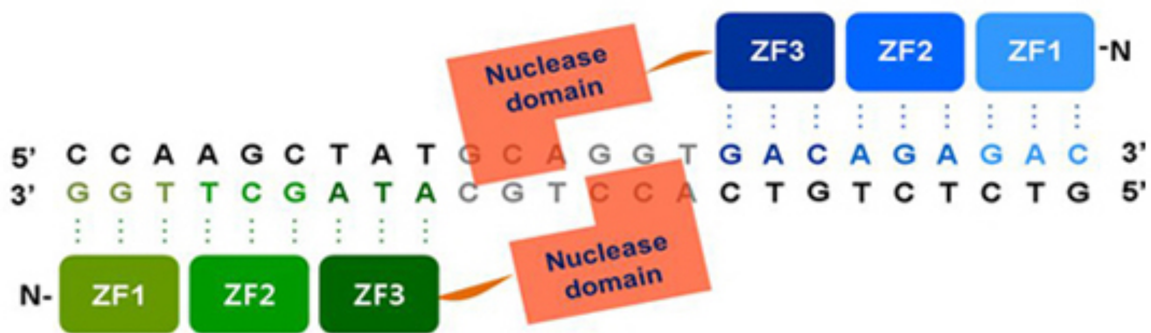
Zinc Finger Nucleases

Term Paper Biology
Genetic Engineering



Yvonne Aitken
Lisa Schiess
Sophie Stebler

4d bZ
Gymnasium Kirschgarten
Patrick Ruggle

27.04.2016

Table of Content

1. Preface 3
Motivation, Interests, Questions with respect 3

2. Introduction 4
1.1 Genome Editing 4
1.2 Scientific History 4
1.3 Where and why is the technique used 4
1.4 Alternative treatments: TALEN and CRISPR/Cas9 5

3. Zinc Finger Nucleases 6
Technique explanation 6

4. D-BSSE Basel 8
Interview and Pictures of the Department of Biosystems Science and Engineering (D-BSSE) 8

5. Discussion 10
5.1 Progress 10
5.2 Future 10
5.3 Ethical aspects 10

Summary 4

References 1

1. Preface

We chose to delve into the topic of zinc finger nucleases (ZFN) for our 4th year biology term paper for a number of different reasons. The first being that we wanted to learn more about how diseases like haemophilia or disabilities like the Down Syndrome or even miscarriage can be prevented. Through this, we discovered the topic “genome editing”. However the topic was far too complex, so we decided to focus on a smaller scale topic. CRISPR/Cas9, Zinc Finger Nucleases and TALEN came into consideration. The reason why we chose the topic ZFN was because it is more precise in its function and targeting than the others. And this technique is still considered cutting edge in gene engineering today, although a number of new and significant advances and techniques have been discovered and developed in recent years as mentioned above. We are interested in comparing the tried and tested, reliable ZFN to these new approaches, their respective differences and the prospects for the future.
Another aspect of interest in the potential (theoretical) application of ZFN to human genomes and the ethical divisions this could create in our society.
Finally the research and further understanding of techniques in a field of biology we have recently dealt with in school is fascinating and helps us understand the greater importance of genetic engineering.

The most important points of interest in ZFN are first and foremost the technique itself and the fascinating fact that it is made possible for us to intervene in our genetic health and manipulate our cells. ZFN has extreme specificity in treating the genome and therefor has great potential for clinical use.
Another great advantage is that this method can be used to treat adults, contrary to other kinds of gene technologies that are only able to change the genome in the embryonic state.

The questions that arise while studying ZFN are why it is so precise and how it will develop in the future. Also how it is possible to create the zinc finger nucleases and how this procedure is preformed in the lab.
Another important question regarding ZFN is whether this might finally be the solution to previously incurable diseases such as HIV and Dementia, and if so, how this is possible.
After hearing about the more recent method CRISPR-Cas9, we wanted to learn more about the differences and possible improvements that have been put into place and compare these two types of gene engineering.

2. Introduction

1.1 Genome Editing (Context)

Genome Editing is the umbrella term for new bio molecular methods, by which it is possible to induce changes and mutations in specific sections of the DNA . With this, scientists can turn on, off or even insert and delete genes. Genome editing is not only used in animal cells, but also in plant cells. These changes in the genome are very precise and efficient. The following three steps are a simple way to understand it.

(1) At the beginning, a specific sequence on which you preform the mutation is found, in the large genome, which consists of milliards of base pairs (DNA building blocks). To do this, there is a kind of „sensor“ constructed in the lab, which perfectly fits to the appropriate sequence. This could be e.g. TALENs, **ZFNs***,* or as in the CRISPR/Cas9-System the *RNA-Sections*
(2) Afterwards at this specific sequence - and only there – the DNA strand is cut with a molecular „scissor“, which is in most cases a protein (restriction enzyme). Both elements, „scissor“ and „sensor“ are induced into the cell.
(3) The double stranded cut at the respective location in the DNA, can be repaired with the natural repair mechanism of the cell. Depending on the used mechanism, there are different variations oft the genotype possible, For example:

- *Homologous recombination*: The broken sequence of the DNA is repaired with the help of a homologous sequence, which serves as a model for the sequence to be replaced. The homologous sequence can be used, to induce a specific sequence into the genome. To do this, a sequence is synthesised, which fits between the cut sequences of the DNA. Available sequences can also be replaced or removed. Through the inserting resp. removing of these sequences, genes can be turned on- or off selectively (*Gene-Knocking/ Gene-Knockout*).

