Using Evolutionary Conserved Modules in Gene Networks as a Strategy to Leverage High Throughput Gene Expression Queries

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Abstract

Background: Large-scale gene expression studies have not yielded the expected insight into genetic networks that control complex processes. These anticipated discoveries have been limited not by technology, but by a lack of effective strategies to investigate the data in a manageable and meaningful way. Previous work suggests that using a pre-determined seed-network of gene relationships to query large-scale expression datasets is an effective way to generate candidate genes for further study and network expansion or enrichment. Based on the evolutionary conservation of gene relationships, we test the hypothesis that a seed network derived from studies of retinal cell determination in the fly, Drosophila melanogaster, will be an effective way to identify novel candidate genes for their role in mouse retinal development.

Methodology/Principal Findings: Our results demonstrate that a number of gene relationships regulating retinal cell differentiation in the fly are identifiable as pairwise correlations between genes from developing mouse retina. In addition, we demonstrate that our extracted seed-network of correlated mouse genes is an effective tool for querying datasets and provides a context to generate hypotheses. Our query identified 46 genes correlated with our extracted seed-network members. Approximately 54% of these candidates had been previously linked to the developing brain and 33% had been previously linked to the developing retina. Five of six candidate genes investigated further were validated by experiments examining spatial and temporal protein expression in the developing retina.

Conclusions/Significance: We present an effective strategy for pursuing a systems biology approach that utilizes an evolutionary comparative framework between two model organisms, fly and mouse. Future implementation of this strategy will be useful to determine the extent of network conservation, not just gene conservation, between species and will facilitate the use of prior biological knowledge to develop rational systems-based hypotheses.

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Introduction

The emergence of system-wide approaches (‘-omics’; e.g., genomics, proteomics, metabolomics, etc.) and related technologies has quantified changes that accompany biological processes or disease states has resulted in an explosion in the amount of data collected by researchers. Investigators across all areas of biology have designed large-scale experiments to capture a broader systems-based understanding of gene or protein expression changes that accompany their process of interest. However, many have found that such datasets are too large to be immediately informative, and extracting useful information from these datasets is dependent upon additional analysis.

One strategy to analyze such data is to generate gene network models using one of several analytical frameworks [1–5]. In theory, these network approaches have two advantages: they should accelerate the rate of novel discoveries by automating data analysis and they should be more immune to experimenter bias. This use of computational strategies will potentially lead to discoveries from omics data without a priori knowledge of the system. However, these computational approaches require a tremendous amount of biological data. For example, if an investigator wants to understand which genes function together during a particular developmental process, she might profile changes in gene expression over developmental time. Ideally the number of conditions (e.g., ages, experimental perturbations) under which gene expression is measured should be much larger than the number of genes being profiled in order to obtain an accurate estimate of the covariance matrix upon which the network of all genes is based [6]. Thus, for a microarray experiment that measures the expression of 5000 genes, one should measure the expression of each gene under more than 5000 different conditions. Even collection of 20% of the ideal amount of data for robust analyses is both time and cost prohibitive for most
investigators. As a consequence, the majority of biologists collect datasets that are too small for effective computational analysis and too large for systematic and efficient consideration of candidate molecules. This data limbo is a limiting factor to the growth of the field of systems biology.

While it is essential that the development of computational tools and approaches continue, it is also essential that efforts are made to establish ‘biological heuristics’ that will allow benchtop investigators to perform meaningful analyses on the sometimes limited amounts of data they are capable of collecting. A key first step in this process is to consider the development of strategies to efficiently query omics data, as opposed to exhaustively analyzing it. The use of biological heuristics is a flexible strategy, which utilizes prior biological knowledge of the system to design queries. These queries ask specific questions about relatively small groups of interacting genes and return manageable numbers of candidate genes for further analysis at the bench.

Our approach to querying high-throughput data utilizes prior biological knowledge by starting with a ‘seed-network’ of genes, and is based on the paradigm that the expression of genes that function together will change in similar ways over time (i.e., their expression will be correlated). The basic assumption is that if a gene is correlated with one member of the seed network, it may be involved in the process of interest; however, if the same gene is correlated with multiple members of the seed-network it much more likely to be involved in that process (e.g., retinal cell fate determination). One of us has demonstrated previous success identifying gene candidates in development of rod photoreceptors by using a seed-network-based heuristic to query high throughput data [7], and this success motivated our efforts to further develop strategies to identify effective seed networks to query large datasets.

Here we employ our seed-network approach to a genetic comparison of two important models in the study of retinal development: the fly, Drosophila melanogaster, and the mouse, Mus musculus. Despite the morphological and developmental disparity of the fly compound eye [8,9] and the mouse camera-type eye [10,11], gene conservation during both fly and mouse retinal development is well-documented [12–16] and there is an implicit assumption of gene regulatory network conservation as well [17,18]. However the networks are not completely congruent [19]. We test the hypothesis that gene relationships established in the developing fly retina can be identified in correlation networks generated using gene expression data from the developing mouse retina. Further, we hypothesize that the resulting mouse network will be an effective tool to discover candidate genes and gene networks that function during mammalian retinal development. In this report, we take advantage of two biological systems by constructing a ‘comparative seed-network’ based on studies of retinal determination in fly and use it to query gene expression data from the developing mouse retina. Our study was guided by three objectives: 1) to construct a literature-based seed network representing the relationships between genes involved in retinal determination in the fly; 2) to determine whether the network relationships of fly genes are identifiable among homologous mouse genes in expression correlation networks generated from the developing mouse retina; and 3) to assess whether this strategy, based on evolutionary comparison between model organisms, is a useful method to identify biologically relevant candidate genes important in retinal determination. Based on these objectives, our results demonstrate successful application of this strategy within our experimental system and provide a clear framework to evaluate this approach in other biological areas.

