microbial library belonging to Bristol-Myers Squibb.

The new technology is possible, according to Dr Michael Dickman, President of ChromaXome, because of the development of very large vectors for transferring foreign DNA into bacteria. ChromaXome uses standard cosmids to transfer up to 30 to 40 kb of DNA and newly developed bacterial artificial chromosomes to transfer up to 300 to 400 kb of DNA. Assuming that, on average, an enzyme can be encoded in 1 kb of DNA, the genetic information for 30 to 400 enzymes can be transferred at one time into the commercial vector.

In many combinatorial experiments, the DNA from up to 100 different organisms is combined and then randomly transferred into the commercial microbe. To ensure that 90% of the total DNA is represented in the library requires the production of 100–200 million distinct clones. Approximately 1% of the clones produce compounds through pathways transferred from the marine organisms. Screening such a large number of clones is a major challenge, which ChromoXome has met with novel screening assays that use fluorescence-activated cell sorting.

ChromaXome is seeking to establish agreements with several other companies who have expressed an interest in the technology. According to Dickman, "ChromaXome intends to remain small, seek collaborative relationships with major pharmaceutical companies and focus on what we do best – the use of our novel technologies to discover new drug leads. We do not intend to get involved in drug development".

Robert W. Wallace

New Alzheimer's model from Japan

Efforts to discover the underlying mechanism in Alzheimer's disease have been hampered by the lack of a suitable animal model. Attempts have been made to mimic Alzheimer's disease by producing brain lesions surgically or chemically, feeding animals on a choline-deficient diet and using aged animals. However, only humans and chimpanzees are known to suffer from the condition naturally, and the models have achieved very limited success. Now a group led by Professor Toshitaka Nabeshima of the Department of Neuropsychopharmacology at the Nagoya University School of Medicine, Japan, have produced a new model, which appears to be more effective and to mimic the real condition more accurately. They have induced memory impairment and neural dysfunction in rats by infusing β-amyloid protein, the core of the plaques that are so characteristic of the disease, into the cerebral ventricles. The protein is administered by mini-osmotic pump continuously for a period of 2 weeks at doses of 3, 30 and 300 pmol per day.

The performance of the treated animals in standard behavioural tests was impaired, and there was an associated reduction in choline acetyltransferase activity in the frontal cortex and hippocampus. Oral administration of agents known to be potent *in vitro* stimulators of nerve growth factor (NGF) synthesis, such as propentofylline, idebenone and trimethylquinone, produced significant improvements in the behavioural deficits, increased levels of NGF protein and mRNA, and stimulated choline acetyltransferase activity.

The results support the hypothesis that β-amyloid protein deposition in the brain is linked to impaired learning and cholinergic neuronal deterioration. The model should therefore be suitable for rapid screening of the many novel agents in development as potential treatments for this disease.

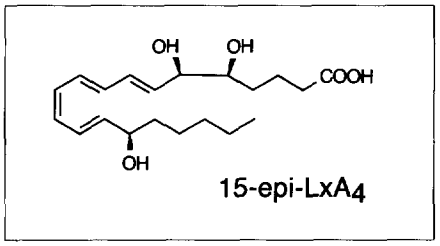
David B. Jack

Better use aspirin

Researchers in the USA have discovered a new mechanism that could help explain some of the pharmacological properties of aspirin (acetylsalicylic acid) that have so far been poorly understood. This may ultimately lead to improved treatment for inflammation and arthritis based on aspirin but without its side-effects, notably gastrointestinal inflammation.

Dr Joan Clària and Professor Charles Serhan at the Department of Medicine, Brigham and Women's Hospital and Harvard Medical School (Boston, MA, USA) have found that aspirin triggers the release of a new group of eicosanoids by a previously unknown biosynthetic pathway [Clària, J. and Serhan, C.N. (1995) *Proc. Natl Acad. Sci. USA* 92, 9475–9479].

Other researchers have demonstrated that the biosynthesis of eicosanoids is influenced by transcellular and cell–cell interactions. Such interactions can amplify and control the release of new mediators in the inflammatory response.



15-epi-LxA$_4$

"We are interested in elucidating the natural 'stop signals' of the inflammatory response – namely chemical mediators that naturally downregulate leukocyte functions" says Serhan. The team were looking at the effects of aspirin on the interaction between white blood cells and blood vessel endothelial cells. They found that they could isolate four members of a new group of compounds belonging to a lipoxin sub-class from their culture. Further biosynthetic studies with human leukocyte and endothelial cells in culture revealed that the production of these lipoxin subtypes resulted from the

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interaction of cyclo-oxygenase (type 2), known to be involved in prostaglandin production, and 5-lipoxygenase, an enzyme that is involved in leukotriene biosynthesis.

