SWISS NATIONAL CENTRE OF COMPETENCE IN RESEARCH

KIDNEY
CONTROL OF HOMEOSTASIS

NEWSLETTER ::: NO. 5 ::: DECEMBER 2012

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NEW TOOLS FOR GENOME ENGINEERING

The development and application of new technologies for the advancement of research in renal-related diseases and the control of homeostasis are of great importance for the scientific community within the NCCR Kidney.CH. One of these technologies involves newly developed engineered nucleases such as Zinc Finger and TALEN that allow the rapid and precise generation of knock-out and knock-in mice and rats. The advantage of doing so is to bypass the use of embryonic stem cells. Great efforts are currently being made to develop these new tools, and also to customize and promote them so that all researchers within the Kidney.CH network can benefit.

RODENT MODELS FOR THE STUDY OF PHYSIOLOGICAL PROCESSES

Rodent models are crucial for the understanding of physiological and homeostatic renal processes. The laboratory rat has been used as an animal model for physiology, pharmacology, toxicology, nutrition, behaviour, immunology and neoplasia for over 150 years. Because of its size, ease of manipulation, and breeding characteristics, it remained the preferred choice for most of these fields, while the mouse became the leading mammalian model organism for experimental genetics. While traditional breeding approaches are very time consuming, the new engineering technologies for rats offer a powerful alternative in terms of speed, cost and labour.

ARTIFICIAL NUCLEASES

Geneticists and genetic engineers have been searching for means of increasing the rate of homologous recombination in cells, and have found a solution that takes advantage of the cellular DNA repair system. Inspired by homing endonucleases and naturally occurring DNA binding molecules, they have engineered artificial nucleases called Zinc Finger (ZFN) and

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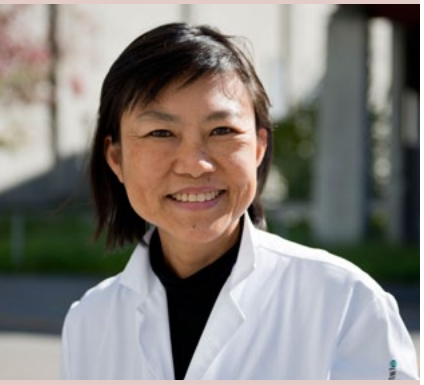
EDITORIAL



Uyen Huynh-Do is senior consultant and Professor at the University Hospital Berne. She is member of the steering committee at Kidney.CH and leads the education programme and advancement of women.

I didn't expect the first question of the European Research Council (ERC) representative to be how many pregnant fellows we had in your Marie Curie co-funded postdoctoral programme. This happened during the recently held evaluation meeting of the education programme in integrative kidney physiology and pathophysiology (IKPP). 'Two fellows during the past two years' was my answer. This accounts for around 10% of our, in total, eighteen postdoctoral fellows. And I was happy to tell the ERC representative that, after one year, both were back working at 80%. Further, one of them has proudly announced that her study has been accepted for publication in Kidney International, a leading journal in our field. The successful continuation of her study was, in part, possible due to her professor having hired a technician as a replacement to carry out her lab work while she was on maternity leave.
At Kidney.CH we support female scientists in their academic career through two pillars: first, a good infrastructure with networking and funding opportunities such as junior grants and mini sabbaticals, and second, comprehensive training encompassing not only scientific topics but also transferable skills.
Ten years ago I would have told young female scientists that the most important thing is to choose the right partner. Today I add without hesitation: '...and also the right mentor'.

Uyen Huynh-Do

Transcription Activator Effector Nucleases (TALEN). Both ZFNs and TALENs are made of two parts: the DNA binding domain and the nuclease domain. Although both ZFNs and TALENs have the same nuclease, namely FokI, their DNA binding domain varies; ZFNs are made of up to 6 Zinc Finger modules of 28 amino acids each. Each binds to 4 nucleotides of both DNA strands (figure 1a). TALENs on the other hand are made of 13 to 33 repeats also known as RVDs (repeat variable di-residues) of 34 amino acids each. Each repeat is almost identical except for two amino acids in positions 12 and 13 of the 34 (figure 1b) (Table 1).

