

Original article

Manual Gene Ontology annotation workflow at the Mouse Genome Informatics Database

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The Mouse Genome Database, the Gene Expression Database and the Mouse Tumor Biology database are integrated components of the Mouse Genome Informatics (MGI) resource (<http://www.informatics.jax.org>). The MGI system presents both a consensus view and an experimental view of the knowledge concerning the genetics and genomics of the laboratory mouse. From genotype to phenotype, this information resource integrates information about genes, sequences, maps, expression analyses, alleles, strains and mutant phenotypes. Comparative mammalian data are also presented particularly in regards to the use of the mouse as a model for the investigation of molecular and genetic components of human diseases. These data are collected from literature curation as well as downloads of large datasets (SwissProt, LocusLink, etc.). MGI is one of the founding members of the Gene Ontology (GO) and uses the GO for functional annotation of genes. Here, we discuss the workflow associated with manual GO annotation at MGI, from literature collection to display of the annotations. Peer-reviewed literature is collected mostly from a set of journals available electronically. Selected articles are entered into a master bibliography and indexed to one of eight areas of interest such as 'GO' or 'homology' or 'phenotype'. Each article is then either indexed to a gene already contained in the database or funneled through a separate nomenclature database to add genes. The master bibliography and associated indexing provide information for various curator-reports such as 'papers selected for GO that refer to genes with NO GO annotation'. Once indexed, curators who have expertise in appropriate disciplines enter pertinent information. MGI makes use of several controlled vocabularies that ensure uniform data encoding, enable robust analysis and support the construction of complex queries. These vocabularies range from pick-lists to structured vocabularies such as the GO. All data associations are supported with statements of evidence as well as access to source publications.

Introduction

Mouse Genome Informatics (MGI) is the primary international database resource for the laboratory mouse, providing integrated genetic, genomic and biological data to facilitate the study of human health and disease. The MGI team curates the biomedical literature (11 000 publications per year) and normalizes and integrates sequence and functional data about mouse genetics and genomics from almost 50 other external database and informatics resources. MGI organizes curation teams around particular types of data including sequence data,

phenotypes, embryonic expression data, comparative and functional information, mouse tumorigenesis and mouse models for human diseases. MGI utilizes multiple bio-ontologies and is the authority for mouse gene and strain nomenclature.

Five projects contribute to this resource. The 'Mouse Genome Database' (1) includes data on gene characterization, nomenclature, mapping, gene homologies among mammals, sequence links, phenotypes, disease models, allelic variants and mutants and strain data. The 'Gene Expression Database' (2) integrates different types of gene expression information from the mouse and provides

a searchable index of published experiments on endogenous gene expression during development. The 'Mouse Tumor Biology (3) Database' provides data on the frequency, incidence, genetics and pathology of neoplastic disorders, emphasizing data on tumors that develop characteristically in different genetically defined strains of mice. The MGI group is a founding member of the 'Gene Ontology Consortium' (GO, www.geneontology.org, (4)). MGI fully incorporates the GO in the database and provides a GO browser for access to mouse functional annotation. Finally, the 'MouseCyc' database (5) focuses on 'Mus musculus' metabolism and includes cell level processes such as biosynthesis, degradation, energy production and detoxification. It is part of the BioCyc collection of pathway databases created at SRI International (6).

Here, we outline the workflow process for 'one' component of the MGI data acquisition and integration process—that associated with the 'Gene Ontology Project at MGI (Figure 1)'. MGI assigns functional annotations (GO terms) to genes and protein products through semi-automated

methods and manual curation. Semi-automated annotation strategies include mapping and translating data from the Enzyme Commission, Swiss-Prot, InterPro (see <http://www.geneontology.org/GO.indices.shtml>), rat and human

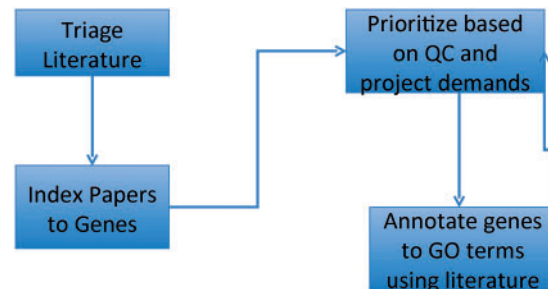


Figure 1. GO curation workflow: papers of interest are identified and entered into the database system (triage) and associated with genes (indexed). GO annotations are made using papers selected based on quality control reports and projects. The quality control reports in turn are revised daily based on added annotation.

