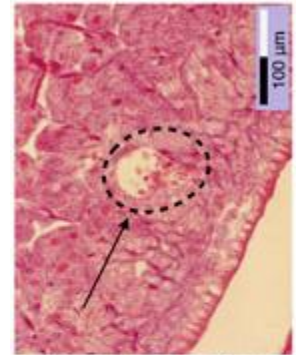
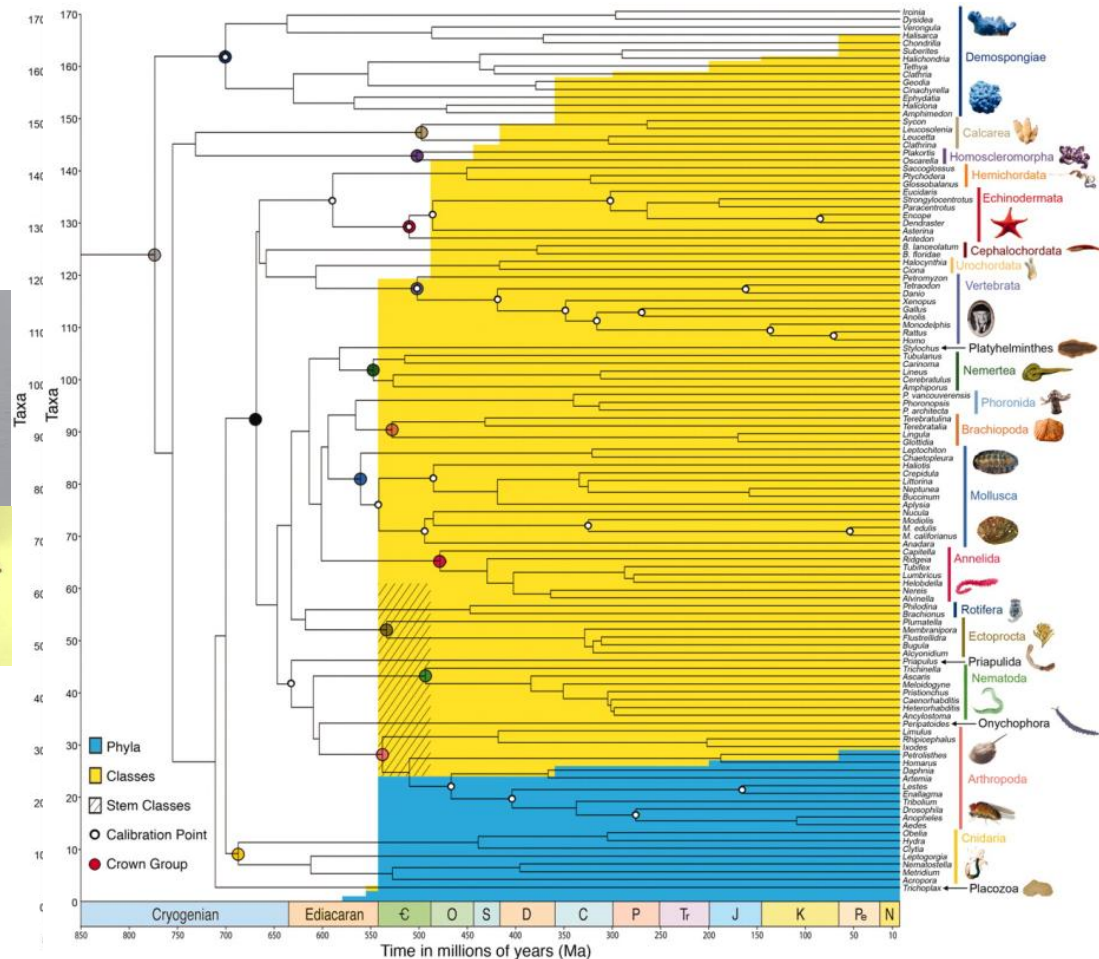
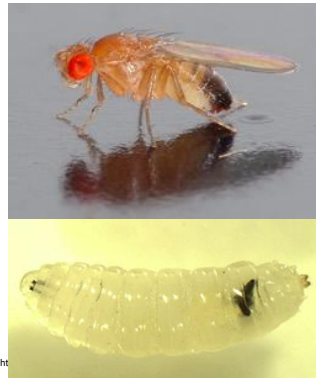


Invertebrate Immunology

Bi5615en

M 08.00-09.50h; D36-212, Masaryk University



etch/UBER1/ZI-SAMP-2016-JAN00-IDS-260-1

TODAY


HK

EXAM 1, MARCH 24. M/C questions for topics 1-4

RECAP

CLOTTING, PPO, COMPLEMENT

Schedule, **NOTE** exams timing

Invertebrate Immunology Bi5615en, tentative schedule, M 08.30-10.00h; D36 212			
	date	lecture	topic
	17-Feb	1	the mammalian standard, phylogeny
	24-Feb	2	absence of invert lymphocytic immunity, red queen
	3-Mar	3	lectins, PRRs and PAMPs
	10-Mar	4	Immune cascades: clotting, PPO, complement
	17-Mar	5	AMPs: discovery, mechanisms, distribution
	24-Mar	(-)	EXAM topics 1-4
	31-Mar	6	hemocytes: phagocytosis, cytotoxicity
	7-Apr	7	Toll and IMD pathways
	14-Apr	8	Cytokines
	21-Apr	9	Diversification of immune factors/memory/novel immune mechanisms
	28-Apr	10	EXAM 2 lectures 5-9

Makeup possibility by ZOOM

Exam 1 Monday 24 March 2025, Invertebrate Immunology Bi5615en
40 total points (2 points for each of 20 questions)

Closed book, do your own work
Time available 08.30h-09.50, (1hour, 20 minutes).

Enter your answer to each of the 20 m/c questions on this answer sheet.
ALSO ENTER YOUR NAME.

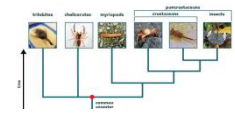
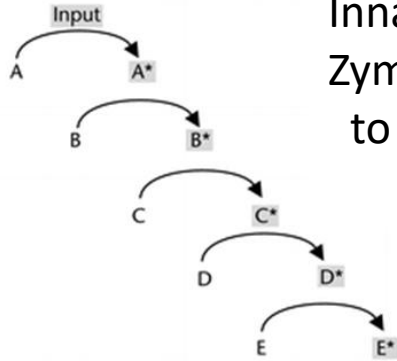
NAME _____

Circle the correct answer for each question and return this exam for grading.

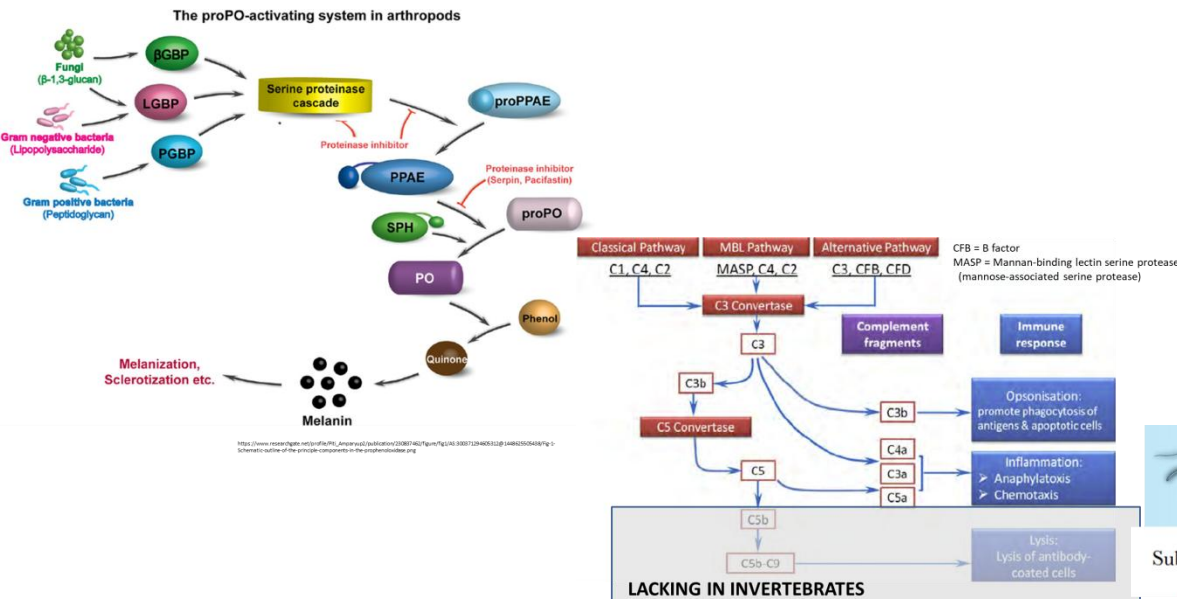
RECAP

Innate immune cascades
Zymogens are activated
to activate the next step

Interactions of
serine protease and
Serine protease inhibitors (serpins)
Fine control



Clotting
Melanization (Phenol Oxidase)
Complement



Substrate specificity of phenoloxidase-like activity in an ecoimmunological model species
Lymnaea stagnalis

Ecoimmunological research on molluscs and other invertebrates frequently quantifies phenoloxidase (PO) activity to estimate the strength of the immune function. PO enzymes form different families whose relative roles in oxidative reactions are typically unknown. Understanding this could allow enzyme-specific assays with higher accuracy than in commonly used nonspecific assays. We tested the contribution of different PO enzyme families to haemolymph PO-like activity in *Lymnaea stagnalis* snails using substrates specific to enzymes detected in *L. stagnalis* transcriptome data (*p*-phenylenediamine, specific to laccases; L-tyrosine, specific to tyrosinases) and compared the reactions to those with a nonspecific substrate (L-dopa). We found laccase-like but no tyrosinase-like activity. However, reactions with L-dopa were the strongest, possibly due to other oxidative enzymes in snail haemolymph. Laccase-like activity is common in molluscs, and we propose the use of enzyme-specific assays in future ecoimmunological studies of this taxon. The lack of tyrosinase-like activity in *L. stagnalis* contradicts earlier transcriptome data, which calls for investigating the expression of PO enzymes in *L. stagnalis* at the proteome level.