ZFNs and TALENs cut DNA and generate double-strand breaks (DSBs). The DNA repair machinery is immediately activated and closes the gap by using a DNA template (usually the sister chromatid) to copy the missing nucleotides by "Homology Derived Recombination" (HDR). In some instances, the cut DNA is directly glued back in a mechanism called Non-Homologous End-Joining (NHEJ). This usually causes small insertions or deletions also called "indels". Indels can lead to a frame shift in the protein sequence resulting in premature stop codons, or nonsense-mediated mRNA decay; this will finally lead to a truncated protein or a knock-out for this gene. This technique can now be developed further by offering to the DNA repair machinery a so-called "donor plasmid" containing a mutated sequence to be inserted in the genome (point mutation, reporter gene, LoxP sequence, tag) and the repair proteins can take this foreign DNA as a template for its repair. The chances of HDR increase with the amount of donor molecules present in the cell.

PORTRAIT

THE KIDNEY.CH NETWORK IS OF GREAT IMPORTANCE

Interview with Sophie de Seigneux Matthey

Sophie, you're the proud mother of Alicia your nine-month-old daughter, you work as a clinician and you are involved in research. How do you manage all this?
I've definitively had to organize my day differently and in a manner better adapted to this new challenge. Before, I was seeing patients every day at the University Hospital of Geneva's nephrology unit. Now, we organized it so that I see mostly ambulatory patients on three half-days. This helps a lot to better structure and deal with my current work-life-situation. It also allows me to predict and plan my day in terms of being home at a reasonable hour to look after my daughter. However, without professional child-care and the help of my parents this still wouldn't be possible.
And how does this new situation affect your research?
At the moment I might even have a bit more time for research than before when I was seeing patients every day. I think my current arrangement is perfect now, and will be for a certain period of time, and is also necessary to balance family with my clinical duties and laboratory research work. I always wanted to do research and I love it. For me and for my research it's also very important that I work as physician and am in regular contact with patients.
When did you decide to become a medical doctor?
Honestly, I decided quite late, at the end of secondary school, to become a physician. And research was the trigger. It could also have been biology or chemistry, but I decided to do medicine. I am, however, very happy with my choice.
During my studies I realized that in addition to research I wanted to care for patients. My decision to choose nephrology came after the courses on electrolytes given by Prof. Pierre-Yves Martin. I was fascinated by electrolyte disequilibria and the way you could study these problems in patients and in research. So I started a three-year internship at the department of internal medicine. Later, thanks to the support of the Swiss National Science Foundation (SNSF) and of Prof. Martin, I was able to join Prof. Søren Nielsen's lab at Aarhus University in Denmark. This, in fact, was my first real laboratory work experience and confirmed my interest in kidney pathophysiology. It was excellent working in Prof. Nielsen's lab and I learnt a lot, also by seeing his enthusiasm for everything he was doing. In the end, I even did a PhD there, which wasn't the plan. But I'm very happy I did.
In early 2011 you were awarded the first Kidney.CH Junior Grant. And now an Ambizione Grant from the SNF – congratulations! What's your experience so far of the NCCR Kidney.CH?
I very much appreciate the network of established Swiss experts in this field. It helps me both in my NCCR project and beyond with all my current research projects. Such networks are highly valuable constructs and I hope that, in the future, participant interaction will even increase over time.
Talking about the future, what are your plans?
Developing some independent research is definitely one of my big goals, and this includes my Kidney.CH Junior Grant project on proteinuria and phosphate excretion. With the help of the Ambizione Grant and the Kidney.CH Junior Grant I hope to succeed in my current research projects. The Ambizione Grant covers my salary and provides funds for research for a total of three years, allowing me to hire a PhD student. In addition, the Junior Grant enables me to pay part of a post doc salary and provides research funds for work on the Kidney.CH project. And if, in the NCCR's future, there is an opportunity to develop another project beyond this Junior Grant, that would be marvellous. In any case, I will do my best to always be able to do clinical work and research. And last but not least, it would be nice for Alicia to have a brother or sister.

