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Oligonucleotide Formation Catalyzed by Mononucleotide Matrices

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Summary. Pb^{2+}-containing precipitates of mononucleotides form matrices which catalyze the self-condensation of nucleotide 5'-phosphorimidazolides and their condensation with nucleosides. The reactions exhibit base-pairing specificity between matrix nucleotide and substrate, and usually follow the Watson-Crick pairing rules. Although purine polynucleotides do not facilitate the oligomerization of pyrimidine nucleotide monomers in solution, it is interesting that purine-containing matrices do catalyze such a reaction. The significance of the results in the context of the prebiotic evolution of polynucleotides is discussed.

Key words: Matrix-catalyzed Condensation – Phosphorimidazolides – Oligonucleotides – Prebiotic

Abbreviations: Im, imidazole; EDTA, ethylenediaminetetraacetic acid; A, adenosine; C, cytidine; C^{Me}, 5-methylcytidine; C^{ara}, 9-β-D-arabinofuranosylcytosine; G, guanosine; I, inosine; U, uridine; U^{ara} 9-β-D-arabinofuranosyluracil; dA, 2'-deoxyadenosine; dC, 2'-deoxycytidine; dG, 2'-deoxyguanosine; T, thymidine; pN (N = A, C, G, I, U, U^{ara}), ribonucleoside 5'-phosphate; pdN (dN = dA, dC, dG, T), deoxynucleoside 5'-phosphate; Np, ribonucleoside 2'(3')-phosphate; N>p, nucleoside cyclic 2',3'-phosphate; ImpN, 5'-phosphorimidazolide of a nucleoside N; NppN, P_1,P_2-dinucleoside 5'-diphosphate; pNp, 5'-phosphonucleoside 2'(3')-phosphate; $N(pN)_n$ (n = 1, 2...) oligonucleotide terminated by a free 5'-OH group; $(pN)_n$ (n = 2, 3...) oligomers of pN; $(Np)_n$ (n = 2, 3...) oligomers of Np; p_2N, nucleoside 5'-diphosphate; p_3N, nucleoside 5'-triphosphate; poly(N), polynucleotide derived from N. (Pb-pN) or (Np-Pb), matrix formed by precipitation of a nucleoside pN or Np, respectively, with Pb^{2+} ions. The numbers given as superscripts between the symbols of a nucleoside and a phosphate indicate the type of internucleotide linkage e.g. A^2pA is adenylyl-[2' → 5']-adenosine; a star above the symbol for a nucleoside indicates the position of a radioactive label, e.g. $\overset{*}{U}\,^3pU$, [2-^{14}C]-uridylyl-[3' → 5']-uridine; OD, optical density units measured at 259 nm.

Introduction

It is often assumed that the evolution of self-replicating polynucleotides on the primitive Earth proceeded in two stages. In the first, activated monomers combined to form primary oligonucleotides. In the second, those oligomers served as templates for the synthesis of complementary molecules. These early replication processes probably resembled the reactions of RNA polymerases in some respects, but must have lacked their rate, efficiency and accuracy.

In the past, most of our work was concerned with finding models for RNA polymerase reactions. Relatively, little of our effort went into studying how primary templates might have been formed on the primitive Earth. It is known that activated mononucleotides like N > p can polymerize in the semi-dry state (Moravek et al 1968); Tapiero and Nagyvary 1971; Orgel and Lohrmann 1974). It is also known that various metal ions catalyze the self-condensation of ImpA. The Pb^{2+} ion was found to be a particularly effective catalyst in these reactions (Sawai and Orgel 1975; Sawai 1976; Sleeper and Orgel 1979).

A number of years ago (Lohrmann 1968 unpublished) we investigated the self-condensation of activated mononucleotides on precipitates of metal salts of mononucleotides to see whether base complementarity between the matrix nucleotides and the activated monomers had any influence on the condensation. Preliminary experiments were performed with mononucleotides precipitated, for example, with Pb^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+} and La^{3+}. We found that the Pb^{2+}- containing precipitates were particularly effective catalysts for the condensation of nucleoside 5'-phosphorimidazolides with nucleosides and that base complementarity between matrix and substrate often had a favorable influence on the condensations. Precipitates

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formed with the other metal ions had no catalytic activity.

Recent results (Sleeper and Orgel 1979; Sleeper et al 1979) on metal ion catalysis of oligonucleotide formation have renewed interest in these earlier findings. Here we report the early studies and some more recent extensions of them.

