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# Rewriting the book of life

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The recent advent of CRISPR-Cas9—an unparalleled and precise DNA-editing technology—has changed the way in which molecular biology research is performed. From basic blue skies research to the study of human congenital disorders, the application of this technology is revolutionizing how scientists are conducting their research. This article introduces the basics of genome biology, the neural crest and the potential for CRISPR-Cas9 in a specific human congenital disorder.

## Molecular life story

**You started life as a single cell, approximately the size of this full stop.**

A solitary cell; containing a DNA instruction manual on how to grow and divide into specialized daughter cells, which over the course of time worked together to build your body.

Nearly every cell in your body has inherited a complete copy of this original instruction manual, known as your genome, irrespective of its function. This inheritance ensures all your cells are on-board with the same overall programme: making you! Your skin cells only differ from your liver cells because they effectively 'read' different 'chapters'—known as genes—from within identical DNA instruction manuals.

Thus, your genome, or DNA instruction manual, must cover all the topics that every different type of cell in your whole body will ever require over the course of your entire life. It is encoded by approximately 3.2 billion DNA bases, or 'letters', and is organized into 23 separate 'volumes', known as chromosomes. Given the importance of these instructions, each cell also has a complete backup set. If some of the pages of the precious instruction manual get damaged, DNA repair proteins can remove the faulty section, and replace it with the correct segment from the backup copy.

Genes are the discrete parts of your genome that are read by cells, and give the directions on how to build the proteins they need to behave and function as they should. Errors in genes can lead to mutated proteins being created. These mutant proteins can wreak havoc within a cell, and this often results in a change in the behaviour of the cells. Many human diseases can be thought of as groups of poorly behaving cells, causing systemic disruption by not being on-board with the overall plan like the rest of the cells within the body.

**The human genome** is comprised of two sets of 23 chromosomes per cell. The average human adult body contains 100 trillion cells. Getting the diversity of cells required to build that body requires some tens of thousands of proteins as coded for by about 30,000 genes found in the 3.2 billion letter genome. Chromosomes range in size from 300–50,000,000 DNA base pairs. The data generated during the Human Genome Project is public property and more information can be found at: www.genome.gov/10001772/all-about-the--human-genome-project-hgp/

