

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

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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).

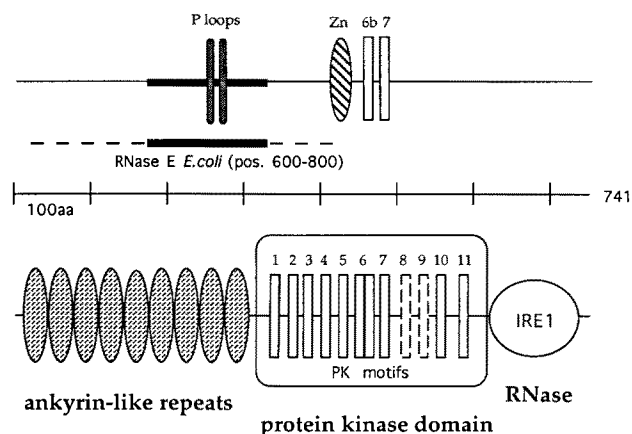


Fig. 1. Molecular architecture of 2-5A-dependent RNase. (Top) Original assignments [1]. (Bottom) Revised assignments, following extensive sequence analysis using a number of methods [3]. Notation for original assignments: P-loops (phosphate binding loops), Zn (zinc finger), 6b,7 (protein kinase motifs [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.

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ANKS: G TPLHhAht thht LLt GAT t
 cons: G T Lh Aht thht LLtt GAT t

24 EDNHLLIKAVQNEDEV...DLVQQLLEG.GANVNFOEEE
 58 GGWTPPLHNVAQMSRE...DIVELLRRH.GADPVLRRK
 91 NGATLFIILAAIAGSV...KLLKFLSK.GADVNECDF
 124 YGTAFAFMAAVYGVK...KALKFLYKR.GANVNLRRKTKEDQERLRK
 167 GGATALMDAAERGHV...EVLKILLDEM.GADVNAACDNM
 202 GRNALIHALLSSDDSDVEAITHLLLDH.GADVNVGRGE
 238 RGKTPLILAVEKKHL...GLVQRLLEQEHIEINDTDS
 272 DGKTALLLAVELKLLK...KIAELLCKR.GASTD...
 301 CG.DLVMTARRNYDH...SLVKVLLSH.GAKEDFHPAEDWKPQS..

[ANK repeat]

Fig. 2. Alignment of the 9 internal repeats in the N-terminal region of human 2-5A-dependent RNase. In the top two lines the consensus derived from the alignment (cons) is compared with the consensus of about 650 ANK repeats (ANKS) [5]. Numbers on the left are residue positions of the beginning of the repeat in the 2-5A-dependent RNase sequence.

