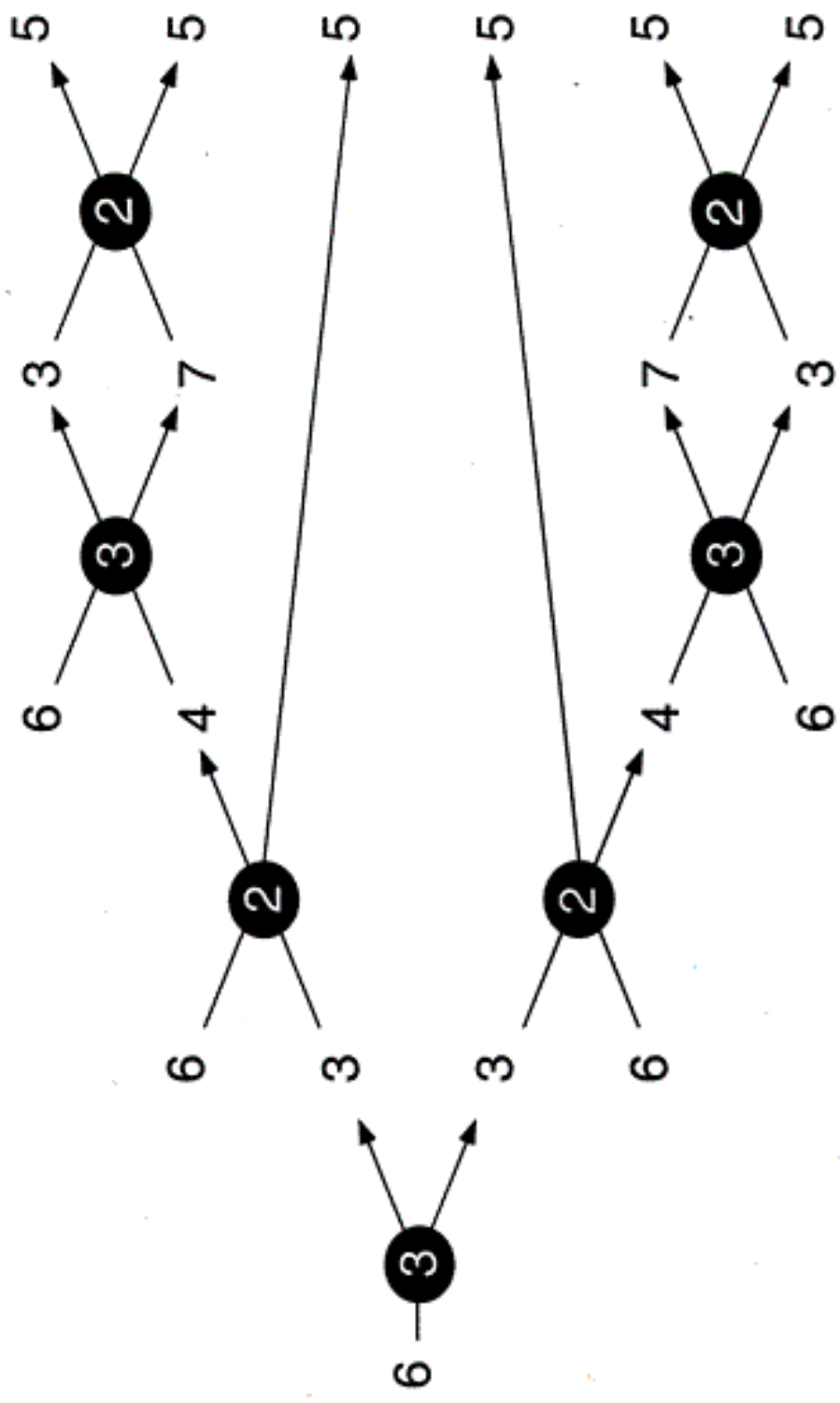


The pentose phosphate cycle



Adapted from Montero et al. J.theor. Biol. (1994)

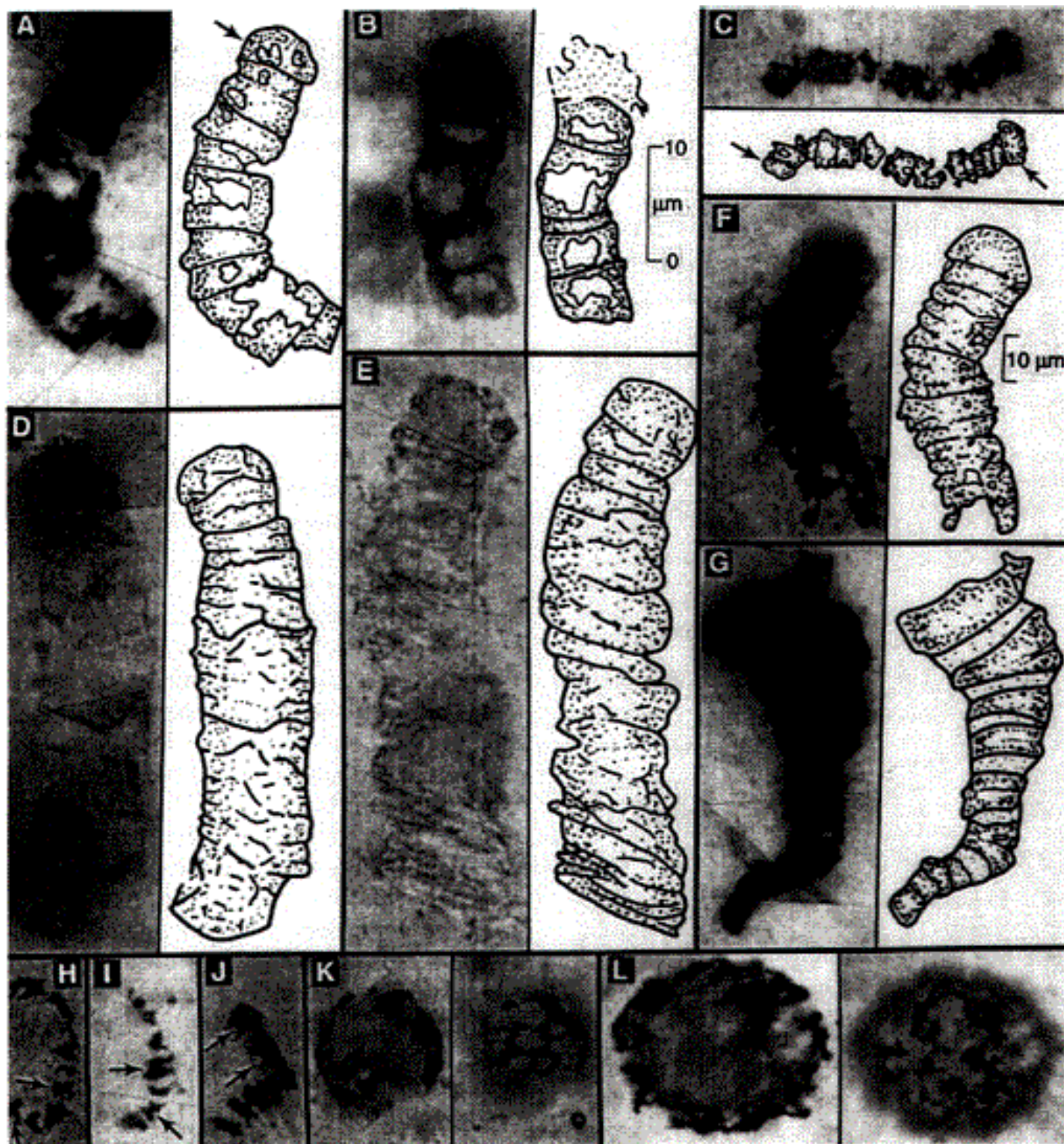
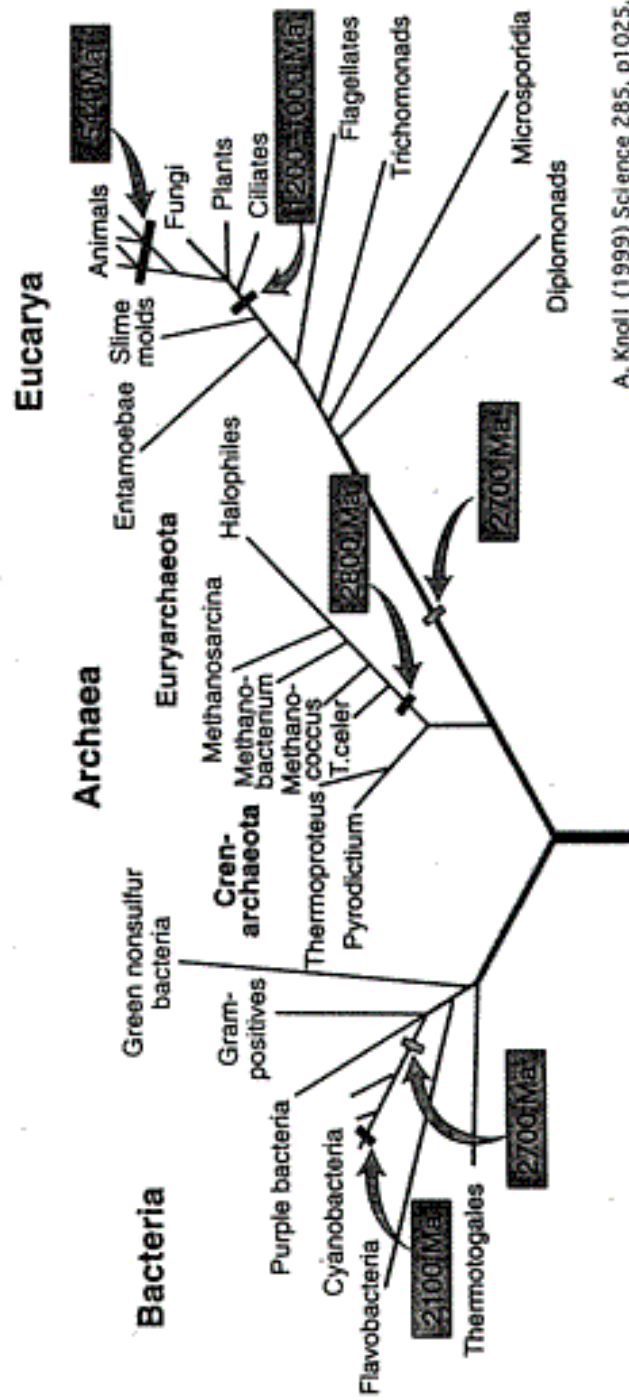
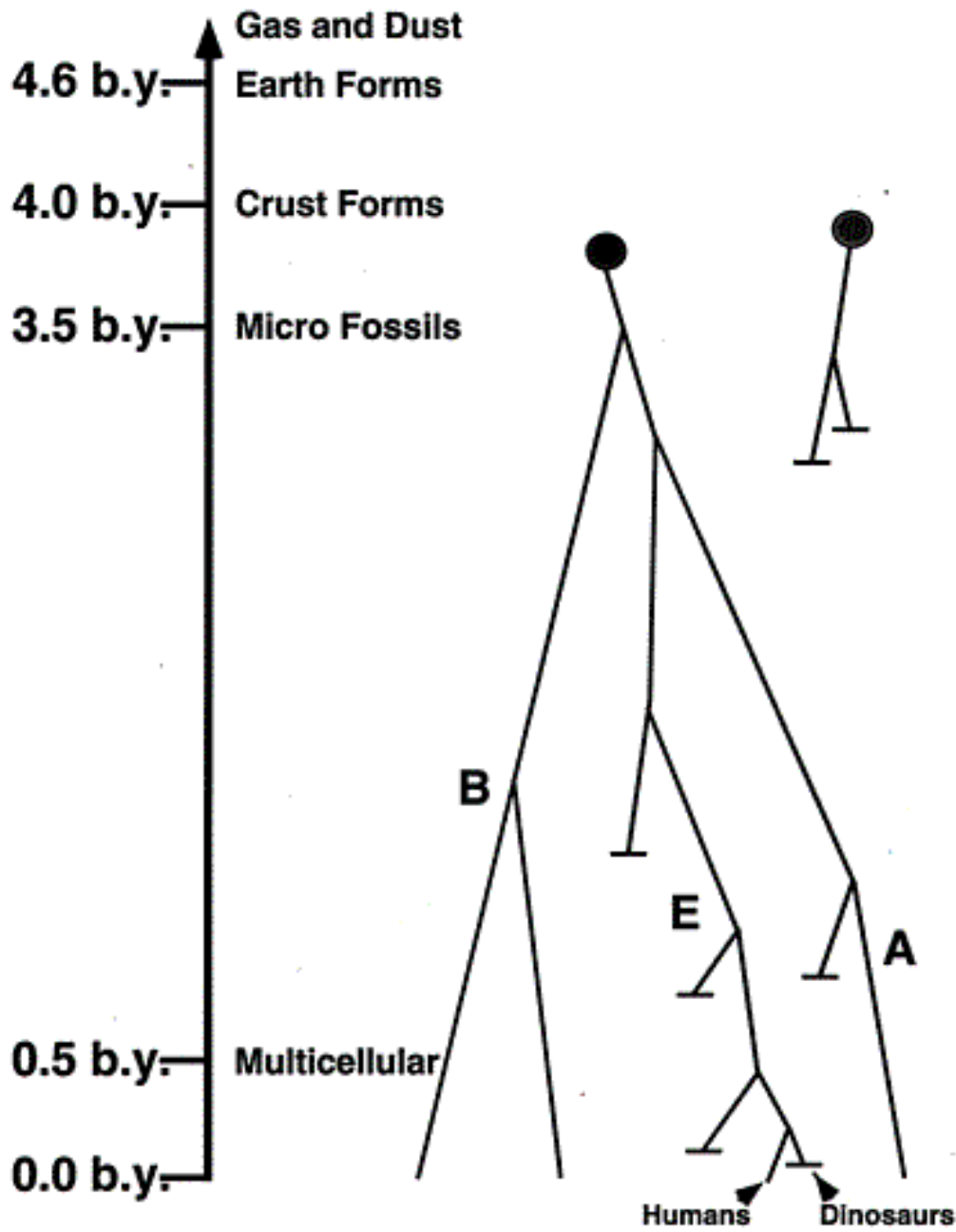


Fig. 5. Carbonaceous and iron-stained (D, E, and F) microfossils (with interpretive drawings) and possible microfossils (K and L) shown in thin sections of the Early Archean Apex chert of Western Australia. Magnification of (C, F, H, I, and J) denoted by scale in (F); magnification of all other parts shown by scale in (B). (A, B, and C) *Primaevifilum laticellulosum*, n. sp. (A, holotype); pillow-shaped terminal cells are indicated by arrows in (A) and (C). (D and E) *Archaeoscillatorioopsis grandis*, n. gen., n. sp. (D, holotype). (F) *Archaeoscillatorioopsis maxima*, n. gen., n. sp. (holotype). (G) *Primaevifilum attenuatum*, n. sp. (holotype). (H, I, and J) Poorly preserved trichomes showing bifurcated cells and cell pairs (at arrows). (K and L) Solitary unicell-like possible microfossils, in equatorial (left) and polar views (right).



