Available online at www.sciencedirect.com
ScienceDirect
Genomics 90 (2007) 421–423
GENOMICS
www.elsevier.com/locate/ygeno

Minireview

Transcriptional regulation in eukaryotic ribosomal protein genes

Haiyan Hu [a], Xiaoman Li [a,b,*]

[a] Center for Computational Biology and Bioinformatics, School of Medicine, Indiana University, 410 W. 10th Street, HS5013, Indianapolis, IN 46202, USA
[b] Division of Biostatistics, School of Medicine, Indiana University, 410 W. 10th Street, HS5013, Indianapolis, IN 46202, USA

Received 12 April 2007; accepted 3 July 2007
Available online 17 August 2007

Abstract

Understanding ribosomal protein gene regulation provides a good avenue for understanding gene regulatory networks. Even after 5 decades of research on ribosomal protein gene regulation, little is known about how higher eukaryotic ribosomal protein genes are coordinately regulated at the transcriptional level. However, a few recent papers shed some light on this complicated problem.

Keywords: Ribosomal protein genes; Transcription factor binding sites; Gene regulation

Introduction

In this postgenomic era, one of the most important problems is to construct gene regulatory networks, that is, to decipher how different genes in one organism are regulated to perform different functions. With thousands of genes in one species, however, constructing gene regulatory networks is extremely difficult. In this aspect, the ribosomal protein (RP) genes may provide a better starting point for understanding gene regulation and for constructing gene regulatory networks.

The ribosome, the protein-synthesizing machinery in all cells, is composed of 53 or so RPs and 3 ribosomal RNAs (rRNA) in *Escherichia coli* and about 80 RPs and 4 rRNAs in mammals [1]. It is commonly believed that RP genes in the ribosome of one species are coordinately regulated under various cellular states [2,3]. Therefore, RP genes in one species form a gene regulatory network. Due to the small size of this regulatory network and the high conservation of RP genes in terms of sequences, expression, and functions, understanding the basic principles behind coordinate regulation in this gene regulatory network will greatly enhance our understanding of other gene regulatory networks.

* Corresponding author. Division of Biostatistics, School of Medicine, Indiana University, 410 W. 10th Street, HS5013, Indianapolis, IN 46202, USA. Fax: +1 317 278 9217.
E-mail address: shawnli@iupui.edu (X. Li).

0888-7543/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2007.07.003

Early research on RP gene regulation

How RP genes are coordinately regulated in prokaryotes was extensively studied by the early experiments in *E. coli* [4]. It is known that RP genes in prokaryotes form operons in which the expression of multiple genes is controlled by a single promoter. Moreover, some RPs can bind to the cistronic mRNA from which these RPs are translated, causing the process of translation to cease, thus forming a feedback network for the regulation of RP genes at the translational level.

Although the known mechanism of coordinate regulation in prokaryotes seems simple and straightforward, the mechanism in higher eukaryotes such as mammals is considerably more complicated and much less understood. In higher eukaryotes, the RP genes are scattered throughout the genome with no operon structure [5], but surprisingly, it was still observed that the RP genes are coordinately expressed [3].

To address why RP genes in higher eukaryotes have similar expression patterns under various conditions, before the inception of the Human Genome Project, several experiments were performed to identify motifs (transcription factor binding sites) in individual eukaryotic RP genes. Some of these experiments were performed by Perry and colleagues, who worked extensively on the regulation mechanism of three RP genes (i.e., *Rpl32*, *Rpl30*, and *Rps16*) in mouse [6–10]. They found four or five motifs associated with each of the three genes but none was shared by all three genes. Interestingly, they have shown that the

promoters of the three genes are of equal strength by in vivo measurements of polymerase loading and by the relative efficiency of the three promoters in driving the expression of a linked reporter gene. This shows that all RP gene promoters may have similar regulatory ability, even though no motif is shared by most RP genes.

After the human genome was sequenced, attempts to identify common motifs in the regulation of RP genes in human were made by searching the upstream sequences of 73 human RP genes with known motifs [11]. Results showed that there was no shared known motif except a weak oligopyrimidine tract around the transcription start sites in every RP gene. A recent study [12] further confirmed that no known motif is common among all RP genes in human by carefully searching 79 pairs of human and mouse RP gene upstream sequences. Note that these computational studies [11,12] may be affected by the current limited knowledge of the mammalian motifs.

