Evolution *in vitro* and Evolutionary Biotechnology

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RNA Secondary Structures in Dijon

Dijon, 24.– 26.06.2002

	Generation time	10 000 generations	10^6 generations	10^7 generations
RNA molecules	10 sec 1 min	27.8 h = 1.16 d 6.94 d	115.7 d 1.90 a	3.17 a 19.01 a
Bacteria	20 min 10 h	138.9 d 11.40 a	38.03 a 1 140 a	380 a 11 408 a
Higher multicelluar organisms	10 d 20 a	274 a 20 000 a	27 380 a 2×10^7 a	273 800 a 2×10^8 a

Generation times and evolutionary timescales

Evolution of RNA molecules based on Qβ phage

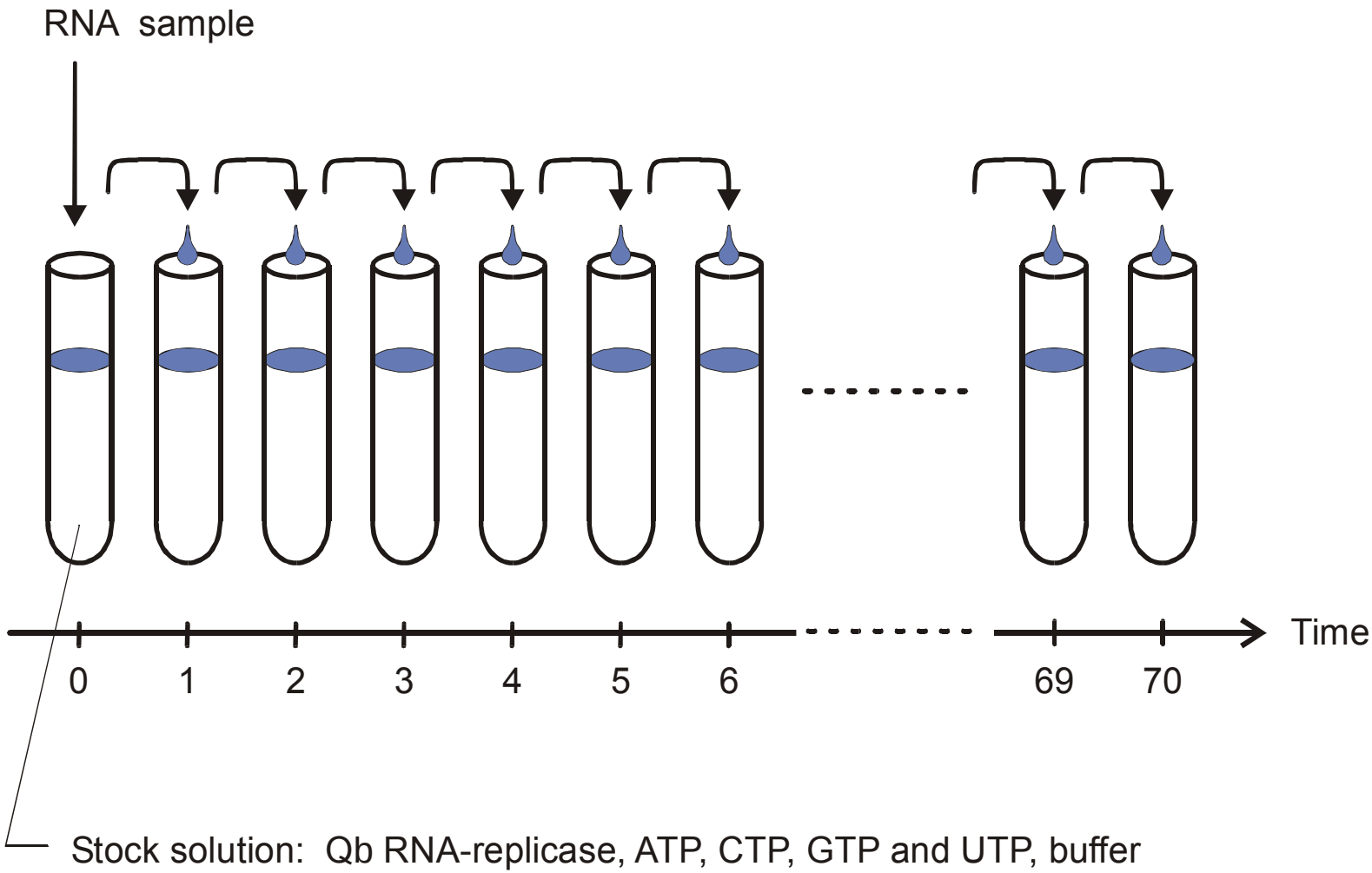
D.R.Mills, R,L,Peterson, S.Spiegelman, ***An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule***. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224

S.Spiegelman, ***An approach to the experimental analysis of precellular evolution***. Quart.Rev.Biophys. **4** (1971), 213-253

C.K.Biebricher, ***Darwinian selection of self-replicating RNA molecules***. Evolutionary Biology **16** (1983), 1-52

C.K.Biebricher, W.C. Gardiner, ***Molecular evolution of RNA*** **in vitro**. Biophysical Chemistry **66** (1997), 179-192

G.Strunk, T. Ederhof, ***Machines for automated evolution experiments*** **in vitro** ***based on the serial transfer concept***. Biophysical Chemistry **66** (1997), 193-202



The serial transfer technique applied to RNA evolution ***in vitro***

Reproduction of the original figure of the serial transfer experiment with Qβ RNA