A. Knoll (1999) Science 285, p1025.

Time Line for Life on Earth



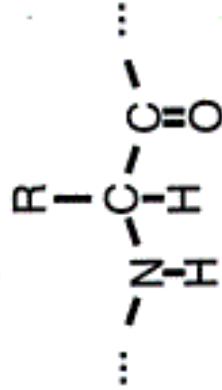
Replication

Nucleic Acid



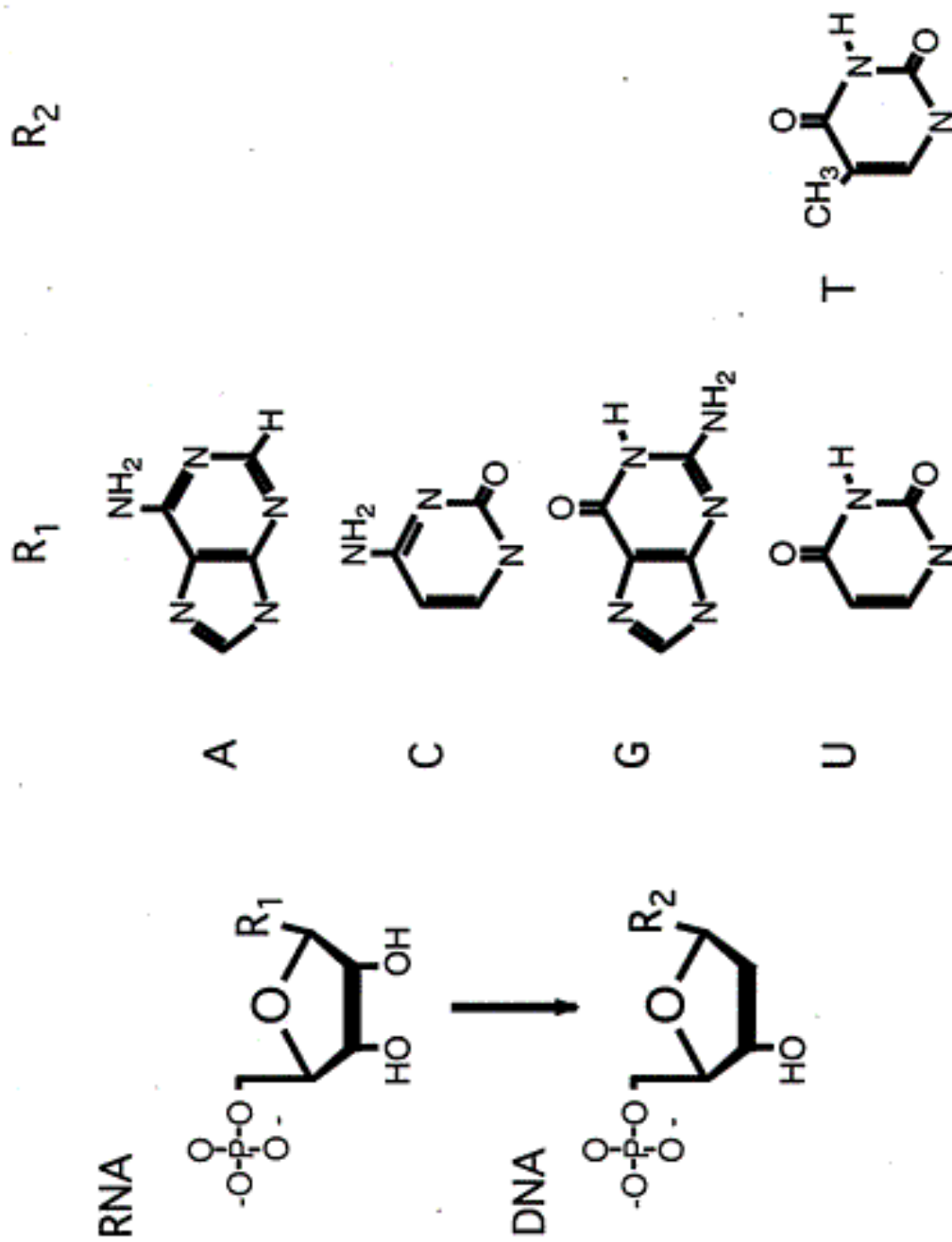
- 4 basic functional groups
- Replication by default at the unit level.

Amino Acid

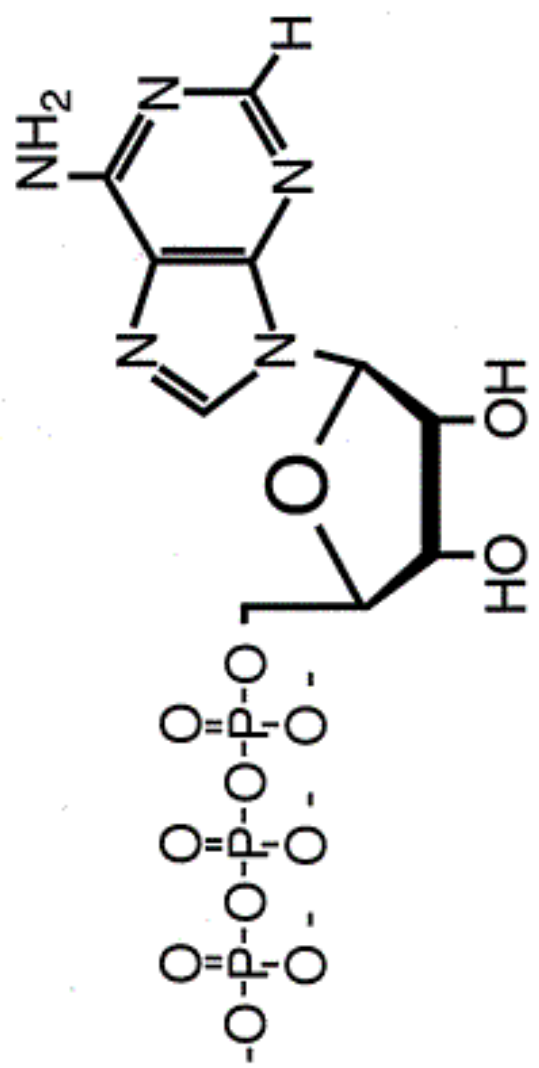


- 20 different functional groups.
- Certain structures (coiled coils etc.) can be made to pair.
- Recognition is not at the unit repeat level.

Nucleic acids

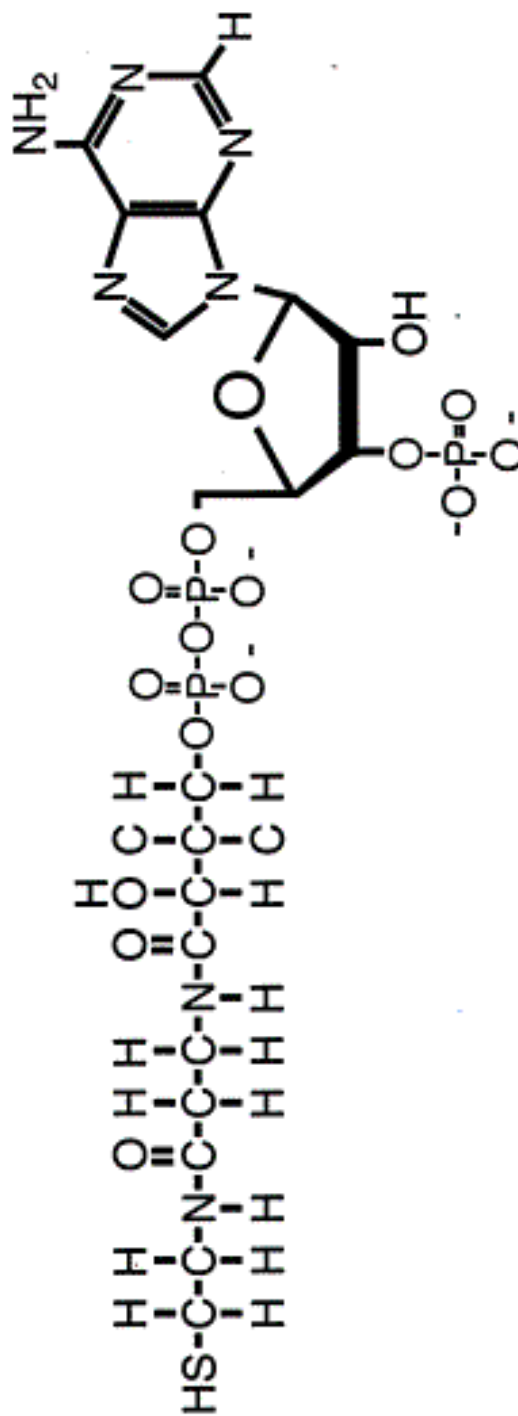


ATP



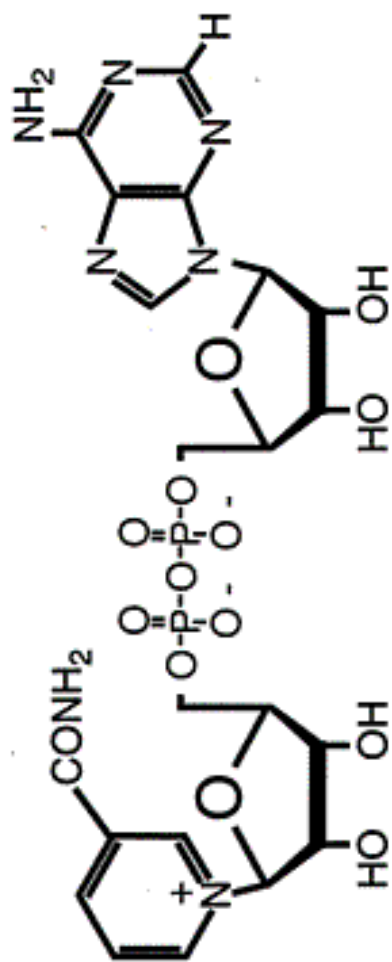
Used by at least 402 different enzymes.