Experimental

Materials

Reagent grade chemicals were used throughout. Imidazole, purchased from Matheson, Coleman and Bell, was recrystallyzed from benzene before use. Triphenylphosphine and 2,2'-dipyridyl disulfide were obtained from Aldrich. 2,2'-Dipyridyldisulfide was recrystallized from petrolether before use. dA and pdA were purchased from Calbiochem, pG from Boehringer, pA and pC from Terra-Marine Bioresearch. All the other nucleosides and nucleotides as well as the [2'–5'] and [3'–5']-linked dinucleoside phosphates and poly(G) were obtained from Sigma. Poly(A), poly(C), poly(I) and poly(U) were prepared by the procedure of Steiner and Beers (1958). The [^{14}C]-labeled nucleosides and nucleotides were purchased from Schwarz-Mann. The ImpN's and Imp$\overset{*}{N}$'s were prepared by slight modifications of procedures described previously for ImpU (Lohrmann and Orgel 1978). The pNp's (Saffhill 1970) and the NppN's (Smith and Khorana 1958) were prepared by published procedures. The oligomers A(pA)$_n$ used as chromatographic markers were prepared as described previously (Lohrmann and Orgel 1979). The U(pU)$_n$'s and C(pC)$_n$'s were obtained from Miles. The oligomers (pA)$_n$, (pC)$_n$ and (pU)$_n$ were prepared by partial digestion of the corresponding poly(N)'s with Nuclease P$_1$, and the (Gp)$_n$'s by partial degradation of poly(G) with KOH followed by acid treatment to open the cyclic phosphate termini (Lohrmann and Orgel 1980). The (Ap)$_n$'s were prepared as described earlier (Lohrmann and Orgel 1979).

Bacterial alkaline phosphatase (BAPF grade) was purchased from Worthington, pancreatic RNase from Boehringer, ribonuclease T$_2$ from Sigma and Nuclease P$_1$ from P-L Biochemicals.

Chromatography and Electrophoresis

Paper chromatography was carried out on Whatman 3MM paper by the descending technique. The following solvent systems were used:

n-propanol, concentrated ammonia and water (55:10:35) (System I). Chromatography in the presence of borate was performed by first applying a band (2.5 cm wide) of 0.1M sodium borate at the origin of the chromatogram. After drying, the compounds were spotted and chromatographed in System I.

95% Ethanol and 1M ammonium acetate made up to 2 x 10^{-3}M in EDTA and brought to pH 5.0 with glacial acetic acid (7:3) (System II).

Saturated ammonium sulfate, 0.1M sodium acetate (pH 6.5), and isopropanol (79:19:2) (System III)

Paper electrophoresis was performed on Whatman 3 MM paper at 3000 Volts (55 volts/cm) using varsol as coolant. The following buffer was used:

0.03M potassium phosphate pH 7.1 (System IV)

The chromatographic and electrophoretic mobilities of relevant compounds are listed in Table 1.

Quantitative estimates of product yields were obtained by running paper chromatograms or electropherograms through a Baird Atomic RSC 363 scanner with integrator. Results from several chromatographic and electrophoretic systems were collated to estimate the yields of individual compounds, when necessary. Yields are expressed as the percentage of the total radioactivity on the paper, after allowing for the background. When, in the course of the studies,the amount of radioactivity on a chromatogram became too low to permit us to make reliable estimates of the counts with the strip scanner, we cut

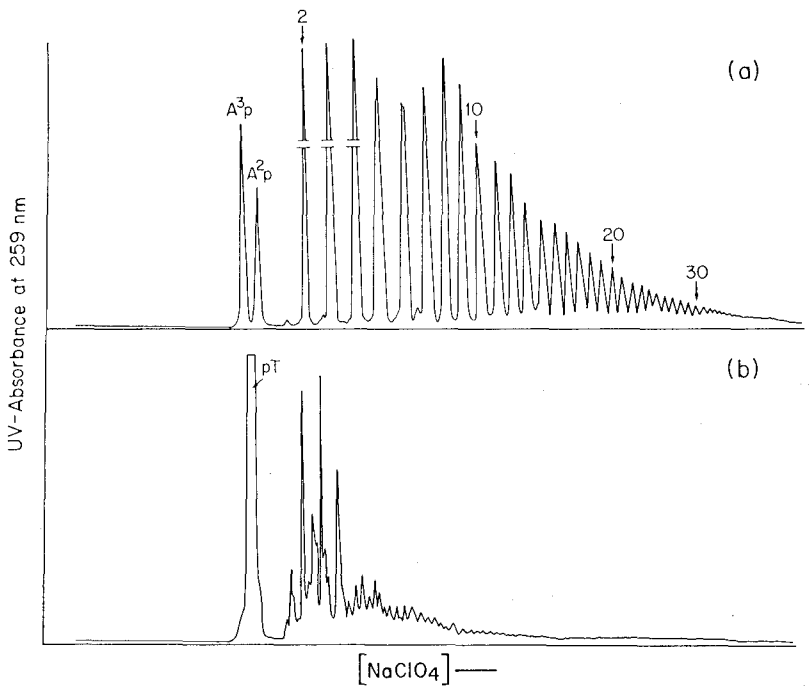

Fig. 1 a and b. Elution profiles of oligoadenylates. a (Ap)$_n$'s formed by partial alkaline hydrolysis of poly (A). b (pA)$_n$'s formed from Imp $\overset{*}{A}$ on (Pb-pT)
Reaction conditions: pT(Na$^+$ salt), 4 μmole; Imp$\overset{*}{A}$ (7.7 mCi/ mmole), 0.59 μmole; $NaNO_3$, 10 μmole; 1-methylimidazole-HNO_3 (pH 8.2), 15 μmole, Pb $(NO_3)_2$, 4 μmole; in 25 μl H_2O at 0°C for four days

out the radioactive zones and counted their radioactivity more accurately in a Beckman liquid scintillation counter.