INSECT "VACCINATION" WITH INJECTED BACTERIA?

**Drosophila survives primary injection with *Enterococcus cloacae*
then survives secondary injection
with normally lethal *Pseudomonas aeruginosa***

INFECTION AND IMMUNITY, July 1974, p. 136-145
Copyright © 1974 American Society for Microbiology

Vol. 10, No. 1
Printed in U.S.A.

Insect Immunity

I. Characteristics of an Inducible Cell-Free Antibacterial Reaction in Hemolymph of *Samia cynthia* Pupae

HANS G. BOMAN, INGRID NILSSON-FAYE, KERSTIN PAUL, AND TORGNY RASMUSON, JR.

A large, brown and white moth, likely a species of Saturniidae, pinned to a light-colored surface. The moth's wings are spread, showing intricate patterns of brown, white, and yellow. A small, rectangular label is attached to the bottom of the moth, containing text: "SATURNIIDAE: Cynthia (Drury, 17 et al. No. 77".



VACCINATION?

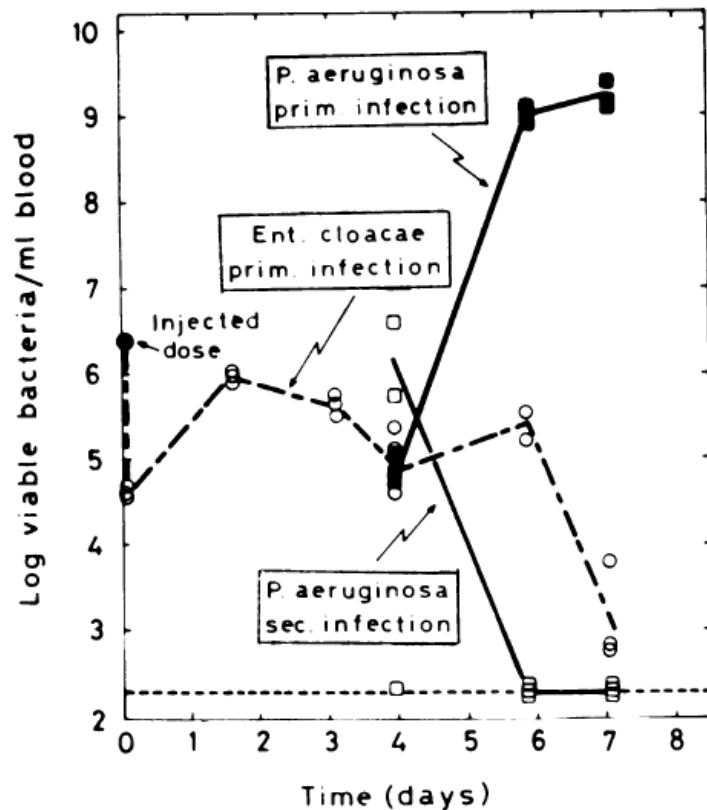


FIG. 1. Vaccination of *S. cynthia* pupae with *E. cloacae* and test for immunity by a secondary infection with *P. aeruginosa*. At the start of the experiment three pupae were injected with *E. cloacae* (○), three control pupae were given W-saline. On day 4 all pupae were given injections with about 10^6 viable *P. aeruginosa* (control pupae, ■; vaccinated pupae, □). At the times indicated 5- μ liter samples of hemolymph were withdrawn from the pupae and assayed for viable bacteria by plating on media containing the selective antibiotics given in Table 1. Each individual pupa is represented by one point and the dotted line in bottom part of the figure indicates the lowest level of bacteria that could be detected.

LYSIS

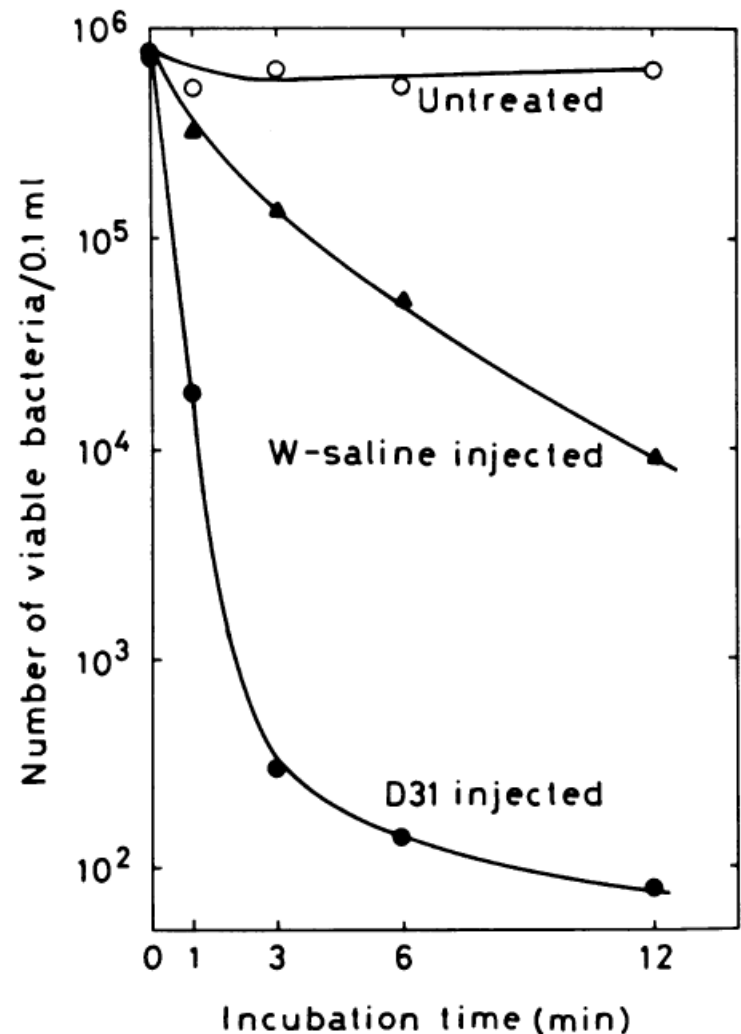


FIG. 2. *In vitro* assay of the antibacterial activity of 1% of hemolymph from *S. cynthia*. The test organism was *E. coli*, strain D31, and the reaction conditions are given in detail in Materials and Methods. Symbols: pupa vaccinated with living *E. coli* strain D31, ●; pupa injected with an equal volume of sterile W-saline, ▲; untreated pupa, ○.