Coenzyme A

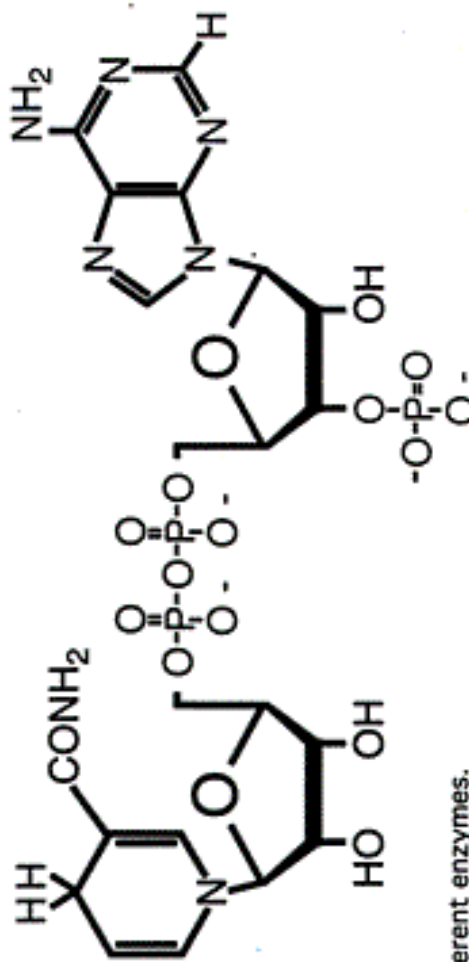


Electron carriers

Oxidizing: NAD⁺



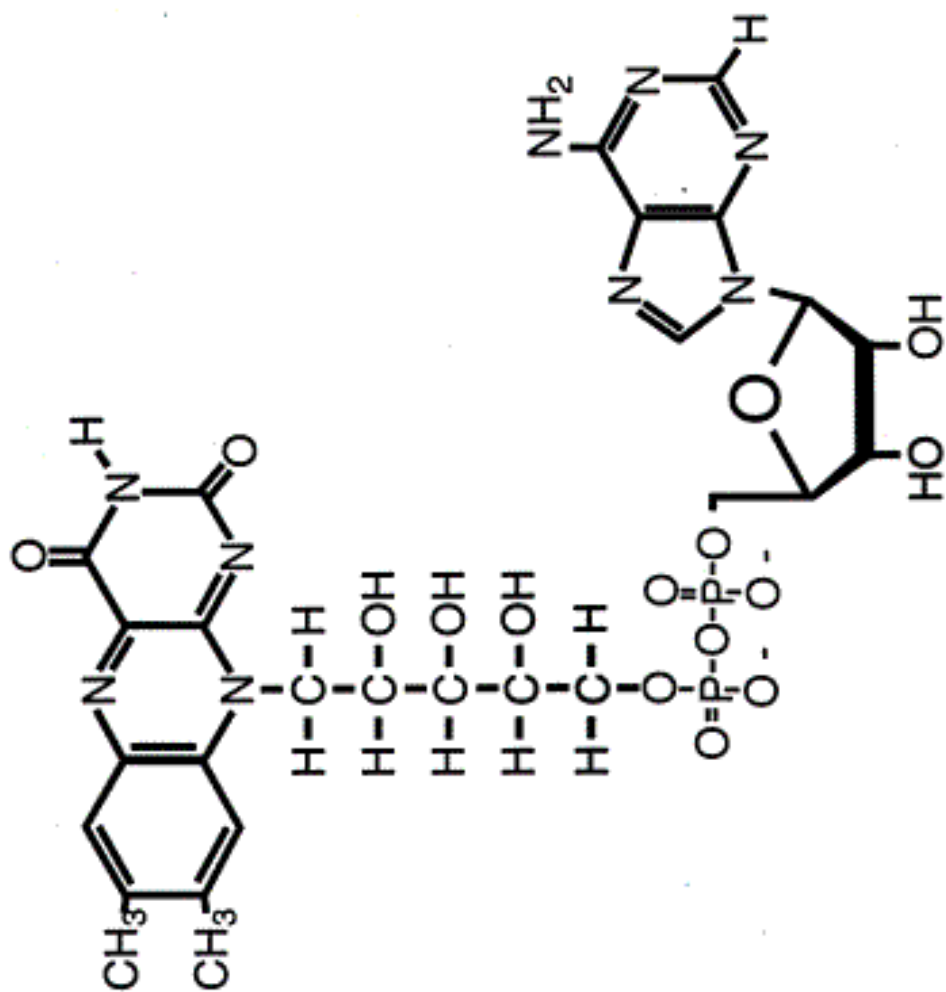
Reducing: NADPH

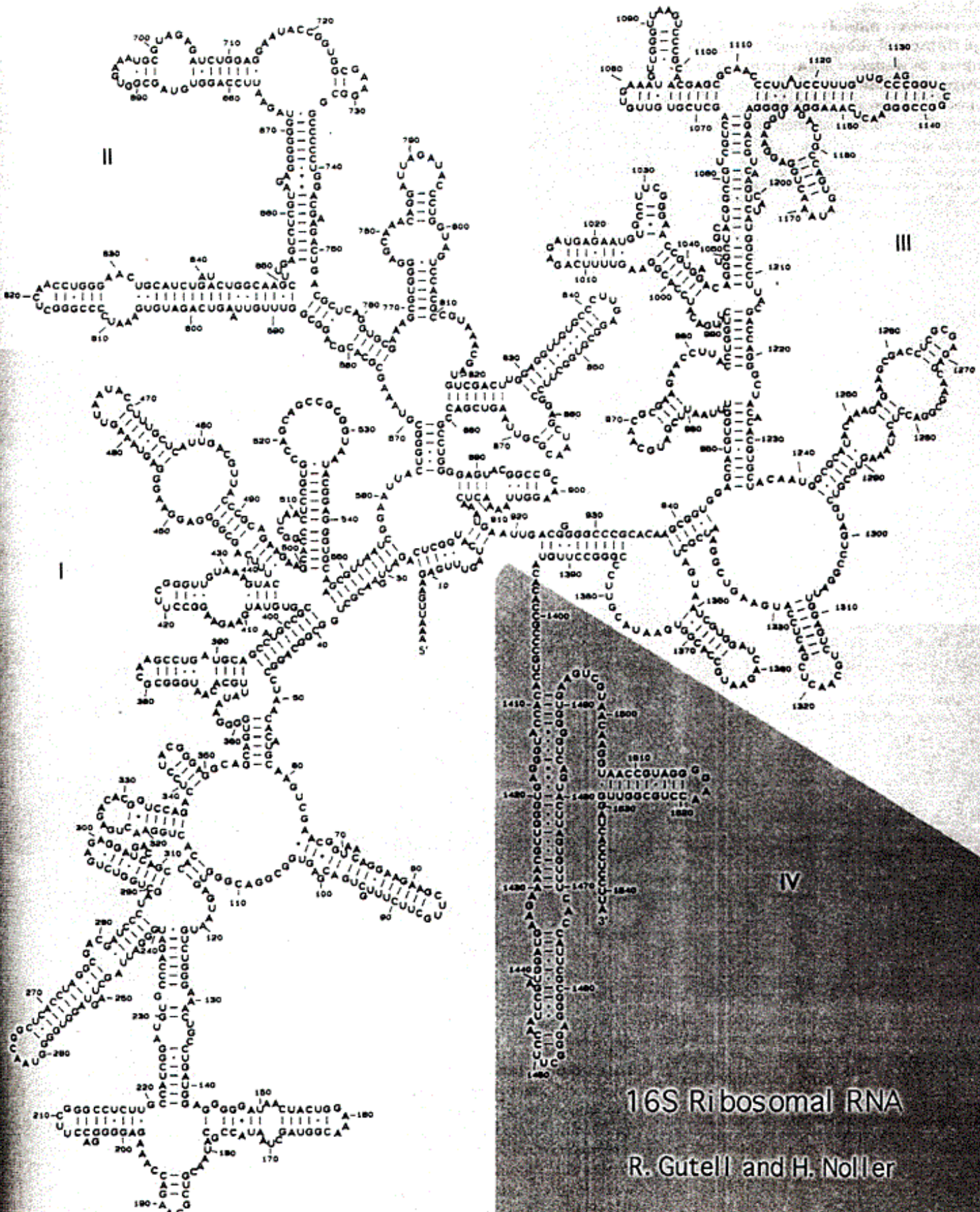


-0.32V

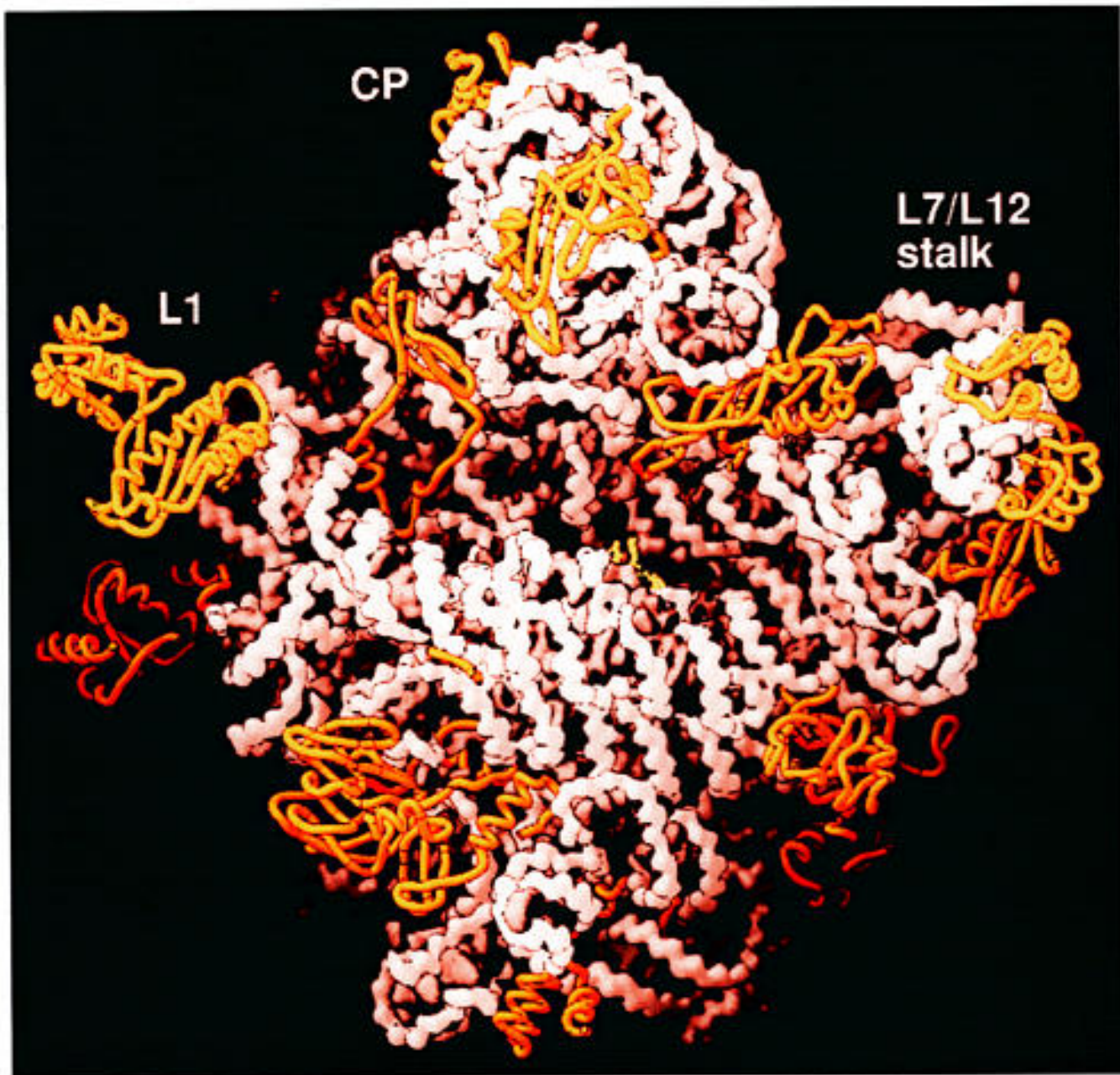
Used by at least 376 different enzymes.

