Reproductive Toxicology 28 (2009) 245–253

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Zebrafish embryos as models for embryotoxic and teratological effects of chemicals

Lixin Yang[a], Nga Yu Ho[a], Rüdiger Alshut[b], Jessica Legradi[a], Carsten Weiss[a], Markus Reischl[b], Ralf Mikut[b], Urban Liebel[a], Ferenc Müller[a,c], Uwe Strähle[a,*]

[a] Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe in the Helmholtz Association, Karlsruhe Institute of Technology, PO Box 3640, 76021 Karlsruhe, Germany
[b] Institute of Applied Computer Science, Forschungszentrum Karlsruhe in the Helmholtz Association, Karlsruhe Institute of Technology, PO Box 3640, 76021 Karlsruhe, Germany
[c] Institute of Biomedical Research, Department of Medical and Molecular Genetics, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, B15 2TT, Edgbaston, Birmingham, United Kingdom

ARTICLE INFO

Article history:
Received 2 March 2009
Received in revised form 7 April 2009
Accepted 20 April 2009
Available online 3 May 2009

Keywords:
Zebrafish embryo
Toxicology
Toxicity testing

ABSTRACT

The experimental virtues of the zebrafish embryo such as small size, development outside of the mother, cheap maintenance of the adult made the zebrafish an excellent model for phenotypic genetic and more recently also chemical screens. The availability of a genome sequence and several thousand mutants and transgenic lines together with gene arrays and a broad spectrum of techniques to manipulate gene functions add further to the experimental strength of this model. Pioneering studies suggest that chemicals can have in many cases very similar toxicological and teratological effects in zebrafish embryos and humans. In certain areas such as cardiotoxicity, the zebrafish appears to outplay the traditional rodent models of toxicity testing. Several pilot projects used zebrafish embryos to identify new chemical entities with specific biological functions. In combination with the establishment of transgenic sensor lines and the further development of existing and new automated imaging systems, the zebrafish embryos could therefore be used as cost-effective and ethically acceptable animal models for drug screening as well as toxicity testing.

Contents

1. Introduction

We are confronted with a large and steadily growing number of bioactive compounds, including drugs, pesticides, industrial by-products and waste. Low-dose effects and synergisms between this plethora of distinct chemical entities add even further complexity to their toxic potential. Early life stages are particular susceptible to adverse effects of chemicals. But these stages are the most inaccessible in the traditional mammalian models of toxicology.

The assessment of chemicals for potential toxic effect on human health and the environment generates a strong demand for robust and cost-effective assays with high predictive power. The availability of whole genome sequences and powerful methods to analyse the expression of thousands of genes and proteins simultaneously provide novel routes to evaluate toxic effects of chemicals [1]. While these holistic (or omics) methods provide usually a vast set of

* Corresponding author.
E-mail address: uwe.straehle@itg.fzk.de (U. Strähle).

0890-6238/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2009.04.013

descriptive data, the derived knowledge concerning the molecular mechanism remains suggestive at the best [2]. The strength of global approaches depends therefore on whether they can be combined with hypothesis-driven follow-up studies that elucidate the molecular mechanisms of toxicant action [3]. A thorough mechanistic understanding of how chemicals affect organisms will in the future facilitate biomonitoring and prediction of toxicity of novel compounds.

Although studies in cultured cells are a very important and productive avenue to gain understanding of toxic mechanisms, *in vitro* systems are limited by the availability of appropriate cell lines or primary cells and by the *in vitro* culture conditions that do not reflect the natural environment of cells in the body. Whole organism approaches provide the most comprehensive picture of the toxic effect. In particular, if the animal system permits manipulation of gene function, studies of animals will not only be the most sensitive means to discover toxic effects but will also allow unravelling the underlying genetic and cellular mechanisms. The use of mammals is expensive, labour-intensive and they attract increasingly ethical concerns, limiting an application in large scale screening programs. In contrast, embryos and non-feeding larvae of the vertebrate zebrafish (*Danio rerio*) offer a cheap, effective alternative which also represents an advance towards the aim of reducing and refining animal use in research. We will summarise in this review the technical merits of the zebrafish system and discuss the current-state-of-the-art of this experimental model in toxicological studies.

1.1. The zebrafish is a vertebrate model for phenotypic screens

The zebrafish was introduced almost three decades ago as a model to study development and neurobiology [4]. In particular, its small size, the transparency of its embryos and the fact that development occurs entirely outside of the body of the mother made it an attractive system to study developmental processes (Fig. 1A–D). Therefore, developmental geneticists, who had previously employed forward genetics screens in the fruit fly *D. melanogaster* and the nematode *C. elegans* to investigate the gene networks controlling development of these organisms, rapidly introduced zebrafish into their laboratories. Like the invertebrate species, zebrafish allow phenotypic screens to identify gene function on a large scale. However, they offer in addition a vertebrate body plan that is in its basic structure not much different from a mouse or a human [4,5].

