



Database Update

SFMetaDB: a comprehensive annotation of mouse RNA splicing factor RNA-Seq datasets

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Abstract

Although the number of RNA-Seq datasets deposited publicly has increased over the past few years, incomplete annotation of the associated metadata limits their potential use. Because of the importance of RNA splicing in diseases and biological processes, we constructed a database called SFMetaDB by curating datasets related with RNA splicing factors. Our effort focused on the RNA-Seq datasets in which splicing factors were knocked-down, knocked-out or over-expressed, leading to 75 datasets corresponding to 56 splicing factors. These datasets can be used in differential alternative splicing analysis for the identification of the potential targets of these splicing factors and other functional studies. Surprisingly, only ~15% of all the splicing factors have been studied by loss- or gain-of-function experiments using RNA-Seq. In particular, splicing factors with domains from a few dominant Pfam domain families have not been studied. This suggests a significant gap that needs to be addressed to fully elucidate the splicing regulatory landscape. Indeed, there are already mouse models available for ~20 of the unstudied splicing factors, and it can be a fruitful research direction to study these splicing factors *in vitro* and *in vivo* using RNA-Seq.

Database URL: <http://sfmetadb.yubiolab.org>

Introduction

Due to the lack of fully structured metadata, the wide use of the valuable RNA-Seq datasets in public repositories such as ArrayExpress (1) and Gene Expression Omnibus (GEO) (2) may be restricted, despite structured metadata having been used elsewhere for raw data usability (3). For example, ArrayExpress is only a repository of datasets, and the completeness of metadata information relies on dataset submitters. Although submission facilities have been improving, metadata information of many datasets in ArrayExpress is still not well structured (1). To fill this gap, manual curation has been devoted to developing and maintaining metadata databases (4). For example, microarray and RNA-Seq datasets have been curated for the downstream analyses in Expression Atlas (5). We previously launched the RNASeqMetaDB (6) database to facilitate the access of the metadata of public available mouse RNA-Seq datasets. Here, we present a new database, SFMetaDB, as an update with metadata of RNA-Seq datasets related with splicing factors with either loss- or gain-of-function experiments.

RNA splicing is a fundamental biological process in eukaryotes that substantially contributes to the overall protein diversity in a cell. According to GENCODE (Release 25) basic transcript annotation, 19 903 human protein-coding genes encode 54 896 isoforms by alternative splicing. The importance of alternative splicing is underscored by the distinct biological functions played by splicing isoforms. Recently, the splicing isoform function of a number of genes has been tested experimentally in a variety of biological contexts, including cancer. For example, two isoforms of *CD44*, a widely expressed cell surface marker, have recently been shown to be important in cancer development. The first isoform CD44V6 is required for the migration and generation of metastatic tumors in colorectal cancer stem cells and can initiate the metastatic process (7). The second isoform of *CD44*, CD44V8-10, is an important marker for human gastric cancer and increases tumor initiation in gastric cancer cells (8). Another example is *NUMB*, a gene that is critical for cell fate determination. Two splicing isoforms varying in the length of proline-rich region (PRR), PRR^L and PRR^S, were recently found to have opposite roles in hepatocellular carcinoma (HCC), suggesting that the alternative splicing of *NUMB* can serve as an important biomarker for HCC (9). In particular, PRR^L promotes proliferation, migration, invasion and colony formation while PRR^S generally works in the opposite way.

Splicing isoforms may also play some critical roles in biological processes other than cancer. For example, *MICU1* is a gene encoding an essential regulator of mitochondrial Ca²⁺ uptake, a process that is critical for energy

