RNA Motifs Required for Maintaining Metabolic Stability of M1 RNA

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The cell rapidly and precisely regulates gene expression for optimal cellular metabolism in response to various metabolic or environmental changes. One way of achieving regulation of gene expression is by controlling RNA stability that changes mRNA levels in the cell.[1,2] In *Escherichia coli*, although bulk mRNA decays with a half-life of about 5 min, individual mRNA half-lives can vary widely in the range of several seconds to an hour.[3–5] Furthermore, there are many stable RNAs, such as rRNAs and tRNAs. Trans-acting factors, such as RNA-binding proteins or *cis*-acting RNA motifs, are known to play a role in determining metabolic stability of RNA,[6] but it is still difficult to explain why some RNAs are stable while others are unstable. As an initial step to answer this question, in this study, we used M1 RNA, the RNA component of RNase P,[7] which is a highly stable RNA,[8,9] as a model RNA and examined *cis*-acting RNA motifs affecting its metabolic stability. As M1 RNA has a secondary structure consisting of 18 stems (P1–P18)[10] (Figure 1), we examined the effects of stem deletion or stem mutation using plasmids that could ectopically express various deletion or mutant derivatives. We determined half-lives of the derivatives by analyzing total RNAs prepared from rifampicin-chased cells containing each plasmid (Figures 2 and 3 and Table 1). First, we tested plasmids pLMd23, pLMdd5-23, and pLMdd5-50 that generated large deletion derivatives: deletion of P3-P18 in pLMd23, P4-P7 and P10-P18 in pLMdd5-23, and P2 and P10-P18 in pLMdd5-30.[11] The derivatives decayed with half-lives of 4–8 min, which are comparable to an average half-life of mRNA.[5] As ectopically overexpressed M1 RNA decayed with a half-life of 50 min, the data suggest that RNA domains in those large deletions are essential for maintaining M1 RNA stability. We then examined the metabolic stability of various derivatives lacking each stem formed by four or more consecutive base-pairs, excluding P10 and P11. P9 (having three base-pairs) was deleted together with P8 to generate a P8/P9 deletion derivative.

The P1 mutation gave rise to an M1 RNA derivative much less stable than its precursor form. As the P1 mutant derivative did not accumulate over endogenous M1 RNA due to its rapid decay, we were not able to determine its half-life. We estimated that the half-life would be much less than 1 min because precursor M1 RNA (pre-M1 RNA) had a half-life of about 1 min.[11] The largest decrease of metabolic stability, exclusive of P1, was observed with the P15 mutation, which showed a half-life of 4 min. An initial cleavage of overexpressed M1 RNA occurs at position 296,[12] which lies within P15. This cleavage may be related to the rapid decay of the P15 mutant derivative. Then, the P6 mutation, P18 mutation, P2 mutation, P4 mutation, P17 mutation, and P14 deletion followed, destabilizing M1 RNA with half-lives of 5–8 min. However, the P8 deletion and the P5 mutation had much less effect with half-lives of more than 20 min.

As all the examined deletion or mutant derivatives of M1 RNA showed less metabolic stability than the intact M1 RNA, it is likely that the integrity of the M1 RNA structure is crucial for M1 RNA to remain as a stable RNA. The P1 stem particularly plays the most crucial role in maintaining M1 RNA stability in that the decay rate of the P1-disrupted M1 RNA was so fast that its cellular levels could be difficult to detect. A previous finding that a M1 RNA derivative lacking P3–P18 and carrying an additional P1 disruption decayed too rapidly to be detected also supports the fact that P1 is the most important motif for M1 RNA stability.[11] The deletion or disruption of many stems such as P2, P4, P6, P14, P15, P17, and P18 changes M1 RNA from a stable RNA to an unstable RNA with half-lives of 4–8 min that would be similar to that of canonical mRNA, suggesting that these stems may contribute in a concerted manner to M1 RNA stability. One may argue that the presence of the regions interacting with C5 protein, the protein component of RNase P, could affect M1 RNA stability. Although P3, not P12, interacts with C5 protein,[13–15] the P3 deletion derivative was more stable than the P12 deletion derivative. C5 protein may have little effect on the stability of M1 RNA derivatives in such a condition where they are overexpressed. This is probably because there is not enough C5 protein to bind to overexpressed M1 RNA derivatives in the cell. It is also noteworthy that RNA stability was significantly lowered by the disruption of P4 and P6, both of which are formed through pseudoknots, suggesting that the pseudoknots may contribute to the metabolic stability of M1 RNA.

