Interaction of Zebrafish FF1 Nuclear Receptors with SOX9a, WT1 and SHP

Sue-Ing Quek¹, and Woon-Khiong Chan²

Department of Biological Sciences, The National University of Singapore
Blk S2, 14 Science Dr 4, Singapore 117543.

ABSTRACT

Previous work in our lab established that FF1b and FF1c are homologues of mammalian SF-1 and LRH in zebrafish. SF-1 was previously shown to interact with WT1 (Whim’s tumor 1) and SOX9 (SRY HMG box related gene 9) in regulation of the male sex determination pathway whereas LRH was shown to interact specifically with SHP (Small Heterodimer Partner). In this study, results of LexA yeast two-hybrid system in yeast suggest that FF1b and FF1c interact with WT1, SOX9, and SHP. The truncated mutants of FF1b up to AF2 core domain or heptad 9 domain lost their ability to interact with these proteins. Based on these results, we confirm the close relation of zebrafish FF1b and FF1c to mammalian SF-1 and LRH and the importance of Heptad 9 and AF2 domains in the interaction of FF1b with the three tested proteins. The yeast-two hybrid assay done in this project will be moved into a mammalian system for a better understanding of protein-protein interactions profile of zebrafish FF1s in comparison to that of SF-1 and LRH.

INTRODUCTION

The Ftz-F1 (FF1) family of orphan nuclear receptors (NRs) represents a very ancestral class of NRs that are found in both invertebrates and vertebrates. Several fushi tarazu factor 1 (FF1) isolated from zebrafish are established as homologues of mammalian SF-1 and LRH (which are also members of FF1 family). Thus, zebrafish FF1 homologues may exhibit characteristics that are indicative of both LRH and SF-1-like genes. The interaction of human SF-1 with SOX9 (de Santa Barbara et al., 1997), mouse SF-1 with WT-1 (Nachitigal et al., 1998) and human LRH with SHP (Goodwin et al., 2000; Lu et al., 2000) have been extensively studied. In this project, we aim to determine if the same interaction pattern is conserved in zebrafish FF1b and FF1c using yeast-two hybrid assay. We included the FF1b heptad 9 and AF2 truncated mutants to test their influence in the ability of FF1b to interact with the three proteins.

MATERIALS AND METHODS

Yeast-two Hybrid Interaction Assay.

Yeast two-hybrid interaction assays were performed as described in Clontech Yeast Protocol Handbook. Briefly, LexA fused FF1s or deletions and B42 SOX9a, WT1 and SHP, were

¹ Student
² Supervisor
transformed into *Saccharomyces cerevisae* EGY48 strain containing the β-galactosidase reporter plasmid 8op-lacZ, and the transformants were selected on plates with appropriate selection markers.

**RESULTS AND DISCUSSION**

Loss of autonomous activation functions in zebrafish FF1b deletion mutants.

A mild level of autonomous activity (18.3 Miller’s unit) was detected in the liquid culture assay using ONPG as substrate. Deletion mutations managed to knock out this autonomous activation indicating that the two domains may play a role in the autonomous activation function of FF1b.

FF1b interact with SOX9a, WT1 and SHP.

FF1b interacts most strongly with SHP followed by SOX9a, and WT1 in yeast. These interactions are anticipated as FF1b were shown to be close relatives of SF-1 based on sequence homology analysis. SHP was shown to interact strongly with both FF1b in oppose to our expectation. The interaction of FF1b with hSHP should be tested to confirm the cause of this differential interaction.

The heptad 9 and AF2 domains of FF1b are required for interaction with SOX9a, WT1 and SHP.

The FF1b deletion mutants had lost their ability to interact with WT1 completely. In contrast, the FF1b mutants were still able to interact weakly with SOX9a and SHP but the β-galactosidase activity was substantially brought down (> 90% reduction in interaction strength). These results suggest that FF1b has an absolute requirement for both intact heptad 9 and AF2 in its interaction with SOX9a, WT1 and SHP.

Point mutations by site-directed mutagenesis might be a better choice in knocking out the activation function of FF1s.

A very low level of β-galactosidase activity (~20 Miller’s unit) was still retained for interaction of SHP and SOX9a with the deletion mutants indicating that there might be other domain(s) in SHP that is interacting weakly with FF1b. Alternatively, it may due to contamination during experimental procedure or there might be some intermediary factors that contributes to the observed protein-protein interaction between FF1b and SHP. In addition, it may due to the fact that deletion mutations are not as powerful as point mutations (which resulting in amino acids substitutions) to effectively knock out the activation function of FF1b.

FF1c physically interact with SOX9a and WT1 at different strength in yeast in comparison to FF1b.

FF1c interacts more strongly with WT1 (~50% stronger) than FF1b and so as the case of SOX9a (smaller difference). This indicates that the two FF1s might be using different domains or different molecular mechanisms to interact with WT1. This observation underlies the possible differences between the two FF1 isoforms despite their sharing of a high sequence homology to each other.
The observed physical interactions of FF1b and FF1c with SOX9a, WT1, and SHP confirm the close relation of the two FF1 isoforms to mammalian SF-1 and LRH. The other isoforms of FF1s as well as the mammalian SF-1 and LRH should be included in the experiments for a better comparison. Mutants of FF1s generated by site-directed mutagenesis (point mutations) within the same region (heptad 9 and AF2) should be included in the assay as comparison to the wild type FF1s and the FF1s deletion mutants. These mutants have actually been constructed in our lab where the leucine (LL) residues within the heptad 9 and AF2 region were mutated into alanine (AA) residues (using the Quickchange Mutagenesis kit) to disrupt the two helices. Lastly, there is still an undeniably significant difference in the microenvironment of yeast in comparison to that in mammalian cells. Thus, the yeast-two hybrid assay done on the tested NRs and co-regulators need to be moved into a mammalian system so that the assay can be done in a more native microenvironment of proteins for a better understanding of the interaction and coordination among them.

REFERENCES


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