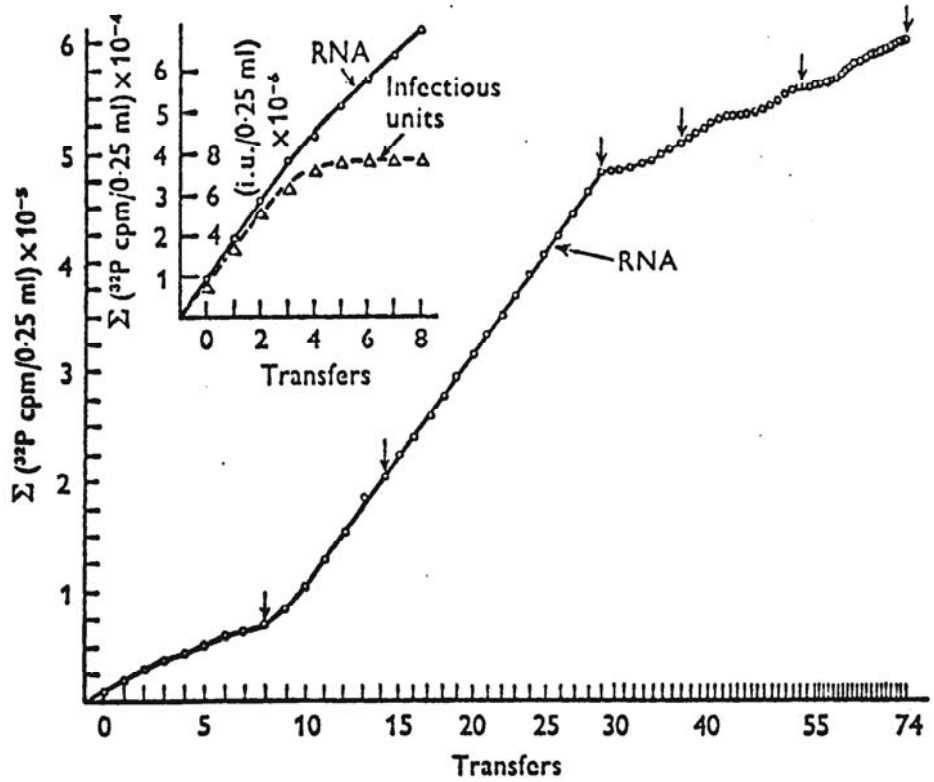
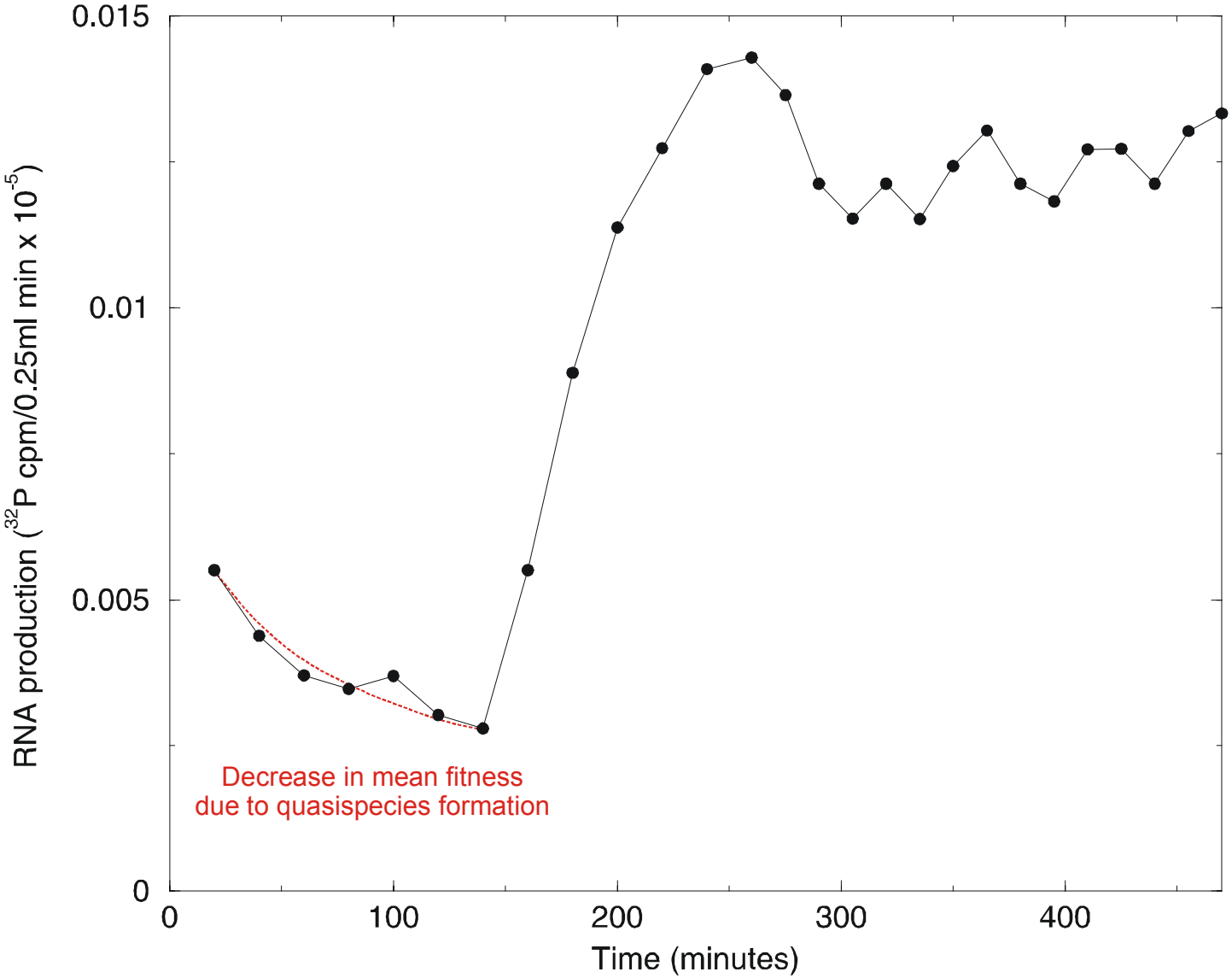
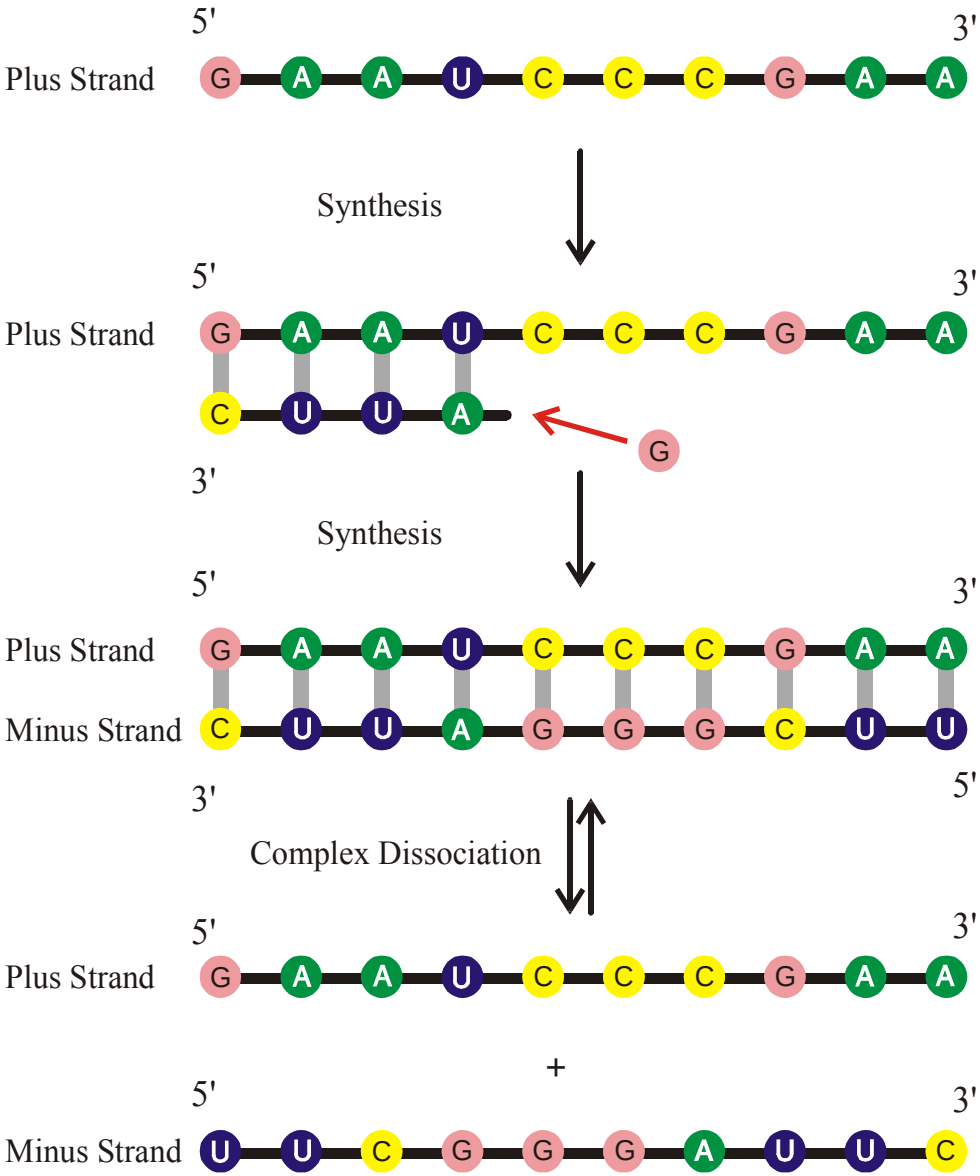


Fig. 9. Serial transfer experiment. Each 0·25 ml standard reaction mixture contained 40 μg of Qβ replicase and ^{32}P-UTP. The first reaction (0 transfer) was initiated by the addition of 0·2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 °C for 20 min, whereupon 0·02 ml was drawn for counting and 0·02 ml was used to prime the second reaction (first transfer), and so on. After the first 13 reactions, the incubation periods were reduced to 15 min (transfers 14–29). Transfers 30–38 were incubated for 10 min. Transfers 39–52 were incubated for 7 min, and transfers 53–74 were incubated for 5 min. The arrows above certain transfers (0, 8, 14, 29, 37, 53, and 73) indicate where 0·001–0·1 ml of product was removed and used to prime reactions for sedimentation analysis on sucrose. The inset examines both infectious and total RNA. The results show that biologically competent RNA ceases to appear after the 4th transfer (Mills *et al.* 1967).

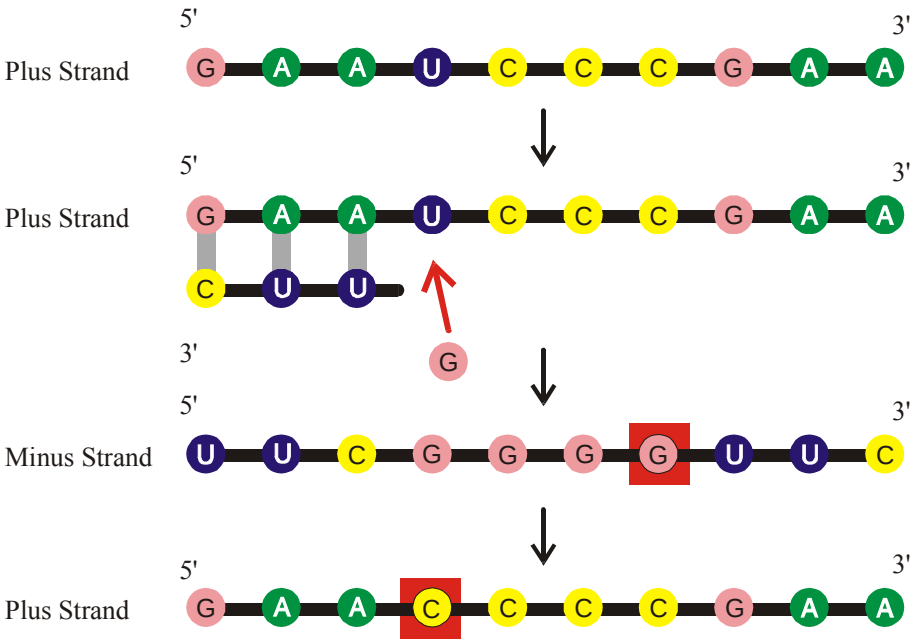
D.R.Mills, R,L,Peterson, S.Spiegelman, *An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule*. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224