DIFFERENT SMALL PROTEINS (PEPTIDES), ACTIVITIES

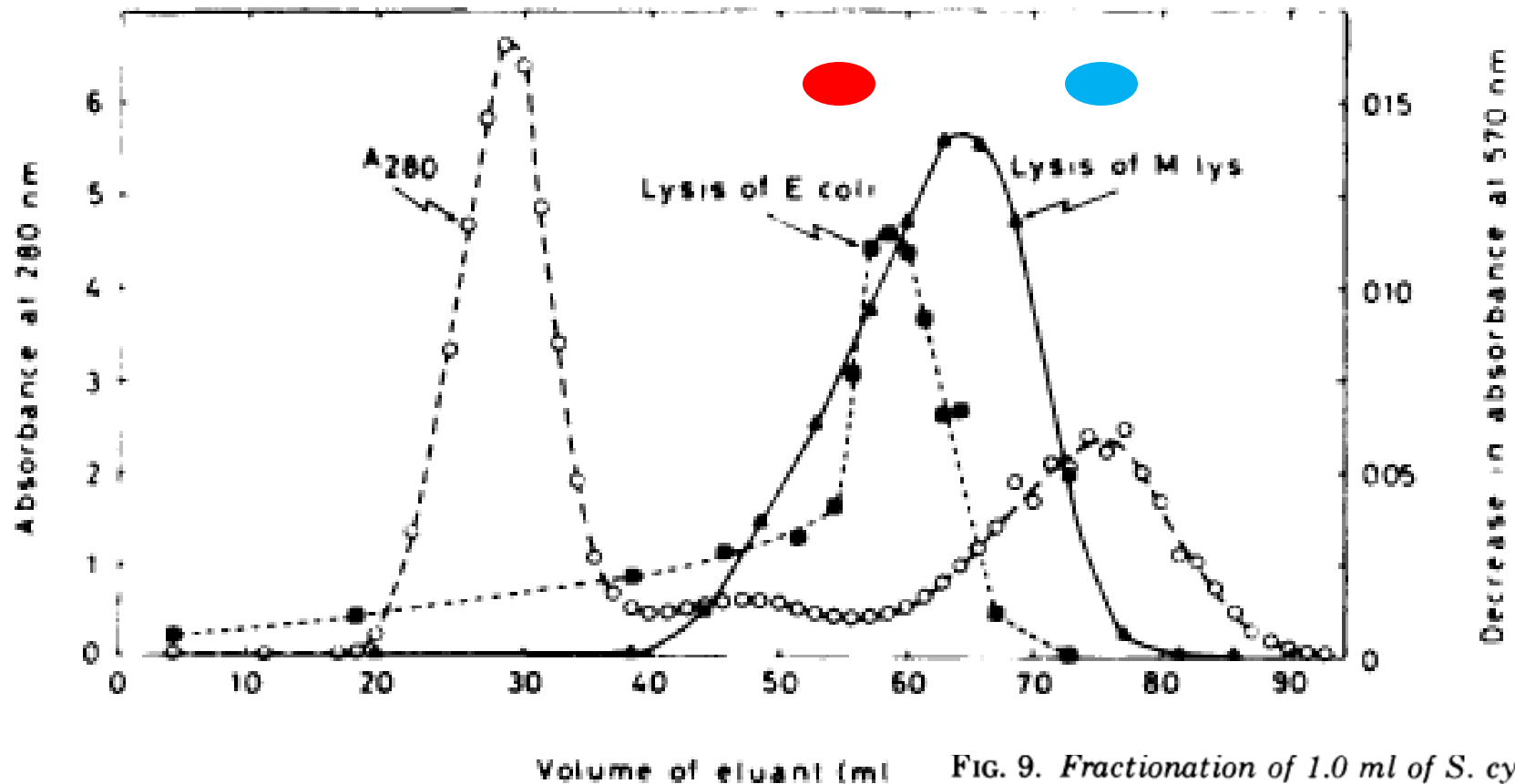


FIG. 9. Fractionation of 1.0 ml of *S. cynthia* hemolymph obtained from two pupae vaccinated with *E. coli* strain D31. The column (44 by 1.35 cm) with Sephadex G-200 (Pharmacia, Uppsala, Sweden) was equilibrated with 0.1 M potassium phosphate buffer, pH 6.4, with 2×10^{-3} M β -mercaptoethanol. The eluant from the column was assayed for absorbance at 280 nm (O) for lysis of *E. coli* strain D31 (■, and for lysis of *M. lysodeikticus* (▲). Both determinations of bacterial lyses were made after incubation of 0.1 ml of sample and 1 ml of cell suspension at 37 C for 45 min.

WHAT IS NEW?

Samia cynthia vs *Hyalophora cecropia*

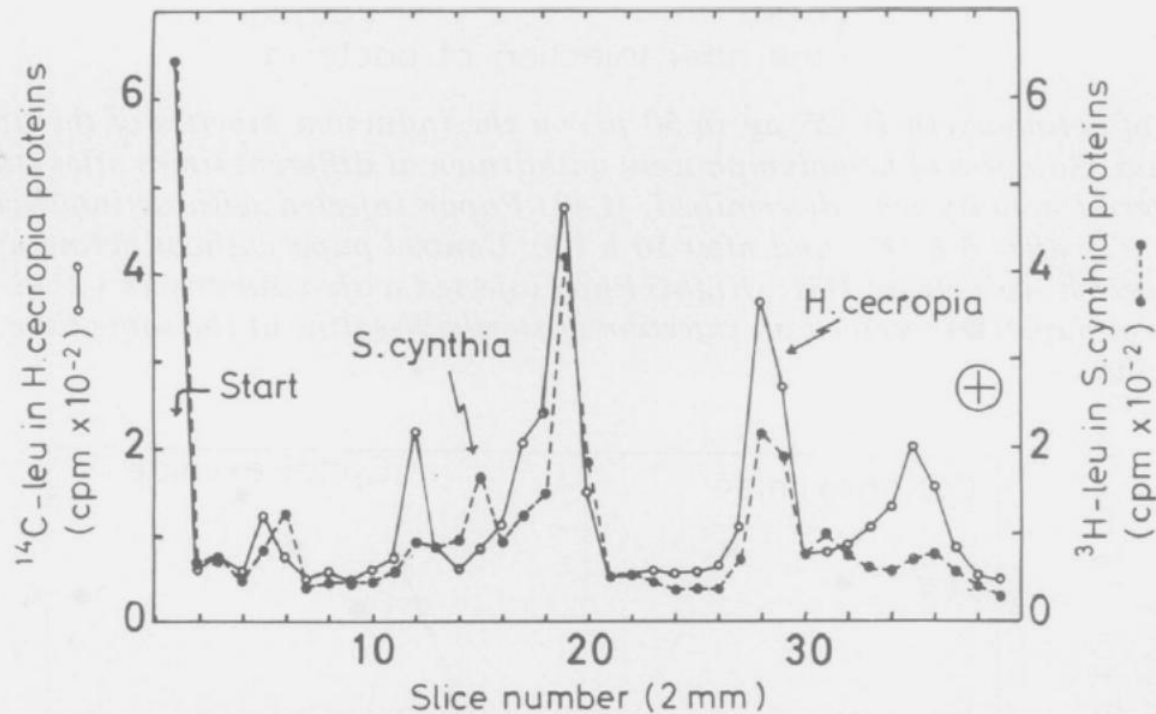




FIG. 5. Co-electrophoresis experiment of hemolymph proteins from *S. cynthia*, labeled with 45 μCi of [^3H]leucine (\bullet), and *H. cecropia*, labeled with 33 μCi of [^{14}C]leucine (\circ). The immunity reaction was induced by viable cells of *E. cloacae*. (Upper) Isotope distribution obtained after cutting the slab into 2-mm slices and burning each slice in a sample oxidizer. (Lower) Photograph of the slab gel electrophoresis showing the two hemolymph samples separate.

LOTS OF P roteins

TABLE 1. Ammonium sulfate fractionation of hemolymph from pupae of *H. cecropia*^a

Ammonium sulfate fractionation		Labeled proteins								Phenoloxi- dase (units/ml of sample)	Lysozyme (units)	Killing of:  	
Fraction	"Cut" as % of saturation	P1	P2	P3	P4	P5	P6	P7	P8			<i>E. coli</i>	<i>B. subtilis</i>
A	0-38	—	—	—	—	L	—	—	S	8,300	0	0.6	<0.01
B	38-50	S	S	S	S	S	S	?	—	2,300	0	0.7	<0.01
C	50-62	S	S	S	S	—	?	S	—	0	20	0.9	<0.01
D	62-75	—	—	S	L	—	S	S	—	0	61	0.7	0.3
A + B + D		S	S	S	L	L	S	S	S	—	—	3.7	0.7

^a Hemolymph was fractionated by adding solid ammonium sulfate as indicated. The precipitates were washed and dissolved as described in Materials and Methods. The activities for phenoloxidase and lysozyme are given in units, defined in reference 24 and in Materials and Methods. Antibacterial activity is given as the log of the number of viable cells at zero time minus the log of the number of viable cells at 3 min. The concentration of each fraction was 1 and 5% in the killing of *E. coli* and *B. subtilis*, respectively. Evidence for the distribution of the labeled proteins were from Fig. 9 and 11, where (L) indicates a large peak and (S) a small peak. The protein concentration in fractions A through D varied between 8 and 25 mg/ml and was always highest in fraction C.

Insect Immunity.

Purification and Properties of Three Inducible Bactericidal Proteins from Hemolymph of Immunized Pupae of *Hyalophora cecropia*

Dan HULTMARK, Håkan STEINER, Torgny RASMUSON, and Hans G. BOMAN

Three inducible bacteriolytic proteins, designated P7, P9A and P9B, from the hemolymph of immunized pupae of the giant silk moth *Hyalophora cecropia* have been purified using a two-step procedure with cation-exchange chromatography. Purified protein P7 has a molecular weight of 15000 and its amino acid composition shows a great similarity to that of the lysozyme from the wax moth *Galleria mellonella*. Moreover, heat stability, pH-rate profile and bacteriolytic specificity also indicate that protein P7 is a lysozyme. The other purified proteins, P9A and P9B, are highly potent against *Escherichia coli* and some other gram-negative bacteria. The amino acid compositions of proteins P9A and P9B are very similar, although the contents of glutamic acid and methionine were different. The molecular weights of these very basic proteins are around 7000. The P9 proteins are heat stable; their activities were retained after 30 min incubation at 100 °C. Both forms of protein P9 clearly differ from the lysozyme class of enzymes and they may represent a new type of bacteriolytic protein.

