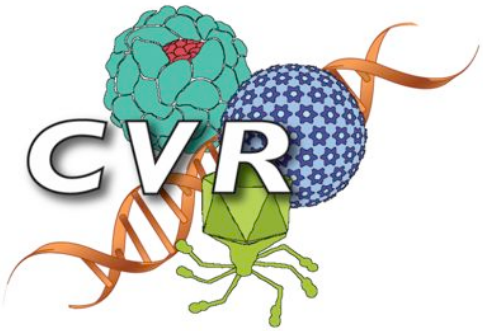


Viral consortia: A social force for ancient and recent life

Subtitle: A 'gangen' of stem-loop RNA originates and diversifies life

Luis P. Villarreal

Center for Virus Research (CVR)
University of California, Irvine

Salzburg, July 3, 2014

Competition

(individual fittest type, lytic virus, selfish)

vs

Cooperation

(symbiosis, group selection, persistent cryptic virus, synergistic social genomes)

Lytic vs persistent virus as a necessary set?!

Silence is golden (and small RNA often involved)

The virosphere is both!

Addiction modules: need for sets of opposites

Persistence & survival in the virosphere

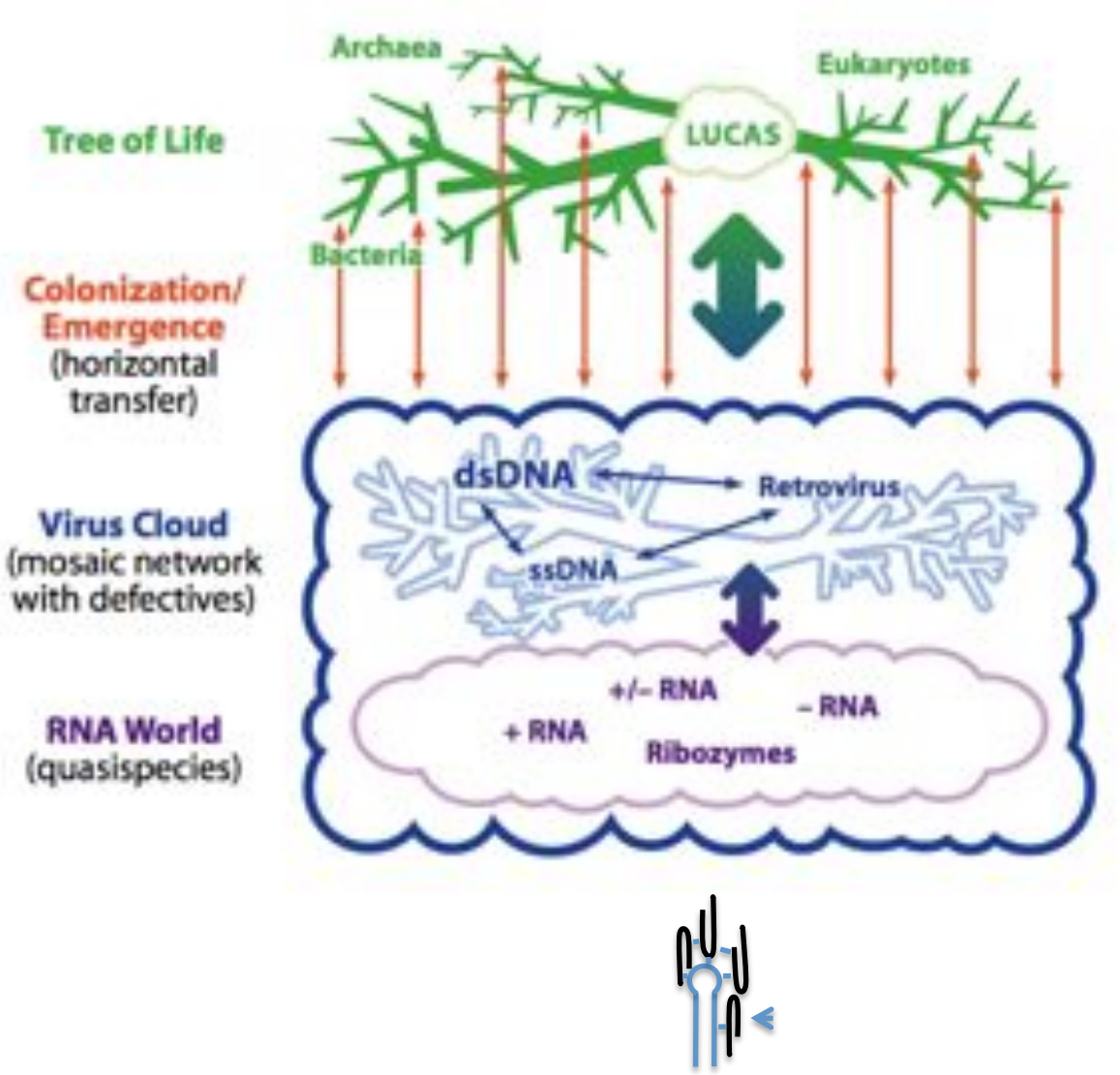
Group identity via cryptic virus sets

Cooperative RNA via quasispecies

Group identity for RNA populations

Stem-loop RNA populations as basal

Origins of networks, virus and life



No life is fit unless
it is in the virosphere

DNA HABITATS AND ITS RNA INHABITANTS

(IDA: social RNA sub-agents with group identity, Szathmáry, Eörs. 1992)

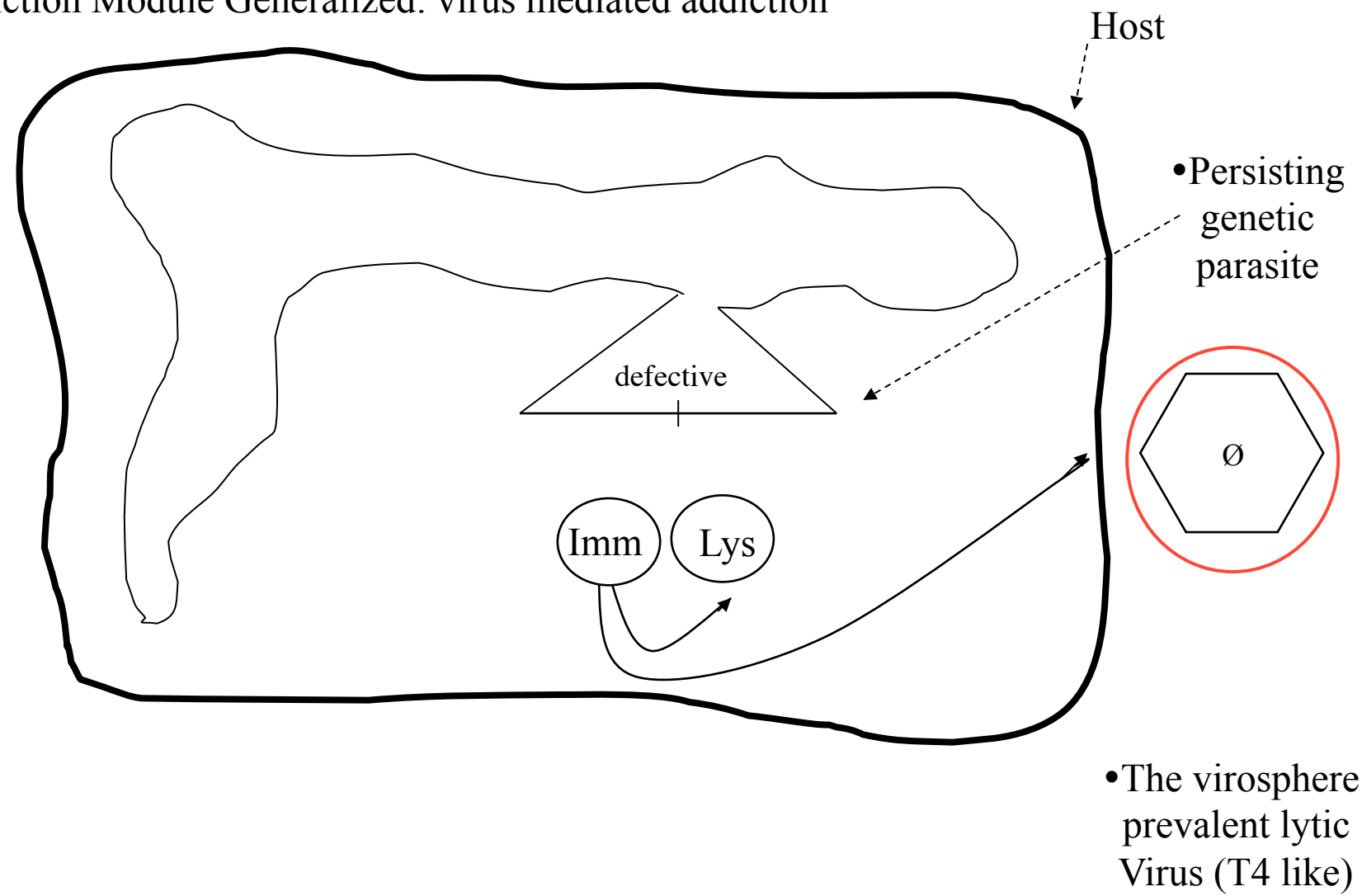
Addiction modules and parasite stability (persistence)

- P1 is a highly prevalent and stable episomal phage of E. coli
- Not integrated – how to be stable?
- Michael Yarmolinsky (1993) proposes term 'addiction module' to describe killing by P1 phage as an explanation of post segregation killing
- Composed of toxin/antitoxin (T/A) P1 gene pair, lost antitoxin (genome) results in death of uninfected daughter. Host is P1 addicted
- Affects sexual compatibility, kills uninfected partners
- Also kills cells infected by other phage! Complex immunity

Addiction module examples

- Persistent virus as addiction module. Viral or host immunity function prevents lysis by various viruses (cryptic, de-effectives, satellite mixes can provide immunity).
- Holins/antiholins. Diverse viral pore proteins that lyse bacteria: kept in check by viral 2nd protein that binds pore
- Restriction/modification enzymes. Destructive stable endonuclease prevented by transient DNA modification - compels maintenance of parasite
- Toxin/antitoxins (TA)- bacteria and fungal viruses express stable toxin (often pore) and unstable antitoxin to compel maintenance- origin of apoptosis
- Two examplars presented: E. coli K12 & O157:H7
- A general strategy to link individuals into a group (RNA)

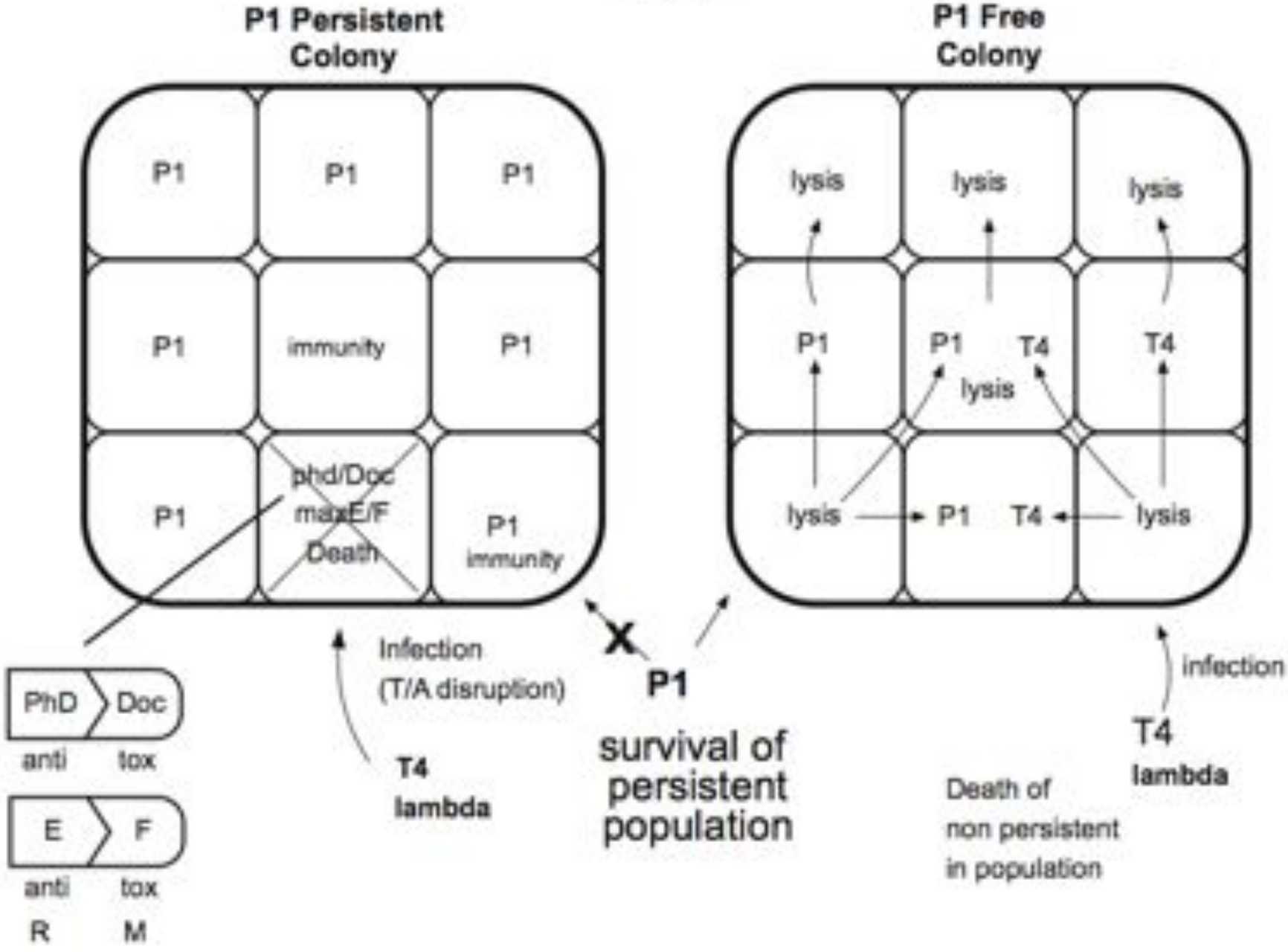
Addiction Module Generalized: virus mediated addiction



