

From DNA transposition and site-specific recombination mechanisms to the molecular biology toolbox

SENIOR SCIENTIST:

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Research Field and Subjects

Transposition and site-specific recombination are two categories of DNA rearrangements that are mediated by specialised enzymes generically referred to as transposases and site-specific recombinases. Transposition is the process by which genetic elements move between different genome locations, whereas site-specific recombination is a reaction in which DNA strands are broken and rejoined at determined positions of two target DNA sequences.

Studies in the laboratory are focused on the transposition and site-specific recombination machinery encoded by the bacterial transposon Tn4430. A multidisciplinary range of genetical and biochemical approaches are used to investigate the molecular interactions involved in the assembly of the transposition/recombination complex and the coordination of the DNA cleavage and rejoining reactions. Specific features of Tn4430 transposition/recombination mechanisms are also used to develop new tools for genetic engineering and *in vitro* cloning or mutagenesis technologies.

Pentapeptide scanning mutagenesis (PSM) is a new method based on Tn4430-mediated insertion of 5-amino acids cassettes at random positions of a target protein. This method was successfully used to study the functional organisation a number of proteins in different laboratories.

The site-specific recombination reaction catalysed by Tn4430-encoded tyrosine recombinase, Tnpl, has been reconstituted *in vitro*.

Recombination was also shown to function in different organisms, including mammals, demonstrating its potential for the development of tools in a wide range of biotechnological applications.

Products and Services

- ▶ Bacterial strains
- ▶ DNA substrates
- ▶ Purified proteins

Main Equipment

- ▶ Basic molecular biology and biochemistry equipment

Representative References

- ▶ HALLET B., AND D. J. SHERRATT (1997) Transposition and site-specific recombination: adapting DNA cut and paste mechanisms to a variety of genetic rearrangements. *FEMS Microbiol. Rev.* 21:157-178.
- ▶ HALLET B., D. J. SHERRATT, AND F. HAYES (1997) Pentapeptide scanning mutagenesis: random insertion of a variable five amino acid cassette in a target protein. *Nucleic Acids Res.* 25:1866-1867.
- ▶ HAYES F., AND B. HALLET (2000) Pentapeptide scanning mutagenesis: encouraging old proteins to execute unusual tricks. *Trends Microbiol.* 8:569-574.
- ▶ VANHOOFF V., C. GALLOY, B. RÉVET, AND B. HALLET. Topologically relaxed and constrained site-specific recombination catalysed by the Tnpl tyrosine recombinase of Tn4430. *In prep.*
- ▶ VANHOOFF V., C. NORMAND, C. GALLOY, AND B. HALLET. Relative contribution of the recombination core site and accessory sequences in the directionality of the recombination reaction catalysed by the tyrosine recombinase Tnpl. *In prep.*

Patents

- ▶ STEWART A. F., Y. ZHANG, AND B. HALLET. (2001) A new tyrosine recombinase for genetic engineering. US Patent Application # 09/895.435.

Partnership

- ▶ Member of *Institut des Sciences de la Vie* (ISV), Louvain-la-Neuve, Belgium
- ▶ EMBL, Heidelberg / Max Planck Institute, Dresden (A. F. Stewart lab.)
- ▶ *Actions de recherche concertées* (ARC) with J. Fastrez / P. Soumillion (ISV, BIO) and J.-P. Declercq (CHIM, CSTR)

STAFF

Total: 5

KEY WORDS FOR R&D

DNA recombination
genetic engineering
gene targeting
mutagenesis
protein engineering
site-specific recombination
transposition
tyrosine recombinase

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