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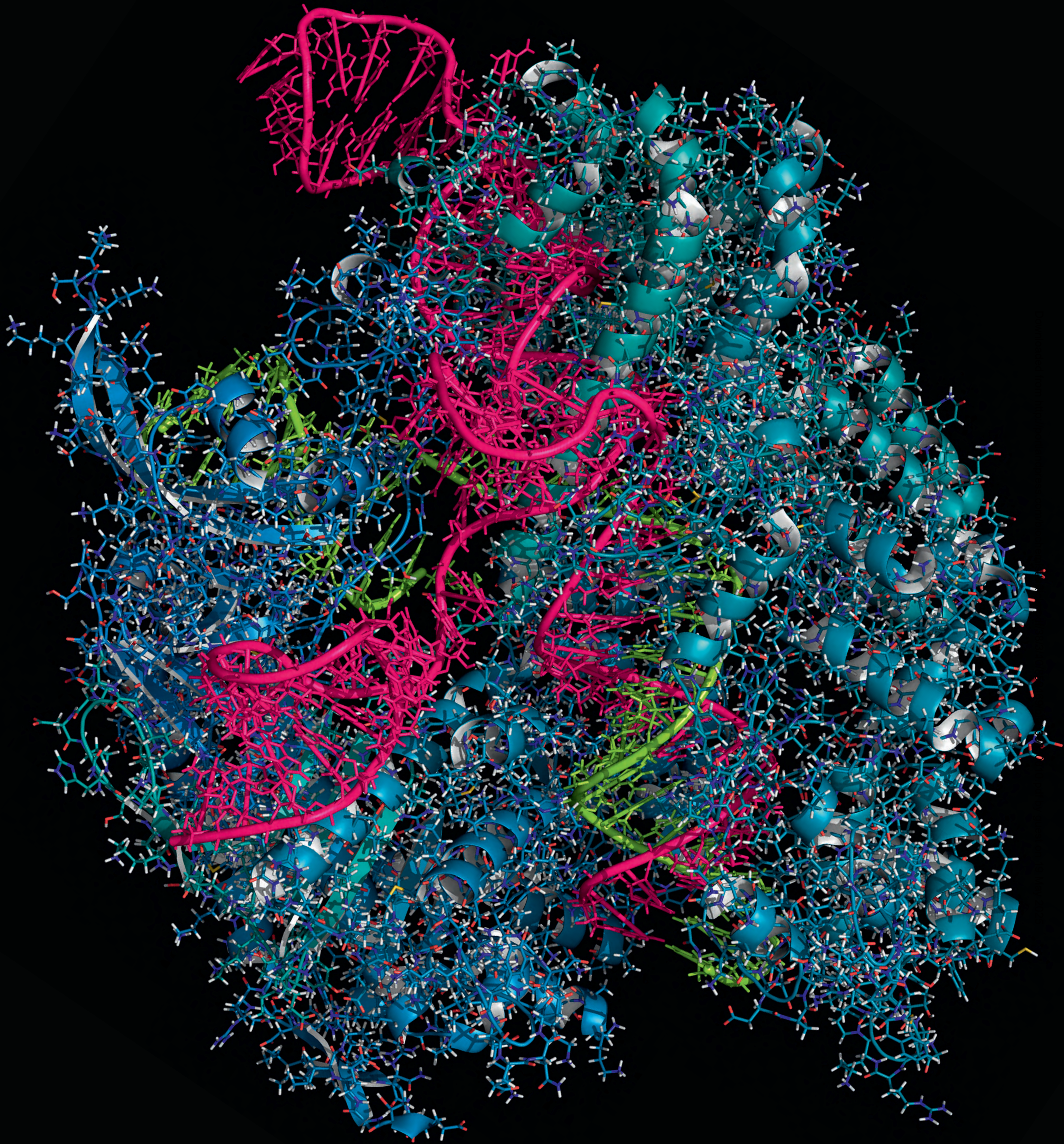
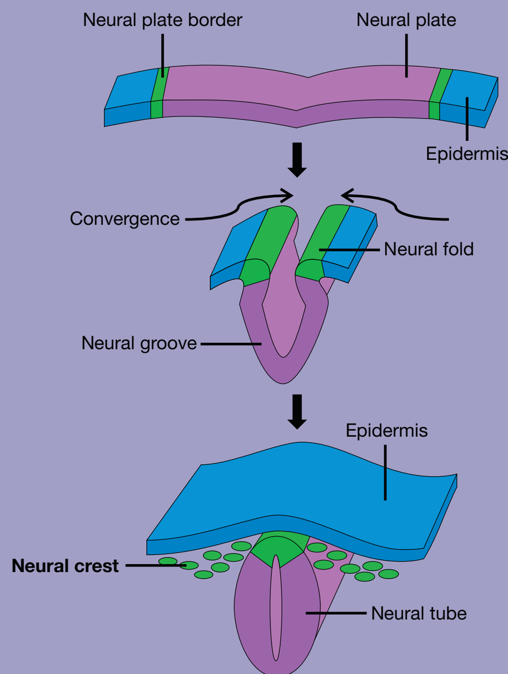


CRISPR-Cas9 gene editing complex from Streptococcus pyogenes. The Cas9 nuclease protein uses a guide RNA sequence to cut DNA at a complementary site. Cas9 teal/blue; RNA magenta; RNA lime green. *Shutterstock*

**The neural crest** is a transient and migratory population of embryonic progenitor cells. The neural crest is exceptional in its capacity to differentiate into a wide range of derivatives and contribute skeletogenic, muscle, neuronal, secretory and pigment cell types to a plethora of unrelated tissues and organs. These include cartilage, bone and connective tissue elements of the cranio-facial skeleton, smooth muscle cells in all blood vessels of the face and the forebrain, catecholamine-releasing chromaffin cells in the adrenal gland medulla, neurons and glia in the cranial and dorsal root ganglia, the entire enteric nervous system and the quasi-totality of the body's pigmentation. They achieve this by undergoing a process known as 'epithelial-to-mesenchymal transition' (EMT), which allows them to delaminate from the neural tube, invade the surrounding tissues and migrate to their final destinations.