VIRUS ADDICTION

(usually mixed wit defectives)

Fig 2.8


P1 Persistent Colony
P1 Free Colony
P1
P1
P1
P1
immunity
P1
P1
phd/Doc
maxE/F
Death
P1
immunity
lysis
lysis
lysis
P1
P1
T4
T4
lysis
lysis
P1
T4
lysis
PhD
Doc
anti
tox
E
F
anti
tox
R
M
Infection
(T/A disruption)
T4
lambda
X
P1
survival of
persistent
population
infection
T4
lambda
Death of
non persistent
in population

nature COMMUNICATIONS

OPEN

ARTICLE

Received 26 May 2010 | Accepted 25 Nov 2010 | Published 21 Dec 2010

DOI: 10.1038/ncomms1146

Cryptic prophages help bacteria cope with adverse environments

Xiaoxue Wang[1], Younghoon Kim[1], Qun Ma[1], Seok Hoon Hong[1], Karina Pokusaeva[2], Joseph M. Sturino[2] & Thomas K. Wood[1]

Phages are the most abundant entity in the biosphere and outnumber bacteria by a factor of 10. Phage DNA may also constitute 20% of bacterial genomes; however, its role is ill defined. Here, we explore the impact of cryptic prophages on cell physiology by precisely deleting all nine prophage elements (166 kbp) using *Escherichia coli*. We find that cryptic prophages contribute significantly to resistance to sub-lethal concentrations of quinolone and β-lactam antibiotics primarily through proteins that inhibit cell division (for example, KilR of rac and DicB of Qin). Moreover, the prophages are beneficial for withstanding osmotic, oxidative and acid stresses, for increasing growth, and for influencing biofilm formation. Prophage CPS-53 proteins YfdK, YfdO and YfdS enhanced resistance to oxidative stress, prophages e14, CPS-53 and CP4-57 increased resistance to acid, and e14 and rac proteins increased early biofilm formation. Therefore, cryptic prophages provide multiple benefits to the host for surviving adverse environmental conditions.

NATURE COMMUNICATIONS | DOI: 10.1038/ncomms1146

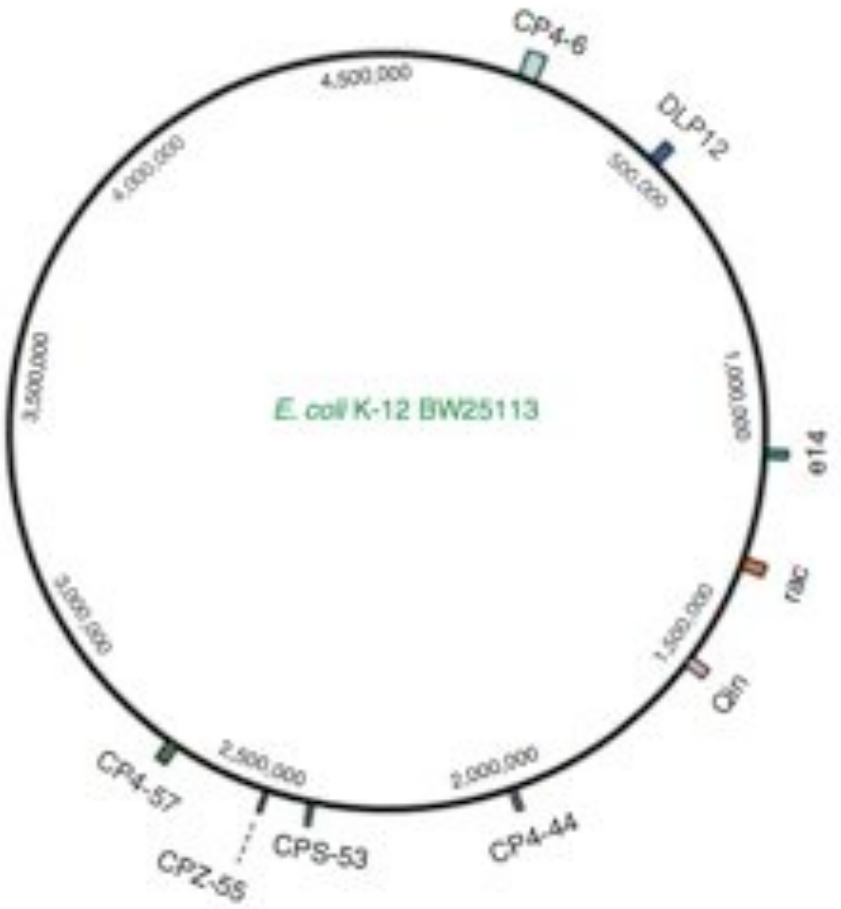


Figure 1 | Size and position of nine cryptic prophages in the *E. coli* K-12 BW25113 genome. Map of the *E. coli* K-12 BW25113 genome demonstrating the cyptic prophages deleted in this study.

~20% of bacterial genomes from virus (166 kbp)

K-12 has 9 prophage (def)
a coherent and interacting set

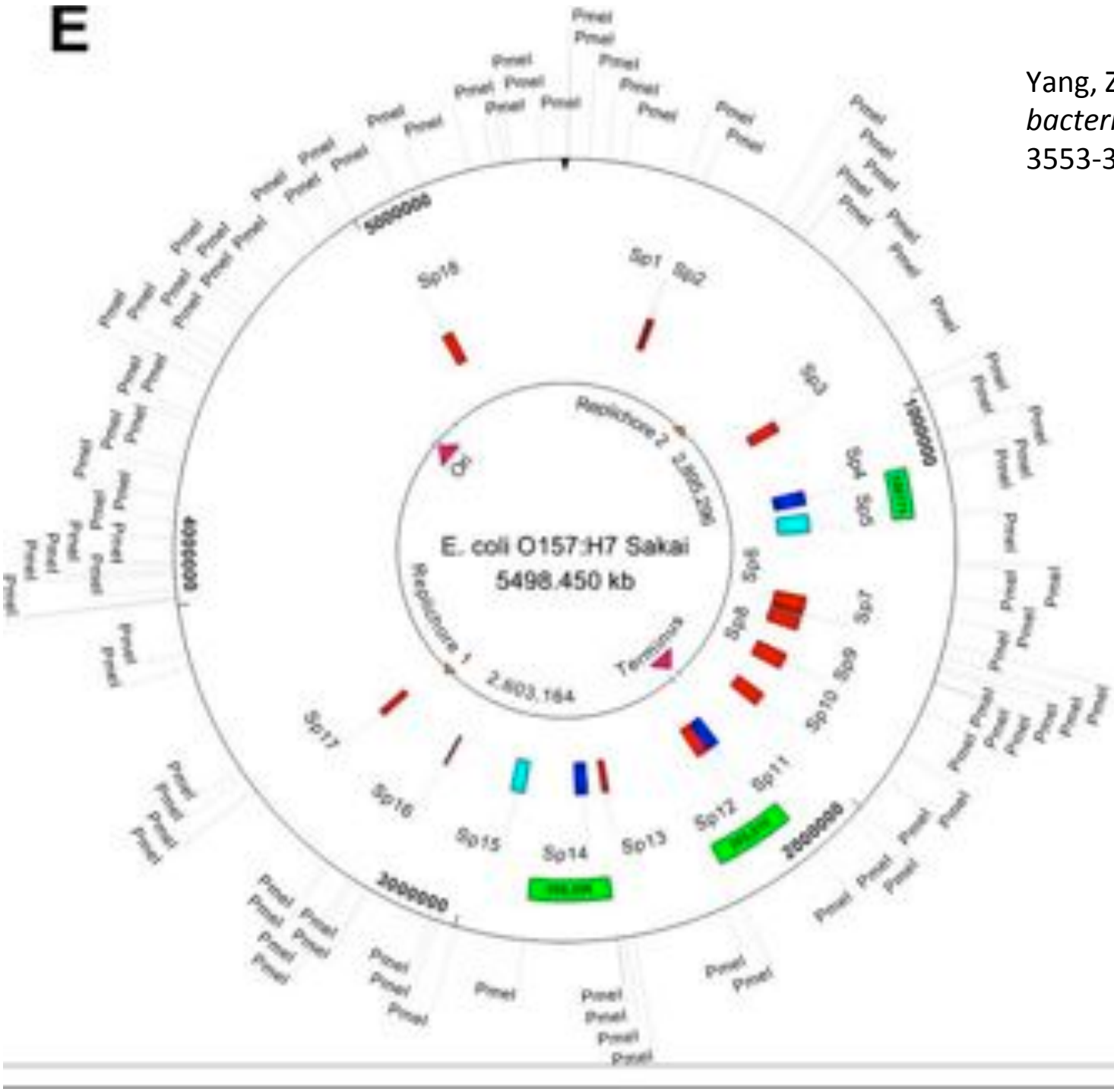
has 4 phage T/A modules
(virus addiction?)

Provide stress resistance via:
antibiotics, osmotic shock,
oxidative stress, acid stress

Eliminates biofilm formation
(group ID)

When stressed, only e14ø made
others stable

VIROSPHERE SURVIVAL?



Yang, Zhijie, et al. *Journal of bacteriology* 191.11 (2009): 3553-3568.

OPEN ACCESS Freely available online PLOS PATHOGENS

The Defective Prophage Pool of *Escherichia coli* O157: Prophage–Prophage Interactions Potentiate Horizontal Transfer of Virulence Determinants

Md Asadulghani[1,2], Yoshitoshi Ogura[1,3], Tadasuke Ooka[3], Takehiko Itoh[4,5], Akira Sawaguchi[6], Atsushi Iguchi[1], Keisuke Nakayama[3], Tetsuya Hayashi[1,3]*

1 Division of Bioenvironmental Sciences, Frontier Science Research Center, University of Miyazaki, Miyazaki, Japan, **2** Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan, **3** Division of Microbiology, Department of Infectious Disease, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan, **4** Advanced Sciences Innovation Group, Mitsubishi Research Institute, Inc., Tokyo, Japan, **5** Bio-Frontier Research Center, Tokyo Institute of Technology, Kanagawa, Japan, **6** Department of Anatomy, Ultrastructural Cell Biology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

In conclusion, many of the prophages of O157 Sakai that contain a wide range of genetic defects show unexpectedly high potential activity as mobile genetic elements, and this mobility is probably achieved through various types of inter-prophage interactions that occur in the O157 prophage pool. Thus, these apparently defective prophages are not simply remnants generated in the course of O157 evolution, but instead should be regarded as genetic elements that are potentially capable of spreading virulence determinants and other genetic traits to other bacterial strains. Similarly to *E. coli*, many other bacteria contain multiple prophages with genetic defects, and the potential of these sequence elements to function as mobile elements has been largely ignored. Our findings suggest that more attention should be paid to their

Subfunctional consortia

A P1-like strategy in Archaea?