- *Non-homologous end joining* (NHEJ): The DNA- endings at the double stranded break get connected with the help of different enzymes. NEHJ is very error-prone, so that most mutations occur at the repair site.

Recent events: There aren't really any recent events about the ZFN because this system got more or less replaced by CRISPR/Cas-9.

1.2 Scientific History

Previously gene-targeting procedures were dependant on a number of factors, for example homologous recombination, and were not effective on eukaryotic organisms.
When the possibility of double stranded brakes was discovered in the 1980's it was possible to increase the frequency of recombination and accelerate research and practise in gene engineering. ZFNs were the first genome editing nucleases to be discovered and became the favoured tool for gene modification until the development of more the modern technologies CRISPR/Cas9 and TALEN. ZFNs were able to produce knock-out genes (developed 1990's), which greatly helped the research of diseases, mutations and overall gene functions.

1.3 Where and why is the technique used?

Where

It is among other things applied in genome editing.
ZFN is a quick and efficient technological solution for the modification of the embryonic genome. This can be beneficial for the treatment and prevention of genetic diseases such as haemophilia. These proteins are created to generate gene specific double stranded cuts.

By application of this technique it is possible to modify genes quickly and precisely without endangering the overall stability of the genome. ZFN cuts can result in Gene disruption (gene deletion/-insertion), tag ligation, large deletion (>>100bp), gene correction, targeted gene addition, trans gene stacking. These applications will be further explained in the course of this paper.

Another area of its applications is found in plant cultivation:
In the 1990's a new "smart breeding" project for modern plant cultivation was started. This projects goal was to breed certain plant varieties (which aren't found in nature) by using the traditional crossing, chemical and physical processes or the new breeding option ZFN.
There are three variations of this technique:

1) The designated location of the double stranded plant DNA is cut and the natural DNA repair mechanism leads to precise mutations in one or more base-pairs, to short deletions or insertions, so the changing or blocking of specific Genes are the result.
2) After a double stranded cut in the DNA the natural gene repair mechanism produces point mutations e.g. the changing of one or more bp through homologous recombination and copying of the DNA-template.
3) A sequence of DNA (<1000bp) with identical endings to the cutting site is inserted into the cell. This piece of DNA is the integrated into the genome of the plant cell.

By using the ZFN in plant cultivation the process of breeding is speeded up because of the predictability of the cutting site.

Why

ZFN solves the problem to knock-out genes in the human cells. Since this technique of ZNF/ TALEN exists, it would be possible to apply this on human cells. Before it was only possible for scientists to study genes by turning them off and examine the outcome with the basis of gene-knockout, the disadvantage of it: it only worked on mice. On other animals, there wasn't really a technique to turn off genes specifically. That's what made the genetic research on animals like rats, clawed frogs, lancelet etc. very complicated and frustrating.
But ever since the existence of the methods of ZNF and TALEN, this problem is solved smartly. Both use protein constructions with special cutting proteins (nucleases), which cuts the DNA at a specific site. The trick lies within the second component: protein structures, which are modified exactly to recognise any DNA sequences and cut them. It's not new, that proteins can bind on DNA sequences, but so far they were too exact. You couldn't change them on one or two places, so that they could fit into another, slightly changed sequence. Even for a minimal other base sequence, you need a completely new protein. The new constructs develop in modular constructions. They contain multiple DNA binding proteins, which build together the binding site for one sequence. If one part of the DNA is different, you can just change the corresponding module and it fits again.

1.4 Alternative treatments

TALEN: They don't recognise base-triplets but each domain recognises a single nucleotide. The interaction between the binding domains of TALEN and their specific nucleotide are less complex than the ones of the ZFN and the production of TALEN is simpler than ZFs.

- ADVANTAGE of both: It changes the genes in the embryo of the mice
- Both techniques were modified in fruit flies, nematodes, rats, zebra fish and livestock

CRISPR/Cas-9:

It is the newest technique (CRISPR: clustered, regularly interspaced, short, palindromic repeats)/Cas9. It is a defence mechanism of bacteria, which is based on RNA . They recognise and eliminate foreign DNA. The "Cas" is an endonuclease and cuts the DNA sequence, which is targeted by a guide RNA (gRNA). CRISPR/Cas-9 can be applied to insert genes, introduce random mutations, etc.

- ADVANTAGE: It is simply designed because the targeting is dependent on the gRNA
- They are very efficient and multiple genes can be introduced at the same time

3. Zinc Finger Nucleases

Zinc finger nuclease (ZFN) is a type of laboratory-synthesised restriction endonuclease, which is a protein, composed of a DNA binding-domain (zinc finger) and a DNA cleaving-domain (FokI-Restriction enzyme/endonuclease).
The zinc finger is made up of a α-helix and a β-sheet that are connected to a zinc-atom by two hystedines and two cystedines. The zinc finger detects and binds to a specific three-nucleotide group. The more zinc fingers are added to the chain, the more specific the binding site becomes, as the required recognition of a greater number of base pairs has to be present for the binding process. Once the zinc finger nucleases has located the designated binding area, the DNA-cleaving nucleases cuts the strands, causing double stranded damage to the DNA strand (Fig. 1).

