Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 352 (2007) 733–738
BBRC
www.elsevier.com/locate/ybbrc

MicroRNAs preferentially target the genes with high transcriptional regulation complexity

Qinghua Cui [a,1], Zhenbao Yu [a,1], Youlian Pan [b], Enrico O. Purisima [a], Edwin Wang [a,*]

[a] *Biotechnology Research Institute, National Research Council Canada, Montreal, Que., Canada H4P 2R2*
[b] *Institute for Information Technology, National Research Council Canada, Ottawa, Ont., Canada K1A 0R6*

Received 2 November 2006
Available online 27 November 2006

Abstract

Over the past few years, microRNAs (miRNAs) have emerged as a new prominent class of gene regulatory factors that negatively regulate expression of approximately one-third of the genes in animal genomes at post-transcriptional level. However, it is still unclear why some genes are regulated by miRNAs but others are not, i.e. what principles govern miRNA regulation in animal genomes. In this study, we systematically analyzed the relationship between transcription factors (TFs) and miRNAs in gene regulation. We found that the genes with more TF-binding sites have a higher probability of being targeted by miRNAs and have more miRNA-binding sites on average. This observation reveals that the genes with higher *cis*-regulation complexity are more coordinately regulated by TFs at the transcriptional level and by miRNAs at the post-transcriptional level. This is a potentially novel discovery of mechanism for coordinated regulation of gene expression. Gene ontology analysis further demonstrated that such coordinated regulation is more popular in the developmental genes.

Keywords: MicroRNA; Transcription factor; Gene expression regulation; MicroRNA target; Transcription factor-binding site

Gene expression is largely regulated by action of *trans*-factors on the *cis*-elements aligning on the regulatory regions of the genes. Among these *trans*-factors and the *cis*-elements, transcription factors (TFs) and their binding sites (TFBSs) play the most important role in gene expression regulation. Recently, another group of molecules, namely microRNAs (miRNAs), have been found to regulate gene expression at the post-transcriptional and translational levels through base-pairing with target messenger RNAs (mRNAs).

MicroRNAs are non-coding RNAs of ~22-nucleotides in length that are encoded in the chromosomal DNA and transcribed as longer stem-loop-like precursors [1,2]. Upon transcription, the miRNA precursors are converted to mature miRNA duplexes through sequential processing by the RNaseIII family of endonucleases Drosha and Dicer [3,4]. One strand of the processed duplex is incorporated into a silencing complex and guided to target mRNA sequences by base-pairing, resulting in the cleavage of target mRNAs or repression of their productive translation [5,6]. Roughly, 1% of the genes in each respective animal genome are miRNAs [7]. A growing body of evidence has revealed that miRNAs are involved in a variety of biological and pathological processes [1,2,8], such as embryonic development, cell proliferation, cell differentiation, apoptosis, insulin secretion, and carcinogenesis.

Recent computational studies indicate that approximately one third of human genes are potentially regulated by miRNAs and each miRNA on average could target more than 200 genes [7]. Similar to TFs and their regulated genes, miRNAs and their targets appear to form a complex regulatory network. However, it remains unclear whether

Abbreviations: GO, gene ontology; miRNA, microRNA; TF, transcription factor; TFBS, TF-binding site.

* Corresponding author. Fax: +1 514 496 5143.
E-mail address: edwin.wang@cnrc-nrc.gc.ca (E. Wang).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.080

the gene expression regulation by miRNAs at the post-transcriptional level is coordinated with that by TFs at the transcriptional level. In this study, we systematically analyzed the relationship between the abundance of the TFBSs and miRNA targeting sites of each gene in the human genome. We found a positive correlation between these two groups of transcriptional regulators.

Materials and methods

Datasets used in this study. A dataset representing three transcription factors, OCT4, NANOG, and SOX2 and their target genes in human embryonic stem cells was obtained from Boyer et al. [9]. The regulatory relationships of the three transcription factors and their target genes are listed in Supplementary Text File S1.

The genome-wide computationally predicted human miRNA target genes were obtained from Krek et al. [10]. There were a total of 6243 genes regulated by 168 miRNAs. The miRNAs and their targets are listed in Supplementary Text File S2.