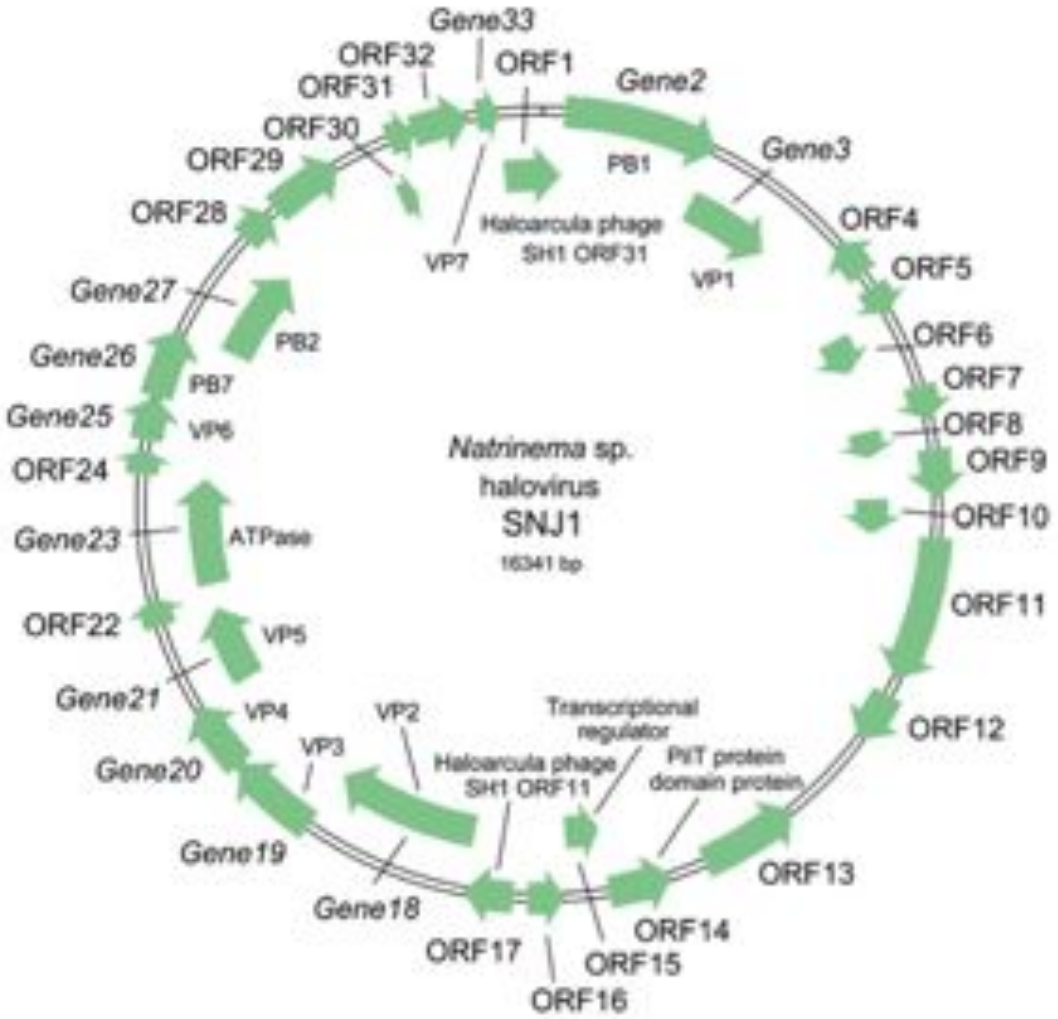


Fig. 3. The genome of SNJ1 is a 16,341-bp-long circular dsDNA molecule that encodes thirty-three putative proteins. The genes with putative annotated functions are indicated.

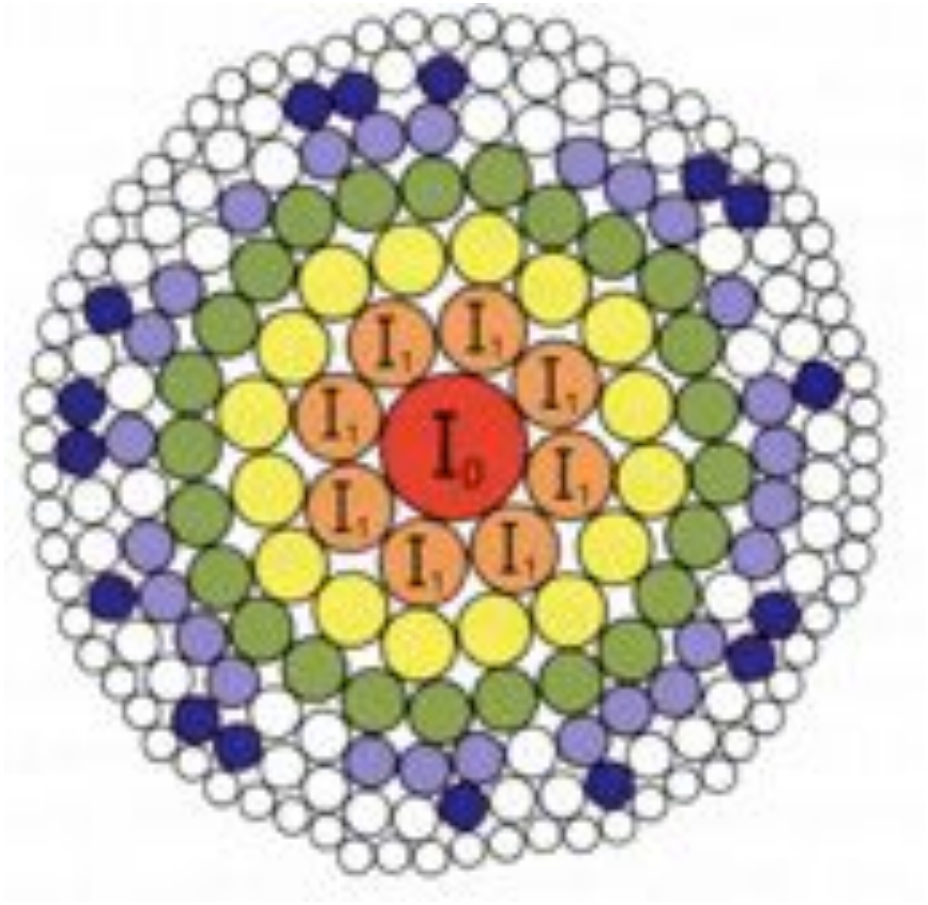
SNJ1; episomal persisting virus (plasmid pHH205) in <u>all</u> isolates of Natrinema sp. (a haloarchael)

Will lyse unlysogenized isolates
Induced by stress
No spontaneous induction
Competes with and excludes other version of episomal virus

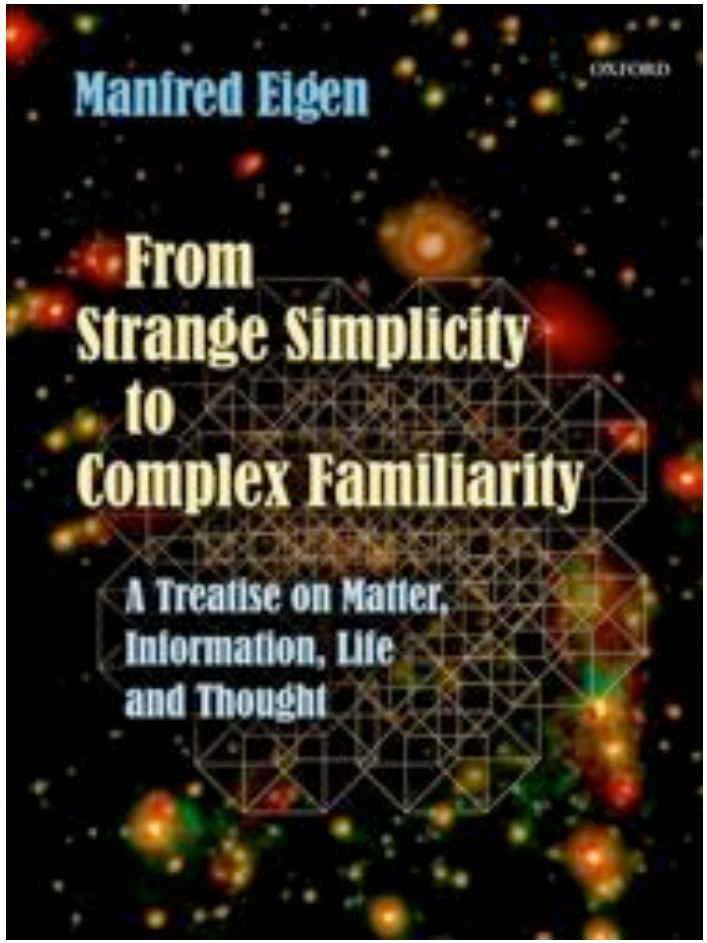
An addiction module?

Zhang, et al. 2012. 1012. Virology 434 (2)

	Experimental virology	RNA evolution theory	Language/code theory
1970's:	extensive variation *DI's JJ Holland (LPV)	M. Eigen – RNA virus quasispecies Origin of code, errors Master fittest type	basal syntax
1980's	RNA population measures	hypercycles, error catastrophe, QS selection	pragmatics
1990's	Observed QS behavior: minority types, DIs, MOI dep. non-consensus adaptions	cooperation problem membership problem	agents, editors
2000's	Observed cooperation: consensus is not master QS competition/exclusion	theory remains master fittest type based	communication meaning and social agents



Eigen's QS: mutant halo around master fittest type
A theoretical construct



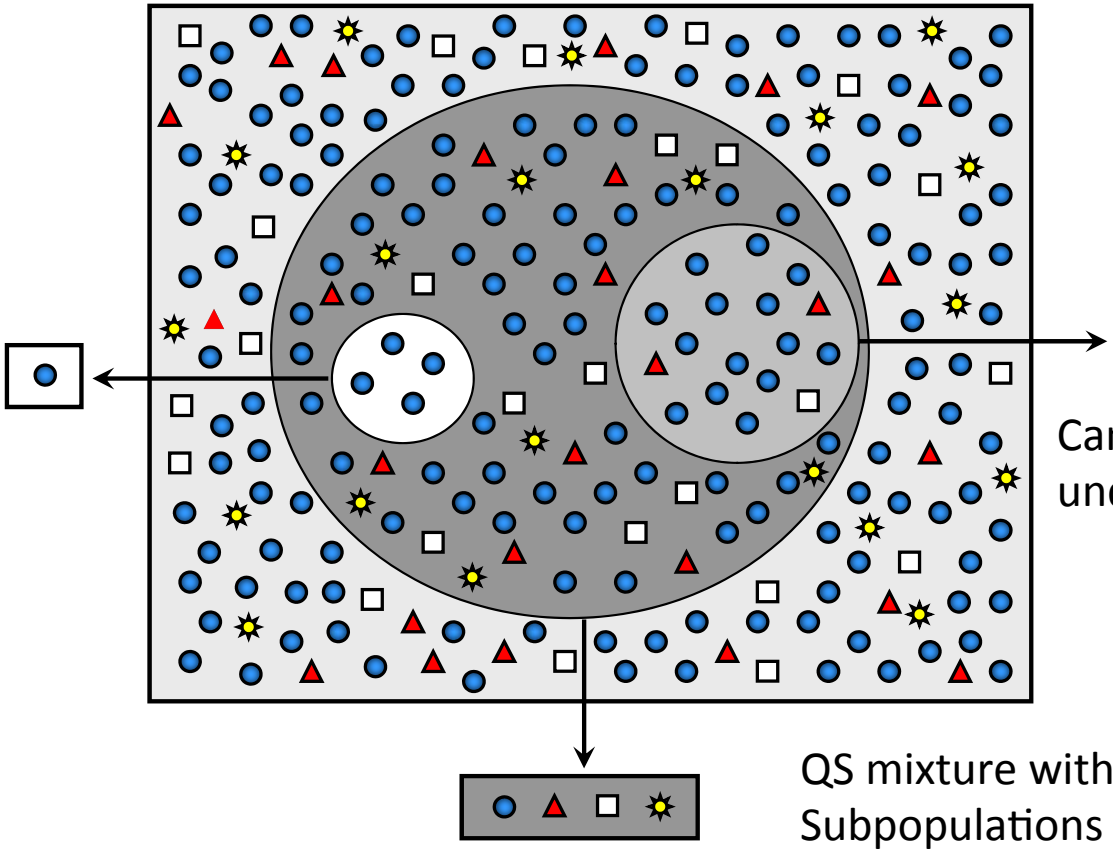
Established characteristics of quasispecies evolution

- Not fittest type; a **consortia** that needs diversity (a form of group selection)
- Participation of defectives and mutants important (the lethal/unfit)
- Complementation, cooperation, preclusion, competition all occur
- Diversity per se provides fitness

Not yet incorporated into RNA world thinking

Fig. 7

Esteban Domingo, J. Sheldon and C. Perales. 2012.
"Viral Quasispecies Evolution." *Microbiology and Molecular Biology Reviews* 76 (2): 159–216:



