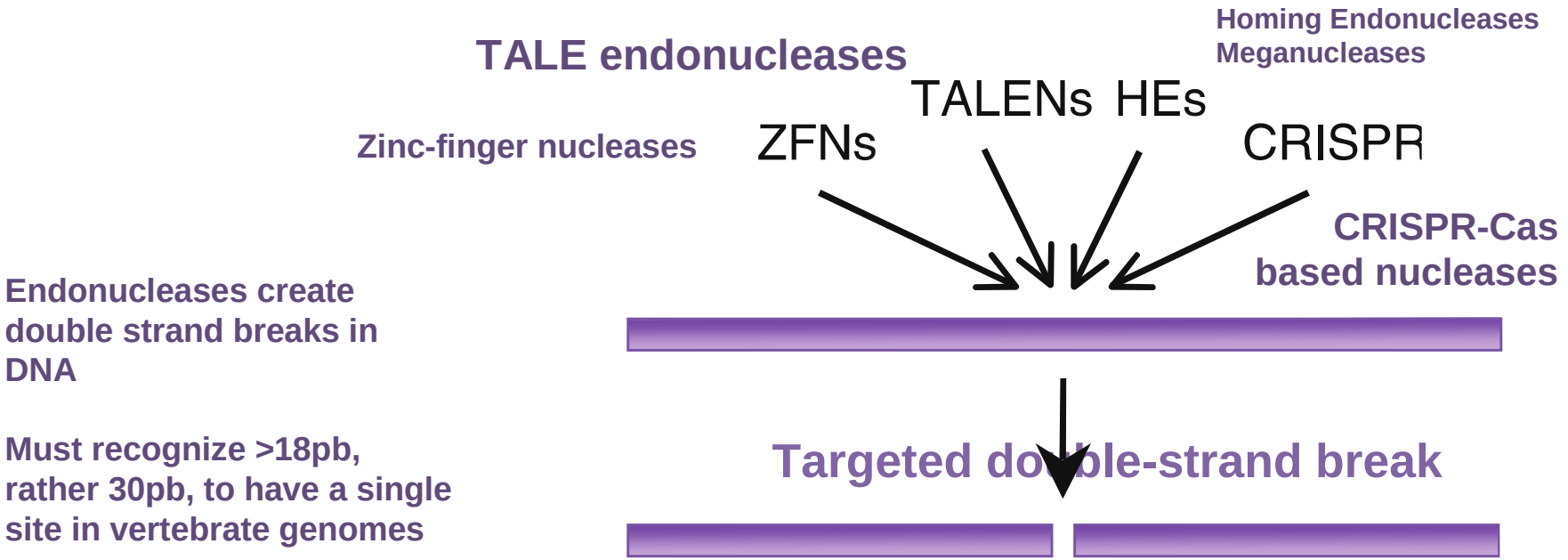
Jean-Stéphane Joly
INRA group
CNRS NED Unit
TEFOR infrastructure
CNRS Gif sur Yvette

- Introduction on genome engineering
- Genome editing in the model fish zebrafish
- Applications in aquaculture ,

Marc Vandeputte

Short history of endonucleases with long sites: four generations



- Meganucleases: not enough versatile
-Zinc fingers: design not predictible, prescreens requested
-TALENs: predictible design, better rates of success
- Crispr: increased ease of production, but off targets

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

15 FEBRUARY 2013 VOL 339 **SCIENCE**

Faster progress at each generation:
TALENs, only one year from in vitro to in vivo applications!

TALEN

- **Modifications in cultured cells**

- cultured cell lines	*Februray 2011, Nature Biotechnol*
- ES and iPS	*July 2011, Nature Biotechnol*