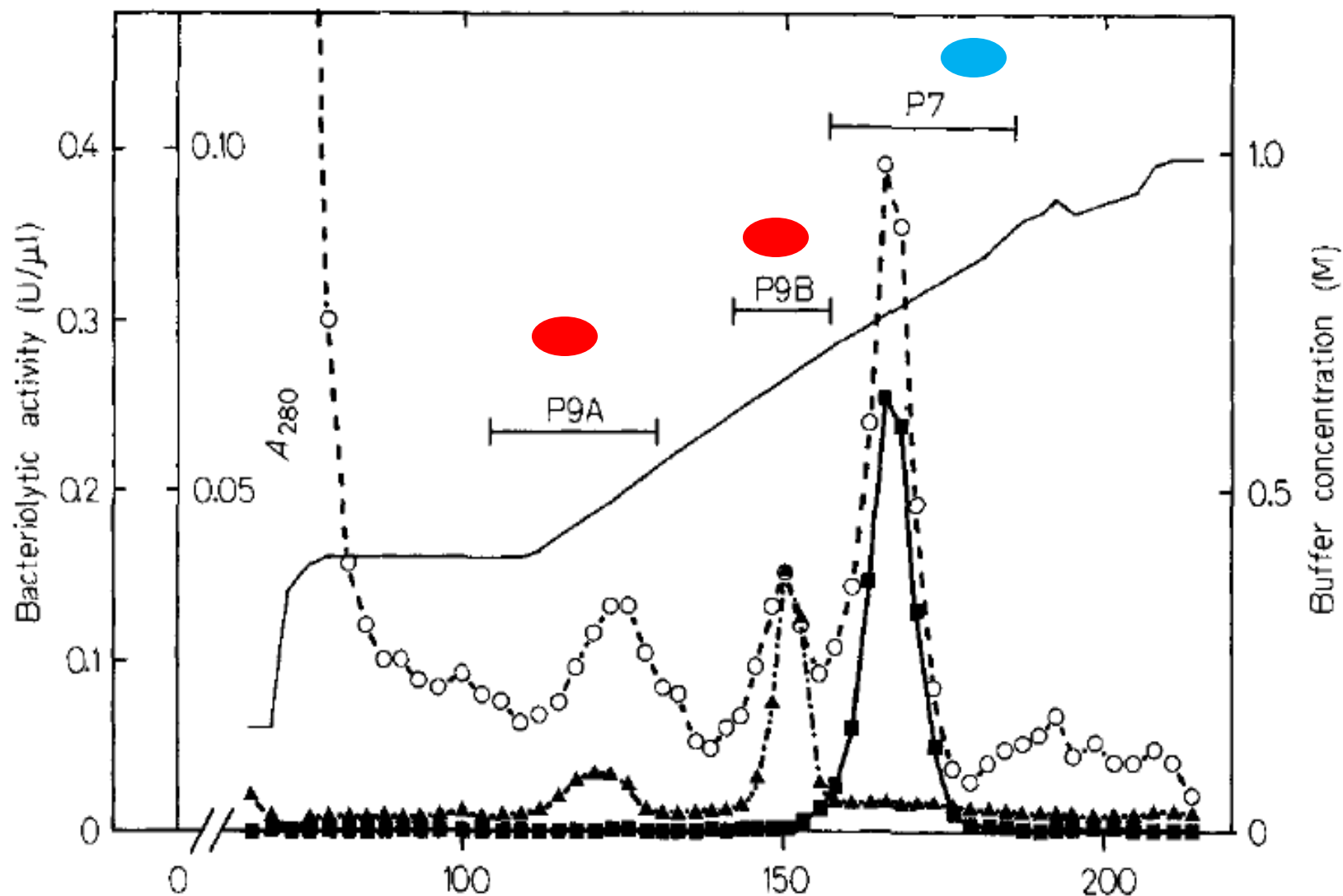


Fig. 2. Step 1 in the chromatographic purification of proteins P7, P9A and P9B from immune hemolymph from *H. cecropia*. Immune hemolymph, 3 ml, was applied to a column of CM-Sepharose (6.5 × 1.5 cm) which, after washing, was eluted with a gradient of 0.4–1.0 M ammonium acetate pH 5.1, as described in Materials and Methods. The fractions were assayed for bacteriolytic activity against *M. luteus* (■—■) and *E. coli* (▲—·—▲); absorbance at 280 nm (○—○); buffer concentration (—). The active fractions were pooled as indicated by the horizontal bars

Peptide PURIFICATION AND ACTIVITY

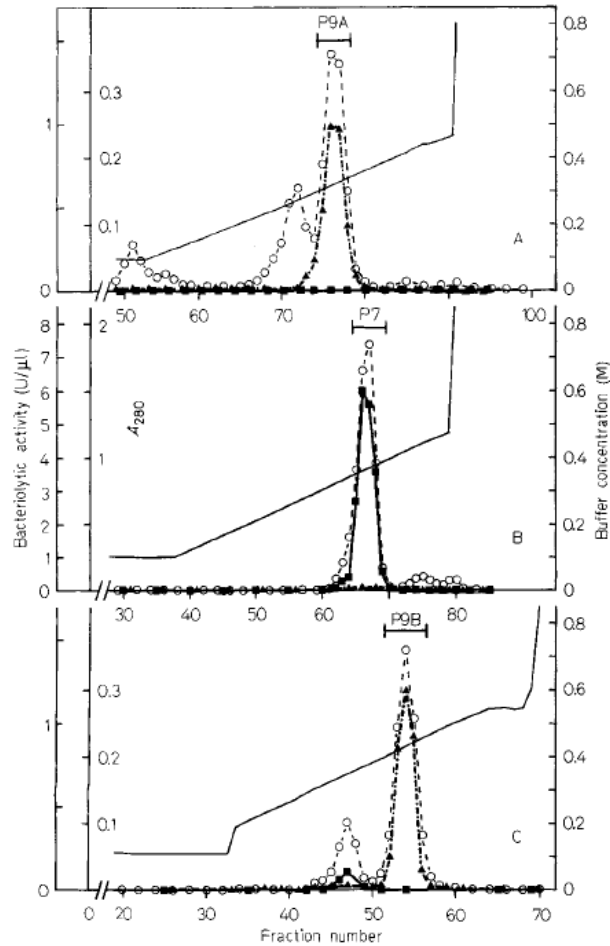


Fig. 3. Step 2 in the chromatographic purifications of proteins P7, P9A and P9B. The starting materials were obtained from 21.6 ml of immune hemolymph, fractionated and pooled as in Fig. 2. The respective components were diluted and separately applied to a column of CM-Sepharose (17.5 × 0.5 cm) equilibrated with 0.1 M ammonium formate pH 6.6. Elutions were performed for protein P9A (A) and protein P7 (B) with a 0.1–0.5 M gradient of ammonium formate pH 6.6, and for protein P9B (C) with a 0.2–0.6 M gradient of the same buffer. Bacteriolytic activity against *M. luteus* (■—■) and *E. coli* (▲—▲); absorbance at 280 nm (○—○), buffer concentration (—). The active fractions were pooled as indicated by the horizontal bars. For further details see Step 2 in Materials and Methods

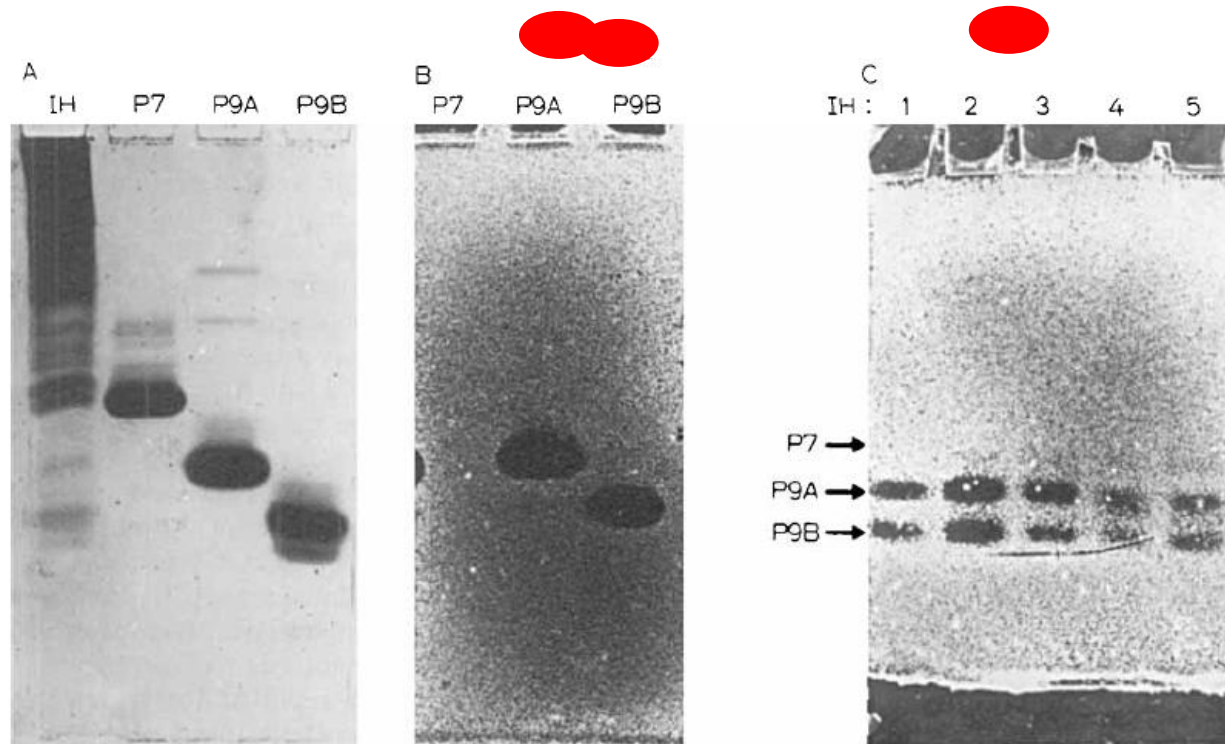


Fig. 4. Electrophoresis of native antibacterial proteins in acidic polyacrylamide gels. Samples were run at 200 V towards the cathode in 15% polyacrylamide gels pH 4, as described in Materials and Methods. Samples of immune hemolymph (IH) were acidified by adding 0.5 vol. of 1 M acetic acid. Gel A was stained for protein, gels B and C were overlaid with viable *E. coli* to detect antibacterial activity as described in Materials and Methods. The known positions of the protein bands in gel C are indicated by arrows. In gel A the samples were from left to right: immune hemolymph (10 μl), protein P7 (18 μg), protein P9A (6 μg), protein P9B (8 μg); in gel B: protein P7 (0.4 μg, 2 units), protein P9A (0.1 μg, 0.6 units), protein P9B (0.3 μg, 0.5 units). All positions in gel C contained 1 μl of immune hemolymph (approx. 1 unit); in position 1 from a batch of 20 pupae, in positions 2–5 from individual pupae