Condensations: General Procedure

Reactions (Tables 2–11) were carried out in glass tubes with rubber stoppers. The mixtures were usually made up by addition of the components in the following order: Inorganic salts, buffer, the matrix compound (Na^+ salt), N and/or ImpN. The Pb^{2+} salt (lM solution) was added last at 0°C. For further details see Tables.

Identification of Products

Reaction products with chain lengths up to 3 were characterized by a sequence of enzymatic and alkaline degradations. The method was an adaption of the identification procedures described previously for $(pA)_n$ and $A(pA)_n$ oligomers (Lohrmann and Orgel 1979). Longer oligomers were less completely characterized by comparing their chromatographic mobilities with those of authentic markers.

Column Chromatography on RPC5

An HPLC analysis of an oligomer mixture obtained by polycondensation of Imp$\overset{*}{A}$ on (Pb–pT) was carried out as a separate experiment using Imp$\overset{*}{A}$ of high specific activity (see Fig. 1 for experimental details). We used the Waters Associates' solvent delivery system model 6000A fitted with a U6K injector and the solvent programmer model 660. The stainless steel column (0.39 x 30 cm) was packed with RPC5 material in the perchlorate form (Lohrmann and Orgel 1980). For elution we used a linear gradient, pump A delivered 0.002M Tris-$HClO_4$ (pH 8.0), while pump B delivered 0.002 M Tris-$HClO_4$ and 0.5M $NaClO_4$. The gradient went from 0.002M to 0.12M in 36 min at a flow rate of 1 ml/min and a pressure of ca. 1500 lb/in^2. The effluent was monitored at 259 nm with a model 450 variable wavelength detector (Waters Assoc.) connected to a Linear Instruments recorder. If effluate samples were re-analyzed by HPLC they were diluted 10 times with water before injection. Samples (1 ml) of the effluent were monitored for radioactivity by mixing them with Aquafluor (New England Nuclear) (10 ml) and counting them in a Beckman Scintillation Counter.

Discussion and Results

Table 2 shows the influence of various uracil (thymine) containing Pb^{2+} precipitates on the reaction between $\overset{*}{A}$ and ImpA. Two control reactions were used, one in which no matrix was present and another in which poly(U) served as template. Without a matrix (or a template) most of the adenosine remained unreacted. Also, the presence of uracil or uridine in the reaction mixture clearly had no effect on the incorporation of adenosine. However, when a nucleotide matrix was used, the incorporation increased. Judged by the extent of incorporation and the length of the labeled oligomers that were formed, the most efficient nucleotide matrices were those derived from pU, pdU, pT and Tp. The similarity between the oligomer distributions obtained in the presence of pU, pdU or pT and that obtained in presence of poly(U) is remarkable. The high yields of dimer formed on a pU- or pT-matrix are also noteworthy. Matrices derived from Up, pUp, UppU and pU^{ara} were much less efficient. Other experiments showed that matrices derived from p_2U, p_3U, p_2T and p_3T were almost inactive too.

The influence of the matrix is further reflected in the [3'–5'] to [2'–5']-isomer ratio of the $\overset{*}{A}$pA formed. In the absence of a matrix, the 3'-isomer content of $\overset{*}{A}$pA was 20%, while in the efficient matrix reactions it was reduced to as low as 2.8% (with pU). This might

Table 1. Chromatographic and electrophoretic mobilities

Compounds	System I[a]	System I[a] + borate	System II[a]	System III[a]	System IV[b]
A	1.68	2.28	1.77	0.45	0.00
C	1.46	2.04	1.93	2.18	0.00
G	1.22	1.67	1.68	1.19	0.03
U	1.44	2.10	1.98	2.02	0.00
pA	1.00	1.00	1.00	1.00	1.00
pC	0.98	0.98	0.90	2.28	1.12
pG	0.69	0.76	0.63	1.49	0.99
pU	0.96	1.04	1.30	2.40	1.13
A^2p	1.10	1.68	0.88	0.85	1.08
A^3p	1.10	1.68	0.88	0.62	1.04
C^2p	1.16	1.53	0.97	2.50	1.12
C^3p	1.16	1.53	0.97	2.28	1.12
G^2p	0.92	1.34	0.80	1.38	1.05
G^3p	0.92	1.34	0.80		1.02
U^2p	1.11	1.51	1.27	2.35	1.17
U^3p	1.11	1.51	1.27	2.22	1.17
ImpA	1.59				0.55
ImpC	1.56				0.65
ImpG	1.36				0.61
ImpU	1.46				0.68

Table 1. Cont.