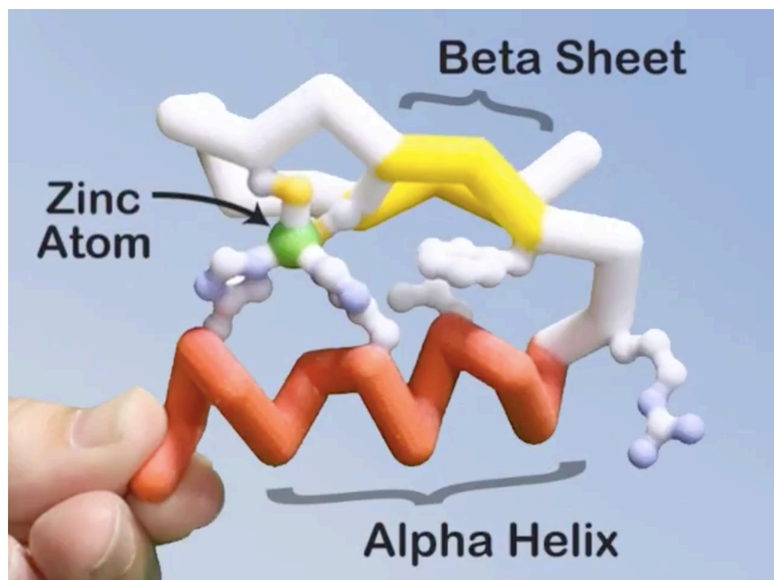


Fig. 1: Molecular parts of a Zinc Finger Nucleases.

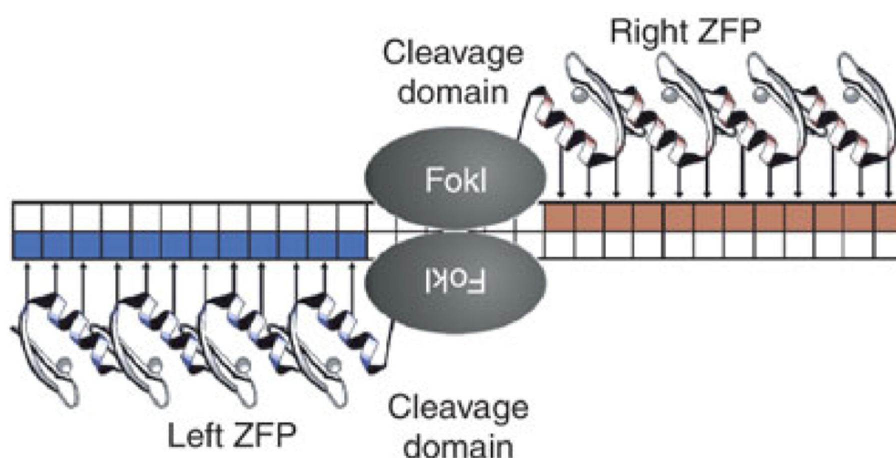


Fig. 2: The blue and red parts represent the DNA base pairs (bp). The arrows above or below of them, show the bonds between the ZFP and the bp's. FokI represent the "scissors" that cut the DNA at the specific loci. The particular ZFP next to the two FokI's are attached to them.

Once the cut has been made, there are several possibilities to repair the damage:

- Non-homologous end joining (NHEJ) is where gene knockouts can occur due to flaws on the mechanism
- Combination with a Donor vector through homologous recombination (HDR: homology-directed repair), which can trigger a precise exchange of genes (insertion/deletion)
- Using the undamaged second chromosome (if healthy) as a template for the repair of the damaged one

Using these methods, a quick and exact modification of the genes is possible without destabilising the genome.

There are 6 outcomes from cutting and repairing DNA with ZFN:

NHEJ:

- Gene disruption: ligation of two ends, often resulting loss or gain of genetic information due to small insertions or deletions
- Tag ligation: production of possible tagged alleles
- Large deletion: two simultaneous double stranded cuts on the same chromosome can result in large scale deletion

HDR:

- Gene correction: single base pair change
- Targeted gene addition: addition of an ORF (open reading frame) or a transgene
- Transgene stacking: multiple linked transgenes inserted producing a “stacked trait”

So far however there have been considerable difficulties in the binding and cutting of DNA as multiple ZFNs influence each other and impede the ideal binding to the foreseen sight. Because of this the ZFNs have to be laboriously optimised, although their basic construction principal is very simple. This process makes them accordingly expensive and has decreased their possible uses and importance in the field. (This disadvantage is eliminated in the more recent technology TALEN)

