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New Applications of Gene Transfer Technology

Use of Transgenic Fish Possessing Special Genes as Model Organisms

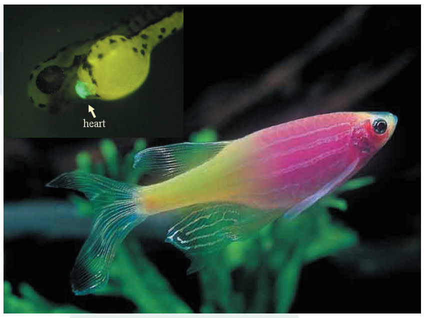
Apart from improving economic fish and shellfish varieties, gene transfer research involving aquatic organisms (such as finfish and shellfish etc.) has also succeeded in using transgenic fish bearing certain special genes of interest as model organisms. Supported by the NSC, the laboratory of Prof. Tsai Huai-jen of the Institute of Molecular & Cellular Biology, National Taiwan University, has developed new strains of zebrafish containing green fluorescent protein (GFP) in the eyes, heart, muscles, or throughout the body, and has used these strains in research on embryonic development, biological toxicity of pollutants or ornamental pet fish. This work has attracted interest in collaboration from many laboratories in Europe and the US.

Small freshwater fish such as the zebrafish (*Danio rerio*) and medaka fish (*Oryzias latipes sinensis*) have become very popular research model over the last few years. These fish are vertebrates but possess many advantages over other model organisms: Their eggs are numerous and relatively large in diameter, their bodies are transparent (allowing direct observation the expression of transferred GFP gene driven by tissue-specific promoter), their ovulations can be controlled by light, they have year-round spawning time, gene transfer is carried out easily, they reach maturity in only 2~3 months, their genome size is only 20 - 40% of that of mammals, and they can be mutated. These features ensure that model fish are widely used for the analysis of vertebrate-specific gene functions and regulations.

According to Prof. Tsai, zebrafish strains possessing special genes are generated by first using molecular biotechnology to clone gene promoters that are highly tissue-specific for the eyes, heart or muscles. GFP gene (originally from jellyfish) serving as a reporter gene is ligated downstream to the promoter. This genetic construct is then transferred into one-celled fertilized zebrafish eggs. After selection and breeding, the germ-line of transgenic fish are generated, in which the expression of transferred gene should mimic the corresponding endogenous gene. Because the transferred gene is reliably transmitted from generation to generation, this special transgenic fish can become valuable material for modern biomedical research and applications.

Transgenic fish are one of the best experimental models for research on the molecular embryology and development. Generally speaking, it is impossible to track the specification and differentiation of specific precursor cells into a tissue or organ in a living mammal unless an individual is sacrificed and tissue sections are made. In contrast, because fish embryos are transparent, it is possible to directly observe and track the development of cells bearing green fluorescent markers in living fish embryos. This ultimately can enable us to understand the organogenesis of the eyes or heart, etc.

Taking the heart as an example: (1) the zebrafish heart consists of one atrium and one ventricle, and can serve as a prototype for mammal hearts with two atria and two ventricles; (2) the zebrafish heart is completely developed within two days after fertilization; (3) early in its development, a zebrafish embryo can survive for roughly a week on dissolved oxygen in the water even when its cardiovascular system is



defective. The latter attribute allows research on many deleterious changes in heart development, which can lead to the discovery of genes controlling the development of the heart. Prof. Tsai's laboratory recently developed the world's first transgenic zebrafish expressing GFP only in its heart, which enables its use in tracking the origin, migration, and regeneration of heart cells. And, because the transparent fish embryos do not need to be sacrificed, it is actually possible to see cells develop into a heart. This is something that is unattainable using conventional research materials. Furthermore, the green fluorescing cells can be isolated and used to discover and determine the function of new genes involved in heart development.

