



Regulated by Notch: the pattern of bristles

Molecular signalling in Vienna

On the Back of a Fly

Beautiful Vienna, Austria, world famous for its music, theatre, art and architecture, is gathering recognition as a different form of art takes centre stage. At the Institute of Molecular Biology, 'artist' Jürgen Knoblich and colleagues are performing, for the first time ever, genome-wide analyses to study signalling pathways in specific tissues – and their model, the fruit fly *Drosophila melanogaster*.

As research scientists, why do we do what we do – the seemingly endless hours, the disappointment of another failed experiment, the constant search for funding, with success measured by the number of high impact publications? Is the motivation money, fame or being the first to elucidate a pathway or cure a disease? German-born scientist Jürgen Knoblich insists that “curiosity should be the driving force because we want to know how something works.” Certainly, an idealistic viewpoint but this philosophy has resulted in an enviable career and a publication record that most scientists would love to boast about.

Knoblich, currently the deputy scientific director at the Institute of Molecular Biology of the Austrian Academy of Sciences (IMBA), in Vienna, Austria, began his career at the University of Tübingen in 1983 studying Biochemistry. He remained in Tübingen for his doctoral studies in the laboratory of scientist, Christian Lehner, who was at that time just starting out. The decision to stay in Tübingen is somewhat serendipitous as he describes attending Lehner's interview seminar and, despite having already accepted

a position elsewhere, he recalls he “was blown away by his job talk that I contacted him immediately to see if he was looking for students.” Lehner was very influential in shaping Knoblich as a scientist. “I was extremely fascinated both by his way of doing science and by the field of cell cycle control, which was at that time a very hot topic,” he exclaims. Therefore, in addition to scientific skills, young Knoblich was introduced to his future topic of study, cell division and the model which he continues using today – the fruit fly, *Drosophila melanogaster*.

At a time when most funding agencies look for human applicability, the fly seems rather unimportant. Knoblich continues to use this model because, “the time it takes from having an idea to testing this idea is reasonably short, in that you can still actually remember the idea when you get the results.” Additionally, results with *Drosophila* are easier to interpret compared to more evolved models, such as rodents. Finally, he enjoys the environment and non-controversial nature of members of the “fly community”. The power of using *Drosophila* is seen in his laboratory's most recent publication in *Nature* (Mummery-Widmer *et. al.*, currently in press) where



Jürgen Knoblich:
Knocking down fly genes

a transgenic RNA interference (RNAi) library targeting almost 90% of all protein-coding genes in *Drosophila* was used to study complex developmental processes mediated by the Notch signalling pathway in a tissue-specific manner.

RNAi has gained popularity in recent years as an experimental tool to study gene function and phenotype by systematically knocking down genes in cells and whole organisms but most experiments have been conducted using genome-wide RNAi screens. Because complex biological processes in organisms are usually tissue-dependent, a challenge for many years was to inactivate genes in a tissue-specific manner in a whole organism. This was achieved in the laboratory of Barry Dickson (*Nature* vol. 448: 151-56) at the Institute of Molecular Pathology (IMP), a neighbouring institute of the IMBA, where Knoblich himself began as a group leader in 1997. Dickson used the

binary GAL4/UAS expression system, developed by the Harvard lab of Norbert Perrimon (*Development* vol. 118: 401-15) to create an RNAi library that generated hairpin RNA constructs targeting every single gene in the *Drosophila* genome. The GAL4/UAS system works because it allows for tissue-specific targeting of genes.

As the RNAi library was created literally next door, Knoblich and his team began collaborating with Dickson, thus having first access to the lines as they were being generated. The goal was to use the library to study a complex developmental process in a whole organism in a tissue-specific manner. To achieve this, the pattern of bristles, the fly's external sensory organs, on the back of the fly was studied. Bristle formation begins from a single sensory organ precursor (SOP) cell specified by a process called lateral inhibition. Rounds of asymmetric cell division (ACD) follow, resulting in outer cells (hair and socket) and inner cells (neuron and sheath). Notch signalling is very important to both processes. According to Knoblich, “Loss of bristles could mean too much Notch or too little Notch. It can also indicate that actin is not polymerized or there is a lack in cell division. Therefore, the connection between the phenotype and the actual physiological function of the gene you knock down is much more complicated, thus making it difficult to predict whether such a transgenic approach would work to study a signalling pathway.”