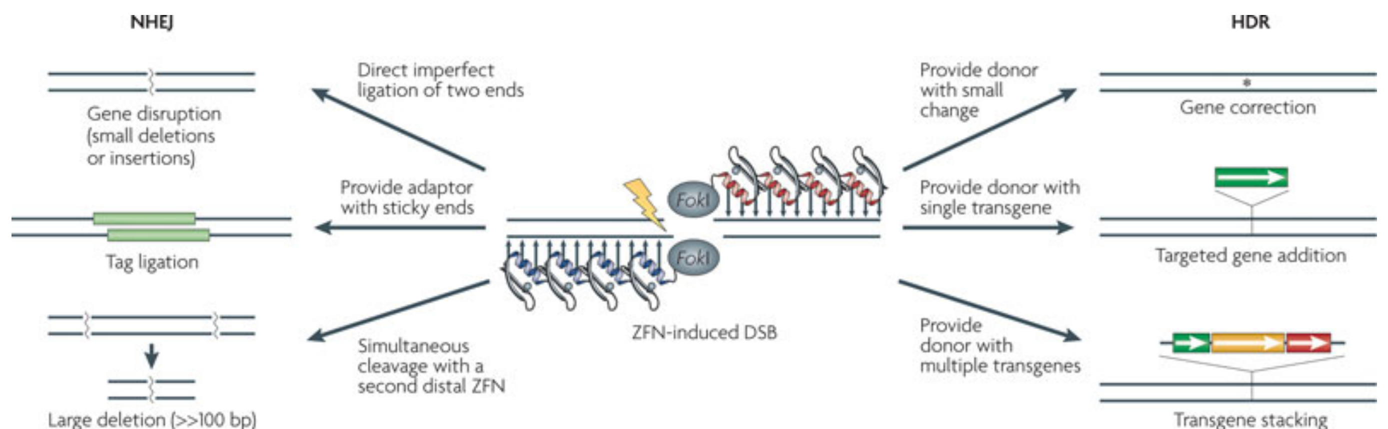


Fig. 3: Visual explanation of the above (NHEJ and HDR)

4. D-BSSE Basel

Insight of the Interview with Prof. Dr. Paro, 24/03/16

(Q: Question; A: Answer)

Q: What and when was your first contact with ZFN?

A: [...] And after a lot of work they were able to deduce the composition of the zinc fingers in terms of the amino acid sequence that is required to recognize a particular DNA sequence and with that knowledge you can produce a code such that you know that this particular zinc finger will be able to recognize a particular DNA sequence.

Q: What is ZFN used for? (-Only theoretically? Treating diseases? Used for animals or plants (gnome editing)?)

A: Exactly, this is the most important question. “Why do you cut DNA?”, you might ask, well you certainly know that if you cut DNA you produce a mutation. So you don’t want to produce mutations, you want to heal a person with a particular mutation. How do you do that?
Well then you have to know another process, that occurs in our cells and we have a so-called DNA repair mechanism. [...] Nature, evolution has developed a mechanism to make sure that whenever there is a mistake in the DNA, it will get corrected. [...]
You have a mutation in one chromosome and you would like to repair and replace it with a normal sequence, now also the other chromosome has a mutation and so you cannot use the other chromosome to repair and produce a normal sequence. Now what you do is you bring in artificial DNA, which is complementary to, that region, that you want to repair, except that mutation. If it’s a point mutation you would replace it with the base that represents the normal function of the gene. And so after cutting the DNA the cell now tries to repair it, but is has no normal function, no normal DNA there, so it will use to foreign DNA the you introduce, and will repair the mutated sequence, with the wild type sequence. And so you corrected that mutation, because you replaced the mutated sequence with a normal sequence. And this is one part of the genetic engineering goals, which you could achieve with ZFN. [...]

Picture of D-BSSE Basel

Fig. 4: Scientist who is working with CRISPR/Cas-9 at the moment; Here you can see their location in the laboratory, where they work almost daily.

5. Discussion

5.1 Progress

One error in the function of the ZFN is the large-scale effect it can have on the organism. Even though it has great specificity in the exact section of DNA it locates, that is to say the precise sequence of codons, the problem is that every single section of DNA with that sequence will be cut. This may cause unwanted cuts throughout the entire DNA.
This known risk is the reason why the technique has not been applied to human beings. In theory it could insure massive progress in disease- and birth defect/disability prevention and reduce the numbers of miscarriages immensely if the embryonic genome were to be treated with ZFN.
However, in the plant cultivation and other areas of agriculture it has been put into action with great success. Gene manipulated crops (GM-crops) can be modified to produce greater yields, longer storage life after harvest and disease prevention or elimination in the plants themselves etc. Also new, not naturally found plant-species can be created due to gene engineering techniques such as ZFN.
As mentioned by Prof. Dr. Paro ZNF-methods are being applied and researched in many firms and businesses even though TALEN and CRISPR/Cas9 are slowly replacing them.

5.2 Future

Because of the development of TALEN and CRISPR/Cas-9 the future prospects of ZFN are not particularly spectacular. The new methods – once fully developed – will override ZFN both in efficiency, simplicity and especially in budget. However, thanks to the knock-out genes and the mechanisms specificity, ZFN will be continuing to work towards progress in the medical and developmental fields (health and agriculture) in the near future.

5.3 Ethical aspects

Advantages:

- It prevents life threatening sicknesses from unborn children
- It enables a longer life
- It helps to grow organs, replace brain cells & burnt skin and transplant bone- marrow cells etc.
- It is a way to reduce mutations in the human genome
- It is a new advancement in genetic engineering
- It helps to prevent miscarriages, ZNF makes life possible, that might otherwise be critically endangered

Disadvantages/ Dangers:

- We don’t have any knowledge of the long-term consequences for the future generation
- It is dangerous to shape the human race in such a way (Stigma, discrimination, false habituation to such unnatural norms)
- There are risks in the engineering process/safety issues (e.g. new mutations possible if
- it is used wrong)
- If it would get to a norm, sick people will experience far greater discriminations
- Huge marketing profit by which only rich people are involved and make profit, increased gap between rich and poor people
- Application only available to the rich, to expensive for the low-middle class
- GM- organisms released into the wild can disrupt the delicate gene-pool of a population and cause problems to the ecological balance in nature
- New allergies against GM-foods/-organisms might appear
- GM-seeds/germs could be misused for military purpose
- Scientists could disregard the value and respect for animals and plants