Prof. Tsai said that joint research involving him and Prof. Hsieh Fon-jou, Dr. Ho Yi-lwun and Dr. Jou Tzuu-shuh of the College of Medicine, National Taiwan University, recently generated a transgenic zebrafish in which cardiac troponin C was conditionally knocked down after addition of an inducer. It was found that some of the transgenic fish displayed inconsistent beating of the atrium and ventricle; this type of arrhythmia is very similar to human heart disease caused by incomplete atrio-ventricular block. This was the first time anywhere that arrhythmia could be seen in a living specimen with the naked eye (no heart monitor was needed). This transgenic strain can be used in the development and assessment of heart drugs, and for research on the molecular mechanism of drugs.

In addition, transgenic fish are also excellent experimental materials for determining whether pollutants can influence early embryonic development. Current tests of the effect of environmental pollutants on living organisms generally attempt to determine the lethal dose, survival rate or deformity rate. But while many pollutants may affect the formation of tissues or organs at an early stage of organisms' embryonic development, there were previously no convenient and direct observation methods or materials that could sensitively detect such changes as they occurred. The newly-developed transgenic fish, however, make it relatively easy to observe whether pollutants affect specific important genes during embryonic development.

Finally, Prof. Tsai's laboratory also made large contributions to Taiwan's ornamental fish industry by developing many unique transgenic fish lines. These fish glow brilliantly under dark-blue light because of the fluorescent proteins contained in each body cell, and their beauty makes them a desirable new aquarium pet fish with strong commercial potential and high economic value. This sensational result was reported in *Science* (May 2003), and chosen by *TIME* magazine as one of the "coolest inventions" of 2003. The Animal Planet television channel and the "France 3" television station also interviewed the researchers and broadcasted reports on the fish.

Integration of Computer and Medical Technology

Taiwan's First Virtual Reality Endoscopic Simulator System Is Ready for the Market

Endoscopic examination is an uncomfortable experience for many patients, and, when used carelessly, may cause complication. Every patient who lies on a chilly table tensely awaiting the endoscope's invasion is anxiously hoping that the examination will be safely and skillfully executed.

A research project led by Prof. Chang King-jen MD., Ph.D., of the National Taiwan University Hospital Surgical Department, and involving the National Taiwan University (NTU) Computer Department and the Vivava Technology Co., Ltd. announced Taiwan's first virtual reality endoscopic simulator system on November 8. Prof. Chang thanked the NSC for its integrated project support and also expressed his gratitude to Vivava Technology for its assistance in making this advanced virtual reality system an actual reality. The system is a highly effective means of training physicians, and is expected to greatly increase young doctors' interest in endoscope operation while boosting their skills. When the system goes on the market, which will probably be some time next year, its price will be only one-fifth to one-third that of comparable foreign products. The research team looks forward to seeing many of these systems in use, benefiting both patients and medical education.

Endoscopic examination, including bronchoscopy, panendoscopy, and colonoscopy, is performed several tens of thousands of times every year in Taiwan. Because these invasive procedures involve the insertion of endoscopic instruments inside the patients' bodies, they cause a large amount of discomfort. And if the operator's skill and experience are insufficient, endoscopy can easily endanger the patient. Furthermore, the use of endoscopic equipment may endanger the health of medical personnel when patients suffer from infectious diseases such as SARS or tuberculosis. And the lack of appropriate instructional materials means that the only way many young doctors can gain experience is by practicing on live patients. Finally, patients have no way of knowing whether a doctor has had sufficient endoscopy training.

The virtual reality simulator system developed by Prof. Chang's team includes modalities of bronchoscopy, panendoscopy, and colonoscopy; users can perform virtual endoscopy employing a virtual model of the human body. The system's computer interface allows users to practice on different pathological conditions in different parts of the body. Since the interactive animation provides a very realistic feeling of performing endoscopic examination on a real patient, a doctor can gain endoscopy experience on over a hundred simulated patients in a relatively short period of time – greatly boosting that doctor's practical expertise. Preliminary testing has shown that the system can dramatically enhance young doctors' endoscopy skills and interest in endoscopy. The system thus can multiply the effectiveness of endoscopy instruction, while protecting doctors' safety and easing patients' discomfort. The NTU College of Medicine has arranged for space in which to test the system, and plans to use it on a large scale in the instruction and training of medical students and doctors next year.

