



## ACHEMA 2003 19–24 May 2003 Frankfurt am Main/Germany

*The opening ceremony of the 27th Achema was performed in the Congress Center at the Frankfurt Exhibitions Grounds. From Monday, May 19 2003 the gates to the world's biggest chemical engineering Exhibition/Congress and International Meeting on Chemical Engineering Environmental Protection and Biotechnology were opened to the public for 6 days.*

*The special technical and trend reports of Achema 2003 exhibition were prepared for publication and released by internet as press information by authorities from DECHEMA. Trend reports covering present and future state of RNA Interference – The New Magic Wand of Biotechnology and, Proteomics ad Protein Expression are present in this issue.*

### RNA Interference – The New Magic Wand of Biotechnology

*Despite the current financial bottlenecks for young biotech SMEs, the sector perpetually creates novel and innovative technologies responsible for some substantial growth rates in the research and laboratory equipment markets.*

*One particular highlight is the world-class Biotechnology Exhibition & Conference featuring the cutting-edge themes which currently concern the biotechnology community. 10 plenary lectures, 100 other lectures and daily panel discussions with distinguished experts in the new Forum provided an opportunity to find out the latest on the "hot" topics in modern life sciences research and on topical business trends.*

*In the bio-sciences, alongside functional genomics and proteomics a new emerging growth technology is set to conquer the research and drug discovery market – namely RNA interference, or RNAi.*

So far, there have been only very few technologies that have immediately captured scientists, pharmaceutical companies and laboratory suppliers alike, quite as RNAi has succeeded in the first five years of its existence. The scientific reference journal 'Science' nominated RNAi the 'breakthrough technology' of the last year. Since between 2000 and 2002 the number of relevant publications has increased more than threefold (from 63 in 2000 to 203 in 2002). Now biotechnology companies are racing for patents to exclusively exploit RNAi for drug discovery, target validation and the development of a new class of drug compounds.

Laboratory equipment companies are experiencing a surge in demand for research reagents that routinely enable RNAi experiments. The reason for that is very simple: for the first time it is possible by means of RNAi to switch-off single genes faster and more efficiently than with any other method without impairing the protein expression of neighbouring genes. Also, the new method allows functional high-throughput analysis of genes for the first time. Hence there is a real

chance to economically exploit the DNA sequences that have been identified in various genome projects. At the same time according to experts RNAi technologies are around 80% cheaper than the creation of knock-out-mice – which is currently used for functional analysis of genes.

"This way virtually every gene can be looked at" explains Dr. Christophe Echeverri who in 1998 founded one of the very first companies exclusively geared towards RNAi – namely Cenix Bioscience, Dresden. "RNAi opens completely new perspectives to post-genomic research. At the same time RNAi drugs would offer all the key features that are expected of sequence-specific therapeutics." Applying proprietary RNAi technologies researchers at Cenix screen the genomes of model organisms like *C. elegans* and the human genome for new validated drug targets that will subsequently be sold to the pharmaceutical industry.

However, the first RNAi medicaments are still a long way off. According to a topical study by US-based Front Line Strategic Consulting, Inc. the first approved drugs will be released in 2008, the earliest. The same study shows that in the meantime laboratory suppliers are cashing in most, and more and more of them are heading for this lucrative RNAi market that is only some two years old. For the current year the market analysts have forecasted sales in the region of \$38 million. Within the next five years this market will reach a volume of \$185 million, according to the study.

The foundations for this already profitable growth market have been laid five years ago by US scientists Andrew Fire and Craig Mello with their work on the nematode worm *Caenorhabditis elegans*. They were the first to describe a new technique that allowed by means of a few double-stranded RNAs (dsRNA) to specifically inhibit the expression of certain proteins in a living cell. To achieve this simply one strand of the dsRNAs had to have the same base sequence as the protein-coding gene that was to be "silenced". Even though the dsRNAs did not inhibit the transcription of

the gene, they managed to activate a mechanism inherent in living cells that destroys transcribed mRNAs thus inhibiting the translation of the respective proteins (by post-transcriptional gene silencing – or PTGS).

According to our current knowledge, targeted degradation of mRNA is initiated by short double-stranded RNA molecules that are 21 to 23 base pairs long (small interfering RNAs, or siRNAs). These are homologous to the mRNAs whose protein translation is to be inhibited. One of the siRNA's strands – the so-called antisense-strand – binds to the cells' own RISC protein complex and the targeted mRNA. In the resulting complex a nuclease enzyme is responsible for the sequence-specific mRNA degradation, thus inhibiting protein expression.