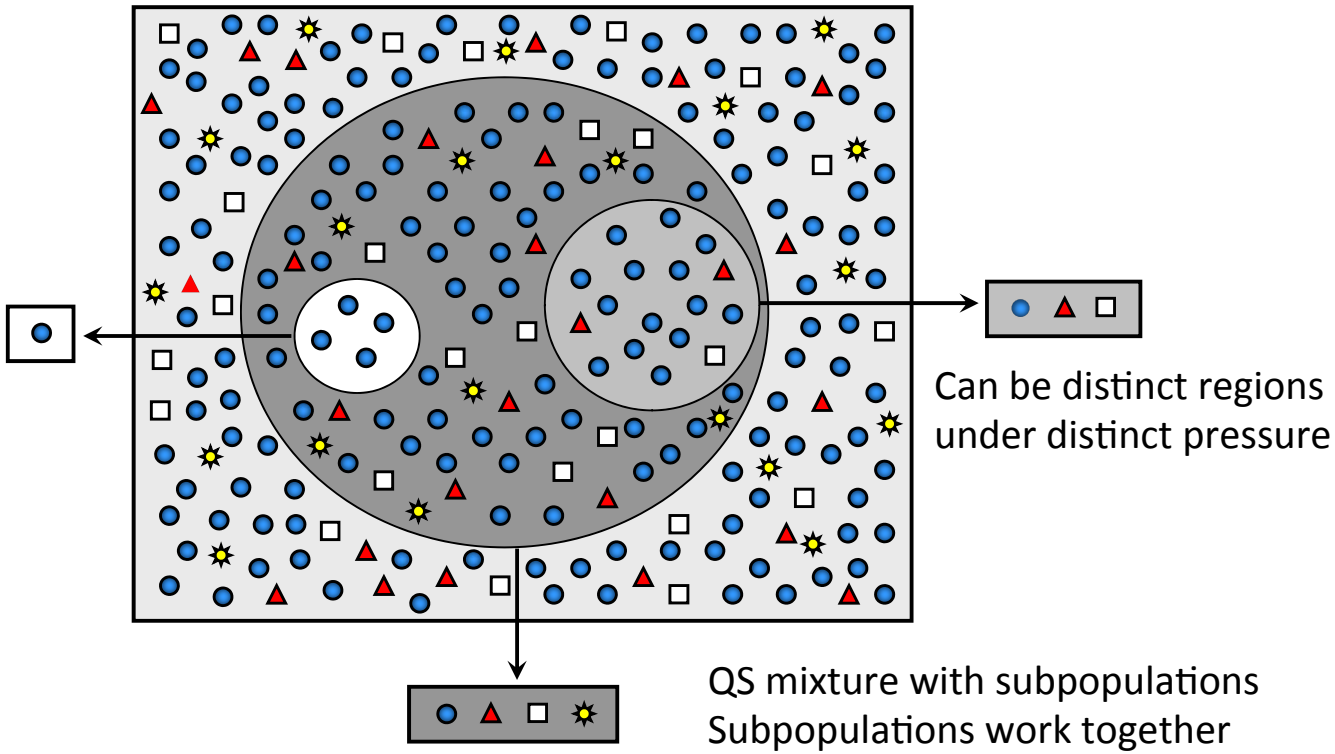
Can be distinct regions
under distinct pressure

QS mixture with subpopulations
Subpopulations work together
and against each other
Not needing consensus change
Can suppress 'Master fittest type"

QS-C (cooperative)
Polio/YFV vaccine: HCV transplants

Fig. 7

Esteban Domingo, J. Sheldon and C. Perales. 2012.
"Viral Quasispecies Evolution." *Microbiology and Molecular Biology Reviews* 76 (2): 159–216:

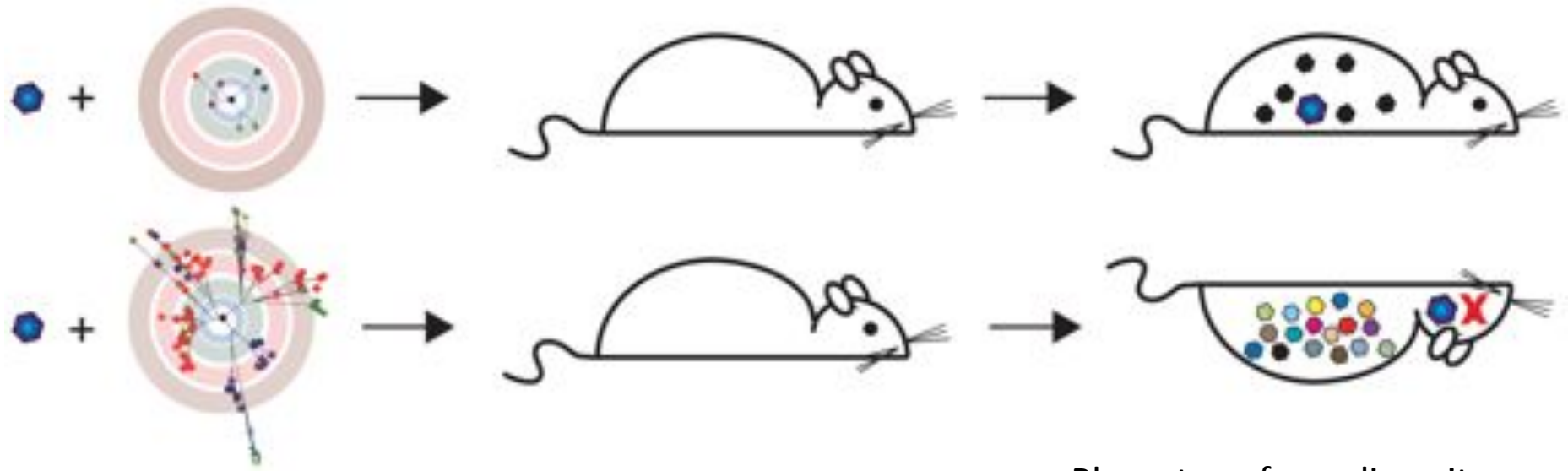


Can be distinct regions
under distinct pressure

QS mixture with subpopulations
Subpopulations work together
and against each other
Not needing consensus change
Can suppress 'Master fittest type"

QS-C (cooperative)
Polio/YFV vaccine: HCV transplants

many remarkable insights. The most basic, far-reaching, awesomely predictive tenet of quasispecies theory will never be overshadowed; numerous variant genomes are bound together through extreme mutation rates, forming obligatorily co-selected partnerships in a vast, error-prone mutant spectrum from which they cannot escape, and from which they inevitably and coordinately may exert myriad, changing, ultimately unforseeable effects on all life forms. This tenet has been unquestionably and elegantly confirmed recently by the U. C. San Francisco, Stanford and Penn State groups (as reviewed above and elsewhere in this volume).

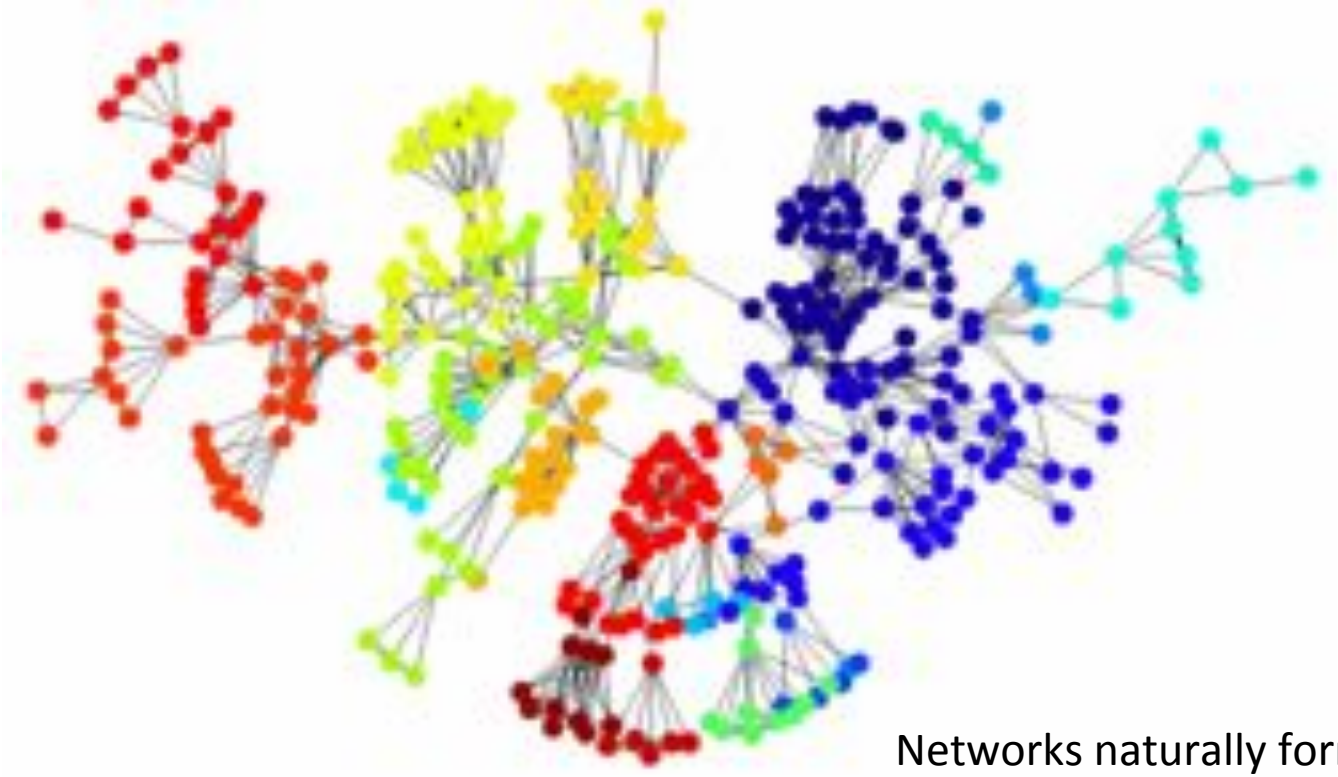


Phenotype from diversity,
not master type: Vignuzzi et al

Figure 4. Population diversity is a virulence determinant.

Live vaccine strains show similar diversity restriction

Lauring, Adam S., and Raul Andino. "Quasispecies theory and the behavior of RNA viruses." *PLoS pathogens* 6, no. 7 (2010): e1001005.



Networks naturally form clades
Clades differentiate
Compete and preclude each other
Acquire group identity
To create or modify, colonization
by diffuse agents

MEMBERSHIP/SECURITY ?

The HCV examplar:
QS based identity/exclusion

Dynamic persistence via ongoing RNA synthesis

Quasispecies determined biology

Group preclusion (identity)

The role of stem-loop RNA - consortia

Laskus T, Wang L-F, Radkowski M, Vargas H, Nowicki M, et al. 2001. Exposure of hepatitis c virus (hcv) rna-positive recipients to hcv rna-positive blood donors results in rapid predominance of a single donor strain and exclusion and/or suppression of the recipient strain. *J. Virol.* 75(5):2059–66

Endogenous retroviruses regulate periimplantation placental growth and differentiation

Kathrin A. Dunlap*, Massimo Palmarini†, Mariana Varela†, Robert C. Burghardt‡, Kanako Hayashi*, Jennifer L. Farmer*, and Thomas E. Spencer*§

*Center for Animal Biotechnology and Genomics, Department of Animal Science, and ‡Image Analysis Laboratory, Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843; and †Institute of Comparative Medicine, University of Glasgow Veterinary School, Glasgow G61 1QH, United Kingdom

Edited by George E. Seidel, Colorado State University, Fort Collins, CO, and approved August 8, 2006 (received for review May 10, 2006)

Endogenous retroviruses (ERVs) are fixed and abundant in the genomes of vertebrates. Circumstantial evidence suggests that ERVs play a role in mammalian reproduction, particularly placental morphogenesis, because intact ERV envelope genes were found to be expressed in the syncytiotrophoblasts of human and mouse placenta and to elicit fusion of cells *in vitro*. We report here *in vivo* and *in vitro* experiments finding that the envelope of a particular class of ERVs of sheep, endogenous Jaagsiekte sheep retroviruses (enJSRVs), regulates trophectoderm growth and differentiation in the periimplantation conceptus (embryo/fetus and associated extraembryonic membranes). The enJSRV envelope gene is expressed in the trophectoderm of the elongating ovine conceptus after day 12 of pregnancy. Loss-of-function experiments were conducted *in utero* by injecting morpholino antisense oligonucleotides on day 8 of pregnancy that blocked enJSRV envelope protein production in the conceptus trophectoderm. This approach retarded trophectoderm outgrowth during conceptus elongation and inhibited trophoblast giant binucleate cell differentiation as observed on day 16. Pregnancy loss was observed by day 20 in sheep receiving morpholino antisense oligonucleotides. *In vitro* inhibition of the enJSRV envelope reduced the proliferation of mononuclear trophectoderm cells isolated from day 15 conceptuses. Consequently, these results demonstrate that the enJSRV envelope regulates trophectoderm growth and differentiation in the periimplantation ovine conceptus. This work supports the hypothesis that ERVs play fundamental roles in placental morphogenesis and mammalian reproduction.

development | placenta | sheep | trophectoderm

The sheep genome contains ≈20 copies of endogenous retroviruses (ERVs) highly related to the exogenous and pathogenic Jaagsiekte sheep retrovirus (JSRV) (1–3). Endogenous JSRVs (enJSRVs) are abundantly expressed in the epithelia of the female genital tract (4). In the placenta, enJSRVs are expressed in the mononuclear trophectoderm cells of the conceptus (embryo/fetus and associated extraembryonic membranes) and are most abundant in the trophoblast giant binucleate cells (BNCs) and multinucleated syncytial plaques of the placentomes (5–7). The temporal expression of the enJSRV envelope (*env*) gene in the trophectoderm is coincident with key events in the development of the sheep conceptus (8). enJSRV *env* mRNAs are first detected at day 12 (5), when the blastocyst begins the process of elongation, involving the intense proliferation and outgrowth of mononuclear trophectoderm cells producing IFN-δ, the antiluteolytic signal for pregnancy recognition in ruminants (9, 10).

Hyaluronidase 2 (HYAL2) is a glycosylphosphatidylinositol-anchored cell-surface protein that can serve as a cellular receptor for exogenous JSRV Env as well as for retroviral vectors pseudotyped by enJSRV Env (13, 14). By RT-PCR analyses, *HYAL2* mRNA is first detected in the conceptus on day 16, which is associated with the onset of BNC differentiation (5). Throughout pregnancy, *HYAL2* mRNA can be detected in the BNCs and multinucleated syncytia of sheep placentomes but not in the mononuclear trophectoderm cells of the conceptus or any cells of the endometrium.