Summary

Zinc Finger Nucleases (ZFN) are restriction enzymes, which were one of the first techniques used in "genome editing". With the help of ZFN we are able to turn on or off genes, as well as cause mutations like insertion, deletion, tag ligation etc. It is composed of a Zinc Finger (ZF), which is the "binding-domain" and is capable of recognising a specific sequence in the DNA. Each ZF recognises triplet base pairs to locate the site where we want to change something in the DNA. In addition, a cutting tool, the "cleaving-domain" is needed: the nuclease. It cuts precisely at the spot, where the mutation has to be made and produces a double stranded break. Together the protein can find and cut at any desired location. After a cut is produced, it has to be repaired. There are two possible ways of doing that: Either by homologous recombination (the DNA is repaired, aided by a homologous sequence) or non-homologous end joining (The endings of the DNA are connected at the double stranded break).
The ZFN was developed as a simplification for previous gene editing. Double stranded breaks, which are caused by ZFN, were discovered in the 1980s and knock-out genes in the 1990s, these greatly improved and sped up studies in the gene engineering field. This technique is used in the lab for scientific- and medical research and in plant cultivation. Alternative treatments to ZFN are CRISPR/Cas9 and TALEN as mentioned in this paper.
The future prospects of ZFN are less hopeful in comparison to CRISPR/Cas9 because it is very time-consuming to synthesise and expensive. Besides this, the application of ZFN on humans would be very dangerous because it isn't precise enough in the mutation site targeting for a single change in the genome.

References

- **Books**:
  Kleinert R., Ruppert W., Strahil F.X. (2013). Abitur- Training plus Biologie. Genetik. München: Langenscheidt, p.132- 133, 155-156.

  Nordheim A., Knippers R. (2015). Molekulare Genetik. Stuttgart: Georg Thieme Verlag KG, p. 46.48

  Mackau J.P., Segal J.J. (2006). Engineered Zinc Finger Proteins, Methods and Protocols. New York, Dordrecht, Heidelberg, London: Springer, p.3-4, 31-33,281-282

- **Internet**:
  Nemudryi A. A., Valetdinova K.R., Medvedev, S.P. & Zakian S.M. (2014). TALEN and CRISPR/ Cas Genome Editing Systems: Tools of Discovery.
  http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4207558/
  (26/04/16)

  Author Unknown (2011). biotechnologie.de. Gen- Chirurgen überprüfen die Schärfe ihrer Scheren..
  http://www.biotechnologie.de/129148
  (23/04/16)

  Author Unknown (2014). Epigenie. Zinc Finger Nucleases.
  http://epigenie.com/epigenetics-research-methods-and-technology/genome-editing/zinc-finger-nucleases-zfns/
  (17/04/16)

  Author Unknown (2013). Trends in biotechnology. ZFN, TALN and CRISPR/ Cas-9 based on the methods for genome engineering.
  http://www.cell.com/trends/biotechnology/abstract/S0167-7799%2813%2900087-5
  (24/03/16)

  Author Unknown (2013). MicrobeWiki. Zinc Finger Nucleases.
  https://microbewiki.kenyon.edu/index.php/Zinc_Finger_Nuclease_%28ZFN%29
  (17/04/16)

  Author Unknown (2016). Zinc Finger Nucleases.
  http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology.html
  (17/04/16)

  Author Unknown (2016). Transpaenz Gentechnik. Zinkfinger Nukleasen.
  http://www.transgen.de/lexikon/1848.zinkfinger-nukleasen.html
  (01/03/16)

  Berlin, Vivan (2016). Zinc-finger nucleases: determining and improving target sequence specificity.
  http://otd.harvard.edu/explore-innovation/technologies/zinc-finger-nucleases-determining-and-improving-target-sequence-specificity/
  (17/03/16)

CASPION (2010). Biology Online. Answer to all you biology questions.
http://www.biology-online.org/dictionary/Vector
(17/04/16)

Caroll, Dana (2011). Genome engineering with zinc finger nucleases.
http://www.genetics.org/content/188/4/773
(17/04/16)

Davis, Ed. (2014). Genome editing: Which should I choose Crisp or TALENs?
http://www.genecopoeia.com/resource/genome-editing-talen-or-crispr/
(26/04/16)

Fischer, Lars (2012). Die neuen Genom- Schreibmaschinen
http://www.zeit.de/wissen/gesundheit/2012-11/gen-knockouts-molekularbiologie
(26/04/16)

Nature America, Inc. (2011). Method of the year. Zinc Finger Nucleases.
http://www.nature.com/nmeth/focus/moy2010/nmeth.f.328.pdf
(23/04/16)

Perkel, Jeffrey M. (2013). Biocompare. Genome editing with CRISPRs, TALEN, ZFNs.
http://www.biocompare.com/Editorial-Articles/144186-Genome-Editing-with-CRISPRs-TALENs-and-ZFNs/
(26/04/16)

Pflanzenforschung .de (2011). Zinkfingernuckleasen. Molekulare Werkzeuge für Pflanzenzucht.
http://www.pflanzenforschung.de/de/journal/journalbeitrage/zinkfinger-nukleasen-molekulare-werkzeuge-fuer-die-pfla-1486
(17/03/16)

Waghmare, Priyesh (2013). Zinc finger nucleases technology.
http://de.slideshare.net/PriyeshWaghmare/zinc-finger-nuclease-technology
(06/03/16)

Yeadon, Jim (2014). Pros and Cons of ZFNs, TALENs and CRISPRs/CAS.
https://www.jax.org/news-and-insights/jax-blog/2014/march/pros-and-cons-of-znfs-talens-and-crispr-cas
(17/04/16)