Flavin adenine dinucleotide



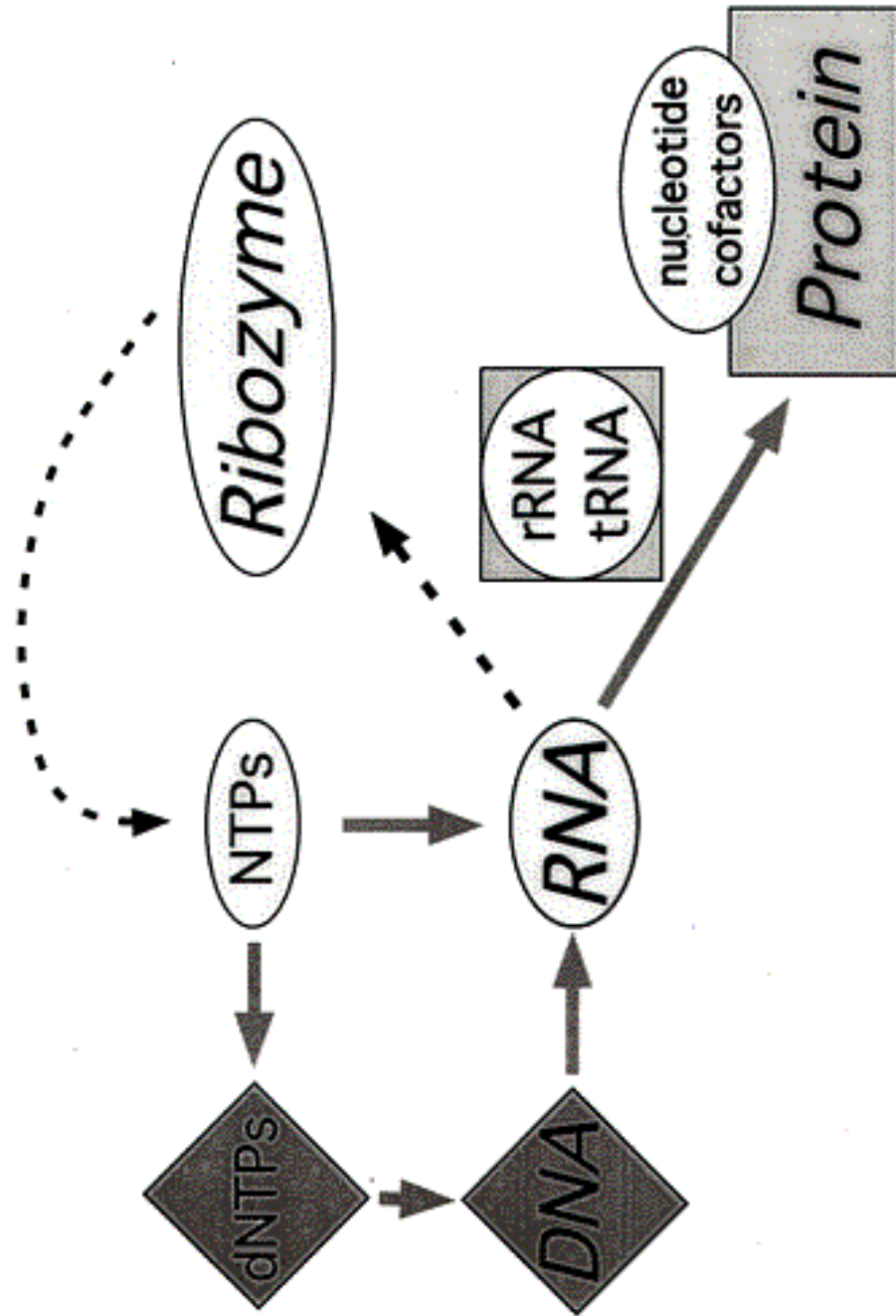


16S Ribosomal RNA
R. Gutell and H. Noller

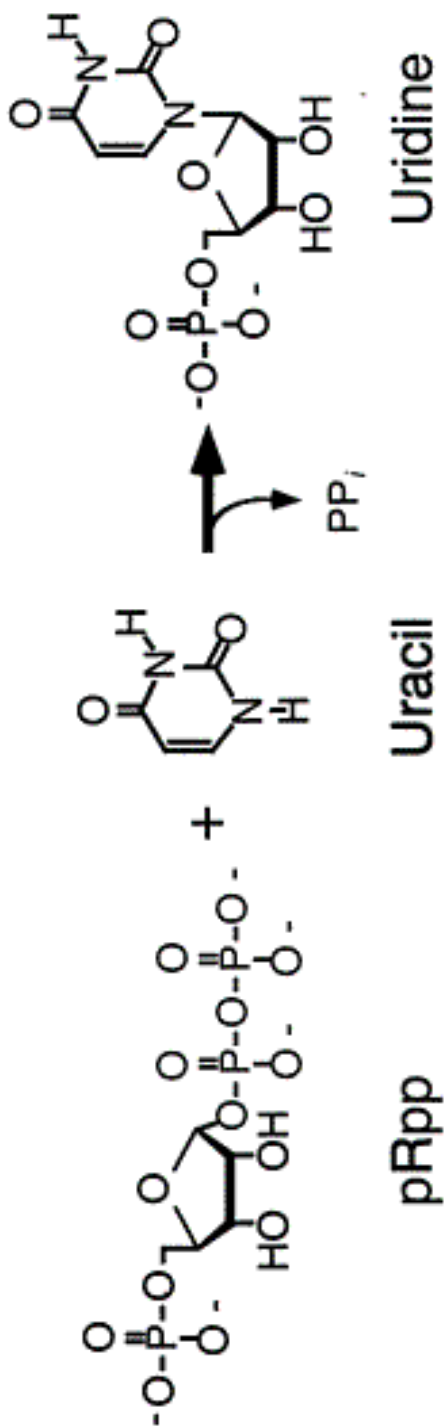


Shatz, Science

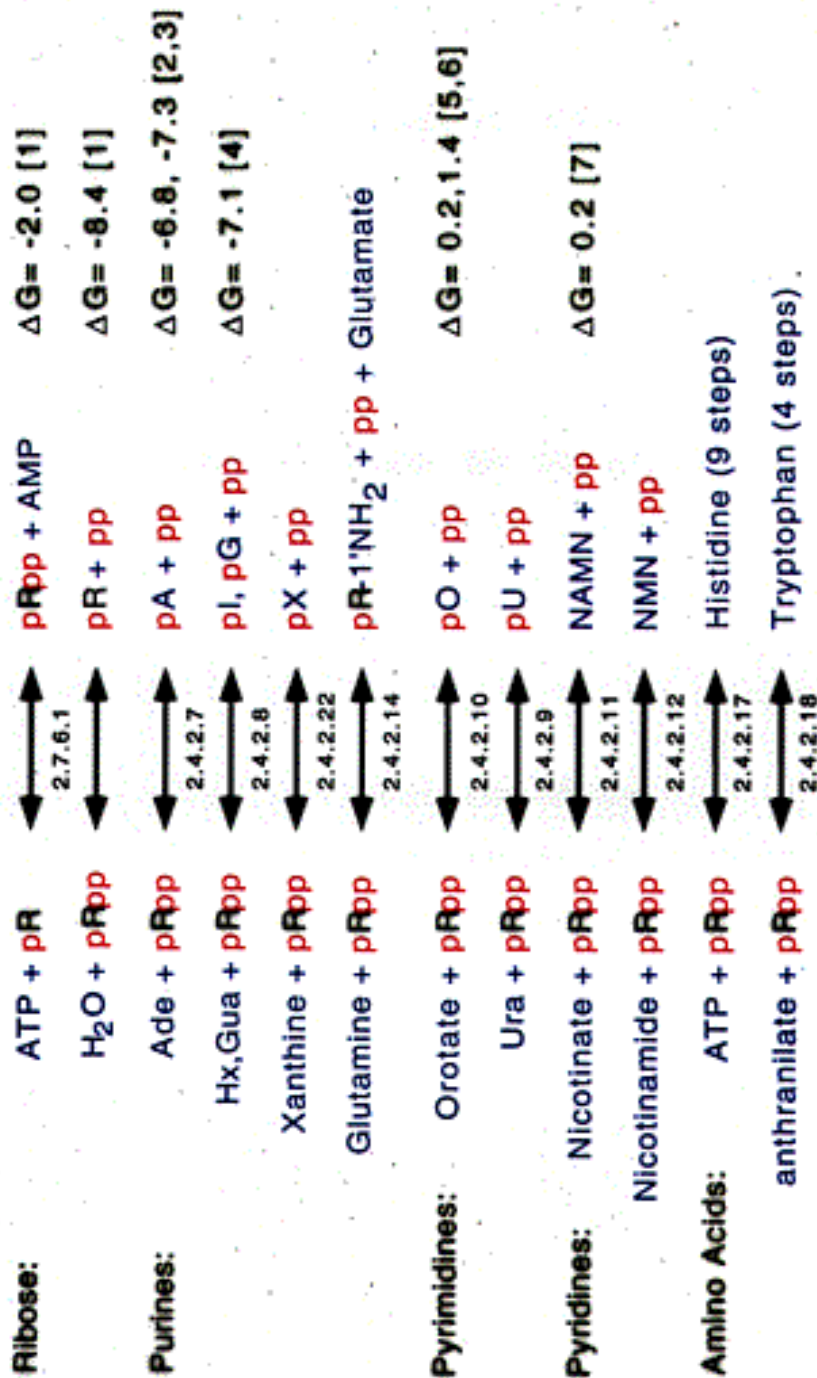
*A possible progression
towards modern metabolism*



Uracil PRTase

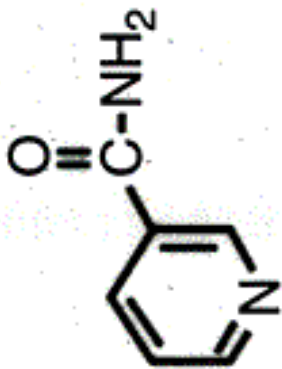


Reactions using pRpp

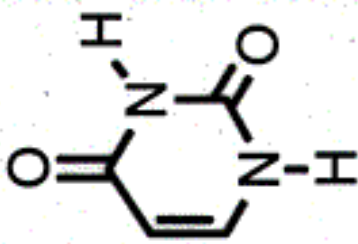


1. Frey et al. *Biochemistry*, 34 (1995) p11307-11310.
2. Kornberg et al. *J. Biol. Chem.*, 215 (1955) p417-427.
3. DeWolf et al. *Biochemistry*, 25 (1986) p4132-4140.
4. Xu et al. *Biochemistry*, 36 (1997) p3700-3712.
5. Tavares et al. *Biochimica et Biophysica Acta*, 913 (1987) p279-284.
6. Bhatia et al. *Biochemistry*, 29 (1990) p10480-10487.
7. Vinitzky et al. *J. Biol. Chem.*, 268 (1993) p26004-26010.

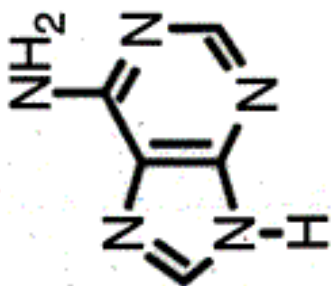
Nucleobases used in metabolism



Pyridine

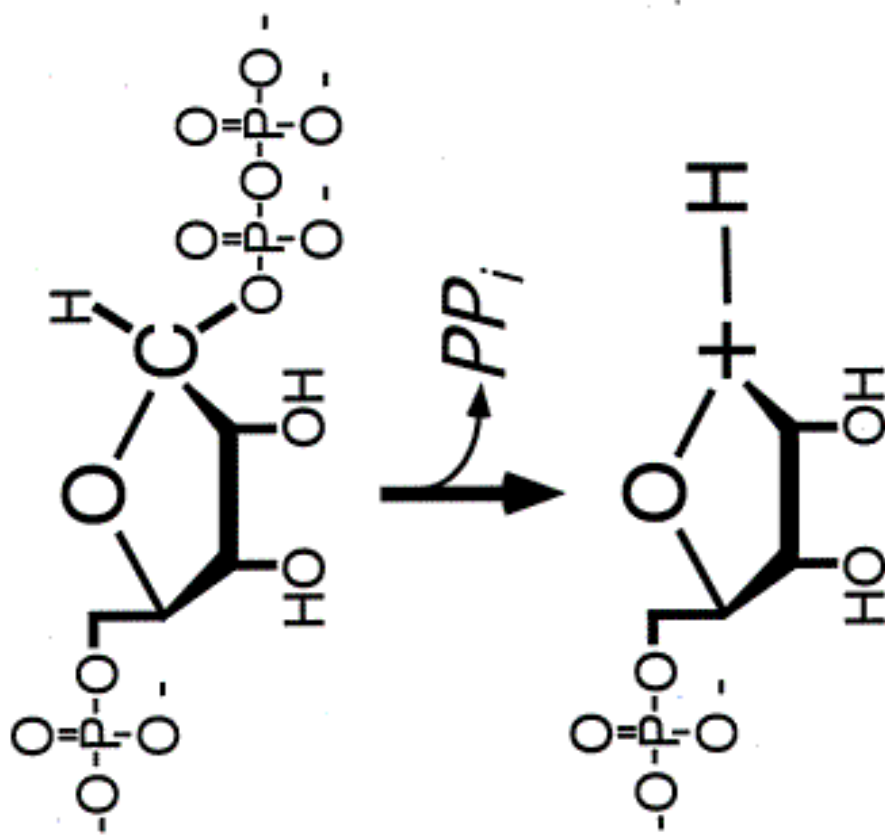


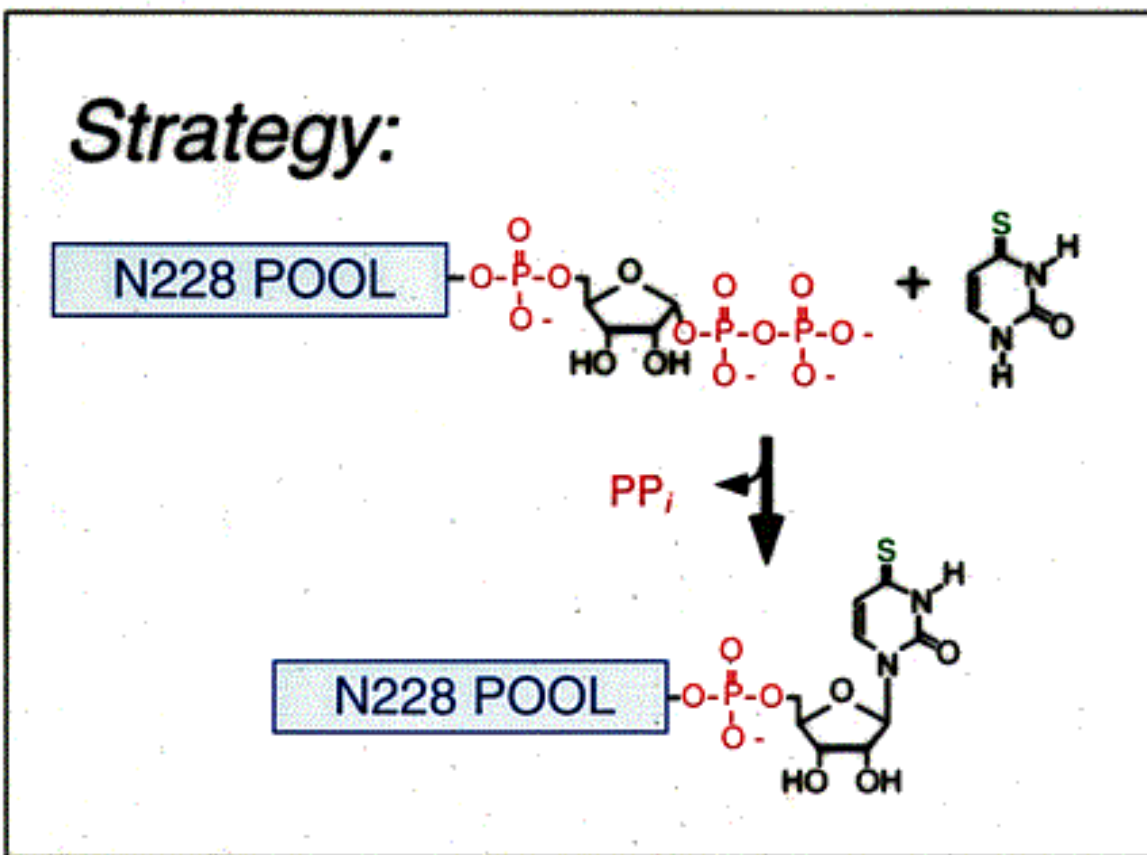
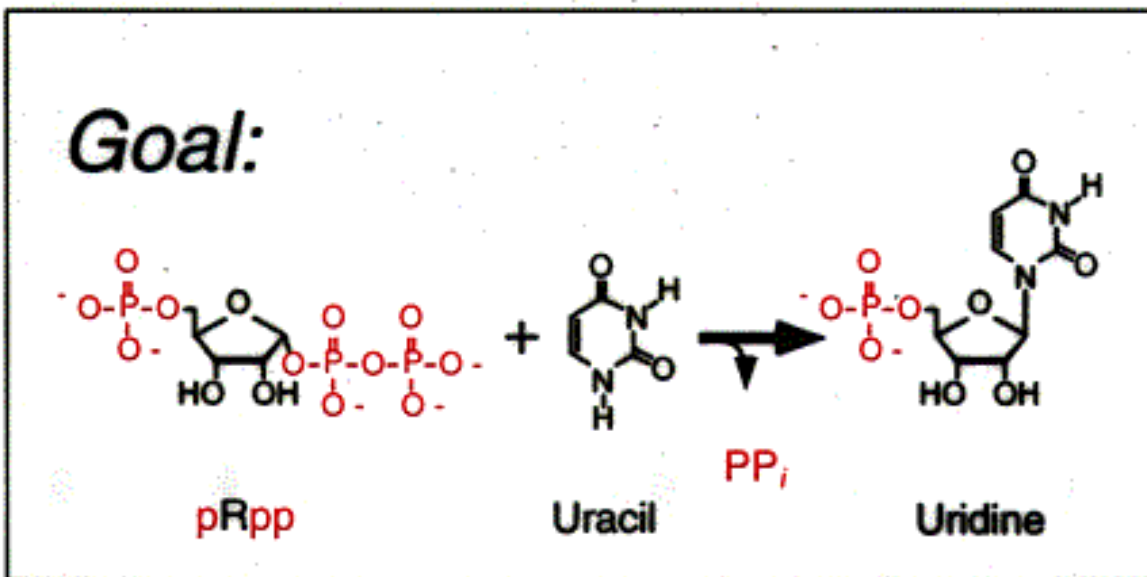
Pyrimidine