Of great interest for comparative physiology is that enJSRV *env* expression in the developing ovine placenta is strikingly similar to that observed for syncytin 1 and 2, products of human ERV (HERV)-W *env* in humans and primates (15–19) and possibly of two related *env* genes (*syncytin A* and *syncytin B*) in mice (20). Syncytins encode highly fusogenic retroviral envelope proteins that are expressed in the syncytiotrophoblast layer generated by mononuclear cytotrophoblast cell fusion at the maternal–fetal interface. Syncytins are fusogenic when expressed *in vitro*, thereby advancing the hypothesis that they are involved in placental morphogenesis (15–19). Thus, circumstantial evidence gleaned from studies of primates, sheep, and rodents supports the concept that independently acquired ERVs have been positively selected for a convergent physiological role in placental morphogenesis (21, 22).

In these studies we tested the hypothesis that enJSRV Env has a biological role in periimplantation ovine conceptus development and placental morphogenesis by using an *in vivo* morpholino loss-of-function approach (23) to block enJSRV Env production *in utero*.

Results

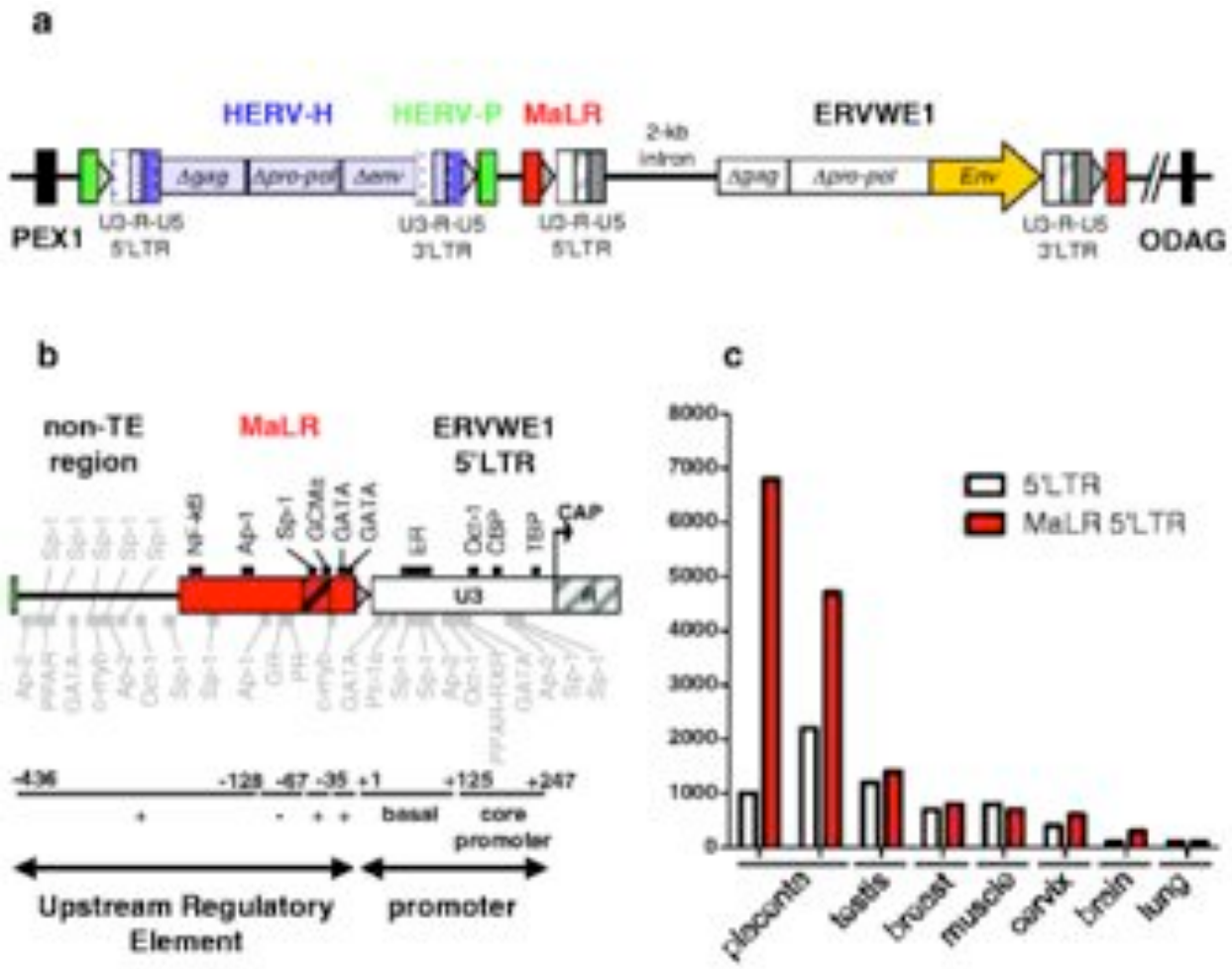
A morpholino antisense oligonucleotide (MAO) was designed to specifically inhibit expression of enJSRV *env* mRNAs (MAO-*env*) (Fig. 1*A*). MAOs inhibit RNA splicing and/or translation by a steric block mechanism that is RNase H-independent (23). Morpholinos are effective only when designed to complement the nucleotide region around the start codon and/or possible splicing sites of a given gene mRNA. The nucleotide sequence around the splice acceptor and start codon of the exogenous JSRV *env* and the known enJSRV loci are highly conserved, indicating that one common MAO should inhibit splicing and translation of most enJSRV proviral loci expressing an intact *env* gene (6). To examine mor-

Author contributions: K.A.D., M.P., M.V., and T.E.S. designed research; K.A.D., M.P., M.V., R.C.B., K.H., J.L.F., and T.E.S. performed research; M.P., M.V., R.C.B., K.H., J.L.F., and T.E.S. contributed new reagents/analytic tools; K.A.D., M.P., M.V., and T.E.S. analyzed data; and

DEVELOPMENTAL BIOLOGY

Network examplar:

Distributed collective of mixed defective virus providing new regulatory complexity



Pérot, Philippe, Pierre-Adrien Bolze, and François Mallet. 2012. Mixed LTRs provide complex control. "From Viruses to Genes: Syncytins." In *Viruses: Essential Agents of Life*, ed. G. Witzany

nature genetics

Endogenous retroviruses function as species-specific enhancer elements in the placenta

Edward B Chuong[1], M A Karim Rumi[2], Michael J Soares[2] & Julie C Baker[1]

The mammalian placenta is remarkably distinct between species, suggesting a history of rapid evolutionary diversification[1]. To gain insight into the molecular drivers of placental evolution, we compared biochemically predicted enhancers in mouse and rat trophoblast stem cells (TSCs) and found that species-specific enhancers are highly enriched for endogenous retroviruses (ERVs) on a genome-wide level. One of these ERV families, RLTR13D5, contributes hundreds of mouse-specific histone H3 lysine 4 monomethylation (H3K4me1)- and histone H3 lysine 27 acetylation (H3K27ac)-defined enhancers that functionally bind Cdx2, Eomes and Elf5—core factors that define the TSC regulatory network. Furthermore, we show that RLTR13D5 is capable of driving gene expression in rat placental cells. Analysis in other tissues shows that species-specific ERV enhancer activity is generally restricted to hypomethylated tissues, suggesting that tissues permissive for ERV activity gain access to an otherwise silenced source of regulatory variation. Overall, our results implicate ERV enhancer co-option as a mechanism underlying the extensive evolutionary diversification of placental development.

we sought to investigate the regulatory landscape of early placental development in two closely related species—mouse and rat. Despite the similarities between mouse and rat placentation, genes expressed by the mature placenta show clear signs of rapid evolution since rodents diverged[9], suggesting that evolution at the regulatory level may also be detected. We cultured mouse and rat TSCs, which represent the first cell population to give rise to the fetal placenta[11], and performed 3′-end RNA sequencing (3′ RNA-seq)[12] and chromatin immunoprecipitation with sequencing (ChIP-seq) for histone marks indicative of promoters (trimethylation of histone H3 at lysine 4 (H3K4me3)), enhancers (H3K4me1 and H3K27ac) and repressed regions (trimethylation at histone H3 lysine 27 (H3K27me3) and trimethylation at histone H3 lysine 9 (H3K9me3))[13]. Only high-quality, uniquely mapping reads were retained, and histone-marked regions were identified using MACS (v2.09) with false discovery rate (FDR) < 0.05. We predicted 9,460 mouse and 7,932 rat TSC promoters on the basis of H3K4me3 enrichment over gene transcriptional start sites (TSSs), which were associated with expressed genes (**Fig. 1a,b**). We predicted 52,476 mouse and 41,142 rat TSC enhancers on the basis of distal enrichment of H3K4me1 (>5 kb from a gene TSS) and 25,736 mouse and 4,471 rat

Lynch, Vincent J., Robert D. Leclerc, Gemma May, and Günter P. Wagner. 2011.
"Transposon-Mediated Rewiring of Gene Regulatory Networks Contributed to the Evolution of Pregnancy in Mammals."
Nature Genetics 43 (11): 1154–59

Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53

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*Center for Biomolecular Science and Engineering, and †Howard Hughes Medical Institute, University of California, Santa Cruz, CA 95064; ‡Division of Hematology/Oncology, Departments of Medicine and Biological Chemistry, University of California, Irvine, CA 92697; and §Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892

Edited by Eric H. Davidson, California Institute of Technology, Pasadena, CA, and approved September 26, 2007 (received for review April 27, 2007)

The evolutionary forces that establish and hone target gene networks of transcription factors are largely unknown. Transposition of retroelements may play a role, but its global importance, beyond a few well described examples for isolated genes, is not clear. We report that LTR class I endogenous retrovirus (ERV) retroelements impact considerably the transcriptional network of human tumor suppressor protein p53. A total of 1,509 of ~319,000 human ERV LTR regions have a near-perfect p53 DNA binding site. The LTR10 and MER61 families are particularly enriched for copies with a p53 site. These ERV families are primate-specific and transposed actively near the time when the New World and Old World monkey lineages split. Other mammalian species lack these p53 response elements. Analysis of published genomewide ChIP data for p53 indicates that more than one-third of identified p53 binding sites are accounted for by ERV copies with a p53 site. ChIP and expression studies for individual genes indicate that human ERV p53 sites are likely part of the p53 transcriptional program and direct regulation of p53 target genes. These results demonstrate how retroelements can significantly shape the regulatory network of a transcription factor in a species-specific manner.

Deciphering gene regulatory networks in the postgenomic era will provide pivotal insights into genome function and human disease, but it will require a much improved knowledge of evolutionary forces that shape transcriptional networks. One key will be understanding the ~5% of the human genome that is under purifying selection and hence likely to contain functional segments (1, 2). Two-thirds of these segments do not code for protein and likely harbor essential regulatory information. Some of these derive from transposable elements, and thus lie in the 45% of our genome once deemed "junk DNA." Although there are examples where transposable elements played important roles in the evolution of gene regulation (3–5), and certain families have deposited putative regulatory elements that are now subjected to purifying selection (6–10), it is unclear how extensively these mobile elements have shaped gene regulatory networks.

Human endogenous retroviruses (ERVs), remnants of exogenous retroviruses [illegible] and is considered a pleiotropic master regulator. Intense computational and experimental efforts have determined p53 DNA binding specificity, mapped many genomic binding sites, and identified numerous target genes (16–19). However, no studies have examined a relationship between p53 and transposable elements to our knowledge.

We report that human ERVs actively shape the p53 transcriptional network in a species-specific manner. p53 sites are highly enriched in LTRs of a few ERV subfamilies. These p53 site-containing LTRs are *in vivo* binding sites for p53 and account for >30% of p53 sites found in a genomewide ChIP analysis (16). Expression of many genes close to these LTRs is regulated by p53, based on published data and our experimental validation. These ERVs likely entered the primate ancestral genome and transposed within it ~25 Mya to 63 Mya. Their proviruses were probably responsible for introducing a p53 site. In general, ERV insertions near genes (including those with p53 sites) were selected against (11, 12), but a significant fraction of p53 site-containing ERVs may have been exapted as regulatory sequences to expand the p53 transcriptional network. At least one ERV insertion likely reshaped the transcriptional landscape of its surrounding genomic area and was instrumental in creating a new gene that became part of the human-specific p53 regulatory network.

Results

p53 Sites Are Enriched in LTRs of Several Human ERV Subfamilies. A genomewide yeast-based screen identified certain ERV LTR elements with a p53-responsive site (J.Z. and R.K.B., unpublished data). This finding triggered a computational survey of the human genome for p53 sites in ERV LTR elements. Using RepeatMasker (44), 319,106 ERV LTR fragments were identified, accounting for 5% of the human genome and belonging to >500 families and subfamilies of LTR-containing retroelements defined in RepBase (21). Only 1,509 fragments had a near-perfect p53 site based on our stringent criteria [see *Materials and Methods* and supporting information (SI) *Text*]. Copies with a p53 site were strikingly overrepresented in the LTR10 and

What allows network membership, editing?

