From the archive

Reflections on the discovery of the double helix, and Charles Darwin investigates a curious case of primrose punishment.

50 years ago

We celebrate in this issue the twenty-first anniversary of the appearance of … *A Structure for Deoxyribose Nucleic Acid* in *Nature* … [F]ew would carp at the … announcement being given pride of place as a starting point of something absolutely new … with Medawar … calling it "the greatest achievement of science in the twentieth century".

From *Nature* 26 April 1974

150 years ago

I have observed every spring in my shrubberies and in the neighbouring woods, that a large number of the flowers of the primrose are cut off, and lie strewn on the ground close round the plants … I once saw some greenfinches flying away from some primroses, I suspect that this is the enemy … One of my sons … suggested that the object was to get the nectar of the flowers; and I have no doubt that this is the right explanation … [N]o animal that I can think of, except a bird, could make two almost parallel clean cuts, transversely across the calyx of a flower. The part which is cut off contains within … the nectar. I have never heard of any bird in Europe feeding on nectar; though there are many that do so in the tropical parts of the New and Old Worlds, and which are believed to aid in the cross-fertilisation of the species. In such cases both the bird and the plant would profit. But with the primrose it is an unmitigated evil, and might well lead to its extermination; for … flowers … destroyed … cannot produce a single seed. My object in this communication to NATURE is to ask your correspondents in England and abroad to observe whether the primroses there suffer … If the habit of cutting off the flowers should prove, as seems probable, to be general, we must look at it as inherited or instinctive … If, on the other hand, the evil is confined to this part of Kent, it will be a curious case of a new habit or instinct arising in this primrose-decked land. CH. DARWIN

From *Nature* 23 April 1874

long timescales, the authors' clocks were only narrowly outperformed in terms of the rate of change of the ticks — a metric known as frequency drift. This means that, after many hours of operation, the relatively small iodine optical clocks would be expected to acquire time errors at levels comparable to those of much larger hydrogen masers.

Roslund and colleagues then took the same two optical clocks, together with a third, smaller, optical clock, to Hawaii, where they tested all three continuously for 20 days aboard a naval ship. Despite experiencing substantial accelerations and vibrations as the ship moved over the ocean waves, the two optical clocks that had been tested at NIST maintained performance comparable with that observed in the laboratory. The frequency drift of the third clock was similar to those of the larger two, but it did not quite reach the same levels of stability. Notably, the two larger optical clocks maintained extremely low time errors (less than 400 picoseconds, where 1 ps is 10^{-12} s) over a 24-hour period (Fig. 1). Thus, even if the ship had lost access to external timing sources for an entire day, it could have used either optical clock to keep time to within 400 trillionths of a second per day.

> **"Optical atomic clocks have so far been confined to laboratories, owing to their size and sensitivity."**

The work reported by Roslund and colleagues represents a crucial step towards optical atomic clocks transitioning from the laboratory to the real world, where they could support maritime, airborne and space-based systems. To facilitate this transition, it will be important to conduct more testing under a wide range of varying environmental and physical conditions and to enable mass production of the necessary lasers and optical frequency combs. With the high performance and small sizes reported by the authors, optical atomic clocks could support precision timekeeping across many platforms, alleviating reliance on external timing sources such as GPS. Future portable optical atomic clocks could even enable innovative ways of studying fundamental physics and metrology, including tests of general relativity and geodesy[4].

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The authors declare no competing interests.

Drug discovery

Targeting RNA in efforts to treat Timothy syndrome

Silvia Velasco

A therapeutic strategy that alters gene expression in a rare and severe neurodevelopmental condition has been tested in stem-cell-based models of the disease, and has been shown to correct genetic and cellular defects. **See p.818**

Scientists understand more about the genetic causes of human diseases than ever before, but there are still many neurological conditions that do not have effective therapies. One barrier is the lack of adequate model systems that can be used to understand disease mechanisms and test therapeutics. *In vitro* models of the human brain hold a great potential to overcome this. Using stem cells, it is now possible to generate complex 3D neural tissues. These structures, known as brain organoids, can be made from a type of cell called induced pluripotent stem cells, which are reprogrammed from an individual's blood or skin cells and have the potential to become any cell type in the body. On page 818, Chen *et al.*[1] describe their use of brain organoids made from patient-derived stem cells to identify a potential therapeutic strategy for the treatment of a severe developmental condition called Timothy syndrome.