**Figure 1. The fly seed network based on experimental results in the literature.** Positive correlations between genes are represented by blue edges and negative correlations are represented by red edges. Full names and their abbreviations for Drosophila genes are provided in Table 1. doi:10.1371/journal.pone.0012525.g001

**Results**

Seed network construction in fly

Seed networks are graphs that represent relationships among genes during a biological process, such as retinal determination. These relationships may be physical interactions or causal relations by direct or indirect means, and are represented as edges in the graph or connections (links) in the gene network. We used the results of published experimental studies on eye differentiation in fly to identify a set of 18 genes implicated in fly retinal development, which was built off of the fly retinal determination gene network (RDGN) [12]. We integrated these data into a comprehensive fly ‘seed network’ (Figure 1) based on the work described in File S1.

| Table 1. Fly genes from the seed network and their putative mouse homologs. |
|---|
| **Fly gene** | **Mouse homologs** |
| toy (twin of eyeless) | Pax6 |
| ey (eyeless) | Pax6 |
| so (sine oculis) | Six1/Six2 |
| eya (eyes absent) | Eya1/Eya2/Eya3 |
| dach (dachshund) | Dach1 |
| dpp (decapentaplegic) | Bmp4 |
| tsh (teashirt) | Sdc2ag33 z |
| hth (homothorax) | Meis2 (Meis homoobox 2) |
| hh (hedgehog) | Shh (Sonic hedgehog) |
| N (notch) | Notch1 |
| wg (wingless) | Wnt4 |
| optix | Six3/Six6 |
| ato (atonal) | Atoh7 (Atonal7) |
| h (hairy) | Hes1 |
| eyg (eye gone) | Pax6;Soa |
| Ci (Cubitus interruptus) | Gli1 (GLI-Kruppel family member) |
| oc (ocelliless) | Otx1 |
| shf (Shifted) | Wif1 (Wnt inhibitory factor 1) |

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# Table 2. Correlation of network seed genes in each of the four expression datasets of mouse.

| Mouse network seed genes | I     | II             | III            | IV              |
|--------------------------|-------|----------------|----------------|-----------------|
| Pax6                     | -     | Notch1 (0.891) | Tshz1 (1.0)    | Notch1 (0.786), Six3 (0.652), Six6 (0.667), Gli1 (0.810) |
| Six1                     | NA    | NA             | NA             | -               |
| Six2                     | NA    | NA             | NA             | Eya1 (−0.667), Bmp4 (−0.714) |
| Eya1                     | NA    | NA             | -              | Six2 (−0.667), Bmp4 (0.905), Notch1 (−0.762), Six3 (−0.690) |
| Eya2                     | Atoh7 (−0.826) | NA | NA | Eya3 (0.761), Dach1 (0.667), Six6 (0.738) |
| Eya3                     | -     | NA             | NA             | Eya2 (0.761), Dach1 (0.667), Wnt4 (0.667) |
| Dach1                    | NA    | NA             | NA             | Eya2 (0.66), Eya3 (0.762) |
| Bmp2                     | NA    | NA             | NA             | Gil2 (0.66) |
| Bmp4                     | NA    | NA             | Hes1 (1.0)     | Six2 (−0.714), Eya1 (0.905), Notch1 (−0.810) |
| Tshz1                    | NA    | NA             | Pax6 (1.0)     | -               |
| Meis2                    | NA    | NA             | NA             | NA               |
| Shh                      | NA    | NA             | NA             | Six6 (−0.66), Gli1 (−0.69), Atoh7 (−0.922) |
| Notch1                   | NA    | Pax6 (0.891)   | Six3 (0.9)     | Pax6 (0.786), Eya1 (−0.762), Bmp4 (−0.810), Six3 (0.881), Six6 (0.881) |
| Wnt/wnt4                 | NA    | NA             | NA             | Eya3 (0.667), Gil2 (−0.667) |
| Six3                     | Hes1 (0.706), Gli1 (0.923), Atoh7 (0.690) | - | Notch1 (0.9) | Pax6 (0.762), Eya1 (−0.690), Six6 (0.833), Notch1 (0.881), Gli1 (0.667) |
| Six6                     | Hes1 (0.696), Atoh7 (0.658) | - | NA | Pax6 (0.667), Eya2 (0.738), Shh (−0.66), Six3 (0.833), Notch1 (0.881) |
| Atoh7                    | Eya2 (0.825), Hes1 (0.799), Six3 (0.690), Six6 (0.658) | - | NA | Bmp2 (0.731), Shh (−0.922) |
| Hes1                     | Gli1 (0.705), Six3 (0.706), Six6 (0.696), Atoh7 (0.799) | NA | Bmp4 (1.0) | NA |
| Gli1                     | Hes1 (0.705), Gli1 (0.924) | NA | NA | Pax6 (0.810), Shh (−0.69), Six3 (0.667) |
| Gli2                     | NA    | NA             | NA             | Bmp2 (0.66), Wnt4 (−0.667) |
| Otx2                     | NA    | -              | NA             | NA               |
| Wif1                     | NA    | NA             | NA             | NA               |

The mouse expression datasets are I [20], II [21], III [22], IV [23]. Numbers in parentheses are the positive or negative correlation coefficient of seed genes in each mouse datasets. "-" indicates that the seed gene is present in the dataset, but is not correlated with other seed genes. "NA" indicates that the seed gene is not present in the dataset. doi:10.1371/journal.pone.0012525.t002
### Table 3: List of 46 candidate genes found in three or more seed gene lists.