However, until two years ago all experiments failed that tried to use longer dsRNAs (>50 base pairs) to silence mammalian genes that would have been interesting for drug discovery. This was due to the so-called interferon answer, an immune reaction to counter virus infections in mammalian cells, that causes the seizure of all protein production by the unspecific degradation of all mRNA.

Finally, the German scientist Tom Tuschl achieved a breakthrough: by means of artificially created siRNAs that were 21 to 23 base pairs long his team succeeded for the first time in silencing mammalian genes without causing the interfering interferon answer. Since that date research is claiming one success after another. For example, in the targeted silencing of a range of HIV genes (*nef*-, *rev*-, *gag*-, *pol*-Gene), or in the fight against viruses causing influenza and hepatitis C. Even cancer is on the list of future indications, on which RNAi is to cause progress. Since it has been observed that siRNAs work most effectively in cells that divide very rapidly.

Tuschl who currently moved from his Max Planck Institute in Goettingen to Rockefeller University, New York, was 34 years old at the time of his discovery. He quickly established contact with Dharmacon, Lafayette, USA – the world market leader in the field of synthetic RNA synthesis. After this, last summer he and a handful of other RNAi pioneers together with Biogen's co-founder Philip Sharp founded the company Alnylum Pharmaceuticals in Boston, USA, which in a first financing round could already raise some \$17 million in institutional investments.

Alnylum is among the very few pioneering RNAi companies that intend to apply siRNAs in the treatment of so far incurable diseases or to use RNAi to discover new druggable targets. Already, these companies hold the patents that are vital for future commercial success. German Ribopharma AG (Kuimbach) that is focussing on the cancer market holds a German and a European patent whilst Australian Benitec (St. Lucia) which applies its proprietary technologies to discover drugs against cancer, HIV, hepatitis C, and auto-immune diseases

holds two Australian patents. In mid-February Fire and Mello who had discovered RNAi in the first place have been granted a comprehensive US patent which they intend to out-license to the pharmaceutical and laboratory supply industry. Even though the development of novel and innovative drugs promises the highest revenues in the long term, at the moment most companies concentrate on target discovery for economic exploitation by the pharmaceutical industry – either as their core business purpose or alongside their own drug developments.

Dresden-based Cenix BioScience holds already patents on 16 genes discovered in the nematode worm *C. elegans* that play an important role in the development of cancer in humans, claiming a "head-start of four years" to its competitors. Similar approaches are applied by Scottish Cyclacel (Dundee) which is specialized in the identification of mitosis genes in *Drosophila*, and Belgian DeVGen (Gent) that is focussing on gene function analysis in *C. elegans*. Similar RNAi services for drug discovery are offered by the US-based companies Sequitur (Natick) and Intradigm (Rockville) which have already entered into several strategic industry partnerships with big US pharmaceutical and biotechnology corporations – amongst which are Merck, Pfizer, GlaxoSmithKline, Amgen and Genentech.

However, since Tuschl's discovery the profits are made in a very different market segment: the one of siRNA tools and applications. Looking at the recent research publications on siRNAs, which have seen a six-fold increase in 2002 (from 24 in 2001 to 160 in 2002), it becomes clear that the \$38 million forecasted by Front Line Consulting for 2003 can only be a rough estimate for the market development to be expected during the current year. A recent survey of some 800 top scientist that was published by US Bioinformatics LLC at the end of February showed that 80% of the surveyed intend to substantially increase the scope of their siRNA work during the upcoming years. According to Front Line's figures laboratory supply companies achieve the greatest revenues in the US (45%), in Europe (40%) and in Japan (12%) predominantly in the three market segments: 'synthetic siRNAs', 'siRNA kits and vectors' and 'drug discovery through RNAi and RNAi-active compounds'.

Front Line's study also shows that the biggest share of these sales (71%) can be attributed to suppliers of synthetic siRNA oligo-nucleotides and the appropriate kits that allow the *in vitro*-creation of siRNAs against all sorts of genes that could be introduced into the relevant cells subsequently. Currently, the key players in the market of synthetically created siRNAs and kits are the US companies Dharmacon Research – which is linked to German MWG Biotech AG (Ebersberg) since last year through a distribution agreement – and Ambion Inc. (Austin). Ambion currently teamed up with

Cenix to build up a bank of validated siRNAs targeting the whole human genome, "which is a market of several 10 million US-\$", according to a Cenix spokesman.