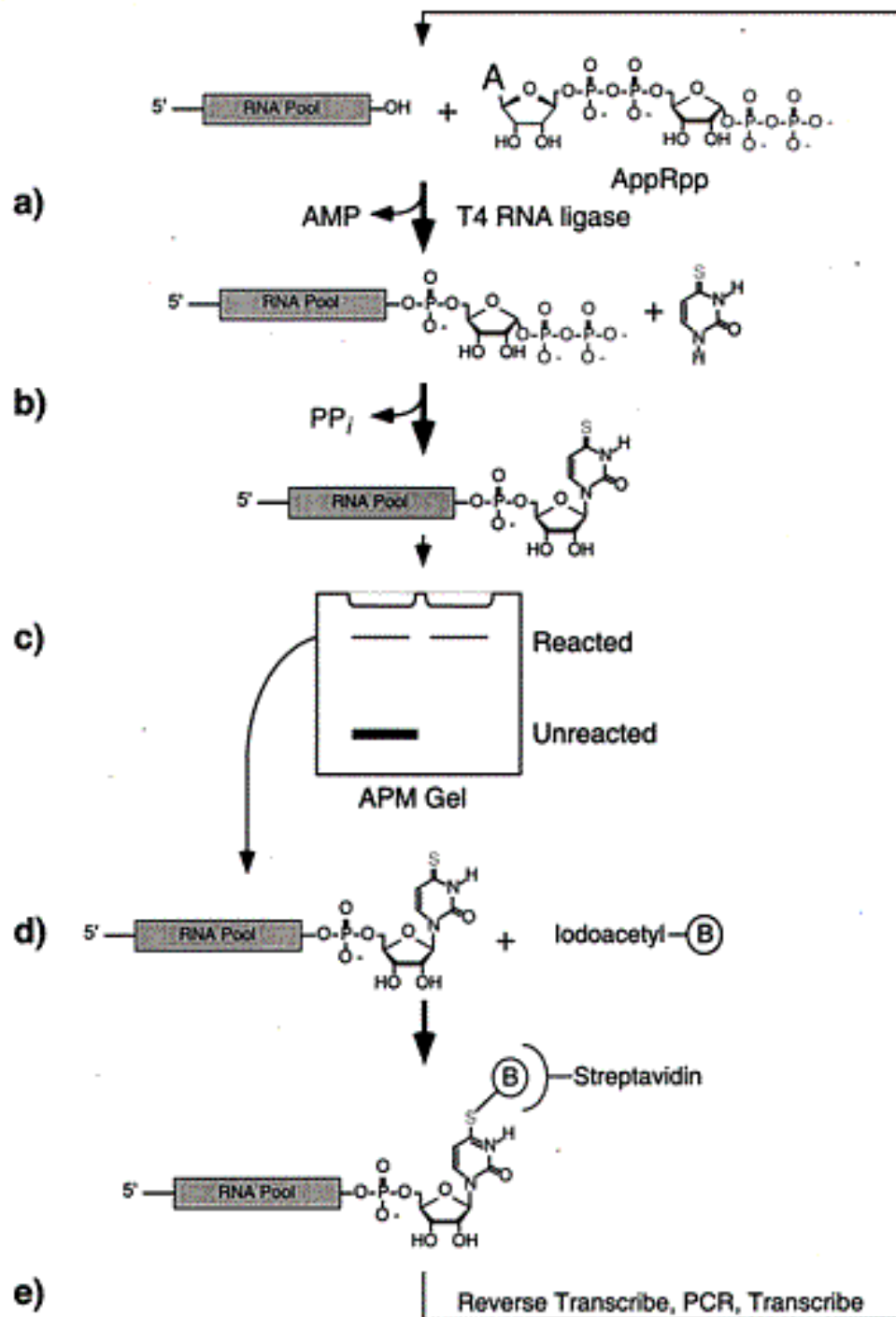
Purine

Oxocarboxation intermediate formed from pRpp



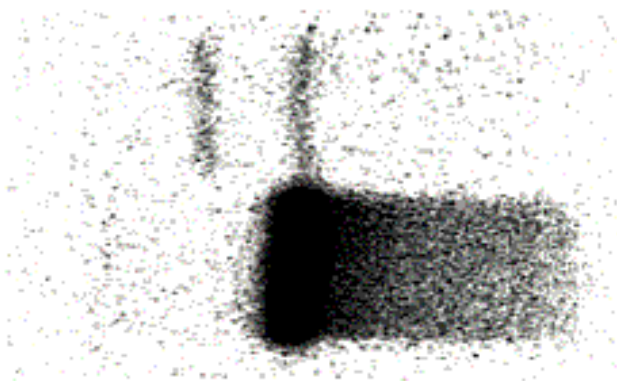


De Novo Selection Scheme



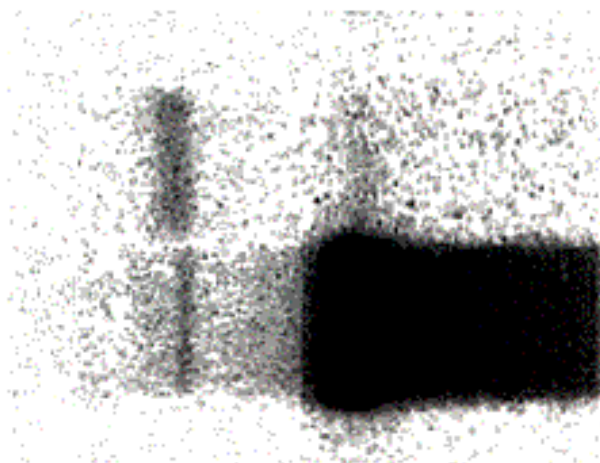
Mercury Gel Selection

Round 4

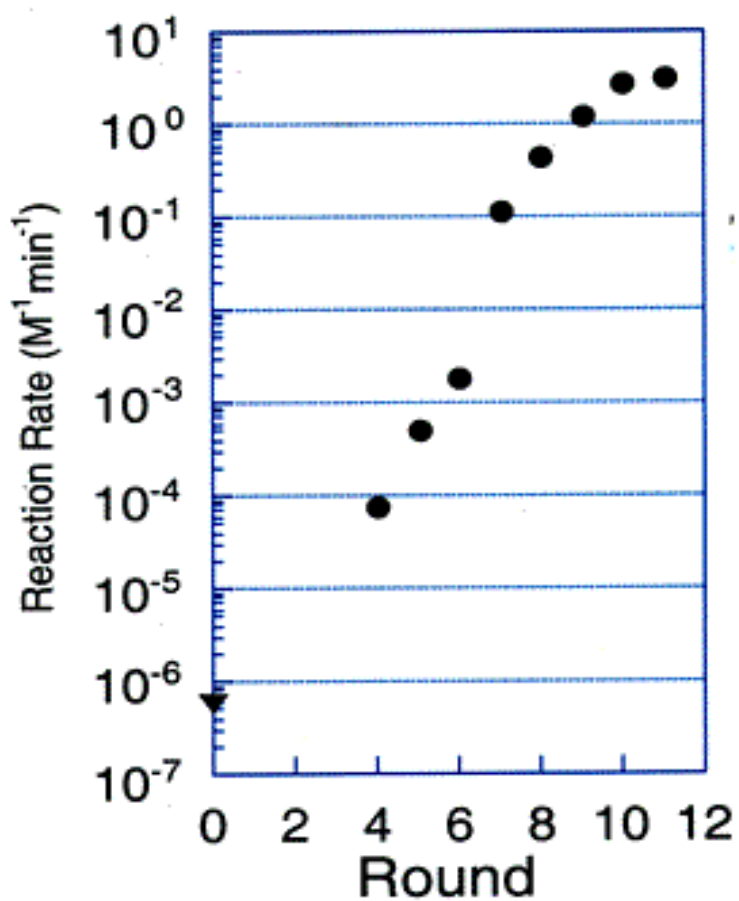


***Mercury
Gel Selection***

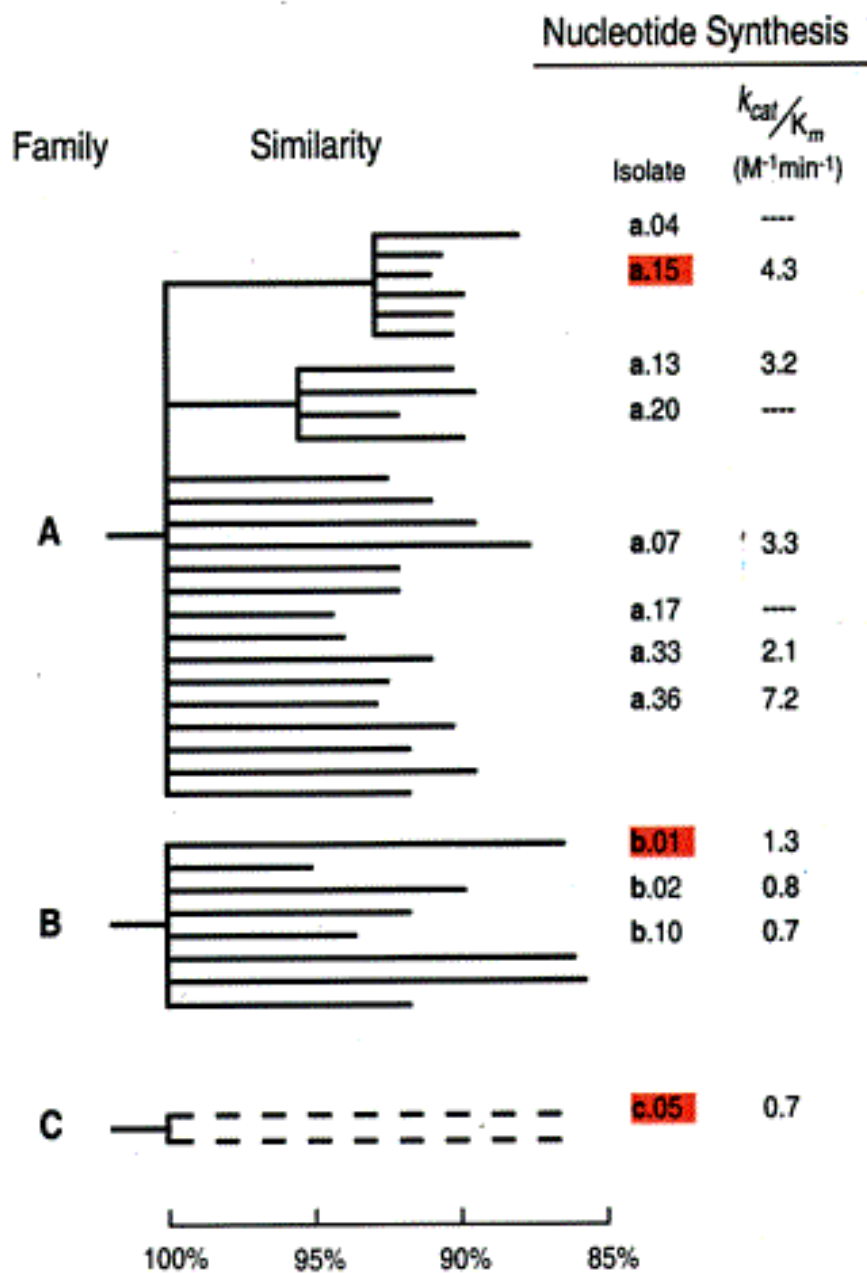
Round 5



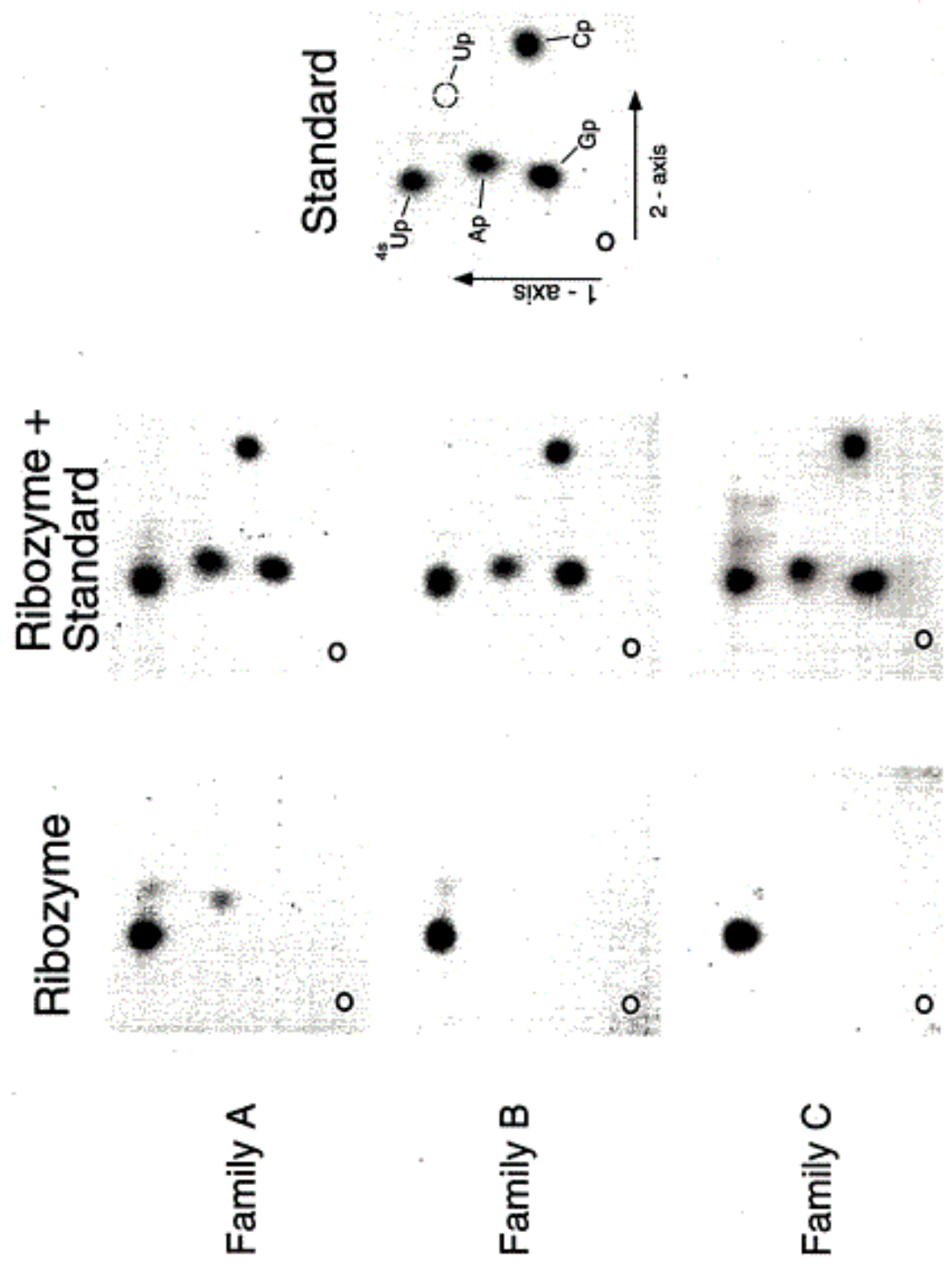
Activity per round



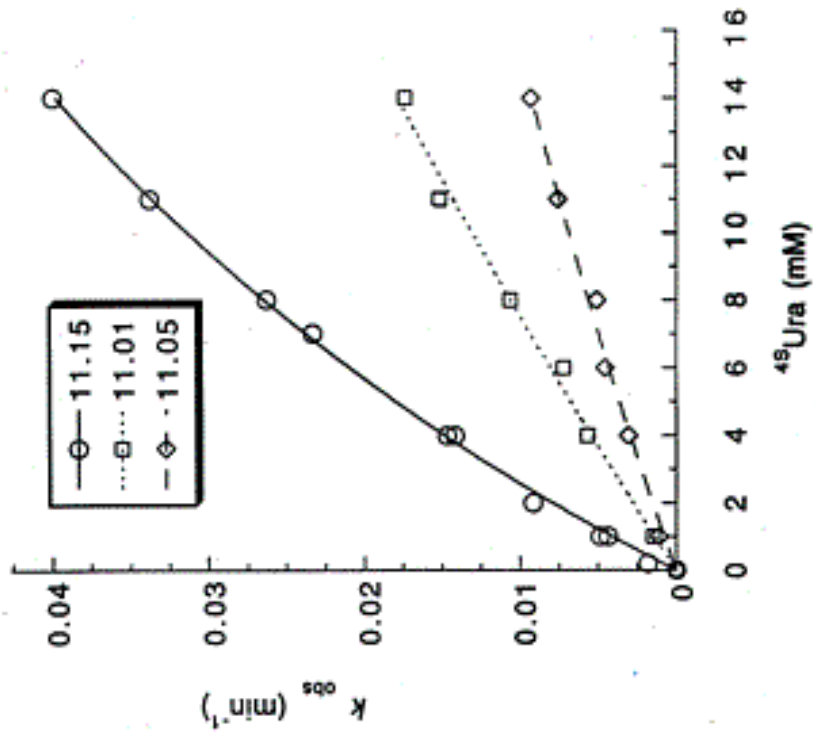
Isolated families



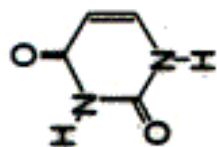
TLC Analysis of Ribozyme Product



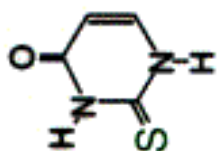
K_m and k_{cat} Measurements



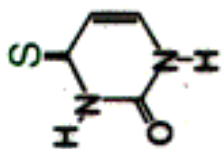