| Candidate gene | Description | Function | Reported links | Fly Homolog |
|----------------|-------------|----------|----------------|-------------|
| Appl²          | Amyloid precursor protein family member in the Alzheimer's disease amyloid beta protein superfamily | Embryonic development of several brain regions [35,45,86] | ***CNS development | γl amyloid protein precursor-like (Appl) |
| Blvra²         | biliverdin reductase A | Heme catabolic process [42,69] | Brain development | None; Only identified in vertebrates |
| Bola²          | Stress-induced morphoprotein, Bola type superfamily | Involved in cell proliferation or cell-cycle regulation [51] | Development | CG33672 |
| Capn2²         | calpain 2 | Calcium-activated neutral proteases; blastocyst development [29,50,66,74] | Retina | CalpA-RB |
| cdk1⁵c (= CDK1, Kip2, p57Kip2)⁵ | cyclin-dependent kinase inhibitor 1C family | Cell cycle arrest [39,40,65,77] | Retina/CNS development | dap (dacapo) CG1772 |
| crmp²          | collapsin response mediator protein 1 | Hydrolase activity [34,82] | Retina/Brain development | CRMP |
| dpsvl⁴ (= CRMP-3, Crmp3, DPY4, Dop-4, Ulp4, unc-33-like phosphoprotein 4)⁴ | dihydroxypirimidinase-like 4 in the cyclic amidohydrolases protein superfamily | Plays a role in dendrite arborization, guide-posts navigation, and neuronal plasticity [46,70,71,83] | Brain development | CRMP |
| dynl1b (= Dynl1, Tctex-1, Tctex1)⁴ | dynein light chain, Tctex-type protein superfamily | Multicellular-based processes [54,85] | Brain development | DyC90F |
| ebf1 (= O/E-1, Olf-1, Ofl1)⁴ | early B-cell factor 1 in the transcription factor, collier type protein superfamily | Cell cycle arrest [39,40,65,77] | Retina/CNS development | kn (knot) |
| Ephb²          | Eph receptor B2, part of tyrosine-protein kinase protein superfamily | Axon guidance in RGC [30] | ** ***Retina development | eph receptor tyrosine kinase (eph) |
| fabp5 (= E-FABP)⁴ | fatty acid binding protein 5, epidermal | Expressed in neurons during axonal growth in development and nerve regeneration; involved in RGC differentiation and axon growth [26,58] | Retina/Brain development | Cg6783 |
| Fyn (= SLK, SYN)⁴ | Protein tyrosine kinase | Involved in axon guidance; RGC targeting in the Superior colliculus [57] | ***Retina/Brain development | Btk family kinase at 29A (Btk29A); CG8049 |
| gng5 (= Gji5S, Ggamma5)⁴ | guanine nucleotide binding protein (G protein), gamma 5 | G-protein coupled receptor protein signaling pathway; expressed in precurser cells during neurogenesis [20,28] | Retina/Brain development | G protein γ 1 (Ggamma1) |
| gstm5 (= GST)⁴ | glutathione S-transferase, mu 5 | Transferase activity; Expressed in developing lens and retina and may play a role in apoptosis suppression [24,25] | Retina | Probably distantly related to glutathione S transferase S1 (GstS1); no mu homolog in Drosophila |
| Hsf1²          | Heat shock factor 1 | Expressed in RGC [48] | Retina | Heat shock factor (Hsf) |
| isoc1²         | isochorismatase domain containing 1 | Metabolic processes; expressed in medulla oblongata of postnatal adult [68] | Brain development | CG3663, CG11333 |
| knob2 (= Kvbeta2)² | potassium voltage-gated channel, shaker-related subfamily, beta member 2 | Modulates action potential propagation and neurotransmitter release in hippocampal formation [63] | Brain development | Hook (Hk) |
| krtp2²         | keratinocyte associated protein 2 | Protein amino acid N-linked glycosylation via asparagine [20] | Retina/CNS development | CG31460 |
| lsm3²          | LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) | mRNA processing, nuclear mRNA splicing, via spliceosome [60] | Retina/CNS development | CG31184 |
| mapre² (= Eb1) | microtubule-associated protein, RP/EB family, member 1 | Kvbeta2 axonal targeting depends on its ability to associate with the microtubule plus-end tracking protein EB1 [44,75] | Brain development | Eb1, CG15306, CG32371, CG18190, CG43055, CG31907, CG2955 |
| Ndn*           | Required for development of GnRH secreting neurons [61] | ***Brain Development | None |
| Candidate gene | Description | Function                                                                 | Reported links | Fly Homolog                  |
|----------------|-------------|--------------------------------------------------------------------------|----------------|-------------------------------|
| nme2 (= nm23-M2)<sup>b</sup> | non-metastatic cells 2, protein (NM23B) expressed in mRNA levels increased during retinal degeneration [27,49] | Retina/Brain development | abnormal wing discs (awd) |
| nsmce1<sup>b</sup> | non-SMC element 1 homolog (S. cerevisiae) | DNA recombination and repair | NA; No papers found in Pubmed under nsmce1 | CG11329 |
| Pafah1b3<sup>b</sup> | platelet-activating factor acetylhydrolase, isoform 1b, alpha1 subunit | May play a role in neuronal migration (based on identified human mutations associated with brain malformation) [79] | Brain development | Platelet-activating factor acetylhydrolase alpha (Paf-Aholpha) |
| Pcyt2<sup>d</sup> | phosphate cytidylytransferase 2, ethanolamine | Biosynthesis of ethanolamine phospholipids, KO of pcyt2 embryonic lethal [43] | Development | Phosphoethanolamine cytidylytransferase (Pect) |
| prps1<sup>f</sup> | phosphoribosyl pyrophosphate synthetase 1 | X-linked enzyme mediates the biochemical step critical for purine metabolism and nucleotide biosynthesis; loss of function associated with optic atrophy [38,52] | Retina | CG6767 |
| prrt1<sup>b</sup> | proline-rich transmembrane protein 1 | Expressed in mouse retina [108] | Retina | None; homologs only in vertebrates |
| psme1 (= PA28a)<sup>b</sup> | proteasome (prosome, macropain) 28 subunit, alpha | Component in the ubiquitin-proteasome system that may play an important role in neuronal apoptosis [41] | Brain | REG |
| Rac1<sup>b</sup> | Small gtpase RAS-related C3 botulinum substrate 1 | Involved in actin cytoskeleton regulation; expressed in developing mouse retina, involved in RGC axon behavior; essential for brain development [36,62,81] | **Retina/Brain development** | Rac1, Rac2 |
| rpl10<sup>b</sup> | ribosomal protein 10 | Translation | NA | Qm |
| rpl27a<sup>a</sup> | ribosomal protein L37 | Expression decreases during maturation of cultured human fetal astrocytes [56] | Brain development | Rpl27A |
| rpl32<sup>b</sup> | ribosomal protein 37 | Dimorphic expression in developing zebra finch brain [80] | Brain development | Rpl37A, Rpl37B |
| rps11<sup>b</sup> | ribosomal protein S11 | Translation | NA | Rps11 |
| rps26<sup>b</sup> | ribosomal protein S26 | Translation | NA | Rps26 |
| rps3<sup>h</sup> | ribosomal protein S3 | Neuroprotective effect in the brain (hippocampus) exposed to ischemia [47] | Brain | Rps3 |
| rps5<sup>b</sup> | ribosomal protein S5 | Translation | NA | Rps5a, Rps5b |
| Smrpe<sup>b</sup> | small nuclear ribonucleoprotein E | mRNA processing; expressed in mouse eye and brain on embryonic day 13.5 and postnatal day 0 [60] | Retina/CNS development | CG18591 |
| Snrpg (= SMG)<sup>b</sup> | small nuclear ribonucleoprotein polypeptide G | mRNA processing; expressed in mouse eye and brain on embryonic day 13.5 and postnatal day 0 [60] | Retina/CNS development | Small ribonucleoprotein G (SmG) |
| Stmn2 (= SCG10)<sup>b</sup> | stathmin-like 2 in Op18/stathmin protein superfamily | Microtubule destabilization; RGC growth and cone behavior; expression in mature RGCs and amacrine cells in rat retina [67,78] | Retina development | stathmin (stai); CG11298 |
| tex261 (= TEG-261)<sup>d</sup> | testis expressed gene 261 | Positive regulation of apoptosis | NA | CG3500 |
| tmsb10 (= Ptmbs10, TB10)<sup>b</sup> | thymosin, beta 10 | Actin cytoskeleton organization; involved in the dynamics of actin polymerization during migration and extension of neurons in the cerebellum [31,33] | Brain/CNS development | Ciboulot (cib) |
**Table 3. Cont.**