This rare genetic disorder is caused by a single-nucleotide alteration (variant) in the *CACNA1C* gene, which encodes the calcium

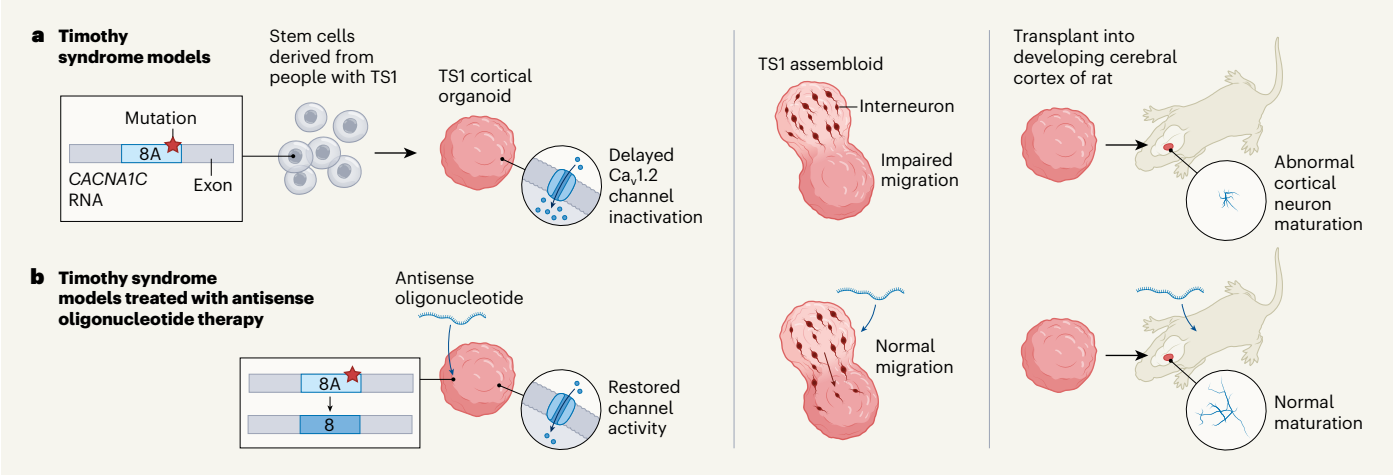

Figure 1 | Testing an RNA-targeting therapy using stem-cell-based models of Timothy syndrome. a, Timothy syndrome type 1 (TS1) is a genetic developmental condition caused by a mutation in the *CACNA1C* gene, which codes for the calcium-ion channel, $Ca_V1.2$. The mutation is in a protein coding-sequence called exon 8A (shown here between exons 7 and 9 in a portion of the RNA for *CACNA1C*). Using stem cells derived from people who have TS1, Chen *et al.*[1] created 3D neural tissues, called cortical organoids, which have been shown to recapitulate key features of the disease[4,5,8]. Neurons from the TS1 cortical organoids had defective $Ca_V1.2$ channels, which exhibited delayed inactivation leading to excess calcium-ion influx. An 'assembloid' in which a cortical organoid is fused to another type of brain organoid was used to model the migration of interneurons (a type of neuron) to the cerebral cortex. This migration would normally occur during development, but it was impaired in the TS1 assembloid. Finally, the authors transplanted the TS1 cortical organoids into the developing cerebral cortex of newborn rats, and the maturation of neurons that typically occurs was impaired. **b**, Chen *et al.* used antisense oligonucleotides — short nucleic acid sequences that bind to RNA — to induce expression of a version of *CACNA1C* containing exon 8, which does not bear a mutation. Treatment with antisense oligonucleotides restored $Ca_V1.2$ function, and corrected the abnormal interneuron migration and neuronal maturation.

channel $Ca_V1.2$. The variant leads to an excessive increase of calcium ions inside cells because inactivation of the channel is delayed, resulting in a wide range of conditions, including life-threatening heart defects, autism and epilepsy[2]. *CACNA1C* is a large gene that contains alternative protein-coding sequences (exons), which allow distinct proteins to be produced. The expression of different *CACNA1C* exons is dependent on the tissue, brain region and stage of embryonic development[3], and is regulated by alternative splicing — a process that happens after transcription and enables several protein products to be generated from the same gene. The variant responsible for Timothy syndrome type 1 (TS1) occurs in the alternatively spliced exon 8A of *CACNA1C*.

To examine the expression of different *CACNA1C* exons during typical brain development and in TS1, Chen *et al.* generated 'cortical' organoids that resemble the cerebral cortex (the outer layer of the brain) using induced pluripotent stem cells from people who have TS1 (Fig. 1). The authors showed that, typically, exon 8A is highly expressed during early brain development and is later replaced with exon 8, but in the TS1 cortical organoids, expression of exon 8A with the TS1-associated variant remained high throughout development.

The authors therefore explored the possibility of inducing a switch from exon 8A to 8 as a strategy to treat Timothy syndrome. For this, they used antisense oligonucleotides (ASOs) — short nucleic acid sequences that bind to precursor RNA before splicing occurs, interfere with splicing, and thus modify exon expression. Introducing ASOs to TS1 organoids reduced exon 8A expression in a dose-dependent manner without affecting the expression of exon 8 and $Ca_V1.2$. ASOs also corrected the delayed inactivation of the $Ca_V1.2$ channel and restored the typical flow of calcium ions in neurons from TS1 organoids, without generating any adverse effects *in vitro*.