		I										II										III										IV										V													
		hG G G Vh										hAhK h										h E thh										hh hh										hah ht h t th thht													
Kapa_Mouse	43	FDRIKT	LGTS	FGFRV	LVKHK	ESGNH	YAMKIL	DKQ	-7-	GIEHTL	NEKRIL	-4-	FPFLVK	LEFS	FKD	NSN	LYM	MEY	VAGG	EMF	SHLR	-4-	FPFLVK	LEFS	FKD	NSN	LYM	MEY	VAGG	EMF	SHLR	-4-	FPFLVK	LEFS	FKD	NSN	LYM	MEY	VAGG	EMF	SHLR	-4-	FPFLVK	LEFS	FKD	NSN	LYM	MEY	VAGG	EMF	SHLR				
Weel_Schpo	566	FRNVTL	LGSG	FESE	VEFV	QVED	PVEK	TLKV	AVK	KL	VVK	-6-	GRNRL	LQEV	SIQ	-5-	HDHIV	ELMDS	WEHGG	FYLM	QVCL	ENGSL	DRFLE	-5-	HDHIV	ELMDS	WEHGG	FYLM	QVCL	ENGSL	DRFLE	-5-	HDHIV	ELMDS	WEHGG	FYLM	QVCL	ENGSL	DRFLE	-5-	HDHIV	ELMDS	WEHGG	FYLM	QVCL	ENGSL	DRFLE	-5-	HDHIV	ELMDS	WEHGG	FYLM	QVCL	ENGSL	DRFLE
Ki82_Yeast	324	FEKIRL	QGQD	VGVK	VYLV	RER	DTNQ	IFALK	VLN	K	-8-	KIKRV	LTQE	EIL	-4-	HPFIV	TLYHS	FQTK	DYLY	LCME	YCMG	GEFF	FRALQ	-4-	HPFIV	TLYHS	FQTK	DYLY	LCME	YCMG	GEFF	FRALQ	-4-	HPFIV	TLYHS	FQTK	DYLY	LCME	YCMG	GEFF	FRALQ	-4-	HPFIV	TLYHS	FQTK	DYLY	LCME	YCMG	GEFF	FRALQ					
Pkgc/Rat	24	YDPKDI	IGRG	SVVVR	RCVHR	ATGD	FAVK	IMEVS	-12-	VRDAT	RRRM	HIL	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE			
Irel1/Yeast	674	VYSEKI	LGYS	SGTV	VVFGS	FGGR	EVAV	KRM	LID	-8-	NMLL	ALME	IKLL	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE		
Cc2_Xenla	3	YTKIEK	IGEG	TYGV	VYKGR	HK	ATGQ	VAMK	KIR	L	-5-	VPSTA	IREIS	LL	-4-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	-5-	HPNVI	RYCSE	TTDR	FLYI	IAELC	NLNL	QDLVE	
Rn25/Human	365	IDEKYK	IAAT	SEGG	IYLG	F	YEQE	VAVK	TPEG	-0-	VSPRA	QREV	SCL	-5-	NSHLV	TFYGS	ESH	RGH	LFVC	VTLC	EQTLE	ACLD	-5-	NSHLV	TFYGS	ESH	RGH	LFVC	VTLC	EQTLE	ACLD	-5-	NSHLV	TFYGS	ESH	RGH	LFVC	VTLC	EQTLE	ACLD	-5-	NSHLV	TFYGS	ESH	RGH	LFVC	VTLC	EQTLE	ACLD						
Rn25/Mouse	364	IHDDYK	IACT	SEG	AVYLG	I	YDN	REV	AVK	VFREN	-0-	LSPRG	CKEV	SCL	-5-	HSNLV	AFYGR	EDDK	GCLY	VCVSLC	EWTL	EEFLR	-5-	HSNLV	AFYGR	EDDK	GCLY	VCVSLC	EWTL	EEFLR	-5-	HSNLV	AFYGR	EDDK	GCLY	VCVSLC	EWTL	EEFLR	-5-	HSNLV	AFYGR	EDDK	GCLY	VCVSLC	EWTL	EEFLR	-5-	HSNLV	AFYGR	EDDK	GCLY	VCVSLC	EWTL	EEFLR	
Hser_Human	491	TDDDR	RL	RTIQ	RLR	OCK	YDK	KRV	ILK	DLKHN	-4-	ITEKQ	KIEL	NKL	-4-	YYNLT	KFYGT	VKLD	TM	IFG	VIEY	CERGS	LEVLN	-4-	YYNLT	KFYGT	VKLD	TM	IFG	VIEY	CERGS	LEVLN	-4-	YYNLT	KFYGT	VKLD	TM	IFG	VIEY	CERGS	LEVLN	-4-	YYNLT	KFYGT	VKLD	TM	IFG	VIEY	CERGS	LEVLN					