| Candidate gene | Description | Function | Reported links | Fly Homolog |
|----------------|-------------|----------|----------------|-------------|
| vam2b          | torsin family, member A | TOR2A mRNA expression and is spliced into preprosalasin; Salasin-beta stimulates the release of arginine-vasopressin from rat pituitary [76] | Brain | torp4a |
| Txn1b          | Thioredoxin 1 | Redox activity; expressed by RGC; protective against oxidative insult [64] | Retina | Thioredoxin2 (txn-2); Thioredoxin T (TxrT); CG13473; deadhead (dhd) |
| unc13b (= Munc13-2, Unc13b2)* | unc-13 homolog B (C. elegans) | Responsible for vesicle priming in glutamatergic nerve cell and gamma-aminobutyrategic (GABAergic) synapses of the hippocampus [72,84] | Brain | unc-13 |
| wdr78b         | WD repeat domain 78 | Expressed in mouse eye during embryonic day 12.5,13.5, and 14.5 and mouse retina [108] | Retina | CG7051; CG13930 |
| Zic2b          | Zinc finger protein of cerebellum 2 | DNA binding; guidance of RGC axons (Zic2 expressed by ipsilateral projecting neurons-by inducing expression of ephH1); important for forebrain formation; shown to interact with Gli proteins [53,55,73] | **Retina/Brain Development** | Odd-paired (opa) |

Groups of correlated seed genes are given a letter designation:
- *Six3, Notch1, Tsh21;
- *Six3, Eya1, Notch1;
- *Eya1, Notch1, Bmp4;
- *Six3, Eya1, Notch1, Bmp4;
- *Six3, Notch1, Dach1;
- *Eya2, Eya3, Notch1, Tsh21;
- *Eya1, Notch1, Tsh21;
- *Six3, Eya1, Notch1, Pax6.

Candidate gene synonymies are provided in parentheses. “RGC” are retinal ganglion cells. “NA” indicates no previous report of the candidate gene specifically involved in retina, retinal development, CNS development, brain development, or development as searched in PubMed.

*CRMP3 is a direct target of calpain that cleaves CRMP-3 at the N terminus [46].

**Biological Process GO Annotation of Neural Retina Development (0003407).

***Biological Process GO Annotation of Nervous System Development (0007399).

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Extraction of homologous seed network from mouse datasets

To determine whether the gene relationships represented in the fly seed network are represented in the developing mouse retina, we first converted the literature-based fly seed network into a mouse gene network of putative homologs (Table 1). Then we used BioNet Workbench [http://bionetworkbench.sourceforge.net/] to query four previously published gene expression datasets (I-IV) from mouse [20–23]. The datasets were queried for pairwise correlations of >0.65] between all mouse genes that are homologous to fly seed network members (“seed genes”). A summary of the seed genes and their pairwise correlation values (if >0.65] in each of the mouse datasets are given in Table 2. The result was a mouse seed network “extracted” from published gene expression datasets for mouse.

Based on the finding that a subset of relationships from the fly seed network appear to be conserved in the developing mouse retina, we hypothesized that the extracted seed-network (ESN) of mouse gene relationships would be useful for querying the mouse gene expression data to identify additional candidate gene network members. To identify candidates, genes correlated with each gene in the extracted seed-network were retrieved from each dataset and the lists were compiled. Lists of genes correlated with each ESN gene were analyzed to identify genes that correlated with more than one gene in the ESN. Based on the paradigm that genes correlated with multiple ESN genes are likely to have a functional relationship to the gene network, we focused our analysis on 46 candidate genes that were correlated with three or more ESN members (Table 3). Among these 46, 39 genes were correlated minimally with *Eya1, Notch1 and Six3*. We evaluated the relevance of candidate genes identified by this comparative seed-network approach in three ways.

First, we performed a manual literature search to find reports of candidate genes’ association with the retina, retinal development, brain development or other developmental processes. Results from this manual search are given in Table 3. Forty out of 46 (86%) candidate genes have been previously reported to be associated with one or more of these topics [20,24–36]. Additionally, eight candidate genes (17%) are associated with retinal ganglion cells (RGCs) or RGC development in previous experimental studies [26,30,36,48,53,55,58,62,64,67,73,78,81] (Table 3).

Second, we examined the spatial and temporal expression of six candidate genes in the developing mouse retina. We chose to examine candidates that had been previously reported to be associated with the developing brain, but not the developing retina, and had commercially available antibodies. Using immunohistochemistry in retinal tissue sections from mice ages embryonic day E13, E15, E17 and postnatal day P0, P5 and P10, we characterized the expression of APLP2, DPYS14, NDN,
PAFAH1B3, PSME1 and TMSB10. Candidates were considered highly relevant if they were: 1) expressed in the developing retina, 2) exhibited specific (as opposed to diffuse) localization in the developing retina, and/or 3) the localization of the immunoreactivity changed as the retina matured. Based on these criteria, five of the six candidate genes tested that had not been previously associated with retinal development (aplp2, ndn, pafah1b3, psme1 and tsmb10) were considered good candidates for further investigation (Figures 2, 3, 4, 5 and 6).

Third, we used the biological process GO annotations Nervous System Development (0007399) and Neural Retina Development (0003407), to statistically evaluate our candidate list. In the list of 46 candidates identified by using our seed-network approach, seven of the genes had a Nervous System Development GO annotation. By using a Fisher’s exact test we determined that Nervous System Development is over-represented among the group of candidate genes. The p-value for this test was 0.026, which represents the probability of seeing seven or more Nervous System Development genes in a list of 46 genes randomly selected from the 8344 genes represented on the Murine Genome U74Av2 array. Because it would be unlikely to see so many Nervous System Development genes in our candidate list of 46 genes by chance, our results suggest that Nervous System Development genes were overrepresented in our candidate list.

In summary, our analysis identified a network of 46 highly correlated candidate genes. Expression of 22 (~47%) of these candidate genes has been previously reported in the retina or developing retina (see references in Table 3), although their specific relationship to genes within the retinal determination gene network has not been reported. We examined six candidate genes that had previously been associated with brain development, and determined that five of these genes have dynamic spatial and temporal expression in the developing mouse retina. Finally, of these 46 mammalian genes, 42 (~91%) have homologs in fly, making them potential candidates for studies of fly retinal development as well. These findings demonstrate the powerful advantages of integrating evolutionary comparisons and systems approaches, even when approaching well-studied biological questions.