The increase in RNA production rate during a serial transfer experiment



Complementary replication as the simplest copying mechanism of RNA



Point Mutation

GAAUCCCGAA → GAAUCCCGUCCCGAA

Insertion

GAAUCCCGAA → GAAUCCGA

Deletion

Mutations represent the mechanism of variation in nucleic acids

$$dx_j / dt = \Sigma_i f_i Q_{ji} x_i - x_j \Phi$$

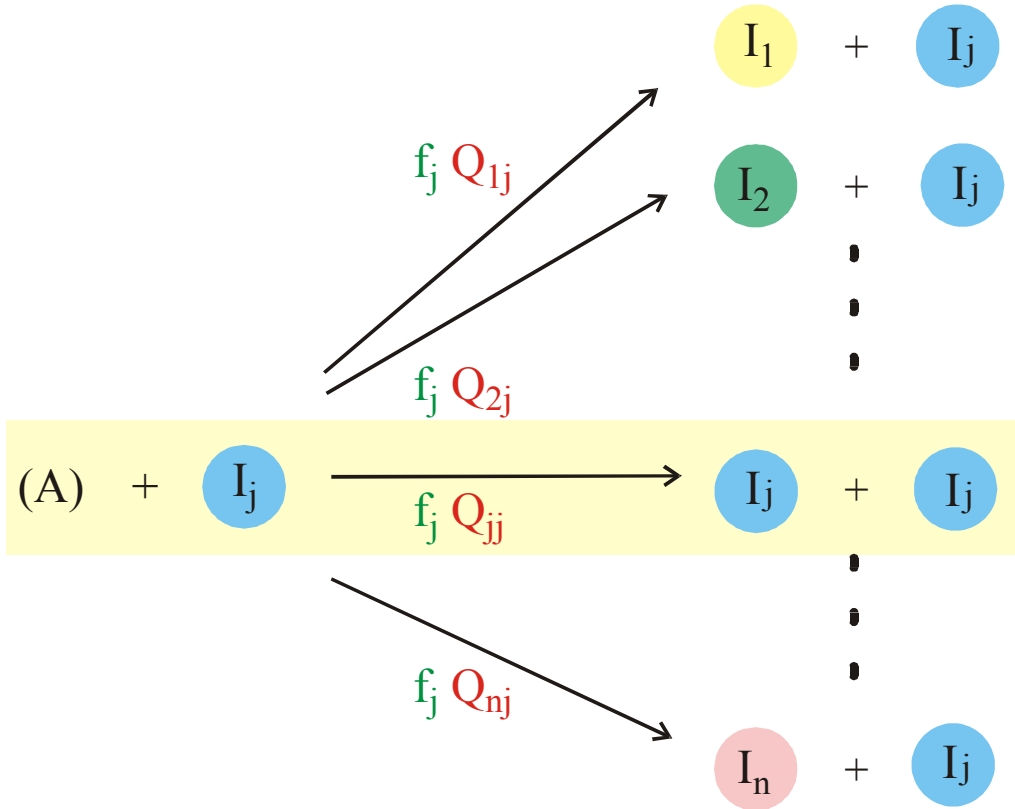
$$\Phi = \Sigma_i f_i x_i ; \quad \Sigma_i x_i = 1 ; \quad \Sigma_i Q_{ij} = 1$$

$$Q_{ij} = (1-p)^{n-d(i,j)} p^{d(i,j)}$$

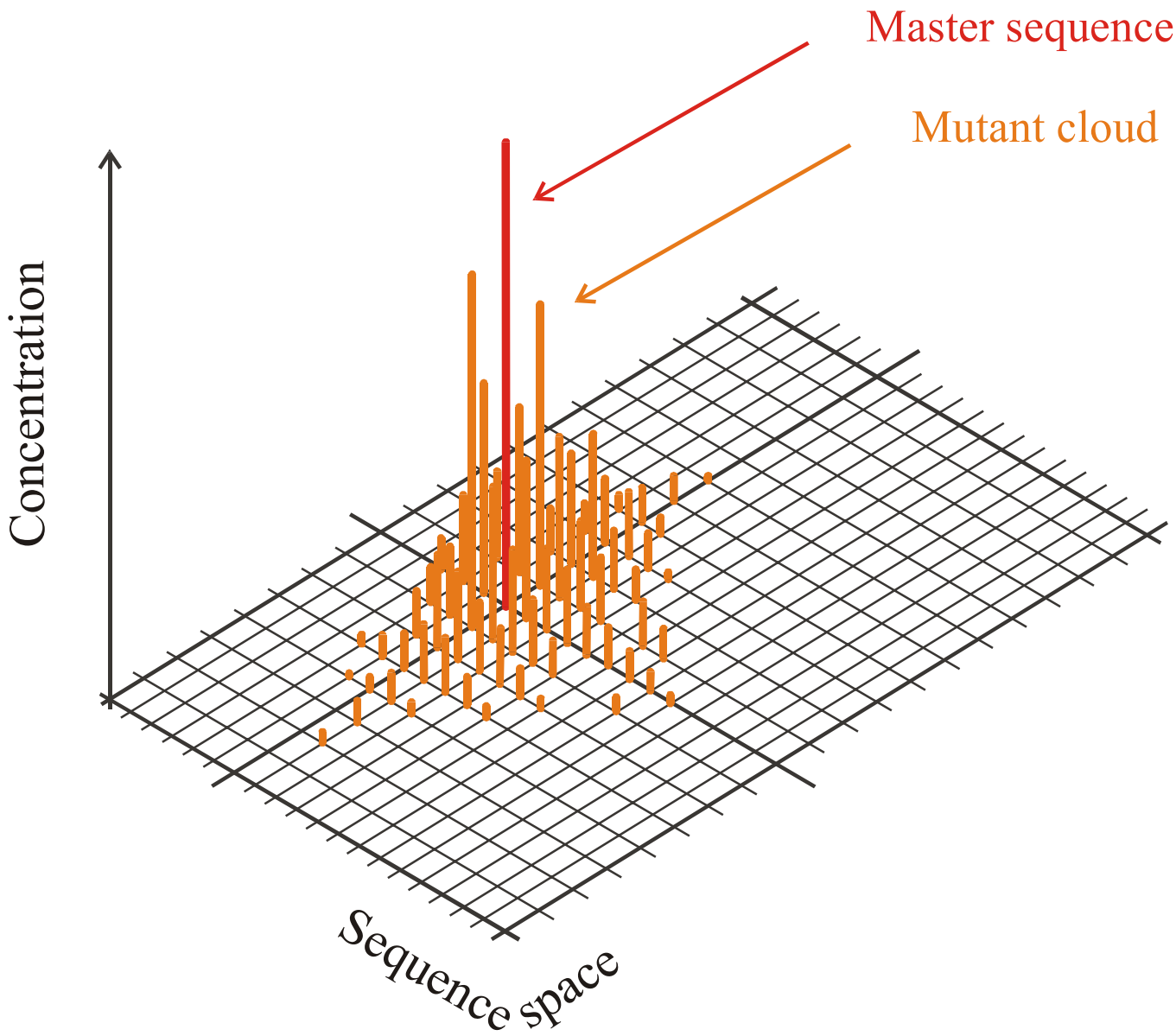
p ..... Error rate per digit

d(i,j) .... Hamming distance between I_i and I_j

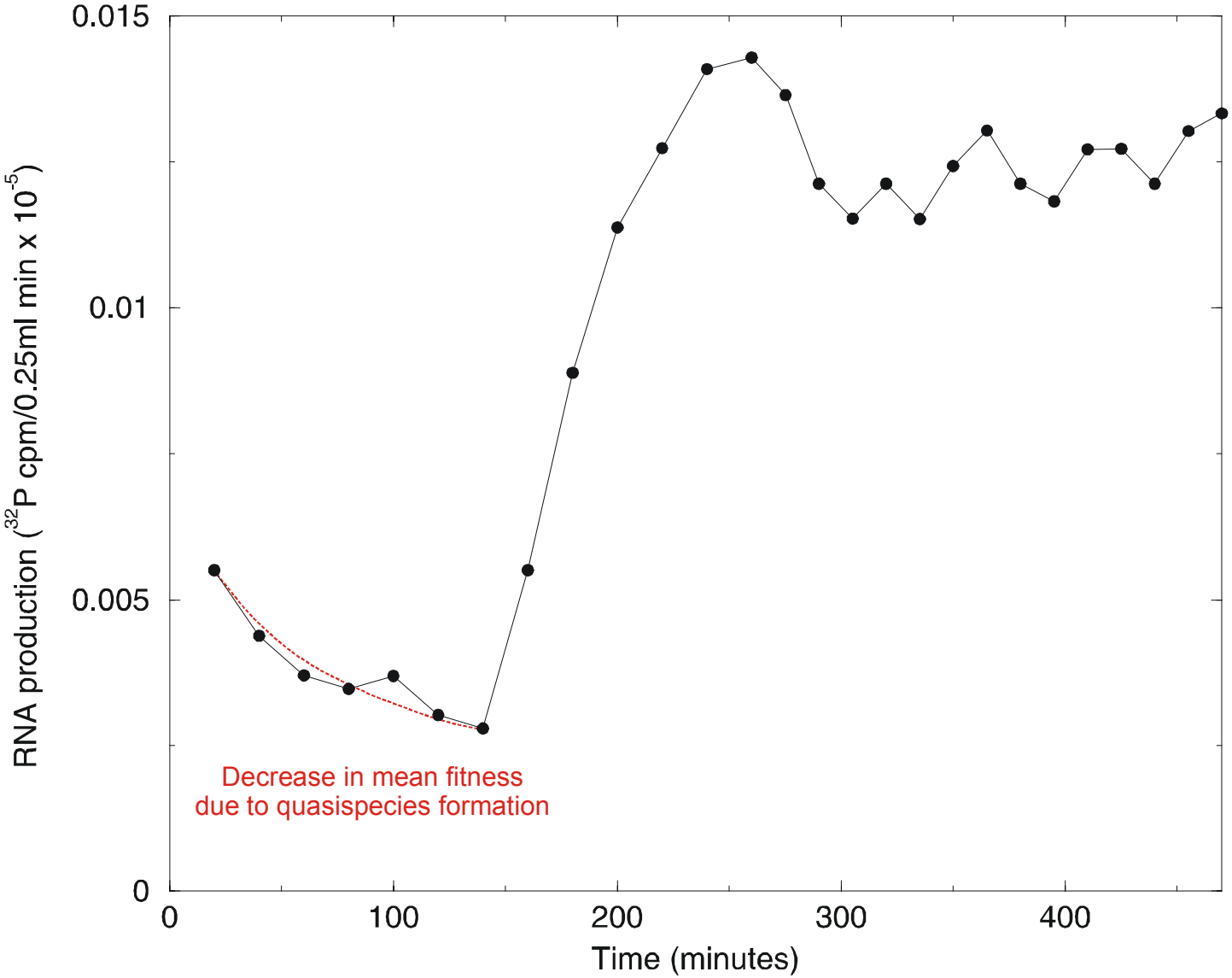
[A] = a = constant



Chemical kinetics of replication and mutation



The molecular quasispecies in sequence space



The increase in RNA production rate during a serial transfer experiment

Ronald Fisher‘s conjecture of optimization of mean fitness in populations does not hold in general for replication-mutation systems: In general evolutionary dynamics the mean fitness of populations may also decrease monotonously or even go through a maximum or minimum. It does also not hold in general for recombination of many alleles and general multi-locus systems in population genetics.