Current Channel: PubMed

Search: mouse murine mice 55 Results (Search In Results)

Pin	No.	Type	Authors	Published	Title	Source
	1		Mori T, Rezi	2012 Feb 24	Tannic Acid is a Natural beta-Secretase In	J Biol Chem. 2012 Feb
	2		Blumbach K	2012 Feb 24	Dwarfism in Mice Lacking Collagen-bindir	J Biol Chem. 2012 Feb
	3		Zhang L, Do	2012 Feb 24	Role of Integrin-beta3 Protein in Macroph	J Biol Chem. 2012 Feb
	4		Luo H, Wu Z	2012 Feb 24	Receptor tyrosine kinase ephb6 regulates	J Biol Chem. 2012 Feb

Role of Integrin- β 3 Protein in Macrophage Polarization and Regeneration of Injured Muscle^[5]

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Background: Integrin- β 3 is important for the cell migration and proliferation linked to muscle regeneration.
Results: In mice with global integrin- β 3 KO, an initial macrophage polarization impairs muscle regeneration and stimulates fibrosis via TGF- β 1 production.
Conclusion: In bone marrow cells, integrin- β 3 expression is necessary for macrophage-dependent processes of muscle repair.
Significance: Stimulating integrin- β 3 could improve muscle regeneration.

Following injury, skeletal muscle achieves repair by a highly coordinated, dynamic process resulting from interplay among numerous inflammatory, growth factors and myogenic regulators. To identify genes involved in muscle regeneration, we used a microarray analysis there was a significant increase in the expression of a group of integrin genes. To verify these results, we used RT-PCR and Western blotting and found that 12 integrins were up-regulated from 3 h to 15 days following injury. Following muscle injury, integrin- β 3 was initially expressed, mainly in macrophages. In integrin- β 3 global KO mice, the expression of myogenic genes was decreased and muscle regeneration was impaired, whereas fibrosis was enhanced versus events in wild type (WT) mice. The mechanism for these responses in integrin- β 3 KO mice included an infiltration of macrophages that were polarized into the M2 phenotype. These macrophages produced more TGF- β 1 and increased TGF- β 1/Smad signaling. *In vitro*, we confirmed that M2 macrophages lacking integrin- β 3 produced more TGF- β 1.

between cells and extracellular matrix proteins are mediated primarily by integrin genes. Integrins are transmembrane receptors that bind the extracellular matrix and the intracellular cytoskeleton. Consequently, integrins, by transducing signals from outside of cells into cells and vice versa, could play important roles in regulating cell adhesion, spreading, migration, proliferation, and differentiation as well as tissue remodeling. The importance of integrin signaling in influencing the repair of injured muscle is emphasized by reports of muscular degenerative disorders in mice with specific integrin deficiencies (1, 2). Integrins can also affect muscle growth indirectly. In response to muscle overloading, integrin- β 2 knock-out (KO) mice (integrin- β 2 is exclusively expressed by hematopoietic cells) exhibited decreased myofiber size, a lower muscle mass, and decreased satellite cell activation and proliferation (3). The pathophysiological changes stimulated by muscle injury also involve integrins (4).

Downloaded from www.jbc.org at JACKSON LAB, on

Figure 2. QUOSA Information Manager showing part of a paper from an issue of JBC with mouse, murine or mice highlighted. Curator quickly looks at context to select appropriate area of MGI that the paper best fits. The paper shown has been 'tagged' for alleles and GO.

