

*Hypothesis***A hybrid protein kinase-RNase in an interferon-induced pathway?**

Peer Bork*, Chris Sander*

EMBL, Meyerhofstr. 1, D-69012 Heidelberg, Germany

Received 31 August 1993; revised version received 24 September 1993

The sequence of RNase L has been re-examined by computer analysis. We propose a molecular architecture of RNase L, with an unusual combination, in one protein chain, of 9 ankyrin-like repeats, a functional active protein kinase and a C-terminal catalytic RNase similar to the yeast protein, IRE1. The protein kinase may be involved in a new signal transduction pathway which remains to be discovered.

RNase; Protein kinase; Homology; Ankyrin-like repeat; Domain

1. INTRODUCTION

Sequence analysis using computers is a powerful tool for elucidating the molecular function of proteins, but it can also lead to pitfalls when interpreting similarities detected in database searches. An example of the latter problem is provided by the recently sequenced 2-5A-dependent RNase [1], an interferon-induced enzyme that is activated by 5'-phosphorylated, 2'-5'-linked oligoadenylates (2-5A). The protein is also called RNase L and may be involved in the inhibition of viral replication and/or in tumor suppression [1,2]. Zhou et al. [1] report several sequence motifs within the approximately 740 residue long sequences of human and murine 2-5A-dependent RNases. These are two nucleotide triphosphate binding sites (P-loops), a zinc finger, motif VI and VII of protein kinases, and a region with similarity to *Escherichia coli* RNase E. We have re-analyzed the sequences of both these 2-5A-dependent RNases by a variety of methods [3], combining the results of standard database searches with information from multiple sequence alignments and known 3D structures. Surprisingly, we have found a molecular architecture of the enzyme that is almost completely different from that proposed in [1]. The 2-5A-dependent RNases each consist of (i) 9 ankyrin-like repeats (ANK), (ii) a complete protein kinase-like domain and (iii) a C-terminal, 130 residue-long region which we presume to contain the RNase activity (Fig. 1).

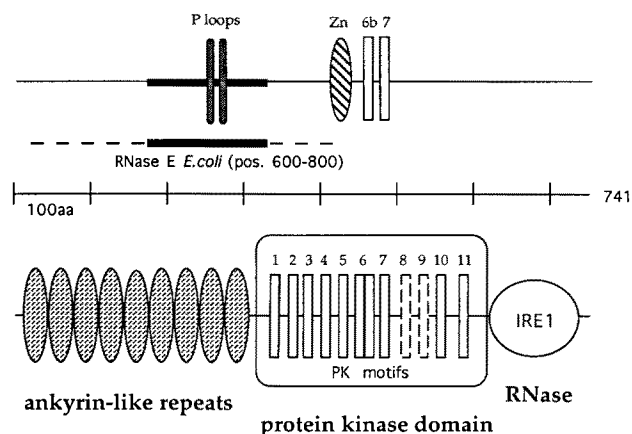


Fig. 1. Molecular architecture of 2-5A-dependent RNase. (Top) Original assignments [1]. (Bottom) Revised assignments, following extensive sequence analysis using a number of methods [3]. Notation for original assignments: P-loops (phosphate binding loops), Zn (zinc finger), 6b,7 (protein kinase motifs [13]); for revised assignments: IRE1 (region similar to yeast IRE1 protein). The highly significant similarity of 2-5A-dependent RNase to ANK-repeats (e.g. BLASTP P values $<10^{-20}$) contradicts the presence of P-loops which have been proposed on the basis of the very frequently occurring and therefore not significant tripeptide GKT [1]. The reported similarity to RNase E from *E. coli*, which also falls within the ANK repeat region, is contradicted as well by the fact that the region of RNase E proposed to match 2-5A-dependent RNase is (i) biased towards negatively charged residues (composition-biased regions can lead to spuriously high search scores) and (ii) likely to have a coiled coil region, as predicted by the method of Lupas et al. [17] and is therefore not homologous to the ANK region of 2-5A-dependent RNase. In positions 395–334 a zinc finger was proposed by Zhou et al. [1]. A detailed analysis of this region, however, did not reveal any significant similarity to known zinc fingers. Furthermore, human and mouse 2-5A-dependent RNase differ in the positions of the cysteines and the cysteine pattern in the human enzyme is atypical of that observed in classical zinc fingers.