Compounds	System 1[a]	System 1[a] + borate	System II[a]	System III[a]	System IV[b]
A^2pA	1.18	1.72	0.86	0.30	0.37
A^3pA	1.21	1.85	0.92	0.11	0.33
A^5pA	1.04	0.88	0.80	0.35	0.37
$A(pA)_2$	0.99		0.29		0.57
$A(pA)_3$	0.75		0.06		0.68
$A(pA)_4$	0.50		0.02		0.79
$A(pA)_5$	0.34		0.01		0.84
$(pA)_2$	0.88		0.41		1.05
$(pA)_3$	0.63				
$(pA)_4$	0.45				
$(pA)_5$	0.30				
$(pA)_6$	0.20				
C^2pC	1.19	1.80	1.17	1.66	0.51
C^3pC	1.20	1.74	1.17	1.41	0.49
$C(pC)_2$	0.88		0.42		0.74
$C(pC)_3$	0.77		0.15		0.91
$C(pC)_4$	0.60		0.04		
$C(pC)_5$	0.45		0.01		
$(pC)_2$	0.80		0.45		
$(pC)_3$	0.59		0.16		
$(pC)_4$	0.44		0.05		
$(pC)_5$	0.32		0.01		
G^2pG	0.86	0.94			
G^3pG	0.69	0.79			
$G(pG)_2$	0.42				
$(Gp)_2$	0.47				
$(Gp)_3$	0.26				
$(Gp)_4$	0.14				
$(Gp)_5$	0.07				
U^2pU	1.11	1.62	1.46	1.64	0.55
U^3pU	1.19	1.70	1.52	1.48	0.52
U^5pU	1.08	0.96	1.17	1.49	0.54
$U(pU)_2$	0.87		0.77		0.82
$U(pU)_3$	0.72		0.35	1.02	
$U(pU)_4$	0.55		0.19		
$(pU)_2$	0.73		0.67		
$(pU)_3$	0.59		0.32		
$(pU)_4$	0.48		0.14		
$(pU)_5$	0.38		0.07		
$(pU)_6$	0.28				
AppA	1.14		0.52		0.78
CppC	1.07		0.55		0.91
GppG	0.52		0.21		0.80
UppU	0.99		0.77		0.93

[a] R_f values are given relative to pA.
[b] Electrophoretic mobilities are given taking $R_{adenosine} = 0.0$ and $R_{adenylic\ acid} = 1.0$

be due either to the formation of a higher proportion of [2'–5']-bonds or to the preferential incorporation of the $\overset{*}{A}^3pA$ isomer into longer oligomers.

Table 3 shows the influence of increasing amounts of (Pb–pU) on the reaction between $\overset{*}{A}$ and ImpA. The incorporation of $\overset{*}{A}$ at first rises steadily and then levels off when the pU:ImpA ratio reaches about 6. With ribonucleotides other than pU in the matrix, the $\overset{*}{A}$ incorporation is increased only moderately relative to the control reaction. Furthermore, the presence of a pU-matrix increased the maximum chain length of the radioactive oligomers detected by paper chromatography up to 7 or higher (Table 3). Longer oligomers are probably present but cannot be detected by paper chromatography (see discussion of ImpA self-polymerization below).

Table 4 gives the product yields from the reaction between $\overset{*}{A}$ and ImpA, in presence of increasing amounts of (Pb–pT). Again, the incorporation of $\overset{*}{A}$ peaks at a matrix to substrate ratio of 6. The other deoxyribonucleotides have only a marginal effect on the reaction.

Table 2. Reaction of ImpA with $\overset{*}{A}$ in presence of an insoluble matrix derived from various uracil or thymine compounds. The yields are based on total radioactivity

Reaction products	Matrix containing –	Uracil	U	Up	pU	pU^{ara}	pUp	UppU	pdU[a]	pT	Tp[b]	poly(U)[c]
$\overset{*}{A}$	93.8	93.7	92.3	84.7	6.3	79.4	79.7	73.3	41.5	24.3	5.7	36.8
$\overset{*}{A}pA$[d]	6.2	6.3	7.2	13.3	72.7	18.7	17.6	23.3	34.1	63.1	73.6	32.4
	(20.1)	(20.6)	(18.9)	(15.6)	(2.8)	(17.5)	(6.0)	(18.4)	(20.8)	(4.7)	(3.8)	(16.2)
$\overset{*}{A}(pA)_2$	tr.		0.5	1.5	11.7	1.9	2.8	3.5	10.4	8.1	15.7	15.1
$\overset{*}{A}(pA)_3$				0.5	4.0				5.2	2.3	2.9	8.1
$\overset{*}{A}(pA)_4$					2.7				3.7	1.4	2.1	4.3
$\overset{*}{A}(pA)_5$					1.8				2.7	0.9	tr.	2.7
$\overset{*}{A}(pA)_{6+}$					0.9				2.5			0.5