production in skeletal muscle. Through the inclusion of a micro-exon (<15 bp) of this gene, an alternative splice isoform named MICU1.1 can be generated. It was found that the exclusion of this microexon causes a ~10× decrease of the Ca²⁺-binding affinity of MICU1 proteins. Therefore, alternative splicing is essential for the sustainability of Ca²⁺ uptake and ATP production of mitochondria, the energy source of skeletal muscle (10). For another example, *FANCE* is a part of the Fanconi anemia complex, which functions in DNA interstrand crosslink repair. *FANCE* plays a critical role to regulate *FANCD2*, which is required in *FANC*–*BRCA* functions. Overexpression of an alternative splicing isoform *FANCEΔ4* promotes degradation of *FANCD2* and causes dysfunction of DNA repair (11). Furthermore, *VEGF-A* is a gene that functions in angiogenesis, vasculogenesis and endothelial cell growth. Two alternative splicing isoforms, *VEGF-A_{xxx}a* and *VEGF-A_{xxx}b*, are critical in nociception (12). *VEGF-A_{xxx}a* is increased with nerve injury and promotes nociceptive function. On the contrary, the overexpression of *VEGF-A_{xxx}b* reduces neuropathic pain. In addition, the *Fas/CD95* gene is critical in the physiological regulation of programmed cell death. *Fas/CD95* has two splicing isoforms with inclusion or exclusion of exon 6, a membrane-bound receptor or a soluble isoform (13). The membrane-bound receptor isoform promotes apoptosis while the soluble isoform inhibits apoptosis.

Alternative splicing is commonly mediated by RNA splicing factors (14). For example, the splicing factor *NOVA1* regulates the alternative splicing of a series of genes in pancreatic beta cells, and knockdown of *Novo1* suppresses insulin secretion and promotes apoptosis (15). Moreover, the splicing factor *NOVA2* uniquely mediates the alternative splicing of many axon guidance-related genes during cortical development (16). As another example, the splicing factor *PTBP1* suppresses *Pbx1* exon 7 and the neuronal *PBX1A* isoform in embryonic stem cells during neuronal development (17).

In this article, we describe our recent effort in curating the metadata of RNA-Seq datasets from ArrayExpress and GEO, which were derived from studies using cell or animal models with a specific splicing factor being knocked-out, knocked-down or overexpressed. We further launched SFMetaDB to facilitate access to the metadata of these datasets and share them with the biomedical community.

Results and discussion

The launch of SFMetaDB focuses on RNA-Seq datasets with perturbed splicing factors. Users can query a given splicing factor to identify the relevant datasets. A use case

for MBNL splicing factors is shown as follows. MBNL1 is an important RNA splicing factor (18), thus we use MBNL1 to demonstrate the usage of SFMetaDB, which confirms the advantage of SFMetaDB over ArrayExpress. As shown in Figure 1a, a query of MBNL1 on SFMetaDB returns the accurate datasets related with *Mbnl1* loss- or gain-of-function experiments. Figure 1a shows that five datasets could be used for the alternative splicing analysis for MBNL1, and the potential targets of MBNL1 can be concluded from the datasets. For example, the dataset GSE39911 (i.e. E-GEOD-39911) includes biological replicates of various tissues, such as brain, heart and muscle, from *Mbnl1*-knockout mice and *Mbnl1*-knockdown C2C12 mouse myoblasts (Figure 1b).

However, as shown in Figure 1c, ArrayExpress returned a total of 13 mouse RNA-Seq datasets with the query *Mbnl1*, and 8 of them were not from *Mbnl1* gain- or loss-of-function experiments. Therefore, these datasets were eliminated in SFMetaDB. For example, the dataset E-GEOD-76222 is retrieved by ArrayExpress because of the appearance of *Mbnl1* in its description, 'Changes in the expression of alternative splicing factors *Zcchc24*, *Esrp1*, *Mbnl1/2* and *Rbm47* were demonstrated to be key contributors to phase-specific AS.' However, this dataset is about an *ESRP* knock-out, thus it is not suitable for MBNL1 related alternative splicing analysis (Figure 1d). The rest of eight retrieved datasets were considered not appropriate for RNA splicing analysis of MBNL1 by our manual curation of metadata information. In summary, no irrelevant datasets of a given splicing factor are shown in SFMetaDB, and SFMetaDB returned more specific results than ArrayExpress.

Guided by SFMetaDB, users can perform potential target identification for a specific splicing factor. In addition, by integrating multiple datasets curated on SFMetaDB, users can form a more comprehensive view on how a splicing event is regulated across different biological contexts. As another use case, we show below a Pfam domain analysis among splicing factors (see Materials and methods).