Uracil Analogs



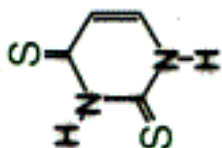
Uracil



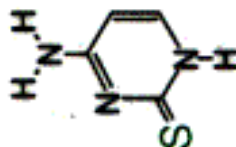
2^s Uracil



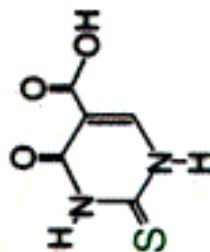
4^s Uracil
4.3 M⁻¹min⁻¹



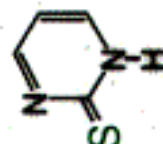
2,4^s Uracil



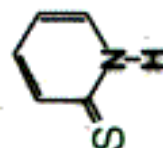
2^s Cytosine



2^s, 5 carboxy-
Uracil



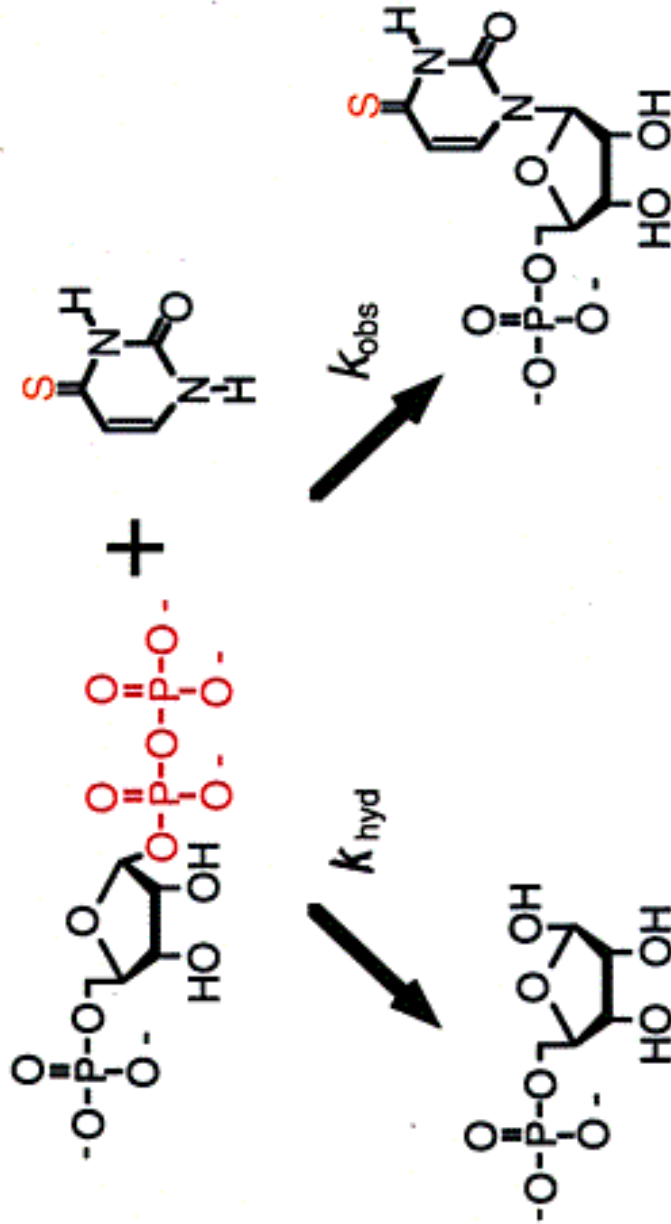
2^s Pyrimidine



2^s Pyridine

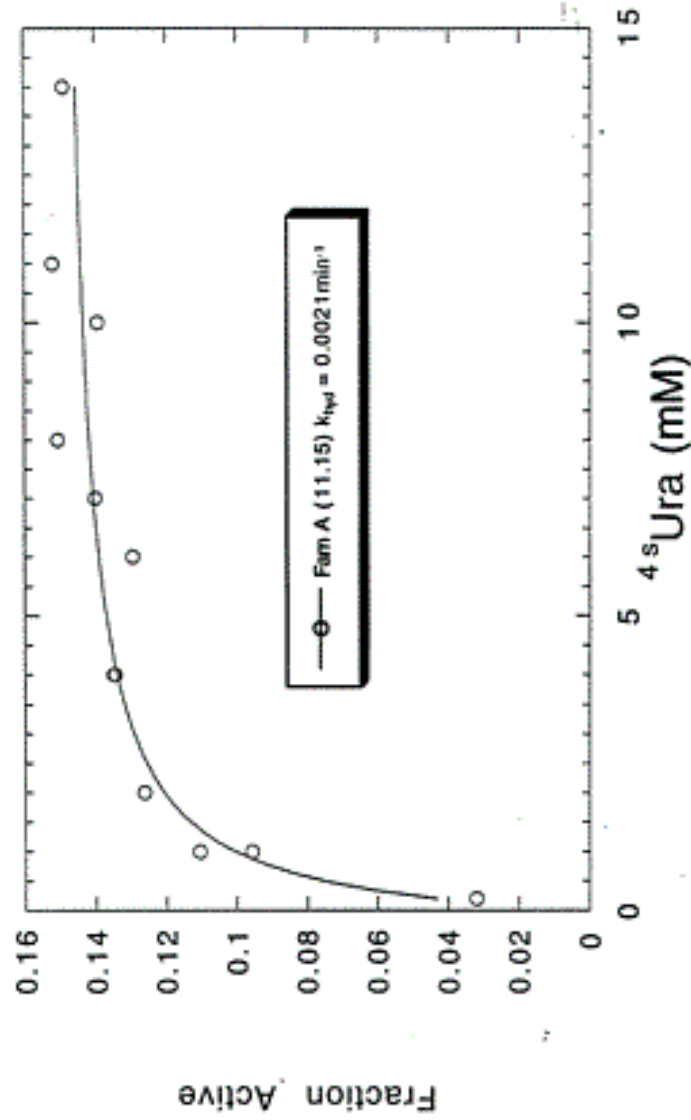
This analogs react at less than $4 \times 10^{-3} - 1 \times 10^{-4} \text{ M}^{-1} \text{ min}^{-1}$

PRPP Hydrolysis

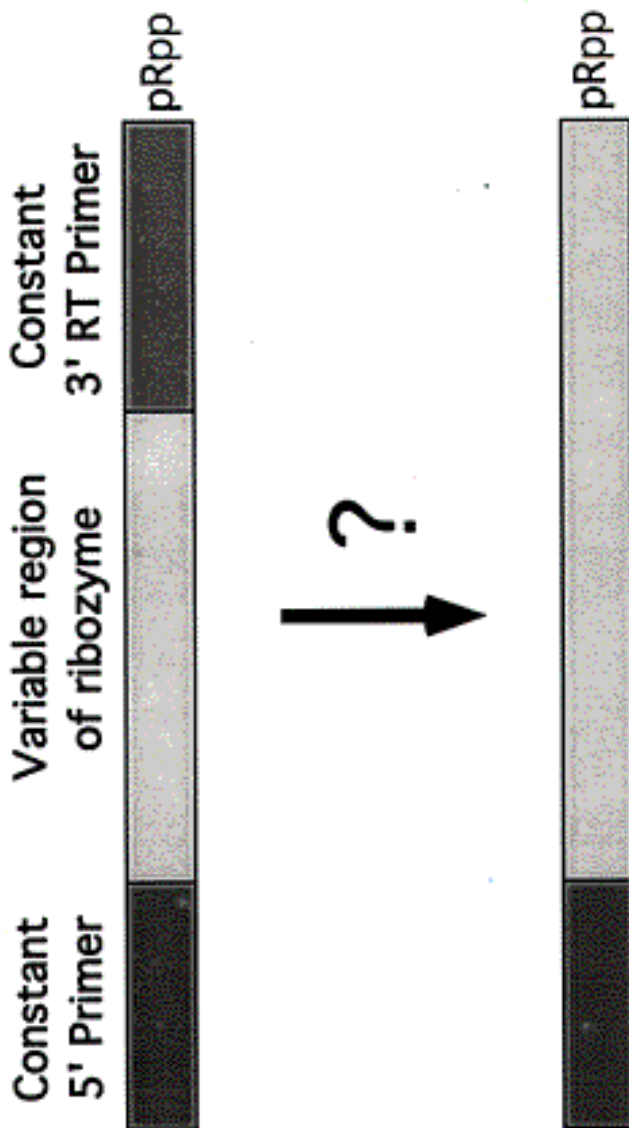


$$F = \frac{F_{Max} k_{obs}}{k_{obs} + k_{hyd}} (1 - e^{-(k_{obs} + k_{hyd})t})$$

