Biochemical Genetics, Vol. 45, Nos. 1/2, February 2007 (© 2007)
DOI: 10.1007/s10528-006-9059-5

Relationship Between mRNA Stability and Length: An Old Question with a New Twist

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Received 31 March 2006—Final 23 June 2006
Published online: 13 January 2007

The half-life of individual mRNA plays a central role in controlling the level of gene expression. However, the determinants of mRNA stability have not yet been well defined. Most previous studies suggest that mRNA length does not affect its stability. Here, we show significant negative correlations between mRNA length and stability in human and Escherichia coli*, but not in* Saccharomyces cerevisiae *or* Bacillus subtilis*. This finding suggests the possibility that endonucleolytic attacks by RNA endonuclease and/or mechanical damage may strongly influence mRNA stability in both prokaryotes and eukaryotes.*

KEY WORDS: mRNA half-life; mRNA-decay rate; mRNA length; mRNA degradation.

INTRODUCTION

Since the steady-state level of mRNA is determined by both synthesis and degradation, mRNA decay is as important as transcription in regulating gene expression (Khodursky and Bernstein, 2003; Meyer *et al*., 2004; Wilusz and Wilusz, 2004; Mata *et al*., 2005). Experiments have shown that the half-life of mRNA transcripts extends over a wide range between and within organisms (Meyer *et al*., 2004). In *Escherichia coli*, mRNA half-life ranges from 1 min to over 10 min (Bernstein *et al*., 2002; Selinger *et al*., 2003). In yeast *Saccharomyces cerevisiae*, mRNA half-life also varies widely, ranging from approximately 3 min to more than 90 min (Wang *et al*., 2002). In humans, unstable mRNA has a half-life of just several minutes, whereas stable mRNA may exceed 10 h (Raghavan *et al*., 2002; Yang *et al*., 2003; Meyer *et al*., 2004).

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In eukaryotes, the majority of mRNAs are shown to be degraded from two ends by deadenylation, decapping, and exonuclease hydrolyzing reactions (Meyer *et al*., 2004; Parker and Song, 2004; Yamashita *et al*., 2005). By contrast, recent advances in *E. coli* show that the degradation of bacterial mRNA starts from endonucleolytic cleavage by RNase E (Jain, 2002). Long bacterial mRNAs are expected to have more putative sites of cleavage, thus may be degraded more quickly than short mRNAs. This recalls an old question: What is the relationship between mRNA length and mRNA stability?

Pioneering analyses of a limited number of genes show that long-lived mRNA tends to be short in various eukaryotes including humans, mice, insects, and the yeast *S. cerevisiae* (Santiago *et al*., 1986, and references therein). The reverse relationship between mRNA length and half-life was not found in later studies in either eukaryotes (Shapiro *et al*., 1988; Herrick *et al*., 1990; Wang *et al*., 2002) or bacteria (Bernstein *et al*., 2002). Advances in genome sequencing and microarray analysis of gene expression make it possible to re-examine that relationship on a genomewide scale. In this study, we collected genomewide data on mRNA length and stability (half-life or decay rate) in two eukaryotic species and two bacterial species. We found significant negative correlation between mRNA length and stability in human and *E. coli*, whereas no simple correlation was found in *S. cerevisiae* or *Bacillus subtilis*.

MATERIALS AND METHODS

The genome annotation files of human (build 35 version 1), yeast *S. cerevisiae* (build 1 version 1), *E. coli* (strain K12), and *B. subtilis* (subspecies subtilis, strain 168) were downloaded from the NCBI genome database (ftp://ftp.ncbi.nih.gov/genomes/). Programs written in Perl were used for genome features extraction and manipulation. Genes with alternative splicing sites or with obvious annotation errors were removed from our analysis. As the genome annotation files of yeast, *E. coli*, and *B. subtilis* contain only information on coding sequence (CDS), we used CDS lengths to represent the mRNA lengths in these organisms.

The data on mRNA stability were collected from online supplemental materials of previous publications, including the decay rates of human mRNAs in HepG2 cells and Bud8 cells (Yang *et al*., 2003), mRNA half-lives in human T lymphocytes under three growth conditions (Raghavan *et al*., 2002), mRNA half-lives of yeast (Holstege *et al*., 1998; Wang *et al*., 2002), and mRNA half-lives of *E. coli* (Bernstein *et al*., 2002, 2004). The data on mRNA half-life in *B. subtilis* (Hambraeus *et al*., 2003) were obtained from Dr. L. Hederstedt.