Optimization of fitness is, nevertheless, fulfilled in most cases, and can be understood as a useful heuristic.

Selection of **Q**b-RNA through replication in a capillary

G.Bauer, H.Otten, J.S. McCaskill,
Proc.Natl.Acad.Sci.USA **90**:4191, 1989

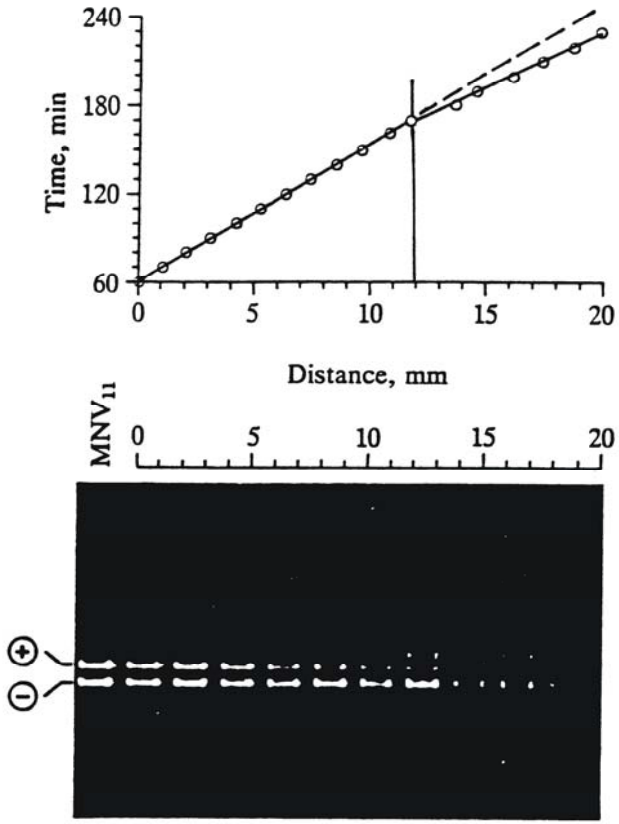


FIG. 3. Evolution of a new quasi-species along the capillary. (*Upper*) Front position measured using setup B. (*Lower*) Gel containing the fractions at 2.5-mm intervals. Regression lines are shown for the periods before and after 170 min. Aliquots (2 μl) of the fractions were withdrawn after 240 min, mixed with 2 μl of loading buffer, boiled for 3 min to melt the double strands, immediately chilled on dry ice, and loaded into the gel slots. The polyacrylamide gel contained 13% (wt/vol) acrylamide and 0.26% *N,N'*-methylenebisacrylamide in running buffer (100 mM Tris borate, pH 8.3). Electrophoresis was for 6 hr at 5 V/cm at 4°C (16). Lane MNV_{11} contains MNV_{11} single strands (plus and minus strands) as reference. The concentration shift to new bands is centered at 12 mm where the velocity changes.

Bacterial Evolution

S. F. Elena, V. S. Cooper, R. E. Lenski. ***Punctuated evolution caused by selection of rare beneficial mutants***. Science **272** (1996), 1802-1804

D. Papadopoulos, D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, M. Blot. ***Genomic evolution during a 10,000-generation experiment with bacteria***. Proc.Natl.Acad.Sci.USA **96** (1999), 3807-3812

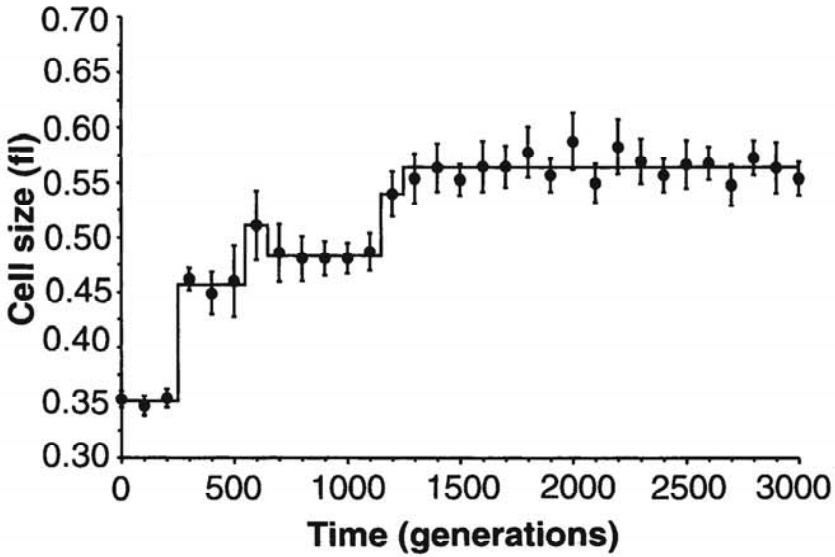


Fig. 1. Change in average cell size (1 fl = 10^{-15} L) in a population of *E. coli* during 3000 generations of experimental evolution. Each point is the mean of 10 replicate assays (*22*). Error bars indicate 95% confidence intervals. The solid line shows the best fit of a step-function model to these data (Table 1).

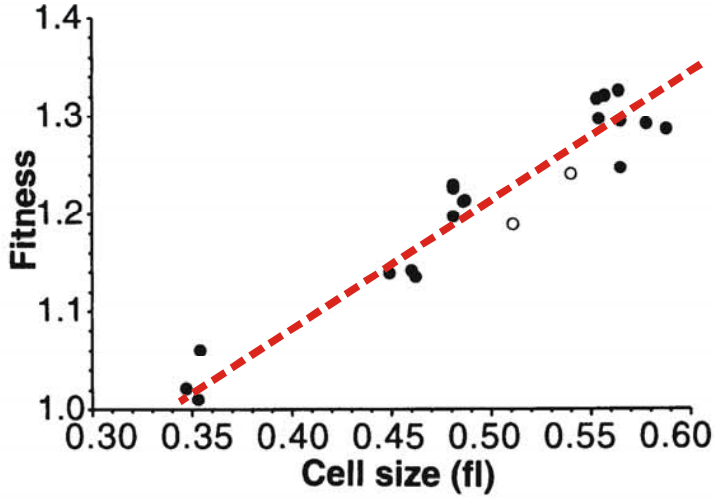
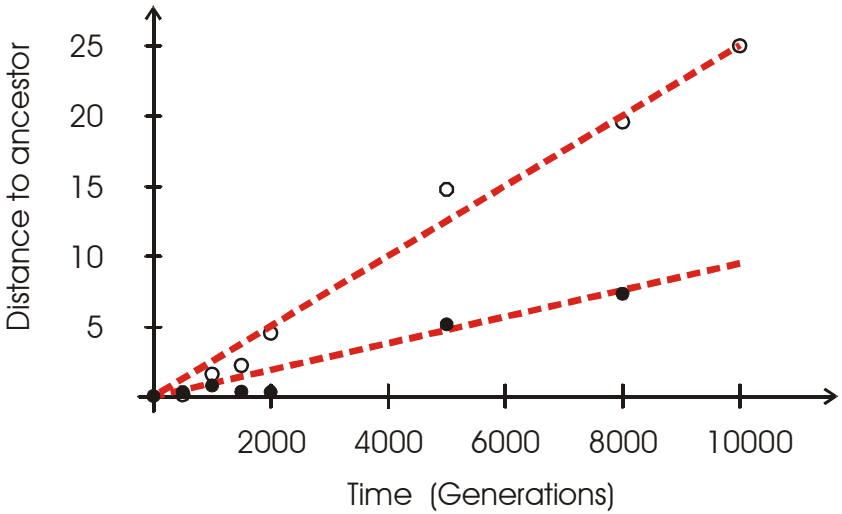
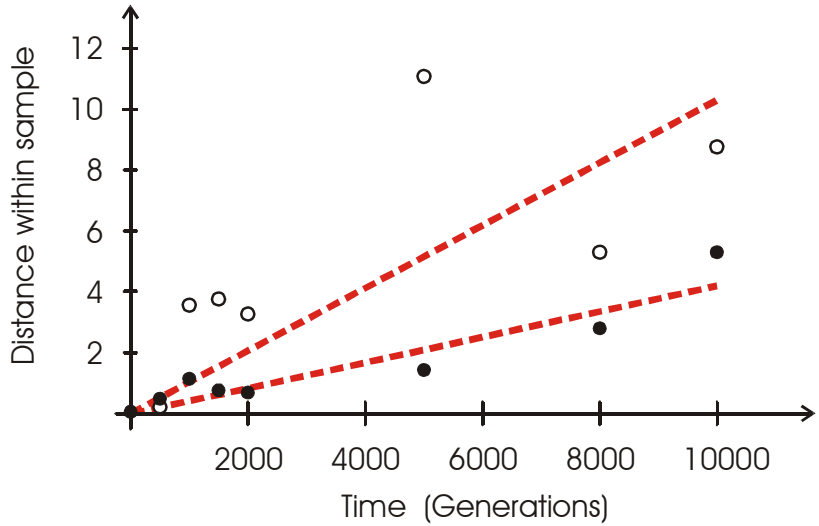


Fig. 2. Correlation between average cell size and mean fitness, each measured at 100-generation intervals for 2000 generations. Fitness is expressed relative to the ancestral genotype and was obtained from competition experiments between derived and ancestral cells (*6*, *7*). The open symbols indicate the only two samples assigned to different steps by the cell size and fitness data.