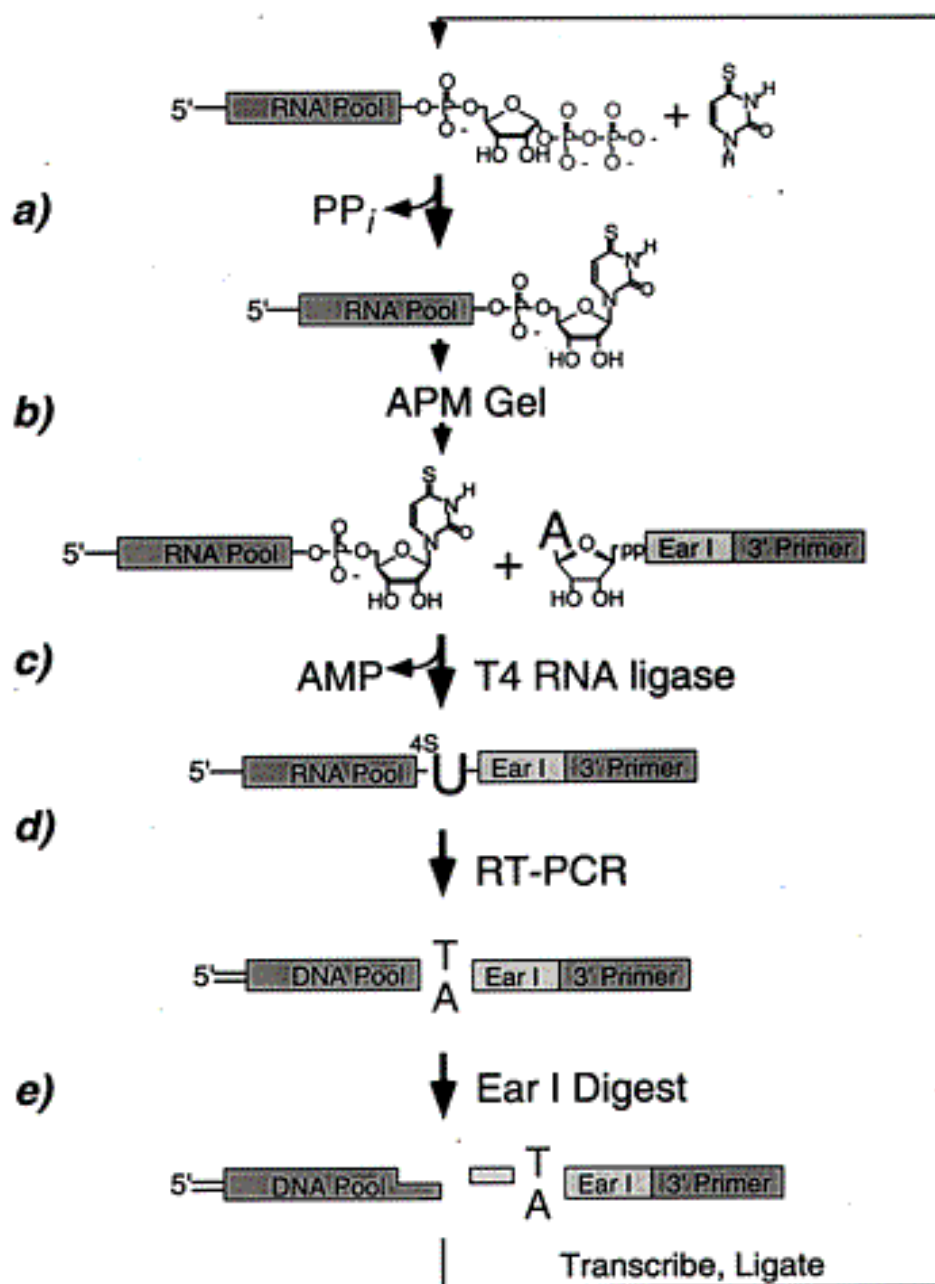
Asymptotic Fraction Reacted



Optimizing the 3' end of a 4-thiouracil synthase



Doped Selection Scheme



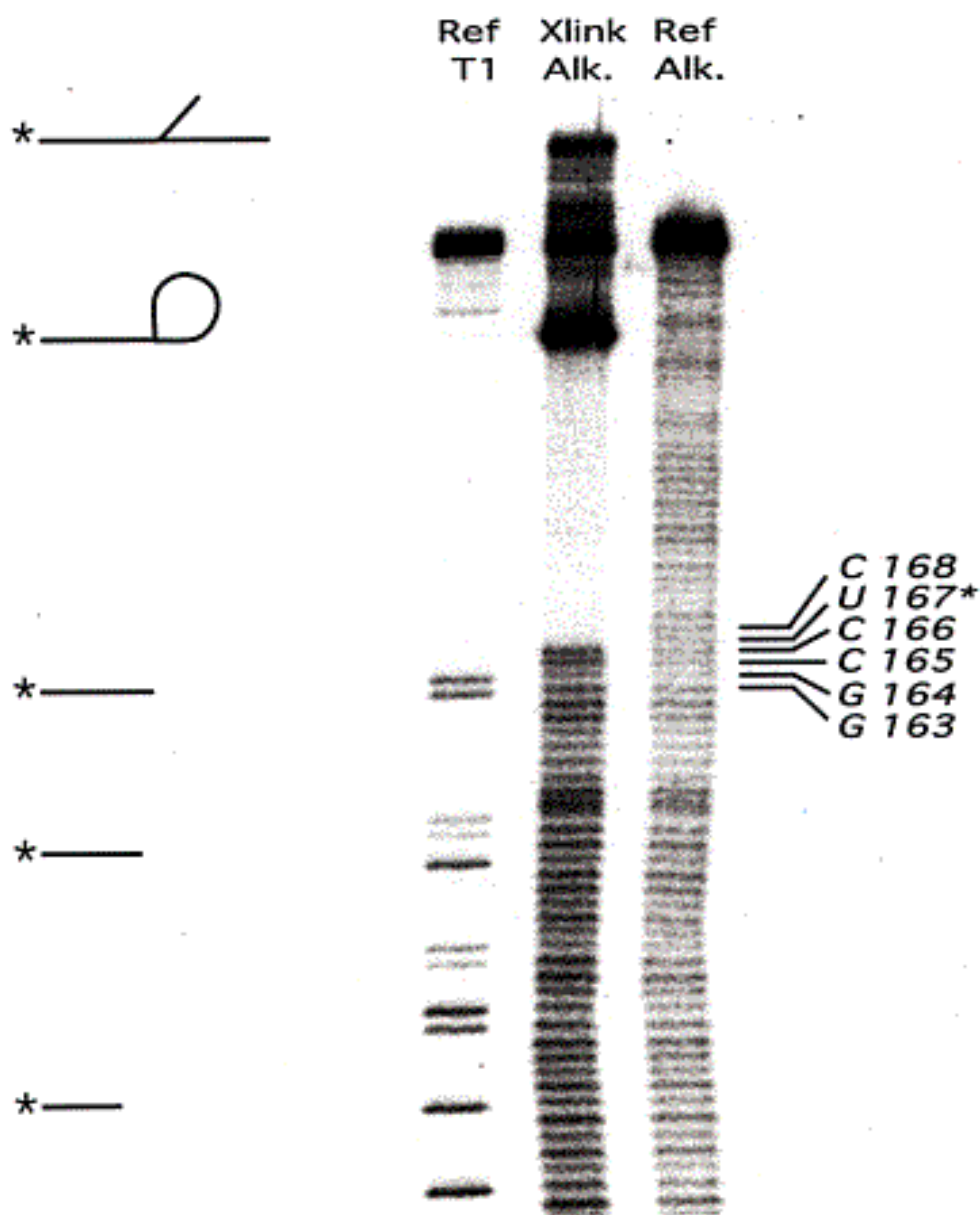
Alignment details

III				IV		III		VI		IV		Isolate	1mM ⁴⁵ Ura (min ⁻¹)	Frac. Reacting
70	80	90	100	110	230									
GCAATGGA- AACGCGGT GGC- T- A- *	GGTTGCCGTTACTACCAT	CACATCGCAGCAACC												
GCAATGGA- AACGCGGT GGC- T- AG	G- TTGCGCGTTACTACCAT	CACATCGCAGCAA- C								*	a.15	4.3	0.15	
ACAATGGA- AACGCACT GGC- T- A-	GGTTGCCAGTTCCATGCCAG	CACATCGCTGCAAGC									a.6.30	97.1	0.36	
GTAATGGA- AACGAGGGGGA- T- A-	GGTTGCTCGTTACTCCCAT	CACATCGCAGCAACC								*	a.6.10	3 68.5	0.27	
GATATGGA- AACGGAGGT G- - DAA-	GGTTGCCGTTAATCCAT	CACATCGCAGCAACC									a.6.13	67.7	0.17	
CATATGTA- STAGCGGTG- - TTCA-	GGTTGCCGTTACTACCAT	CACATCGCAGCAACC									a.6.03	4 66.3	0.09	
GAAATGGG- AACGCGGGG- - TTA-	GGTTGCCGTTCTCCAT	CACATCGCAGCAACC									a.6.45	2 65.5	0.13	
GAAATGGA- AACGCGGGGA- T- A-	GGTGC GCGTTACTACCAS	CACACCGCAGCGACC								*	a.6.29	62.2	0.16	
GCAATTTGT AACGCGGT GGC- T- A-	GGCTGCCGCTACAATAAA	CACACCGCAGCAG- C								*	a.6.11	48.7	0.19	
TCAATGGA- AACGCGGT GGA- T- G	GCT- GC GCGTTGCTCCAT	CACACCGCAGCAAGC								*	a.6.02	43.4	0.25	
GACAGGGA- ACGCGGT GGC- T- G	GGC- GC GCGT GACTATGAA	CACATCGTAGC- GCC								*	a.6.07	3 39.5	0.31	
GCAACAGA- AACGTGGTAG- - DAA-	GGTTGCCGTTACCGACAS	CACATCGCAGCAACC									a.6.55	3 38.2	0.20	
GAAATGGA- AACGCGGT GGA- DCA-	GGTTGCCGTTAATACAT	CACATCGCAGCAACC									a.6.37	34.4	0.19	
GAAATGGA- AACGCGG- - - T- - G	GCTTGCCGTTACTCCAT	CACATCGCAGCAAGC									a.6.43	33.1	0.25	
GAAATGGT- AACGCGGT A- - TTCA-	GGCTGCCGCTACTACCAT	CACGTCCGAGCAGCC								*	a.6.09	15 31.1	0.29	
GCAATGGA- AGCGCGGG- C- T- AG	G- CTGCCGTTACTCCAT	CACACCGCAGCAG- C									a.6.61	30.8	0.28	
ATAATGTGAAACGTCATAGA- T- A-	GGTTGCCGTTTCTACCAT	CACATCGCAGCAACC									a.6.48	29.8	0.04	
GCTATGGA- CACGCGGATG- - DAA-	GGTTGTCCGTTCTACCAT	CACACCGCAGCAACC									a.6.04	27.8	0.36	
GAAATGGG- AACGCGGGG- - TTA-	GGTTGCCATTCTCCAT	CACATCGCAGCAACC									a.6.36	27.2	0.22	
GCAASGGA- ATCGCGGGGA- T- A-	GGTTGCCGATATAACCT	CACATCGCAGCAACC									a.6.01	7 25.4	0.27	
GCAATGGA- AGCAGCGGT GGA- TAA-	G- CTGCCGTTTCTACAT	CACACCGCAGCAG- C									a.6.35	3 24.1	0.29	
GTCATGG- AACGCGGTAAGCT- A-	GGTCCGCGTTCTACCAT	CACATCGCGCAACC									a.6.47	24.1	0.18	
ACAATGGA- GACGOTGTAGA- T- AG	G- CTGCCGTTACTACCAT	CACCGCGCAGCAG- C									a.6.63	22.7	0.25	
GCTATGGAAGCGCGGGG- - TTA-	GGTTGCCGTTACTACCAT	CACATCGCGCAACC									a.6.53	22.1	0.24	
GCAAGGGA- GACGACSTGGC- T- AG	GGTTGCCGTTACTACCAT	CACATCGCTGCAACC									a.6.26	20.4	0.21	
CAATGGA- AACGCGGTAGC- T- AG	G- CTGCCGTTACTACCAT	CACACCGAGACAS- C									a.6.08	19.9	0.31	
AAAAGGG- AACGCGGTAGC- T- AG	G- CTGCCGTTACTACCGG	CACACCGGCGCAG- C									a.6.65	19.4	0.22	
GCATGGAAACGCGGTGGC- T- G	G- CTGCCGTTACTCCAT	CACATCGCAGCAG- C									a.6.33	14.9	0.42	
ACTATGGCAACGCGGTAGC- T- AG	G- CTGCCGTTACTCCAT	GCATCGCAGCAG- C									a.6.57	13.4	0.28	
GCAATAGG- AACGCGGGG- - T- A-	GGTTGCCGTTCTACCAT	CACATCGCAGCAACC									a.6.25	0.8	0.44	

