Major Scientific Achievements of Walter Fiers and the Laboratory of Molecular Biology of the University of Ghent.

(listed according to research project)

Bacteriophages

Bacteriophage ΦX174

- *For the first time proof was provided that genomic DNA can occur as an uninterrupted, physical ring. Later-on it was shown that also the genome of bacteria, mitochondria, plant organelles and many viruses usually occurs in the form of such an uninterrupted, covalently-closed ring structure.*

FIERS, W. & SINSHEIMER, R.L. The structure of the DNA of bacteriophage ΦX174. III. Ultracentrifugal evidence for a ring structure. J. Mol. Biol. 5, 424-434, 1962.

Bacteriophage MS2

- *A new method to separate hundreds of intermediate length RNAs.*

DE WACHTER, R. and FIERS, W. Preparative two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled RNA. Anal. Biochem. 49, 184-197, 1972.

- *The first complete nuleotide sequence of a gene was reported.; this was the Rosetta Stone of Molecular Biology. For the first time it became possible to compare directly the sequence of the nucleotide building blocks in the RNA with the sequence of amino acids in the corresponding polypeptide. The presumptive "Code Word Table" was confirmed by data from a real, in vivo functioning system.*

MIN JOU, W., HAEGEMAN, G., YSEBAERT, M. & FIERS, W. Nucleotide sequence of the gene coding for the Bacteriophage MS2 coat protein. Nature 237, 82-88, 1972.

- *First complete nucleotide sequence of a genome: this corresponds to the total genetic information carried by the virus: it includes not only information for the structure of viral proteins, but also information for regulation of gene expression, signals for replication, etc. For the first time the complete amino acid sequence of a protein, not yet isolated in pure form, was deduced from its gene sequence.*

FIERS, W., CONTRERAS, R., DUERINCK, F., HAEGEMAN, G., ISERENTANT, D., MERREGAERT, J., MIN JOU, W., MOLEMANS, F., RAEYMAEKERS, A., VAN DEN BERGHE, A., VOLCKAERT, G. & YSEBAERT, M. Complete nucleotide sequence of Bacteriophage MS2 RNA: Primary and secondary structure of the replicase gene. Nature 260, 500-507, 1976.

- *Virus genes were individually transferred to plasmids, and expressed. This allowed to study their interdependent regulation of expression.*

REMAUT, E., DE WAELE, P., MARMENOUT, A., STANSSENS, P. and FIERS, W. Functional expression of individual plasmid-coded RNA bacteriophage MS2 genes. EMBO J. 1, 205-209, 1982.

SV40 (a primate-derived, oncogenic virus)

- *The research groups of S. Weissman in Yale and W. Fiers in Ghent finished at about the same time the sequence of the complete genome of SV40. This virus replicates in monkey cells and causes tumor formation in rodents. By mutual agreement both groups decided to publish separately but at the same time, one in "Science" and the other in "Nature". It was the first time that the primary structure of an oncogen (a gene which causes tumor-formation in vivo) was established. A related provirus, known as JC, occurs in the human genome; occasionally, e.g. under conditions of immuno-suppression, this JC provirus becomes activated which leads to deadly "progressive leucoencephalopathy". This SV40-system was also one of the first in which splicing of messenger RNA was established.*

FIERS, W., CONTRERAS, R., HAEGEMAN, G., ROGIERS, R., VAN DE VOORDE, A., VAN HEUVERSWYN, H., VAN HERREWEGHE, J., VOLCKAERT, G. & YSEBAERT, M. Complete nucleotide sequence of SV40 DNA. Nature 273, 113-120, 1978.

HAEGEMAN, G. & FIERS, W. Evidence for 'splicing' of SV40 16S mRNA. Nature 273, 70-73, 1978.

Influenza

- *For the first time, the sequence was established of an hemagglutinin (HA)- gene from a human influenza virus. HA is the most important, but highly variable, antigenic component of the virus. Influenza vaccines are mainly directed against HA. By comparison of HA-genes derived from different virus strains a molecular explanation was provided why successive influenza epidemics emerge almost once a year, while pandemics (a novel HA-subtype gene) occur once a decade or less.*

MIN JOU, W., VERHOEYEN, M., DEVOS, R., SAMAN, E., FANG, R., HUYLEBROECK, D., FIERS, W., THRELFALL, G., BARBER, C., CAREY, N. & EMTAGE, S. Complete structure of the hemagglutinin gene from the human influenza A/Victoria/3/75 (H3N2) strain as determined from cloned DNA. Cell 19, 683-696, 1980.