"At first it was very difficult to find suppliers that were specialized in RNAi", explains Jörg Wadzack, PhD, of the German Human Genome Project. However, the market quickly responded to the increased demand by scientists. "Within a single year the sales of RNA oligo-nucleotides jumped from 0 to \$15 million", estimates Eric Ladder of Dutch Qiagen NV (Venlo). Following Qiagen's acquisition of US Xeragon (Germantown) the company was able to address the lucrative market of synthetic siRNAs. Oligo-nucleotide manufacturers like Prologo and Eurogentec (both based at Hamburg), and kit manufacturers like New England Biolabs, Stratagene (La Jolla), Cruachem (Glasgow), Orbigen Inc. (San Diego) and ChemGenes (Ashland, USA) have followed suit. Until the year 2008 sales are prospected to rise. However, according to Front Line the market share is bound to fall to 50%.

The reason for this is the relatively young field of so-called 'vector systems'. These are viral and DNA transport vehicles for the siRNA sequences, that are only produced after transforming the living cells with the sequences. Behind a relevant promoter the vectors carry the DNA sequence of the siRNA's sense and anti-sense strand that are cut *in vivo* by the cell's own DICER complex to exactly the right length. By means of these vectors that are produced by an ever growing number of companies genes can be silenced much longer than with their counterparts that have been synthesized outside of the cell. A brand-new method allows the silencing of several genes by transforming the cell with a single vector. Besides, vectors are much cheaper to produce than synthetic oligo-nucleotides. Consequently, the market share of vectors is expected to rise from the current 18% to some 29% in 2008.

Looking at the number of competitors the US companies are clearly dominating the scene: Ambion holds a license agreement with the RNAi developers Mello and Fire and offers respective vectors. Californian Imgenex Corp. (San Diego) is able to offer adenovirus and DNA vectors. Similar systems are available from the US companies Invitrogen, New England Biolabs and Oligo-Engine. Still, the only company that claims to have succeeded in expressing siRNA in so-called primary cells, like haematopoietic or nerve cells, by means of a DNA vector is Cologne-based amaxa GmbH having applied its proprietary 'Nucleofector Technology'. According to experts DNA vectors are also more suitable to be used in future therapeutic applications.

Since market introduction for these applications is still such a long way off the siRNA market share in this segment is the smallest with only 11%. However according to Front Line, because of their high potentials in target validation the market shares of siRNA technologies is going to rise to 21% over the next five

years. Still, before siRNAs could be looked-at in terms of active compounds, several problems like potential side effects and tdrug delivery of these highly potent molecules need to be addressed.

'But to get there, we still have to go a very long way – at least for another ten years' explains US Nobel Prize laureate and Alnylum co-founder Philip Sharp. But beyond the proof of efficacy of novel siRNA therapeutics and the hurdles that every new class of compounds have to tackle there grows the potential that RNA-Interference is aiding the real breakthrough for gene function analysis.

## Proteomics and Protein Expression

*During the current year laboratory suppliers and biotechnology companies will generate revenues around \$1.52 billion with proteomics research instrumentation and services, according to a market survey recently published by US-based market analysts Front Line Strategic Management Consulting. Sales of the proteomics sector will rise up to around \$2.68 billion until 2008 the study says. Another report by US corporate consultants Frost & Sullivan is more optimistic: It forecasts a volume of \$5.6 billion for the world market to reach already in 2006.*

The reason for the technology surge lies in the ever growing demand of the research and drug discovery market for new instrumentation that enable proteome analyses. Researchers and the pharmaceutical industry, as well as the diagnostics companies are fostering high hopes in proteome techniques. These are expected to cause a new thrust in efficiency in finding new drug targets, diagnostic markers and biological lead compounds. The novelty is: proteome analyses offer for the first time a real prospect to quantitatively detect all proteins expressed by a cell – the so-called proteome. Thereby creating a protein profile that could act almost like a "fingerprint" of the cell's physiological state. A direct comparison of these individual protein profiles from diseased and healthy cells allows the identification of the key proteins that decide whether the body remains healthy or whether it develops a disease – a truly promising market.