Sophie de Seigneux Matthey is senior consultant at the Dept. of Nephrology at the University Hospital of Geneva. At Kidney.CH she leads a Junior Grant project on the effects of proteinuria on renal phosphate handling.

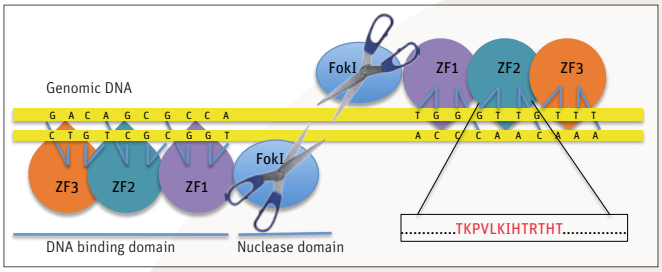


Fig. 1a: ZFN structure
Each ZF module is made of 28 amino acid residues, 3 of which bind to one DNA strand and 1 to the opposite strand. ZFNs can be made of 3 to 6 modules and the length of the linker between the modules can increase the binding efficiency of the whole nuclease.

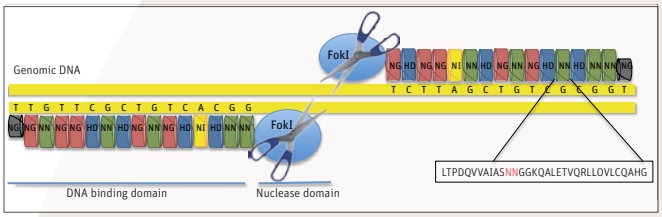


Fig. 1b: TALEN structure
TALENs are made of 13 to 33 repeats of 34 amino acid residues. Only the amino acid residues in positions 12 and 13 bind to one nucleotide. The TALEN code is the following: NG bind to T, HD bind to C, NI bind to A, and NN bind to G.

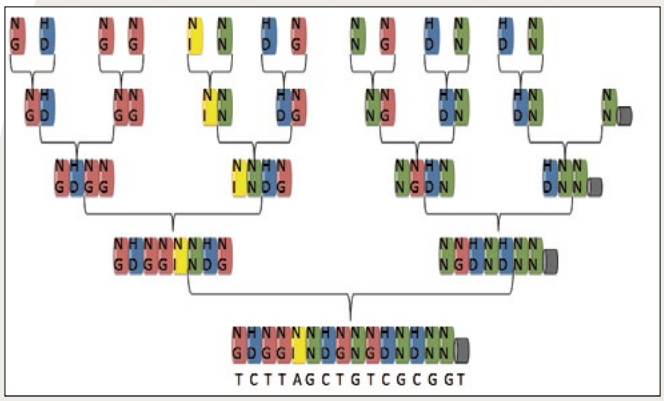


Fig. 2: TALENs cloning strategy
TALENS are assembled by multiple cloning of TALEN libraries each containing one RVD. Single-step cloning of each repeat is carried out with the same complementary enzymes BbsI and BsaI. Today, a faster method made of a larger library containing dimers, trimers and tetramers of RVDs can be obtained. RVD, repeat-variable di-residues.

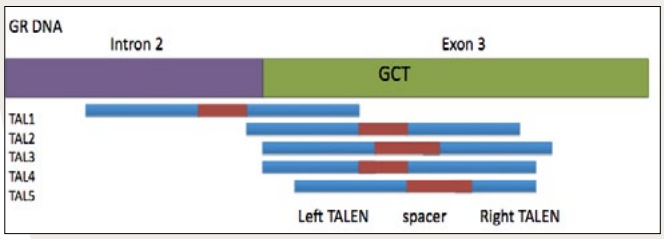


Fig. 3: Guidelines for choosing TALEN candidates for GR A476T mutation in rat
Talengineering software gives many TALEN candidates for cleavage around the mutation (GCT). We chose the candidates that share some guidelines from naturally occurring TALEs from Xanthomonas ssp. We also chose a candidate that was 25 bp away from the triplet GCT (TAL1 in figure) to increase the chances of success.

