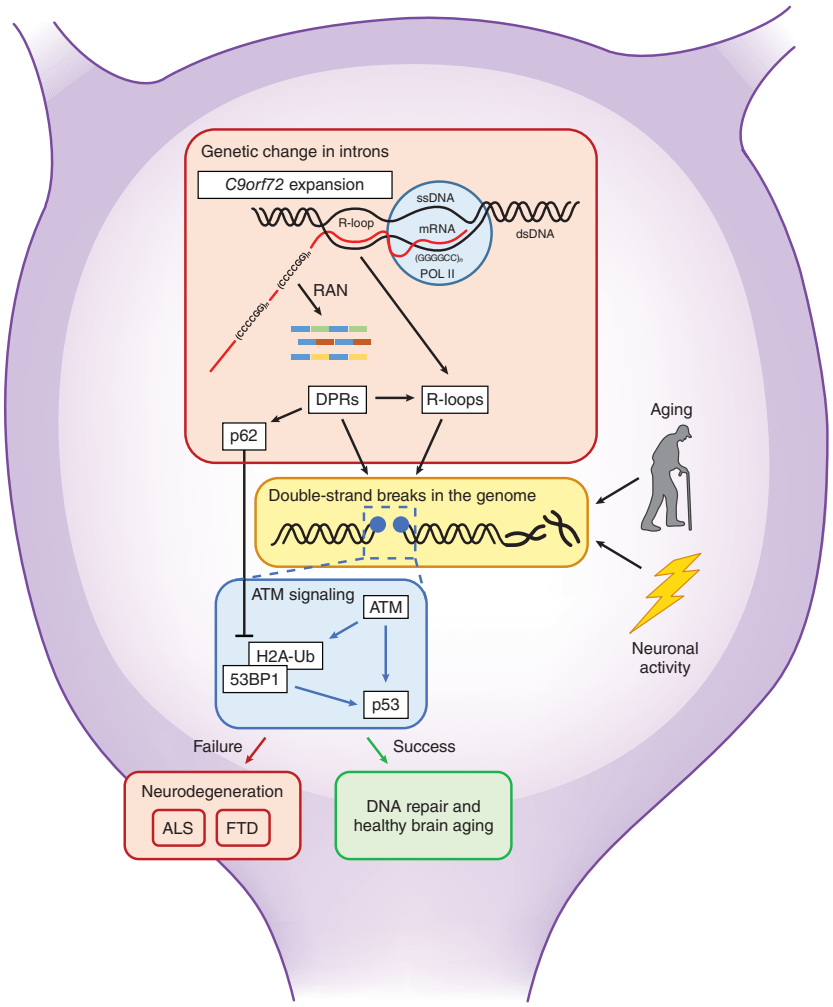

Figure 1 The complex pathways from *C9orf72* repeats to neuronal death. The effects of the expansion diverge, causing both R-loop formation and the RAN translation of DPRs. Three different dipeptides can be generated from the sense strand: poly(GR), poly(GA) and poly(GP). DNA helix disruption by R-loops leads to double-strand breaks, which are repaired by ATM and its signaling network. When successful, such repairs allow cell survival and healthy brain aging. Unfortunately, the DPRs inhibit histone H2A ubiquitination (H2A-Ub), which in turn inhibits the work of ATM. When ATM signaling fails, the result is ectopic neuronal cell cycling and cell death. This sequence of events can be enhanced or diminished by unrelated changes such as aging and neuronal activity. p53, tumor protein p53; 53BP1, p53-binding protein 1; p62, ubiquitin-binding protein p62 (sequestosome 1); ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; POL II, RNA polymerase II.

ubiquitination of histone H2A at sites of DNA damage is inhibited[4,5], as is downstream DNA repair signaling. The result is that not only do the R-loops directly cause new DNA damage but their translation product also actively inhibits DNA repair through an independent pathway.

The authors next ask whether the ataxia observed in patients with the *C9orf72* expansion is linked in any way to the ATM (ataxia-telangiectasia mutated) kinase, a DNA repair protein whose loss also leads to an ataxic phenotype. The authors find that in the absence of H2A ubiquitination, ATM fails to activate and fails to form nuclear foci. This blocks the nonhomologous end joining DNA repair process, as shown by increased levels of γH2AX and by the cell's failure to recruit other repair proteins such as 53BP1 to foci of DNA damage. Suppression of ATM activity also increases the formation of heterochromatin, possibly through nuclear migration of histone deacetylase 4, as well as through enhanced EZH2-mediated methylation[6]. This creates yet another problem for the cell, as condensed chromatin hampers double-strand break repair[7]. The end result of the expansion, then, is a vicious downward spiral of enhanced DNA damage and compromised DNA repair that eventually leads to neuronal death (**Fig. 1**).

As satisfying as this story might be as an explanation for the genetic forms of ALS and FTD caused by the *C9orf72* expansion, the authors suggest, and we agree, that the R-loop and DNA damage story, and the companion saga of the DPR inhibition of DNA repair, has potentially important implications beyond these two relatively uncommon diseases. Although the *C9orf72* locus has some very specific traits (GC-rich content and RAN translation), these conditions might be replicated at other genetic loci and thus could be responsible for neurodegeneration in a broad range of situations. Also, there is no obvious reason why transcription-coupled DNA damage caused by R-loop formation would be unique to motor neurons or to a specific set of cells in frontal cortex. C9orf72 is expressed in many parts of the brain and throughout the body. The normal function of the protein encoded by this gene is only now being uncovered. Some reports suggest that it is a vesicle-associated protein whose haploinsufficiency causes vesicle trafficking deficits[8]. Other groups have shown that it affects stress granules whose functions are critical to normal RNA metabolism[9]. It therefore seems reasonable to expect that other cellular or neural network stresses could lead to an ectopic burst of transcription from this gene locus in cells where it is normally only weakly expressed. This could drive the sequence of events described by Walker *et al.*[2] in cells other than motor neurons. Furthermore, while it might be tempting to dismiss this scenario as irrelevant to individuals who carry only normal repeat lengths at the *C9orf72* locus, somatic expansion of nucleotide repeats is increasingly recognized as a byproduct of defective DNA repair[10]. Thus, the mechanism identified for this rare familial form of the disease might yet be relevant to the more common sporadic cases in people with no known genetic risk.