IN B, C,

DARK = dead (Gram -) bacteria

P7 (Lysozyme) better against Gram +
(peptidoglycans less hidden)

Sequence and specificity of two antibacterial proteins involved in insect immunity

H. Steiner*, D. Hultmark*, Å. Engström†,
H. Bennich† & H. G. Boman*

Nature Vol. 292 16 July 1981

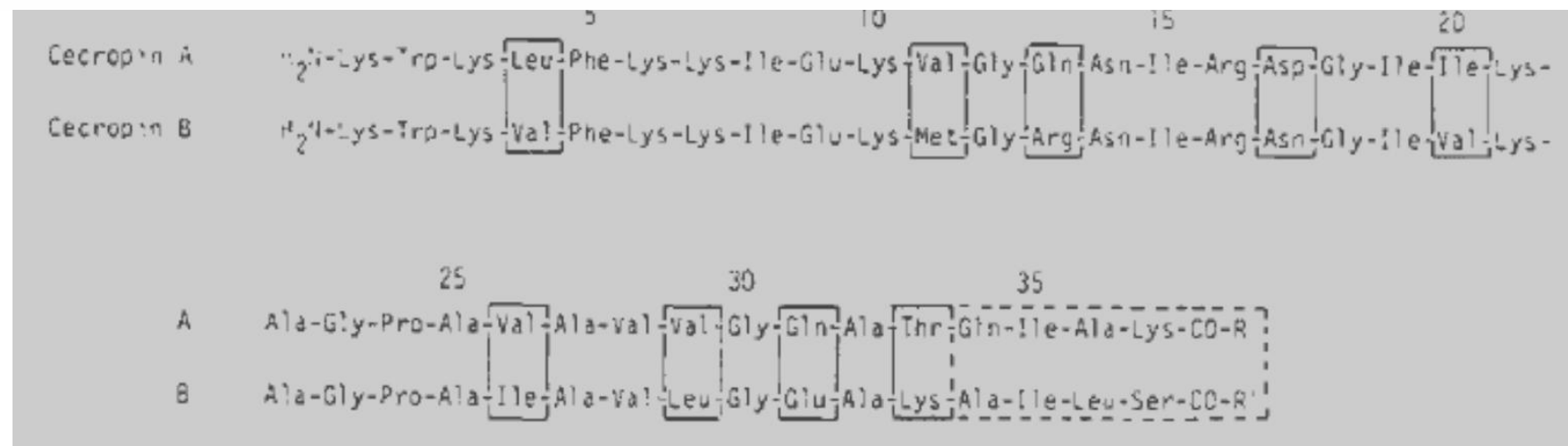


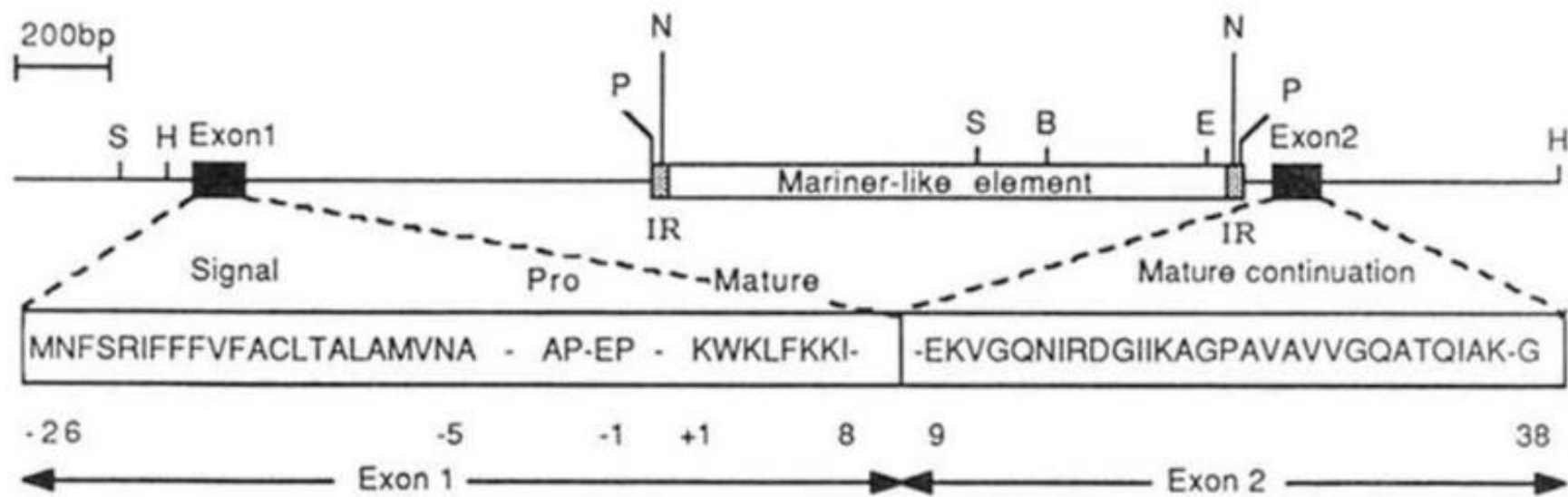
Fig. 1 The tentative amino acid sequence for cecropins A and B. Replacements of amino acids within the first 33 residues are boxed by solid lines. The C-terminal part boxed by a broken line is tentative. R and R' are unidentified blocking groups. The purified proteins used in sequence determination were obtained by collecting immune haemolymph 7 days after vaccination with live *Enterobacter cloacae* as previously described⁷. The haemolymph was applied to a Sephadex G-100 column equilibrated with 0.15 M ammonium acetate buffer, pH 5, containing phenylthiourea. The cecropins eluted together with the lysozyme. This material was pooled and further purified by ion-exchange chromatography⁷.

The Cecropin Locus

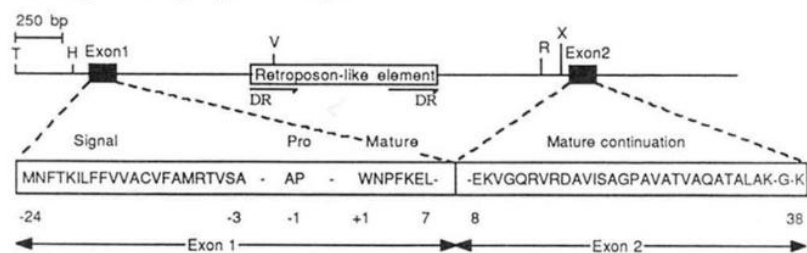
CLONING AND EXPRESSION OF A GENE CLUSTER ENCODING THREE ANTIBACTERIAL PEPTIDES IN *HYALOPHORA CECROPIA**

Gudmundur H. Gudmundsson, Dan-Anders Lidholm, Bengt Åsling, Renbao Gan‡, and Hans G. Boman

a) The gene for prepro-cecropin A



b) The gene for prepro-cecropin D



Stored as inactive propeptide
Released as active peptide,

Cecropin A Transcript induced after 2h, maximum at 48h, mature protein 10-24h
Cecropin D Transcript induced after 48-96h, peak at 144h, mature protein 48h

MANY ANIMALS HAVE CECROPINS

Cecropin A,B,D

Moths

CECD

Aedes aegypti (Yellow fever mosquito).

Papiliocin

Papilio xuthus (Asian swallowtail butterfly).

Attacins

Houseflies

Cecropin P1

Ascaris suum a parasitic nematode Peptide Sequence (SWLS-KTAKKLENSAKKRISSEGIAIAIQGGPR).

LOGO PLOT (CONSENSUS)



THERE ARE OTHER CATEGORIES OF AMPs (AntiMicrobial Peptides)

DEFENSIN, first identified from *Hyalophora cecropia*

Defensins are a well known family of cationic antibacterial peptides (AMPs) isolated from fungi, plants, insects, mussels, birds, and various mammals. They are predominantly active against gram (+) bacteria, and a few of them are also active against gram (-) bacteria and fungi. All insect defensins belonging to the invertebrate class have a consensus motif, C-X₅₋₁₆-C-X₃-C-X₉₋₁₀-C-X₄₋₇-CX₁-C. Only seven AMPs have already been found in different lepidopteran species. No report was published on the isolation of defensin from the Egyptian cotton leafworm, *Spodoptera littoralis*.