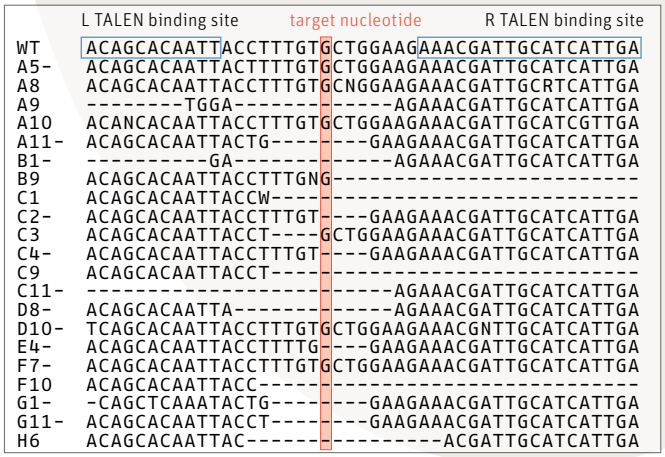


Fig. 4: TALEN screening
Amplicons around the GCT were cloned into standard vectors and transformed into bacteria. Colonies were screened by clone picking from 96 well plates. 17.4% of the colonies presented deletions of one or several nucleotides, supporting the T7-Endo1 assay.

ZFN	TALEN
Established over a decade ago. Efficient in cells of plants, zebrafish, rats, mice, pigs, rabbits, cattle, frogs and humans.	New technique (3 years old). Efficient in zebrafish, rats and mice. Works also in pig, human and cattle cells.
100 amino acid residues, each ZF module has 7 to 8 amino acid residues.	700 amino acid residues, each RVD has 34 amino acid residues.
Unknown engineering mechanism (private companies or complex algorithms).	Uses a straightforward DNA base recognition cipher.
Limited number of ZFN target sites per sequence (1 per 200 bps).	1 target sequence every 30 bps.
Single-step cloning of the ZFN sequence into an expressing vector.	8 to 16 cloning steps.

Table 1:
Comparing ZFNs and TALENs

STUDYING SODIUM RETENTION IN RATS

At Kidney.CH we started a project in 2010 to develop and implement this new technology and to generate transgenic knock-out/-in mice and rats using ZFNs and TALENs. Our project aims to understand the role of the aldosterone- and glucocorticoid-dependent pathways in the control of sodium retention in a rat model. ZFNs and TALENs are used to generate glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) dimerization deficient rats by mutating a G in the dimerization domain of the receptors (GR, A476T). We designed and cloned two pairs of ZFNs and assembled five pairs of TALEN nucleases (figure 2), which were designed using ZiFit, a publically available software package by the Zinc Finger- (zincfingers.org) or TALEN Consortium (talengineering.org) (figure 3). Following transfection into rat C6 cells, TALENs demonstrated a cutting efficiency of up to 20% using the T7-Endo-1 assay that detects mismatched DNA. Cells transfected with the most efficient TALEN candidate were sequenced (figure 4).

CONCLUDING REMARKS

Zinc Finger and TALEN nucleases have considerably increased the possibility to mutate nearly any sequence in most of the organisms used today in basic research. Using open Web platforms, these tools are flexible and become affordable for research with cells, animals or even as a therapeutic treatment in humans. In 2007, a first patient (the “Berlin” patient) was cured of HIV by a blood stem cell transplant modified by TALENs. A gene mutation had been introduced that provided natural resistance to HIV. This was a first step towards a clinical application of this technique.

Edith Hummler
is Senior Lecturer at the Departement of Pharmacology and Toxicology at the University of Lausanne, and principal investigator at the Kidney.CH.
She is member of the Kidney.CH steering committee and leads the transgenic animal facility of the University of Lausanne / CHUV at Lausanne.

Verónica Ponce de León
is scientist in Edith Hummler’s group at the University of Lausanne. At Kidney.CH she is responsible for the development of rodent models.

FIRST SWISS-WIDE KIDNEY STONE COHORT

Renal colic accounts for up to 1% of all hospital admissions internationally. Most emergency departments treat an average of at least one patient with acute renal colic every day. Costs of kidney stone disease in the US have been estimated, based on data from 2000, to be around US$ 2.1 billion per year. Despite the high prevalence of kidney stones, very little is known about their aetiology and there is a clear need for high quality research to guide treatment and prevention strategies in this field.