VERHOEYEN, M., FANG, R., MIN JOU, W., DEVOS, R., HUYLEBROECK, D., SAMAN, E. FIERS, W. Antigenic drift between the haemagglutinin of the Hong Kong influenza strains A/Aichi/2/68 and A/Victoria/3/75. Nature 286, 771-776, 1980.

FANG, R., MIN JOU, W., HUYLEBROECK, D., DEVOS, R. & FIERS, W. Complete structure of A/duck/Ukraine/63 influenza hemagglutinin gene: Animal virus as progenitor of human H3 Hong Kong 1968 influenza hemagglutinin. Cell 25, 315-323, 1981.

.

Interferon-β

T.Tanigushi was the first to clone the human interferon-β gene. W. Fiers and his group were the first to clone and also express this gene. Expression in a eukaryotic cell provided human interferon-β with demonstrable biological activity in human cells. This established beyond any doubt the identity of the cloned gene. Expression also opened the way to commercial production. L. Jacobs found that human interferon-β reduces the exacerbations of multiple sclerosis patients. But only interferon-β made by recombinant DNA-technology can provide sufficient material for medical use. Human interferon-β is now the major treatment for Multiple Sclerosis (Biogen is the main manufacturer of human interferon-β, sold under the trade name "Avonex"). In 2009 a dominant patent, with W. Fiers as inventor, was granted in the USA and several other countries; it covers the use of human interferon-β to treat immunomodulatory diseases (such as Multiple Sclerosis).

DERYNCK, R., CONTENT, J., DE CLERCQ, E., VOLCKAERT, G., TAVERNIER, J., DEVOS, R. & FIERS, W. Isolation and structure of a human fibroblast interferon gene. Nature 285, 542-547, 1980.

DERYNCK, R., REMAUT, E., SAMAN, E., STANSSENS, P., DE CLERCQ, E., CONTENT, J. & FIERS, W. Expression of human fibroblast interferon gene in Escherichia coli. Nature 287, 193-197, 1980.

GHEYSEN, D. & FIERS, W. Expression and excretion of human fibroblast β1 interferon in monkey cells after transfection with a recombinant SV40 plasmid vector. J. Mol. Appl. Genet. 1, 385-394, 1982.

Cytokines

- Several human cytokine genes were cloned and expressed, such as Interferon-γ, Interleukin-2, Interleukin-6, etc. Mainly used for scientific research.

.

***DEVOS, R., CHEROUTRE, H., TAYA, Y., DEGRAVE, W., VAN HEUVERSWYN, H. &* FIERS, W. Molecular cloning of human immune interferon cDNA and its expression in eukaryotic cells. Nucl. Acids Res. 10, 2487-2501, 1982.**

DEVOS, R., PLAETINCK, G., CHEROUTRE, H., SIMONS, G., DEGRAVE, W.,TAVERNIER, J., REMAUT, E. & FIERS, W. Molecular cloning of human Interleukin-2 cDNA and its expression in E. coli. Nucl. Acids Res. 11, 4307-4323, 1983.

HAEGEMAN, G., CONTENT, J., VOLCKAERT, G., DERYNCK, R., TAVERNIER, J. & FIERS, W. Structural analysis of the sequence coding for an inducible 26-kDa protein in human fibroblasts. Eur. J. Biochem. 159, 625-632, 1986.

STEIDLER, L., HANS, W., SCHOTTE, L., NEIRYNCK, S., OBERMEIER, F., FALK, W., FIERS, W. and REMAUT, E. Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. Science 289, 1352-1355, 2000.

TNF

- Originally "Tumor Necrosis Factor" (TNF) was believed to be a natural compound which could selectively kill cancer cells. In combination with interferon-γ, this is often true. But this combination of cytokines is also quite toxic. In fact, presumably the real role of TNF is to kill selectively weakened cells, such as pathogen-infected cells. Programmed cell death developed into an important research topic of the laboratory; on the one hand mechanisms to escape cell death, as observed in cancer cells, and on the other hand premature cell death as in Parkinson and a number of other diseases; programmed cell death is also important in adaptive immune responses and in inflammatory processes.

