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## Integrative Biology

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Most of the work in JCSMR is laboratory-based and can be classified in one or other of the various broad topics used in the earlier part of Part II. Some of the research such as population genetics and arbovirus research has been done in the field and laboratory, and is included in the sections on Human Genetics and Microbiology respectively. Two of our essays are better characterized as 'integrative biology', the first entirely by field observations and the second in the laboratory and Animal House.

### Urban Biology: the Human Ecology of Hong Kong

*by Stephen Boyden*

This was a new program of work focusing on the impacts of civilization on the biology and ecology of humankind throughout human history, but with special emphasis on the modern world. It was carried out between 1968 and 1973 by Stephen Boyden (see p. 73), with the assistance of PhD students Sheila Millar and Ken Newcombe and Research Assistants Rosemary Brissenden, Brazey de Zalduondo, Jeremy Evans, Sheila Faulkner and Beverley O'Neill.

The Program was based on appreciation of the fact that all human situations involve continual interplay between cultural and biophysical aspects of reality, and on the view that a better understanding of this interplay is a prerequisite for wise decision making at all levels in society – from the individual and family to society as a whole. Early on, considerable effort was put into the development of an inter-relational conceptual framework to facilitate thinking and communicating about this interplay.

The Program, while recognizing the cru-

cial roles of cultural, economic and social factors in determining the characteristics of human situations, took the life sciences as its intellectual starting point. This conceptual approach simply reflects the reality that the processes of life permeate, underpin and make possible the whole system and everything that happens within it. Without them, no human situation would exist. If they go wrong – then the whole system goes wrong. Keeping them healthy must, in the long run, be our first priority, because everything else depends on them. Besides the main topic of this essay, Boyden launched two other important initiatives in this field: in 1968, an International Symposium at the Australian Academy of Science on 'The Impact of Civilization on the Biology of Man', and in 1972 an experimental undergraduate program in the School of General Studies on 'Human Sciences', based on his conceptual approach. He was made responsible for the first three years of its existence, most of it after he had left JCSMR. The Human Sci-

ences Program survived for some 25 years and, although no longer going by that name, its main components are still in existence.

In 1972 the Urban Biology Group initiated a program of research on the human ecology of Hong Kong. This project provided an opportunity to study a real human situation in terms of the inter-relational conceptual framework developed by the Group. This research involved collaboration with the Social Research Centre at the Chinese University of Hong Kong, various departments of the University of Hong Kong, the Government of Hong Kong and CSIRO in Australia. The design of the Hong Kong study reflected the conceptual framework of conventional ecology, in that it consisted of two parts, one examining the urban system in terms of *autoecology* – that is, the ecology of a certain species within the system (in this case *Homo sapiens*) – the other examining it from the standpoint of *synecology* – that is, the ecology at the level of the system as a whole.

The autecology part of the study focussed on the changing conditions of life of people in different parts of Hong Kong (e.g., population density in homes, commuting patterns, air quality, family experience, noise levels) and their changing patterns of health and disease (physical and mental). The final Report of the study included, for

example, information on the quality of air that people breathed, the noise levels that they experienced, their diet, and the physical characteristics of the dwellings in which they lived, as well as discussion about the implications for their health of changes that were taking place in their environment and in their life styles. One of the outcomes of this research was a greater appreciation of the enormous importance in people's life conditions of experiences of a kind that promote a sense of enjoyment – 'meliors' – which help to protect people from the undesirable consequences of environmental stressors (of which there are plenty in Hong Kong). It was interesting that people living in squatter areas were better off, in terms of measures of psychological well-being, than people of the same socio-economic group who were living in new blocks of flats.

The synecology component described changing patterns of flow of energy, nutrients and water in the system, as well as the overall population dynamics, the built environment and the transport system. This work included the first analysis of the 'metabolism' of an urban ecosystem. The Hong Kong Human Ecology Program gave rise to numerous scientific publications. It was the first study of its kind, although ecological studies of urban settlements have become common in recent years.