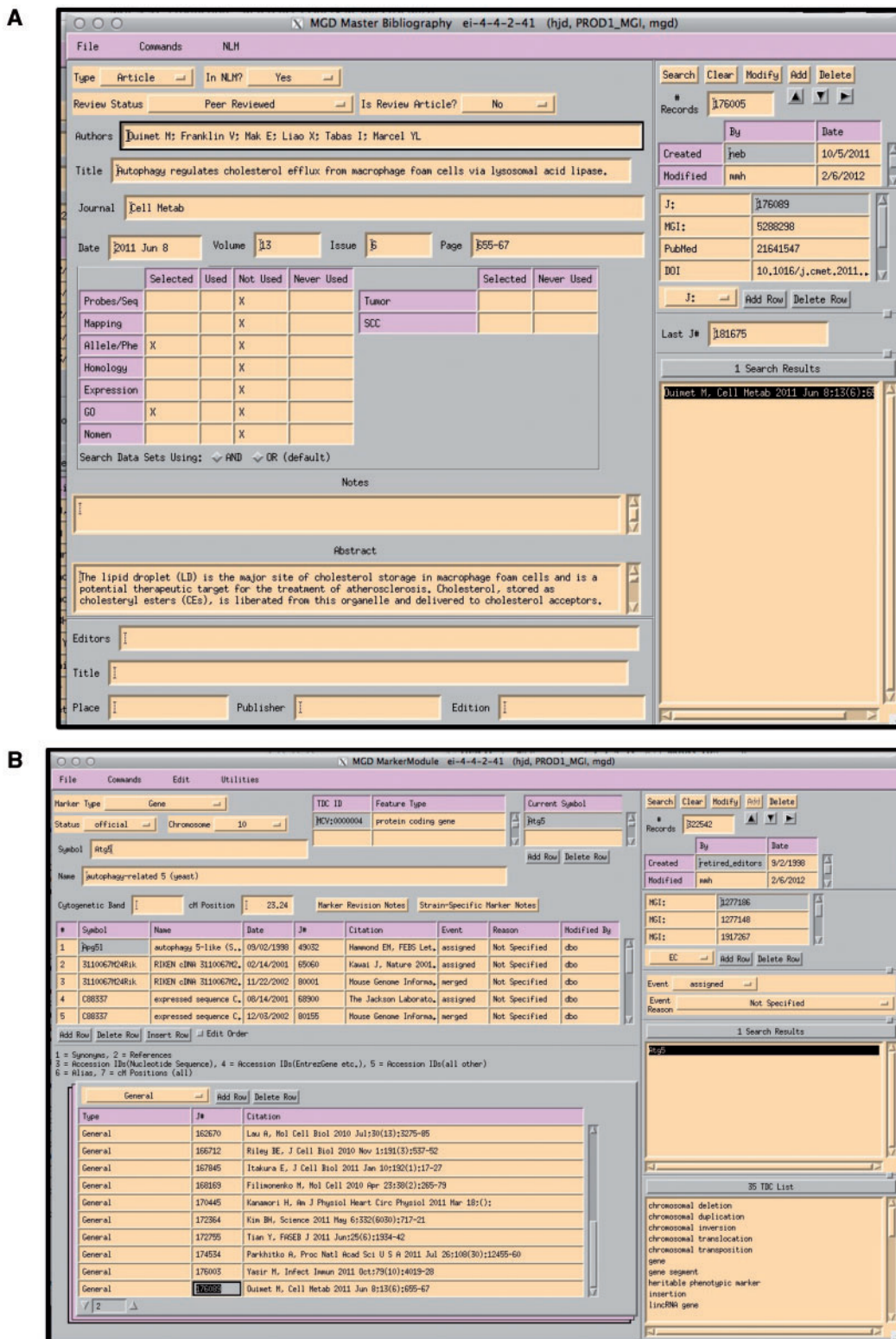


Figure 3. (A) MGI Master Bib EI, showing a record for a paper that has been selected for alleles/phenotypes and GO. The paper has not yet been curated for GO (indicated by an X in the selected and X in Not Used). **(B)** MGI Gene Feature Detail Module. References to be associated with this gene are entered into the lower left hand panel. If a paper is associated with multiple genes, the same paper is entered for each gene. All EIs are TeleUSE graphical user interface applications running under X-windows.

ortholog experimental data and others. Curation of these data sets includes review and resolution of Quality Control Reports generated through the process of data loading and comparisons to existing data in MGI. For the purpose of this paper, we will not discuss these semi-automated integration methods, but rather concentrate on the manual literature curation, which is a vital source of experimental mouse functional data. While we focus on the GO component of MGI in this description, the literature curation process is very similar for other MGI components that curate literature.

The subcomponents of the literature curation process include the following:

- (a) Literature triage: identifying and obtaining relevant scientific literature

Each curator is assigned a specific subset of journals from a set of 160 relevant journals out of ~650

subscriptions carried by the Jackson Laboratory. Journals are chosen for triage based on the numbers of articles that have been identified and curated for that journal over the previous years. The manuscripts accepted for curation come from a variety of sources. Most are selected from the journals that are regularly triaged. Others are selected on the basis of particular annotation processes such as full curation of the Wnt family of proteins, or in another example, full curation of genes implicated in human diseases. On a yearly basis, the number of papers selected from all journals is tabulated, and the selection of which journals to regularly curate the next year is determined based on the relevancy of the journal publications during the previous year and the number of full time equivalents available for this task. Typically, a few journals are

PROMINER used for indexing aid

fXII and PKK immunoblot analysis

Plasma anticoagulated with sodium citrate was fractionated on 4%-12% gradient SDS-polyacrylamide gels (Invitrogen Life Technologies) followed by immunoblotting with human **fXII** (Accurate Chemicals), mouse **PKK** (R&D Systems) or mouse $\alpha 2$ -antiplasmin (**A2AP**; R&D Systems) antibodies. Blots were incubated with secondary fluorophore-labeled antibodies (LI-COR) and imaged on Odyssey Imager (LI-COR). **PKK** and **fXII** relative plasma protein levels were determined by densitometry analysis (ImageJ 1.43).