If we assume that stems causing shorter half-lives when deleted or disrupted are more crucial for maintaining

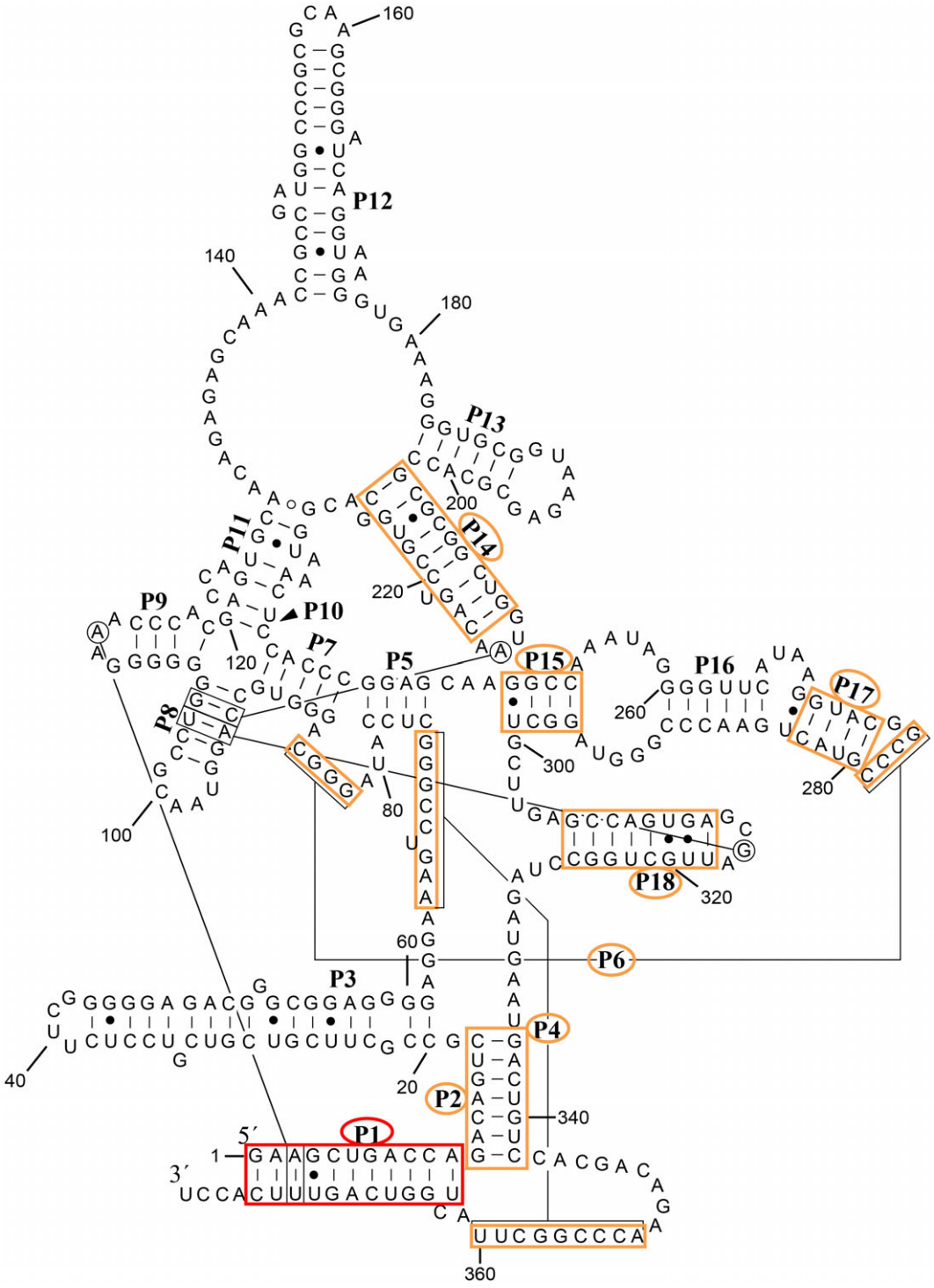

Figure 1. Eighteen stems in a secondary structure model of M1 RNA.[10,18] Stem structures whose deletion or mutation severely reduced metabolic stability of M1 RNA are highlighted in red (the most severe reduction) or orange.

metabolic stability of M1 RNA, the order of importance of each stem to M1 RNA stability would be P1 > P15 > P6 ≃ P18 > P2 ≃ P4 > P17 > P14 > P12 > P13 > P16 > P7 > P3 > P8/9 > P5. RNA motifs identified as essential for M1 RNA stability in this study may offer a system where the control mechanism of RNA stability can be studied in detail.