Reaction conditions: $\overset{*}{A}$ (4 mCi/mmole), 0.1 μmole; ImpA, 1.15 μmole; $MgCl_2$, 10 μmole; NaCl, 20 μmole; Im-HCl (pH 8.0), 20 μmole; a uracil (thymine) derivative, 7 μmole (based on base content); $Pb(NO_3)_2$, 10 μmole; in 50 μl H_2O kept at 0°C for 6 days

[a]Reaction conditions as above except NaCl, 25 μmole; Im-HCl (pH 8.0), 25 μmole

[b]Reaction conditions as above except with Im-HCl (pH 8.2), 30 μmole and $Pb(NO_3)_2$, 15 μmole

[c]The amount of poly(U) was 3.5 μmole

[d]The numbers in parentheses give the percentage of $\overset{*}{A}pA$ that is exclusively [3'-5']-linked

A reaction between a radioactive nucleoside $\overset{*}{N}$ and an activated nucleotide pN leads to a single series or radioactively labeled oligomers $\overset{*}{N}(pN)_n$ (n = 1, 2 ...), since side products are not radioactive. This is not the case when the activated nucleotide itself is labeled. In Table 5 we show the condensation products formed with Imp$\overset{*}{A}$. Besides the expected series of $(p\overset{*}{A})_n$ oligomers we find pyrophosphates of the type $N^5p(p\overset{*}{A})_n$ in which the 5'-terminal phosphate is linked to a nucleotide pN which can be pA itself or the matrix nucleotide. With a ribonucleotide pN other than pA as matrix an additional series of the type $pN(p\overset{*}{A})_n$ arises, in which a $(p\overset{*}{A})_n$ oligomer is linked to pN by a phosphodiester bond. It should be noted, however, that only one terminal matrix ribonucleotide or pyrophosphate bond can be present in an oligomer. The yields of higher oligomers listed in Table 5 contain some contributions from the $(p\overset{*}{A})_n$'s, the N^5 $p(p\overset{*}{A})_n$'s and $pN(p\overset{*}{A})_n$'s, when ribonucleotide matrices were used. When deoxynucleotide matrices were employed the amounts of $pdN(p\overset{*}{A})_n$'s formed were negligible. The data in Table 5 show that with most matrices only small amounts of oligomers up to the tetramer were formed. The degree of oligomerization on pU or pT, however, is remarkable. The yield of pentamer and higher oligomers formed in presence of pT (25.3%) is very close to that obtained on a poly(U) template.

In a separate experiment with Imp$\overset{*}{A}$ on (Pb–pT) performed under somewhat different conditions (see Fig. 1) we found the following precentage yields by paper chromatography: ImpA (4.7%) ImpApA (9.1%), pA (20.7%), $(pA)_2$ (16.3%), $(pA)_3$ (19.9%), $(pA)_4$ (11.3%), $(pA)_5$ (9.7%) and $(pA)_{6+}$ (8.3%). When an aliquot of the reaction mixture was subjected to analysis by HPLC, a complicated pattern of UV-absorbing peaks was obtained (Fig. 1). For comparison we ran a mixture of $(Ap)_n$ markers on RPC 5 under identical condition. Comparison of the traces shows that UV-absorbing material from the reaction mixture is eluted up to the position of oligomers 20–30 units long. For confirmation we cochromatographed an aliquot (0.12 OD) of the reaction product with ca. 0.12 OD of the $(Ap)_n$ oligomers and counted the radioactivity that co-eluted with members of the $(Ap)_n$ series. We found that 89.2% of the counts were eluted with $(Ap)_{1-5}$, 2.9% with $(Ap)_6$, 2.2% with $(Ap)_7$, 1.3% with $(Ap)_8$, 1.1% with $(Ap)_9$, 2.3% with $(Ap)_{10-15}$, 0.5% with $(Ap)_{16-20}$, 0.29% with $(Ap)_{21-30}$, and 0.13% with $(Ap)_{31-40}$. We then reinjected aliquots of each of the eluted fractions into the HPLC and then reran them under the same conditions as before. In each case a large portion of the radioactivity cochromatographed with the corresponding UV-absorbing peaks of the appropriate $(Ap)_n$.

Previous experiments have shown that deoxynucleotides are poor acceptors in template-directed reactions, presumably because isolated hydroxyl groups are less nucleophilic than cis glycols (Lohrmann and Orgel 1977). We were not surprised, therefore, to find that the (Pb–pU)- or (Pb–pT)-directed reactions between $d\overset{*}{A}$ and ImpA or ImpdA produced very little oligomeric product.