We obtained two TFBS datasets, one from Cora et al. [11] and the other from Xie et al. [12]. We counted the number of TFBS for each gene from these two datasets and presented them in Supplementary Text Files S3 and S4, respectively.

The original gene ontology (GO) term file was downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/). Human GO terms were extracted and listed in Supplementary Text File S5.

Analysis of the relationship between miRNA target rate and TFBS number of human genes. We define the miRNA target rate as a ratio of the number of genes that are miRNA targets to the total number of genes in a dataset. To examine the relationship between miRNAs and TFs in gene regulation, the 6243 human miRNA target genes reported by Krek et al. [10] were mapped onto 9348 human genes whose numbers of corresponding TFBSs were determined by Cora et al. [11]. We call the number of TFBSs associated with a gene its TFBS-count. We divided these 9348 human genes into two groups. One group contains miRNA targets (Supplementary Text File S6) and the other group contains the rest of the genes (Supplementary Text File S7). We calculated the average TFBS-count of the genes in each group. A Wilcoxon Ranksum test was performed to determine whether the average number of TFBSs was significantly different between the two groups.

In order to investigate the relationship between the miRNA target rate and the TFBS-count of genes in more detail, we calculated the number of TFBSs for each gene in the dataset of Cora et al [11] (Supplementary Text File S3) and grouped the genes according to their TFBS-count. The genes in each group have the same number of TFBSs. Since some groups contain a limited number of genes, we set a threshold of 100 genes for each group and regrouped the genes if a group contained less than 100 genes. For example, when a group contained N genes ($N < 100$), we randomly selected 100-N genes from the adjacent group that contained the genes with one less TFBS-count. If the number of the genes in the adjacent group (M) was not enough to make a new group ($M + N < 100$), we continued this procedure until the number of genes reached to the threshold. This ensured that each group had at least 100 genes. Then, we calculated the miRNA target rate for each gene group (Supplementary Text File S8). We performed a similar analysis based on the genes' TFBS information from the dataset of Xie et al. [12] (Supplementary Text File S9).

Analysis of the relationship between the number of TFBSs and the number of miRNAs that regulate the same gene. As many genes are regulated by several different TFBSs and different miRNAs, we analyzed the relationship between the TFBS-count and the miRNA-count of the human genes. To do so, we divided the genes in Supplementary Text File S3 into subgroups based on the miRNA-count. The grouping information and genes in each group are listed in Supplementary Text File S10. For example, Group one contains the genes that are not regulated by miRNAs at all, Group two contains the genes that are regulated by one miRNA, and so on. The average TFBS-count in each group was calculated using a similar method as described above and listed in Supplementary Text File S11. A similar analysis was conducted using the genes' TFBS information from Xie et al. [12] (Supplementary Text File S12).

Analysis of gene ontology categories with overrepresented genes that have both a high TFBS-count and miRNA rate. To investigate which biological processes and functional categories are more regulated by TFs and miRNAs, we assembled genes into functional groups based on the gene ontology (GO) annotations. Among the 9348 genes in the dataset of Cora et al. [11], 6994 were found to have GO terms. We took the top 200 genes that had both a high TFBS-count and high miRNA target rate, and found the GO terms that were statistically overrepresented in these genes using the method reported by Beißbarth and Speed [13].

All intermediate results and data files are accessible at: http://www.bri.nrc.ca/wang/mirna3.html.

Results and discussion

To study how miRNAs and TFs coordinately regulate genes in the human genome, we first took a dataset which represents the regulatory relations between the three TFs, OCT4, NANOG, and SOX2, and their target genes in human embryonic stem cells [9]. The regulatory relationships between the three TFs (OCT4, NANOG, and SOX2) and their target genes were determined through ChIP-chip analysis (chromatin immunoprecipitation coupled with DNA microarray) [9]. The three TFs regulate a total of 2046 genes. Among these 2046 genes including the three TFs, 341 are regulated by only one of the three TFs, 1314 are co-regulated by two of the TFs and 391 are co-regulated by all of the TFs (Table 1). Meanwhile, we obtained a set of computationally predicted human miRNA target genes from Krek et al. [10]. The current miRNA target prediction methods [10,14–19] are mainly based on the principle of miRNA-target interactions [20], and the accuracy of these methods has been confirmed by experimental validation of randomly selected miRNA targets [21] and by large-scale gene expression profiling studies [22,23]. Up to 90% of the randomly selected miRNA targets from the predictions by Krek et al. [10] have been validated as true targets [21]. Accordingly, the predicted miRNA targets have been used for genome-wide analysis of miRNA target expression [20,24–26] and cellular signaling network regulation [27]. We mapped the miRNA targets onto the target genes of the three TFs. We then divided the genes into three groups, in which they are regulated by one, two or all three of the TFs, respectively, and