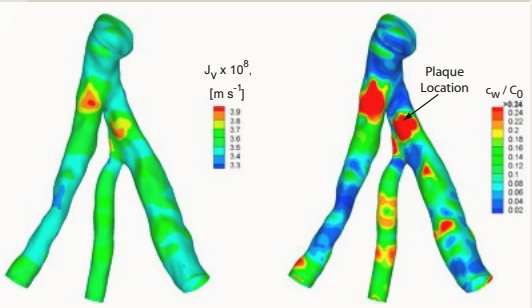
In Switzerland today there is no nationwide initiative that coordinates the recruitment of kidney stone patients, sampling, the analysis of samples following standard protocols, and storage of samples under identical conditions. Even worldwide, few such initiatives exist. A new project from Kidney.CH is the establishment of a Swiss Kidney Stone Cohort initially comprising the five academic centres: Basel, Berne, Geneva, Lausanne and Zurich.

KIDNEY.CH SYMPOSIUM 2012

The 2nd International "Kidney - Control of Homeostasis" Symposium took place on June 14, 2012 at the University of Geneva. Speakers from the US, France, England, Spain and Switzerland provided excellent insights into sodium and potassium homeostasis. We warmly thank all the speakers and attendees who made this event such a great success.

Kidney – Control of Homeostasis is a Swiss research initiative, headquartered at University of Zurich, bringing together leading specialists in experimental and clinical nephrology and physiology from the Universities of Basel, Berne, Fribourg, Geneva, Lausanne and Zurich and corresponding University Hospitals.

COMPUTATIONAL MODELLING OF TRANSPORT PROCESSES



Vartan Kurtcuoglu joined Kidney.CH this September as an assistant professor of computational and experimental physiology. Based at the Institute of Physiology of the University of Zurich, he will apply his expertise in biofluidics to investigating transport processes in the kidney, bringing to Kidney.CH valuable know-how in the computational modelling of biophysical processes.

Dr Kurtcuoglu was awarded his PhD in Biomedical Engineering by ETH Zurich in 2006 and was a group leader and lecturer in biofluidics at the same institution until joining Kidney.CH. In 2011, he was a visiting scientist at Harvard Medical School and Brigham and Women's Hospital, where he dedicated himself to investigating the influence of hemodynamics on atherosclerotic plaque formation. His main area of research has been the dynamics of cerebrospinal fluid and associated transport processes. At Kidney.CH, he will adapt and expand the computational tools he has developed for blood and cerebrospinal fluid to study flow and transport processes in kidneys.

Vartan Kurtcuoglu

PROGRESS REPORT APPROVED

The second project review of Kidney.CH by the Swiss National Science Foundation (SNSF) together with an international review panel comprising eight leading experts took place over two days in June 2012. The Research Council of the SNSF followed the recommendations of the review panel and approved the second progress report of the NCCR Kidney.CH, and granted funding for the 3rd year.

EVENTS

INHERITED KIDNEY DISORDERS: AN UPDATE FROM THE CME–ERA-EDTA WORKING GROUP
December 14–15, 2012
Zurich, Switzerland

RETREAT NCCR KIDNEY.CH
February 8–9, 2013
Morat, Switzerland

WORLD KIDNEY DAY 2013
March 14, 2013
Worldwide

1ST KIDNEY.CH INNOVATION WORKSHOP
March, 2013
Switzerland

50TH ERA-EDTA CONGRESS
May 18–21, 2013
Istanbul, Turkey

WCN 2013 – WORLD CONGRESS OF NEPHROLOGY
May 31 – June 4, 2013
Hong Kong, China

3RD KIDNEY.CH SITE VISIT
June 17–18, 2013
Zurich, Switzerland

OUTLOOK

3RD INTERNATIONAL KIDNEY.CH SYMPOSIUM
June 20, 2013

Imprint
Editor: Matthias Meier
Design: bigfish.ch
Print: SuterKeller Druck AG

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