When we started to investigate the reaction between $\overset{*}{G}$ and ImpG we performed a preliminary study similar to that reported in Table 2 for $\overset{*}{A}$ and ImpA. We used Pb-matrices containing the following nucleotides: pdC, pC, pC^{ara}, pCp, Cp and pC^{Me}. We also checked whether cytosine or cytidine in presence of Pb^{2+} ions had any effect on the reaction. Our results showed that of the above-mentioned compounds only pC, pdC and pC^{Me} were active.

Table 6 gives the results on some reactions of $\overset{*}{G}$ and ImpG in presence of various matrix nucleotides. Without a nucleotide, only 22% of the radioactivity was

Table 3. Reaction of ImpA with $\overset{*}{A}$ on a matrix containing increasing amounts of Pb-pU and comparison of Pb-pU with other nucleotide matrices. The yields are based on total radioactivity

	(Pb-pU) matrix (μmole)										(Pb-pN) matrix (10 μmole)					
Reaction	0	1	2	3	4	5	6	7	8	9	10	12[a]	pA	pC	pG	Ap	Cp	Gp	Up
$\overset{*}{A}$	91.8	87.7	78.4	66.0	37.3	15.6	9.3	7.1	4.9	4.4	5.4	9.4	73.0	78.3	83.2	62.4	79.0	66.7	82.2
$\overset{*}{A}$pA	8.2	11.3	19.2	25.1	45.7	61.3	64.9	56.0	60.8	62.3	58.4	60.9	22.0	19.2	14.7	21.1	19.0	27.8	14.7
$\overset{*}{A}(pA)_2$		0.9	2.4	5.9	11.6	16.3	17.9	24.1	22.4	21.7	24.2	17.2	5.0	2.5	2.1	13.8	2.1	5.6	2.5
$\overset{*}{A}(pA)_3$			tr.	1.7	2.1	2.5	2.7	3.6	4.2	3.6	3.4	3.9				2.8			0.6
$\overset{*}{A}(pA)_4$				1.3	2.3	1.9	2.0	3.5	2.1	2.2	2.7	3.1							
$\overset{*}{A}(pA)_5$					1.3	1.5	2.0	4.3	4.2	4.4	4.7	3.9							
$\overset{*}{A}(pA)_6^+$					tr.	1.0	1.3	1.4	1.4	1.5	1.3	1.6							

Reaction conditions: $\overset{*}{A}$ (3 mCi/mmole), 0.1 μmole; ImpA, 1.15 μmole; $MgCl_2$, 10 μmole, NaCl, 25 μmole; Im-HCl (pH 8.0), 25 μmole; a nucleotide for matrix formation as indicated; $Pb(NO_3)_2$, 10 μmole; H_2O, 50 μl; kept at 0°C for 6 days

[a]Performed in presence of 12 μmole $Pb(NO_3)_2$

incorporated. With increasing amounts of (Pb–pC) the yield of $\overset{*}{G}(pG)_n$ oligomers rose. The optimal matrix to substrate ratio was about 6, at which ca. 60% of the $\overset{*}{G}$ reacted. With the same amount of pdC in the matrix over 90% of the radioactivity was incorporated. However, it is remarkable that only 6.6% of the $\overset{*}{G}$ remained at the origin of the chromatogram when poly(C) was used as a template. Clearly, the (Pb–pC)-directed reaction yields large amounts of dimer and short oligomers, while the yields of longer oligomers are moderate compared with those obtained in poly(C). pU, pG and pA matrices seem to have an inhibitory effect on the reaction. In another series of experiments using pdA, pdG and pT as matrix components a similar inhibition was observed. Finally, it should be noted that the matrix-directed dimer has a lowered [3'–5']-isomer content.

In Table 7 we show the condensation products obtained from Imp$\overset{*}{G}$ on Pb^{2+} matrices. As in the case of Imp$\overset{*}{A}$ (Table 5) there are 3 series of oligomers: $(p\overset{*}{G})_n$, $N^5p(p\overset{*}{G})_n$ and $pN(p\overset{*}{G})_n$. The data show that the self-condensation of Imp$\overset{*}{G}$ even in absence of a complementary matrix or template proceeded well, giving oligomers in ca. 40% yield. Poly(A) gave 38%, and matrices derived from pA, pG and pU gave similar oligomer distributions. With dpC as matrix 51% of Imp$\overset{*}{G}$ reacted to give oligomers and with pC up to 60%. For comparison, poly(C) gave 72% incorporation with 58.5% of the counts at the origin. Details of the latter reaction are reported elsewhere (Lohrmann and Orgel 1980). It should be noticed that poly(U) also facilitated condensation to some extent.

In Table 8 we show the results obtained from the reaction between $\overset{*}{C}$ and ImpC on matrices. In the control without a matrix, ca. 70% of the $\overset{*}{C}$ remained unreacted. Among the nucleotides, pdG formed the most effective matrix, with 77% of the radioactivity being incorporated into oligomers. Surprisingly, pG had little effect on $\overset{*}{C}$ incorporation. Several variations of the original experiment with pG did not show a significant effect on incorporation.

