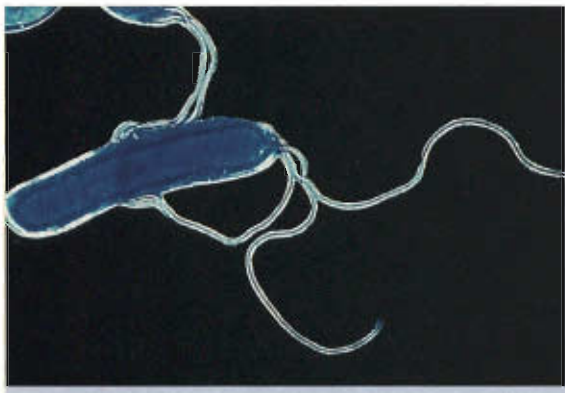

An early prototype for the multidisciplinary, parallel approach to drug discovery and development was the quest for a monotherapy against the stomach bacterium *Helicobacter pylori*. This was a project that was both scientifically innovative and benefited from a recognition of the needs of the market place, drawing on many of the new technologies that are currently revolutionizing drug research.

Following three years of preliminary work, a task force was set up in the summer of 1991 to work on *H. pylori* with the aim of understanding the organism, the environment it lives in, and its genetics. Although they came from different disciplines, the team members focused entirely on finding a therapy to eradicate *H. pylori* in patients with ulcers, and thus prevent recurrence.

Specific advances made by the team included new methods of culturing *H. pylori*, new mouse models of *H. pylori* infection, and a greater understanding of the organism's biochemistry. The bacterium survives in a strange limbo, half way between being aerobic and anaerobic: though it needs oxygen, it dies in air, but it also requires a high concentration of carbon dioxide.

From these and other observations it became clear that there were some fundamental quirks in the biochemistry and metabolism of *H. pylori* which might provide novel targets. The result was perhaps the world's first application of pathogen genome sequencing for target identification. This was initiated in January 1992 in collaboration with the molecular genetics department. Gene sequencing was used to pick out the 'aerobic' and 'anaerobic' genes, and in less than six months all the information needed to understand electron transport in *H. pylori* came together.

An early benefit of the team's expertise was the ability to support the preclinical research needed to develop ranitidine bismuth citrate, which has been internationally launched for use with an antibiotic to eradicate *H. pylori*. But the *H. pylori* project was also one of the first in Glaxo Wellcome to take advantage of technological advances such as combinatorial chemistry, high-throughput screening, gene sequencing and bioinformatics.

Over four years, more than 500,000 entities were screened against *H. pylori*. Many compounds that were highly efficient *in vitro* did not work *in vivo*, and penetration of a drug through mucus, bioavailability, emergence of resistance and the ability of an agent to act on dormant or coccoid forms have also proved extremely important in the search for a monotherapy that will completely eradicate *H. pylori*.

Robotic screening

Three years ago, high-throughput screening was largely a manual process. Today, researchers can test over 30 times as many samples for basic biological activity, largely due to investment in robotics and miniaturization.

Screening large numbers of chemicals obtained from natural products and in-house catalogues of previously synthesized compounds has long been a mainstay of drug discovery. When no chemical starting point presented itself from experience or the literature, a screen was the natural route to leads for an eventual medicinal chemistry programme. The numbers involved grew steadily, so that it was not unusual for a target such as an enzyme or a receptor to be bombarded by up to 90,000 randomly chosen compounds a year.

At the beginning of the 1990s, when the term "high-throughput screening" was coined, a department of 20 would typically be able to screen around 1.5 million samples in a year, each researcher handling around 75,000 samples. Today, four researchers using Glaxo Wellcome's fully automated robotic technology can screen 50,000 samples a day, or around 2.5 million samples each a year.

At Glaxo Wellcome, high-throughput screening is largely used to test compounds in primary activity screens, identifying lead compounds for further biological testing and chemical optimization. Typically, 190,000 compounds will be tested during this phase. Using the robotic high-throughput screening system, this now takes only a few weeks, compared with the two years that sometimes elapsed just three years ago.

Some of these gains have come from miniaturization; the new robot at Stevenage, UK, will use 384-well microtitre plates, replacing the standard 96-well plate and quadrupling the number of samples that can be tested at once. As well as enhancing throughput, miniaturization lowers the cost of screening, as the cost of running assays depends substantially on sample volumes. With current high-throughput technology, a single assay can cost up to fifty pence; this becomes a significant cost when screening several hundred thousand samples. Further miniaturization is possible, and 864-well formats are currently being evaluated together with other higher density sample plates.

Successful high-throughput screening relies heavily on the strategy used for combinatorial chemistry. In an ideal world, biologists would prefer that each assay contained only one compound, as this simplifies identification of hits, but the huge number of compounds produced by combinatorial chemistry makes pooled compounds inevitable. In the past, each pool might have contained several hundred compounds; today, pools of about 40 are normal, but a clear strategy for separating the individual compounds in any active well is nevertheless needed from the outset. Members of the active pool can then be retested in the screen, to identify the active compound itself.

To ensure that the libraries produced can be efficiently screened and deconvoluted, Glaxo Wellcome has integrated the biology and chemistry teams working at the Medicines Research Centre in Stevenage. Now, combinatorial chemists work side by side with the biologists developing assays for drug activity, so that the chemists understand how the screens work and can devise libraries of compounds designed to allow easy identification of active entities. □