Plasma fXIIa-antithrombin complex ELISA

fXIIa-antithrombin plasma complex levels were measured by sandwich ELISA. Briefly, assay plates were coated with anti-human **fXII** antibody (Accurate Chemicals) and blocked with 2% BSA before incubation with diluted mouse platelet poor plasma. After extensive washing, fXIIa-antithrombin complex was detected by incubation with HRP-conjugated **antithrombin** antibody (Enzygnost human TAT Micro ELISA kit, Siemens). Relative levels of fXIIa-antithrombin complex were calculated using serial dilutions of control mouse plasma as a standard.

Ferric chloride-induced inferior vena cava thrombosis

Antithrombotic activity was studied using a well-established ferric chloride (FeCl₃) induced inferior vena cava (IVC) thrombosis model.^{17,18} Total mRNA was purified from vena cava tissue samples and analyzed by RT-PCR for **Platelet Factor 4** (**PF4**) mRNA levels. **PF4** mRNA levels were used to determine the effect of treatment on platelet deposition as a measure of thrombus formation.¹⁹ **PF4** mRNA levels in the IVC tissue exposed to FeCl₃ was normalized to nonexposed vena cava tissue.

Stenosis-induced IVC thrombosis

The St Tomas model which uses a combination of reduced blood flow and endothelial damage, was used to study stenosis-induced IVC thrombosis.¹⁸ Briefly, the IVC of male BALB/c mice anaesthetized with 2.5% inhalant isoflurane was exposed via a midline abdominal incision below the left renal vein, and separated from the abdominal aorta. A 6-0 silk tie (Ethicon) was placed behind the vessel and a metal 4-0 suture (Ethicon) was placed

Data were analyzed using SPSS software package for Windows Version 14.0 (SPSS Inc). Graphics were constructed using GraphPad Prism Version 5 for Windows (GraphPad Software Inc).

Results

Systemic delivery of ASO results in suppression of fXII and PKK levels in mice

The role of the intrinsic pathway and contact system in thrombosis in mice has been studied using several approaches, including knockout strategies and specific inhibitors.^{9,11,13,20,21} It is clear that **fXII** is involved in thrombus formation in these models. In vitro, **PKK** is required for optimal **fXII** activity. **PKK** may have a pro-thrombotic role as **PKK** knockout mice are not currently available. **fXII** ASO treatment was used to explore the importance of **fXII** in thrombus formation. **fXII** mRNA in liver, and levels of **fXII** in plasma were shown in Figure 1. Systemic delivery of **fXII** ASO (twice per week for 3 weeks) resulted in a 92% reduction of target mRNA levels (92% and 96% reduction for **fXII** and **PKK** expression, respectively) at the highest tested ASO dose (Figure 1A). ASO treatment produced dose-dependent reduction of the target plasma protein, with maximum reductions of 83% and 85% for **PKK** and **fXII**, respectively (Figure 1B-D). Both **PKK** and **fXII** ASOs were highly specific for their targets, as demonstrated by unchanged liver mRNA expression of several nontargeted coagulation factor mRNAs. Specifically, **fXII** ASO did not change mRNA expression of **coagulation factors II** (**prothrombin**), V, VII, VIII, IX, X, XI, as well as **fibrinogen** and **PKK**. **PKK** ASOs did not change mRNA expression of **coagulation factors II** (**prothrombin**), VII, XI and XII (Figure 1A and data not shown). Both **PKK** and **fXII** mRNA levels correlated with plasma protein levels of the respective factors (supplemental Figure 1A-C, available on the

CLASS: Mouse
 Found: fXII
 F12
 Q80YC5@SWISSPROT
 FA12_MOUSE@SWISSPROT
 58992@ENTREZGENE

Figure 4. Output from PROMINER: PROMINER is used to assist in the gene indexing process. Utilizing official nomenclatures and synonym lists for mouse/rat/human gene names and gene symbols, PROMINER marks up papers for review by a curator, who then associates genes-to-papers in the MGI EI.