- **Modifications in model organisms**

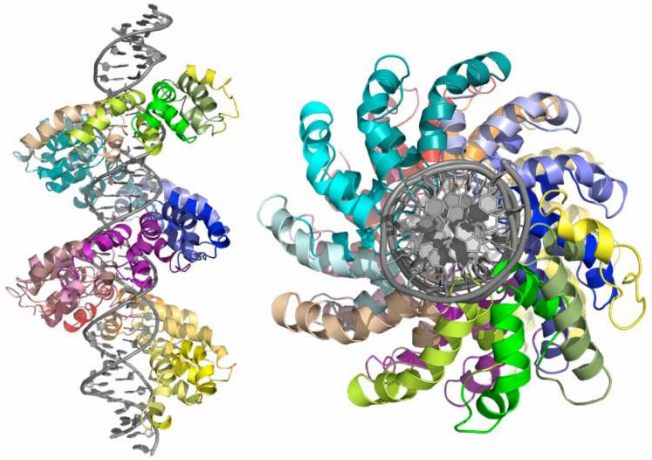
- rat	*July 2011, Nature Biotechnol*
- zebrafish	*July 2011, Nature Biotechnol*
- rice	*May 2012, Nature Biotechnol*
- Xenopus	*October 2012, PNAS*
- Livestock	*October 2012, PNAS*
- mouse	*January 2013, Nature Biotechnol*

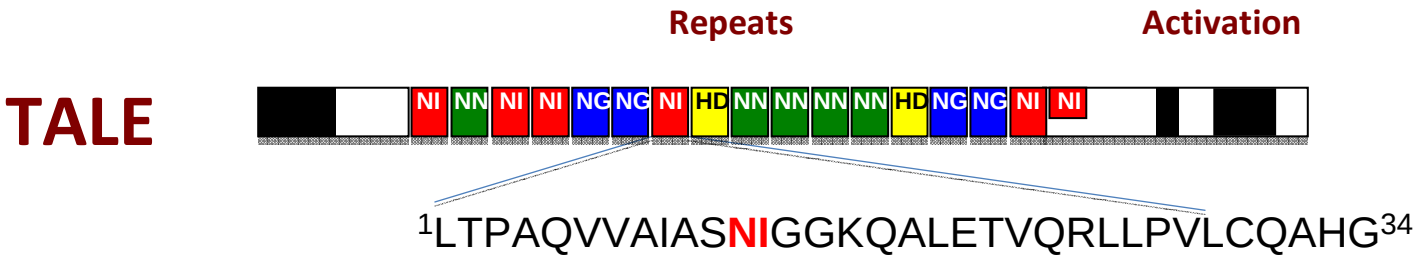
How TALEN work

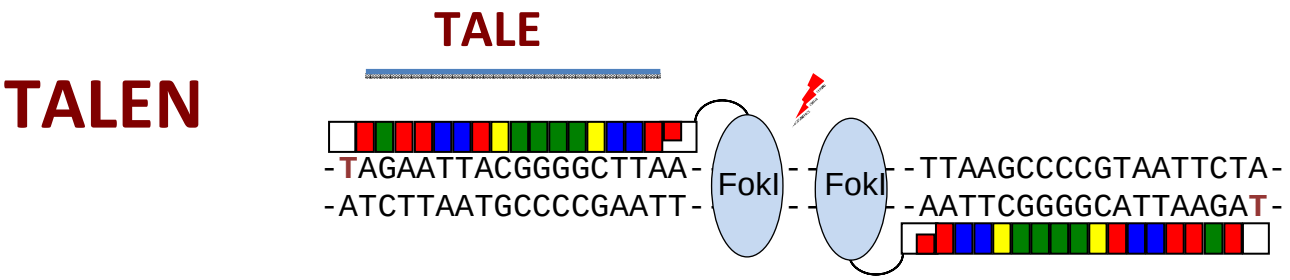
DNA-binding code of TALE transcription factors from *Xanthomonas*

(Boch et al, Science, 2009)