*Corresponding authors. Fax: (49) (6221) 387–306. Email: bork@embl-heidelberg.de.

Wt tt+ht L tVt-t -It Rt S hL h- tt tS D WT Kh-t hMth-+ YtK attth DLL+hhRN Hh - ttt htL t Pt YFtKcFP-LhI VY
 Ire1/Yeast WPKSKKLEFLKVSRLREIENRDPSPALLMKFDAGSDVIPSGD. WTVKFKDTFMDNLER. YRK. . . YHSSKLMDDLRLRNKYHFMDLPEIAELMGVPDGFYDYFTKRPFNLLIGVYM
 Rn25/Human WTWESRYRTLRLNVGNESDIKTRKSESEILRLQLQGPSEHSKSFQDKWTKINECVMKMKNFYEKR. GNFYQNTVGDLLKFIIRNLGEHIDEKHKMKLKGIDPSL. . . YFQKTFPDLVIVYT
 Rn25/Mouse WTWENRYRTLRLNVGNESDIKVRKCKSDLLRLQLQHTLEPPRSPFQDKWTSKIDKNVMDENHFEKRKNFYQDVTGDLKFIIRNIGEHEINEKKRG>>>

Fig. 4. Alignment of the C-terminal, probably catalytic, domain of 2-5A-dependent RNases with the C-terminus of yeast IRE1 protein [10]. Bold residues are conserved between IRE1 and at least one of the two 2-5A RNases. Mouse 2-5A RNase has not entirely been sequenced yet. Invariant charged and polar residues in conserved regions are likely to participate in catalysis.

and are thought to mediate protein-protein interactions [4,6]. An involvement of ANK in DNA binding was shown for the GA-binding protein complex [7] and for transcription factor complex SWI4/SWI6 [8]. The ability of ANK repeats in 2-5A-dependent RNase to bind oligoadenylates would explain the abrupt loss of 2-5A binding affinity when truncating the 7th and 8th repeat, i.e. position 265–294 [1].

The putative protein kinase domain of 2-5A-dependent RNase is located immediately following the ANK repeats (Figs. 1 and 3). The closest protein kinase relative appears to be yeast KIN82 [9], with 30% amino acid sequence identity over the entire kinase domain. Other kinases, such as yeast IRE1 [10], are also significantly similar, with high FASTA opt scores >150 [11] and/or BLASTP P values <10⁻⁶ [12]. These similarities are verified by a multiple sequence alignment which indeed reveals the presence of all 11 boxes conserved in the protein kinase family [13], with some modifications (Fig. 3). Further evidence (data not shown) comes from mapping conserved residues onto the known 3D structure of mouse cAMP-dependent kinase [14]. It confirms that the changes are neither in functionally nor in structurally essential positions. Not only are all essential hydrophobic 3D contacts conserved (in particular near the active site), but also all residues known to participate in ATP- and peptide binding in protein kinases (Fig. 3).

So, if most of the protein sequence consists of ANK repeats and a protein kinase domain, then the RNase activity is most probably located in the C-terminal 130 residues. Several facts support this hypothesis. (i) The C-terminal domain of 2-5A-dependent RNase is more conserved between mouse and human (73% identical residues) than the two proteins are on average (64%), indicating strong selective pressure on this domain, typical of catalytic function. (ii) Another protein family, the membrane-bound guanylyl cyclases [15], with similar modular construction (protein kinase plus C-terminal domain), has its catalytic domain also at the C-terminus. (iii) Interestingly, the C-terminal domain of 2-5A-dependent RNase has 29% sequence identity (FASTA opt. score of 155 [11]) to the C-terminal domain of yeast IRE1 protein (Fig. 4). (Yeast IRE1 protein is involved in inositol phototrophy and has been identified by genetic complementation of *myo*-inositol auxotrophic yeast mutants [10].) (iv) The IRE1 protein also contains a protein kinase domain (Fig. 1, bottom panel) and the two putative catalytic domains are in the same relative