dropped and a few are added each year to the formal triage process. The QUOSA application (<http://www.quosa.com>) is used as an aid to access and identify recent papers as represented in PUBMED containing data about the mouse and determining which component of the database will be curated from the paper (GO, expression, mutant alleles, phenotypes, mapping, tumor). QUOSA is used to retrieve full text PDFs for a journal issue or time frame of interest. Because the experimental organism 'mouse' is often not mentioned in the abstract (3–34% depending on the journal), a curator selects an issue of a journal and then searches the full text of papers containing the keywords 'mouse', 'murine' or 'mice'. The application highlights these terms showing the context of the search keywords, which enables curators to quickly determine whether the experiments described are of a suitable nature to be used for GO annotation (i.e. do the experiments aid in determining the normal function of the gene?) (Figure 2). The number of papers examined and the number selected are somewhat journal dependent. For example, out of ~80–90 papers per each weekly issue of Journal of Biological Chemistry, roughly 60 contain one or more of the three keywords, and of those, the curator may select 10–15 as being relevant for some area of the database. For an issue of Nature, which may have 15–20 research articles per issue, up to 5 may have the keywords and all of them are relevant. Papers selected are uploaded to an in-house server for the next steps.

- (b) Adding the publications to the MGI system through the editorial interface

Papers selected as containing data appropriate for GO annotation are entered into a 'master bibliography' module. Figure 3a shows the module's data entry screen. Each record is tagged for the area of use of the database for which it has information. Information about journal, volume, pages and the abstract are automatically obtained nightly from PUBMED using the PMID. The tag for the database area is added manually. At this stage, the paper is not associated with any specific gene. The area tag will automatically change when the paper is actually used for curation. For example, if this paper was used for a GO annotation, the 'used' box would then have an X added automatically.

- (c) Indexing the papers to determine the genes being studied

A paper is then associated or indexed to the genes discussed within by adding the paper to each gene's detail module (Figure 3b). PROMINER,

a natural language processing (NLP) application, is used to assist in the gene indexing process (7). Utilizing official nomenclatures and synonym lists for mouse/rat/human gene names and gene symbols, PROMINER marks up papers for review by a curator, who then associates genes-to-papers in the MGI editorial interface (EI) (Figure 4).

Sometimes a paper discusses several genes, but not all of them may be objects for direct GO annotation. For example, a paper describing the effects of a knock out of a particular gene may use analysis of other gene products to analyze the particular processes being affected, but the annotation to involvement in the process would only be made to the gene being knocked out. Currently, the topical areas selected for each paper are not directly tied to the genes associated with the paper. Thus, a paper selected for GO for one of the genes will appear in the GO EI interface of unused papers for each of the genes indexed to the paper.

Curation triage: selecting what genes to annotate

Ideally, all papers would be immediately used for GO annotation, but on average 300 new papers are added to the database each week, only less than half of these are curated each week due to resource limitations. Therefore, various priority selection criteria are used to choose which genes and papers warrant immediate attention. Reports of interest are generated, such as 'genes with no GO annotation but have new indexed literature selected for GO' or 'genes with mutant alleles that have literature selected for GO' (Figure 5). Additionally, participation in various collaborative projects, such as the Reference Genome project (8), or the Protein Ontology (8) defines primary sets of genes to work based on community input.

Data entry: creating a GO annotation using the EI module for GO

A curator at MGI uses the GO EI module to enter annotations (Figure 6). Curators use the annotation guidelines set forth by the Gene Ontology Consortium (<http://www.geneontology.org/GO.annotation.shtml>). The MGI interface is gene centric. It is divided into two main data entry sections: the annotation area and the annotation properties area. A list of papers selected for GO that are associated with this gene is shown in the lower right panel. Individual protein isoforms or modified forms can be indicated using annotation properties where an id for a specific isoform can be indicated. After reading a paper, the curator selects the appropriate GO id (1). Next, the reference number

Partial list of QC reports used to triage GO curation

- [Mouse Genes that have Rat/Human Homologs but no GO Annotations](#)
- [Mouse Genes with no GO Annotations](#)
- [Mouse Genes that have Alleles but no GO Annotations](#)
- [Genes with no GO Annotations with references that are selected for GO but have not been used](#)
- [Genes with references that are selected for GO but have not been used](#)
- [Genes with GO Annotations of evidence IEA only and with references that are selected for GO but have not been used](#)
- [Genes with OMIM Annotations and either GO Annotations of evidence IEA only or no GO Annotations](#)
- [All genes with 'root' annotations with new indexed literature](#)
- ["Done" Genes with New Literature](#)
- [Non-Gene Markers with GO Annotations](#)
- [Markers with Annotations to Obsolete GO Terms](#)

Figure 5. GO QC reports used for annotation triage and quality control.