Table 1
MiRNA target rate in each subgroup of the genes in human embryonic stem cell

Group[a]	1	2	3
Number of total genes	341	1314	391
Number of miRNA target genes	141	550	192
Number of non-miRNA targets	200	764	199
P value	0.031		

[a] The genes were grouped according to the number of TFs by which they are regulated.

counted the number of genes that are miRNA targets and the number of genes that are not miRNA targets, respectively, in each group. We found that the miRNA targets are enriched in the group of genes targeted by more TFs (Table 1). Fisher exact test confirmed the significance of the observation ($P = 0.031$). This result indicates that a gene that is regulated by more TFs is also more likely to be a target of miRNAs.

Table 2
Average TFBS-counts of miRNA targets and non-miRNA targets[a]

	Total genes	miRNA targets	Non-miRNA targets
Number of genes	9348	3942	5406
Average TFBS-count	20.4	24.2	17.4
P value[b]		1.9×10^{-55}	

[a] TFBS data were obtained from Cora et al.'s report [11].
[b] Wilcoxon Ranksum test.

A gene is usually regulated by more than three TFs. To validate and expand the above observation, we examined the relationship between TFs and miRNAs for gene regulation in a genome-wide scale. In the past few years, genome-wide identification of TFBSs has been extensively studied by using various bioinformatics methods. Among these methods, the comparative genomics approach emerges as one of the most effective and relatively accurate approaches for identifying potential *cis*-regulatory ele-