Epochal evolution of bacteria in serial transfer experiments under constant conditions

S. F. Elena, V. S. Cooper, R. E. Lenski. *Punctuated evolution caused by selection of rare beneficial mutants*. Science **272** (1996), 1802-1804





Variation of genotypes in a bacterial serial transfer experiment

D. Papadopoulos, D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, M. Blot. *Genomic evolution during a 10,000-generation experiment with bacteria*. Proc.Natl.Acad.Sci.USA **96** (1999), 3807-3812

Evolutionary design of RNA molecules

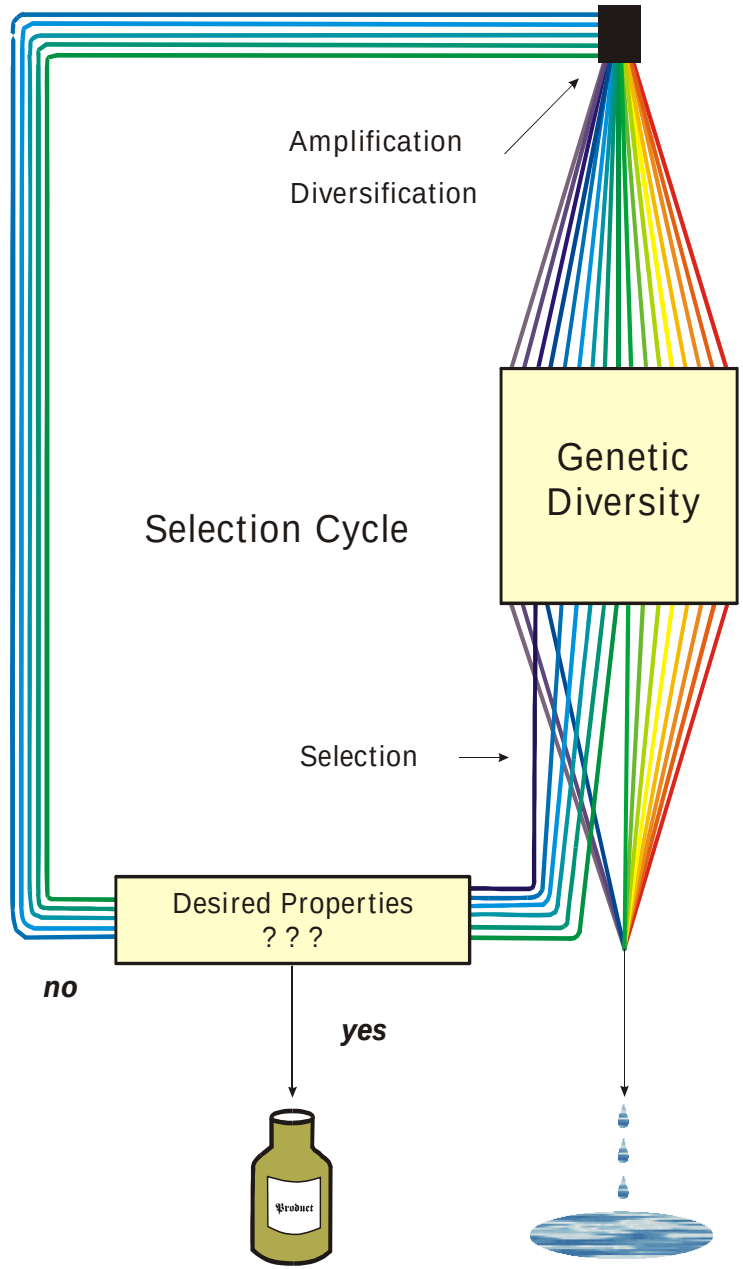
D.B.Bartel, J.W.Szostak, **In vitro** ***selection of RNA molecules that bind specific ligands***. Nature **346** (1990), 818-822

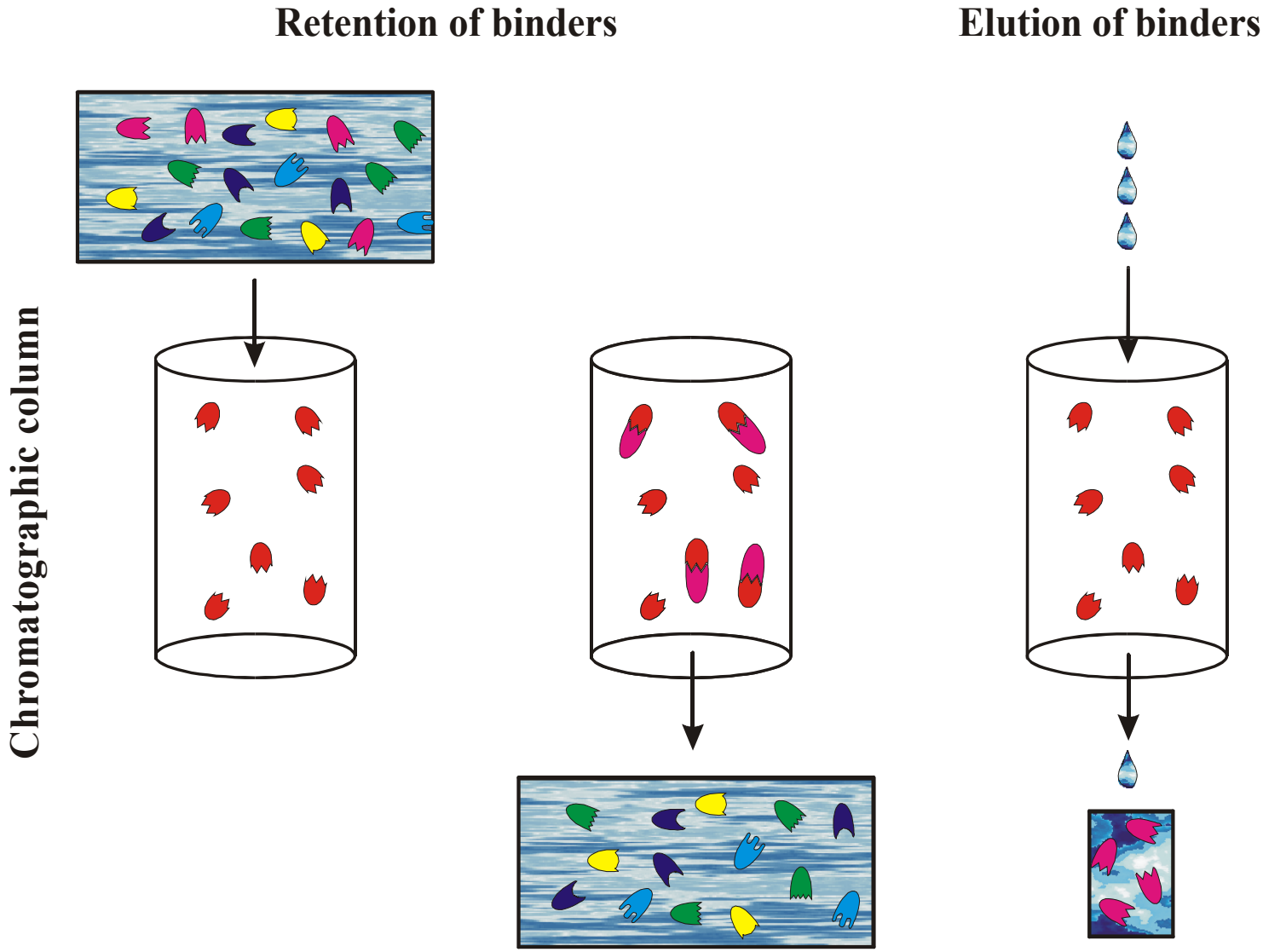
C.Tuerk, L.Gold, **SELEX** - ***Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage*** **T4** ***DNA polymerase***. Science **249** (1990), 505-510

D.P.Bartel, J.W.Szostak, ***Isolation of new ribozymes from a large pool of random sequences***. Science **261** (1993), 1411-1418

R.D.Jenison, S.C.Gill, A.Pardi, B.Poliski, ***High-resolution molecular discrimination by RNA***. Science **263** (1994), 1425-1429

Selection cycle used in applied molecular evolution to design molecules with predefined properties