Thus, without the operon structure and without any shared motifs by most RP genes, how RP genes in higher eukaryotes such as mammals are coordinately regulated still remains a mystery. Recently, studies of RP genes in yeast [13–21] and mammals [3] have shed some light on this problem from different angles. Some observed principles behind coordinate regulation in these studies may provide insights for understanding RP gene regulation in higher eukaryotes.

Recent progress in RP gene transcriptional regulation

In contrast to the above-mentioned results in mammals in which no motifs were found to be shared by most RP genes, ChIP–chip experiments in *Saccharomyces cerevisiae* showed that Rap1 [22,23] and Fhl1 [23] bind to almost all the RP gene promoters. In fact, the upstream region of almost all RP genes in *S. cerevisiae* contains one or two Rap1 binding sites, which was originally thought to be the basis of the coordinate regulation of RP genes in *S. cerevisiae* [22,24]. However, Rap1 is responsible for the transcription of many other genes as well and Rap1 alone cannot explain the coordinate regulation of RP genes. Thus, the specificity of Rap1 in the regulation of RP genes presumably lies in its recruitment of specific cofactors. Now it is known that Fhl1 and Ifh1 are some of the key cofactors in the transcription regulation of RP genes [14,17–21]. When the transcription of RP genes is activated, Rap1 binds to its binding sites, and the binding of Rap1 with DNA may recruit Fhl1, which in turn recruits Ifh1 that interacts with Fhl1 through the forkhead-associated domain of Fhl1 and may bind with the IFHL motif [18–20]. When the transcription of RP genes is repressed, only Rap1 and Fhl1 are recruited to the promoters of RP genes [19–21]. But how is the activation and repression of the transcription of RP genes determined? There are at least three models. First, Crf1, a cofactor of Fhl1, can compete with Ifh1 for the binding to Fhl1 at RP gene promoters and thereby switch the transcription between repression and activation [16]. Second, Hmo1 may be the switch from activation to repression and vice versa, since Hmo1 is required for both the assembly of transcription factor complexes containing Fhl1 and Ifh1 at RP promoters and the maximal transcription of rRNA genes by Pol I [14]. Third, the CURI complex is shown to contribute to the switch, because Ifh1 is absent from the PR gene promoters and becomes part of the CURI complex when the transcription is repressed [19]. Other proteins such as Sfp1 are also indicated to control the switch by binding to the RP gene promoters in the nucleus and helping promote RP gene expression under optimal growth conditions and leaving the nucleus in response to stress and nutrient deficiency [13,15]. In all these models, it is clear that the RP genes in *S. cerevisiae* are regulated mainly at the transcriptional level by the combinations of many transcription factors that have not been found in higher level species such as the mammals. Since all these models are supported by experimental evidence, they could be the components of a more complete regulatory network of the RP genes in *S. cerevisiae*.

In addition to the common motifs shared by most of the RP genes and the combinations of motifs in *S. cerevisiae*, Tanay et al. studied the evolution of the motifs regulating RP genes in yeast [25]. By looking at the RP gene upstream sequences in 17 fungal genomes, they showed that: (i) the IFHL motif is conserved across the 17 species, (ii) the Rap1 motif is conserved in 11 species, and (iii) as evolutionary divergence of species increases compared to *S. cerevisiae*, the Rap1 motif is replaced by the Homol-D motif, which is conserved in 8 species. This study of motif evolution indicates that the transcription factors regulating RP genes evolve, although RP genes and their functions are highly conserved. Thus, the common transcription factors that regulate RP genes in higher eukaryotes, if there are any, should be different from those of yeast.