Discussion

The compound eye of Drosophila is an outstanding model system to study the molecular basis of eye specification, in part, because retinal development is an organized, step-wise process with clearly demarcated regions of cell differentiation and patterning [8,87]. These properties of the fly model have facilitated the elucidation of genetic networks involved in retinal cell differentiation and the

Figure 2. Dynamic protein expression of APLP2 in developing mouse retina. APLP2-IR in the E13 mouse retina was slightly more intense in cells in the inner and outermost retina (A). In the E15 mouse retina (B), APLP2-IR was observed throughout the thickness of the retina, though the most intense immunoreactivity remained localized to cells in the in the inner and outermost retina. By E17, APLP2-IR was largely restricted to cells and processes in the inner one-third of the retina (C). By the day of birth (P0), APLP2-IR was restricted to cell bodies in the ganglion cell layer (GCL), the IPL and the inner nuclear layer (INL). In the P5 retina, APLP2-IR was most prominent in the IPL, GCL and OFL, though some punctate APLP2-IR remained in the INL (D). By P10, APLP2-IR was further restricted to the IPL and OFL, with punctate immunoreactivity only present in the GCL. Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NBL, neuroblastic layer; OFL, optic fiber layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium. Bars, 30 μm.

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identification of key genes required for retinal development in fly. Comparative studies between model organisms [12,18] led to discoveries that homologous genes play important and similar roles in fly and mammalian retinal development and many of these key genes have similar connectivity in gene networks [19]. This principle of gene network conservation has motivated our development of the seed-network strategy, which we have presented here, and provides a way to validate our novel heuristic approach.

We tested our strategy using gene expression datasets from the developing mouse retina. The results from this study support our hypothesis that gene relationships in the developing fly retina are identifiable in correlation networks generated using gene expression data from the developing mouse retina. While not all gene relationships in the fly network were identified in the mouse ESN, this is not unexpected. Our results provide support for the assumption that there will be a degree of conservation within genetic networks of homologous genes, even between highly divergent species such as fly and mouse. Complete congruence between the RDGN of fly and mouse would be surprising given that these organisms possess highly divergent eye morphologies. Our results also support the second hypothesis that the mouse network derived from relationships between homologous genes from the fly RDGN [i.e. our extracted seed network (ESN)], would be an effective way to discover high quality candidate genes involved in retinal development in mouse. Our queries identified a reasonable number (46) of candidates, when compared to the hundreds or thousands of genes that correlate with a single gene of interest. The majority of our candidate genes were correlated (positively or negatively) with the same three seed genes (Notch1, Eya1 and Six3) suggesting that these three seed genes are at the functional core of this network regulating retinal development in mouse.

At the heart of our approach is the development of biological heuristics to focus queries of relatively sparse (albeit typical) expression datasets from the developing mouse retina. It is important to note that this approach is intended to facilitate the formulation of hypotheses by providing a mechanism to integrate prior biological knowledge, but not intended to make conclusions about the function or assign significance to the candidates. The use of relationships among genes as a biological heuristic to query high-throughput data, as opposed to queries based on single genes, appears to be more profitable and efficient for the identification of additional candidates. Thus, the candidate genes we identified in this study are not end points, but are the basis of hypotheses to guide future experimental work. Traditional wet-lab experiments will be required to test these hypotheses of the specific role of each candidate gene and its placement in the gene regulatory network during mouse retinal development.

From a comparative evolutionary perspective, our results underscore the importance of looking for conservation of networks, and not just conservation at the level of individual genes. While gene orthologs may function in a similar way in a complex process or a disease state in different organisms, it is the
conservation of not only the gene, but of its relationships to other genes in a network, that dramatically increases the likelihood that the gene, in fact, functions similarly. Although it has been directly demonstrated in only a few cases [19,88–91], regulatory network conservation has long been the rationale for the use of model organisms to study human diseases. Comparative studies that investigate the extent of conservation in developmental regulatory networks (and of characteristics, such as modularity, connectivity, etc.) are beginning to identify common themes in networks that direct organogenesis, e.g., [92]. While it is unreasonable to expect that genetic regulatory networks controlling the development of organs in highly divergent organisms will be conserved in their entirety, application of the approach proposed here to identify conserved network modules should allow systems biologists to better capitalize on what is known in one species to advance discovery in another.

Materials and Methods

Our biological heuristic strategy is described below and summarized in Figure 7.

Construction of the fly seed network

We identified 18 genes in *Drosophila* that are involved in the retinal determination gene network (RDGN), based on published literature. Previous researchers have identified individual relationships of these genes to one another and these experimentally-determined relationships among the 18 genes were the basis of our fly seed network (see descriptions and citations in File S1).

Homolog identification of seed network genes

Homolog identification can be difficult when comparing genomes across great evolutionary time as a result of sequence evolution and paralogous duplication events within a lineage. Because of these issues, we identified putative mouse orthologs of the *Drosophila* seed network manually, using a combination of approaches, including examination of the genomic databases FlyBase [http://flybase.org/] [93] and Mouse Genome Informatics [MGI; http://www.informatics.jax.org/] [94], phylogenetic methods presented in TreeFam [http://www.treefam.org/] [95,96], and HomoloGene [http://www.ncbi.nlm.nih.gov/homologene] [97]. Additional assignment of orthology between fly and mouse genes was based on experimental data. For example, the mouse has three *Teashirt* (*tsh*)-like genes, *Tshz1*, *Tshz2* and *Tshz3*, all of which can rescue *tsh* null mutants and induce ectopic eyes in the fly [98]. Likewise, we designated the *Pax6* isoform, *Pax6* (*5a*), found in humans and mouse, as the ortholog for fly *eyg* because the genes are structurally related [99], and we treated the mouse gene *Math5* (*Atonal7*) as the homolog to the fly gene *Atonal* based on others’ work [100,101] reviewed in [19]. Finally, qualitative and functional comparisons of the mouse genes *Six3* and *Six6* to *so* and *optix* in fly, suggest that *optix* should be treated as an ortholog of *Six3* and *Six6* [102], reviewed in [19]. Table 1 lists fly seed network genes and their mouse homolog assignment based on these data.
Description of mouse data sets and construction of ESN

Published, freely available datasets measuring gene or protein expression in the developing mouse retina at multiple time points were collected and preprocessed as described in Hecker et al. [7]. Each dataset represents expression data collected from developing mouse retinae at multiple time points and includes: a SAGE (serial analysis of gene expression) of whole retina from Blackshaw et al. [20] was downloaded from online supplementary material; one cDNA microarray of whole retina from Zhang et al. [21] was downloaded from online supplementary material; and two Affymetrix microarrays of whole retina, the Mu74Av2 chip from Liu et al. [22] was downloaded from GEO (GDS 1845) and the Mu74Av2_1 chip from Dorrell et al. [23] was downloaded from http://www.scripps.edu/cb/friedlander/gene_expression/). These mouse datasets were designated as I, II, III, and IV, respectively, and were saved in BioNet Workbench [http://bionetworkbench.sourceforge.net/] for analysis.