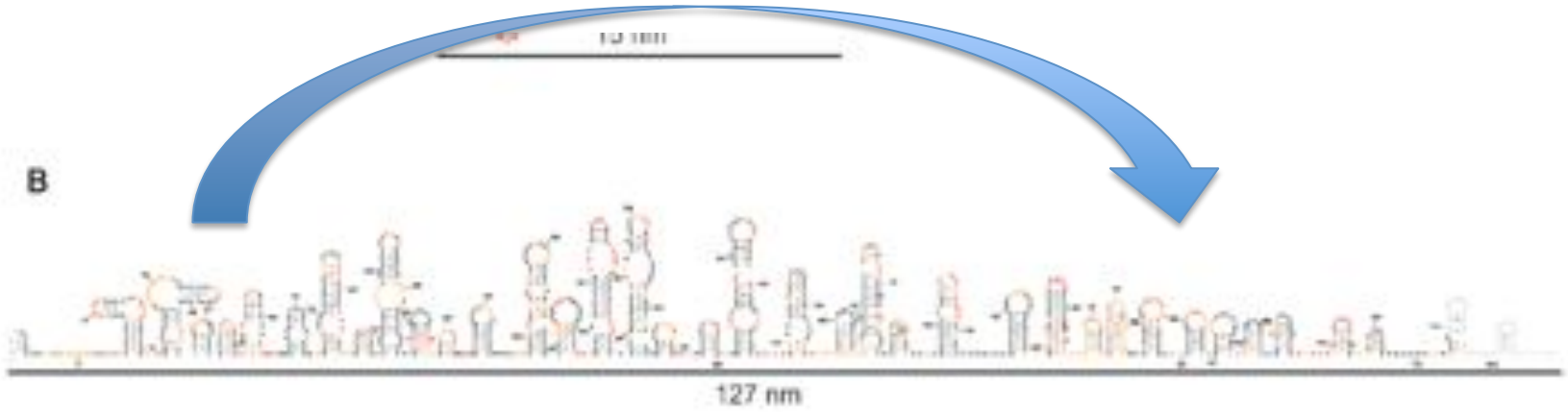


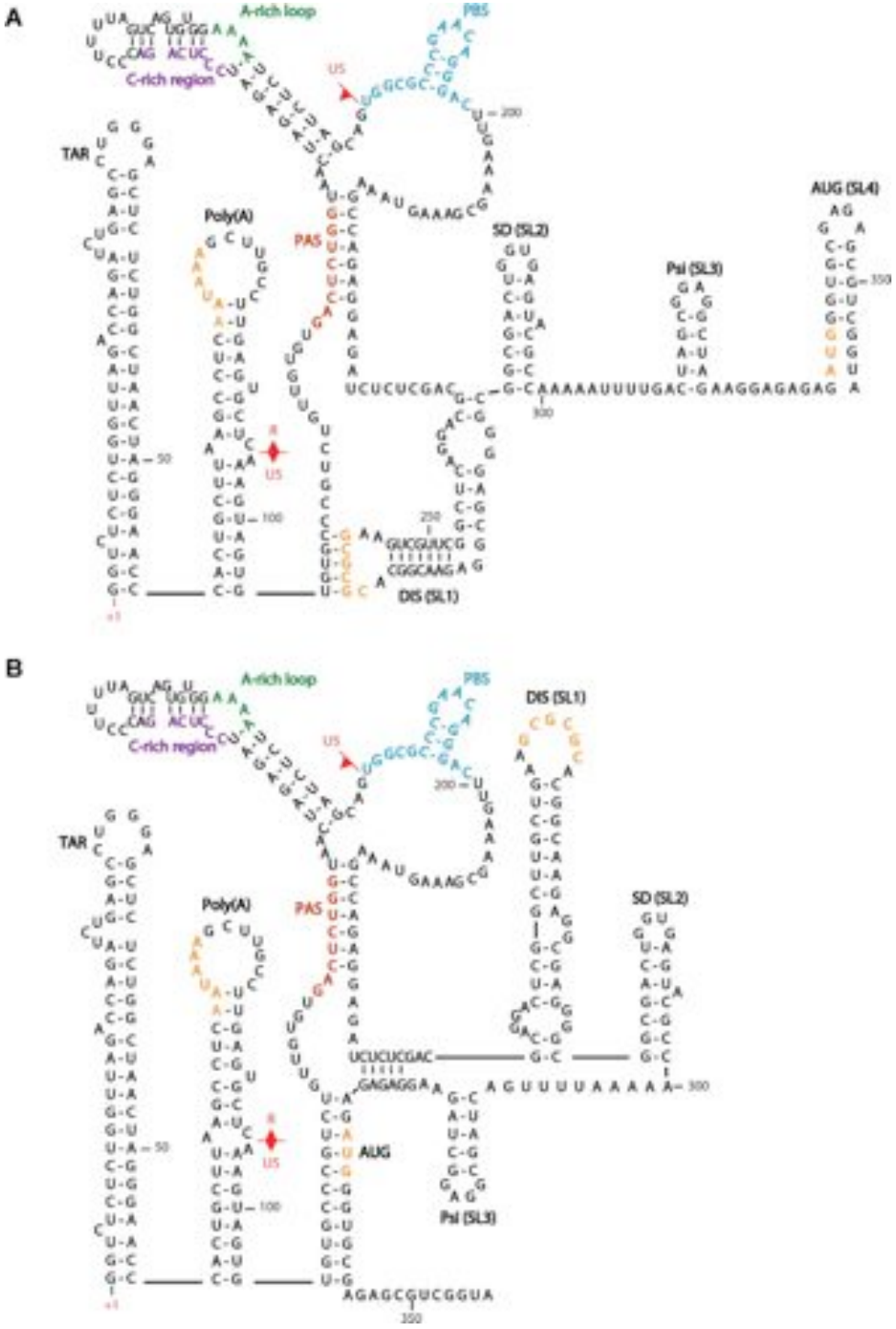
Figure 3. Secondary structure models for the STMV RNA *ex virio*. (A) SHAPE-directed model. Maximum allowed base pairing distance was 600 nucleotides.[22] The start and stop codons for the capsid protein are boxed. (B) Linked stem-loop model, created using SHAPE data and parameters designed to force formation of short stem-loop motifs by restricting the maximum base pairing distance to ≤50 nucleotides. Nucleotides are colored by SHAPE reactivity (see legend); gray indicates no data were obtained. Calculated lengths of major structural features in each structure are shown (in nanometers).

Archer, Eva J., et al. "Long-Range Architecture in a Viral RNA Genome." *Biochemistry* (2013).

STMV as the 'hydrogen' of + RNA viruses: core role for stem-loop RNAs

Strong evidence that 'cooperative' long distance RNA-RNA interactions needed for gene expression and replication. Recall HCV preclusion.

RNA stem-loops as predecessors – a way to create interacting networks



Secondary structure of the 5’-UTR of the HIV-1 genomic RNA

Sleiman, Dona, et al. "Initiation of HIV-1 reverse transcription and functional role of nucleocapsid-mediated tRNA/viral genome interactions." *Virus research* (2012)

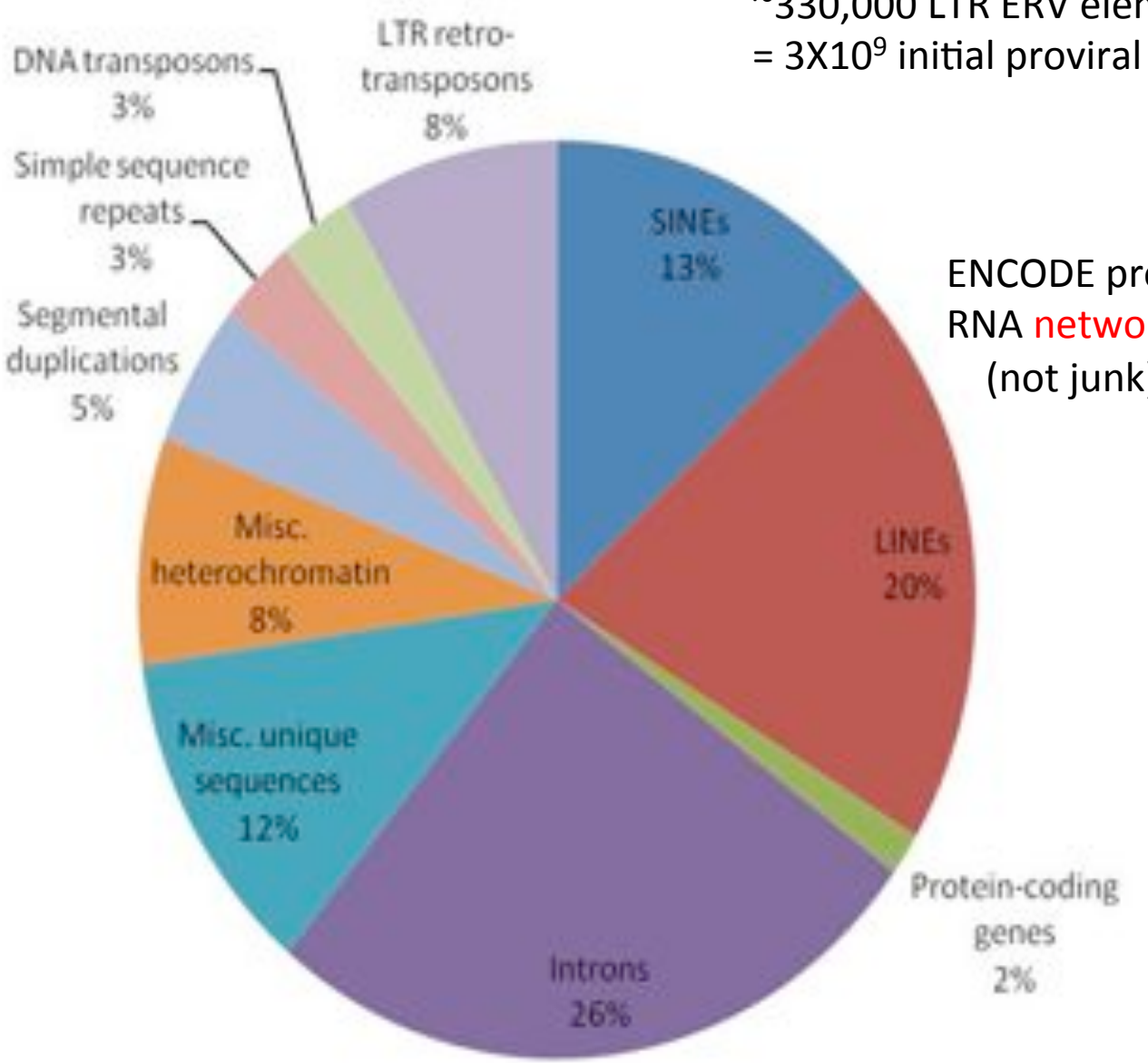
OK! Stem loops crucial
For RNA virus replication, regulation, packaging (ID)

What if virus provides them en mass (via LTRs) to host DNA? Virus addiction?

New QS based RNA networks?

What about defective DNA viral colonization events (i.e. Maverick)? Also provide diffuse stem-loop RNAs.

~330,000 LTR ERV elements
= $3X10^9$ initial proviral DNA



Components of the Human Genome

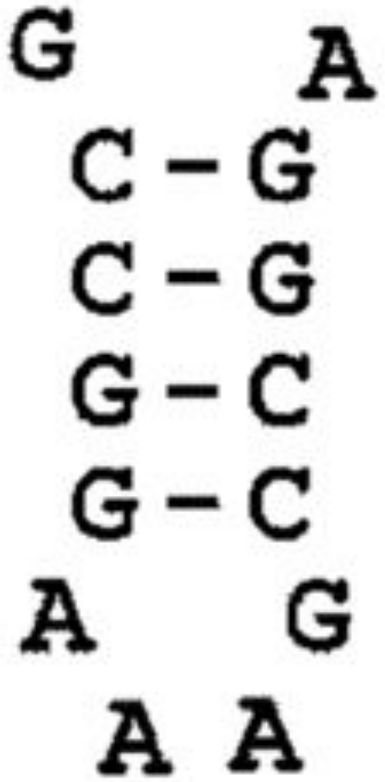
ENCODE project?
RNA networks
(not junk)

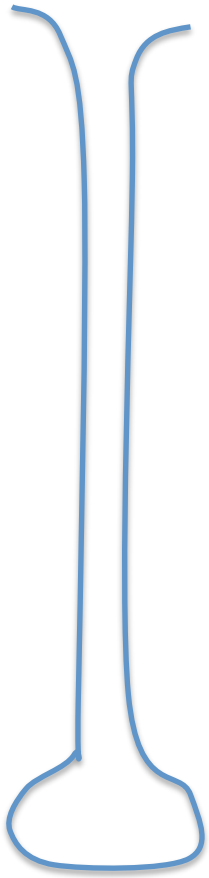
Most eukaryotic stem-loop RNA derived from retroposons

- Retroposons as DNA editors via stem-loops
- Sources of new regulatory regimes, new identity networks
- Promote big adaptations: p53, stem cells, brain, placenta
- DNA viruses (transposons) also use sRNA (stem-loops) as regulators of replication and persistence

- So RNA viruses are consortia of stem-loops. Are similar consortia needed for the Origin of life?