According to Serhan, aspirin acetylates the 5-lipoxygenase in endothelial cells, allowing it to generate an intermediate, which is picked up by leukocytes and is rapidly converted to the new 15-epilipoxins. "This is an entirely new route, showing how aspirin can 'pirate' endogeneous biosynthetic mechanisms to trigger new mediators", says Serhan. He explains further, "In the absence of aspirin, the traditional pathway is in place; here aspirin 'pirates' by changing the enzyme's activity to generate new compounds by transcellular biosynthesis". Hence, aspirin enables the production of mediators with potential beneficial cellular actions. The finding that some of these compounds belong to the family of eicosanoids was serendipitous, according to Serhan, and the pathway that gives rise to them is totally novel.

This new interaction may contribute to the therapeutic effects of the drug and could provide a novel target for the development of nonsteroidal anti-inflammatory drugs without side-effects. "We have already synthesized analogues of these compounds that are bioactive and in some cases more potent" adds Serhan. These analogues should now serve as lead structures for drug design.

David Bradley

Modified transcriptional activators as therapeutic tools

The second annual meeting on *Drug Discovery for the Modulation of Signal Transduction and Gene Transcription*, organized by the Cambridge Healthtech Institute, was held in October in San Diego. The meeting highlighted some recent advances in the development of modified transcriptional regulators as potential therapeutic agents for selective regulation of gene expression. There is a clear need for an exquisitely specific signal transducer, or genetic switch, to control the expression of therapeutic transgenes[1]. Such a switch would respond to an otherwise physiologically inactive signal, and, when activated, would modulate the expression of a single or defined set of genes.

Three groups presented results of studies on the transcriptional activation properties of modified steroid receptors. Steroid receptors are ideally suited in many respects as prototypes for engineered signal transducers that would control the expression of transgenes. Unlike membrane receptors, where signals are transduced to components of the cell membrane and the cytoplasm, as well as the nucleus, steroid receptors are transcriptional regulators that function directly in the nucleus to modulate the transcription of a specific set of target genes[2,3].

Steroid receptors are composed of a series of conserved domains. The DNA-binding domain recognizes specific palindromic DNA sequences, known as hormone response elements, found in the vicinity of target genes. Hormone binding is controlled by a separate functional domain. Many *in vitro* studies have suggested that steroid receptors may be cytoplasmic in the absence of hormone, bound to the 90 kDa heat shock protein, HSP90. The hormone-bound receptor is dissociated from HSP90, is nuclear, and is competent for transcriptional activation. Past results have shown that steroid receptors can efficiently activate the transcription in a strictly hormone-dependent manner of transgenes whose regulatory sequences are composed solely of hormone response elements[4], indicating that they do not require other signal transduction pathways for function.

Ideally, both the DNA-binding and hormone-binding specificities of receptors would have to be engineered to generate an exquisitely specific signal transducer. Data presented in San Diego provide advances towards both of these goals. Two groups used similar approaches to generate steroid receptors with altered DNA-binding specificities. Dr V. Allgood and coworkers (GeneMedicine Inc., Woodlands, TX, USA) replaced the DNA-binding domain or the progesterone receptor with that of the yeast transcriptional activator GAL4. A McGill University group has generated a similar chimera of the estrogen receptor. GAL4 recognizes a specific DNA sequence that is distinct from those recognized by mammalian transcriptional activators.

Using gene transfer experiments in tissue culture cells, the GAL4-receptor chimeras were shown to activate transcription in a hormone-dependent manner of model transgenes whose regulatory sequences contained multiple binding sites recognized by GAL4. The GeneMedicine group went further and showed that the GAL4-progesterone receptor chimera could function *in vivo* after administration of 5 μg/kg of activator. One striking aspect of the GAL-estrogen receptor chimeras is that they failed to interact with HSP90, suggesting that the estrogen receptor–HSP90 interactions may not be important for controlling receptor activity *in vivo*. The above experiments demonstrated that modified steroid receptors could be generated that function through recognition of DNA sequences distinct from those bound by all other classes of human transcriptional activators.

The GeneMedicine group and Dr J. Carlstedt-Duke and coworkers (Karolinska Institute, Stockholm, Sweden) presented studies of steroid receptors with altered ligand-binding specificities. The GeneMedicine group exploited the observation that a modified progesterone receptor lacking the C-terminal 42 amino acids of the protein can be strongly activated by compounds that are antagonists of the wild-type receptor[5]. Carlstedt-Duke and coworkers used affinity labelling techniques to identify key residues in the glucocorticoid and progesterone receptor–ligand binding domain. Based

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