NI->A **NG**->T **NN**->G **HD**->C

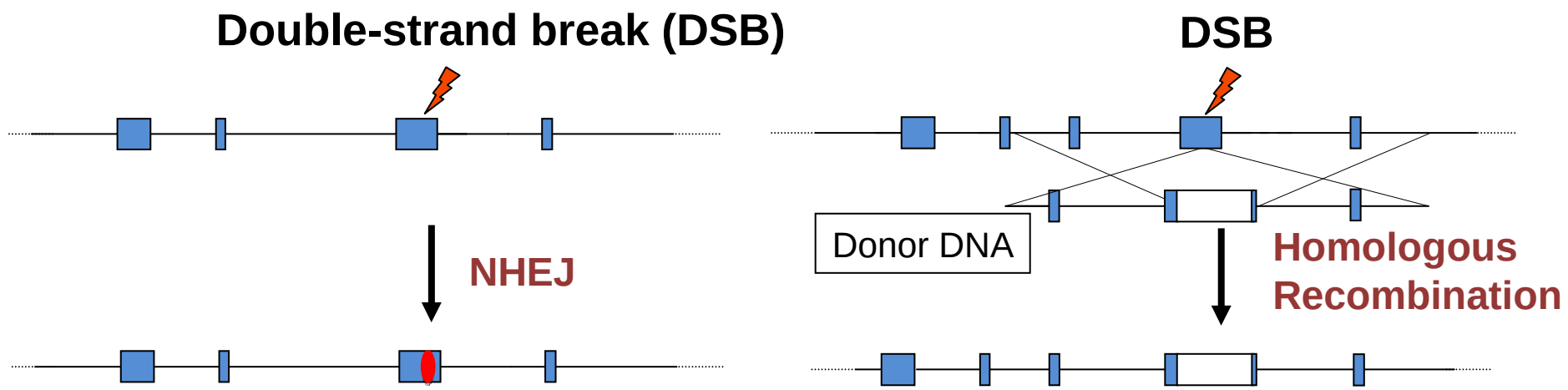






What do they do?

Two types of targeted genome modifications



Gene Inactivation (KO)

Deletions / Insertions

Controlled gene modification (KI)

Point mutations, gene replacement

Which revolutionary modifications?

- **Introduce short deletions: mutants to study gene function**

- **Introduce long DNA sequences: targeted transgenesis, landing pads**

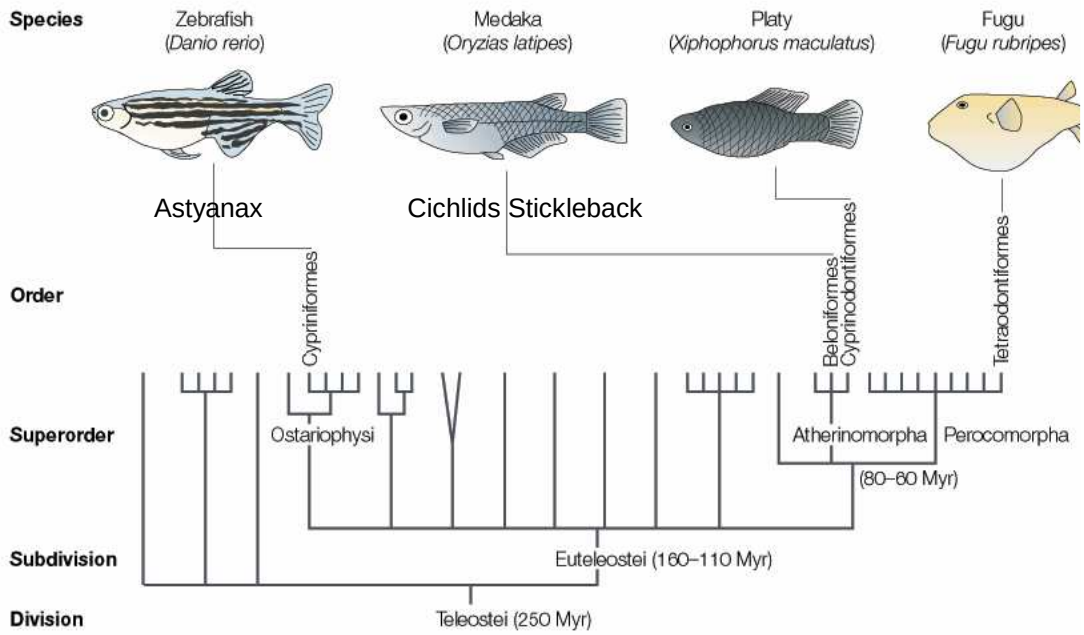
- **Introduce point mutations: similar to natural SNP mutations, not tractable, not GMO!**

- **Diversifying applications: modify genes or regulatory elements, DNA modification, targeted transposition, monitor DNA loops**