The screenshot shows the MGI GO data entry module interface. At the top, there are fields for 'Annotation Type' (GO/Marker), 'MGI Accession ID' (MGI:94924), and 'Marker' (Drd2, dopamine receptor 10, Chr 9). Below this is a table of GO annotations with columns for Term Acc ID, ING, Vocabulary Term, Qual, J#, Citation, Evi, Inferred From, Modified By, Date, Created By, and P. A callout labeled '1' points to the 'Term Acc ID' column. A callout labeled '2' points to the 'Citation' column. A callout labeled '3' points to the 'Evi' column. A callout labeled '4' points to the 'Inferred From' column. Below the table is a 'GO Marker Notes' section with a callout labeled '5' pointing to the 'Property' column. To the right of the table, there are three callouts: 'GO annotation' pointing to the table, 'Annotation extensions' pointing to the 'GO Marker Notes' section, and 'Unused references selected for GO' pointing to a search results table at the bottom. The search results table has columns for '#', 'Citation', and 'Evi'. A callout labeled '5' points to the 'Property' column in the 'GO Marker Notes' section. At the bottom, there are several drop-down menus for 'Evidence Property', 'Qualifier', and 'Evidence Code', with callouts pointing to them.

Figure 6. MGI GO data entry module: the interface is divided into three main sections: GO annotation, annotation properties and search and reference tracking. Drop-down menus display pick-lists of allowed entries in various fields (evidence property, GO qualifier and evidence codes). Numbered areas: 1, GO ID entry; 2, reference entry; 3, evidence code entry; 4, 'inferred_from' entry required for certain evidence codes and 5, annotation properties entry.

is added (2), as well as the evidence code (3). If required by the type of evidence code, additional information is added to the inferred from column (4). Once the annotation is saved, information about the cell type that the experiment was done in, or the specific isoform, or tissue, is entered into the annotation properties section (5). Additional ontologies such as the Cell Ontology (9), Mouse Adult (10) and Embryonic Anatomies (11), Protein Ontology (12), and psi-Mod (13) are used in the properties fields. Several of these are used to supply an extension to the annotation which are used in the gene association file (GAF) (5). Curators can use the OBO-EDIT tool (14) to load multiple ontologies to aid in searching for appropriate terms, as well as viewing the chosen term in the context of the rest of the ontology (Figure 7). The data entry module has several built-in features to aid in QC. The GO vocabulary is refreshed daily from the GO site, and only current GO terms can be used, otherwise data entry is prohibited. Only reference identifiers previously entered into 'master bibliography' can be used. Incorrect evidence codes are automatically rejected. There are other mechanisms, such as data loads, that provide GO annotation. In all cases,

provenance is provided. The GO EI also has a built-in report generator that highlights words matching GO terms found in the abstracts of papers selected for GO as an aid to suggesting the type of information and evidence present in a paper (Figure 8).

Tracking metrics and quality control measures to set priorities for upcoming work

GO annotation metrics in MGI are generated daily. MGI GO curators add on average 200 new annotations per week. Annotations are tracked based on a variety of criteria such as annotation source (MGI curation or data load) and evidence (experimental or predictive, such as through orthology or functional domain). Scripts review changes to the GO structure and provide QC reports for curators noting genes whose annotations may be affected by these changes (Figure 9). Additionally, we use the master bibliography tables and the GO annotations to keep track of various areas that need focus, such as 'genes with

Figure 7. OBO-Edit ontology tool used to browse multiple OBO ontologies. The far left panel shows the vocabularies that have been loaded for searching and viewing. The right panel displays the terms in all of the vocabularies that contain the word 'kidney'. The GO term 'kidney mesenchyme morphogenesis' is selected and is visible as a tree view showing its children (middle panel), and as a graphical view showing its parents (lower left).

The screenshot shows the MGD GOVocAnnot interface. The 'Reports' menu is highlighted. A report titled 'Potential New GO References' is displayed for the gene 'Drd2, dopamine receptor D2, Chr 9'. The report includes a table of GO terms, a list of references, and abstract excerpts with highlighted text.

Term	Acc ID	DAWG	Vocabulary Term
	GO:0016021	C	integral to membrane
	GO:0016021	C	integral to membrane
	GO:0001669	C	acrosomal vesicle
	GO:0043679	C	axon terminus
	GO:0031410	C	cytoplasmic vesicle
	GO:0043197	C	dendritic spine
	GO:0019861	C	flagellum
	GO:0030672	C	synaptic vesicle
	GO:0005625	C	soluble fraction
	GO:0005887	C	integral to plasma membrane

Potential New GO References
Symbol: Drd2, dopamine receptor D2, Chr 9
 Start Date/Time: Tue Mar 27 11:10:49 2012