Only ~15% of known splicing factors have been studied with loss- or gain-of-function RNA-Seq experiments. Because splicing factors sharing similar domains tend to regulate common splicing targets, we determined what additional splicing factors may be prioritized for study by investigating the domain structures of the splicing factors using UniProt (19). Among the 353 splicing factors, 299 of them contained one or multiple conservative domains. Of these 299 splicing factors, 190 have a single domain that belongs to a Pfam domain family, and the rest have domains that belong to more than one Pfam domain family.

RNA splicing factors have highly conserved functional domains, and some domains are dominant among all the splicing factors. In Figure 2, the domain families are ranked by their number of occurrences in all the splicing factors. Pfam family PF00076 (RNA recognition motif) is the most dominant, and the splicing factors with domains from this family are relatively well studied (25 over the total 87). Splicing factors from five additional Pfam families are fairly well-studied (≥ 3 splicing factors annotated), consisting of PF00271 (Helicase conserved C-terminal domain), PF00270 (DEAD/DEAH box helicase), PF00013 (KH domain), PF00642 (Zinc finger C-x8-C-x5-C-x3-H type) and PF12414 (Calcitonin gene-related peptide regulator C terminal). However, three highly dominant families are not. Specifically, none of the 17 splicing factors with the Pfam family PF01423 (LSM domain) (Figure 2) have been studied yet (20), and these splicing factors provide feasible candidates for future studies. For example, the splicing factor SNRPN has two mouse models from the International Mouse Strain Resource (IMSR) (21) that can be used for splicing analysis. In fact, 25 unstudied splicing factors (Supplementary Table S1) have been identified with more than one mouse model from IMSR. Therefore, splicing factors that are non-homologous with already studied ones constitute promising candidates for comprehensive studies of splicing regulation.

Materials and methods

RNA-Seq dataset curation and SFMetaDB web server deployment

We extracted 353 RNA splicing factors annotated in Gene Ontology (GO) (accession GO:0008380) (22) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (entry mmu03040) (23) for mice. Then, we queried ArrayExpress (1) and GEO (2) using the official symbol of each splicing factor to search for related mouse RNA-Seq datasets and obtained a total of 214 datasets. Note that due to the limitation of the search function in ArrayExpress and GEO, many of these datasets were not directly relevant to the manipulation of these splicing factors despite that the symbols were mentioned in the metadata of these datasets. We chose to manually curate each dataset, providing a total of 75 datasets that have biological replications in which at least one splicing factor was knocked-out, knocked-down or overexpressed (along with the corresponding wild types/controls) (Supplementary Table S1). Because some splicing factors were studied in more than one dataset, a total of 56 splicing factors were found (Supplementary Table S1).

To facilitate the access to these datasets, we launched the database SFMetaDB (<http://sfmetadb.yubiolab.org>).

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Show 10 entries Search: Mbn1

Accession ID	Title	Samples	RNA Splicing Factor	Perturbation	PubMed
GSE39911	Transcriptome-wide Regulation of Splicing and mRNA Localization by Muscleblind Proteins	55	Mbn1	KO	PMID: 22901804
GSE60487	Loss of MBNL Leads to Disruption of Developmentally Regulated Alternative Polyadenylation in RNA-Mediated Disease	36	Mbn1, Mbn2	DKO	PMID: 25263597
GSE67828	Profiling of soma and neurite transcriptomes	48	Mbn1, Mbn2	KD	PMID: 26907613
GSE68890	MBNL Sequestration by Toxic RNAs and RNA Mis-Processing in the Myotonic Dystrophy Brain	39	Mbn1, Mbn2	DKO	PMID: 26257173
GSE79095	Alternative splicing regulation by homologous Muscleblind proteins	12	Mbn1, Mbn2	DKO	PMID: 27557707

Showing 1 to 5 of 5 entries (filtered from 75 total entries) Previous 1 Next

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Scope: Self Format: HTML Amount: Quick GEO accession: GSE39911 GO