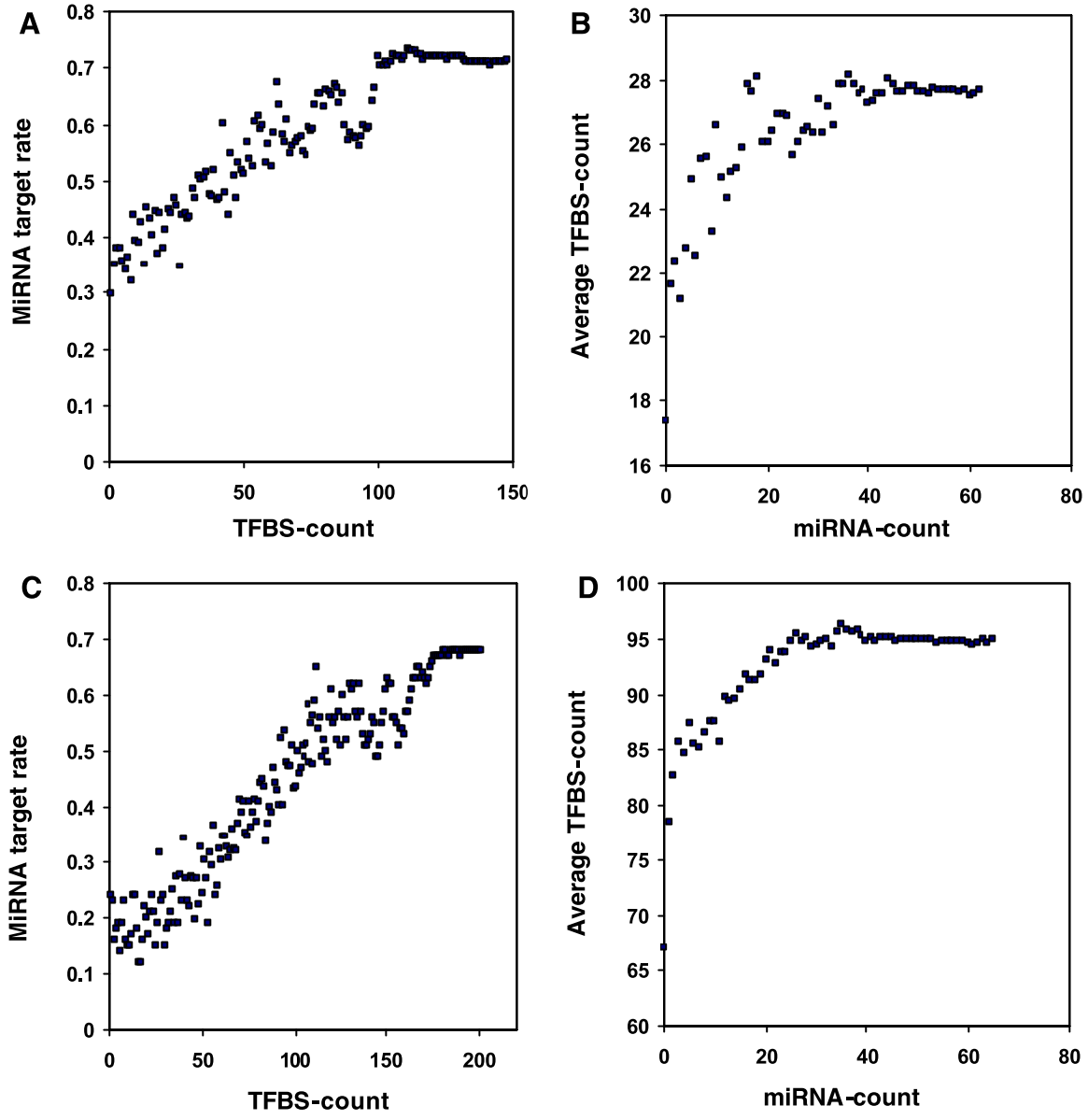

Fig. 1. Correlation between TFs and miRNAs in gene regulation. The analysis was performed using two TFBS datasets from Cora et al. [11] (A and B) and Xie et al. [12] (C and D), respectively, and one miRNA target dataset from Krek et al. [10] (A–D). (A,C) Genes were grouped based on the number of TFBSs. MicroRNA targets were mapped onto these genes and the miRNA target rate in each group was calculated. (B,D) Genes were grouped based on the number of targeting miRNAs. The average number of TFBSs was calculated in each group.

ments in genomes [28,29]. *cis*-Regulatory elements have been used to study gene expression divergence [30–33] and tissue-specific gene expression profiles [12,34]. TFBSs have also been used to reconstruct gene regulatory networks, find co-regulated genes and infer evolutionary insights [35–38]. One report found that 98% of known TFBSs of skeletal muscle-specific TFs are confined to 19% of the most conserved sequences between human and mouse [39]. Furthermore, several integrative methods that combine phylogenetic footprinting with others such as gene microarray and GO have been developed to filter out false positives in the TFBS identification [11]. TFBSs, or *cis*-regulatory elements are normally located in the promoter region of a gene. TFs regulate a gene through binding to the TFBSs of the gene. Basically, the more TFBSs a gene has, the more complex its regulation can be as provided by various possible combinations of TFs. We took two datasets of putative human TFBSs identified through comparative genomics [11,12]. In the first dataset [11], each gene on average contains 20 putative TFBSs in its promoter region. After mapping miRNA targets reported in [10] onto the 9348 genes, we found that 42.16% of them are miRNA targets and the average TFBS-count of the miRNA target genes is significantly higher than that of the non-target genes (24.2 vs. 17.4, $P < 1.9 \times 10^{-55}$, Wilcoxon Ranksum test, Table 2). A similar result (86.3 vs. 67.1, Wilcoxon Ranksum test, $P < 1.5 \times 10^{-216}$) was obtained based on the other TFBS dataset [12].