Experimental Section

Bacterial Strains and Plasmids. The *E. coli* K-12 strain JM109 was used for plasmid propagation and RNA analysis. Plasmid pLM1, a derivative of the pGEM3 vector, contains the *rnpB* (encoding M1 RNA) sequence of positions −270 to

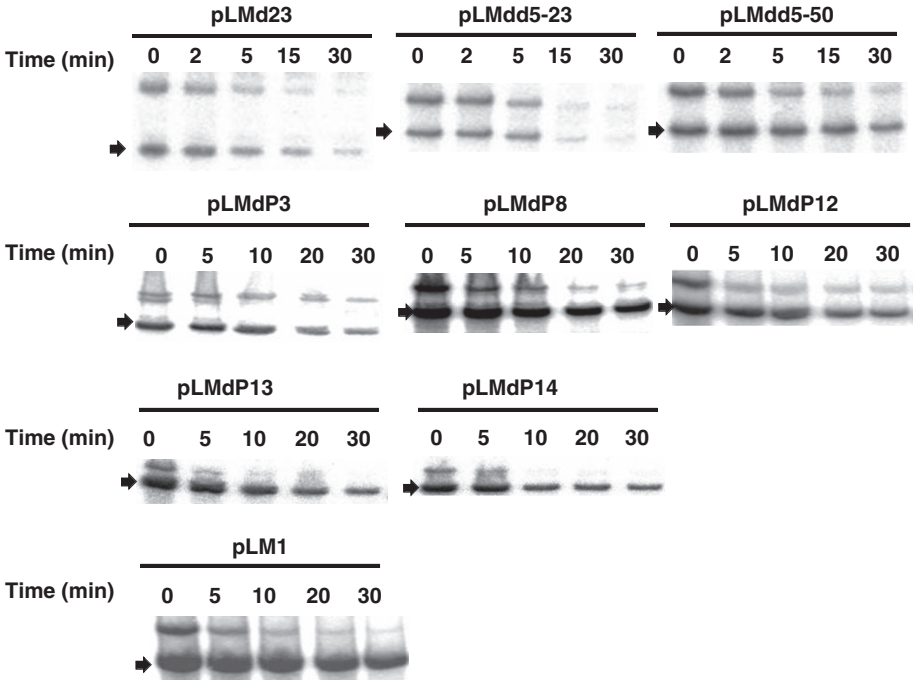

Figure 2. Metabolic stability of M1 RNA deletion derivatives. Total cellular RNA was isolated from cells containing deletion plasmids at the indicated times after the rifampicin treatment. RNAs were subjected to Northern blot analysis using an antisense M1 RNA riboprobe. M1 RNA and its derivatives are indicated by arrows.

