



Neurogenesis in Marseille

Finding your Way

Harold Cremer is a German in Marseille; an appropriate place to study the migration of neuronal precursors from their place of birth to their site of function.

Marseille's harbour is a busy place. Ships come and go from all over the world. While countless people from the Maghreb (North Africa) have arrived in Marseille full of anticipation and hope, others experienced hell on earth just a few kilometres offshore in the fortress on the tiny Île d'If. People like the shoemaker François Picaud (the inspiration behind Alexandre Dumas' legendary duke of Monte Christo) wished nothing more than to escape from here and even today most immigrants proceed to other French cities. However, one man approached Marseille from the opposite direction, the north, more than ten years ago and actually stayed, voluntarily. His name is Harold Cremer. Don't be fooled by his Christian name; he is not English. His mother does not speak a single word of English, he maintains. In fact, he is a German who, after his studies in Neurogenetics in Cologne, decided to do a postdoc in Europe rather than the US.

"I got an EMBO fellowship and my plan was to stay for only one year in the south," says Cremer. But he liked his work at the Developmental Biology Institute of Marseille (IBDML) so much that when he was offered a group leader position he accepted immediately. Together with his team, including staff scientist Marie-Catherine Tiveron, postdoc Simone Diestel, PhD student Camille Boutin, masters students Elise Martinez and Marion Rame and, last but not least, an "incredibly good technician", Angélique Desoeuvre, he is determined to understand how neurogenesis and cell migration in the brain are regulated on the cellular and molecular level.

Important immigrants

Cremer has actually been interested in this subject since his PhD when, back in the early nineties, he constructed a knock-out mouse model for NCAM, the Neural Cell Adhesion Molecule. This was an emotional time. During his PhD Cremer's supervisor,

a friend as well as his boss, died. Thanks to the commitment of Klaus Rajewsky (who, despite retirement, is still not tired of research and left the University of Cologne for Harvard a couple of years ago), Cremer finished his PhD and even landed a *Nature* paper with his NCAM model (vol. 367, p. 455).

NCAM belongs to the immunoglobulin superfamily, exists in various isoforms and plays an important role in a plethora of cell-cell and cell-substrate interactions. Cremer observed that mice without NCAM develop a much smaller olfactory bulb (OB), where odours are processed, than normal mice. This structure in the forebrain is very important. For a long time people thought it impossible for adult brains to produce new neurons. But as early as the 1960s the observations of Joseph Altman questioned this assumption. Altman showed that in mature brains some cells are indeed able to divide.



Cell migration: Transplanted stem cells (upper right) generate neuronal precursors that migrate into the olfactory bulb (left), where they differentiate into interneurons.

But he did not manage to prove that these cells were neurons. Conclusive proof was still 30 years away. Since the late 1980s it has emerged that, at least in the OB and the hippocampus, the production of neurons takes place throughout life.

However, new neurons do not appear in the OB. Their precursors are generated elsewhere and have to find their way there. This is where NCAM comes into the game and where the answer to the reduced size of

the OB in NCAM knock-out mice lies. Cremer's team discovered that the presence of NCAM, and in particular its polysialic acid (PSA)-bearing form, is crucial if the precursors of an important group of neurons, the interneurons, are to migrate to the OB (*Journal of Neuroscience* 20, p. 1446). These relatively small neurons link projection neurons, which make up the majority of neurons. They possess dendrites and long axons and are therefore able to activate each other over long distances.

Cellular hiking guides...

From their place of birth in the subventricular zone to their final destination, the interneuron precursors follow a well-defined "immigration" route, the rostral migration stream. "They travel in a form resembling a big cable," says Cremer. "The precursors synthesize NCAM, which is important for them to recognize each other. They thereby generate their own hiking trail." NCAM is thus a signpost instructing the neuron precursors to stay together and to crawl forward on top of each other. If there is no NCAM, many of the cells will end their journey somewhere on the way to the OB and will never arrive at their new home.