The cerebral cortex contains two broad types of neuron: excitatory neurons and inhibitory interneurons. Excitatory neurons are generated from neural precursor cells (progenitors) in the top (dorsal) part of the forebrain, whereas inhibitory interneurons originate from a separate population of progenitors outside the developing cortex in the bottom (ventral) part of the forebrain, and then migrate to the dorsal forebrain. In TS1, this migration is disrupted[4]. The cortical organoids generated by Chen *et al.* mostly contained cells derived from the dorsal forebrain. Therefore, to model interneuron migration, the authors fused dorsal and ventral forebrain organoids into a structure known as an assembloid. In TS1 assembloids, they found that treatment with ASOs corrected the impaired interneuron migration.

Finally, to test the delivery of ASOs and their ability to correct genetic and functional defects *in vivo*, Chen and colleagues used a transplantation technique, developed by researchers in the same laboratory, to engraft TS1 cortical organoids into the developing cerebral cortex of newborn rats[5]. They found that treating rats with ASOs reduced exon 8A expression and corrected $Ca_V1.2$ function in the grafted human cortical neurons. Previous work has shown that transplanted organoids integrate into the host's neural circuits and that cortical neurons mature more readily in the engraftment than they do *in vitro* because of the more physiological environment[5]. Chen *et al.* confirmed previous observations that the integrated neurons from the TS1 cortical organoids had dendrites (neuronal processes that conduct electrical impulses) with reduced length and complexity compared with non-TS1 neurons, indicating an abnormality in neuronal-activity-dependent maturation[5]. The authors showed that treatment with ASOs corrected this.

Chen and colleagues' study illustrates a promising therapeutic avenue for the treatment of Timothy syndrome. Strategies that target exon splicing with ASOs have been the foundation of clinical trials conducted in the past few years for treatments for a neurodegenerative condition called spinal muscular atrophy and for Dravet syndrome, a neurodevelopmental condition[6]. Because dysregulation of splicing has been proposed as a key mechanism underlying neuropsychiatric disorders[7], strategies that use ASOs to target splicing could be applied to a large spectrum of neurological diseases.

Although the *in vitro* results are encouraging, translating the findings from the current study into the clinic would require further refinement of the specificity of ASOs to the TS1-associated version of exon 8A, and an evaluation of the ability of ASOs to target specific cell types across different brain regions. It would also require an extensive assessment of the potential toxic side effects of ASOs — which is best done in *in vivo* models. Future studies might use the transplantation model described in this study

to assess the toxicity of ASOs in both human brain cells and rodent organs. It is also important to consider that ASOs will not be able to correct developmental defects that present before birth, such as abnormalities in the generation of specific cell types[8]. ASOs would only be able to address functional defects that present after birth, such as aberrant activity of the $Ca_V1.2$ channel. This challenge, which is true for most neurodevelopmental disorders, might be addressed by therapies that combine several strategies.

Despite the great therapeutic promise of ASO strategies for the treatment of genetic disorders, treating neurological conditions is particularly challenging. This is because ASOs cannot cross the blood–brain barrier and need to be administered directly into the cerebrospinal fluid by injection into the spinal canal. The procedure is invasive and not always an option for people. Also, it would be desirable to find ways to improve the cost-effectiveness of ASOs, especially considering that their intended use is to treat neurological diseases that are relatively rare.

Over the past 10 to 15 years, 90% of drug candidates that showed promise in preclinical tests failed in clinical trials, with candidates for cardiovascular and nervous-system conditions having the lowest probability of success[9]. The development of models that can better replicate human diseases and accurately predict human responses promises to improve the outcomes of drug-development programmes. As shown by the current study, stem-cell-based models are well suited for validating drug targets, and for screening and optimizing drug candidates. Notably, because of their increased cellular complexity compared with 2D neural cell cultures, brain organoids could be useful for testing interventions that target specific cell types affected in certain diseases, enabling more effective therapies to be developed.

"Brain organoids could be useful for testing interventions that target specific cell types."

In 2022, a US bill called the Food and Drug Administration (FDA) Modernization Act 2.0 recognized the value of *in vitro* cell-based approaches for evaluating therapeutic candidates[10]. Organoids that model the retina of the human eye have been used in the preclinical development of ASO-based therapies, which reached clinical trials, that aim to correct aberrant splicing seen in inherited neurodegenerative conditions that cause childhood blindness[11,12]. However, the application of brain organoids for similar purposes has not been fully explored yet. Therefore, Chen and colleagues' study is timely and makes a strong case for the implementation of brain organoids into drug-discovery efforts for the treatment of currently untreatable neurodegenerative and neurodevelopmental disorders.

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The author declares no competing interests.