In Table 9 we show the reaction products obtained from Imp$\overset{*}{C}$ under conditions similar to those of Table 8. A comparison of yields on an absolute basis is not possible because in some cases considerable amounts of Imp$\overset{*}{C}$ remained unreacted. From the data described, one can conclude that the self-condensation of Imp$\overset{*}{C}$ proceeded more rapidly on matrices derived from guanine containing nucleotides than on those derived from other bases. Among the deoxynucleotides, pdG clearly formed the most efficient matrix. When ribonucleotides were used in the matrix a considerable amount of the Imp$\overset{*}{C}$ reacted with the matrix material leading to the oligomers of the type $pN(p\overset{*}{C})_n$. Once again, pG is inactive as a matrix for the incorporation of Imp$\overset{*}{C}$ into oligomers.

Table 10 shows the results of the reactions between $\overset{*}{U}$ and ImpU on various matrices. Without a matrix nucleotide, only 13% of the $\overset{*}{U}$ condensed. The $\overset{*}{U}$ incorporation was improved by the addition of pA. Again, the best matrix to substrate ratio was 6 at which ca. 45% of the $\overset{*}{U}$ reacted. The similar efficiencies of pA and Ap as matrices are noteworthy. Oligomers up to the pentamer were detectable. Poly(A) showed little, if any, activity as template.

Table 4. Reaction of ImpA with $\overset{*}{A}$ on various Pb^{2+}-containing deoxyribonucleotide matrices. The yields are based on total radioactivity

	Pb-nucleotide matrix (μmole)													
	pT											pdA	pdC	pdG
Reaction products	0	1	2	3	4	5	6	7	8	9	10	7	7	7
$\overset{*}{A}$	95.6	91.2	58.8	35.2	16.5	32.9	16.2	4.1	6.4	8.9	9.8	83.9	80.0	86.0
$\overset{*}{A}$pA[a]	4.4	8.8	33.2	51.8	60.8	49.4	61.0	61.2	53.5	53.6	52.8	14.3	17.3	14.0
	(14.9)		(4.5)					(1.6)				(25.4)	(26.3)	(22.6)
$\overset{*}{A}(pA)_2$		tr.	3.2	5.7	9.3	6.3	8.1	8.3	9.6	7.8	8.6	1.8	2.7	tr.
$\overset{*}{A}(pA)_3$			3.2	4.2	6.2	7.6	4.4	11.6	13.4	14.5	13.5			
$\overset{*}{A}(pA)_4$			1.6	2.1	4.1	2.6	5.3	5.8	5.7	6.2	6.8			
$\overset{*}{A}(pA)_5$+				1.0	3.1	1.3	5.0	9.1	11.5	8.9	8.6			

Reaction conditions: $\overset{*}{A}$ (2.1 mCi/mmole), 0.125 μmole; ImpA, 1.12 μmole; $MgCl_2$, 10 μmole; NaCl, 20 μmole; Im-HCl (pH 8.0), 20 μmole; the matrix nucleotide as indicated; $Pb(NO_3)_2$, 10 μmole; H_2O, 50 μl; kept at 0°C for 3 days

[a]The numbers given in parentheses give the percentage of the $\overset{*}{A}$pA which is exclusively [3'-5']-linked

Table 5. Polycondensation of Imp$\overset{*}{A}$ on various Pb^{2+}-containing matrices. The yields are based on total radioactivity

	Pb-nucleotide matrix										
Reaction products	–	pA	pC	pG	pU	pdA	pdC	pdG	pT	poly(U)	poly(C)
ImpA	27.6	3.5	13.1	12.9	5.0	14.5	19.1	21.2	4.0	1.8	20.2
ImpApA	6.7	3.8	9.2	16.1	6.4	13.7	18.4	15.2	8.6	5.5	17.9
AppA AppN	7.0	13.8	6.6	14.5	4.0	7.6	6.6	6.9	4.7	2.6	4.5
pA	49.4	53.8	36.0	25.0	21.4	53.5	36.5	40.4	21.6	15.0	30.6
$(pA)_2$[a]	7.4	15.3	2.4	6.0	10.4	8.3	12.0	11.8	9.6	14.7	17.9
	(25)	(16)			(12)						
pNpA[a]			21.7	9.5	18.4						
					(25)						
trimer	2.0	7.7	8.3	9.7	21.4	2.4	5.7	3.0	16.6	20.0	6.7
tetramer		2.1	2.6	3.9	8.5		1.8	1.5	9.6	13.7	1.5
pentamer +				2.3	4.3				25.3	26.8	0.7

Reaction conditions: Imp$\overset{*}{A}$ (0.51 mCi/mmole), 1.25 μmole; $MgCl_2$, 10 μmole; NaCl, 20 μmole; Im-HCl (pH 8.0), 20 μmole; ± pN or pdN, 7 μmole, or poly(N), 2.5 μmole; $Pb(NO_3)_2$, 10 μmole; in H_2O, 50 μl, kept for 5 d at 0°C