- Video:
Garside, Ben (2014). Zinc Finger Nuclease.
https://www.youtube.com/watch?v= 9yBP0b8zIc
(17/03/16)

Hoelzer, Mark (2014). National Smart Teams- History of Zinc Finger Nucleases
https://www.youtube.com/watch?v=iZxvmHw5XGU
(26/04/16)

- Pictures:
Fig. 1: Hoelzer, Mark (2014). National Smart Teams- History of Zinc Finger Nucleases
https://www.youtube.com/watch?v=iZxvmHw5XGU
(26/04/16)

Fig. 2: Author Unknown (2007). Nature biotechnology.

http://www.mdpi.com/1999-4915/4/2/309/htm
(24/03/16)

Fig. 3: Fyodor D. Urnov, Edward J. Rebar, Michael C. Holmes, H. Steve Zhang & Philip D. Gregory (2010). Nature Reviews Genetics 11, 636-646. Genome editing with engineered zinc finger nucleases. Types of genome editing made possible using zinc finger nucleases. http://www.nature.com/nrg/journal/v11/n9/fig_tab/nrg2842_F3.html
(26/04/16)

Fig. 4-6: Aitken. Y. (2016). Pictures of the Institution. Self made. (06/04/16)

- **Magazine**:
  Christner, Jürgen (2000). Genetik. In: Genetik. Stuttgart, Düsseldorf, Leipzig: Ernst Klett Verlag, p 40-41

Attachement:

1. Interview with Prof. Dr. R. Paro

Q1: You are working at the department of Biosystems Science and Engineering (D-BSSE) and you studied biology,
what and when was your first contact with genetic engineering?

A1: Actually my first contact was already as a gradient student, bc genetic engineering was at that time the revolution of a new technology, called recombinant DNA engineering, which was started in the middle of the 70s and this is when I started to study here in Basel and to do my Phd work, so that's the time when genetic engineering the actual technology started and I got expose to that very early.

Q2: What and when was your first contact with ZFN?

A2: ZFN, that obviously came much later because the invention of the ZFN was I think in the 90s or so.
The point being that ZFNs are DNA binding proteins, so they can recognize the sequence of DNA in a specific manner. This is a normal function that cells have. They have so called transcription factors which can bind DNA sequences specifically and regulate the expression of genes and different transcription factors have different motives, protein domains that allow them to bind DNA in a sequence specific manner, as for example the homeodomain, lucine zipper and the so called zinc fingers. So after the discovery of these transcription factors, people tried to identify how these protein motives can recognize DNA in a sequence specific manner. And after a lot of work they were able to deduce the composition of the zinc fingers in terms of the amino acid sequence that is required to recognize a particular DNA sequence and with that knowledge you can produce a code such that you know that this particular zinc finger will be able to recognize a particular DNA sequence. And another zinc finger will be able to recognize another DNA sequence and so you can predict which zinc finger will bind which DNA sequence. And with this prediction you can engineer a zinc finger yourself with the hope that this engineered protein then will be able to bind exactly to the sequence you would like it to bind so that you don't only take naturally occurring proteins with zinc fingers where the DNA sequence is fixed but that you take an engineered zinc finger protein where you recombine the zinc fingers and hands have the possibility to generate the sequence specificity of these factors at will at your need.

Q3: If you would describe ZFN to us, what would you say? How would you describe it? What is the easiest way to understand it?

A3: In principal, the most important issue about these proteins is, that you have to understand what a transcription factor is. A transcription factor is a protein, which is mostly found in the nucleus that can bind to a particular DNA sequence, and this DNA sequence is something like between 15 to 20/30 bases in length. And this ability of a protein to recognize the information of the genetic material, namely the sequence of the DNA allow this factors to bind to specific genes, to the so called promoter regions, regulatory region of the genes. And so they can turn on very specific genes in specific cells and you probably know that in all our cells the DNA is the same. (because it's all derived from the fertilized egg, it's just duplicated in all the cells). But to make cells different the interpretation of the genetic information meaning which gene has to be active and which gene has to be repressed, thats the task mostly of these transcription factors. And the difference between a blood cell and liver cell or a skin cell is the difference in which gene is expressed/ active and which gene is repressed. So transcription factors have an important task in regulating and interpreting the genetic information, which then results in the production of

specific proteins and in the functioning of cells for particular tasks like for example a neuron has a different task than a liver cell. Now ZF proteins are as I mentioned before transcription factors and they have this section of the protein motif called the zinc finger. And the zinc finger is called a zinc finger because the protein backbone, the amino acids complex with a zinc atom and they form a 3 dimensional structure which like fingers interacts with the DNA. So these fingers interact with the DNA and touch the bases of the DNA and read the bases of the DNA and so it's kind of a factor that can interpret the sequence information that is encoded in the DNA.

Q4: Do they bind to the DNA?

A4: Yes they bind, they bind to the bases of the DNA. It's not a covalent interaction because these factors in one cell they have to bind to one gene and in another cell they have to bind to another gene, so the have to be mobile, they have to move along the DNA, they have to move to another chromosome. Which means, that they cannot form covalent bonds with the DNA, the interaction are van der Waals interaction and therefore not covalent.

Q5: What is a restriction enzyme and how is it connected to ZFN?