We calculated Spearman Rank pairwise correlations in each mouse expression dataset using BioNet Workbench to construct the extracted seed network (ESN) for mouse. Correlation networks provide a visual representation of pairwise associations between genes in large data sets consisting of expression measurements for hundreds or thousands of genes. In a gene or protein expression correlation network, the nodes represent the genes or proteins and weighted links model interactions between them. The weight associated with a link between a pair of nodes models the correlation estimated from measurements of expression (e.g., mRNA or protein) levels of the corresponding genes across a set of experimental conditions or time points. The Spearman rank correlation measure, which assumes only an arbitrary monotonic, not necessarily linear, relationship between variables being correlated, has been demonstrated to be effective for detecting functional relationships between genes [103]. Correlation coefficients using time-course expression data are calculated by a measure of how the expression levels between any given pair of genes changes over time. Genes that are perfectly correlated with one another have a correlation coefficient of 1. Gene pairs whose expression is exactly the opposite of one another have a correlation coefficient of −1. Two genes whose expression is not correlated (no different than random) have a correlation coefficient of 0. In cases where multiple mouse paralogs for a single fly gene are present, each paralog was queried separately. Not all mouse seed genes were present in all datasets.

A link between a pair of seed network genes is supported by a dataset if the corresponding genes are positively or negatively correlated in that dataset, with the absolute value of correlation greater than or equal to 0.65 in one of the mouse datasets (I–IV). Our choice of the threshold of 0.65 for correlation was influenced by similar choices in previous studies [104–106] that have revealed biologically relevant links between co-expressed genes. It should be noted that we do not assign statistical significance to the value of the correlation coefficient, but rather consider the value a flexible tool that can be used at the discretion of the investigator to filter gene lists to a manageable number of candidates. This is appropriate, as our strategy is designed to facilitate the generation of hypotheses, not conclusions.

Figure 5. Dynamic protein expression of Psmel in developing mouse retina. PSME1-IR in the E13 mouse retina was diffusely distributed throughout the E13, E15 and E17 retinas (A–C). However, by P0, PSME1-IR was no longer detectable above background (D). Similarly, no PSME-IR was detected in the P5 or P10 retinas (E, F). Abbreviations same as in Figure 2. Bars, 30 μm.

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Generating candidate gene lists

To identify candidate genes that may be involved in the gene network controlling mouse development, we used genes from the extracted seed-network (ESN; mouse homologs of fly RDGN genes whose pairwise expression correlation coefficients were $|0.65|$ in at least one dataset) to query large-scale gene expression datasets of the developing retina (I–IV). Each of the 17 genes from the ESN (a.k.a. seed genes) in mouse was examined separately in datasets I–IV to develop candidate gene lists. Lists were compiled by identifying genes that were correlated with individual seed genes, with a correlation coefficient greater than $|0.65|$. Then, gene lists for all seed genes were compared to identify candidate genes that correlated with more than one seed gene. Genes that correlated with more than three seed genes were investigated for potential biological relevance in mouse retinal development.

Biological relevance of candidate genes was assessed using manual PubMed searches with the following search terms: retina, retinal development, CNS development, brain development, development. Gene synonymies used in the literature searches of the candidate mouse genes are listed in Table 3. Putative Drosophila homologs of the mouse genes from the candidate gene list were identified using FlyBase, the Mouse Genome Database (MGD), and TreeFam. When necessary, paralogous genes were included to more fully capture gene homology between the two organismal models.

In order to determine if the GO annotation Nervous System Development was over-represented among the 46 candidate genes, a Fisher’s exact test was used. Over-representation was declared if the number of genes with the GO annotation of interest on our candidate list was significantly higher than would be expected by chance, i.e., if the observed number of Nervous System Development genes was greater than would be expected when randomly selecting 46 genes from a collection of 550 Nervous System Development genes mixed with 7994 other genes. Information from version 2.4.1 of the R statistical software annotation package mgu74av2.db and release 30 of the NetAffx annotation file for the Murine Genome U74Av2 array was combined in order to perform the Fisher’s exact test. Due to relatively frequent changes in probe set annotations and gene symbols, and also due to slight disagreements between the two annotation sources, a conservative analysis was performed. Although there are likely more genes represented on the Murine Genome U74Av2, the total number of genes represented was declared to be 8544. Similarly, the number of genes with Nervous System Development annotation was declared to be 550, although this number is likely high. Using an under-estimate of the total number of genes and an over-estimate of the genes with Nervous System Development annotation results in a higher p-value and thus more conservative results than if the true values were used.

It should also be noted that no probe set was mapped to the gene Pnt1 in either of the current annotation sources (although it

Figure 6. Dynamic protein expression of TSMB10 in developing mouse retina. TSMB10-IR in the E13 and E15 mouse retina was distributed throughout the retina (A, B). In the E17 mouse retina, TSMB10-IR was more intense in the inner one-third of the retina (C). By P0 TSMB10-IR in the mouse retina was largely restricted to the IPL, GCL and OFL (D). Similarly in the P5 and P10 retinas, TSMB10-IR was observed in the IPL, GCL and OFL (E, F). Abbreviations same as in Figure 2. Bars, 30 μm.

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was included in the analysis because of its inclusion in the analysis of previous papers. Removing this gene from the analysis does not affect the significance of our results.

Examining candidate gene expression with immunohistochemistry

To investigate the biological relevance of the candidate genes correlated with the ESN, we examined the spatial and temporal expression of six candidate genes in the developing mouse retina. Tissue was prepared from C57BL/6 mice in a colony maintained at Iowa State University. The gestational period of C57BL/6 mice is approximately 19 days and date of birth is designated as postnatal day 0 (P0). The developmental time series investigated included pups from embryonic days 13, 15 and 17 and postnatal days 0, 5, and 10. Mice were euthanized and their heads were removed and immersion fixed in 4% paraformaldehyde in 0.1M PO4 buffer (pH 7.5). The tissue was cryoprotected in a 30% sucrose solution in 0.1 M PO4 buffer (pH 7.4) and embedded in OCT mounting media. Tissue was sectioned at a thickness of 20 μm on a cryostat and the sections were thaw-mounted onto microscope slides and stored at −20°C. All animal procedures had the approval of the ISU committee on animal care.