It is therefore no surprise that across the world the race is on for the most profitable economic exploitation of proteome research. Recently the British government has approved increases in the spending on proteomics over the next two years by 10% each. German proteome researchers have joined ranks with the Ministry of Research to draft a new research funding strategy in order to secure Germany's proteome research an internationally leading role. Also, a major portion of the EU's FP6 billions will go into proteomics projects and networks. And the US-dominated Human Proteome Organization (HUPO) has already initiated several major international research programmes that are aimed at analyzing and identifying all brain and blood serum proteins.

### Research- and drug discovery-market realise a profit through high priced instrumentation

All proteomics technologies aim to determine the complete protein contents of a cell at a given point in time – the proteome – or that of cell components – a so-called sub-proteome. To fully accomplish this, preferably all of the cell's proteins have to be separated first by electrophoresis or chromatography and after that identified by mass spectrometry. However, the most potent technologies available to date are only capable to separate up to 60% of a cell's proteins. Consequently, there has been an emerging trend over the last three years to concentrate on specific parts of the proteome (fractions, or sub-proteomes) and to standardize separation techniques that are difficult to reproduce and to automate.

"It simply doesn't matter which of the projections for the future market volume will become reality. The market is big enough!" rejoices Dr. Christoph Eckerskorn, CSO of Tecan Munich GmbH. Tecan is one of the companies that opted for the automation of proteome analysis since manual separation of cell proteins has proved to be too time-consuming and too difficult to be reliably reproduced. Alongside automation several techniques that have been developed only a few years ago are already establishing, namely protein chip technologies and methods to elucidate protein-protein interactions. "In the future a whole range of technologies for the separation and analysis of cell proteins will be successfully and permanently established on the market", projects Prof. Dr. Klaus Unger, proteomics expert of the German Society for Proteome Research.

### Demand for new technologies

In this relatively young growth market for instruments that are capable to perform proteome analyses the technology providers are faring most successfully. According to the Front Line report the biggest sales at the moment are generated with techniques for the separation of cell proteins. Sales in this market segment are going to rise from this year's \$780 million (51% market share) to some \$1.19 billion in 2008. Still, despite the success with protein separations this business is by far not an easy one. Instrumentation and technology providers are faced with a huge challenge: to reproducibly separate and quantify proteins with most diverse physical properties – e.g. water soluble and insoluble, acidic or extremely basic, abundant and extremely rare, minute or gigantic – by physical methods. Because most often the changes in the amount of protein that decide about health or disease are minute.

The best separations of proteins are achieved by two-dimensional gel-electrophoresis (2D-GE) that has been developed already in 1975. Of the 20,000 different proteins within a cell that are present in 10 or up to a million copies the method is capable of separating some

60%. In a first step proteins in a gel travel against a pH gradient and are separated based on their net charge. For the second step the gel is turned 90° and subjected to an electric field. Again proteins travel differently and are now separated according to their charge and size. Upon suitable staining of the gel a characteristic protein profile becomes visible that can subsequently be captured by a camera and assessed by sophisticated image processing software. After separation all proteins on the gel will be enzymatically digested and individual spots cut out. By means of a following mass spectrometric analysis of the protein fragments aided by powerful bioinformatics individual proteins are easily identified (peptide mapping). Even though 2D gel-electrophoresis – especially the image processing part – can not be fully automated at the moment, it still offers some very promising results. Pharmaceutical researchers at Bayer AG, Wuppertal, Germany, have recently identified by means of 2D gel-electrophoresis certain protein profiles in bacteria, which have been treated with antibiotics, that allow forecasts for the mode of action of specific antibiotics.

Alongside 2D gel-electrophoresis several liquid chromatographic methods for the separation of proteins are increasingly gaining importance. However, their separation capacity is currently limited to around 6,000 proteins. Though methods like multi-dimensional liquid chromatography can be fully automated and coupled directly to a mass spectrometers that identifies the proteins. "Given a tandem set-up of cationic and reversed-phase separation the potential separation capacity of this method can easily be pushed up to 10,000 proteins in the future", explains Unger, who sees the future in chips that are equally capable of capacities like the space-filling and cost-intensive robot lines. Providers like Agilent Technologies or CIPHERGEN have miniaturized separation methods like capillary electrophoresis or chromatography on so-called microfluidic chips.

