

Comparative functional analysis of ribonuclease 1 homologs: Molecular insights into evolving vertebrate physiology

Jo E. Lomax^{1,†}, Chelcie H. Eller^{2,†}, & Ronald T. Raines^{2,3,*}

¹Graduate Program in Cell & Molecular Biology, University of Wisconsin–Madison, 1525 Linden Drive, Madison, Wisconsin, U.S.A.; ²Department of Biochemistry, University of Wisconsin–Madison, 433 Babcock Drive, Madison, Wisconsin, U.S.A.; ³Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, Wisconsin, U.S.A.

[†]Authors contributed equally to this work.

*Correspondence: Ronald T. Raines (rtraines@wisc.edu)

Table S1 Oligonucleotides used in the cloning of novel ribonucleases.

Gene	Restriction Enzyme	Primer Sequence
Bat RNase 1 (forward)	None	TATGAAGGAATCACGGGCCATGAAGTTC
Bat RNase 1 (reverse)	<i>Sall</i>	CTTATATAGTCGACTCAGCTGGAGGCCTCTACTG
Squirrel RNase 1 (forward)	None	TATGAAGGAATCCGCAGCCAACAAATTC
Squirrel RNase 1 (reverse)	<i>Sall</i>	CTTATATAGTCGACTTAAGTGGACTCCTC
Horse RNase 1 (forward)	None	TATGAAGGAATCACCCGCCATGAAG
Horse RNase 1 (reverse)	<i>Sall</i>	CTTATATAGTCGACTCAAGTGGAGACCTCC
Cat RNase 1 (forward)	None	TATGAAGGAATCCCGGGCCATGAAG
Cat RNase 1 (reverse)	<i>Sall</i>	CTTATATAGTCGACCTAACAGAATCATCAAAGTG

Table S2 GenBank accession numbers for RNase 1 genes used in this study.

Binomial Name	Common Name	Order	GenBank Accession No.
<i>Ailuropoda melanoleuca</i>	Giant panda	Carnivora	AHI58810
<i>Anolis carolinensis</i>	Anole lizard	Reptilia (Class)	XP_003223861
<i>Artibeus jamaicensis</i>	Common fruit bat	Chiroptera	CAD59680
<i>Balaena mysticetus</i>	Bowhead whale	Cetacea	AAP76380
<i>Bos taurus</i>	Cow	Cetartiodactyla	AAI49530
<i>Canis lupus familiaris</i>	Dog	Carnivora	AHI58814
<i>Capra hircus</i>	Goat	Artiodactyla	P67926
<i>Ceratotherium simum</i>	White rhinoceros	Perissodactyla	XP_004421360
<i>Cervus elaphus</i>	Red deer	Artiodactyla	P00663
<i>Colobus polykomos</i>	King colobus	Primates	ACT98220
<i>Equus africanus asinus</i>	Donkey	Perissodactyla	XP_014699959
<i>Equus caballus</i>	Horse	Perissodactyla	NP_001296341
<i>Felis catus</i>	Cat	Carnivora	XP_003987441
<i>Gallus gallus</i>	Chicken	Aves (Class)	ABD60081
<i>Giraffa camelopardalis</i>	Giraffe	Artiodactyla	P00662
<i>Gorilla gorilla</i>	Gorilla	Primates	AAL87050
<i>Hipposideros pratti</i>	Pratt's roundleaf bat	Chiroptera	AGF41055
<i>Homo sapiens</i>	Human	Primates	CAG29314.1
<i>Macaca mulatta</i>	Rhesus macaque	Primates	AFI37916
<i>Microtus ochrogaster</i>	Prairie vole	Rodentia	XP_005371043
<i>Mus musculus</i>	Mouse	Rodentia	NP_035401
<i>Myotis lucifugus</i>	Little brown bat	Chiroptera	AEF13449
<i>Nomascus leucogenys</i>	Gibbon	Primates	Q8SQ11
<i>Odobenus rosmarus</i>	Walrus	Carnivora	XP_004402205
<i>Ovis aries</i>	Sheep	Artiodactyla	P67927
<i>Panthera tigris</i>	Tiger	Carnivora	XP_007091464
<i>Pan troglodytes</i>	Chimpanzee	Primates	NP_001009108.2
<i>Pongo abelii</i>	Orangutan	Primates	NP_001126810
<i>Rana pipiens</i>	Northern leopard frog	Amphibia (Class)	AAL54383.1
<i>Rattus norvegicus</i>	Common rat	Rodentia	EDL88443
<i>Sciurus carolinensis</i>	Eastern gray squirrel	Rodentia	ACV70066
<i>Ursus maritimus</i>	Polar bear	Carnivora	AHI58815
<i>Vicugna pacos</i>	Alpaca	Artiodactyla	XP_006218762

Table S3 Physical parameters of homologous ribonucleases.