Of course, NCAM is not the only molecule needed for the ordered migration of interneuron precursors. Cremer and his group have unravelled the function of others, including one called Reelin. Once the caravan of interneuron precursors has arrived at the OB, the individual cells have to say goodbye to their travelling companions, spread throughout their new home, settle down and develop into GABAergic or dopamine-producing interneurons. Cremer's team has been able to show that Reelin triggers these tearful goodbyes (*Nature Neuroscience* 5, p. 939). Scientifically speaking, it acts as a detachment signal. The interneuron precursors, having so far migrated tangentially, i.e. straight through the brain tissue, change

to individual radial, i.e. brain-outbound migration patterns. “You can imagine that like a bicycle’s spoke,” Cremer explains.

However, Reelin does not tell the precursors to stop at the OB and it does not guide them on their way. Plenty of unknown molecules are implicated in neurogenesis and cell migration. “One of my group’s goals is to identify more of these signalling candidates,” says Cremer. To find them, they perform comprehensive gene expression analysis using SAGE (serial analysis of gene expression) on interneuron and projection neuron progenitors and confirm their results through microarray technology and quantitative real-time PCR. Crucial in accomplishing this task is co-operation with Miltenyi, a company in Cologne that has extensive technical know-how and equip-

on a molecular level during corticogenesis (*Journal of Neuroscience* 26, p. 13273). In contrast to interneuron precursors, projection neuron precursors are born in the cortex. During their tangential travelling, the interneurons collide with radially migrating projection neurons. “We could show that the projection neuron precursors secrete SDF-1, whereby they strongly regulate the interneuron’s travelling route. It tells them to take exactly this route – the subventricular zone,” says Cremer.

Mysterious maturation

The genomic approach has already and will surely continue to reveal interesting information about neurons. However, there is one major limitation: technically, it is only feasible to isolate projection neuron pre-



Following in the footsteps of migrating neurons: Harold Cremer (fourth from right) *et al.*

ment. A joint PhD Student, Olaf Hardt, is central to this collaboration and under the co-direction of Miltenyi’s Head of Research, Andreas Bosio. A nice plus for Cremer is that the cooperation enables him to visit his old homeland, Cologne, fairly regularly. As with every genome-wide scan, there are hundreds of candidates. Using gut feeling and a diligent search of literature these have been restricted to the 20 most promising, which they want to study in more detail, first on the gene expression and then at the functional level. Cremer is not yet ready to disclose who those candidates are but interesting projects are certainly on the way.

...and traffic lights

What he does like to talk about, though, are three other molecules on which they have recently published papers: Cux2, SDF-1 (chemokine stromal-derived factor 1) and BAD-LAMP (brain-associated LAMP-like molecule). “Lately, we have discovered that the transcriptional factor Cux2 defines a special subpopulation of cortical projection neurons – those in the upper layer and their precursors,” he explains. The second molecule, SDF-1, colocalizes with Cux2. It enabled the Cremer group to prove for the first time that projection neuron precursors and interneuron precursors communicate

precursors before they develop their long dendrites and axons. It is thus impossible to analyse the genes that determine the final phases of maturation into projection neurons.

But we are getting ahead of ourselves. The Cremer team first needs to have an inkling of which gene families could play a role, verify the candidates’ expression in the brain by Northern blot analysis and then try to find the cell populations accounting for expression by immunoblotting. In collaboration with another group in Marseille, headed by the cell biologist Philippe Pierre, they have in fact successfully identified a hot candidate. The candidate in question is BAD-LAMP (*Journal of Cell Science* 120, p. 353). “It is a lysosome associated protein found in a type of vesicle that has been completely unknown so far,” explains Cremer. The groups found a correlation between the appearance of BAD-LAMP and the formation of synapses, yet BAD-LAMP’s function is completely unknown. They now want to find out what these vesicles do and the role that BAD-LAMP plays there.

How many immigrants will have landed in Marseille before the mysteries of immigration processes in the brain are solved? Nobody knows.

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