The development of the zebrafish embryo is very fast. Details on the developmental stages of the zebrafish can be found in Ref. [4]. Therefore, we only briefly summarise the developmental stages. Gastrulation is completed by aproximately 10 h post-fertilisation (hpf) followed immediately by the segmentation stage where the somites are formed. By 24 hpf, somitogenesis is completed and many organ rudiments have been laid down. Embryos are motile and motility has become touch evoked by 28–30 hpf resulting in the first behaviour, the startle response. By 5 days post-fertilisation (dpf), embryos start feeding suggesting that most organs have reached a functional state by this time.

When the zebrafish was introduced on a larger scale into the laboratories in the late 1980s and early 1990s, only very few molecular tools and techniques to monitor and manipulate gene function were available in comparison to the more traditional vertebrate systems like the mouse, chicken and the frog Xenopus. With the increasing attention that the zebrafish received, this situation changed rapidly. We have nowadays highly efficient protocols to induce mutations by the alkylating agent ethylnitroso urea (ENU) [6–8]. Moreover, mutations can be generated by retroviral insertion [9,10]. Although the efficiency of insertional mutagenesis is lower than that of chemical mutagenesis, it has the advantage that the mutated gene can be identified easily obviating the need for cumbersome positional cloning of the mutated loci. By these genetic screening approaches, several thousand genes have been identified giving completely novel insights into the processes of vertebrate development and at the same time providing animal models covering a wide range of human diseases from cancer, myopathies, neurodegeneration to heart diseases (for recent reviews see [11–13]). These mutagenesis protocols are complemented by reverse genetic techniques that allow manipulation of gene function specifically [14]. Morpholino antisense oligonucleotides can disrupt the function of genes during early development by transiently blocking the translation of the mRNA [15]. Tilling is a technique that permits the isolation of induced point mutations in specific genes by large scale sequencing [16–18]. Recently, several groups reported the use of zinc finger nucleases to knock-out genes in the zebrafish genome [19–22]. The zinc fingers of these nucleases were engineered specifically so that they recognised a unique target DNA sequence in the gene of interest. Incorrect repair of the double strand cut inflicted by the attached nuclease generated an heritable mutation in the targeted genes. These loss-of-function approaches are complemented by gain-of-function techniques ranging from injection of synthetic mRNAs to transgenes to cells [23,24]. Efficient protocols have been developed to introduce transgenes with the help of transposases, leading to single copy integrations and inheritance of transgenes through the germ line at high frequency [25,26].

By now, we have an almost complete genome sequence (http://www.sanger.ac.uk/Projects/D_rerio/) that facilitates mining for genes and regulatory sequences. Not only the coding sequences of many zebrafish genes but frequently also their regulatory sequences are conserved between zebrafish and humans [27,28]. Several arrays with an almost complete representation of the transcriptome are available to carry out expression profiling [29–31] (Table 1).

In recent years, small chemicals were employed in whole embryo exposure experiments to manipulate specific developmental pathways in a conditional manner. These included inhibitors of Fibroblast Growth Factor [32], Sonic Hedgehog [33], Notch/Delta [34] and Retinoic acid [35] pathways to name just a few examples. These inhibitors were developed against the mammalian orthologues of the various signalling components, but have very similar activities in zebrafish embryos. First large scale screens for bioactive molecules using zebrafish embryos were reported, in which the virtues of the zebrafish as phenotypic screening models were exploited to assess the effects of small molecules and importantly to discover new bioactive compounds [36–43]. We can thus expect in the future a much larger repertoire of specific inhibitors for various pathways. These bioactive molecules are particularly useful to probe the function of signalling pathways and other regulatory processes in a conditional manner at later stages of development.

1.2. The zebrafish as a toxicological model

The many virtues of the zebrafish embryo have also attracted the toxicologist. In particular the transparency of the embryo and the development outside of the mother allow scoring of teratological and embryotoxic effects easily (Fig. 1E–H). In Germany, the zebrafish embryo test was introduced as a standardised ISO assay (recent revision ISO 15088:2007: water quality—determination of the acute toxicity of waste water to zebrafish eggs (*D. rerio*)) for water testing in 2005, replacing traditional toxicological tests with adult fish [44,45].

We need a thorough mechanistic understanding of the toxic mechanisms to make eventually predictions of the toxic potential of novel compounds. Given its experimental advantages, the zebrafish embryo is one of the most promising vertebrate systems for mechanistic toxicology [46–48]. With the targeted gene knock-