More detailed analysis was performed by grouping these genes based on the TFBS-count and calculating the miRNA target rate in each group. As shown in Fig. 1A, the TFBS-count is significantly correlated with the miRNA target rate (Pearson's correlation coefficient $r = 0.9432$, $P < 3.5 \times 10^{-68}$). For example, the miRNA target rate is doubled from the group of genes that have less than 10 TFBSs to those that have more than 100 TFBSs (from ~35% to ~70%). A similar result was obtained using the TFBS dataset from Xie et al. [12] (Fig. 1C, $r = 0.9680$, $P < 3.9 \times 10^{-113}$). These results are consistent with the findings in the human stem cell gene regulation and therefore strongly suggest that miRNAs preferentially target the genes that bear more TFBSs.

Since many genes can be targeted by more than one miRNA, we analyzed the relationship between miRNA-count and TFBS-count of these genes. We found a significant correlation (Fig. 1B, $r = 0.7364$, $P < 6.1 \times 10^{-12}$). A similar result was obtained when using the TFBS dataset of Xie et al. [12] (Fig. 1D, $r = 0.7200$, $P < 9.5 \times 10^{-12}$). These results suggest that genes targeted by more miRNAs have more TFBSs.

Taken together, these results indicate that the complexity of gene regulation by miRNAs at the post-transcriptional level is positively related to the complexity of gene regulation by TFs at the transcriptional level in human genome.

To understand which biological processes and functional categories are more coordinately regulated by both TFs and miRNAs, we took the top 200 genes that had both a high TFBS-count and miRNA target rate and found the top 20 GO terms that were statistically overrepresented in these genes. As shown in Table 3, we found that many of them are involved in development, such as GO:0009653 (development-morphogenesis), GO:0048513 (organ development), GO:0019219 (skeletal development), GO:0007399 (nervous system development), GO:0030154 (development-cell differentiation), and some of them are related to the regulation of different cellular processes. This reflects the fact that gene regulation of development and cellular regulatory processes are highly complex, requiring multiple TFs and miRNAs.

In conclusion, human genes, especially the genes involved in developments, are coordinately regulated by

Table 3
Statistically overrepresented GO terms with genes that have both a high TFBS-count and miRNA target rate

GO term	*P* value	Annotation
GO:0009653	3.74E−24	Development-morphogenesis
GO:0050789	2.02E−22	Regulation of biological process
GO:0050791	4.11E−21	Regulation of physiological process
GO:0050794	1.59E−19	Regulation of cellular process
GO:0051244	5.36E−19	Regulation of cellular physiological process
GO:0048513	2.58E−17	Organ development
GO:0006355	2.76E−17	Regulation of transcription, DNA-dependent
GO:0006351	1.72E−16	Transcription, DNA-dependent
GO:0019222	3.12E−16	Regulation of metabolism
GO:0045449	4.91E−15	Regulation of transcription
GO:0019219	1.18E−14	Skeletal development
GO:0031323	1.60E−14	Regulation of cellular metabolism
GO:0006350	1.60E−14	Transcription
GO:0007399	4.43E−14	Nervous system development
GO:0048731	5.12E−14	System development
GO:0048519	1.97E−10	Negative regulation of biological process
GO:0043118	1.37E−09	Negative regulation of physiological process
GO:0051243	3.95E−09	Negative regulation of cellular physiological process
GO:0030154	4.69E−09	Development-cell differentiation
GO:0007154	7.15E−09	Cell communication

both TFs at the transcriptional level and miRNAs at the post-transcriptional level. Genes that are more complexly regulated at transcriptional level are more frequently turned on and more differentially expressed at the different temporal and spatial conditions and therefore also require to be more frequently turned off. MiRNAs as negative regulators can exert the turning-off function at the post-transcriptional level through repressing mRNA translation and/or mediating cleavage of mRNAs. The coordination between TFs and miRNAs in gene expression regulation found in this research reveals a potential mechanism of gene regulation in the human genome. We also found that such coordinately regulated genes are enriched in certain GO functions, particularly in those involved in developmental processes and cellular regulatory processes.

Acknowledgments

This work was partially supported by the Genomics and Health Initiative, National Research Council Canada. We thank Doreen Harcus for reading the manuscript and correcting grammar mistakes.