	*		*		*	*		*	*	*		*	*	*	
ASABF- α	MKT	--	AIIVLLVIFASTNAAVDFSS	CA	--	RMDVPGL-SKVAQGL	CISS	CKFQNGTGHE	EKR	--	GGRPTCV	EDRC			
ASABF- β	MKT	--	IVVVILFVAVVAVTSAIDFSS	CA	--	RMDVPGL-NKVAQGL	CISS	CKYQNGTGHE	EKR	--	GGRPTCV	EGRC			
ASABF- γ	MKT	--	IIFTVLVAVVAVTSAIDFST	CA	--	RMDVPGL-SKVAQGS	CISS	CKFQNGTGHE	EKR	--	GGRPTCV	ESRC			
ASABF- δ	MRI	--	FTAVVLIVVLVCVNAGIDFST	CA	--	RMDVPGL-SKVARGL	CISS	CKFQNGTGHE	EKR	--	GGRPTCV	ESRC			
ASABF- ϵ	MVT	KGIVLFMLVILFASTDA	----	TOG	--	YDDAKL-NRPTIG	-CIL	SKVQGETGAC	YLR	--	DSRPICV	EKRC			
ASABF- ζ	MKA	--	ILIALLLTTFTVVNGGVLTSE	CA	--	RMDTPVL-SKAAQGL	CISS	CKYQNGTGHE	QKV	--	GGRPTCV	EMRC			
ABF-1	MLY	----	FCLLLVLLLPNNGVSSEAS	CA	--	RMDVPVM-QRIAQGL	CISS	CKYQNGTGHE	QKV	--	DSHPTCV	EGGC			
ABF-2	MFV	RSFLALLLATIVA	--	ADIDFST	CA	--	RMDVPIL-KKAAQGL	CISS	CKYQNGTGHE	QKV	--	SGRPTCV	EYRC		
ABF-3	MNF	--	SFLFFIFAFLIGLNKG	--	SVCLTRRTDWGQLGAI	FTNPV	CDVW	CRIRCGPGQ	CKED	PMTSDEA	QCV	EKRC			
ABF-4	MIC	-NC	FLLIIVTLVISNCDGI	----	CL-NHEGWGNVGSV	FTDPL	ENVW	CEIKLGP	QCI	EDPMTTAPAR	CV	EKRC			
ABF-5	MNY	NFLLLSACIIFLIPEKSE	--	SICVTRRTDWGQFGS	FTDPL	CDVW	CRIRCG	KGQ	CKED	PATSN	TAN	CV	EKRC		
ABF-6	MFR	-KL	IATFVLSLCDLANSV	--	TICS	----	SSLLSTFTDPL	TSW	CKVRF	SSGS	RSV	-MSGSDPT	CE	ESRC	

MANY DIFFERENT CATEGORIES AMPs (in plants, invertebrates, (vertebrates))

Box 1 | **Classes of antimicrobial peptides**

Anionic peptides

- Maximin H5 from amphibians¹⁴⁶.
- Small anionic peptides rich in glutamic and aspartic acids from sheep, cattle and humans³⁰.
- Dermcidin from humans¹⁴⁷.

Linear cationic α -helical peptides

- Cecropins (A), andropin, moricin, ceratotoxin and melittin from insects.
- Cecropin P1 from *Ascaris nematodes*¹⁴⁸.
- Magainin (2), dermaseptin, bombinin, brevinin-1, esculentins and buforin II from amphibians.
- Pleurocidin from skin mucous secretions of the winter flounder.
- Seminalplasmin, BMAP, SMAP (SMAP29, ovispirin), PMAP from cattle, sheep and pigs.
- CAP18 from rabbits.
- LL37 from humans.

Cationic peptides enriched for specific amino acids

- Proline-containing peptides include abaecin from honeybees²⁸.
- Proline- and arginine-containing peptides include apidaecins from honeybees²⁸; drosocin from *Drosophila*²⁸; pyrrhocoricin from the European sap-sucking bug³⁶; batenecins from cattle (Bac7), sheep, and goats¹⁴⁹; and PR-39 from pigs^{73,150}.
- Proline- and phenylalanine-containing peptides include prophenin from pigs¹⁵⁰.
- Glycine-containing peptides include hymenoptaecin from honeybees²⁸.
- Glycine- and proline-containing peptides include coleopteracin and holotricin from beetles²⁸.
- Tryptophan-containing peptides include indolicidin from cattle¹⁵¹.
- Small histidine-rich salivary polypeptides, including the histatins from man and some higher primates¹¹⁶.

ANTIMICROBIAL PEPTIDES: PORE FORMERS OR METABOLIC INHIBITORS IN BACTERIA?

Kim A. Brogden

Anionic and cationic peptides that contain cysteine and form disulphide bonds

- Peptides with 1 disulphide bond include brevinins¹⁵².
- Peptides with 2 disulphide bonds include protegrin from pigs and tachyplesins from horseshoe crabs¹⁵³.
- Peptides with 3 disulphide bonds include α -defensins from humans (HNP-1, HNP-2, cryptidins), rabbits (NP-1) and rats¹⁵⁴; β -defensins from humans (HBD1, DEFB118), cattle, mice, rats, pigs, goats and poultry¹²; and rhesus θ -defensin (RTD-1) from the rhesus monkey⁴⁰.
- Insect defensins (defensin A)⁹⁵.
- SPAG11/isoform HE2C, an atypical anionic β -defensin⁴².
- Peptides with >3 disulphide bonds include drosomycin in fruit flies¹⁵⁵ and plant antifungal defensins¹⁵⁵.

Anionic and cationic peptide fragments of larger proteins

- Lactoferricin from lactoferrin.
- Casocidin I from human casein.
- Antimicrobial domains from bovine α -lactalbumin, human haemoglobin, lysozyme and ovalbumin.

The Antimicrobial Peptide Database

[About](#) | [Database Search](#) | [Calculation & Prediction](#) | [Peptide Design](#) | [Statistics](#) | [AMP Facts](#) | [Contact](#)
[AMP Timeline](#) | [Nomenclature](#) | [Classification](#) | [3D Structure](#) | [My Tools](#) | [Seq Download](#) | [FAQs](#) | [What's New](#) |
[Glossary](#) | [Links](#) | [Opportunities](#) | [Conference](#) | [AMP Antiviral News](#)

The **Antimicrobial Peptide Database (APD)** contains 3257 antimicrobial peptides from six kingdoms (365 **bacteriocins**/peptide antibiotics from **bacteria**, 5 from archaea, 8 from protists, 22 from **fungi**, 360 from **plants**, and 2414 from animals, including some synthetic peptides) with the following activity:

Antibacterial peptides; **Antibiofilm peptides**;
Anti-MRSA peptides; **Anti-TB peptides****New**;
Anti-endotoxin peptides**New**; **Anti-toxin peptides**;
Antiviral peptides; **Anti-HIV peptides**;
Antifungal peptides; **Anti-Candida peptides****New**;
Antiparasitic peptides; **Antimalarial peptides**;
Anticancer peptides;
Anti-diabetic peptides**New**;
Wound healing peptides;
Chemotactic peptides; **Anti-inflammatory peptides** ;
Spermicidal peptides;
Insecticidal peptides;
Ion channel inhibitors;
Protease inhibitors;
Antioxidant peptides;
Surface immobilized peptides;
Two-chain peptides;
Synergistic peptides.

This original database for antimicrobial peptides is manually curated based on a set of **data-collection criteria**. There are 141 **human host defense peptides**, 328 from mammals annotated, 1120 active peptides from **amphibians (1042 from frogs and 74 from toads)**, 136 **fish** peptides, 45 **reptile** peptides, 43 from **birds**, 577 from **arthropods**, [323 from **insects**, 71 from **crustaceans**, 8 from myriapods, 175 from **chelicerata**, (43 from **spiders**, 88 from **scorpions**)], 47 from **molluscs**, 6 AMPs from protozoa, and more.

This comprehensive database consists of a pipeline of search functions for innate immune peptides. You can search for peptide information using APD ID, peptide name, amino acid sequence, peptide motif, **chemical modification**, length, charge, hydrophobic content, PDB ID, **3D structure**, methods for structural determination, peptide source organism, peptide family name, life domain/kingdom (bacteria, archaea, protists, fungi, plants, animals), biological activity (see the links above), synergistic effects, target microbes, molecular targets, mechanism of action, contributing authors, and year of publication.

Of the 443 unique NMR/X-ray diffracted 3D structures annotated for host defense peptides in the APD, 317 with coordinates deposited in the Protein Data Bank (PDB) can be directly rotated, zoomed, and viewed. Top left: **Amphibian α -helical magainin II**; Top right: **bovine β -sheet lactoferricin**; Bottom left: **plant $\alpha\beta$ -PsD1**; Bottom right: **bovine non- $\alpha\beta$ indolicidin**.

Important notice: 2020 FASTA sequence release has been sorted and uploaded for **download**.

