CDB SEMINAR

# Julianne Smith

Cellectis, France

Wednesday, November 19, 2008

16:00~17:00 A7F Seminar Room

## Meganucleases with tailored specificities for genome engineering purposes

**Summary**

Homologous gene targeting is the best way to modify a genome in a precise and rational way; however it has proven to be highly inefficient in the majority of cells examined. This limit can be alleviated by using meganucleases, sequence-specific endonucleases recognizing large (>12 bp) cleavage sites. These proteins can stimulate homologous gene targeting by a 1000-fold factor or induce mutagenesis in the vicinity of their target site, and these findings have opened novel perspectives for genome engineering. However, the use of this technology has long been limited by the repertoire of natural meganucleases: the probability of finding a sequence cleaved by a natural meganuclease in a chosen gene is extremely low. Therefore, the design of artificial endonucleases with chosen specificities is under intense investigation. Given their exceptional specificity, meganucleases should provide ideal scaffolds to derive genome engineering tools.

We have developed a combinatorial approach to redesign the DNA-binding interface of I-CreI, a *Chlamydomonas reinhardti* protein belonging to the LAGLIDADG family of meganucleases. In a first step, we have collected large numbers of locally engineered I-CreI derivatives with altered specificity. Second, we assemble these mutants into entirely redesigned endonucleases binding *a priori* chosen targets. Third, additional refinement steps allow both the activity and specificity of these mutants to be improved. This approach has enabled us to design over a dozen custom meganucleases, including meganucleases that target genes involved in Xeroderma Pigmentosum, SCID, thalassemia, and other genetic diseases. These engineered proteins keep the essential properties of natural meganucleases in terms of folding, activity and specificity and have been used successfully to induce recombination in up to 1% of cells in reporter systems as well as in the endogenous genes. Thus, the combined properties of these proteins (activity and specificity) qualify them as ideal tools for genome engineering (e.g. for the development of new animal models, stem cells and iPS) and as a novel therapeutic approach to treat viral and monogenic diseases through genome surgery.

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