References

[1] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[2] V. Ambros, The functions of animal microRNAs, Nature 431 (2004) 350–355.
[3] V.N. Kim, MicroRNA biogenesis: coordinated cropping and dicing, Nat. Rev. Mol. Cell Biol. 6 (2005) 376–385.
[4] B.R. Cullen, Transcription and processing of human microRNA precursors, Mol. Cell 16 (2004) 861–865.
[5] M.A. Carmell, G.J. Hannon, RNase III enzymes and the initiation of gene silencing, Nat. Struct. Mol. Biol. 11 (2004) 214–218.
[6] G. Meister, T. Tuschl, Mechanisms of gene silencing by double-stranded RNA, Nature 431 (2004) 343–349.
[7] S. Griffiths-Jones, S. Moxon, M. Marshall, A. Khanna, S.R. Eddy, A. Bateman, Rfam: annotating non-coding RNAs in complete genomes, Nucleic Acids Res. 33 (2005) D121–D124.
[8] S.M. Hammond, MicroRNAs as oncogenes, Curr. Opin. Genet. Dev. 16 (2006) 4–9.
[9] L.A. Boyer, T.I. Lee, M.F. Cole, S.E. Johnstone, S.S. Levine, J.P. Zucker, M.G. Guenther, R.M. Kumar, H.L. Murray, R.G. Jenner, D.K. Gifford, D.A. Melton, R. Jaenisch, R.A. Young, Core transcriptional regulatory circuitry in human embryonic stem cells, Cell 122 (2005) 947–956.
[10] A. Krek, D. Grun, M.N. Poy, R. Wolf, L. Rosenberg, E.J. Epstein, P. MacMenamin, P. da Isabelle, K.C. Gunsalus, M. Stoffel, N. Rajewsky, Combinatorial microRNA target predictions, Nat. Genet. 37 (2005) 495–500.
[11] D. Cora, C. Herrmann, C. Dieterich, C.F. Di, P. Provero, M. Caselle, Ab initio identification of putative human transcription factor binding sites by comparative genomics, BMC Bioinformatics. 6 (2005) 110.
[12] X. Xie, J. Lu, E.J. Kulbokas, T.R. Golub, V. Mootha, K. Lindblad-Toh, E.S. Lander, M. Kellis, Systematic discovery of regulatory motifs in human promoters and 3′ UTRs by comparison of several mammals, Nature 434 (2005) 338–345.
[13] T. Beißbarth, T.P. Speed, GOstat: find statistically overrepesented gene ontologies within a group of genes, Bioinformatics 20 (2004) 1464–1465.
[14] A.J. Enright, B. John, U. Gaul, T. Tuschl, C. Sander, D.S. Marks, MicroRNA targets in *Drosophila*, Genome Biol. 5 (2003) R1.
[15] B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, D.S. Marks, Human MicroRNA targets, PLoS Biol. 2 (2004) e363.
[16] M. Kiriakidou, P.T. Nelson, A. Kouranov, P. Fitziev, C. Bouyioukos, Z. Mourelatos, A. Hatzigeorgiou, A combined computational-experimental approach predicts human microRNA targets, Genes Dev. 18 (2004) 1165–1178.
[17] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.
[18] A. Stark, J. Brennecke, R.B. Russell, S.M. Cohen, Identification of *Drosophila* MicroRNA targets, PLoS Biol. 1 (2003) E60.
[19] A. Stark, J. Brennecke, N. Bushati, R.B. Russell, S.M. Cohen, Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3′UTR evolution, Cell 123 (2005) 1133–1146.
[20] J. Brennecke, A. Stark, R.B. Russell, S.M. Cohen, Principles of microRNA-target recognition, PLoS Biol. 3 (2005) e85.
[21] N. Rajewsky, MicroRNA target predictions in animals, Nat. Genet. 38 Suppl. (2006) S8–S13.
[22] J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Silencing of microRNAs in vivo with 'antagomirs', Nature 438 (2005) 685–689.
[23] L.P. Lim, N.C. Lau, P. Garrett-Engele, A. Grimson, J.M. Schelter, J. Castle, D.P. Bartel, P.S. Linsley, J.M. Johnson, Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs, Nature 433 (2005) 769–773.