Simple stem loop as basal



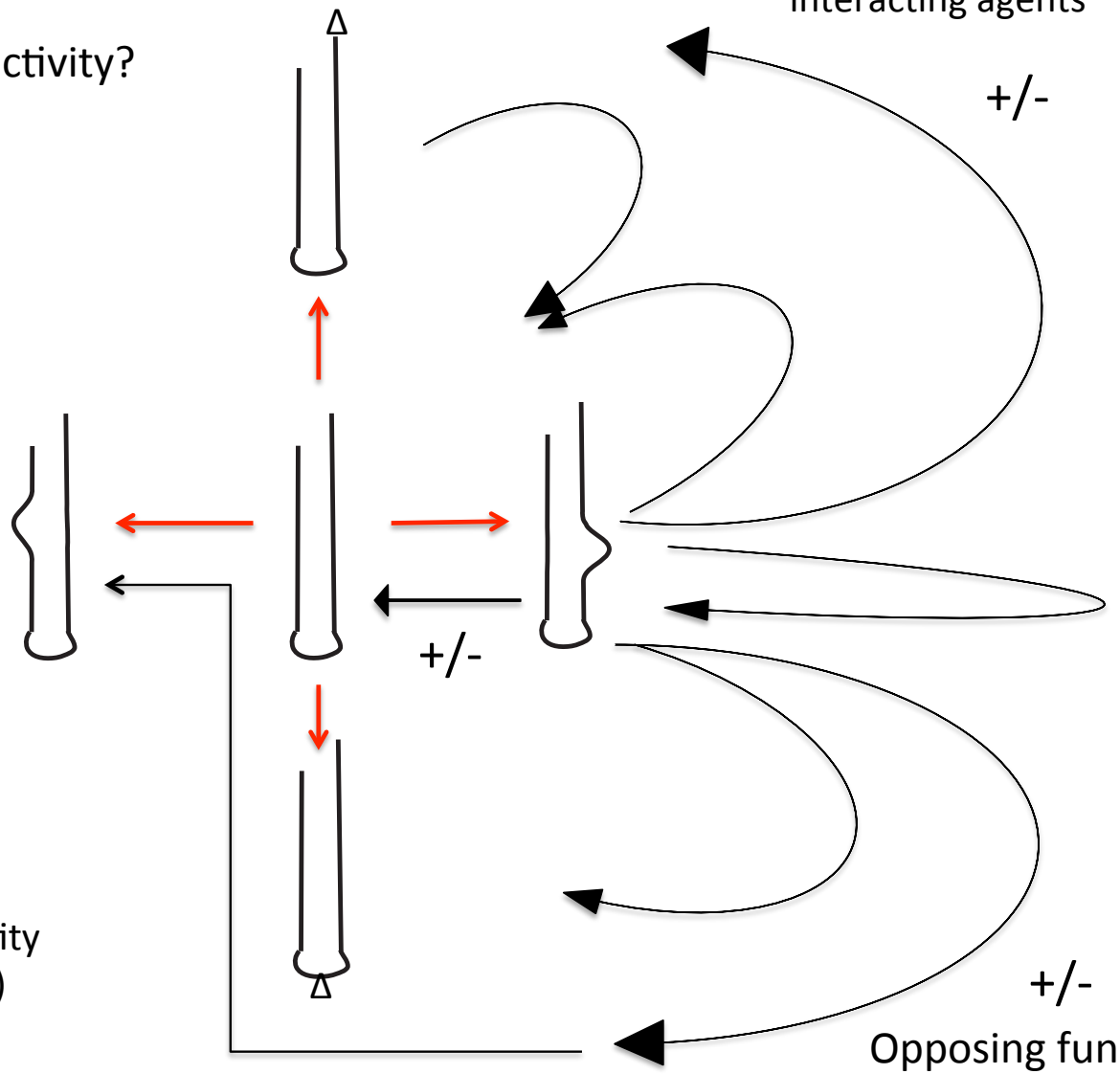


Briones, Stich, and Manrubia. 2009. “The Dawn of the RNA World: Toward Functional Complexity through Ligation of Random RNA Oligomers.” *RNA* 15 (5): 743–49.

Cooperative QS

Stem loop first perspective
Diversity from ribozyme replication

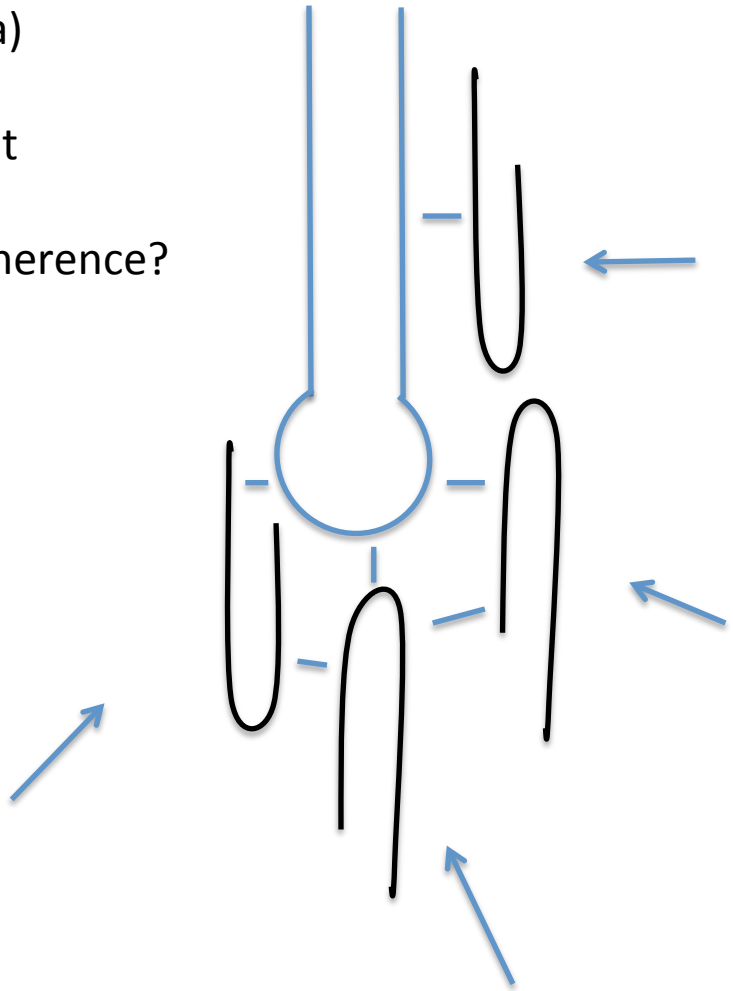
How to initiate ribozyme activity?



No master fittest type: consortia at origin

Need for affinities (ID)
Individuals are subfunctional
Need to interact (consortia)
No common ancestor
Not an error based concept

How to promote group coherence?



Group activities:

Cooperation
Complementation
Repression
Interference
Consortia behavior (T/A)

Ribozyme consortia

ARTICLE

doi:10.1038/nature11549

Spontaneous network formation among cooperative RNA replicators

Nilesh Vaidya[1], Michael L. Manapat[2], Irene A. Chen[3]†, Ramon Xulvi-Brunet[3], Eric J. Hayden[4] & Niles Lehman[1]

The origins of life on Earth required the establishment of self-replicating chemical systems capable of maintaining and evolving biological information. In an RNA world, single self-replicating RNAs would have faced the extreme challenge of possessing a mutation rate low enough both to sustain their own information and to compete successfully against molecular parasites with limited evolvability. Thus theoretical analyses suggest that networks of interacting molecules were more likely to develop and sustain life-like behaviour. Here we show that mixtures of RNA fragments that self-assemble into self-replicating ribozymes spontaneously form cooperative catalytic cycles and networks. We find that a specific three-membered network has highly cooperative growth dynamics. When such cooperative networks are competed directly against selfish autocatalytic cycles, the former grow faster, indicating an intrinsic ability of RNA populations to evolve greater complexity through cooperation. We can observe the
Our experiments highlight the advantages of cooperative behaviour

Vaidya N, Manapat ML, Chen IA, Xulvi-Brunet R, Hayden EJ, Lehman N

Nature 2012, 491:72–77.

Directly compare cooperative ribozymes
to selfish individual ribozymes – cooperation wins

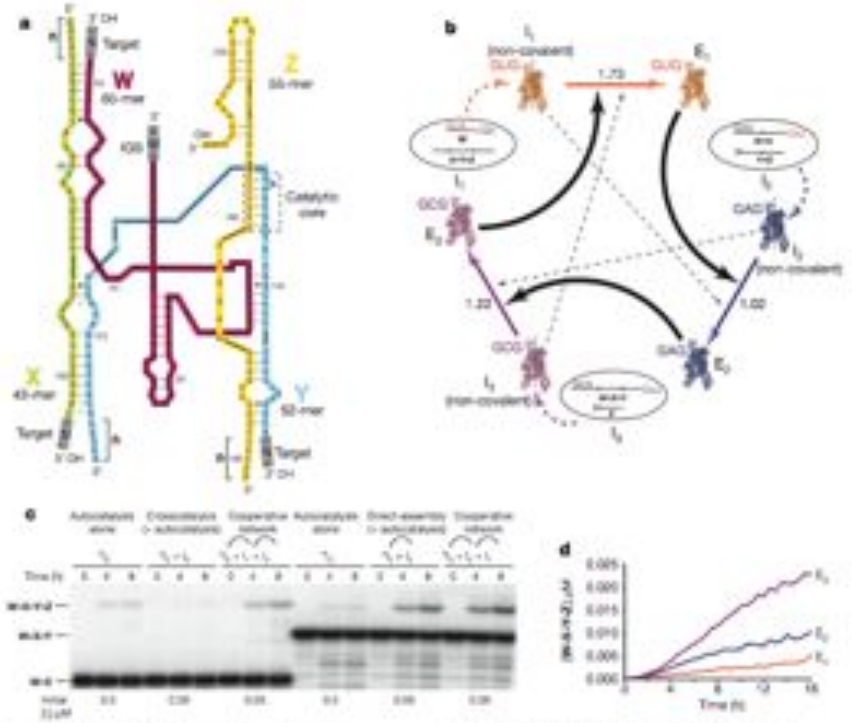
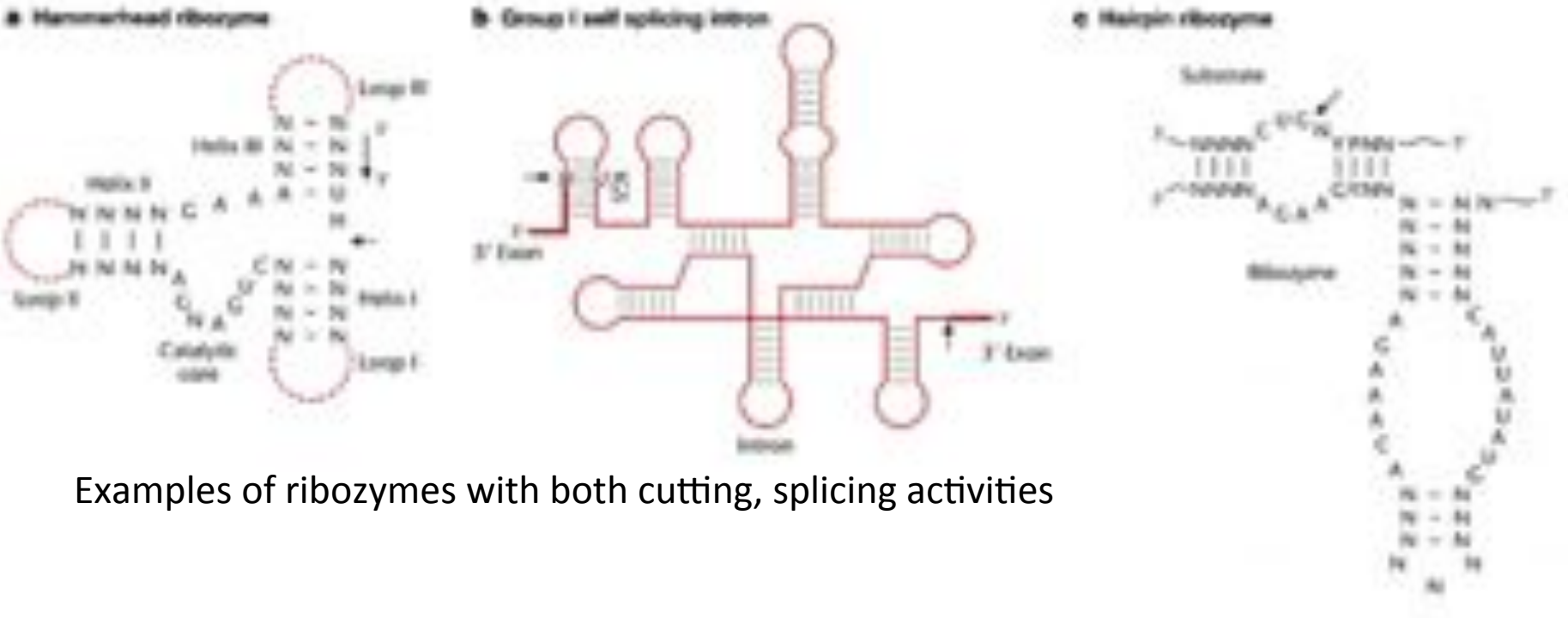