#### *Further Reading*

- Boyden, S. (ed.) (1970). *The Impact of Civilization on the Biology of Man*. ANU Press, Canberra.
- Boyden, S. (1979). *An Integrative Ecological Approach to the Study of Human Settlements*. MAB Technical Note Number 12. UNESCO, Paris.
- Boyden, S., Millar, S., Newcombe, K. and O'Neill, B. (1981). *The Ecology of a City and its People: the Case of Hong Kong*. ANU Press, Canberra.

## The Gene Targeting Facility.

*Klaus Matthaei*

A major aim of modern biology is to understand how normal gene activities give rise to the structure and behaviour of complex organisms. In particular it is important to study the function of genes and their derangements in human diseases. In most cases, it is impossible to achieve these studies directly in humans. It is easier therefore, to carry out such studies in a more manipulable system such as the mouse. Natural mutations occur in a serendipitous manner, i.e., by chance. To find a mutation that mimics a particular human disease is therefore difficult. However, it is possible to manipulate the germline of mice by introducing new genes (transgenic mice) or by deleting an existing gene (knockout mice). This is the major function of the Gene Targeting Facility founded in 1991 and was the vision of Ian Young (see p. 136) who initiated and found funding for the Facility. It is part of the Centre for Molecular Structure and Function (CMSF); a cross-campus initiative involving JCSMR, the Research School of Biological Sciences, the Research School of Chemistry and the Faculty of Science, and is located in the Division of Biochemistry & Molecular Biology, JCSMR.

Transgenic mice are made by the injection of a DNA construct into the male pronucleus of single cell mouse embryos (approximately 12 hours after fertilisation) which are then returned to the uterus of a pseudopregnant foster mother. The DNA becomes integrated into the genome of the embryo and live transgenic mice are born (called 'founder' mice). The gene can then be passed on from the founder to their offspring creating a transgenic mouse strain. This results in the expression (usually over-expression) of a new gene in the transgenic animals. The DNA construct can have a generalised promoter so that almost all cells of the mouse express the introduced gene, or a tissue-specific promoter so that it is

only expressed in specific tissues, say liver or muscle. The function of a gene *in vivo* can therefore be studied. For example, the first transgenic mice were made in 1980 and they expressed the growth hormone gene so that the transgenic mice grew 2 to 3 times larger than their non-transgenic littermates.

The first transgenic mice produced in the JCSMR were in response to a request from Donna Cohen in 1993. We generated two strains of transgenic mice, which over-expressed an anti-sense mRNA to Fra-2 (a transcription factor). One was driven by a testis-specific promoter and the other by a general promoter. Both constructs proved too successful, since the over-expression rendered both strains sterile and they could not breed, hence the strains were lost. In hindsight this was an obvious result but these experiments, although unsuccessful in generating mice that were useful for further work, proved that approaches using anti-sense constructs to delete a gene function *in vivo* could also be used.

Since then we have generated a series of transgenic mice too numerous to list here; however some warrant a special mention, notably systems with better, less destructive methods of detection. Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* emits bright green light after excitation by UV light in the absence of additional co-factors or substrates. This allows GFP to be rapidly and easily detected in living cells without fixation or disruption. Appropriately cloned genes or promoters can therefore be used to track almost any gene or cell type *in vivo*. For example, we have expressed the enhanced form of GFP (EGFP) under a general promoter and have generated mice which express this gene product in almost all cells of the mouse (even the skin of these mice glows green under UV light). These mice will be used to trace specific cell types after adoptive trans-

fer to other mice.