Accordingly, companies like CIPHERGEN (projected market share 2003: 1–4%; 2008: 2–6%) that focus on chip-based separations of protein fractions and binding of proteins to pre-activated surfaces are growing. In due course these companies will gain some of the market share currently held by the market leaders like Applied Biosystems (market share 2003: 20–24%; 2008: 19–23%), Waters (2003: 10–14%; 2008: 9–13%), Amersham Bioscience (2003: 10–14%; 8–12%), Agilent (2003/2008: 8–12%), Bio-Rad/Micromass (2003/2008: 7–12%), and Thermo Lifescience (2003/2008: 6–10%). Many of the established providers try to counter the growing competition by creating joint-ventures or leaner sales and marketing structures through co-operations, like Bio-Rad with Micromass, or Amersham that has acquired certain Thermo business units. According to Front Line service providers are also cashing in the relatively high procurement costs for proteomics

systems: based on Front Line's forecasts their market share is to rise from currently 5% (\$70 million) to around 7% (\$180 million) until 2008.

For the market segment of protein characterization Front Line envisages growth from presently \$600 million (39% market share) to some \$1.17 billion in 2008 (44%). This segment is host to the currently booming mass spectrometric methods for protein identification (MALDI-TOF, ES) and protein interaction analysis (ESI-TOF). It also encompasses other techniques for protein interaction elucidation (Yeast-Two-Hybrid) and so-called protein and antibody chips.

Analysts of US corporate consultants Frost & Sullivan are placing their hopes on the relatively young protein chip technology, which is mostly implemented by young and innovative biotechnology companies. The market experts envisage for arrays that enable highly parallel and extremely sensitive proteome analyses an annual growth rate of more than 50%, to reach some \$665 million in 2007. Protein array developers usually follow by the route to initially create antibodies against human proteins and to immobilize them on glass or plastic chips (so-called capture arrays).

Alternatively, samples of cell or tissue extracts could be spotted onto the array (reverse arrays). In the next step diagnostically interesting proteins could be identified with fluorescence-labelled antibodies, or marker-free methods (Surface Plasmon Resonance, Biacore AB, or Surface Planar Waveguide technology, Zeptosens AG). According to Dr. Michael Pawlak, of Swiss Zeptosens AG, the sensitivity of these protein chips is "in the zepto-molar range – i.e. at around 600 protein molecules". The exceptional market potential of the chips is in their capacity to carry out multi-parameter tests, quickly, economically and highly-parallel. Reutlingen-based protein array expert Dr. Thomas Joos sees future applications in immune diagnostics, drug screening or the monitoring of therapies.

Around the world the race is also on to establish this alternative alongside the instrumentation- and cost-intensive proteome analysis. However, next to solving the most pressing technological problems major investments are necessary to establish adequate and comprehensive protein and antibody libraries. This in turn will create substantial benefits for the market for protein expression: the set-up of a single collection of all proteins plus respective antibodies that are derived from expression of the 30,000 genes of the human genome is estimated to figure at around 80 million euros.

Since genes usually code for more than one protein and since proteins are commonly modified after synthesis the total number of human proteins could realistically be in the range of 100,000. Consequently, the potential for the resource sector for diagnostics and drug discovery is immense. Already during last summer a German consortium of proteome researchers and industry partners – the European Protein Initiative – has submitted a respective project proposal to receive funding as part of the 6th Research Framework Programme of the European Union.

To create proteins and antibodies for application in protein arrays and other research tools several protein expression techniques are available. The most economical and fastest high throughput method for protein expression/translation is the cell-free protein synthesis in bacteria, as implemented in a system offered by Roche Applied Science, Penzberg, Germany (RTS). This method avoids the protein agglomeration that often accompanies protein expression in live bacteria. However, proteins of bacterial origin lack the "post-translational" modifications that are crucial to their proper function in humans, just like the attachment of specific sugar molecules to the expressed protein.

A workable alternative is offered in the format of cell-free systems on the basis of higher cells: e.g. Promega (Madison) offers an expression system based on a reticulocyte lysate. There are also expression systems in higher cells that are capable of post-translational modifications and that possess the necessary enzymes to piece the different parts of conjugated mammalian proteins together by a process called "intron-splicing".

The companies Dyadic International (Jupiter) and Zymark (Hopkinton) offer a system that is based on the mould *Chryso sporium lucknowense*. Vertex Pharmaceuticals (Cambridge, USA) has succeeded in culturing insect cells as a workable alternative to protein expression in mammalian cells (CHO) which up to now has been the basis for the production of therapeutic proteins.

The example of protein chips – an area still in the stage of basic research – shows that the development of the market for proteome research is far from being finished. The future development however will heavily depend on investments of the pharmaceutical companies that will depend on the expected return of investment from their engagements in proteomic research.