Frozen tissue sections were rinsed in 0.5M KPBS and incubated in blocking solution consisting of KPBS containing 1% bovine serum albumin (Fisher, Pittsburgh, PA), 0.4% Triton-X 100 (Sigma), and 1.5% normal donkey serum (Invitrogen) for 2 hours. Cells were incubated in primary antibody overnight at 4°C. The following day slides (tissue or cells) were washed in KPBS containing 0.02% Triton-X 100 after which fluorescent secondary antibody was applied for 2 hours. After washes in KPBS containing 0.02% Triton-X 100, the slides were incubated in 300 μM DAPI diluted in KPBS. The slides were rinsed in KPBS before cover-slipping with Vectashield fluorescence mounting medium (Vector Laboratories, Burlingame, CA). The antibody sources and concentrations used for the immunohistochemical analysis are summarized in Table S1.

Supporting Information

File S1 Seed network construction in fly. Found at: doi:10.1371/journal.pone.0012525.s001 (0.12 MB DOC)

Table S1 The antibody sources and concentrations used for the immunohistochemical analysis. Found at: doi:10.1371/journal.pone.0012525.s002 (0.04 MB DOC)

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Author Contributions

Conceived and designed the experiments: JMS MHWG. Performed the experiments: JMS MHWG. Analyzed the data: JMS MCO MHWG. Wrote the paper: JMS MCO MHWG.
1. Hartemink AJ, Gifford DK, Jaakkola TS, Young RA (2002) Combining location and expression data for principled discovery of genetic regulatory network models. In: Symposium on Biocomputing 2002: 437–449.

2. Markowetz F, Spang R (2007) Inferring cellular networks—a review. BMC Bioinformatics 8 Suppl 6: S5.

3. Magwene PM, Kim J (2004) Estimating genomic coexpression networks using first-order conditional independence. Genome Biology 5: R100.

4. Butte A, Kohane I (2003) Relevance networks: a first step towards finding genetic regulatory networks within microarray data. In: Parmigiani G, Garret ES, Irizarry RA, Zeger SL, eds. The analysis of gene expression data. New York: Springer.

5. Friedman N, Linial M, Nachman I, Pe’er D (2000) Using Bayesian networks to analyze expression data. Journal of Computing Biology 7: 601–620.

6. Johnson RA, Wichern DW (2002) Applied multivariate statistical analysis Prentice Hall.

7. Hecker LA, Alkon TA, Houzagar VG, West Greenlee MH (2009) Using a seed-network to query multiple large-scale gene expression datasets from the developing retina in order to identify and prioritize experimental targets. Bioinformatics and Biology Insights 2008: 91–102.

8. Pupp KS, Mardon G (2004) Genetic control of retinal specification and determination in Drosophila. International Journal of Developmental Biology 48: 913–924 SI.

9. Land MF, Nilsson D-E (2006) Animal Eyes Oxford University Press.

10. L’sevesy F, Cepko CL (2001) Vertebrate neural cell fate determination: lessons from the retina. Nature Reviews 2: 109–118.

11. Walls GL (1967) The vertebrate eye and its adaptive radiation. New York: Haffner Publishing Company. 785 p.

12. Quiring R, Waldford U, Kloster U, Gehring WJ (1994) Homology of the zebus gene of Drosophila to the small eye gene in mice and anura in smooth humans. Science 265: 785–789.

13. Xu P, Woe I, Her HB, D.R., Maas RL (1997) Mouse Eye homologues of the Drosophila eyes absent gene require Pax6 for expression in lens and nasal placodes. Development 124: 219–231.

14. Brown NL, Kanekar S, Vetter ML, Tucker PK, Gemza DL, et al. (1998) Math5 encodes a murine basic helix-loop-helix transcription factor expressed during early stages of retinal neurogenesis. Development 125: 4621–4633.

15. Niiya A, Ohhto H, Kawakami K, Araki M (1998) Localization of Six4/AREC3 in the mouse retina, implications in mammalian retinal development. Experimental Eye Research 67: 699–707.

16. Chen R, Haldor G, Zhang Z, Mardon G (1999) Signaling by the TGF-beta homolog decaentropist function reiteratively within the network of genes controlling retinal cell fate determination in Drosophila. Development 126: 935–943.

17. Wawersik S, Maas RL (2000) Vertebrate eye development as modeled in Drosophila. Human Molecular Genetics 9: 917–923.

18. Gehring WJ, Beo K (1999) Pax6: mastering eye morphogenesis and eye evolution. Trends 15: 371–377.

19. Donner AL, Maas RL (2004) Conservation and non-conservation of genetic pathways in eye specification. Int J Dev Biol 48: 743–753.

20. Blackshaw S, Harpavat S, Trimarchi J, Cai L, Huang H, et al. (2004) Genomic analysis of mouse retina, implications in mammalian retinal development. Experimental Eye Research 67: 621–633.

21. Zhang SS, Xu X, Liu MG, Zhao H, Soares MB, et al. (2006) Biphosphatase pattern of gene expression during mouse retina development. BMC Developmental Biology 6: 48.

22. Liu J, Wang J, Huang Q, Hagedon J, Magdaleno S, et al. (2006) Gene expression profiles of mouse retinas during the second and third postnatal weeks. Brain Research 1098: 113–125.

23. Dorrell MI, Aguilar E, Weber C, Friedlander M (2004) Global gene expression pathways in eye specificiation. Int J Dev Biol 48: 127–132.

24. Birgbauer E, Cosan CA, Sretavan DW, Henkenreuter M (2000) Kinase independent function of Ebf3 ribbons in retinal axon pathfinding to the optic chiasm. Development 127: 1233–1241.

25. Border BG, Lin SC, Griffin WS, Parhase S, Morrison-Rosgova M (1993) Alterations in actin-binding beta-thymosin expression accompany neuronal differentiation and migration in rat cerebellum. Journal of Neurochemistry 61: 2410–2414.

26. Bolt G, Epping JT, Richardson JE, Blake JA, Group annotMGD (2008) The Mouse Genome Database (MGD): mouse biology and model systems. Nucleic Acids Research 36: D724–728.

27. Carpentero P, Anandu R, Gomez-Marquez J (1999) Expression of the thyroïdn beta10 gene in normal and bacitracin acid-treated rat forebrain. Brain Research Mol Brain Res 18: 141–146.

28. Charras E, Mosingier B, Meisselder G, Agraia M, Rogemond V, et al. (2006) Transient alterations in granule cell proliferation, apoptosis and migration in postnatal developing cerebellum of Crmp1-/- mice. Genes to Cells 11: 1317–1352.

29. Shen Y, Tang BL (2006) The amyloid precursor protein and postnatal neurogenesis/neuroregeneration. Biochemical and Biophysical Research Communications 341: 1–5.

