

Structure-led studies of a nuclease central to RNA decay and processing

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Introduction

The *Escherichia coli* endoribonuclease RNaseE is required for the rapid decay of mRNA and the correct processing of precursors of ribosomal and transfer RNAs. It is the archetype of a family that is widespread in bacteria and plastids. *E. coli* RNaseE is a relatively large protein of 1061 amino acids. The N-terminal half (NTH; residues 1 to 529) is the ribonucleolytic centre. RNaseE appears to initiate the decay of many, if not most, transcripts in *E. coli* generating upstream products that have a 3'-OH group and are rapidly attacked by 3' exonucleases, and downstream products that have a 5' monophosphate, which greatly stimulates further cleavages by RNaseE. The C-terminal half (CTH; 530 to 1061) contains an ancillary RNA-binding domain and multiple sites that serve as a platform for the assembly of the degradosome complex, which is arguably the main centre for RNA processing and decay in *E. coli*. The other major components of this non-covalent assembly are the 3' exonuclease polynucleotide phosphorylase (PNPase), the RhlB helicase, and the glycolytic enzyme enolase. Components of this complex have been shown to interact functionally, e.g. RhlB assists PNPase to progress through stable stem-loop structures. Our objective is to understand, at the molecular level, all the factors that contribute to the cleavage of transcripts by RNaseE within the context of the multi-enzyme degradosome complex. Structural and biochemical studies are being integrated with an analysis of RNA processing and decay *in vivo*. Our approach has been to start by thoroughly characterising the structure of the N-terminal catalytic domain and the effects on hydrolysis of modifying functional groups within RNA. These studies provide a solid base from which to analyse in more detail the recognition and hydrolysis of RNA by mutating key amino acids, and to begin to probe the structural and functional interplay of enzymes within the degradosome by extending our analysis to include domains within the C-terminal half of RNaseE.

The zinc link and structure-led studies

Recently we have begun to investigate interactions required for the formation of tetramer, and the role of these interactions in mediating the hydrolysis of RNA. The starting point for this aspect was the observation that in *E. coli* RNaseE there is a sequence, CPxCxGxG, between residues 404 to 410, which is evolutionarily conserved in homologues and found in the metal coordinating sites of the chaperonin DnaJ and the DNA mismatch repair protein MutH (also called MutR). In DnaJ and MutH, two copies of the pattern are used to coordinate a single zinc ion in a tetrahedral arrangement. These observations lead us to test whether the motif

might play a similar role in RNaseE. We made cysteine to alanine substitutions at 404 and 407 and found, using sedimentation velocity AUC and a discontinuous enzyme assay, that mutation of either of these conserved residues resulted in the disruption of the tetramer into dimers and a greater than 200-fold reduction in the rates of hydrolysis of substrates regardless of whether they have a phosphate or hydroxyl group at the 5' end. Additionally, our collaborator, Dr Ben Luisi (University of Cambridge) has shown that the NTH-RNaseE does indeed contain zinc, with a ratio of protomer to zinc of 2:1, and obtained data consistent with a tetrathiol co-ordination scheme expected for metal co-ordination by four

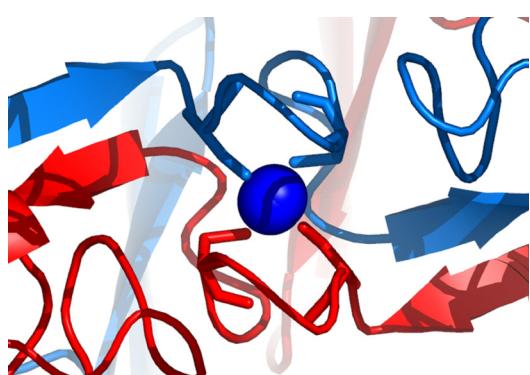


Fig. 1. The Zn Link. Different colours are used to distinguish the two protomers in a pair. Structure solved by the laboratory of Dr. Ben Luisi (University of Cambridge)

cysteine side chains using synchrotron X-ray fluorescence (with Dr. J. Gunter Grossmann, CCLRC Daresbury Laboratory), micro particle induced X-ray emission (with Dr. Elspeth Garman, University of Oxford) and measurements of extended X-ray absorption (with Dr. Lorrie Murphy, University of Bangor), respectively. Taken together, these data are consistent with a model in which the RNase E tetramer is arranged around two, non-equivalent interfaces: The zinc-mediated interface being required for the organisation of the catalytic site but not RNA binding.

We have also started to investigate whether zinc binding may have a role in regulating RNaseE activity *in vivo*. Although the study is incomplete, we are able, through studying the effects of treating preparations of RNaseE and cultures of *E. coli* with diamide (a thiol-specific oxidising agent), to conclude that zinc-binding is redox sensitive and that RNaseE activity is reduced, and mRNA is stabilised in cells during diamide stress. This is a nice example of how structural studies have suggested a possible mechanism by which gene expression is regulated *in vivo*. Dr. Ben Luisi has also been successful in solving crystal structures of the catalytic domain of RNase E as trapped allosteric intermediates with RNA substrates. Four subunits of RNase E associate into an inter-woven quaternary structure that is consolidated through the coordination of two zinc ions that are shared between subunit pairs (Fig. 1). The structure offers explanation for why the quaternary structure is required for activity, and how the recognition of the 5' terminus of the substrate triggers an allosteric transition to initiate catalysis. Mechanistic models are currently being explored through the mutagenesis of the NTH-RNaseE.

Publications

Callaghan, A.J., Redko, Y., Murphy, L., Grossmann, J.G., Yates, D., Garman, E., Ilag, L., Robinson, C.V., Symmons, M.F., McDowall, K. and Luisi, B.F. (2005) The ‘Zn-link’: A metal-sharing interface that organizes the quaternary structure and catalytic site of the endoribonuclease, RNase E. *Biochemistry* – in press.

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