Species	MW (Da)	Number of Residues	Z ^a	T _m (°C) ^b
Human (<i>H. sapiens</i>)	14706	128	+6	56.8 ± 0.8
Bat (<i>M. lucifugus</i>)	14565	128	+6	62.2 ± 1.2
Squirrel (<i>S. carolinensis</i>)	14467	128	+4	56.8 ± 0.4
Horse (<i>E. caballus</i>)	14523	128	+4	54.8 ± 0.5
Cat (<i>F. catus</i>)	14395	124	+2	64.7 ± 1.2
Mouse (<i>M. musculus</i>)	14150	124	+4	65.4 ± 0.9
Cow (<i>B. taurus</i>)	13690	124	+4	62.2 ± 0.6
Chicken (<i>G. gallus</i>)	13354	116	+8	56.7 ± 0.3
Anole (<i>A. carolinensis</i>)	13897	121	+5	51.7 ± 0.6
Frog (<i>R. pipiens</i>)	11820	104	+6	85.1 ± 0.7

^aValues of Z refer to the net molecular charge: Arg + Lys – Asp – Glu.

^bValues of T_m (± SE) are the temperature at the midpoint of thermal denaturation, as determined by DSF.

Table S4 Percent identity/similarity^a between ribonuclease homologs.

	Human	Bat	Squirrel	Horse	Cat	Mouse	Cow	Chicken	Lizard	Frog
Human		84.3%	85.0%	85.0%	61.4%	77.1%	76.4%	42.9%	47.9%	35.7%
Bat	80.2%		82.1%	83.6%	67.1%	79.3%	74.3%	46.4%	47.9%	38.6%
Squirrel	80.5%	76.2%		85.7%	63.6%	81.4%	81.4%	45.7%	49.3%	37.1%
Horse	79.7%	79.4%	80.5%		64.3%	79.3%	80.0%	42.9%	47.9%	36.4%
Cat	62.6%	65.9%	64.2%	62.6%		62.9%	59.3%	35.0%	37.1%	22.9%
Mouse	70.2%	71.8%	74.2%	60.2%	72.6%		77.9%	47.9%	52.9%	37.9%
Cow	70.2%	68.5%	76.6%	57.7%	73.4%	70.2%		45.7%	50.0%	37.9%
Chicken	30.2%	31.9%	31.0%	25.9%	30.2%	33.6%	30.2%		49.3%	39.3%
Lizard	41.3%	41.3%	41.3%	33.1%	41.3%	42.1%	39.7%	35.3%		40.7%
Frog	24.0%	26.0%	27.0%	18.3%	26.0%	25.0%	25.0%	25.0%	21.2%	

^aGray shading denotes percent similarity of residues, as calculated by assuming that G = A = V = L = I; F = Y = W; C = M; S = T; K = R = H; D = E; N = Q.

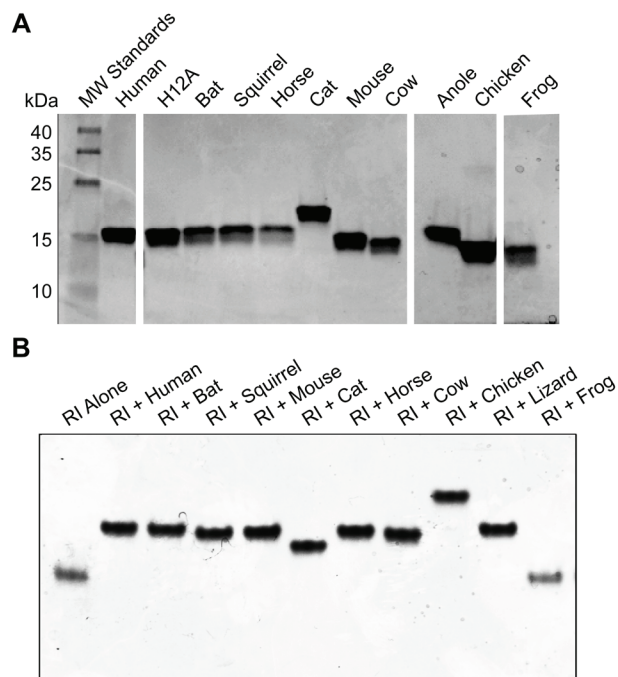


Figure S1. Analyses of ribonucleases with polyacrylamide gel electrophoresis.