The transcription factors of human and yeast were obtained from the TRANSFAC transcription factor database (release 7.0; http://www.gene-regulation.com/pub/databases.html#transfac).

As the data on mRNA stability, mRNA length, and CDS length are not normally distributed, parametric analyses are not applicable. We performed non-parametric Spearman correlation analysis to examine the relationship between mRNA stability and mRNA length (or CDS length). After logarithmic transformation using 10 as the base, the distributions of the data became normal or nearly normal. Parametric Pearson correlation analysis of the normalized data got the same results (data not shown) as the Spearman analysis, except in one case, *E. coli* strain BZ453. The correlation between mRNA half-life and stability in *E. coli* strain BZ453 is insignificant in the Pearson analysis ($P > 0.10$) but is marginally significant with a weak correlation coefficient in the Spearman analysis.

RESULTS AND DISCUSSION

In *E. coli*, a previous study did not find a statistically significant correlation between ORF (open reading frame) length and mRNA half-life of strain NCM3416 in M9 medium (Bernstein *et al.*, 2002). But we found that there is in fact a very weak but significant negative correlation (Spearman's correlation coefficient, -0.041; $P = 0.012$). Furthermore, we observed strong negative correlations between CDS length and mRNA half-life in the same strain grown in LB medium and in several other *E. coli* strains (Table I). In *B. subtilis*, however, similar to a previous report (Hambraeus *et al.*, 2003), no significant correlation was found between CDS length and mRNA half-life ($P = 0.690$).

Our result in *E. coli* is consistent with the current model of *E. coli* mRNA decay, which is initiated by endonucleolytic enzyme cleavage (Jain, 2002). Simple mechanical damage that affects the stability of long mRNA cannot be excluded. There are two possible explanations for the lack of correlation between CDS length and mRNA half-life in *B. subtilis*. The first is that the early stationary phase when the mRNA half-life data were collected (Hambraeus *et al.*, 2003) does not represent the normal growth conditions in natural environments. From the results with *E. coli*, we can see that growth conditions influence global mRNA stability, as is also illustrated in stressed cells (Knapinska *et al.*, 2005). The second possibility is that the mRNA-decay pathway of *B. subtilis* is different from that of *E. coli*. *E. coli* was traditionally assumed to be a typical prokaryote, but now genome biologists realize that prokaryotes display a considerable diversity in genome organization (Brown, 2002).

In yeast strain Y262, we did not find significant correlation between CDS length and mRNA half-life (Table I), which is the same as previously reported (Wang *et al.*, 2002). Strangely, there is a weak but significant positive correlation between CDS length and mRNA half-life of yeast strain *rpb1-1* (Spearman's correlation coefficient, 0.091; $P < 10^{-5}$). In human HepG2 cells and Bud8 cells, mRNA length is positively correlated with mRNA-decay rate and in

Table I. Correlation Between mRNA (or CDS) Length and Stability

Species	Cell types or strains	Stability indicator	mRNA length[a]	CDS length[a]
Homo sapiens	HepG2 cells	Decay rate	0.267***	0.244***
	Bud8 cells	Decay rate	0.246***	0.215***
	T lymphocytes (medium)	Half-life	−0.036**	NS
	T lymphocytes (αCD3)	Half-life	−0.100***	−0.056**
	T lymphocytes (αCD3 + αCD28)	Half-life	−0.105***	−0.062**
Saccharomyces cerevisiae	Strain Y262	Half-life		NS
	Strain *rpb1-1*	Half-life		0.091***
Escherichia coli	Strain NCM3416 (LB medium)	Half-life		−0.258***
	Strain NCM3416 (M9 medium)	Half-life		−0.041**
	Strain BZ453	Half-life		−0.035*
	Strain DF261	Half-life		−0.136***
	Strain K10	Half-life		−0.184***
	Strain N3433	Half-life		−0.135***
	Strain SH3208	Half-life		−0.169***
	Strain SU02	Half-life		−0.110***
	Strain YHC012	Half-life		−0.087**
Bacillus subtilis	Strain BR95	Half-life		NS

[a]Spearman's correlation coefficient; NS, not significant; *marginally significant, $0.1 > P > 0.05$; $^{**}P < 0.05$; $^{***}P < 10^{-5}$.

human T lymphocytes mRNA length is negatively correlated with mRNA half-life (Table I). As a stable mRNA can be measured by lower decay rate or longer half-life, our analyses of different sources of data consistently showed that short mRNAs are more stable than long mRNAs in human cells. This is unexpected from the eukaryotic mRNA-decay pathways discovered up to now (Meyer *et al.*, 2004; Parker and Song, 2004; Yamashita *et al.*, 2005).