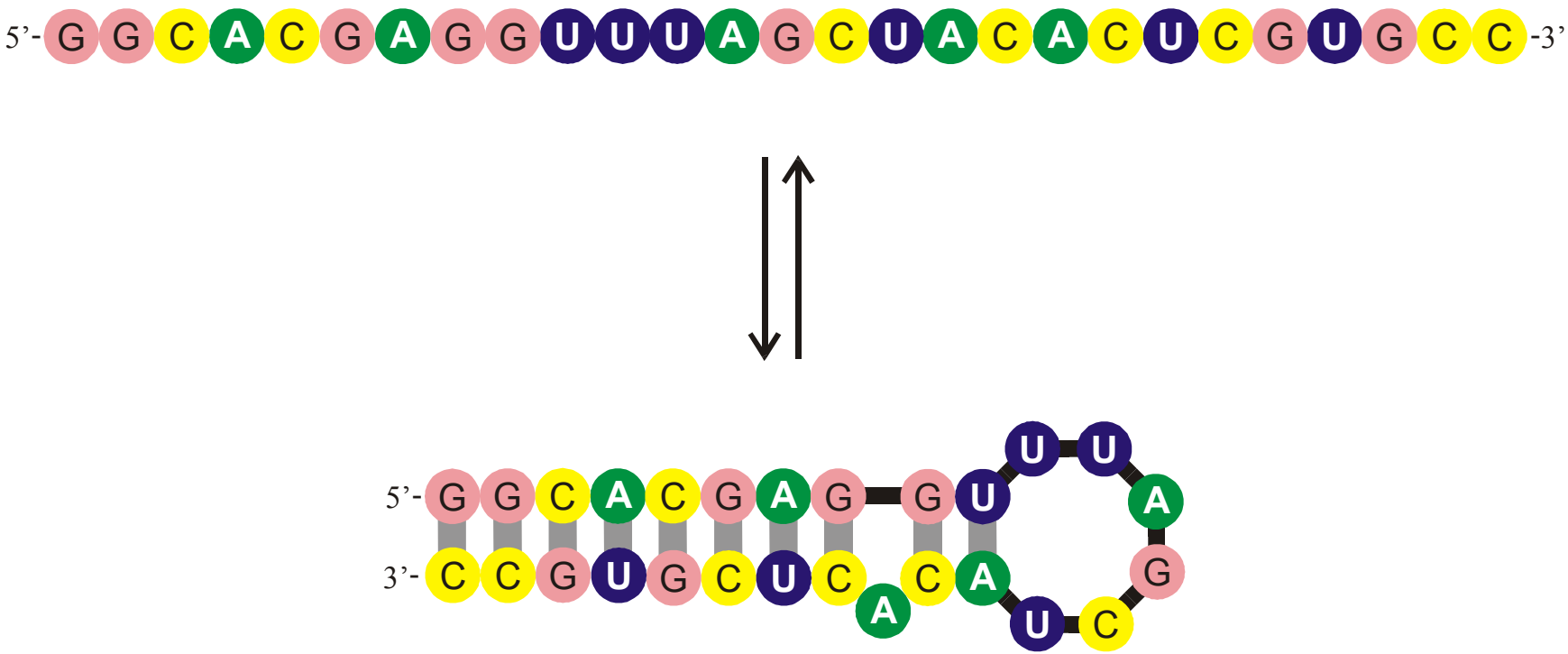
The SELEX technique for the evolutionary design of ***aptamers***

$4^{27} = 1.801 \text{ Ç } 10^{16}$ possible different sequences

Combinatorial diversity of sequences: $N = 4^{\{}$

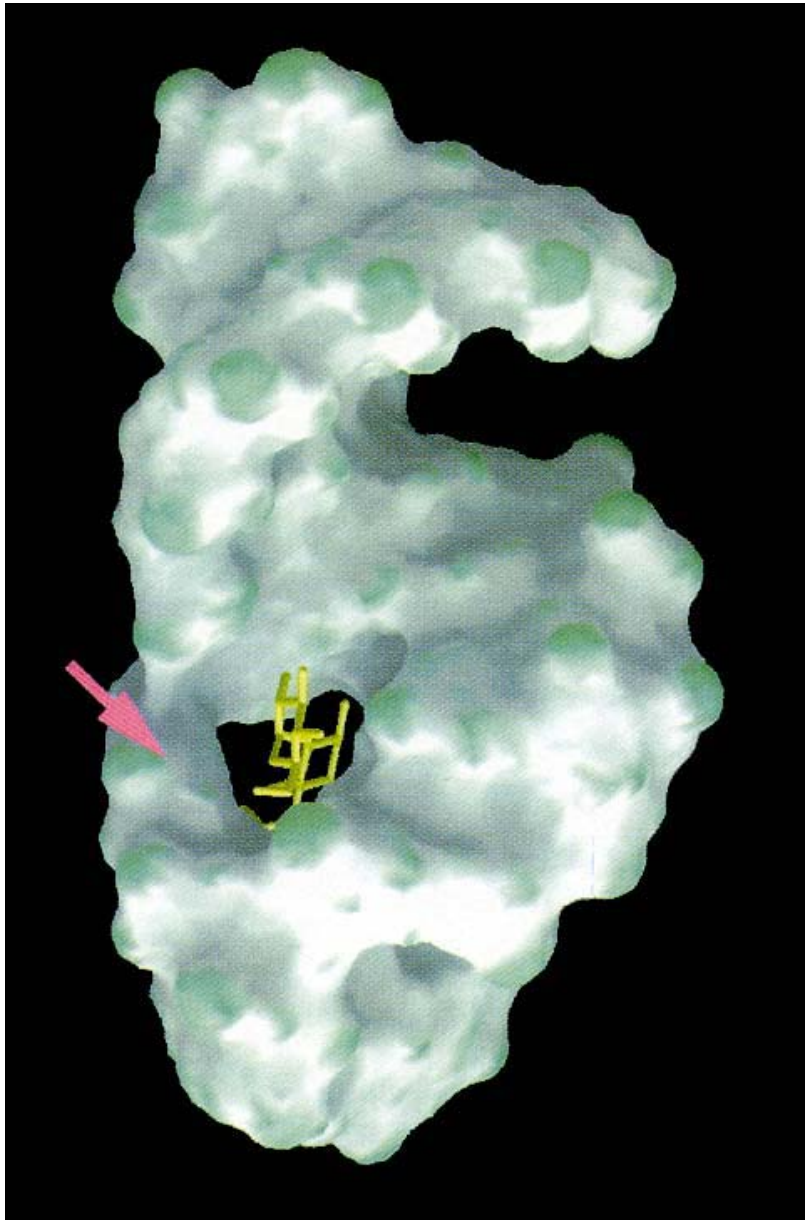
A = adenylate
U = uridylate
C = cytidylate
G = guanylate

Combinatorial diversity of heteropolymers illustrated by means of an RNA aptamer that binds to the antibiotic tobramycin



Formation of secondary structure of the tobramycin binding RNA aptamer

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)

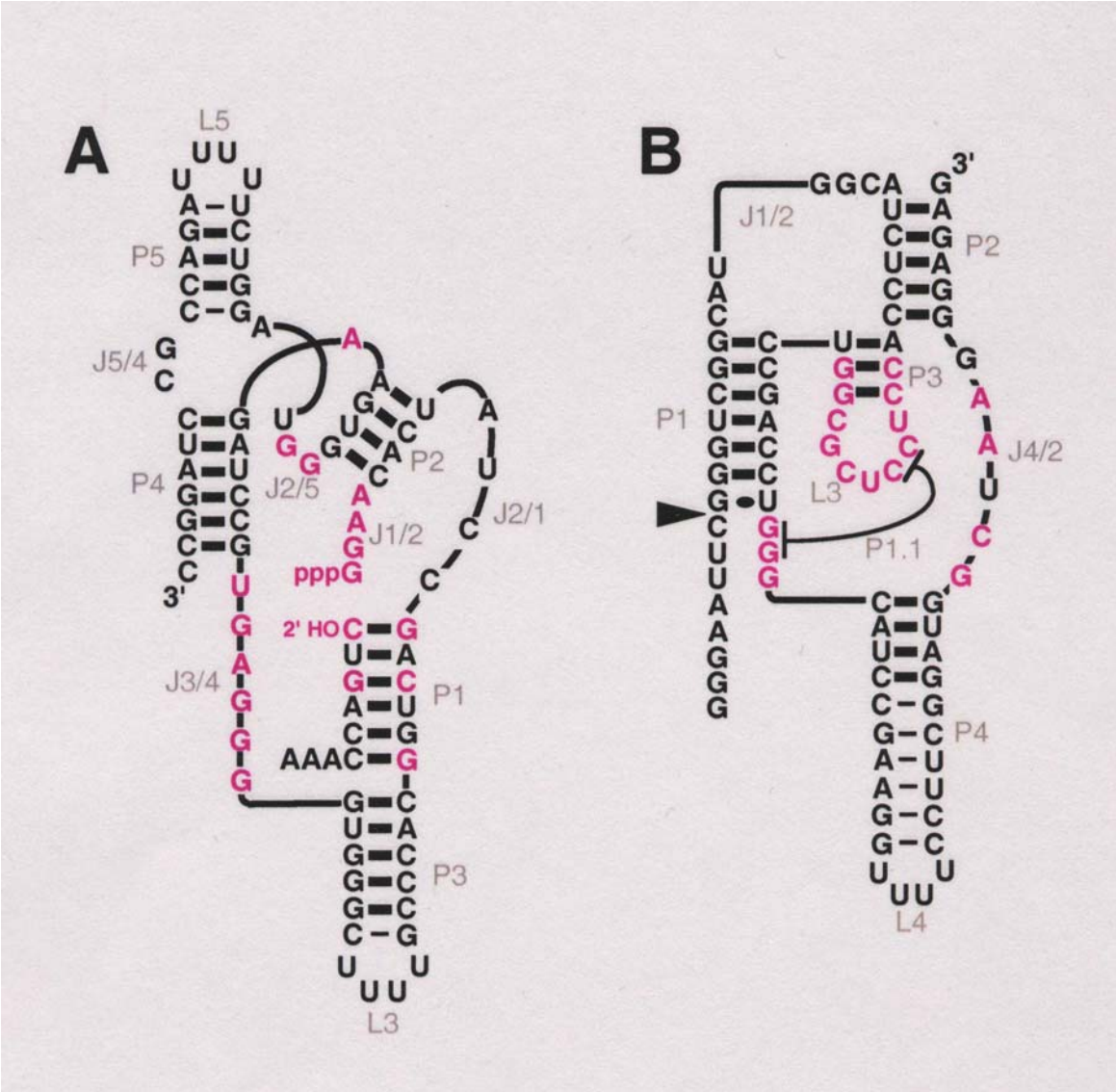


The three-dimensional structure of the tobramycin aptamer complex

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)

