CRISPR tools for the study of embryonic development in zebrafish

Animal models are essential for biomedical research. Zebrafish are widely used, because the animals have a number of advantages, including external fertilization, rapid early embryonic development, and transparency among others. Genetic studies in fish can be informative about human physiology. Most human genes have orthologues (genes of the same evolutionary descent) in zebrafish, and vice versa. Despite several hundred million years of separate evolution, the functions of many genes are still similar in man and fish, and loss of function has similar consequences. The ability to mimic problems with development or health of relevance to humans in fish has greatly profited from a completely unexpected corner.

During the last 20 years, it has become clear that bacteria possess not only generic immunity against their pathogens and invading genetic material, but also a form of adaptive immunity. The adaptive immunity is based on a "blacklist" of samples of unwelcome genetic material. Because of the way the blacklist is arranged, it is known as CRISPR, or "Clustered Regularly Interspaced Short Palindromic Repeats". Bacteria also make proteins that destroy invaders based on information in the "blacklist", among them the now famous Cas9, and the Csm complexes.

The bacterial proteins that take blacklist instructions to destroy invaders have led to a revolution in genetic engineering, because it is easy to "fake" the blacklist to direct the machinery against any nucleic acid of choice. For the carrier of hereditary information, DNA, this works already very well. For messenger RNA, the carrier of instructions to make proteins, the technology is not quite so advanced, but our collaborators, Dr. Gintautas Tamulaitis, and Prof. Virginijus Siksnys, have made key contributions.

More recently, we have done preliminary experiments in zebrafish to test their tools in zebrafish embryos, with encouraging results, but only in somewhat artificial test settings to simplify experiments. We now want to try "real" cases, address some of the shortcomings of our first implementation of the method, test whether the method can be expanded into a screening tool, and ideally use it for a "real" screen to get new insights into early zebrafish development.