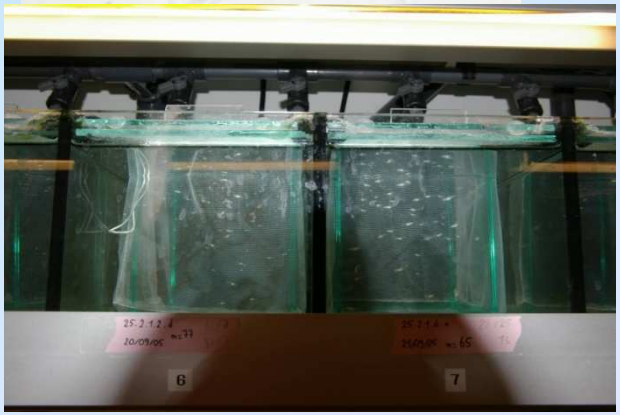
KO in zebrafish

Why tank model fish?

easy breeding, short generation time, zebrafish: third vertebrate genome sequenced with best annotations



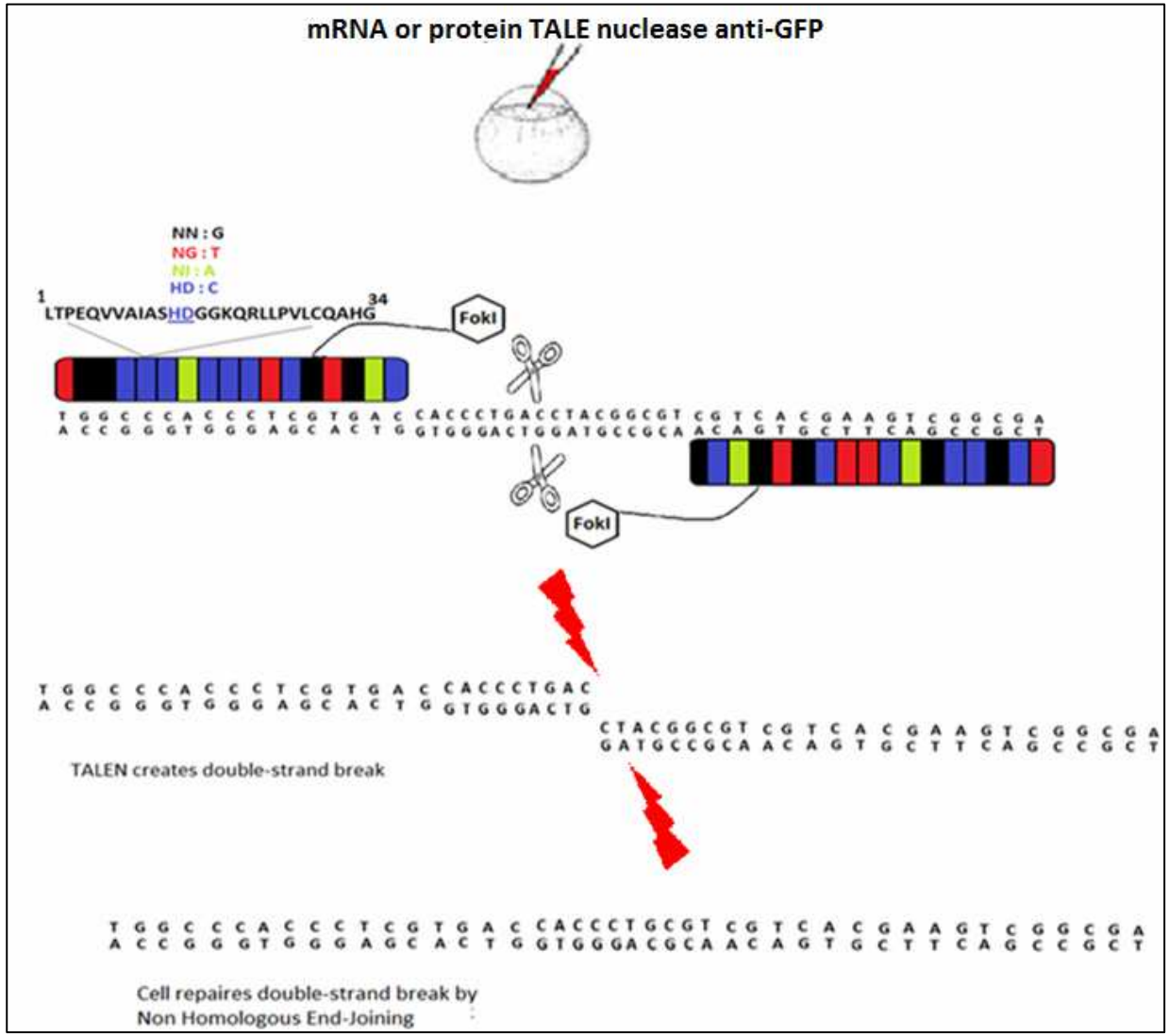
Easy microinjection



5000 fish/15m2

- Big transparent eggs and fertility
- So far only transgenesis with random insertion and chemical mutagenesis availablet

Principle : TALEN RNA injection



Genome modifications in zebrafish by use of TALENs

Range of modifications induced by TALENs

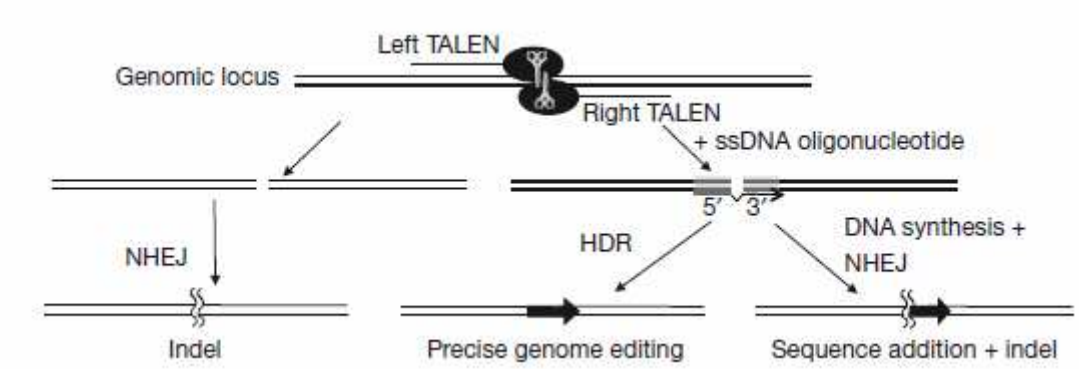
Modification	Range
Mortality + malformation rate	5-85%
Embryos with modified somatic alleles	77-100%
Modified somatic alleles per embryo	2-100%
Embryos with modified germline (transmitting)	5-100%
Modified germline alleles per transmitting embryo	8-100%

KI in zebrafish

In vivo genome editing using a high-efficiency TALEN system

Victoria M. Bedell[1]*, Ying Wang[2]*, Jarryd M. Campbell[1]*, Tanya L. Poshusta[1], Colby G. Starker[3], Randall G. Krug II[1], Wenfang Tan[3], Sumedha G. Penheiter[1], Alvin C. Ma[1,4], Anskar Y. H. Leung[4], Scott C. Fahrenkrug[3,5], Daniel F. Carlson[3,5], Daniel F. Voytas[3], Karl J. Clark[1], Jeffrey J. Essner[2] & Stephen C. Ekker[1]

Nature 2012



In total, 6 out of 58 injected animals transmitted the mloxP oligo through the germline

Numerous perspectives

- Improve further TALEN efficiency (early access to DNA by injecting proteins, TEFOR patent)
- Improve CRISPR specificity
- Couple to other catalytic or targeting agents
- Improve further genotyping/selection methods for KI
- Optimize in other fish: aquaculture fish?

DISTRIBUTED INFRASTRUCTURE
For zebrafish and drosophila communities

Technological research on TALEN
Patent on TALEN protein injection allowing to obtain non mosaic animals

Johanna Djian-Zaouche djian@tefor.net

TALEN service (TALEN from Cellectis):
Jean-Baptiste Pénigault penigault@tefor.net

http://www.tefor.net

NETWORK
Functional studies on model organisms

Johanna Djian-Zaouche
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TALEN/CRISPR call for use in novel model organisms

http://www.efor.fr

- **Jean-Stéphane Joly joly@inaf.cnrs-gif.fr**