In a similar vein, the mechanisms leading to hexanucleotide repeat expansion are not necessarily restricted to *C9orf72*. A separate repeat in a separate gene has been linked to spinocerebellar ataxia type 36 via an RNA gain-of-function mechanism[11], although R-loops were not specifically identified in this study. It may be a spurious connection, but it is at least intriguing that this condition also shows spinal motor neuron involvement. Thus, with *C9orf72*, we might be looking at a situation that is unique to this one locus, or we might be seeing just the tip of a genetic iceberg of vulnerability.

As with all pathways in biology, the sequence of events described by the authors is embedded in a complex cellular environment that interacts with and potentially alters the described course of pathogenesis. **Figure 1** attempts to capture the authors' main points, as well as this cellular context. The G_4C_2 expansion leads to R-loop formation and DNA damage, as well as to dipeptide RAN synthesis and inhibition of DNA repair. These two pathways, acting together, are sufficient to trigger neurodegeneration, but both can be worsened by other events taking place in the cell. Consider, for example, the creation of DNA damage by R-loops. Cells are constantly subjected to DNA damage and so have evolved overlapping layers of repair processes. Despite these restorative efforts, unrepaired DNA damage accumulates and likely serves as a master driver of the aging process in neurons[12] and other cells[13] of the brain. Indeed, neuronal activity itself has been proposed to contribute to DNA double-strand breaks[14]. We may be able to fix our breaks when we are young, but as DNA damage accumulates with age (at this locus and others), we are less and less able to correct the errors. This makes our brain cells increasingly vulnerable to the damage caused by the *C9orf72* expansions. This would lead to the prediction that we should be more vulnerable to ALS and FTD as we age, and this is indeed the case.

A second way in which context could work to enhance the R-loop and DPR story is in regard to DNA repair. Any somatic event that leads to a loss of ATM, for example, would increase the sensitivity of cells to downstream events requiring its activity. Compromised ATM activity has been shown to increase with age. Indeed, the loss of ATM activity can occur on a neuron-by-neuron basis during neurodegenerative disease[15]. If a neuron begins with a deficit of ATM activity, the effects of enhanced G_4C_2 production would be amplified and thus hasten the cell along the road to destruction.

The study by Walker *et al.*[2] thus answers many questions but raises many others, as any good paper should. The interdependent pathways described in detail by the authors offer a compelling model that accounts for many of the known features of *C9orf72*-repeat-driven disease. More importantly, in describing a molecular pathway at work in two uncommon diseases, they potentially pull back the curtains covering explanations for the loss of neurons in a wide range of more common neurodegenerative conditions, including Parkinson's, Huntington's and Alzheimer's diseases.

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Jamais vu all over again

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What is the basis for the feeling that someplace or someone is familiar? Molas *et al.* have identified brain structures involved in signaling familiarity, a necessary element for the expression of preference for novelty.

Most of us have had the experience of encountering a person who looks familiar, yet we cannot recall having met. A related phenomenon is *déjà vu*, a vivid but inaccurate feeling that the current situation is familiar. This strong sense of familiarity occurs in the absence of any explicit evidence that the situation was previously encountered. *Déjà vu* is generally accepted to be a memory-based illusion resulting from a brief bout of anomalous activity in memory-related structures of the medial temporal lobe[1]. *Jamais vu*, sometimes regarded as the opposite of *déjà vu*, is the intense feeling that the current circumstances are novel and strange, despite the objective realization that they have indeed been previously experienced[2]. Both *déjà vu* and *jamais vu* occur in temporal lobe epilepsy[3], as well as in normal individuals under ordinary situations. Compared with *déjà vu*, *jamais vu* is less common in normal populations and much more prevalent in some neuropsychiatric conditions; this difference in prevalence suggests that novelty and familiarity may be signaled by different brain pathways.

Molas *et al.*[4] provide evidence explaining how we differentiate the new and strange from the old and familiar. They have identified a circuit in the midbrain that combines familiarity and novelty signals to allow the expression of novelty preference, a capacity exhibited by virtually all mammals that have been tested. Novelty preference and preferential exploration of novelty have yielded a number of tasks useful in the study of attention, perception, recognition, sociability and cognitive development. The novelty task, originally developed by Fantz[5], has been used to study cognition in nonverbal subjects including chicks, rodents, nonhuman primates and infant humans.

Molas *et al.* employed two versions of the classic novelty task. The first is a social interaction test in which a mouse is first allowed to explore an empty pen and a pen holding an unfamiliar (or novel) juvenile demonstrator mouse (**Fig. 1a**, left). In the test phase, the subject mouse is presented with the now-familiar demonstrator mouse and a novel demonstrator mouse. Normal mice will explore the demonstrator mouse in preference to the empty pen and the novel demonstrator mouse in preference to the familiar demonstrator mouse. The second version of the novelty task is spontaneous object recognition (**Fig. 1a**, right). Here the mouse is presented with two identical objects in the study phase. In the test phase, the mouse is presented with a third copy of the familiar object along with a novel object. Normal mice

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