		VIa										VIb										VII										VIII										IX									
		hh thh tht hH										thh atDLKttNhhht										th h htDFG										t a tPEh										htt h									
Kapa_Mouse	-3-	FSEPHAR	YAAQ	IVLT	FEY	LHSL	LLIY	RD	LKPEN	LLID	-0-	QGGY	IQV	TD	FG	FAKRV	-8-	GTPEY	LAPE	ILS	-1-	GYNKAV	-0-	QGGY	IQV	TD	FG	FAKRV	-8-	GTPEY	LAPE	ILS	-1-	GYNKAV	-0-	QGGY	IQV	TD	FG	FAKRV	-8-	GTPEY	LAPE	ILS	-1-	GYNKAV					
Weel_Schpo	-6-	LDEFVR	WV	ILVE	VALG	LQFI	HHK	NYV	HL	DLKPAN	VMIT	-0-	HEGTL	KIGD	FG	MASVW	-9-	GDCEY	IAPE	VLAN	-1-	LYDKPA	-0-	HEGTL	KIGD	FG	MASVW	-9-	GDCEY	IAPE	VLAN	-1-	LYDKPA	-0-	HEGTL	KIGD	FG	MASVW	-9-	GDCEY	IAPE	VLAN	-1-	LYDKPA							
Ki82_Yeast	-6-	AEDAK	Y	YASE	VVA	LEY	LHSL	CFIY	RD	LKPEN	ILLH	-0-	QSGH	VML	SDFD	LSIQA	-29-	GTPEY	LAPE	VIRG	-1-	GHTAAV	-0-	QSGH	VML	SDFD	LSIQA	-29-	GTPEY	LAPE	VIRG	-1-	GHTAAV	-0-	QSGH	VML	SDFD	LSIQA	-29-	GTPEY	LAPE	VIRG	-1-	GHTAAV							
Pkgc/Rat	-4-	SEKETR	S	IMRS	LLEA	VNPL	HVN	NIVH	RD	LKPEN	ILLD	-0-	INMQ	IRLS	SDFG	F	SCHL	-10-	GTPEY	LAPE	ILK	-7-	GYGKEV	-0-	INMQ	IRLS	SDFG	F	SCHL	-10-	GTPEY	LAPE	ILK	-7-	GYGKEV	-0-	INMQ	IRLS	SDFG	F	SCHL	-10-	GTPEY	LAPE	ILK	-7-	GYGKEV				
Irel1/Yeast	-10-	QKEYNP	IS	LLRQ	IASG	VAH	LHSL	KI	HL	RD	LKPEN	ILLV	-13-	HNLR	ILIS	D	FG	LCKKL	-15-	GTSGW	RAPE	LLIE	-33-	RLTRSI	-0-	HNLR	ILIS	D	FG	LCKKL	-15-	GTSGW	RAPE	LLIE	-33-	RLTRSI	-0-	HNLR	ILIS	D	FG	LCKKL	-15-	GTSGW	RAPE	LLIE	-33-	RLTRSI			
Cc2_Xenla	-7-	IDTMLV	KSY	LYQ	ILQ	GIV	FC	HSR	GV	HL	RD	LKPEN	ILLD	-0-	NKGVI	KLAD	FG	L	ARAF	-11-	VTWLY	RAPE	VLLG	-2-	RYSTPV	-0-	NKGVI	KLAD	FG	L	ARAF	-11-	VTWLY	RAPE	VLLG	-2-	RYSTPV	-0-	NKGVI	KLAD	FG	L	ARAF	-11-	VTWLY	RAPE	VLLG	-2-	RYSTPV		
Rn25/Human	-8-	EEDEFAR	N	VLS	SIFK	AVQ	ELH	SCCY	THQD	LQ	PQNILD	-0-	SKKAA	HLAD	FD	-0-	KS	IKWAGD	PQEV	KR	-0-	DLEDLG	-0-	SKKAA	HLAD	FD	-0-	KS	IKWAGD	PQEV	KR	-0-	DLEDLG	-0-	SKKAA	HLAD	FD	-0-	KS	IKWAGD	PQEV	KR	-0-	DLEDLG				
Rn25/Mouse	-8-	GEDKFA	HS	ILLS	IFEG	VQ	KLH	LH	CY	SHQD	LQ	PQNILD	-0-	SKKAV	RLAD	FD	-0-	QS	IRWMGESQ	MVRR	-0-	DLEDLG	-0-	SKKAV	RLAD	FD	-0-	QS	IRWMGESQ	MVRR	-0-	DLEDLG	-0-	SKKAV	RLAD	FD	-0-	QS	IRWMGESQ	MVRR	-0-	DLEDLG						
Hser_Human	-9-	MDWEFK	IS	VLYD	IAK	GMSY	LH	SKTE	VEVH	DLK	STNC	VVD	-0-	SRMV	VKIT	D	FG	ENSIL	-2-	KKDLW	TAPE	HL	ARQ	-1-	NISQKG	-0-	SRMV	VKIT	D	FG	ENSIL	-2-	KKDLW	TAPE	HL	ARQ	-1-	NISQKG	-0-	SRMV	VKIT	D	FG	ENSIL	-2-	KKDLW	TAPE	HL	ARQ	-1-	NISQKG