Series GSE39911 Query DataSets for GSE39911

Status Public on Aug 17, 2012

Title Transcriptome-wide Regulation of Splicing and mRNA Localization by Muscleblind Proteins

Organisms *Drosophila melanogaster*; *Mus musculus*

Experiment type Expression profiling by high throughput sequencing

Summary The Muscleblind-like (Mbnl) family of RNA-binding proteins plays important roles in muscle and eye development and in Myotonic Dystrophy (DM), where expanded CUG or CCUG repeats functionally deplete Mbnl proteins. We identified transcriptome-wide functional and biophysical targets of Mbnl proteins in brain, heart, muscle, and myoblasts using RNA sequencing and crosslinking/immunoprecipitation-sequencing approaches. This analysis identified several hundred splicing events whose regulation depended on Mbnl function, in a pattern indicative of functional interchangeability between Mbn1 and Mbn2. A nucleotide resolution RNA map associated repression or activation of exon splicing with Mbnl binding near either 3' splice site or near the downstream 5' splice site, respectively. Transcriptomic analysis of sub-cellular compartments uncovered a global role for Mbnls in regulating localization of mRNAs encoding membrane, synaptic and other proteins in both mouse and *Drosophila* cells, and Mbnls also contribute to protein secretion. These findings hold several new implications for DM pathogenesis.

Overall design To assess global functions of Muscleblind proteins, RNA-Seq was performed using WT and Mbn1 KO brain, heart, and muscle (5 mice each). Additionally, C2C12 mouse myoblasts were depleted of Mbn1, Mbn2, or both. Subcellular fractionation experiments were performed to analyze mRNA localization following depletion of Mbn1 and Mbn2 in C2C12 mouse myoblasts, and following depletion of Mbnl in *Drosophila* S-2R+ cells. CLIP-Seq was also performed against Mbn1 in mouse brain, heart, muscle, and C2C12 myoblasts. Finally, ribosome footprinting was performed with C2C12 mouse myoblasts that were depleted of Mbn1, Mbn2, or both.

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Figure 1. A use case of SFMetaDB for the splicing factor Mbn1. We showed a use case of the splicing factor Mbn1 to demonstrate the advantage of SFMetaDB over ArrayExpress. By using the same keyword, Mbn1, SFMetaDB returned five accurate datasets that can be used for the downstream alternative splicing analyses. On the contrary, ArrayExpress returned 13 datasets with 8 that could not be used for the downstream alternative splicing analyses for Mbn1. (a) The result page in SFMetaDB of the query Mbn1. (b) The description page of the dataset GSE39911 in GEO. (c) The result page in ArrayExpress of the query Mbn1. (d) The description page of the dataset E-GEOD-76222 in ArrayExpress.

The screenshot shows the ArrayExpress search results for 'Mbn1'. The search is filtered by organism 'Mus musculus', experiment type 'rna assay', and experiment type 'sequencing assay'. There are 13 experiments listed in a table with columns for Accession, Title, Type, Organism, Assays, Released, Processed, Raw, Views, and Atlas. The table lists various RNA-seq experiments related to alternative splicing regulation and pluripotency induction in Mus musculus and Drosophila melanogaster.