CITE:

- [1] Wang, G., Li, X. and Wang, Z. (2016) APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Research 44, D1087-D1093. [Paper PDF](#)
- [2] Wang, G., Li, X. and Wang, Z. (2009) APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Research 37, D933-D937. [Paper PDF](#)
- [3] Wang, Z. and Wang, G. (2004) APD: the antimicrobial peptide database. Nucleic Acids Research 32, D590-D592. [Paper PDF](#)

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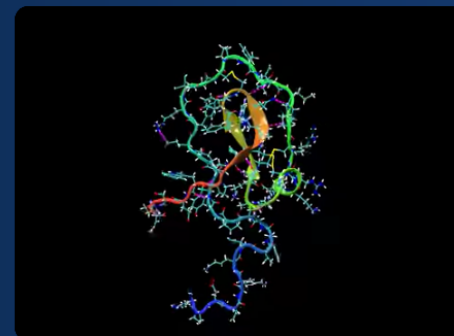
<https://dbaasp.org/home>

NEWS :

DBAASP now offers improved infrastructure for cyclic peptides: see the Intrachain Bond section in [Search](#)

Database of Antimicrobial Activity and Structure of Peptides

[SEE ALL MD MODELS](#)



Monomer

22473

Multimer

386

Multi Peptide

236

Records with the Data on Synergistic
Activities

718

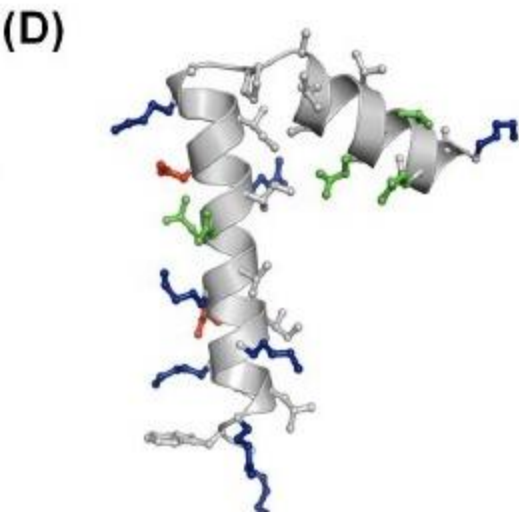
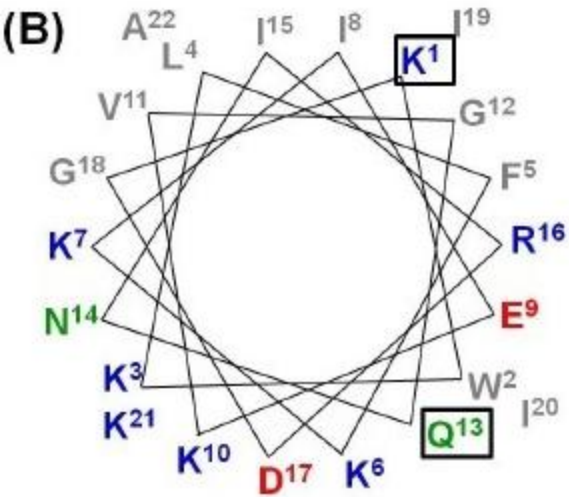
CHARACTERISITICS of AMPs

- 1 **Size**
The size of antimicrobial peptides varies from 6 amino acid residues for anionic peptides to greater than 59 amino acid residues for Bac7. Even di- and tripeptides with antimicrobial activity have been reported.
- 2 **Sequence**
Peptides often contain the basic amino acid residues lysine or arginine, the hydrophobic residues alanine, leucine, phenylalanine or tryptophan, and other residues such as isoleucine, tyrosine and valine. Some peptides contain amino acid repeats. Ratios of hydrophobic to charged residues can vary from 1:1 to 2:1.
- 3 **Charge**
Anionic peptides are rich in aspartic and glutamic acids and cationic peptides are rich in arginine and lysine. Anionic peptides that are complexed with zinc, or highly cationic peptides, are often more active than neutral peptides or those with a lower charge.
- 4 **Conformation and structure**
Antimicrobial peptides can assume a variety of secondary structures including α -helices, relaxed coils and antiparallel β -sheet structures. Amphipathic α -helical peptides are often more active than peptides with less-defined secondary structures. Peptides with a γ -core motif (two antiparallel β -sheets with an interposed short turn in defensin-like molecules) are often very active.
- 5 **Hydrophobicity**
This characteristic enables water-soluble antimicrobial peptides to partition into the membrane lipid bilayer.
- 6 **Amphipathicity**
A trait by which peptides contain hydrophilic amino acid residues aligned along one side and hydrophobic amino acid residues aligned along the opposite side of a helical molecule. For α -helical peptides, amphipathicity is often expressed as a hydrophobic moment, which is the vector sum of hydrophobicity indices, treated as vectors normal to the helical axis. Other peptides often show spatial separation of polar and hydrophobic residues that is less easy to quantify.

Structure-activity relationships of cecropin-like peptides and their interactions with phospholipid membrane

Eunjung Lee,¹ Ki-Woong Jeong,¹ Juho Lee,¹ Areum Shin,¹ Jin-Kyoung Kim,¹ Juneyoung Lee,² Dong Gun Lee,² and Yangmee Kim^{1,*}

cecropin A



Helical-wheel diagram and ribbon diagram of cecropin A. Helical-wheel diagram of N-terminal helix region from Lys1 to Ala22 of cecropin A. Ribbon diagram of cecropin A. Residues are color-coded as follows: gray, hydrophobic; blue, positive; red, negative; green, neutral.

α -helix – hinge - α -helix

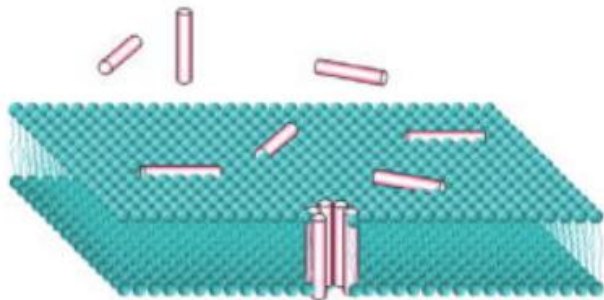
cecropin-like peptides can bind to the negatively charged bacterial cell membrane

AMPS:

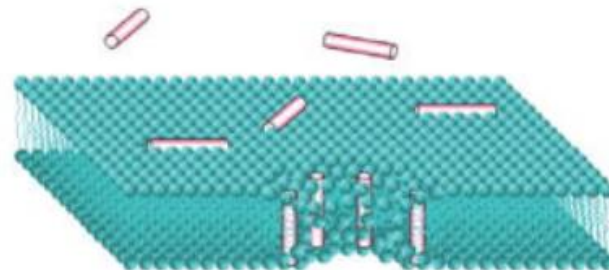
HOW DO THEY WORK?

Describing the Mechanism of Antimicrobial Peptide Action with the Interfacial Activity Model William C. Wimley
ACS Chem Biol. 2010 October 15; 5(10): 905–917. doi:10.1021/cb1001558.

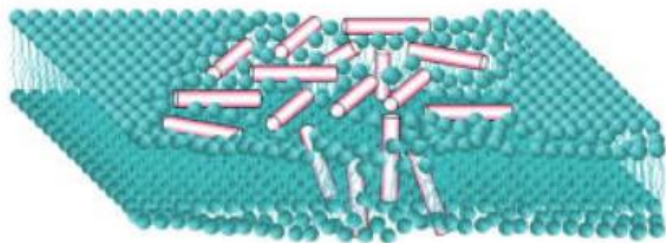
A: Barrel-stave Pore



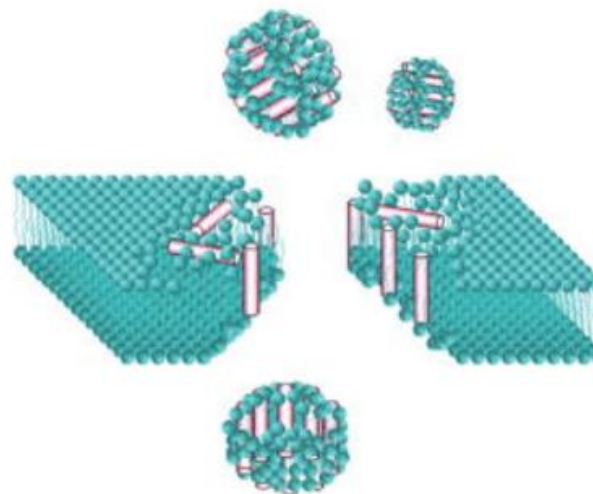
B: Toroidal Pore



C: Carpet Model



D: Detergent Model



AMPS HOW DO THEY WORK?