For the discrepancy observed between human and yeast, we have four hypotheses. The first is that the experimental growth conditions of yeast may not be representative of normal growth conditions in natural environments, and so the mRNA stability data of yeast are not representative. Heat shock, hypoxia, and other stresses were reported to cause stabilization of some mRNA (Knapinska *et al.*, 2005; Kang *et al.*, 2006). We are also not sure whether the human cells studied, like lymphocytes, are representative.

Comparison of the genomes of unicellular eukaryotes and multicellular eukaryotes has shown that the evolution of multicellularity was accompanied by construction of numerous multidomain extracellular matrix proteins and signaling proteins (Patthy, 1999, 2003). Transcripts encoding important regulatory proteins, such as transcription factors, cell cycle regulators, and regulators of apoptosis, turn

Table II. Comparison of Coding Sequence Length Between Human and Yeast

		Homo sapiens	*Saccharomyces cerevisiae*	P^a
All the analyzed genes	*n*	4506	5364	
	CDS lengths[b] (base)	1870 ± 26	1529 ± 15	$< 10^{-10}$
Transcription factors	*n*	214	281	
	CDS lengths[b] (base)	1831 ± 111	1772 ± 60	0.238

[a]Mann–Whitney test.
[b]Mean ± standard error of mean.

over rapidly (Dodson and Shapiro, 2002; Raghavan *et al.*, 2002; Wang *et al.*, 2002; Yang *et al.*, 2003). The second hypothesis is that important regulatory proteins in human may contain more domains in order to perform precise regulation of physiological function than those in yeast; consequently, they tend to be longer than the other proteins in general. Transcripts encoding transcription factors were shown to have short half-lives (Dodson and Shapiro, 2002). But we did not find significant differences between the CDS lengths of transcription factors in human and yeast (Table II).

The third hypothesis is that humans may have an undiscovered mRNA-decay pathway similar to that of *E. coli* (Jain, 2002), but yeast does not. More and more endonucleolytic cleavages of mRNA in vertebrate cells are being identified (Dodson and Shapiro, 2002). For example, Irel, an endoplasmic reticulum-transmembrane protein containing both protein kinase and endoribonuclease domains, probably has a wide range of target sites on mRNA of mammals (Dodson and Shapiro, 2002). A recent study shows that stalling of ribosome by a stem–loop structure triggers endonucleolytic cleavage of mRNA (Doma and Parker, 2006; Tollervey, 2006). Apparently, long mRNAs are more likely to form stem–loop structures than short mRNAs, either by regulation or by chance. The authors suggest other sources of stalled translation that may trigger such a mRNA-decay pathway: damaged mRNAs or ribosomes. Long mRNAs having more nucleotides and more ribosomes attached are thus expected to be more likely to be attacked by such "no-go" decay than short mRNAs. Unfortunately, we have not observed strong negative correlation between mRNA stability and length in budding yeast, in which the "no-go" decay was observed.

The last hypothesis is that human mRNAs are much longer and thus may be more mechanically fragile than yeast mRNA. So we compared the lengths of human and yeast CDSs analyzed in this study. Human CDSs are significantly longer than yeast CDSs (Table II), but still, we are not sure whether the longer CDSs in human are enough to account for the observed difference between human and yeast.

In summary, our study suggests the possibility that endonucleolytic attacks by RNA endonuclease and/or mechanical damage may strongly influence mRNA stability in both prokaryotes and eukaryotes. Once more data on mRNA stability are available, further analysis will be required to reach a conclusion.

ACKNOWLEDGMENTS

We would like to thank an anonymous referee for comments, Dr. L. Hederstedt of Lund University for providing the data of *B. subtilis* mRNA half-lives, Shu-Wei Li for help in parsing mRNA length, and Lei Zhu for discussion. This work was supported by National Natural Science Foundation of China (Grant No. 30270695) and Beijing Normal University.

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