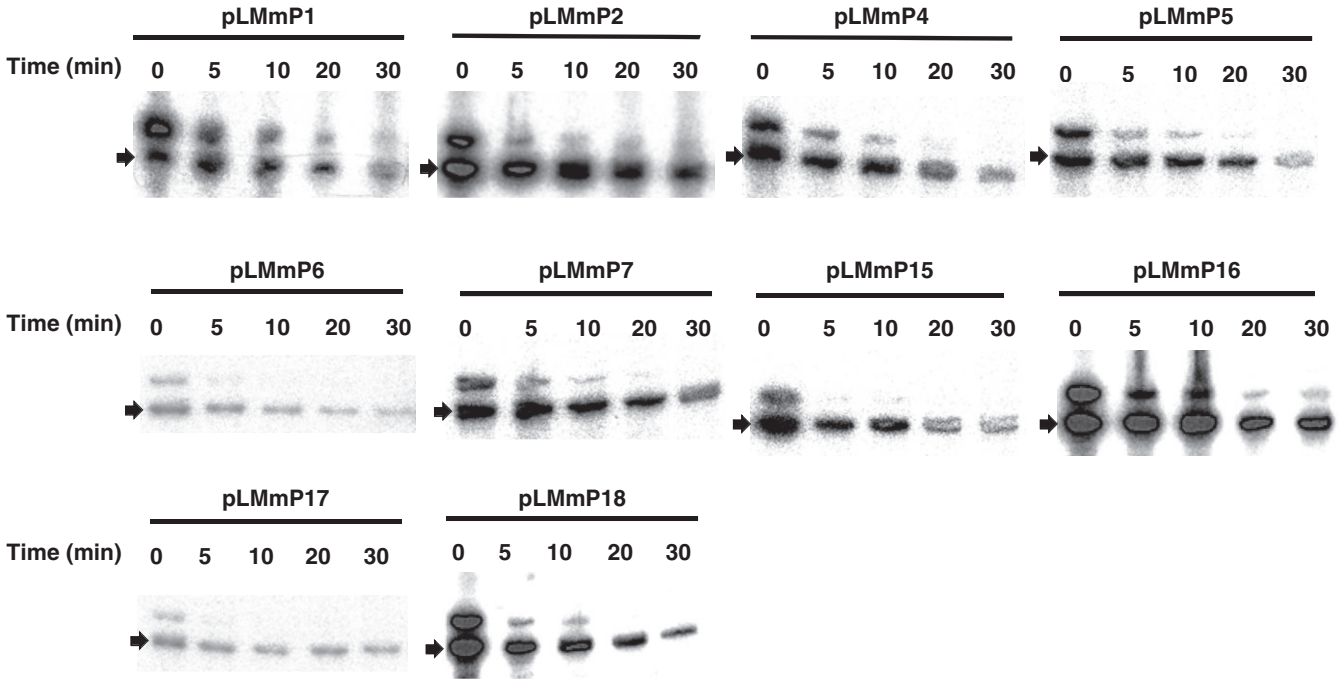

Figure 3. Metabolic stability of M1 RNA mutant derivatives. Total cellular RNA from cells containing mutant plasmids was analyzed as in Figure 2.

+1286.[11] Plasmids pLMd23, pLMdd5-23, pLMdd5-50, pLMdP3, pLMdP8/9, and pLMdP12 are derivatives of pLM1 with various internal deletions of *rnpB*.[11,15] The additional deletions were introduced using pLM1 as a template plasmid by gene splicing via overlap extension (SOEing) PCR, as previously described.[16] The PCR products containing deleted *rnpB* genes were cloned into the *Kpn*I-*Eco*RI sites of the pGEM3 vector. pLM1 mutant derivatives carrying disrupted stems were constructed through site-directed mutagenesis of pLM1, as previously described.[11]

Table 1. Features and half-lives of M1 RNA deletion derivatives.

M1 RNA derivative	Half-life (min)[a]	Plasmid	Deletion	Mutation[b]	Plasmid source[c]
d23	4	pLMd23	57–330		Ref. 11
dd5-23	7	pLMdd5-23	139–330		Ref. 11
dd5-50	8	pLMdd5-50	139–363		Ref. 11
dP3	18	pLMdP3	21–60		Ref. 15
dP8/9	22	pLMd8/9	92–119		Ref. 15
dP12	12	pLMdP12	143–175		Ref. 15
dP13	15	pLMdP13	185–201		This study
dP14	8	pLMdP14	205–225		This study
mP1	<< 1	pLMmP1		8TGGT	This study
mP2	6	pLMmP2		15TCAG	This study
mP4	6	pLMmP4		71GCCC	This study
mP5	23	pLMmP5		75GAGG	This study
mP6	5	pLMmP6		276CGGG	This study
mP7	17	pLMmP7		88CCAC	This study
mP15	4	pLMmP15		250CCGG	This study
mP16	16	pLMmP16		261CCAA	This study
mP17	7.5	pLMmP17		271CATG	This study
mP18	5	pLMmP18		307GGTC	This study
M1 RNA	50	pLM1			Ref. 11

[a] Half-lives were determined by linear regression of Northern data of Figures 2 and 3. In case of mutant derivatives, the remaining RNA was corrected by subtracting the endogenous M1 RNA.
[b] A four-nucleotide change from the indicated number for each construct was made by site-directed mutagenesis.
[c] Some plasmids used for RNA half-life determination in this study were constructed in our previous studies.

RNA Stability Assay. An RNA stability assay was performed, as described previously.[17] Briefly, JM109 cells containing plasmids were grown overnight (37°C) in LB containing ampicillin (50 mg/mL), diluted 1:100 in the same medium, and grown to an A_{600} of approximately 0.5 at 37°C. Rifampicin was added to the culture at a final concentration of 150 μg/mL to terminate further transcription. Cultures were then obtained at different time intervals, and the total RNA was prepared by hot phenol extraction and subjected to Northern blot analysis. Hybridization signals were analyzed using Image Analyzer FLA7000 (Fuji, Tokyo, Japan). Decay rates were calculated by least-squares determination of the slope of a plot of RNA intensity *vs.* time.

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