A moving story

Could transposons in the neural genome be contributing to individual differences in brain and behaviour?

Mobile genetic elements, transposons, make up a significant fraction of the genome – 40% in the case of humans. It is not clear whether they are just 'genetic parasites' or also have some biological function. By looking at transposon expression in individual cells in the fly brain, **Christoph Treiber** has found that their expression is cell type specific, could potentially generate novel neural proteins, and might therefore be contributing to individual variation in brain function and behaviour.

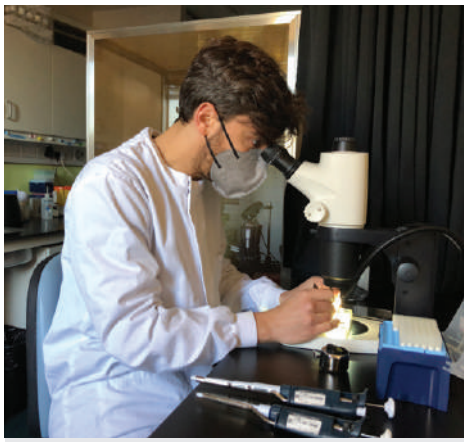
Christoph's interest in this area arose following an experiment analysing gene expression in the brain: "If you look at all of the RNAs in the brain that are expressed, a lot of them map to genes, but a quite large percentage actually don't map to genes but to transposons."

Transposons are families of mobile genetic elements that have colonised the genome of essentially all organisms, filling the genome with huge numbers of nearly identical repeated sequences. Notably, the precise composition of transposons varies between individuals: "If we look at the human genome, we all differ in something like 1000 transposons to any other person on this planet. We each have our own transposon fingerprint."

Although mapping the movement of transposons around the genome is tricky, it is more straightforward to detect RNA transcripts originating from them. Remarkably, in the fly brain, around 40% of transcripts show signs of being derived wholly or partly from transposons: "So it's making up a huge chunk of what's going on in the brains of animals."

Homing in on single cells

A key question, says Christoph, is whether there any patterns in this expression of transposons that might indicate functional relevance: "Are they just randomly or broadly expressed in all cells of the brain, or is there some sort of logic to it, or some kind of control to how they're expressed?



Christoph Treiber

And that's where the single-cell sequencing came in as the perfect technology to really get to the bottom of this."

Looking at the transcriptomes of individual cells had been championed by US researcher Steve McCarroll, who initially focused on the mouse retina. "A few months after he published that he visited Oxford and I had a chance to chat with him and discuss the options for taking this from the mouse to the fly brain."

Christoph discovered that the technology transferred remarkably well: "We're really able to look at basically all the cells in the brain. The fly midbrain consists of something like 100,000 cells. So in one experiment, we analyse the whole brain, every single cell in the whole brain. The other really cool thing is, we can resolve an insane number of cell types. I hesitate to say all cell types, because we don't really know how many cell types there are. But the complexity that we can resolve with our technology, it's just absolutely mind-blowing. And when we do this, we can now plot the expression level of every single gene we can possibly come up with."

Moreover, it is not just genes that can be tracked: "We can also look at transposons. We can ask, what is the expression pattern of transposons throughout the brain? And when we do this, we find this very unexpected observation, which is transposon expression is far from random. In fact, it's highly stereotypical. And many many transposons are very specifically expressed in certain cell types in the brain."

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Not only that, Christoph has also recently explored how transposon expression changes during processes such as learning and memory. "We train a group of flies and we don't train another group of flies. And we see that there are some transposons that are specifically upregulated in flies that have been trained to learn a particular task. So we are very confident that some transposons seem to be specifically expressed as part of the formation of memory in fly brains, which is incredibly cool."

This could simply reflect the fact that gene expression varies between cell types, so the actively transcribed transposons could just be those sitting next to genes switched on in particular cell types. However, by delving even deeper and fully sequencing transcripts, Christoph has found that the picture is more interesting. Many of the transposons sit in introns, the gaps between coding portions of a gene, and are transcribed along with coding sequencing. This can introduce 'cryptic' splice sites, changing which bits of RNA are excised from transcripts.

This could have big implications, Christoph suggests: "We have one very intriguing hypothesis now that these insertions slightly alter the way that the proteins that are encoded by these neural genes function, and thereby alter the functionality of the cell and, potentially, the behaviour of an animal."

Furthermore, given the heterogeneity

in transposons seen across a population, these subtle differences could be contributing to differences between individuals. Given every individual's unique transposon fingerprint, these subtle changes could be contributing to individual differences in brain function: "We're hypothesising this contributes to what makes you, you and me, me."