[J:157250](#), Sahar S, PLoS One 2010;5(1):e8561
 Regulation of **BMAL1** protein stability and circadian function by **GSK3beta**-mediated **phosphorylation**.
BACKGROUND: Circadian rhythms govern a large array of physiological and metabolic functions. To achieve plasticity in circadian regulation, proteins constituting the molecular clock machinery undergo various post-translational modifications (PTMs), which influence their activity and intracellular localization. The core clock protein **BMAL1** undergoes several PTMs. Here we report that the Akt-GSK3beta signaling pathway regulates **BMAL1** protein stability and activity. **PRINCIPAL FINDINGS:** GSK3beta phosphorylates **BMAL1** specifically on Ser 17 and Thr 21 and primes it for ubiquitylation. In the absence of GSK3beta-mediated phosphorylation, **BMAL1** becomes stabilized and **BMAL1** dependent circadian gene expression is dampened. Dopamine D2 receptor mediated signaling, known to control the Akt-GSK3beta pathway, influences **BMAL1** stability and in vivo circadian gene expression in striatal neurons. **CONCLUSIONS:** These findings uncover a previously unknown mechanism of circadian clock control. The GSK3beta kinase phosphorylates **BMAL1**, an event that controls the stability of the protein and the amplitude of circadian oscillation. **BMAL1** phosphorylation appears to be an important regulatory step in maintaining the robustness of the circadian clock.

[J:161807](#), Thompson D, PLoS One 2010;5(6):e11038
 Altered ratio of D1 and D2 dopamine receptors in mouse striatum is associated with **behavioral sensitization** to cocaine.
BACKGROUND: Drugs of abuse elevate brain dopamine levels, and, in vivo, chronic drug use is accompanied by a selective decrease in dopamine D2 receptor (D2R) availability in the brain. Such a decrease consequently alters the ratio of D1R:D2R signaling towards the D1R. Despite a plethora of behavioral studies dedicated to the understanding of the role of dopamine in addiction, a molecular mechanism responsible for the downregulation of the D2R, in vivo, in response to chronic drug use has yet to be identified. **METHODS AND FINDINGS:** ETHICS STATEMENT: All animal work was approved by the Gallo Center IACUC committee and was performed in our AAALAC approved facility. In this study, we used wild type (WT) and G protein coupled receptor associated sorting protein-1 (GASP-1) knock out (KO) mice to assess molecular changes that accompany cocaine sensitization. Here, we show that downregulation of D2Rs or upregulation of D1Rs is associated with a sensitized locomotor response to an acute injection of cocaine. Furthermore, we demonstrate that disruption of GASP-1, that targets D2Rs for degradation after endocytosis, prevents cocaine-induced downregulation of D2Rs. As a consequence, mice with a GASP-1 disruption show a reduction in the sensitized locomotor response to cocaine. **CONCLUSIONS:** Together, our data suggests that changes in the ratio of the D1:D2R could contribute to cocaine-induced behavioral plasticity and demonstrates a role of GASP-1 in regulating both the levels of the D2R and cocaine sensitization.

Figure 8. Report generated using the abstracts of papers selected for GO for the gene being annotated within the GO EI. Text contained in GO terms in each abstract is highlighted.

no GO annotation but have papers that are selected for GO but not used'.

Annotation presentation and usage

GO data for each gene at MGI are displayed to the public in a 'GO Summary' page. This page displays the GO annotations as a table, summary text or graph. Sample views for the gene *Drd2* are shown in Figure 10. All data assertions in MGI are supported by evidence and citation to the source of the information. For assertions that are associated with controlled vocabularies such as the GO, links are provided to vocabulary browsers that provide the relationships between the assertion and other knowledge in that area of the ontology. Using the table and associated information, MGI provides an automatically generated text description of the GO annotations. MGI also provides a graphical display of GO annotations from the GO detail page for each gene.

GO annotations are also shared with the GO Consortium (GOC) through a GAF. This is a tab-delimited file that contains most of the elements of a GO annotation as outlined in the GO EI section above. Presently, only the 'cell type' and 'gene product' annotation properties are included in the GAF. More will be included over time. This file is available on either the GOC web site or along with other data sets, from the MGI FTP site (<ftp://ftp.informatics.jax.org/pub/reports/index.html>). The GAF and the GO vocabulary file are used as input for many analytical tools such as GO TermFinder (15). Instructions for construction of a GO GAF file are found in GO documentation at <http://www.geneontology.org/GO.format.annotation.shtml>.