Accession	Title	Type	Organism	Assays	Released	Processed	Raw	Views	Atlas
E-GEOD-79095	Alternative splicing regulation by homologous Muscleblind proteins	RNA-seq of coding RNA	Mus musculus	12	01/06/2016	-	136	-	
E-GEOD-76222	Multiphasic and dynamic changes in alternative splicing during induction of pluripotency are coordinated by numerous RNA binding proteins [ESRP DKO]	RNA-seq of coding RNA	Mus musculus	6	15/03/2016	-	142	-	
E-GEOD-70022	Multiphasic and dynamic changes in alternative splicing during induction of pluripotency are coordinated by numerous RNA binding proteins [PS]	RNA-seq of coding RNA	Mus musculus	42	15/03/2016	-	123	-	
E-GEOD-67828	Profiling of soma and neurite transcriptomes	RNA-seq of coding RNA	Mus musculus	40	19/02/2016	1	154	-	
E-GEOD-68890	MBNL Sequestration by Toxic RNAs and RNA Mis-Processing in the Myotonic Dystrophy Brain	other RNA-seq of coding RNA	Homo sapiens, Mus musculus	39	30/07/2015	1	299	-	
E-GEOD-60487	Loss of MBNL Leads to Disruption of Developmentally Regulated Alternative Polyadenylation in RNA-Mediated Disease	RNA-seq of coding RNA	Homo sapiens, Mus musculus	36	05/09/2014	-	103	-	
E-GEOD-49906	Transcriptome modulation of ventricles, cardiomyocytes and cardiac fibroblasts during postnatal mouse development	RNA-seq of coding RNA	Mus musculus	13	16/04/2014	1	257	1	
E-GEOD-47794	Muscleblind-like compound knockout models for myotonic dystrophy	RNA-seq of coding RNA	Mus musculus	2	01/12/2013	1	126	-	
E-GEOD-45505	Muscleblind-like proteins regulate embryonic stem cell-specific alternative splicing and reprogramming	RNA-seq of coding RNA	Homo sapiens, Mus musculus	6	26/06/2013	1	163	-	
E-GEOD-45504	Muscleblind-like proteins regulate embryonic stem cell-specific alternative splicing and reprogramming II	RNA-seq of coding RNA	Homo sapiens, Mus musculus	5	26/06/2013	1	177	-	
E-GEOD-45503	Muscleblind-like proteins regulate embryonic stem cell-specific alternative splicing and reprogramming I	RNA-seq of coding RNA	Mus musculus	1	26/06/2013	1	122	-	
E-GEOD-39911	Transcriptome-wide Regulation of Splicing and mRNA Localization by Muscleblind Proteins	RNA-seq of coding RNA	Drosophila melanogaster, Mus musculus	55	17/08/2012	1	298	-	
E-MTAB-414	RNA CLIP-seq for Cugbp1, Mbnl1 and Pttb1 in mouse C2C12 myoblasts	CLIP-seq	Mus musculus	13	04/01/2012	-	702	-	

The screenshot shows the detailed view of experiment E-GEOD-76222. The title is 'E-GEOD-76222 - Multiphasic and dynamic changes in alternative splicing during induction of pluripotency are coordinated by numerous RNA binding proteins [ESRP DKO]'. The status is 'Released on 15 March 2016, last updated on 19 March 2016'. The organism is 'Mus musculus'. The description provides a detailed overview of the study, mentioning alternative splicing (AS) and its role in cell fate transitions, development, and disease. The experiment type is 'RNA-seq of coding RNA'. The contact information is 'Yi Xing <geo@ncbi.nlm.nih.gov>, Russ Carstens'. The files section includes 'Investigation description', 'Sample and data relationship', and 'Additional data (1)'. The links section includes 'GEO - GSE76222, ENA - SRP067650' and 'Send E-GEOD-76222 data to GENOME SPACE'.

Figure 1. Continued.