The transgenic technology allows the study of genes which are overactive, or active in the wrong cell type. In contrast Gene Targeting allows the study of what happens when genes do not function. This process involves the use of recombinant DNA technology to modify a cloned gene (usually to stop its function). This can be achieved by the insertion of a drug resistance gene into an exon (a functional unit of the gene that you want to disrupt). At the same time a cultured cell line of embryonic stem (ES) cells is generated from an early mouse embryo (a blastocyst). The ES cells are totipotent and can be used to regenerate live animals (i.e., it is possible to select a single ES cell and produce a whole mouse from that cell, see below). Whilst in tissue culture the modified gene is introduced into the ES cells and the normal gene is replaced by the mutated (functionally inactive) gene. The modified ES cells are then micro-injected into another mouse embryo and the ES cells become integrated. The injected embryos are re-implanted into pseudo-pregnant mice and give rise to live chimæric offspring which consist of the modified injected cells as well as the normal cells. Since the injected cells can also contribute to the testis of these mice, the breeding of a chimæra with a normal mouse gives rise to an animal carrying half of the genes of the modified stem cell including the mutated gene. Interbreeding of the heterozygous (F1) siblings finally yields transgenic animals homozygous for the desired mutation (usually a deletion or a 'gene knockout' mouse). In this way co-isogenic animals can be generated, i.e., animals which are identical to the original mouse strain except

that the function of a single gene has been deleted, thereby allowing the study of the loss of this gene *in vivo*.

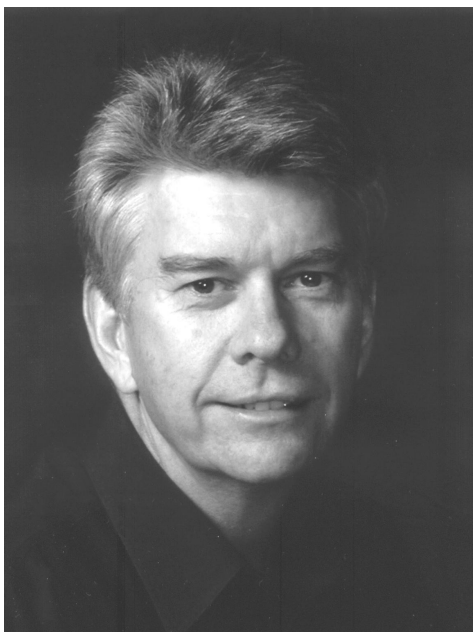
An excellent example of the power of this technology is the interleukin-5 (IL-5) knockout mouse which was our first target (see p. 250). IL-5 is a hormone that specifically regulates the proliferation of eosinophils, a granulated white blood cell, which accumulate in huge numbers around parasite infections in mammals. The eosinophil was hence reputed to be host protective against parasites by attacking and killing them. However, eosinophils and IL-5 are also greatly increased in the human asthmatic lung and could be responsible for the pathogenesis of this disease. Studies with the IL-5 deficient mouse proved not only that IL-5 and eosinophils are indeed closely related to the pathogenesis of allergic lung inflammation but are also host protective against natural parasites.

Gene targeting therefore allows for the first time in a mammal the ability to study the function of a cloned gene in the context of the whole organism by creating mutants defective in that gene. This is particularly important since, with gene targeting and transgenics as shown above, mouse models can be created for studying human genetic diseases and also provides a powerful approach to the development of somatic gene therapy.

In summary, the advent of the Gene Targeting Facility has provided a freely available avenue for a range of investigations to identify, at the molecular level, the critical pathways regulating disease or gene function, *in vivo*.

*Further Reading*

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Klaus Ingo Matthaei (1950–) was born in Marburg, Germany but grew up in the western suburbs of Sydney after the age of nine. He graduated BSc Hons in Biochemistry from the University of New South Wales and gained his PhD in Biochemistry at the ANU. As a postdoctoral fellow at the Roche Institute of Molecular Biology in New Jersey, USA, he worked on mouse embryonal carcinoma cell differentiation (the fore-runners of embryonic stem cells) and early mouse embryology. On returning to Australia he worked with Ken Reed in the Faculty of Science, ANU, on mammalian sex determination using both protein and molecular biological approaches resulting in the world's first licensed use of multiplex PCR for commercial purposes (a PCR-based sex test for pre-implantation cattle embryos). In 1991 he joined the JCSMR to set up a Gene Targeting laboratory and has played an active role in introducing the use of transgenic and gene knockout animals for medical research at the JCSMR.