A5: You probably know that restriction enzymes are also DNA binding proteins. They can also like transcription factors bind DNA in a sequence specific manner. They use different protein motives to interact with the DNA but the principal is the same. And you probably also know that restriction enzymes only occur in bacteria, we don't produce restriction enzymes. Restriction enzymes are proteins that bacteria use to degrade e. g. the DNA from invading viruses, to… it's like an immune system of the bacteria. But you probably also know that restrictions enzymes were discovered here in Basel and so the question is where do the restriction enzymes play a role in ZFN. Now probably the term restriction enzyme is not quite correct because nucleases is not…, they do not need to be sequence specific. The ZF is the part of that specific protein that is sequence specific. Nucleases just means that its an enzyme that cleaves DNA, that cuts it in the middle, like restriction enzymes and how it is cut is dependent on the restriction enzyme. Some produce staggered cut but some also cut it right through.
So what people did is, to combine.. so the reason ZFNs are a fusion between 2 different proteins, one protein that.. or 2 different parts of proteins. One that can bind DNA in a sequence specific manner, which is the zinc finger part. And I already explained to you before why we use ZF, because we can predict to what DNA sequence they will bind. And the other part is the nuclease part which derives from a restriction enzyme, but which has the capability to cut the DNA, both DNA strands and so with that combination you generate a protein which can find a sequence in the huge genome of a human cell for example and find specifically one sequence and it will cut that DNA right in the middle.

Q6: From where does the ZF know to which cell it has to go?
A6: The ZF does not know that, the scientist has to express that fusion protein via a process called transformation, in that particular cell or there are other ways where you have so called viral vectors, that just invade particular cells and then express that fusion protein in just those particular cells so it's not like a drug, that you take up and then somehow it finds cells or all the cells take up the drug but only the disease cells react upon this drug. You need to specifically target the zinc finger vector to express that fusion protein in that particular cell.

Q7: Do you think that ZFN is next to the CRISPR/Cas-9 something that is going to be in the „pharma“ in the future?

A7: Well as I said before there are companies that have a business model on ZFN meaning that there must be quite a number of people that think „I can make a living out of a producing ZFN“ and then obviously selling them to pharma and hospitals and use them as drugs. Indeed this is what people still are attempting to use such a zinc fingers to genome engineer certain mutations, repair genomes with certain mutations using these kinds of approaches. And if you are going to

compare ZF vs. CRISPR/Cas-9 vs. TALEN you will realize that e.g. ZF have a quite a specific target sequences, whereas with CRISPR, at least at the beginning the CRISPR system had what is called a off target effect, so that the specificity of the proteins in the RNA which in that case is necessary, was not high enough and you would not only produce one cut in the DNA but you would produce at the site you wanted it to have you would produce many other cuts. With unknown consequences. It's still a problem that is not solved but people are improving the system so that you get just one cut.

Q8: What is a nuclease respectively an endonuclease?

A8: There's a so-called Exonuclease, and there is also an endonuclease. And the exonuclease… you have linear DNA molecule. The exonuclease nibbles in from one end, and so degrades the DNA from one end and the endonuclease cuts it right in the middle, it doesn't need an end, so an endonuclease can also cut for example a plasmid which is a ring molecule, exonucleases would not be able to attack such ring molecules. You need to cut them in the middle.

Q9: When did ZFN develop? Out of what or how/ who did it develop?

A9: Well, ZFNs are an artificial construct, you will not find them in nature. You will find ZF proteins and you will find nucleases. Nucleases are rather unspecific and the restriction enzyme are sequence specific. But the ZFN as they are used for genome engineering, this is an artificial product from the laboratory. With the purpose that we discuss namely to recognize DNA sequence specifically and so to be able to cut one particular gene among a thousands of genes in the genome and hence you have to combine a protein that can recognize the gene with a protein that can do the cut right there.

Q10: Was this „development" a big step for scientists?

A10: Well in so far a big step, as you know restriction enzymes have a fixed DNA sequence, recognition motif. Meaning that if that sequence motif is in your DNA of interest, it would cut exactly there, and if it is not there, it would not cut. So the goal of producing a protein that you can manipulate at will, such that it will cut exactly at that DNA sequence what you like, is only possible through the combination that I explained to you, namely to use the combination of ZF that you adapt to the sequence you want to cut, otherwise you simply have to take a restriction enzyme which need not to cut exactly at that site.

Q11: What is ZFN used for? (-Only theoretically? Treating diseases? Used for animals or plants?)

A11: Exactly, this is the most important question. Why do you cut DNA?, you might ask, well you certainly know that if you cut DNA you produce a mutation. So you don't want to produce mutations, you want to heal a person with a particular mutation. How do you do that?
Well then you have to know another process, that occurs in our cells and we have a so called DNA repair mechanism. Meaning, because the fidelity of the DNA sequence is so important, that the sequence of the DNA stays the same all the time. Especially during DNA replication or when you produce germ cells, the DNA sequence has to stay the same. Nature, evolution has developed a mechanism to make sure that whenever there is a mistake in the DNA, it will get corrected. And you know, while exposing yourself to the sun, you constantly produce mistakes in the DNA, for example produce so called …dimers, which the repair system cuts out and replaces it with the correct base. Now you can do so, because DNA consist of two complementary strands. So one strand has a mistake, it will take the complementary strand to repair the mistake. Now the genome engineering (being it TALEN, CRISPR, ZFN) uses the same approach, namely if you introduce a cut in the DNA, you trigger the DNA repair system, meaning that (and now it becomes a bit more complicated) if you have a cut then you cannot use the complementary