Information access: NLP and beyond

In general, GO annotation from the mouse experimental literature can be very challenging. Although some groups

Curator Report

DATE: 12/15/2011 03:03:23

Accession ID	Term	Discrepancy
GO:0008418	protein-N-terminal asparagine amidohydrolase activity	Definition change for Term with annotations. Old Definition: Catalysis of the deamidation of an N-terminal asparagine residue in a peptide or protein. New Definition: Catalysis of the reaction: protein-L-asparagine + H2O = protein-L-aspartate + NH3. This reaction is the deamidation of an N-terminal asparagine residue in a peptide or protein. Symbols: Ntan1
GO:0034595	phosphatidylinositol phosphate 5-phosphatase activity	Definition change for Term with annotations. Old Definition: Catalysis of the removal of the 5-phosphate group of a phosphatidylinositol phosphate. New Definition: Catalysis of the removal of the 5-phosphate group of a phosphatidylinositol phosphate. Symbols: Inpp5k Synj1
GO:0035602	fibroblast growth factor receptor signaling pathway involved in negative regulation of apoptotic process in bone marrow	Definition change for Term with annotations. Old Definition: The series of molecular signals generated as a consequence of a fibroblast growth factor receptor binding to one of its physiological ligands, which stops, prevents, or reduces the frequency, rate or extent of the occurrence or rate of cell death by apoptosis in the bone marrow. New Definition: The series of molecular signals generated as a consequence of a fibroblast growth factor receptor binding to one of its physiological ligands, which stops, prevents, or reduces the frequency, rate or extent of the occurrence or rate of cell death by apoptotic process in the bone marrow. Symbols: Fgfr2
GO:0052744	phosphatidylinositol monophosphate phosphatase activity	Definition change for Term with annotations. Old Definition: Catalysis of the reaction: phosphatidylinositol phosphate + H2O = phosphatidylinositol + phosphate. New Definition: Catalysis of the reaction: phosphatidylinositol monophosphate + H2O = phosphatidylinositol + phosphate. Symbols: Synj2
GO:0070773	protein-N-terminal glutamine amidohydrolase activity	Definition change for Term with annotations. Old Definition: Catalysis of the deamidation of an N-terminal glutamine residue of a protein. New Definition: Catalysis of the reaction: protein-N-terminal-L-glutamine + H2O = protein-N-terminal-L-glutamate + NH3. This reaction is the deamidation of an N-terminal glutamine residue of a protein. Symbols: Wdyhv1
GO:0071866	negative regulation of apoptotic process in bone marrow	Definition change for Term with annotations. Old Definition: Any process that stops, prevents, or reduces the frequency, rate or extent of the occurrence or rate of cell death by apoptosis in the bone marrow. New Definition: Any process that stops, prevents, or reduces the frequency, rate or extent of the occurrence or rate of cell death by apoptotic process in the bone marrow. Symbols: Lef1

Figure 9. GO change log report showing changes to the GO and genes with annotations using the term that may need to be looked at.

Gene Ontology Classifications

Symbol: **Drd2**
 Name: Dopamine receptor D2
 ID: MGI:94924

GO Annotations as Summary Text (Tabular View) (GO Graph)

GO curators for mouse genes have assigned the following annotations to the gene product of Drd2. (This text reflects annotations as of Saturday, May 26, 2012.) MGI curation of this mouse gene is considered complete, including annotations derived from the biomedical literature as of July 27, 2007. If you know of any additional information regarding this mouse gene please let us know. Please supply mouse gene symbol and a PubMed ID.

Summary from NCBI RefSeq

[Summary is not available for the mouse gene. This G-protein coupled receptor inhibits adenylyl been associated with schizophrenia. Alternative been described, but it has not been determined]

Summary text based on GO annotations su

- Researchers have inferred from direct
 - participates in the following biolog
 - G-protein coupled receptor
 - activation of phospholipase
 - inhibition of adenylate cyclase
 - positive regulation of neuro
 - response to cocaine [4]

Molecular Function	GO Term	ISO	Count
dopamine receptor activity, coupled via G/Gq	P61169	ISO	1:155856
drug binding	P14416	ISO	1:164563
drug binding	P61169	ISO	1:155856
G-protein coupled receptor activity	IDA		1:88364
ionotropic glutamate receptor binding			
receptor activity			
receptor binding			
signal transducer activity			
Cellular Component	acrosomal vesicle		
Cellular Component	axon		

C

Figure 10. The GO annotation details for Drd2 displayed as summary text (A), table (B) or graph (C). Only a portion of each summary is shown. There are 175 annotations total.

have used NLP to expedite the curation of literature (16), this can be especially difficult to do when applied to mouse biology because of the integration of human and mouse studies within the same description of results. The concepts captured by the GO cannot be gleaned just from simple text matching of terms, but must also take into account inferences that reflect a given context. Additionally, an understanding of the nature of an experimental assay is important to correctly use the information as evidence of a particular result. While we continue to work with NLP developers to design a system to automate identification and tagging of papers (17), it is clear that the complexity of understanding the information in a biomedical publication requires the intervention of an experienced biologist-curator in the process.

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