Figure 1 | Cooperative covalent assembly of recombinase ribozymes. a. Design of recombinase ribozymes capable of spontaneous cooperative (W•X + Δ•Y•Z) and I_2 (W•X•Y + Δ•Z). Numbers over arrows estimate the cooperative advantage for each step (see text). c. Electrophoretic observation of

Lincoln, Tracey A., and Gerald F. Joyce. 2009. “Self-Sustained Replication of an RNA Enzyme.” *Science* 323 (5918): 1229–32

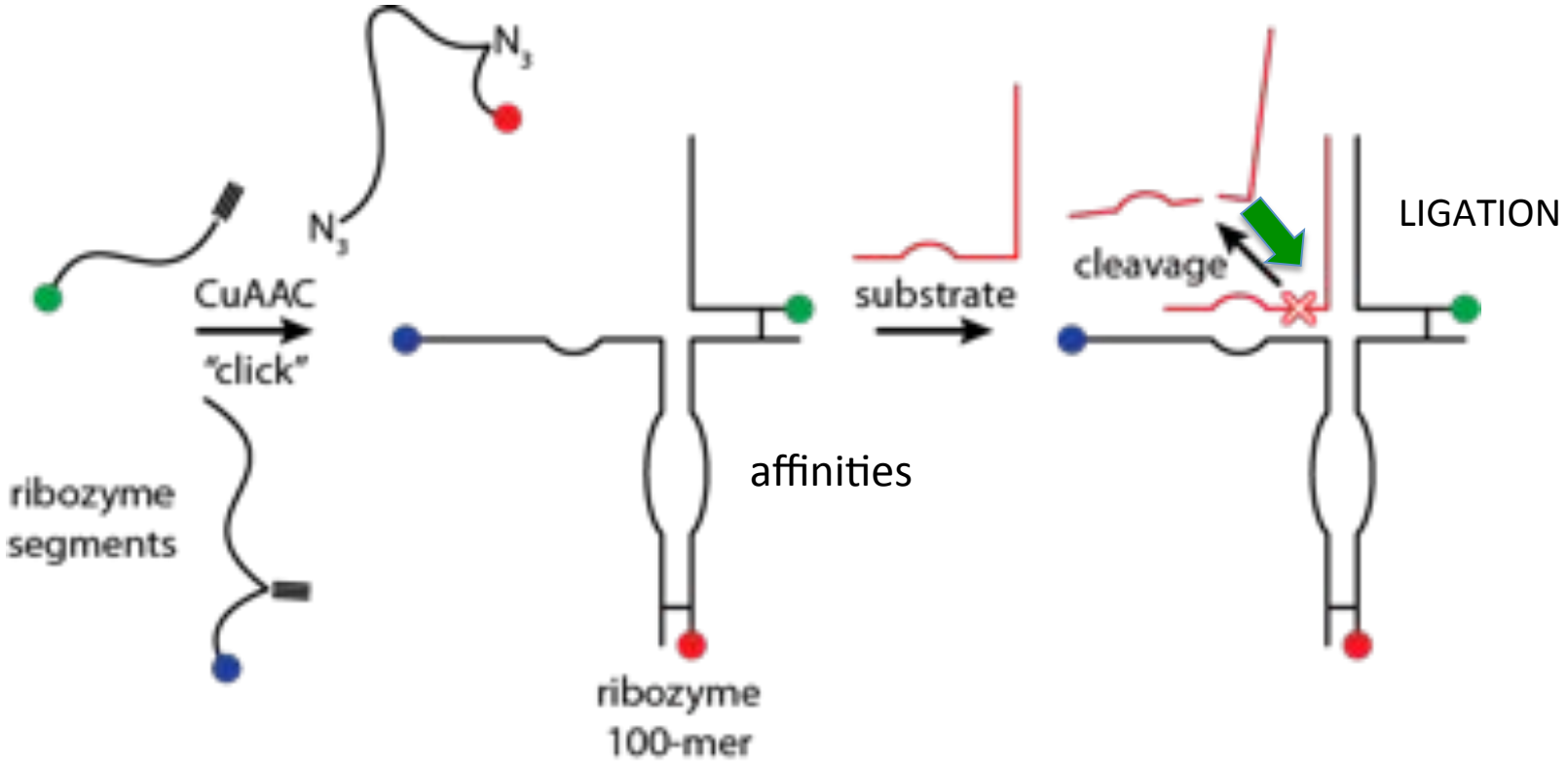


Examples of ribozymes with both cutting, splicing activities

Observed cooperative interactions:

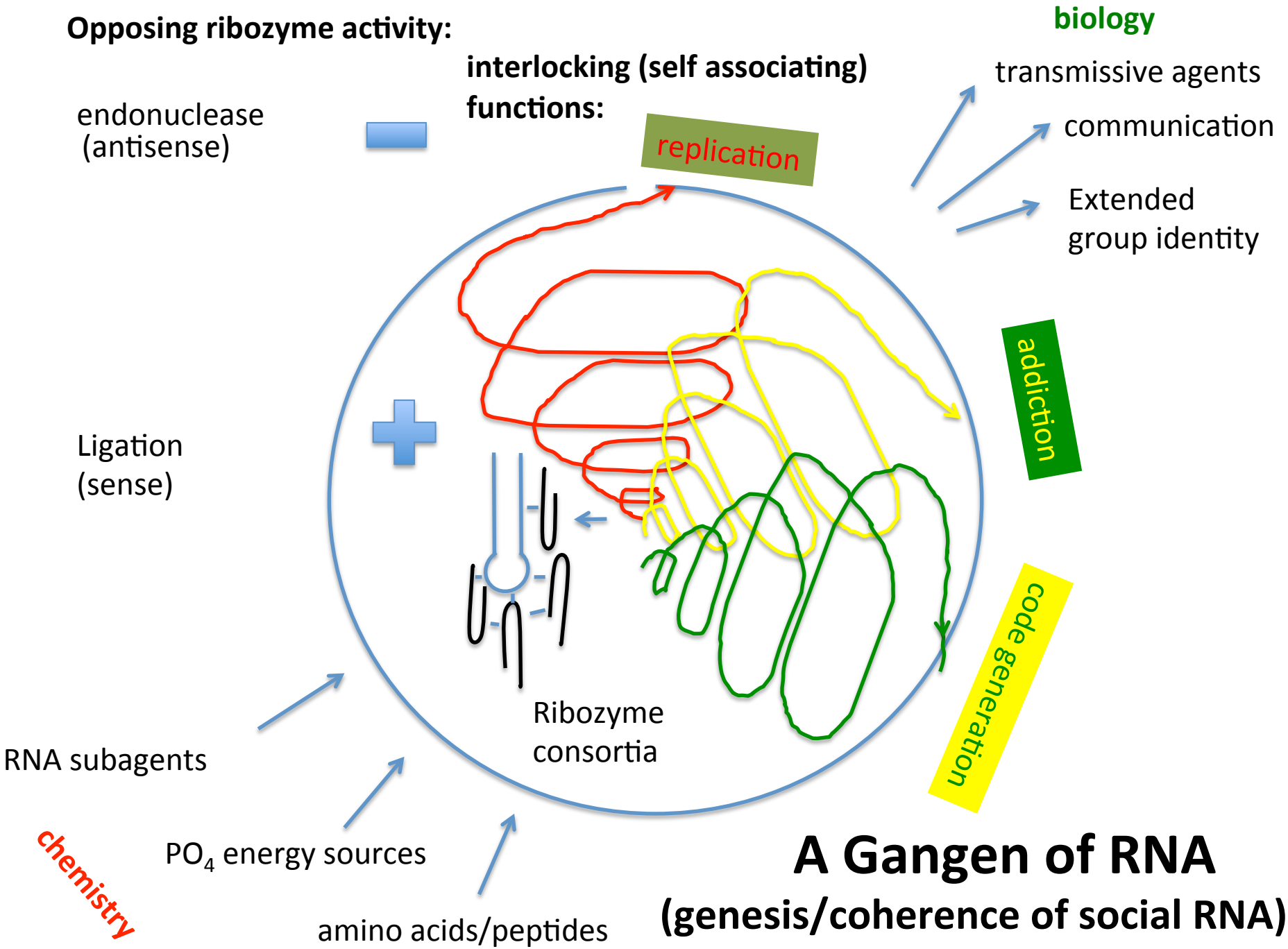
Cooperation
Complementation
Repression
Interference
Consortia behavior (T/A)

Ribozymes do both:



CLEAVAGE/LIGATION: Basis of RNA synthesis (polymerization)

- Group identity via addiction module (toxin/antitoxin)
- Basis of de novo code generation (editing)
- Opposing functions and group affinities all needed to initiate selection


Opposing ribozyme activity:
interlocking (self associating) functions:
biology
transmissive agents
communication
Extended group identity
endonuclease (antisense)
replication
addiction
Ligation (sense)
code generation
Ribozyme consortia
RNA subagents
chemistry
PO_4 energy sources
amino acids/peptides
A Gangen of RNA
(genesis/coherence of social RNA)

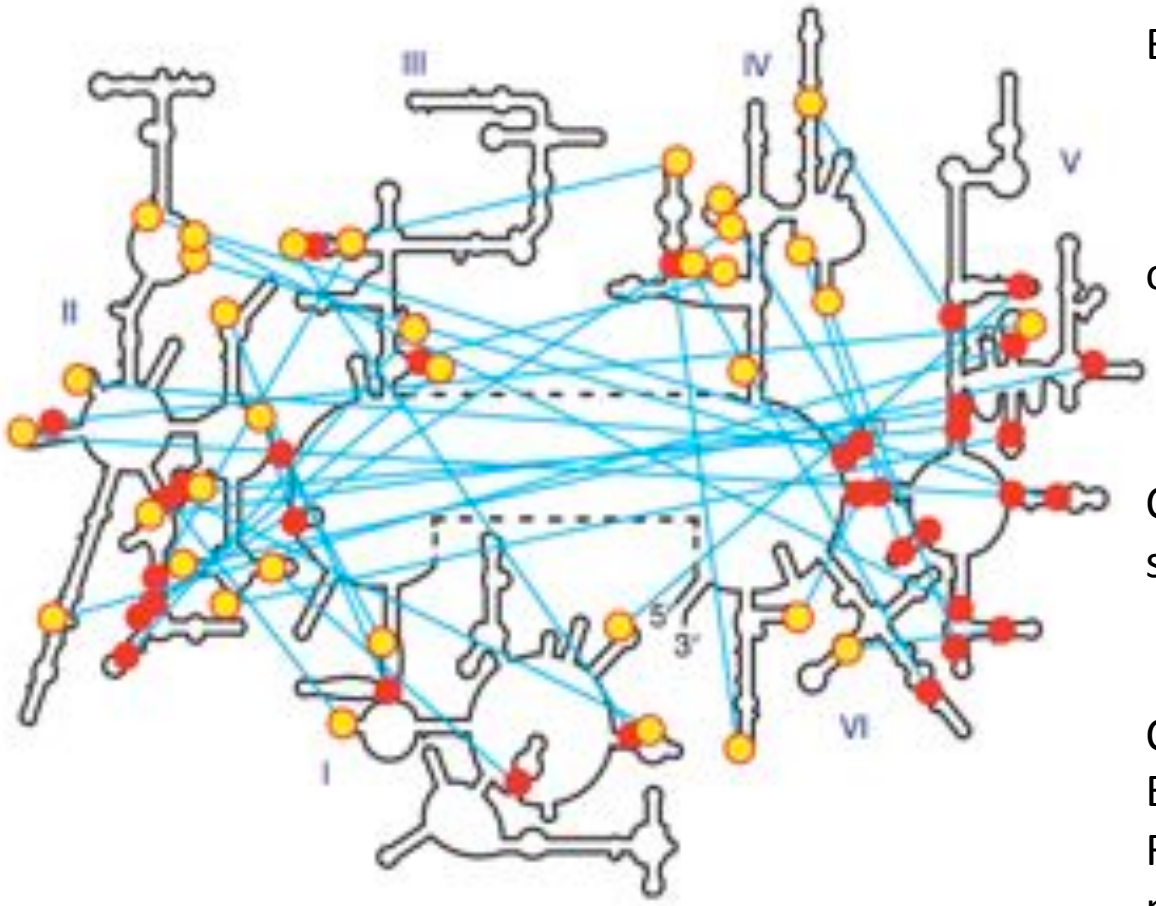


Figure 1 | Location of inter-domain A-minor interactions in the secondary structure of the *E. coli* 23S rRNA. The secondary-structure domains are marked by roman numerals. Each A-minor interaction is shown by a cyan line connecting the double helix (red circle) and the corresponding adenosine stack (yellow circle). Unlike other domains, domain V almost exclusively forms these interactions using double helices and not adenosine stacks.

Bokov, Konstantin, and Sergey V Steinberg. 2009.

A Hierarchical Model for Evolution of 23S Ribosomal RNA. *Nature* 457 (7232). 977–980.

Core interaction with ½ of tRNA stem-loops for catalytic function

Caetano-Anolles, G. “Tracing the Evolution of RNA Structure in Ribosomes.” *Nucleic Acids Res* 30, no. 11 (2002

DNA HABITATS AND ITS RNA INHABITANTS