White paper from the International Zebrafish research community

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Biologists first adopted the zebrafish during the late 1970s. The aim was to provide a a relatively inexpensive vertebrate model system that would permit forward genetic analysis of complex biological processes. Subsequently, this small tropical fish has grown into an internationally popular vertebrate model for answering developmental, biological and health-related questions. This model features a wealth of complementary genetic methodologies, including transgenesis, specific gene knock-downs, thousands of mutants and an almost completed genome sequence.

The experimental features of zebrafish can be exploited to the great benefit of European R&D in a number of biomedical research arenas. However surprisingly, awareness of its potential is still not sufficient in the political, industrial and medical communities. This is sadly reflected in the latest calls for proposals both at the national and at European levels.
At the recent 2nd Strategic Conference held in Asilomar, USA, (Feb. 2007) zebrafish researchers pinpointed the following topics as being ideally suited for study using zebrafish:

Disease models
The ZF-MODELS IP currently funded by the EC has laid the groundwork for the study of zebrafish as disease models in many fields. A continuation of this successful work, in particular the systematic phenotyping of mutant and transgenic fish, is necessary at the level of an IP to strengthen the foundations of European zebrafish research. However, many projects with great potential for biomedical research cannot be covered in-depth by a single IP. So increasingly, zebrafish researchers will aim to join interdisciplinary consortia addressing particular human diseases in order to provide complementary input.

Cancer research
Transgenic models of cancer and genetic screens for cancer phenotypes are adding new insights into cancer. Cancer studies will benefit from a better-established pathology infrastructure that is integrated with cancer pathology of other model organisms. There remain many opportunities for additional genetic and chemical screens relating to cancer.

Regeneration and stem cells.
In contrast to mammals, fish can regenerate many organs including the heart optic nerve, retina, kidney, appendages, and pancreas. The regulatory mechanisms that maintain stem cells and permit the plasticity of remodeling adult tissues are not yet known. Understanding regeneration in zebrafish could provide concepts and

molecules for potential use in human therapy. A concerted effort (IP scale) is necessary to foster and integrate European expertise with the goal of developing the zebrafish for systematic regenerative studies and to elucidate the underlying mechanisms. In parallel, contributions from individual zebrafish groups should be invited to calls on regeneration of specific organ systems in mammals.

Neurobiology
Zebrafish embryos already show basic aspects of behavior during embryonic and larval stages (e.g. startle, optomotor response, circadian activity rhythms, avoidance and attraction). This together with the transparency and small size of the animals provides a unique opportunity to study the neurophysiology of simple behavior. More recently, zebrafish have attracted the interest of sleep researchers - another important biomedical area of the future. A key aspect of zebrafish neurobiology is the similarity of neurotransmitter and receptor systems with those of mammals. Thus, zebrafish are eminently suitable for high-throughput behavioral and pharmacological testing of candidate drugs.

Pain, tolerance and dependence
The zebrafish has been used as a model to analyze the effects of various drugs such as alcohol or cocaine with similar results to those observed in mammals. Behavioural studies have revealed that fishes also have pain perception and respond to painful stimuli with patterns of behaviour similar to those encountered in mammals. The zebrafish has a great potential for studying the molecular mechanisms that control analgesia and to analyze the biochemical changes responsible for the development of drug induced tolerance and dependence. Furthermore, it can be an invaluable model to design new drugs with analgesic properties but without the secondary effects resulting from chronic exposure.

Aging and degeneration
With the tremendous progress in medicine, the Western population faces increasing health issues created by aging. The vast majority of age-related disorders are caused by degeneration of specific neuronal populations in the brain and spinal cord. In recent years, the zebrafish has become a model organism of choice to study these neurodegenerative diseases. Strikingly, in some cases in which it has proven impossible to model degenerative disease in the mouse, a phenocopy of the disease has been successfully obtained in zebrafish. We now need to advance our level of analysis, requiring the collaborative effort of experts in a range of disciplines from biochemistry to neurophysiology. We are also close to the stage of pre-therapeutic studies for identifying compounds able to prevent, cure or control age related disorders. Here again, a consortium is required to focus European expertise on developing the zebrafish for systematic multidisciplinary studies.

Pharmacology and Toxicology
Pharmaceutical companies are increasingly attracted to the zebrafish model. Given the small size of the embryo, high throughput chemical screens can identify molecules with therapeutic potential. In addition, several tens of thousands of chemicals have to be tested under the REACH program of the EC for their toxic potential in the next years, a task generating an enormous ethical and financial burden. Although the zebrafish embryo is a cost-effective and ethically acceptable alternative model for these applications, substantial technology development is still required for its full potential to be realized. Specifically, input from molecular toxicology, toxicogenomics and pharmacokinetics needs to be combined with non-biological disciplines such as high throughput microscopy, liquid handling, 3D rendering etc.

Cell biology, Proteomics, Genomics, and Systems Biology

Due to its transparency the zebrafish embryo allows real time observations at a subcellular resolution to be made in an intact, living vertebrate. Such a capacity for imaging is unmatched by any other vertebrate model system. The integration of lifespan atlas data with the development of appropriate sensor technologies and optical instruments will allow the zebrafish to provide insights into both qualitative and quantitative aspects of biological processes. This is an essential prerequisite for mathematical modeling of developmental and other cellular processes as they operate in an intact organism. This task requires interdisciplinary approaches where biologists, nanotechnologists and physicists will need to interact to develop novel sensor systems and appropriate optical instruments permitting high-resolution and real-time observations.

As a result of its nearly completed genome sequence and well-established technologies for gene expression analysis and proteomics, the zebrafish promises to provide a comprehensive picture of gene regulation networks in vertebrate development. No other vertebrate allows this type of analysis in early development. Priorities in this area will be genome-wide enhancer analysis to be combined with microarray and proteomics data, as well as knock-out by the TILLING method and production of fluorescent transgenes on a genome-wide scale.