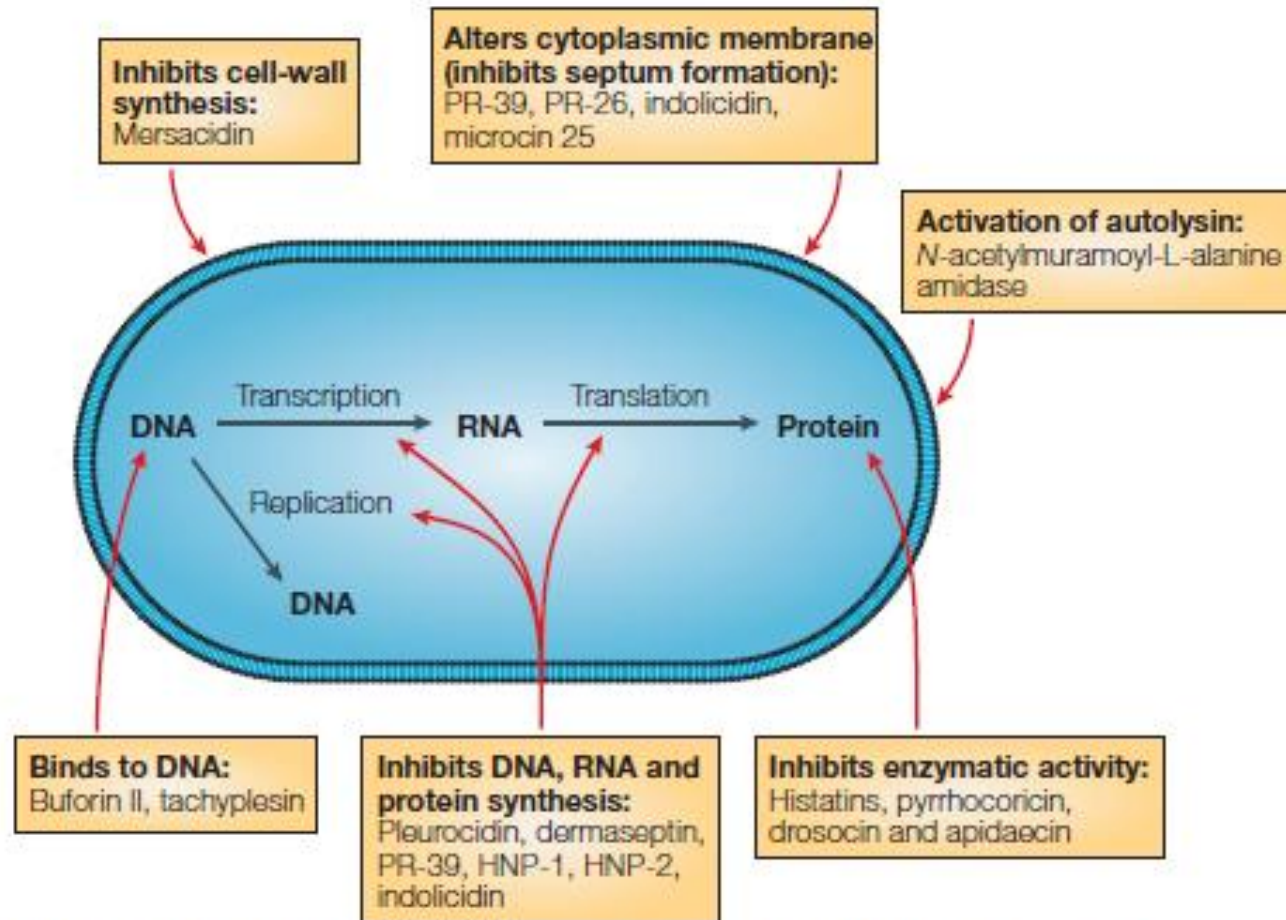


Figure 6 | **Mode of action for intracellular antimicrobial peptide activity.** In this figure *Escherichia coli* is shown as the target microorganism

AMPS ALSO TARGET EUKARYOTE PATHOGENS



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Prolixicin: a novel antimicrobial peptide isolated from *Rhodnius prolixus* with differential activity against bacteria and *Trypanosoma cruzi*

R. Ursic-Bedoya, J. Buchhop, J. B. Joy, R. Durvasula, C. Lowenberger [✉](#)

First published: 12 September 2011 [Full publication history](#)



Experimental Parasitology

Volume 80, Issue 3, May 1995, Pages 401-406



Regular Article

Brugia pahangi: The Effects of Cecropins on Microfilariae *in Vitro* and in *Aedes aegypti*

R. Chalk, H. Townson, [P.J. Ham](#)

FEBS
Letters

Research letters

Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids

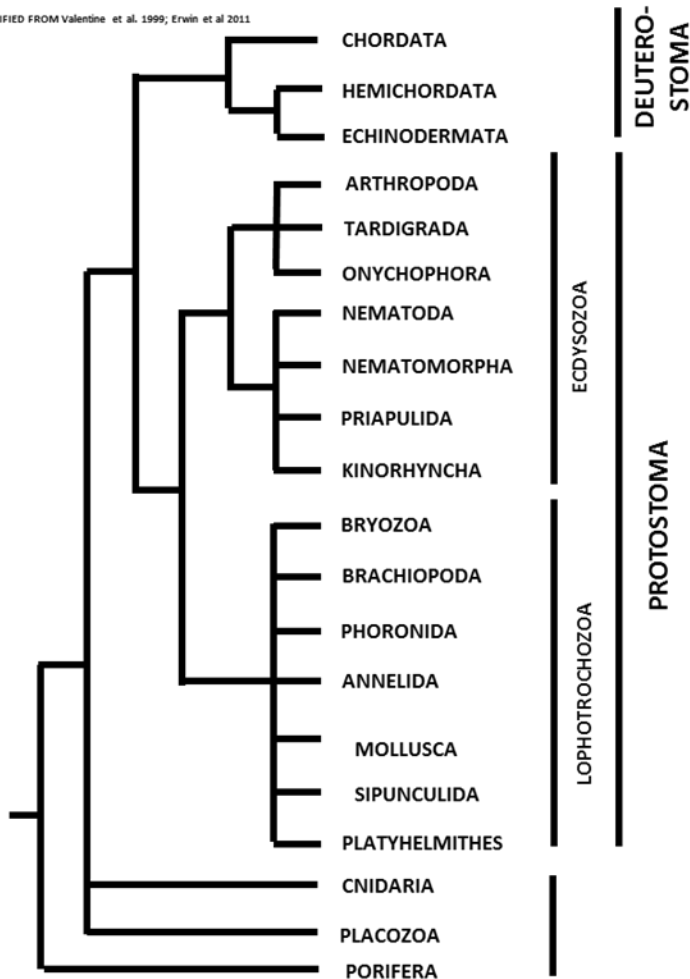
H.G. Boman, D. Wade, I.A. Boman, B. Wählén, R.B. Merrifield



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AMPS OCCUR THROUGHOUT PHYLOGENY

MODIFIED FROM Valentine et al. 1999; Erwin et al 2011



Arthropoda :

D. melanogaster, seven AMP classes
(cecropin, attacin, defensin, drosomycin, dipteracin, drosocin and metchnikowin)

Mollusca:

Mytilus (Mussel, Bivalves), four AMP classes
(defensins, mytilins, myticins and mytimycin)

Biomphalaria (Snail, Gastropods) has only two AMP classes (macins, aranetoxins)

Other factors?

The antimicrobial peptide (AMP) arsenal of *B. glabrata* is surprisingly reduced compared to other invertebrate species (for example, bivalve molluscs have multiple AMP gene families²²); our searches indicated only a single macin-type gene family, comprising six biomphamacin genes. However, *B. glabrata* does possess multigenic families of antibacterial proteins including two achacins, five LBP/BPIs, and 21 biomphalysins (Supplementary Fig. 34; Supplementary Note 18; Supplementary Data 22 and 23).



Article

Glbralysins, Potential New β -Pore-Forming Toxin Family Members from the Schistosomiasis Vector Snail *Biomphalaria glabrata*

Damien Lassalle¹, Guillaume Tetreau^{1,†}, Silvain Pinaud^{1,†,‡}, Richard Galinier¹, Neil Crickmore², Benjamin Gourbal^{1,§} and David Duval^{1,*,§}

Lymnaealysins, Physalysins



Developmental & Comparative Immunology

Volume 57, April 2016, Pages 20–30



The LBP/BPI multigenic family in invertebrates: Evolutionary history and evidences of specialization in mollusks

Olga Lucia Baron^{a, b}, Emeline Deleury^a, Jean-Marc Reichhart^b, Christine Coustau^a

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<https://doi.org/10.1016/j.dci.2015.11.006>

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New Insights Into Biomphalysin Gene Family Diversification in the Vector Snail *Biomphalaria glabrata*

Silvain Pinaud^{1,2,†}, Guillaume Tetreau^{1,2,†}, Pierre Poteaux^{1,2}, Richard Galinier^{1,2}, Cristian Chaparro^{1,2}, Damien Lassalle^{1,2}, Anaïs Portet^{1,2,†}, Elodie Simphor^{1,2}, Benjamin Gourbal^{1,2} and David Duval^{1,2*}

FEBS 26728

FEBS Letters 531 (2002) 509–512

Antimicrobial action of achacin is mediated by L-amino acid oxidase activity

Tatsuya Ehara, Seiji Kitajima, Nobuyuki Kanzawa, Toru Tamiya, Takahide Tsuchiya*

Yes, vertebrates too!

Cell, Vol. 88, 553–560, February 21, 1997, Copyright ©1997 by Cell Press

Human β -Defensin-1 Is a Salt-Sensitive Antibiotic in Lung That Is Inactivated in Cystic Fibrosis

Mitchell J. Goldman,* G. Mark Anderson,[†]
Ethan D. Stolzenberg,[†] U. Prasad Kari,[†]
Michael Zasloff,[†] and James M. Wilson*

Smith et al. (1996) report a defect in host c primary cultures of hur

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Emerging roles of antimicrobial peptides in innate immunity, neuronal function, and neurodegeneration

[Soojin Lee](#)¹ · [Neal Silverman](#) ²  · [Fen-Biao Gao](#) ¹ 