To determine whether some phenomena discovered in yeast also occur in other species, Li et al. recently attempted to identify novel motifs in RP genes of 13 eukaryotic species and found unknown but frequently shared motifs in RP genes of species other than yeast [3]. In every species they considered, except for the 2 worm species, they found at least one motif appearing in almost all RP gene promoters (the two most frequent motifs identified in worms occur in about half of the RP gene promoters under consideration). Moreover, these motifs are conserved across species. For instance, the motifs found in human are conserved in mouse and rat, the motifs found in *Drosophila melanogaster* are conserved in *Anopheles gambiae*, and the motifs found in *Caenorhabditis elegans* are conserved in *Caenorhabditis briggsae.* Furthermore, the motifs do not look similar across distant species. For example, the mammalian motifs are not similar to the insect motifs and the insect motifs are not similar to those of the worm. One of the most surprising findings is that the most frequent motif found in mammals is located in the intronic region of mammalian RP genes—most of them being in the first intron—while motifs found in other species are within 1000 bp upstream of the transcription start sites of the RP genes. The middle part of the predicted binding site of the most frequent motif in the mouse RP gene RPL30 has been experimentally verified to be critical for RPL30 promoter function [6]. However, the authors did not find that this motif is actually shared by the three RP genes they did experiments on [6], because they treated the middle part of this motif predicted by Li et al. as the motif, while the middle part of this motif is very degenerate and the two ends of this

motif are more conserved [3]. These frequent motifs in the regulatory regions of RP genes of human, mouse, and rat together with those in fly and mosquito suggest that the RP genes in higher eukaryotes may be coordinately regulated at the transcriptional level as well, instead of only at the translational level. These results also further confirm the notion that motifs regulating RP genes are evolving although RP gene sequences and functions are extremely conserved across species. Together with the experimental results in mouse [6] and many studies in yeast [13–21], it is likely that the most frequent motifs in the individual species may have roles in RP gene regulation similar to that of the Rap1 motif in *S. cerevisiae,* and there should be other undiscovered motifs that are important for RP gene regulation.

Concluding remarks

Given the aforementioned motifs in every species identified by Li et al. [3], together with the results of yeast RP gene motif studies [13–21,24,25], it is clear that there are motifs shared by almost all RP genes in most eukaryotic species. Moreover, RP genes are more likely to be controlled by multiple transcription factors instead of only a single one. Furthermore, the control mechanism behind RP gene regulation evolves. However, many unanswered questions remain. For instance, what transcription factors will bind to these most frequent motifs? How did these transcription factors and motifs coevolve? What are the other transcription factors and motifs that are also important in regulating RP genes? How were the most frequent motifs transferred from the upstream to the downstream region of the transcription start sites of RP genes in mammals? How different is the mechanism of RP gene regulation between yeast and higher eukaryotes? We anticipate additional research to address these issues and further uncover the secrets of RP gene coordinate transcription in higher eukaryotes, especially mammals.

Acknowledgments

We thank Drs. Robert Perry, Marc Cortese, Arthur Berg, Patrick Monahan, and Peru Narayanan for their valuable comments. This work was supported by the Showalter Trust Award (X.L.) and the Indiana Genomics Initiative (X.L. and H.H.), which is funded in part by the Lilly Endowment.