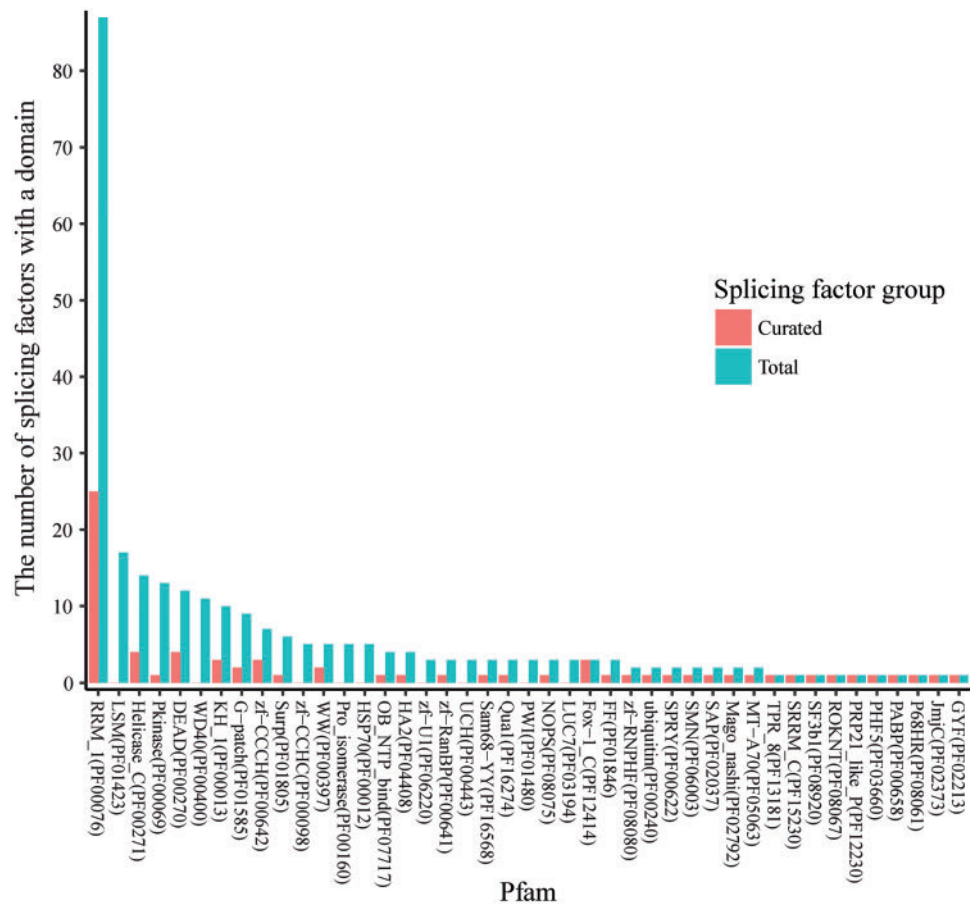


Figure 2. The occurrence of Pfam domain families in splicing factors. The known RNA splicing factors are annotated in UniProt according to the Pfam domain families of the protein domains found in these factors. A splicing factor may have multiple domains that belong to multiple Pfam families, and a Pfam domain family may contain domains in multiple splicing factors. The Pfam annotations were retrieved for each of 353 splicing factors, and the number of splicing factors was calculated for each of the Pfam families. For the 56 splicing factors that have curated datasets in SFMetaDB, the number of splicing factors was also calculated for the associated Pfam families. In the dodged barplots, the Pfam domain families are ranked by the number of the splicing factors which contain domains in the given families. Of the total 217 Pfam domain families annotated in UniProt, 26 Pfam domain families have ≥ 3 splicing factors annotated. The Pfam domain family with the most number of splicing factors is Pfam RRM_1 (PF00076). It contains 87 splicing factors, and 25 of these splicing factors have been studied according to our curation results. However, the splicing factors in the rest of the Pfam domain families have brought relatively less attention in RNA-Seq analysis, and they may be promising candidates for future studies.

When datasets were deposited in GEO, ArrayExpress imported the most metadata information from GEO, and the ArrayExpress description contained the link to the GEO webpage. Therefore, SFMetaDB used GEO accession ID if possible. The web server of SFMetaDB is freely available, and it presents the Accession ID, description, the number of samples, associated curated splicing factors, perturbation and PubMed references of each RNA-Seq dataset.

Domain structures analysis in RNA splicing factors

The domain structures of the RNA splicing factors may guide us to identify the candidate splicing factors for future studies. Known RNA splicing factors are retrieved from GO term (GO:0008380) using R package GO.db (22) and

KEGG pathway (entry mmu03040). UniProt annotates the conservative Pfam domain families for the canonical sequences of the splicing factors (19). From these domain annotations, we calculate the numbers of the splicing factors in Pfam domain families. Figure 2 plots the dodged barplots of the number of splicing factors in Pfam domain families using curated splicing factors and the total splicing factors. By comparing the domain families of the splicing factors with RNA-Seq datasets to the families of all the splicing factors, the splicing factors in not well-studied domain families can be the promising candidates for future RNA-Seq studies.

Supplementary data

Supplementary data are available at Database Online.

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Conflict of interest. None declared.

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