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SignaFish: A Zebrafish-Specific Signaling Pathway Resource

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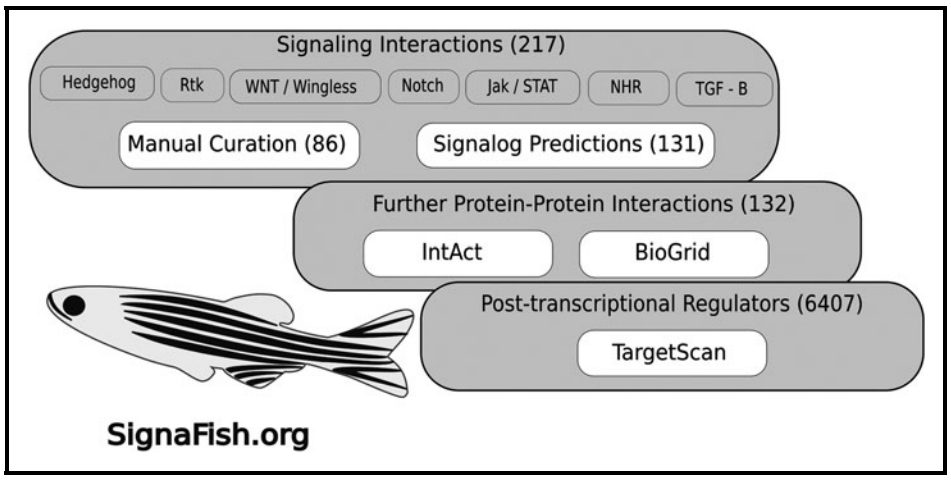

FIG. 1. The structure of SignaFish and its sources. The number of interactions acquired from each source is shown in *parenthesis*. All parts of the SignaFish database can be browsed and downloaded through the website, where users can filter to specific signaling pathways.

Abstract

Understanding living systems requires an in-depth knowledge of the signaling networks that drive cellular homeostasis, regulate intercellular communication, and contribute to cell fates during development. Several resources exist to provide high-throughput data sets or manually curated interaction information from human or invertebrate model organisms. We previously developed SignaLink, a uniformly curated, multi-layered signaling resource containing information for

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human and for the model organisms nematode *Caenorhabditis elegans* and fruit fly *Drosophila melanogaster*. Until now, the use of the SignaLink database for zebrafish pathway analysis was limited. To overcome this limitation, we created SignaFish (http://signafish.org), a fish-specific signaling resource, built using the concept of SignaLink. SignaFish contains more than 200 curation-based signaling interactions, 132 further interactions listed in other resources, and it also lists potential miRNA-based regulatory connections for seven major signaling pathways. From the SignaFish website, users can reach other web resources, such as ZFIN. SignaFish provides signaling or signaling-related interactions that can be examined for each gene or downloaded for each signaling pathway. We believe that the SignaFish resource will serve as a novel navigating point for experimental design and evaluation for the zebrafish community and for researchers focusing on nonmodel fish species, such as cyclids.

Keywords: signaling network, pathway, database, zebrafish, regulation, miRNA

THE BIOLOGY OF LIVING ORGANISMS cannot be interpreted without an in-depth knowledge of the signaling networks that drive cellular homeostasis, regulate intercellular communication, and contribute to establish cell fates, which have an indispensable role both during and after development. Although early research concentrated on deciphering individual signaling pathways, later results demonstrated that these pathways are often unexpectedly interwoven.[1] This particular feature of signaling networks can often contribute to seemingly confusing results upon the analysis of interactions between signaling proteins. Without accounting for the details of an interaction (direction or sign), the interpretation of experimental data will be often erroneous.

A better understanding of signaling network topology allows for a better accounting of the observed phenotypes by recognizing robustness within the examined network or identifying key proteins, which could become potential targets in future treatments or interventions.[2] Hence the need for well-annotated protein–protein interaction (PPI) and signaling databases for biological and biomedical research.

SignaLink (http://signalink.org/),[3,4] a uniformly curated, multi-layered signaling resource, has become over the years a widely used signaling resource for the model organisms nematode *Caenorhabditis elegans* and fruit fly *Drosophila melanogaster*, as well as a gap-filling human database with high coverage. With an easy-to-use interface, customizable download site, and integrated data sets, it had been used in several high-profile studies (e.g., Ref.[5]) and also integrated in model-organism resources, such as FlyBase and WormBase.

However, the use of SignaLink for the zebrafish community was limited. To overcome this limitation, we developed SignaFish (http://signafish.org), a fish-specific signaling resource, built using the concept of SignaLink.

With SignaFish, based on primary literature articles, we have created a manually curated database of seven major signaling pathways, which are biochemically and evolutionary defined, and encompass all major developmental signaling mechanisms[1]: Rtk (receptor tyrosine kinase), Tgf-β (transforming growth factor beta), Wnt/Wingless, Hedgehog, Jak/Stat, Notch, and NHR (nuclear hormone receptor). The signaling interactions in SignaFish were coming from (1) manual curation of zebrafish articles, where we performed review-based literature searches, which identified the primary zebrafish-related article that described the interactions or (2) we used the curated signaling interactions of *C. elegans*, *D. melanogaster*, and *Homo sapiens* from SignaLink2,[4] and based on the interolog and signalog concepts,[6,7] we predicted potential zebrafish signaling interactions. Interologs are interactions detected in one species and predicted to be potentially existing in another species (in this case in zebrafish) as sequence homology shows the presence of the interacting protein pairs in zebrafish. We developed earlier the signalog concept to filter the more relevant interolog predictions by including only those interactions where the interacting proteins in the target species (in this case in zebrafish) have

known signaling pathway-related functions. Accordingly, we included a predicted interaction to the SignaFish database if (1) we found no direct evidence that the given interaction has been verified in zebrafish, (2) the orthologs of potentially interacting proteins were present and interacted in at least one species in SignaLink, and (3) to minimize the false positive interactions, a zebrafish study showed that genetic modification of both potentially interacting proteins produces a phenotype related to the annotated pathway.