FRANSEN, L., MULLER, R., MARMENOUT, A., TAVERNIER, J., VAN DER HEYDEN, J., KAWASHIMA, E., CHOLLET, A., TIZARD, R., VAN HEUVERSWYN, H., VAN VLIET, A., RUYSSCHAERT, M.R. & FIERS, W. Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. Nucl. Acids Res. 13, 4417-4429, 1985.

BROUCKAERT, P.G.G., LEROUX-ROELS, G.G., GUISEZ, Y., TAVERNIER, J. & FIERS, W. In vivo anti-tumour activity of recombinant human and murine TNF, alone and in combination with murine IFN-gamma, on a syngeneic murine melanoma. Int. J. Cancer 38, 763-769, 1986.

VAN OSTADE, X., TAVERNIER, J., PRANGE, T. & FIERS, W. Localization of the active site of human tumour necrosis factor (hTNF) by mutational analysis. EMBO J. 10, 827-836, 1991.

VAN OSTADE, X., VANDENABEELE, P., EVERAERDT, B., LOETSCHER, H., GENTZ, R., BROCKHAUS, M., LESSLAUER, W., TAVERNIER, J., BROUCKAERT, P. & FIERS, W. Human TNF mutants with selective activity on the p55 receptor. Nature 361, 266-269, 1993.

VERCAMMEN, D., BEYAERT, R., DENECKER, G., GOOSSENS, V., VAN LOO, G., DECLERCQ, W., GROOTEN, J., FIERS, W. & VANDENABEELE, P. Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by Tumor Necrosis Factor. J. Exp. Med. 187, 1477-1485, 1998.

FIERS, W., BEYAERT, R., DECLERCQ, W. & VANDENABEELE, P. More than one way to die: Apoptosis, necrosis and reactive oxygen damage. Oncogene 18, 7719-7730, 1999.

CAUWELS, A., VAN MOLLE, W., JANSSEN, B., EVERAERDT, B., HUANG, P., FIERS, W. and BROUCKAERT, P. Protection against TNF-induced lethal shock by soluble guanylate cyclase inhibition requires functional inducible nitric oxide synthase. Immunity 13, 223-231, 2000.

Influenza vaccine

- *Ongoing reseach project. The goal is to develop a broad-spectrum vaccine directed to conserved, antigenic patches on the influenza virus. Such a vaccine would protect not only against successive, yearly epidemics, but also against an occasional pandemic. A vaccine which fulfils these criteria has been tested successfully in a human phase I trial. It is based on the highly conserved external domain of the small viral protein M2. Unlike classical influenza vaccines, M2e-based vaccination is infection-permissive; meaning that infection (silent because of protective M2e-antibodies) still induces cellular immunity, leading to broad and long-lasting protection.*

NEIRYNCK, S., DEROO, T., SAELENS, X., VANLANDSCHOOT, P., MIN JOU, W. & FIERS, W. A universal influenza A vaccine based on the extracellular domain of the M2 protein. Nature Medicine 5, 1157-1163, 1999.

DE FILETTE, M., MIN JOU, W., BIRKETT, A., ET AL. & FIERS, W. Universal Influenza A Vaccine: Optimization of M2-based construct. *Virology*, 337, 149–161, 2005.

DE FILETTE, M., MARTENS, W., ROOSE, K., DEROO, T., VERVALLE, F., BENTAHIR, M., VANDEKERCKHOVE, J., FIERS, W. & SAELENS, X. An influenza a vaccine based on tetrameric ectodomain of matrix protein 2. *J Biol Chem.* 283 (17), 11382-87, 2008.

SCHOTSAERT, M., YSENBAERT, T., NEYT, K., IBAÑEZ, LI., BOGAERT, P., SCHEPENS, B., LAMBRECHT, B.N., FIERS, W. & SAELENS, X. Natural and long-lasting cellular immune responses against influenza in the M2e-immune host. Mucosal Immunol. 2012 Jul 18. doi: 10.1038/mi.2012.69.