(A) SDS-PAGE gel of purified RNase 1 homologs, following their purification. (B) Native PAGE gel of human ribonuclease inhibitor (RI) and an RNase, which had been incubated in a 1:1.2 ratio. A shift is indicative of a binding interaction.

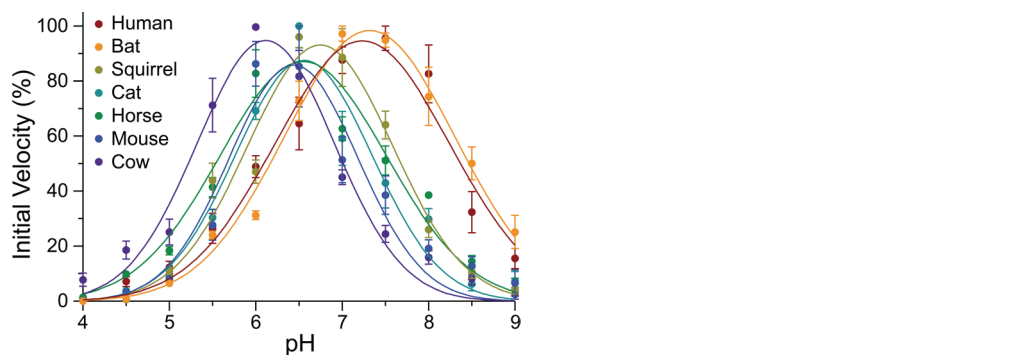


Figure S2. Catalysis of ssRNA cleavage.

pH-rate profile for cleavage of a ssRNA substrate by mammalian RNase 1 homologs. Data points are the mean \pm SEM from 3–10 independent experiments and are normalized to the value at the optimal pH (100%). Values of k_{cat}/K_M at the optimal pH are listed in Table 1.

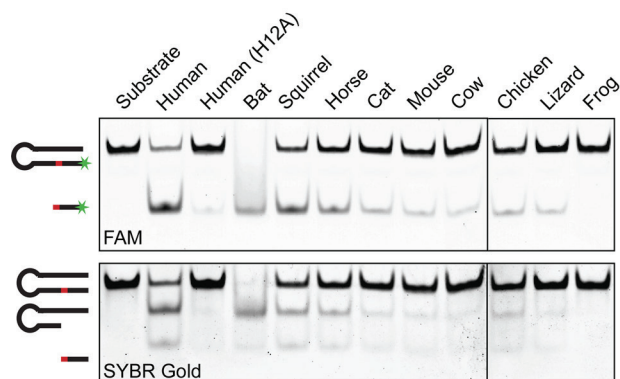


Figure S3. Catalysis of dsRNA cleavage.

Representative native polyacrylamide gel showing RNase 1 cleavage of a DNA hairpin containing a single RNA residue (red). FAM refers to imaging of the fluorophore at the 5' end of the substrate; SYBR Gold refers to imaging of total nucleic acid. Values of production formation (%) are listed in Table 1.

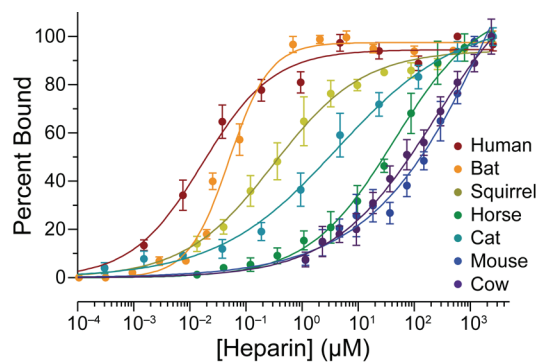


Figure S4. Cellular binding.

Representative binding isotherms for mammalian RNase 1–BODIPY conjugates to heparin as measured by fluorescence polarization. Data points are the mean \pm SEM from 3–6 independent fluorescence polarization experiments. Values of K_d are listed in Figure 1B.