Prof. Chang said that virtual reality simulation training systems have long played important roles in military and aviation training. Virtual reality training systems allow trainees to repeatedly practice operating techniques in advance, so that they can become technically proficient before performing in real situations. While this type of endoscopy system has already been in use in Europe and the US for a number of years, they are still very costly and have not yet been used in Taiwan.

The NTU team's system is the first of its kind in Taiwan, and is a product of purely local ingenuity. The team spent three years and NT$20 million developing the system, which has made Taiwan the fourth country to develop a virtual endoscopic examination teaching system (after the US, Europe, and Israel). Since the system's expected price of approximately NT$1~1.2 million is only one-fifth to one-third that of comparable foreign systems, it will be highly competitive on the market. The virtual reality endoscopic examination system will enhance the quality of medical education while spurring the development of Taiwan's medical information industry, and it is therefore a groundbreaking innovation in this age of computerized learning.

Countdown to Success

Taiwan's Sounding Rocket No. Four Successfully Launched to Conduct Scientific Missions

On Dec. 14, 2004 at 22:30, the the National Space Program Office (NSPO) successfully launched Sounding Rocket No. Four (SR-4) from a launch base in southern Taiwan to perform sub-orbital space experiments. This scientific experiment project was managed by the NSPO and employed launch vehicles developed by the Chung-Shan Institute of Science and Technology (CSIST), in conjunction with a photometer payload developed by the National Central University (NCU) and a global positioning system (GPS) receiver produced by CSIST. The rocket soared an altitude of 265 km as expected and successfully transmitted data back to Earth. NSPO Director General Dr. Lance Wu described the event as a "perfect launch" that marked a new milestone in Taiwan's quest for independent capabilities in sounding rocket and scientific experiment payload technologies.

The SR-4 was on a mission to investigate the distribution of airglow along the upper atmosphere over Taiwan, and for the first time carried scientific experiment instruments developed entirely in Taiwan. The photometer payload and GPS receiver aboard the rocket were respectively produced by NCU and CSIST, and the ground-based GPS antenna was developed by National Cheng Kung University (NCKU). According to SR-4 science project leader Prof. Hau Lin-ni of the NCU Institute of Space Science, airglow is divided into dayglow, twilightglow, and nightglow, and is primarily due to the excitation of oxygen and sodium atoms in the atmosphere caused directly or indirectly by the absorption of solar radiation and resulting in the emission of red, green, or yellow light. Scientists had detected the presence of a green background luminosity over high-latitude regions even in the absence of aurora as early as 1868, and this phenomena was later confirmed at the mid and low latitudes.

Prof. Hau pointed out that airglow appears brightest in a 10- to 20-km thick zone at an altitude of 100 km above the Earth, but cannot be detected with the naked eye from the ground because of the atmospheric absorption of light; atmospheric absorption also renders it impossible to determine the precise luminosity of airglow emissions from Earth. Researchers have therefore been sending up sounding rockets with photometers since the 1960s to obtain in-situ measurement of airglow emission varying with altitudes.

Research on airglow can also contribute to the understanding of solar terrestrial interactions as well as long-term changes in the upper atmosphere. World-wide nations have been conducting airglow measurement by sounding rockets for more than four decades, but Taiwan has yet to produce measurement data or papers in this field; in particular, Taiwan is situated in the equatorial anomaly region where intense physical and chemical activities in the upper atmosphere have been found. The photometer payload for this project developed by the NCU team has enabled domestic researchers to measure actual green line emission at a wavelength of 557.7 nm at altitudes of 70 to 300 km above Taiwan. And thanks to the success of this project, Taiwan has now joined the ranks of the world's airglow research community.