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## CRISPR in action

A particular protein called CHD7 contributes to determining the behaviour of a group of stem-cell like cells during early development. These neural crest cells are responsible for building most of the structures of your head, giving your skin its protective pigmentation and innervating your gastrointestinal tract amongst other important contributions[1]. However, when CHD7 is mutated or entirely missing from the DNA instruction manual, these cells become deregulated and do not behave as they should. The outcome for the affected human is a congenital disorder called CHARGE syndrome[2].

CHARGE syndrome is a sporadic autosomal dominant disorder, characterized by a multitude of aberrations. Patients clinically present with coloboma (an irregular iris within the eye), heart malformations (such as hole in the heart), atresia of the choanae (aberrations of the nasal cavity), retardation of growth, genital hypoplasia and ear abnormalities. This disorder occurs in approximately 1:8,500–15,000 live births worldwide. More than 65% of CHARGE patients only have errors in their *CHD7* gene[3].

To put this into perspective, an unfortunate single letter error in a ~190,000 base pair long gene, accounting for 0.006% of the total genetic material within a given cell, can lead to a significant congenital disorder. However, this also presents an opportunity to have a large positive impact if using modern-day gene-editing technologies.

CRISPR-Cas9 is a revolutionary new molecular DNA-editing technology, which is allowing scientists to edit DNA in live cells with unprecedented precision. The initial work in bacteria during 2012[4], and its subsequent translation into human cells in 2013[5,6], has changed the way molecular biology research is performed.

CRISPR-Cas9 functions much like the 'Find-and-Replace' function in Microsoft Word—it can be used to locate a defined sequence of letters and can either delete or replace it with another user-defined sequence. Technically, a 'guide' RNA molecule complementary to a user-defined target sequence is generated. This guide RNA then directs a Cas9 protein, which acts as a pair of 'molecular scissors', to the target sequence. Here the Cas9 cuts through the DNA, which can be utilized to physically excise the target from the DNA.

In addition to the simple excision of a sequence, if a replacement sequence is provided by the user—with matching sequences to either side of the CRISPR-Cas9 target site—then that is usually incorporated into the target site. This has opened the door to the highly exciting, and potentially medically useful, prospect of being able to modify any sequence within a genome. For example, in vitro fertilization (IVF) embryos could be screened for mutations in *CHD7*; then if an error is found, CRISPR-Cas9 theoretically could be used to replace the entire gene with a functional and non-mutated version, preventing the occurrence of CHARGE syndrome.

CRISPR-Cas9 gene editing also allows scientists to better address basic research questions such as, "What happens to a young neural crest cell if we cut certain genes out of it?" Previously this question has been addressed by various methods to attenuate the production of the proteins made by the genes in question—but attenuation is not the same as complete removal. This has been one of the most exciting uses of the CRISPR-Cas9 system in the study of gene networks. It is additionally possible now to ask more advanced questions such as, "What

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happens to a neural crest cell if we specifically target a single gene for constitutive activation?” Again, this was not possible to do cleanly prior to the emergence of the CRISPR-Cas9 system.

## Future directions

The speed with the CRISPR-Cas9 tools can be generated within the lab is a great strength of the system. It is versatile, and allows the addition of bolt-on style functions, by literally attaching other proteins to Cas9 and using that to direct these other proteins to user-defined locations.

Though the application of CRISPR-Cas9 gene editing in laboratory research has not come under scrutiny, the implications for medical treatments are a point of public debate. At the end of 2015, the first summit in using CRISPR-Cas9 in the human germ line was held. It is worth noting that most research is intended to have some potential impact on healthcare or reveal more of the normal workings of nature.

In a watershed moment, on 1 February 2016, the Human Fertilisation and Embryological Association (HFEA) gave Dr Kathy Niakan, a group leader at The Francis Crick Institute, approval for CRISPR-Cas9 “gene editing of [human] embryos”[7]. This is the first authorization for the use of gene-editing technology in the UK to further the study of development and embryogenesis in human embryos[8].

Since the advent of CRISPR-Cas9 technologies, these directions have become distinct possibilities and face their own challenges. Should we apply the outcomes of academic research to clinical medicine, using CRISPR-Cas9 technologies to precisely make the changes we desire to our genomes? Should we be concerned by the potential risks, or should it be welcomed as the next logical step?

Finally, it should be noted that whilst the use of CRISPR-Cas9 gene editing in humans is increasingly being debated in the public sphere, academic applications of the CRISPR-Cas9 system quietly go on unashamedly developing and evolving. ■

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### Additional reading

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