strand because that is also cut, but you always have two chromosomes, in your cells (from mother and father). So if the one from the father gets the cut, the cell will use the one from the mother to repair that cut, because it know that the one from the mother is still in tact so I can use that template to repair the one from the father. This is the principal of DNA repair. (New DNA strand is replicated). So this is the normal cellular process, that goes on millions and millions of times, in our cells constantly because we are exposed to sunlight, to chemicals, irradiation that destroys our DNA. And so you would die of cancer probably very early in our life if we did not have these repair systems.
There are actually inherited diseases where people have an enzyme of the repair pathway which is mutated and these people, develop very fast skin tumours, because their DNA is no repaired.
Ok, now what is the trigger: The trick is, that you do this cut in the DNA with the ZF, but instead of using the maternal chromosome information, the homologous maternal chromosome information you give foreign DNA to the cell, which has… or lets put it in a different way,
You have a mutation in one chromosome and you would like to repair and replace it with a normal sequence, now also the other chromosome has a mutation and so you cannot use the other chromosome to repair and produce a normal sequence. Now what you do is you bring in artificial DNA, which is complementary to that region, that you want to repair, except that mutation. If it is a point mutation you would replace it with the base that represents the normal function of the gene. And so after cutting the DNA the cell now tries to repair it, but is has no normal function, no normal DNA there, so it will use to foreign DNA the you introduce, and will repair the mutated sequence, with the wild type sequence. And so you corrected that mutation, because you replaced the mutated sequence with a normal sequence. And this is one part of the genetic engineering goals which you could achieve with ZFN.
Now this is obviously the theory behind it, and there has also been in the laboratory implement the model organisms and cell (carges? was? min. 29:49) and so on, that you can replace any DNA sequence of interest with another DNA sequence that is synthesized in the laboratory. A typical example is what I explained to you before, you have a mutation and you want to correct that mutation with a wild type sequence. But you could also imagine that you want to produce a fusion protein, so you have a normal protein sequence encoded in the DNA and you replace that thought this method with another protein sequence producing a completely new protein, which you might use for pharmaceutical goal or for any other task that you might think of, like a new enzyme that degrades plastics, that degrades oil spills or whatever. We just has people talking about bacteria that eat up explosives, so your imagination is the limit of what kind of function you would like to have these proteins and so… normally they don't exist in the cell and you have to engineer those proteins to have these new functions and you do that by changing the DNA sequence with this method. And you can use it for all cells, for all animals, plants that obviously have DNA and the DNA repair system is conserved in all the living cells (even bacteria). So the principal of replacing DNA sequences with other DNA sequences that you introduce to the cell using this nucleases stays the same. (and it is also possible in plants)

Q12.: What do you think how important was Zinc finger Nucleases of for human development/ science

A12: Well it is certainly an important step because genome engineering as such is an important technology. We've been working on genome engineering since the last thirty- forty years. We want to manipulate the genetic information to fulfil tasks, purposes that we need, not only in terms of correcting mutations but for e.g. for bio technology. We want to produce cells or engineer cells that produce certain proteins that we might use for pharmaceutical purposes or in agriculture or in other fields. So manipulating the genetic context of a cell through genome engineering is something very important in biology and in medicine for that.

Q13: What is your personal fascination about ZFN resp. Genetic Engineering?

A13: Well I'm working in basic research meaning that actually I studied a process called cellular memory, so during development cells are programmed for a particular task and that program

needs to be maintained in all the progeny of that cell, so a blood cell has to become a blood cell again, a liver cell has to becomes a liver cell again and this is called cellular memory so the cell memorizes what its identity is and this is what we try to understand, how can a cell achieve that memory, because if it loses the memory it can become a cancer cell for example. And we know that obviously as I mentioned to you also the difference between the cells is not the DNA sequence because that's the same but it is the way that the DNA sequence is read. It's interpreted and which gene is activated and which is repressed. And as we do in research to understand the process we want to manipulate the process and see what are the consequences. If we know a pathway or if we know a particular gene is involved in a particular process if we mutate that gene and that process is stopped somewhere we can understand the sequence of event or ideally if you understand the process you should be able to assemble the parts and reproduce the process. So it means that we are always depend on manipulating the genetic information and genome engineering is exactly that too. That allows us to manipulate the genetic information in order to understand how biological process is work. That's the view of a scientist working in basic science to understand important biological mechanisms and processes. And eventually based on this knowledge to understand how e.g. in the disease case the process is misregulated in order to develop drugs or cures for that disease. But e.g. the bioengineer has a different view why he wants to use genome engineering. The bioengineer will say: "I would like to have a cell that produces a particular protein that is used as a drug or a particular antibody that is used as a drug against cancer molecule. So you have to engineer the sequence for that protein into the cell because that cell normally would never produce that protein or that antibody and so you have to specifically change the sequence of that genome to produce something totally new that nature or that cell would have not produced by its own and this is what is also called synthetic biology. You produce synthetically something that never occurred on this planet but that is new and should be beneficial for humanity. So there are many reason why you would want to do that and e.g. you want to engineer a cell to talk to another cell so that these cells form aggregates or form interaction that produce a new structure. Another hype in biology is e.g. the so-called organic meaning that that you can produce little brains or stomachs, so not only one cell or cells culture with individual cells but cells that form little organs. And e.g. and you can produce pancreatic that would produce insulin that would produce this molecules. So there are many new ideas and possibilities that genome engineering can fulfil.

2. Laboratory Pictures

Fig. 1: Scientist who is showing how they pipette.

Fig. 6: One of their biggest and best microscopes; Here you see the pictures upside down.