location in the two proteins. Taken together, these facts indicate that the C-terminal domain of 2-5A-dependent RNase is a catalytic domain, which very plausibly has RNase function.

As neither 2-5A-dependent RNase nor IRE1 (for which an RNase function can be predicted by analogy) have obvious sequence similarity to other RNase families, they might form a new structural class of RNases. Although the functionality of the kinase domain has not yet been proved, its presence could imply a regulatory function for 2-5A-dependent RNase in the interferon-dependent pathway.

3. RECENT EXPERIMENTAL EVIDENCE

As this manuscript was about to be submitted, a report by Hassel et al. [16] appeared which reports the presence of 9 ANK repeats and provides experimental evidence for the location of the RNase function within the C-terminus (a clone lacking the last 89 residues has no RNase activity) [16]. This confirms parts of our conclusions and focusses attention on experimental verification of the predicted protein kinase function. The C-terminal similarity to IRE1 is useful for identification of conserved polar residues that might be involved in catalysis (Fig. 4).

REFERENCES

- [1] Zhou, A., Hassel, B.A. and Silverman, R.H. (1993) *Cell* 72, 753–765.
- [2] Lengley, P. (1993) *Proc. Natl. Acad. Sci. USA* 90, 5893–5895.
- [3] Bork, P., Ouzounis, C., Sander, C., Scharf, M., Schneider, R. and Sonnhammer, E. (1992) *Protein Sci.* 1, 1677–1690.
- [4] Lux, S.E., John, K.M. and Bennett, V. (1990) *Nature* 344, 36–42.
- [5] Bork, P. (1993) *Proteins* (in press).
- [6] Bennett, V. (1992) *J. Biol. Chem.* 267, 8703–8706.
- [7] Thompson, C.C., Brown, T.A. and Knight, S.L. (1991) *Science* 253, 762–768.
- [8] Sidorova, J. and Breeden L. (1993) *Mol. Cell. Biol.* 13, 1069–1077.
- [9] Wilson, C., Bergantino, E., Lanfranchi, G., Valle, G., Carigani, G. and Frontali, L. (1992) *Yeast* 8, 71–77.
- [10] Nikawa, J.I. and Yamashita, S. (1992) *Mol. Microbiol.* 6, 1441–1446.
- [11] Pearson, W. R. and Lipman D. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2444–2448.
- [12] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) *J. Mol. Biol.* 215, 403–410.
- [13] Hanks, S.K. and Quinn, A.M. (1991) *Methods Enzymol.* 200, 38–62.
- [14] Knighton, D.R., Zheng, J.H., Ten Euck, L.F., Ashford, V.A.,

- Yuong, N.H., Taylor, S.S. and Sowadski, J.M. (1991) *Science* 253, 407–414.
- [15] Schulz, S., Green, C.K., Yuen, P.S.T. and Garbers, D.L. (1990) *Cell* 63, 941–948.
- [16] Hassel, B.A., Zhou, A., Sotomayor, C., Avudaiappan, M. and Silverman, R.H. (1993) *EMBO J.* 13, 3297–3304.
- [17] Lupas A., Van Dyke, M. and Stock, J. (1991) *Science* 252, 1162–1164.
- [18] Bairoch, A. and Boeckmann, B. (1992) *Nucleic Acids Res.* 20 Suppl., 2019–2022.
- [19] de Sauvage, F.J., Camerato, T.R. and Goeddel, D.V. (1991) *J. Biol. Chem.* 266, 17912–17918.