		IX										X										XI										XII										XIII															
		Dha hGhh										h Pa										h D hht hht t t R t										h h aa										h h aa															
Kapa_Mouse		DWAL	GV	L	YEMAA	GYP	PFFA	...	QPIQ	IYEK	IY	-6-	PSHF	-3-	LKD	LLRNL	LQV	DLTK	R	GNL	KNGV	NDIK	NH	KW	FAT	TD	303																														
Weel_Schpo		DIFSL	G	I	T	FEAA	ANIV	LPDNG	...	QSQK	L	RSCD	-9-	TDNG	-10-	GLDR	V	VEW	MLSP	EP	RN	RPTIDQ	...	ILAT	DEV	C	VEM	MR	852																												
Ki82_Yeast		DWNTL	G	I	L	YEMLF	GCT	PFFK	...	INS	NET	F	SNIL	-6-	PHDK	-5-	KD	L	IKK	LLN	KNEA	K	R	L	G	S	K	608																													
Pkgc/Rat		DLWAC	G	V	I	L	YEMLF	GCT	PFFK	...	INS	NET	F	SNIL	-6-	PHDK	-5-	KD	L	IKK	LLN	KNEA	K	R	L	G	S	K	608																												
Irel1/Yeast		DIFSM	G	C	V	Y	I	L	YEMLF	GCT	PFFK	...	INS	NET	F	SNIL	-6-	PHDK	-5-	KD	L	IKK	LLN	KNEA	K	R	L	G	S	K	608																										
Cc2_Xenla		DVNSV	G	T	I	FEAA	...	TKK	PLF	G	DSE	I	D	L	F	R	I	R	-10-	PEVE	-27-	GLD	L	L	S	K	M	L	V	Y	D	P	A	K	R	I	S	...	ARK	A	M	L	H	P	F	D	D	L	292								
Rn25/Human		RLVLY	V	V	K	G	S	I	...	SFED	L	K	A	...	QSN	E	E	V	Q	L	S	...	LSD	L	L	G	H	P	F	F	T	W	E	591																							
Rn25/Mouse		RLVLY	V	V	K	G	S	I	...	SFED	L	K	A	...	QSN	E	E	V	Q	L	S	...	LSD	L	L	G	H	P	F	F	T	W	E	591																							
Hser_Human		DVSYG	I	I	A	...	KEI	...	PFET	L	K	T	...	QNE	V	L	L	T																																							

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 WPKSKKLEFLKVDRLKLEIENRDPSSALLMKFDAGSDVIPSGD. WTVKFKDTFMDNLER. YRK. . . YHSSKLMDDLRLRNKYHFMDLPEIAELMGVPDGFYDYFTKRPFNLLIGVYM
 Rn25/Human WTWESRYRTLRLNVGNESDIKTRKSESEILRLQLQGPSEHSKSFQDKWTKINECVMKMKNFYEKR. GNFYQNTVGDLLKFIIRNLGEHIDEKHKMKLKGIDPSL. . . YFQKTFPDLVIVYT
 Rn25/Mouse WTWENRYRTLRLNVGNESDIKVRKCKSDLLRLQLQHTLEPPRSFQDKWTSKIDKNVMDENHFEKRKNFYQDVTGDLKFIIRNIGEHINEEKKRG>>>

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).

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