30. Cornetta S, Guadoni S, Ciceri G, MONANI M, Zuccaro E, et al. (2009) Essential role of Rac1 and Rac3 GTPases in neuronal development. FASEB J 23: 1347–1357.

31. Davis JA, Reed RR (1996) Role of Olf-1 and Pax-6 transcription factors in mammalian neurodevelopment. Journal of Neuroscience 16: 5042–5049.

32. de Brouwer AP, Williams KL, Dalley JA, van Kulzen AB, Nabuurs SB, et al. (2007) Arts syndrome is caused by loss-of-function mutations in PRPS1. American Journal of Human Genetics 81: 567–578.

33. Dyer MA, Cepko CL (2001) p27Kip1 and p57Kip2 regulate proliferation in distinct retinal progenitor cell populations. Journal of Neuroscience 21: 4259–4271.

34. Dyer MA, Cepko CL (2001) The p37Kip2 cyclin kinase inhibitor is expressed by a restricted set of amacrine cells in the rodent retina. Journal of Comparative Neurology 429: 601–614.

35. El-Kindour BF, Kholodof NG, Yaragina O, Burke RE (2001) The expression of mRNAs for the proteasome complex is developmentally regulated in the rat mesencephalon. Brain Research, Developmental Brain Research 129: 47–56.

36. Eisenh FF, MAINES MD (1998) Immunohistochemical localization of biliverdin reductase in rat brain: age related expression of protein and transcript. Brain Research 672: 29–41.

37. Fullerton MD, Hakimuddin F, Bakovic M (2007) Developmental and metabolic effects of disruption of the mouse CTP:phosphoethanolamine cytidylyltransferase gene (Cpy2). Molecular Cellular Biology 27: 3327–3336.

38. Gu C, Zhou W, Puthenveedu MA, Xu M, Jan YN, et al. (2006) The microtubule plus-end tracking protein EBI is required for Kv1 voltage-gated K+ channel axonal targeting. Neuron 52: 803–816.

39. Guerette S, Chang Y, Hieberger T, Richardson JA, Eckman CB, et al. (2006) Essential roles for the FFE5 amyloid precursor protein-interacting proteins in brain development. EMBO Journal 25: 420–431.

40. Hou SJ, Jiang SX, Deboos A, Huang D, Kelly J, et al. (2006) Calpain-cleaved connexin 36 protein response mediated by Cx36 induces neuronal death after glutamate toxicity and cerebral ischemia. Journal of Neuroscience 26: 2241–2209.

41. Hwang IK, Yoo KY, Kim DW, Kim SY, Park JH, et al. (2008) Ischemia-induced ribosomal protein S3 expression changes and the neuroprotective effect against experimental cerebral ischemic damage. Journal of Neuroscience Research 86: 1823–1835.

42. Ivanov D, Dvojciatkikova G, Barakat DJ, Nathanson L, ShetopaloV VI (2008) Differential gene expression profiling of large and small retinal ganglion cells. Journal of Neuroscience Methods 174: 10–17.

43. Jones NE, Jounay C, Griv J, Newart HJ, Neal MJ (2000) Identification by array screening of altered cell23M2/M2f mRNA expression in mouse retinal degeneration. Molecular Cell Biol Res Commun 4: 20–23.

44. Kanay M, Moseyev G, Agarwal N, Ma JK, Al-Ubaidi MR (2007) Light induces programmed cell death by activating multiple independent proteases in a cone photoreceptor cell line. Investigative Ophthalmology and Visual Science 45: 1009–1019.

45. Ahmad H, Singh SV, Medh RD, Ansari GA, Kurosky A, et al. (1988) Selective localization of G protein gamma3 subunit in the subventricular zones of the lateral ventricle and rostral migratory stream of the adult rat brain. Journal of Neuroscience 19: 1129–1135.

46. Anzuma M, Shearer TR (2008) The role of calcium-activated protease calpain in experimental retinal pathology. Survey of Ophthalmology 53: 150–163.
56. Lee R, Petros TJ, Mason CA (2000) Zip2 regulates retinal ganglion cell axon avoidance of ephrinB2 through inducing expression of the guidance receptor EphB1. Journal of Neuroscience 20: 5910–5919.

57. Lee SS, Seo HS, Choi SJ, Park HS, Lee JY, et al. (2002) Characterization of the two genes differentially expressed during development in human fetal astrocytes. Yonsei Medical Journal 43: 1059–1068.

58. Lim YS, McLaughlin T, Sung TC, Santiago A, Lee KF, et al. (2008) p75(NTR) mediates ephrin-A reverse signaling required for axon repulsion and mapping. Neuron 59: 746–759.

59. Liu JW, Almaguel FG, Bu L, De Leon DD, De Leon M (2008) Expression of E-FABP in PC12 cells increases neurite extension during differentiation: involvement of n-3 and n-6 fatty acids. Journal of Neurochemistry 106: 2013–2029.

60. Lobo MK, Yeh C, Yang XW (2008) Pivotal role of early B-cell factor 1 in development of striatogiral medium spiny neurons in the matrix compartment. Journal of Neurosciences Research 86: 2134–2146.

61. Mitchell DC, Bryan BA, Liu JP, Liu WB, Zhang L, et al. (2007) Developmental role of Munc13-4 in the growth cone response of dorsal root ganglion neurons. Nature 451: 746–749.

62. Miller NL, Wevrick R, Mellon PL (2009) Necdin, a Prader-Willi syndrome candidate gene, regulates gonadotropin-releasing hormone neurons during development. Human Molecular Genetics 18: 240–260.

63. Mitchell DG, Bryan BA, Liu JP, Liu WB, Zhang L, et al. (2007) Developmental expression of three small GTPases in the mouse eye. Molecular Vision 13: 1144–1153.

64. Monaghan MM, Trimmer JS, Rhodes KJ (2008) Autonomous and non-autonomous roles for the Math5 homeodomain gene. Developmental Biology 321: 563–573.

65. Nakajima E, David LL, Bystrom C, Shearer TR, Azuma M (2006) Calpain-mediated apoptosis in photoreceptor cell axons. Development 135: 3531–3541.

66. Nakajima E, David LL, Bystrom C, Shearer TR, Azuma M (2006) Calpain-mediated apoptosis in photoreceptor cell axons. Development 135: 3531–3541.

67. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

68. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

69. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

70. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

71. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

72. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

73. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

74. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

75. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

76. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

77. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

78. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

79. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.

80. Okazaki Y, Furuno M, Kasukawa T, Acachi J, Team. FCRGERGPII (2002) Comprehensive DNA microarray analysis of gene expression in the mouse brain. Nature Reviews Genetics 3: 649–660.