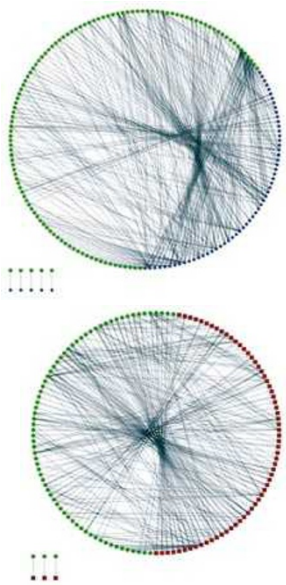
Nature Reviews Genetics | AOP, published online 14 October 2008; doi:10.1038/nrg2472

GENE REGULATION

A tiny missing link for regulatory networks



Top. TF–miRNA transcription regulatory network (blue diamonds, miRNA promoters; green circles, TFs). Bottom. Predicted miRNA–TF post-transcription regulatory network (red squares, miRNAs; green circles, TFs). Figure is reproduced, with permission, from Martinez, N. J. *et al. Genes Dev.* **22**, 2535–2549 © (2008) Cold Spring Harbor Laboratory Press.

Much effort has gone into the identification and prediction of microRNA (miRNA) targets. Transcriptional regulation of protein-coding genes has received similar attention, whereas regulation of miRNA expression has been relatively neglected. Martinez *et al.* now describe the first genome-wide, experimentally mapped transcription factor (TF)–miRNA interaction set in *Caenorhabditis elegans*, as well as a computationally predicted post-transcriptional miRNA–TF interaction set. Properties of these networks offer important insights into the role of miRNAs in regulating gene expression at a global level.

TFs predominate among miRNA targets, suggesting that the two classes of molecule could be connected in regulatory networks. To investigate this further the authors used a yeast one-hybrid method, which they had previously developed, to identify TFs that bind a set of promoters of interest; in this case 0.3–2 kb intergenic regions that were located upstream of annotated miRNAs. The screen yielded 347 high-confidence interactions between 63 miRNA promoters and 116 proteins (see figure, top). Among the most highly connected miRNAs were the familiar *let-7* and *lin-4* families, which are involved in developmental timing. A network model of all high-confidence TF–miRNA interactions revealed an architecture that is similar to that of protein-coding regulatory networks, in that it has characteristic hubs (that is, it is 'scale free').

The authors also created a post-transcriptional miRNA–TF network by retrieving miRNA targets among the TFs found in the yeast one-hybrid screen (see figure, bottom). Among the 252 high-confidence interactions between 67 miRNAs and 73 TFs, *let-7* was again among the most highly connected. Interestingly, this network has no clear hubs. This observation is consistent with previous suggestions and with the recent demonstration that miRNAs fine-tune gene expression (see further reading). Further network analysis revealed that miRNAs and TFs function together in composite feedback loops that consist of mutual regulation as well as higher order network structures that involve, for example, one miRNA and two TFs or two miRNAs and one TF.

The authors propose that composite feedback loops between miRNAs and TFs are common in gene regulation, at least in *C. elegans*. Although predicted to be important in homeostasis and cell differentiation programmes, feedback loops are under-represented in purely transcriptional networks. The work of Martinez *et al.* seems to have identified a missing link. The authors propose that the tight regulation and connectivity within these loops provide a mechanism for the coordination and adaptability of miRNA and TF target regulons.

Magdalena Skipper, Senior Editor, Nature

ORIGINAL RESEARCH PAPER Martinez, N. J. *et al.* A *C. elegans* genome-scale microRNA network contains composite feedback motifs with high flux capacity. *Genes Dev.* **22**, 2535–2549 (2008)

FURTHER READING Alon, U. Network motifs: theory and experimental approaches. *Nature Rev. Genet.* **8**, 450–461 (2007) | Selbach, M. *et al.* Widespread changes in protein synthesis induced by microRNAs. *Nature* **455**, 58–63 (2008) | Baek, D. *et al.* The impact of microRNAs on protein output. *Nature* **455**, 64–71 (2008)