References

[1] I.G. Wool, The structure and function of eukaryotic ribosomes, Annu. Rev. Biochem. 48 (1979) 719–754.
[2] O. Meyuhas, R.P. Perry, Construction and identification of cDNA clones for mouse ribosomal proteins: application for the study of r-protein gene expression, Gene 10 (1980) 113–129.
[3] X. Li, S. Zhong, W.H. Wong, Reliable prediction of transcription factor binding sites by phylogenetic verification, Proc. Natl. Acad. Sci. USA 102 (2005) 16945–16950.
[4] M. Nomura, R. Gourse, G. Baughman, Regulation of the synthesis of ribosomes and ribosomal components, Annu. Rev. Biochem. 53 (1984) 75–117.
[5] N. Kenmochi, T. Kawaguchi, S. Rozen, E. Davis, N. Goodman, T.J. Hudson, T. Tanaka, D.C. Page, A map of 75 human ribosomal protein genes, Genome Res. 8 (1998) 509–523.
[6] N. Hariharan, D.E. Kelley, R.P. Perry, Equipotent mouse ribosomal protein promoters have a similar architecture that includes internal sequence elements, Genes Dev. 3 (1989) 1789–1800.
[7] N. Hariharan, R.P. Perry, A characterization of the elements comprising the promoter of the mouse ribosomal protein gene RPS16, Nucleic Acids Res. 17 (1989) 5323–5337.
[8] M.L. Atchison, O. Meyuhas, R.P. Perry, Localization of transcriptional regulatory elements and nuclear factor binding sites in mouse ribosomal protein gene rpL32, Mol. Cell. Biol. 9 (1989) 2067–2074.
[9] S. Chung, R.P. Perry, Importance of introns for expression of mouse ribosomal protein gene rpL32, Mol. Cell. Biol. 9 (1989) 2075–2082.
[10] R. Moura-Neto, K.P. Dudov, R.P. Perry, An element downstream of the cap site is required for transcription of the gene encoding mouse ribosomal protein L32, Proc. Natl. Acad. Sci. USA 86 (1989) 3997–4001.
[11] M. Yoshihama, T. Uechi, S. Asakawa, K. Kawasaki, S. Kato, S. Higa, N. Maeda, S. Minoshima, T. Tanaka, N. Shimizu, N. Kenmochi, The human ribosomal protein genes: sequencing and comparative analysis of 73 genes, Genome Res. 12 (2002) 379–390.
[12] R. Perry, The architecture of mammalian ribosomal protein promoters, BMC Evol. Biol. 5 (2005) 15.
[13] P. Jorgensen, I. Rupes, J.R. Sharom, L. Schneper, J.R. Broach, M. Tyers, A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size, Genes Dev. 18 (2004) 2491–2505.
[14] D.B. Hall, J.T. Wade, K. Struhl, An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in Saccharomyces cerevisiae, Mol. Cell. Biol. 26 (2006) 3672–3679.
[15] R.M. Marion, A. Regev, E. Segal, Y. Barash, D. Koller, N. Friedman, et al., Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression, Proc. Natl. Acad. Sci. USA 101 (2004) 14315–14322.
[16] D.E. Martin, A. Soulard, M.N. Hall, TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1, Cell 119 (2004) 969–979.
[17] S.B. Schawalder, M. Kabani, I. Howald, U. Choudhury, M. Werner, D. Shore, Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1, Nature 432 (2004) 1058–1061.
[18] J.T. Wade, D.B. Hall, K. Struhl, The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes, Nature 432 (2004) 1054–1058.
[19] D. Rudra, J. Mallick, Y. Zhao, J.R. Warner, Potential interface between ribosomal protein production and pre-rRNA processing, Mol. Cell. Biol. 27 (2007) 4815–4824.
[20] D. Rudra, Y. Zhao, J.R. Warner, Central role of Ifh1p–Fhl1p interaction in the synthesis of yeast ribosomal proteins, EMBO J. 24 (2005) 533–542.
[21] Y. Zhao, K.B. McIntosh, D. Rudra, S. Schawalder, D. Shore, J.R. Warner, Fine-structure analysis of ribosomal protein gene transcription, Mol. Cell. Biol. 26 (2006) 4853–4862.
[22] J.D. Lieb, X. Liu, D. Botstein, P.O. Brown, Promoter-specific binding of Rap1 revealed by genome-wide maps of protein–DNA association, Nat. Genet. 28 (2001) 327–334.
[23] T.I. Lee, N.J. Rinaldi, F. Robert, D.T. Odom, Z. Bar-Joseph, G.K. Gerber, N. M. Hannett, C.R. Harbison, C.M. Thompson, I. Simon, J. Zeitlinger, E.G. Jennings, H.L. Murray, D.B. Gordon, B. Ren, J.J. Wyrick, J. Tagne, T.L. Volkert, E. Fraenkel, D.K. Gifford, R.A. Young, Transcriptional regulatory networks in Saccharomyces cerevisiae, Science 298 (2002) 799–804.
[24] R.F. Lascaris, W.H. Mager, R.J. Planta, DNA-binding requirements of the yeast protein Rap1p as selected in silico from ribosomal protein gene promoter sequences, Bioinformatics 15 (1999) 267–277.
[25] A. Tanay, A. Regev, R. Shamir, Conservation and evolvability in regulatory networks: the evolution of ribosomal regulation in yeast, Proc. Natl. Acad. Sci. USA 102 (2005) 7203–7208.