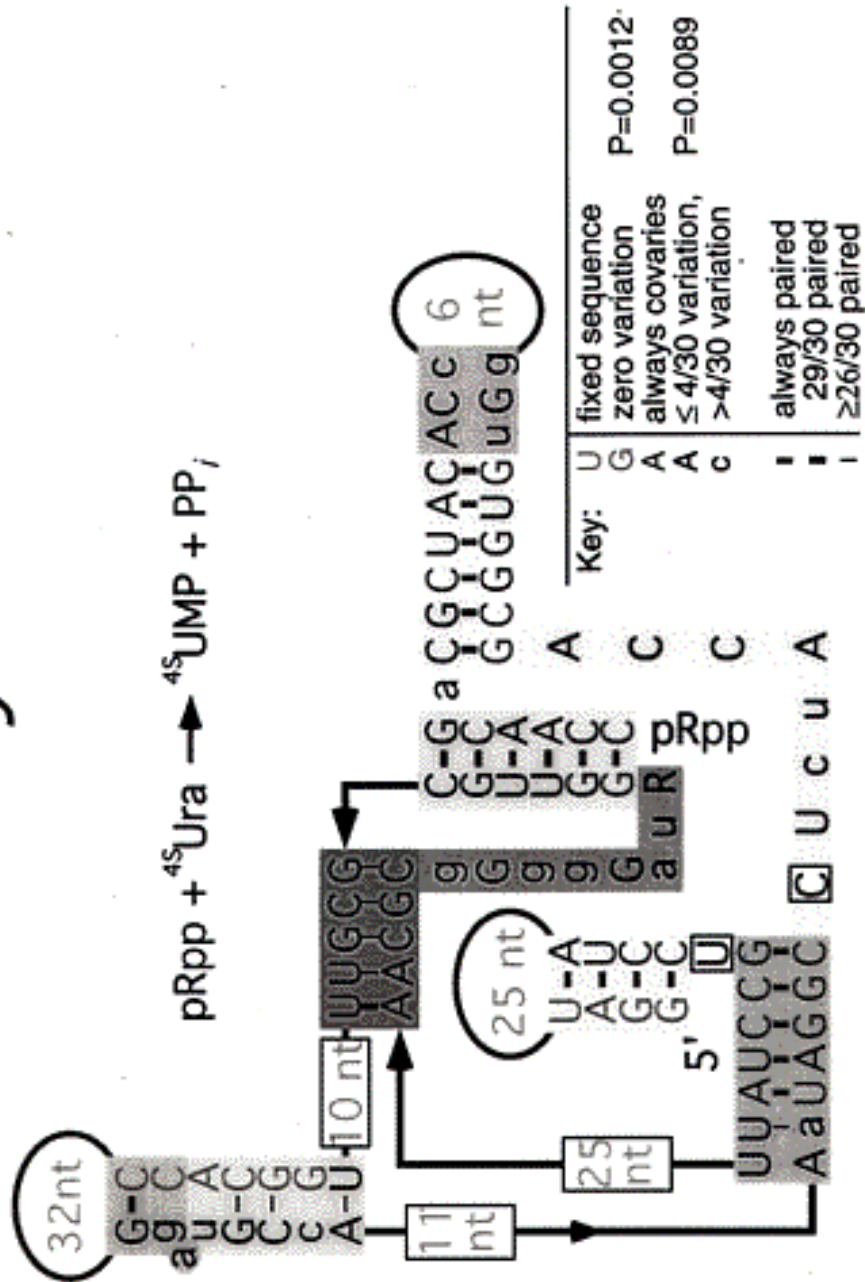
Crosslinking of the active site



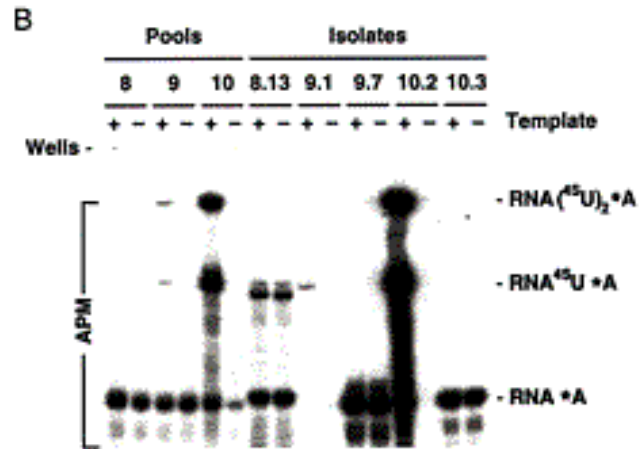
UV crosslinking of reaction product



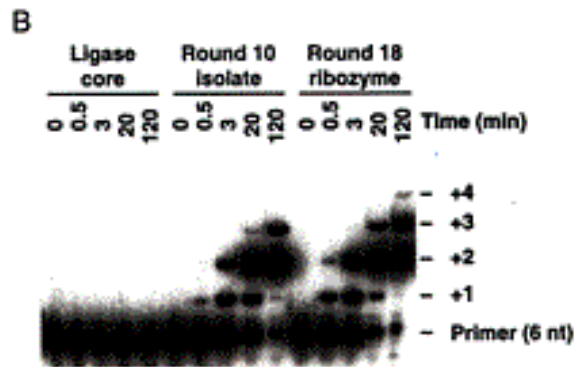
Nucleotide synthase secondary structure



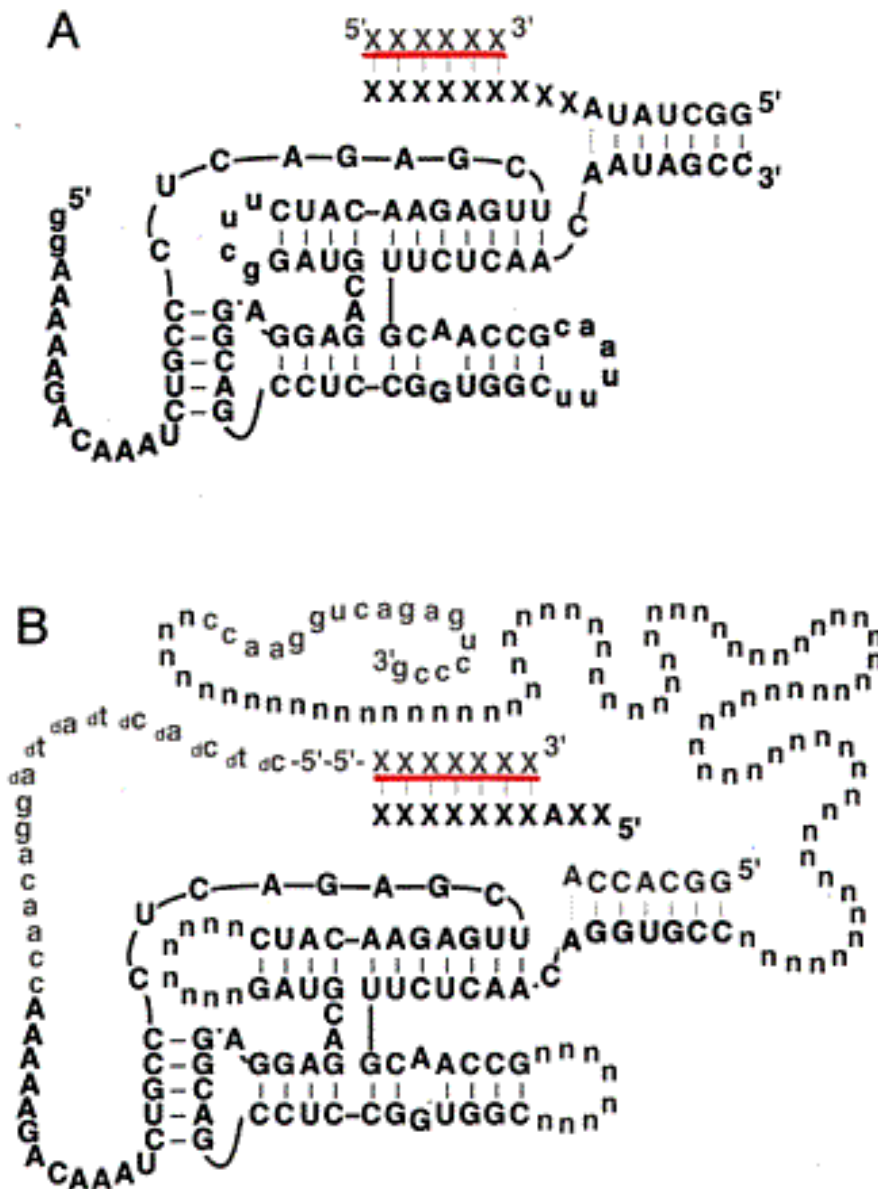
Johnston et al.
Figure 2



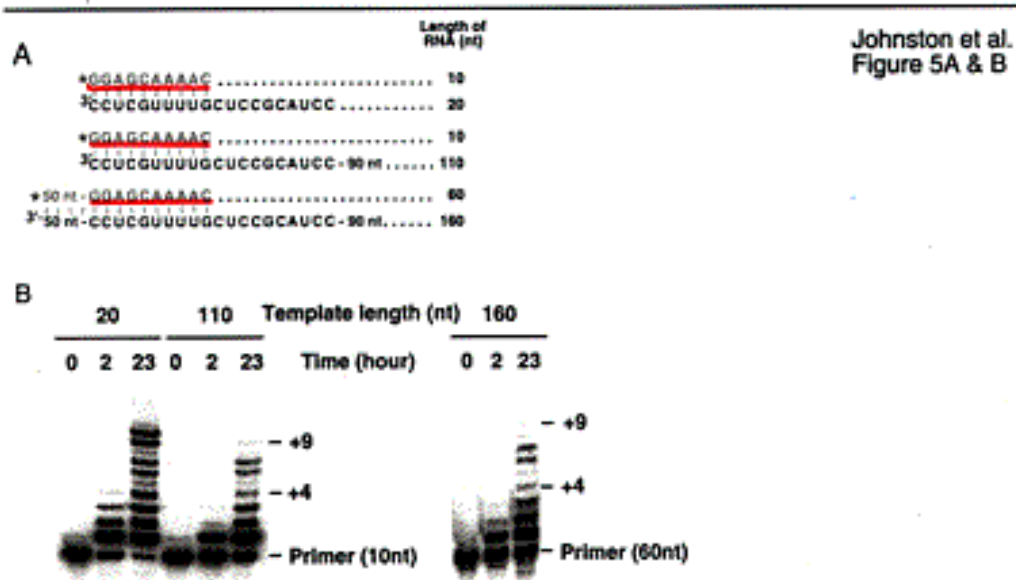
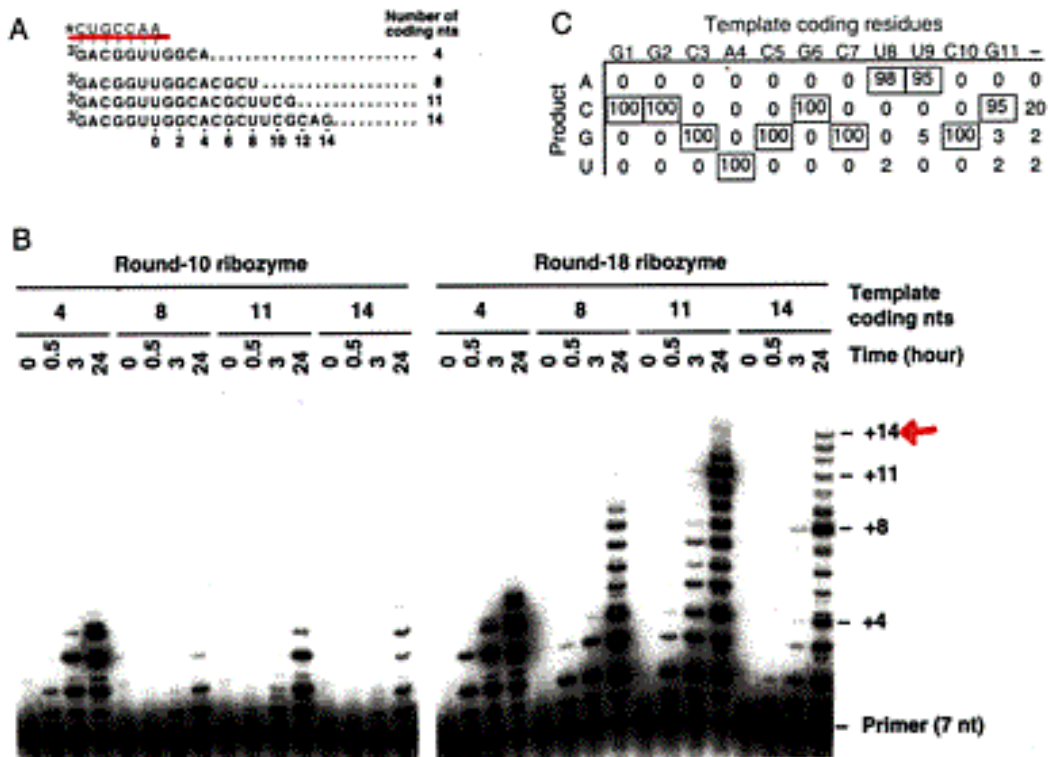
Johnston et al.
Figure 3



Johnston et al.
Figure 1



Johnston et al.
Figure 4



Johnston et al.
Figure 5A & B

Johnston et al.
Supplemental Material
Figure 2