A ribozyme switch

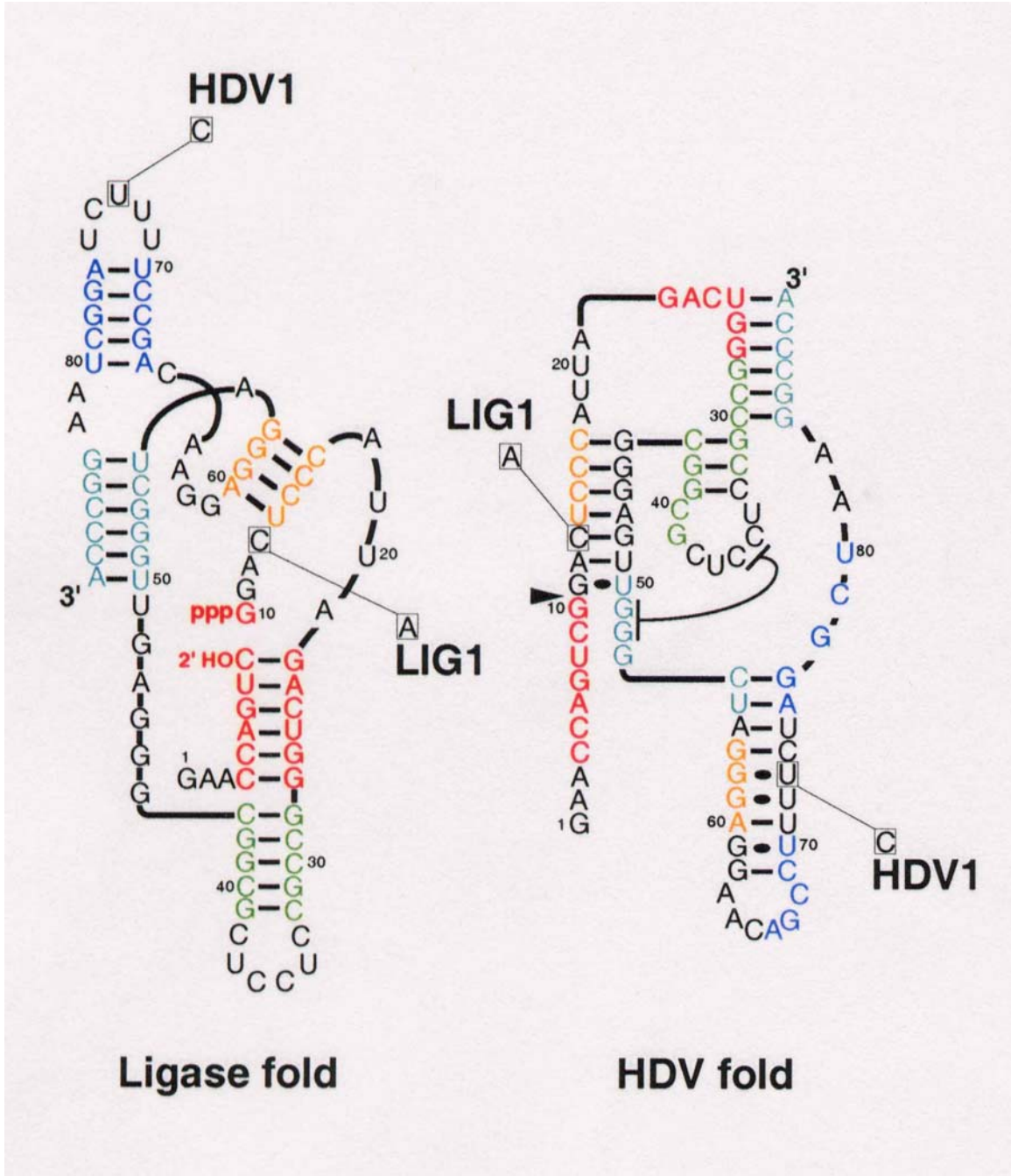
E.A.Schultes, D.B.Bartel, ***One sequence, two ribozymes: Implication for the emergence of new ribozyme folds***. Science **289** (2000), 448-452



Two ribozymes of chain lengths n = 88 nucleotides: An artificial ligase (**A**) and a natural cleavage ribozyme of hepatitis-d-virus (**B**)

The sequence at the ***intersection***:

An RNA molecules which is 88 nucleotides long and can form both structures



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GENERIC PROPERTIES OF COMBINATORY MAPS: NEUTRAL NETWORKS OF RNA SECONDARY STRUCTURES[1]

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Random graph theory is used to model and analyse the relationships between sequences and secondary structures of RNA molecules, which are understood as mappings from sequence space into shape space. These maps are non-invertible since there are always many orders of magnitude more sequences than structures. Sequences folding into identical structures form *neutral networks*. A neutral network is embedded in the set of sequences that are *compatible* with the given structure. Networks are modeled as graphs and constructed by random choice of vertices from the space of compatible sequences. The theory characterizes neutral networks by the mean fraction of neutral neighbors (λ). The networks are connected and percolate sequence space if the fraction of neutral nearest neighbors exceeds a threshold value ($\lambda > \lambda^*$). Below threshold ($\lambda < \lambda^*$), the networks are partitioned into a largest "giant" component and several smaller components. Structures are classified as "common" or "rare" according to the sizes of their pre-images, i.e. according to the fractions of sequences folding into them. The neutral networks of any pair of two different common structures almost touch each other, and, as expressed by the conjecture of *shape space covering* sequences folding into almost all common structures, can be found in a small ball of an arbitrary location in sequence space. The results from random graph theory are compared to data obtained by folding large samples of RNA sequences. Differences are explained in terms of specific features of RNA molecular structures. © *1997 Society for Mathematical Biology*

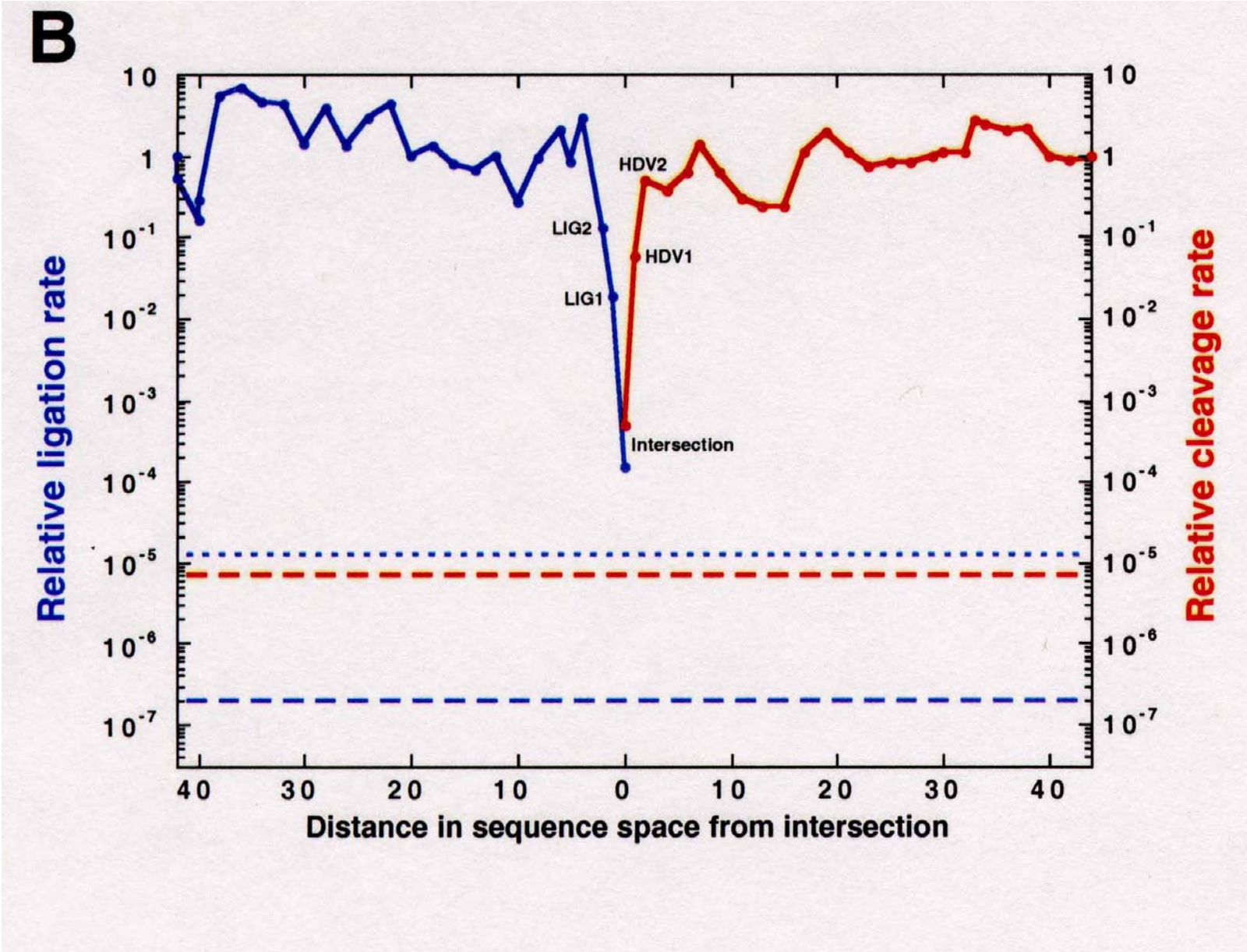
THEOREM 5. INTERSECTION-THEOREM. *Let* s *and* s′ *be arbitrary secondary structures and* **C**[s], **C**[s′] *their corresponding compatible sequences. Then,*