[24] K.K. Farh, A. Grimson, C. Jan, B.P. Lewis, W.K. Johnston, L.P. Lim, C.B. Burge, D.P. Bartel, The widespread impact of mammalian MicroRNAs on mRNA repression and evolution, Science 310 (2005) 1817–1821.
[25] P. Sood, A. Krek, M. Zavolan, G. Macino, N. Rajewsky, Cell-type-specific signatures of microRNAs on target mRNA expression, Proc. Natl. Acad. Sci. USA 103 (2006) 2746–2751.
[26] Z. Yu, Z. Jian, S.H. Shen, E. Purisima, E. Wang, Global analysis of microRNA target gene expression reveals that miRNA targets are lower expressed in mature mouse and *Drosophila* tissues than in the embryos, Nucleic Acids Res., in press, doi:10.1093/nar/gk11032.
[27] Q. Cui, Z. Yu, E.O. Purisima, E. Wang, Principles of microRNA regulation of a human cellular signaling network, Mol. Syst. Biol. 2 (2006) 46.
[28] C.T. Harbison, D.B. Gordon, T.I. Lee, N.J. Rinaldi, K.D. Macisaac, T.W. Danford, N.M. Hannett, J.B. Tagne, D.B. Reynolds, J. Yoo, E.G. Jennings, J. Zeitlinger, D.K. Pokholok, M. Kellis, P.A. Rolfe, K.T. Takusagawa, E.S. Lander, D.K. Gifford, E. Fraenkel, R.A. Young, Transcriptional regulatory code of a eukaryotic genome, Nature 431 (2004) 99–104.
[29] T. Wang, G.D. Stormo, Identifying the conserved network of cis-regulatory sites of a eukaryotic genome, Proc. Natl. Acad. Sci. USA 102 (2005) 17400–17405.
[30] C.I. Castillo-Davis, D.L. Hartl, G. Achaz, *cis*-Regulatory and protein evolution in orthologous and duplicate genes, Genome Res. 14 (2004) 1530–1536.
[31] G. Haberer, T. Hindemitt, B.C. Meyers, K.F. Mayer, Transcriptional similarities, dissimilarities, and conservation of *cis*-elements in duplicated genes of Arabidopsis, Plant Physiol. 136 (2004) 3009–3022.
[32] W.H. Li, J. Yang, X. Gu, Expression divergence between duplicate genes, Trends Genet. 21 (2005) 602–607.
[33] Z. Zhang, J. Gu, X. Gu, How much expression divergence after yeast gene duplication could be explained by regulatory motif evolution? Trends Genet. 20 (2004) 403–407.
[34] A.D. Smith, P. Sumazin, Z. Xuan, M.Q. Zhang, DNA motifs in human and mouse proximal promoters predict tissue-specific expression, Proc. Natl. Acad. Sci. USA 103 (2006) 6275–6280.
[35] D.A. Rodionov, I.L. Dubchak, A.P. Arkin, E.J. Alm, M.S. Gelfand, Dissimilatory metabolism of nitrogen oxides in bacteria: comparative reconstruction of transcriptional networks, PLoS Comput. Biol. 1 (2005) e55.

[36] S. Imoto, T. Higuchi, T. Goto, K. Tashiro, S. Kuhara, S. Miyano, Combining microarrays and biological knowledge for estimating gene networks via Bayesian networks, Proc. IEEE Comput. Soc. Bioinform. Conf. 2 (2003) 104–113.

[37] S. Tang, S.L. Tan, S.K. Ramadoss, A.P. Kumar, M.H. Tang, V.B. Bajic, Computational method for discovery of estrogen responsive genes, Nucleic Acids Res. 32 (2004) 6212–6217.

[38] V.X. Jin, G.A. Singer, F.J. gosto-Perez, S. Liyanarachchi, R.V. Davuluri, Genome-wide analysis of core promoter elements from conserved human and mouse orthologous pairs, BMC Bioinformatics 7 (2006) 114.

[39] W.W. Wasserman, M. Palumbo, W. Thompson, J.W. Fickett, C.E. Lawrence, Human–mouse genome comparisons to locate regulatory sites, Nat. Genet. 26 (2000) 225–228.