European science

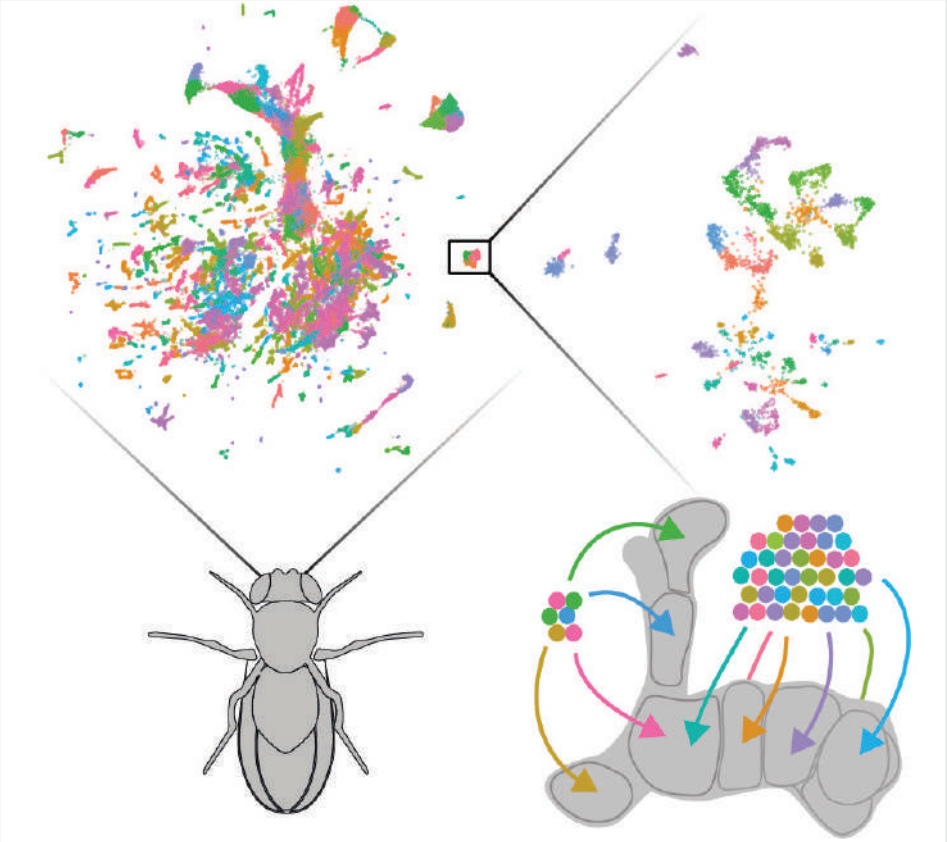
Christoph was recently awarded a European Research Council (ERC) Starting Grant to take his work forward. In part this will mean exploring whether the genetic changes seen do truly lead to the generation of different proteins. He is also interested in pursuing 'transposo-typing' and then carrying out the equivalent of genome-wide association studies to search for correlations between particular transposon signatures and specific behavioural or other characteristics.

The main thrust, however, is to carry out long-read single-cell sequencing. "It sounds like just an incremental sort of step," Christoph acknowledges, "but it's really a game-changer in trying to understand how transposons change genes in the brain."

"We'll take a fly brain and we'll sequence the entire intact mRNAs in each individual cell of that fly brain. That will give us two really cool bits of information. The first one is we'll have all splice isoforms of all the genes in each individual cell. But most importantly, we'll directly see where these transposon gene fusion transcripts are expressed in the brain."

These studies will be technically and computationally demanding, and rely on the detailed wiring diagram that has been created for the fly brain. Through this, the spatial information on gene expression can be mapped onto neural circuitry.

A further advantage of the fly system is that populations of genetically identical animals can be studied. "I call them perfect clone armies," says Christoph. "This allows us to perform behavioural experiments, physiological imaging, functional brain imaging, anatomical analysis of a particular transposon type, comparing results when the transposon is there with when it is not." Implication of particular transposons in functional processes would then open the door to further studies to determine what specific role they are playing.



Schematic showing how iterative sub-clustering of groups of cells leads to the assignment of single-cell transcriptomes onto anatomically distinct dopaminergic neurons.

One obvious question is whether this has any relevance to the human brain. "15% of all the RNAs in the human brain have some transposon sequence," Christoph points out (although studying them is much harder as many human transposons are genetically identical so it can be difficult to identify the chromosomal origin of a transcript). "If we look at the human genome, we do see transposon insertions in introns. So there will be cryptic splice sites introduced by transposons. And I think it's very likely that these transposons will then also change human genes."

Although obviously delighted to have been awarded an ERC grant, Christoph is dismayed by the continuing impasse which sees the UK still outside the EU's Horizon Europe programme: "The UK's association with Horizon Europe is looking less and less likely, but losing out on the ERC's amazing support for ambitious discovery-focused research and large collaborative projects would be an absolute disaster for the UK. I think the scientific community has to fight harder to sway policy-makers and explain to the public how valuable this programme is for our society."

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• Christophe is recruiting – anyone interested in joining his lab can drop him a line at christoph.d.treiber@gmail.com