20,000 transgenic RNAi lines

The primary analysis, which began more than three years ago, involved screening over 20,000 transgenic RNAi lines targeting more than 10,000 genes on the back of the fly. Abnormal phenotypes were painstakingly noted in a carefully organised dataset. “The outcome was quite amazing because about 20% of all the genes in the fly genome resulted in a visible phenotype,”

remarks Knoblich. These genes were categorised as involved in lateral inhibition or ACD. The data are now publicly available where scientists can explore their favourite gene (<http://bristlescreen.imba.oeaw.ac.at>). Following this extensive and surely exhausting screening process, Knoblich commented that now the laborious work began, to try and make sense of all the data collected. Three types of secondary analyses were performed.

The first analysis was to identify new Notch regulators. A candidate set of genes was defined from the primary analysis that appeared to be involved in Notch signalling, that is, when being knocked down caused a loss of bristles, too many bristles or bristles with abnormal morphology. The approximately 200 genes were then knocked down in the wing, the tissue where Notch was originally identified and phenotypic changes were recorded. "We found 30 genes: seven of those were known to be involved in Notch signalling and 23 new ones. Therefore, by using the RNAi screen compared to a classical screen, the efficiency by which these genes were identified has been potentiated and not just doubled," Knoblich proudly explains. These 23 genes are being further investigated for their role in Notch signalling.

With a little software help

The second analysis looked for new genes involved in ACD regulation. An imaging assay was developed to identify both SOP cells and asymmetric protein segregation. Initially, genes that resulted in normal SOP cells but developed no external sensory organs were isolated. The 23 genes were then further analysed to determine which were involved in cellular transformation as opposed to normal differentiation, resulting in the identification of six new genes important for ACD.

The final analysis was perhaps the most ambitious. "We asked whether we can take the entire set of genes we found involved in Notch signalling and integrate them with previously existing data sets and determine pathways with the help of a software programme," says Knoblich, "And we were surprised that it worked!" These previously existing data sets included interaction data from yeast two hybrid, biochemical and genetic analyses. "Therefore," he continues, "For every gene that we found as a potential Notch regulator, we asked whether there are interaction partners." The outcome was an impressive web-like network made up of variously coloured circles, triangles and lines representing genes, gain or

loss of phenotypes and type of interaction. A network topology analysis followed using Molecular Complex Detection (MCODE) to determine whether any regions of highly interconnected nodes represented protein complexes or genetic pathways. Nine complexes were identified including Notch and two new complexes not previously implicated in Notch signalling. One complex consists of proteins involved in nuclear import and the other subunits of the COP9 signalosome, which inhibited Notch signalling when knocked down. Knoblich insists that these new complexes would not have been identified with classical genetic analysis because the phenotypes are too weak due to their role in normal cellular function. Mutant analyses have confirmed them as new complexes involved in Notch signalling.

Not just a list of genes

"The strength of our analysis is not just a list of genes," Knoblich summarises the significance of the findings, "it shows that one can analyse complex biological processes on a genome-wide level and can extract information, even with computer analysis, on individual signal transduction pathways from the data set. One can now use our data set and extract information for other cellular pathways."

Very little time is spent dwelling on their successes. A main interest of the laboratory is stem cell biology and Knoblich and his group have already begun successfully screening a number of stem cell systems in flies. Follow-up analyses are being performed on their favourite genes and processes such as planar polarity and proliferation control are being analysed using the primary data set. Finally, in addition to *Drosophila*, mice are currently being used to corroborate the results in a vertebrate model.

There is little doubt that Knoblich and his international group in Vienna will continue to succeed. Knoblich's excitement and passion for the science he does is palpable. He is thankful to all his mentors throughout his career – Lehner, who taught him how to do science, Lily and Yuh Nung Jan, his post doctoral mentors at the University of California, San Francisco, who taught him independent thinking and how to publish, and the IMP, where he learned to manage. He is also full of praise for the IMDB, which he describes as an ideal environment for science. He now imparts his knowledge to his own students and, in return, asks for dedication, enthusiasm and a lasting curiosity for science's many unanswered questions.

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