SignaFish contains altogether 217 signaling interactions found with manual curation or with high-confident predictions (as previously described). These interactions were extended with PPIs (imported from BioGrid and IntAct databases[8,9]) and potential miRNA–mRNA connections (from TargetScanFish[10]). Altogether, SignaFish contains pathway and interaction information for 389 proteins and 178 miRNAs (Fig. 1). The number of manually curated interactions directly found in zebrafish studies is low (86) compared with the total number of articles available for zebrafish, because we applied a strict curation protocol,[3] which allowed the inclusion of only those studies where the identified interaction was investigated between two zebrafish proteins, and the experiments were carried out in zebrafish. Due to the lack of zebrafish-specific regulatory resources listing transcription factor–target gene connections, we were not able to include transcriptional regulations to this version of SignaFish. The database structure of SignaFish already allows the incorporation of such transcriptional data once a large transcriptional regulation data set becomes available.

To facilitate the exploration of this complex resource, we implemented it to a website with a user-friendly graphical interface, available at http://signafish.org. The website is linked to main web resources including UniProt, ENSEMBL, and ZFIN. SignaFish is the first real zebrafish signaling web resource. Established resources such as KEGG or Reactome[11,12] contain only predicted interactions for zebrafish based on orthology information from human (i.e., they list interologs). In contrast, SignaFish contains (1) direct zebrafish experimental data, (2) predictions filtered for pathway phenotypes known from zebrafish experiments, (3) the predictions are based not only on human data but *Drosophila* and *C. elegans* as well.

We clearly recognize that the list of the included data is far from complete and we are committed in updating the manual curation and the actual databases integrated into SignaFish annually. The annual update allows the users, especially those who are focusing on a set of genes or pathways, to find the same information content for a fixed period. More frequent updates could cause replication problems. We welcome inputs from the wider community of zebrafish researchers; therefore, we included an online form (available from the website) that could be used to submit novel interactions, which—after manual verification—will be included in the next update of SignaFish. In the next curation effort, we plan to extend the pathway set with immune-related pathways and the integrated data content with further information on noncoding elements.

Since its publication, the SignaLink database has proven its usefulness for invertebrate model organism; therefore, we believe SignaFish will also become an important resource for zebrafish research. The signaling interactions that can be examined for each gene, or downloaded for each signaling pathway, will serve as a novel navigating point for experimental design and evaluation for the zebrafish community and for researchers focusing on nonmodel fish species.

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Disclosure Statement

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References

1. Pires-daSilva A, Sommer RJ. The evolution of signalling pathways in animal development. Nat Rev Genet 2003;4:39–49.
2. Csermely P, Korcsmaros T, Kiss HJ, London G, Nussinov R. Structure and dynamics of molecular networks: A novel paradigm of drug discovery: A comprehensive review. Pharmacol Ther 2013;138:333–408.
3. Korcsmaros T, Farkas IJ, Szalay MS, Rovo P, Fazekas D, Spiro Z, *et al.* Uniformly curated signaling pathways reveal tissue-specific cross-talks and support drug target discovery. Bioinformatics 2010;26:2042–2050.
4. Fazekas D, Koltai M, Turei D, Modos D, Palfy M, Dul Z, *et al.* SignaLink 2—A signaling pathway resource with multi-layered regulatory networks. BMC Syst Biol 2013;7:7.
5. Vinayagam A, Zirin J, Roesel C, Hu Y, Yilmazel B, Samsonova AA, *et al.* Integrating protein-protein interaction networks with phenotypes reveals signs of interactions. Nat Methods 2014;11:94–99.
6. Yu H, Luscombe NM, Lu HX, Zhu X, Xia Y, Han JD, *et al.* Annotation transfer between genomes: Protein-protein interologs and protein-DNA regulogs. Genome Res 2004;14:1107–1118.
7. Korcsmaros T, Szalay MS, Rovo P, Palotai R, Fazekas D, Lenti K, *et al*. Signalogs: Orthology-based identification of novel signaling pathway components in three metazoans. PLoS One 2011;6:e19240.
8. Chatr-Aryamontri A, Breitkreutz BJ, Heinicke S, Boucher L, Winter A, Stark C, *et al.* The BioGRID interaction database: 2013 update. Nucleic Acids Res 2013;41: D816–D823.
9. Kerrien S, Aranda B, Breuza L, Bridge A, Broackes-Carter F, Chen C, *et al.* The IntAct molecular interaction database in 2012. Nucleic Acids Res 2012;40:D841–D846.
10. Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120: 15–20.
11. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 1999;27:29–34.
12. Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L, *et al.* Reactome: A database of reactions, pathways and biological processes. Nucleic Acids Res 2011;39:D691–D697.

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