According to the NSPO, the SR-4 also carried a real-time GPS receiver developed and produced by CSIST. This receiver is able to transmit and receive GPS signals in a dramatically dynamical environment and works in conjunction with a ground-based differential global positioning system (DGPS) developed by NCKU to track the rocket's trajectory and make necessary adjustments. The GPS system can pinpoint the photometer's exact positions and times of observation, providing essential references for developing multi-function rockets in the future.

Finally, this project was the first sounding rocket scientific experiment

to employ a payload completely developed in Taiwan. The photometer payload's development process from the stages of design, manufacture, to testing was fully and independently performed by researchers at domestic universities. This represents a momentous achievement by Taiwan's academic community in space science.

A New Way to Analyze Biological Particles

New Microbial Whole Cell Mass Spectrometer Developed in Taiwan

In another excellent success for NSC-funded research, Dr. Chang Huan-cheng, research fellow of the Institute of Atomic and Molecular Sciences, Academia Sinica, and Mr. Peng Wen-ping were awarded the "IJMS Best Student Paper Award 2003 – Best Instrumentation MS Paper" for their innovative development of a nanoparticle ion detector. The team recently achieved yet another success in designing the world's first microbial whole cell mass spectrometer able to measure the mass and charge number of single biological particles. This innovation represents a significant breakthrough where scientists up till now have been unable to precisely determine the mass of very large biological molecules, and offers great promise as the basis for a new generation of high-speed bacterial and viral testing methods. In particular, biomedical researchers are optimistic about the potential use of this technique for stem cell extraction.

Measuring the mass of microbial cells is an integral part of basic research in the life sciences. However, conventional methods of measuring microbial mass, such as flow cytometry and gravimetric determination, are laborious and time-consuming, and the newly-developed superconducting quantum interference method for measuring the mass of single bacterial or viral particles often yields a relatively large error of greater than 10%. In contrast, the device developed by Dr. Chang's team has reduced the error to less than 0.1% for bacterial mass.

According to Dr. Chang, he and his students announced their successful development of the nanoparticle ion detector in the article "Laser-Induced Fluorescence/Ion Trap as a Detector for Mass Spectrometric Analysis of Nanoparticles" on pages 67 - 76 of the *International Journal of Mass Spectrometry*, 229 (2003). The team's detector for nanoparticles is composed of three elements: a laser, a photomultiplier tube, and an audio-frequency quadrupole ion trap. The detector's special design enables it to detect charged fluorescent particles of nanometer size (10 - 100 nm). Both particles that are inherently fluorescent and those that can be fluorescently labeled can be caught by the detector and examined by the mass spectrometer. By combining the ion detector with an audio-frequency quadrupole ion trap developed in-house, the team successfully performed the mass spectrometry of fluorescent polystyrene spheres of 27 nm and 110 nm in diameter. This work proves that the mass spectra of nanoparticles with the mass-to-charge ratio of $m/z > 10^6$ can be readily attained.

The team has applied its newly-developed microbial whole cell mass spectrometer to measuring the mass of *E. coli*, and obtained an average weight of approximately 8.23×10^{-14} g for 60 dehydrated bacteria. The weight distribution is within ±3%. The instrument first uses a pulsed laser to illuminate a dehydrated sample composed of the biological particles mixed with an organic acid. The organic acid absorbs energy from the laser and causes tiny explosions. Like a rocket lifting off, these explosions help bring the ionized biological particles into space, where an ion trap consisting of an alternating electric field captures the particles so that their mass can be measured. The team's next goal is to develop a mass spectrometer able to measure the mass of a single virus and use it to detect the SARS virus and other potentially dangerous viruses.

After the announcement (*Journal of the American Chemical Society* 126 (2004), pages 11766-11767) of the new mass spectrometer developed at the Institute of Atomic and Molecular Sciences, scientists at the Academia Sinica's Genomics Research Center expressed strong interest in the instrument. These scientists hope to use the instrument to solve one of the most challenging problems in current stem cells research, which is how to separate stem cells from an assortment of embryonic cells that are all more-or-less similar in appearance.

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