$$\mathbf{C}[s] \cap \mathbf{C}[s'] \neq \varnothing.$$

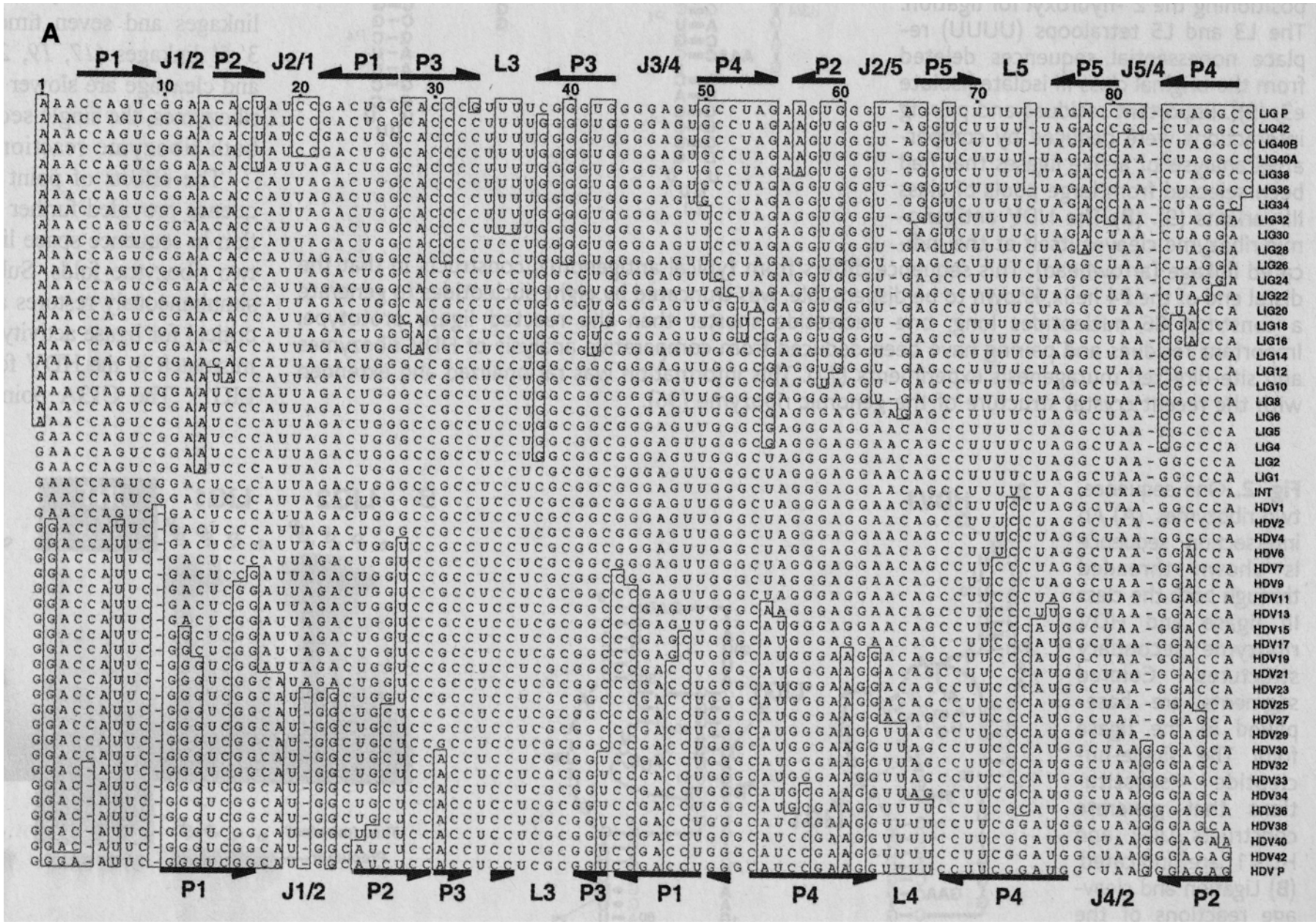
Proof. Suppose that the alphabet admits only the complementary base pair $[XY]$ and we ask for a sequence x compatible to both s and s'. Then $\gamma(s, s') \cong D_m$ operates on the set of all positions $\{x_1, \ldots, x_n\}$. Since we have the operation of a dihedral group, the orbits are either cycles or chains and the cycles have even order. A constraint for the sequence compatible to both structures appears only in the cycles where the choice of bases is not independent. It remains to be shown that there is a valid choice of bases for each cycle, which is obvious since these have even order. Therefore, it suffices to choose an alternating sequence of the pairing partners X and Y. Thus, there are at least two different choices for the first base in the orbit. ■

Remark. A generalization of the statement of theorem 5 to three different structures is false.

Reference for the definition of the intersection and the proof of the ***intersection theorem***



Two neutral walks through sequence space with conservation of structure and catalytic activity



Sequence of mutants from the intersection to both reference ribozymes

From sequences to shapes and back: a case study in RNA secondary structures

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[2] *Institut für Theoretische Chemie, Universität Wien, Austria*
[3] *Santa Fe Institute, Santa Fe, U.S.A.*

SUMMARY

RNA folding is viewed here as a map assigning secondary structures to sequences. At fixed chain length the number of sequences far exceeds the number of structures. Frequencies of structures are highly non-uniform and follow a generalized form of Zipf's law: we find relatively few common and many rare ones. By using an algorithm for inverse folding, we show that sequences sharing the same structure are distributed randomly over sequence space. All common structures can be accessed from an arbitrary sequence by a number of mutations much smaller than the chain length. The sequence space is percolated by extensive neutral networks connecting nearest neighbours folding into identical structures. Implications for evolutionary adaptation and for applied molecular evolution are evident: finding a particular structure by mutation and selection is much simpler than expected and, even if catalytic activity should turn out to be sparse in the space of RNA structures, it can hardly be missed by evolutionary processes.

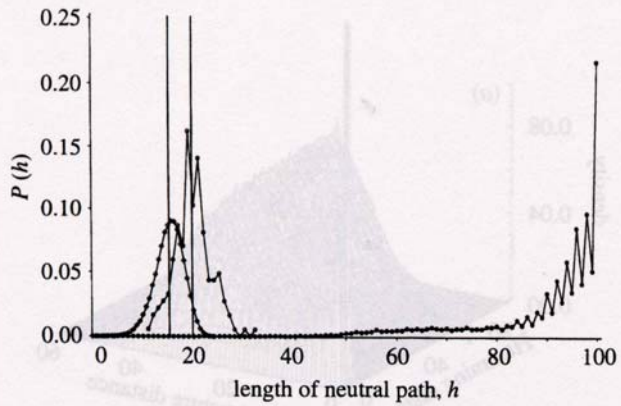

Figure 4. Neutral paths. A neutral path is defined by a series of nearest neighbour sequences that fold into identical structures. Two classes of nearest neighbours are admitted: neighbours of Hamming distance 1, which are obtained by single base exchanges in unpaired stretches of the structure, and neighbours of Hamming distance 2, resulting from base pair exchanges in stacks. Two probability densities of Hamming distances are shown that were obtained by searching for neutral paths in sequence space: (i) an upper bound for the closest approach of trial and target sequences (open circles) obtained as endpoints of neutral paths approaching the target from a random trial sequence (185 targets and 100 trials for each were used); (ii) a lower bound for the closest approach of trial and target sequences (open diamonds) derived from secondary structure statistics (Fontana *et al.* 1993*a*; see this paper, §4); and (iii) longest distances between the reference and the endpoints of monotonously diverging neutral paths (filled circles) (500 reference sequences were used).

Proc. R. Soc. Lond. B (1994) **255**, 279–284
Printed in Great Britain

Reference for postulation and ***in silico*** verification of ***neutral networks***

No new principle will declare itself from below a heap of facts.

Sir Peter Medawar, 1985

Coworkers

Walter Fontana, Santa Fe Institute, NM

Christian Reidys, Christian Forst, Los Alamos National Laboratory, NM

Peter Stadler, Universität Leipzig, GE

Ivo L.Hofacker, Christoph Flamm, Universität Wien, AT

Bärbel Stadler, Andreas Wernitznig, Universität Wien, AT
Michael Kospach, Ulrike Langhammer, Ulrike Mückstein, Stefanie Widder
Jan Cupal, Kurt Grünberger, Andreas Svrček-Seiler, Stefan Wuchty

Ulrike Göbel, Institut für Molekulare Biotechnologie, Jena, GE
Walter Grüner, Stefan Kopp, Jaqueline Weber