[a]The numbers in parentheses give the percentage of dimer that is exclusively [3'-5']-linked

Table 6. Reaction of ImpG with $\overset{*}{G}$ on various Pb^{2+}-containing nucleotide matrices. The yields are based on total radioactivity

	Pb-nucleotide matrix (μmoles)												
	pC									pdC[b]	pU	pG	pA	poly(C)
Reaction products	0	2	4	5	6	7	8	9	10	7	5	5	5	2.5
$\overset{*}{G}$	77.8	75.0	75.4	60.6	46.5	58.1	40.4	45.1	46.3	8.3	83.1	85.5	80.5	26.3
$\overset{*}{G}$pG[a]	14.8	16.7	15.8	29.3	37.4	30.2	43.6	37.0	40.0	47.6	13.0	10.5	16.7	20.7
	(25.7)						(9.4)			(8.3)				(9.6)
$\overset{*}{G}(pG)_2$	4.9	4.8	8.8	7.1	11.1	8.1	10.6	9.8	8.1	22.0	3.9	4.0	2.9	13.9
$\overset{*}{G}(pG)_3$	2.5	3.6		3.1	5.1	3.5	5.3	8.1	5.6	15.5	tr.			2.4
Origin										6.6				36.7

Reaction conditions: $\overset{*}{G}$ (3.6 mCi/mmole), 0.11 μmole; ImpG, 1.13 μmole; $MgCl_2$, 15 μmole; NaCl, 30 μmole; Im-HCl (pH 8.0), 30 μmole; a matrix nucleotide as indicated and $Pb(NO_3)_2$, 10 μmole; in H_2O, 50 μl, for 4 days at 0°C

[a]The numbers given in parentheses indicate the percentage of $\overset{*}{G}$pG that is exclusively [3'-5']-linked

[b]Reaction conditions for pdC: $\overset{*}{G}$ (3 mCi/mmole), 0.10 μmole; ImpG, 1.15 μmole; $MgCl_2$, 10 μmole; NaCl, 20 μmole; Im-HCl (pH 8.0), 20 μmole; pdC, 7 μmole; $Pb(NO_3)_2$, 10 μmole; in H_2O, 50 μl, for 6 d at 0°C

Table 7. Condensation of Imp$\overset{*}{G}$ on various Pb^{2+}-nucleotide containing matrices. The yields are based on total radioactivity

Reaction products	Pb-nucleotide matrix (μmole)								
	–	pC 8	pdC 8	pA 8	pG 8	pU 8	poly(C) 2.5	poly(U) 2.5	poly(A) 2.5
ImpG	1.1	1.5	2.2	tr.	tr.	1.5	tr.		
unknown	4.2	3.1	1.0	3.3	3.9	4.4	3.8	5.1	4.2
pG	46.3	26.8	33.9	46.3	31.4	41.8	24.5	37.3	48.4
NppG		4.0	6.5	4.9		2.7			
GppG	7.4	4.9	5.7	6.2	17.7	3.7	tr.	8.5	9.5
pNpG		21.3		7.7		12.7			
$(pG)_2$ / $pN(pG)_2$	12.6	11.5	21.8	21.1	19.6	22.4	7.6	15.3	12.6
$(pG)_3$ / $pN(pG)_3$	12.6	12.3	12.9	5.7	17.7	7.3	5.7	10.2	12.6
$(pG)_4$	8.4	9.2	9.6	3.3	6.7	3.6	tr.	11.9	6.3
origin	7.4	5.4	6.5	1.6	3.0		58.5	11.9	6.3

Reaction conditions: Imp$\overset{*}{G}$ (0.4 mCi/mmole), 1.25 μmole; NaCl, 30 μmole; $MgCl_2$, 15 μmole; Im-HCl (pH 8.0), 20 μmole; nucleotide or polynucleotide as indicated and $Pb(NO_3)_2$, 10 μmole; in H_2O, 50 μl, kept for 4 d at 0°C

Table 8. Reaction of ImpC with $\overset{*}{C}$ on various Pb^{2+}-containing matrices. The yields are based on total radioactivity

Reaction products	Pb-nucleotide matrix (7 μmole)						
	–	pdG	pG	pdA	pdC	pT	pA
$\overset{*}{C}$	72.2	32.9	93.5	90.1	81.9	83.5	86.9
$\overset{*}{C}pC$[a]	20.9	52.4	5.8	8.1	14.3	13.6	11.2
	(38)	(89)		(45)	(28)	(44)	
$\overset{*}{C}p$							
$\overset{*}{C}(pC)_2$	7.0	12.2	0.7	1.8	3.8	2.9	1.9
$\overset{*}{C}(pC)_3$+	tr.	2.4					

Reaction conditions: $\overset{*}{C}$ (2.7 mCi/mmole), 0.11 μmole; ImpC, 1.14 μmole; NaCl, 15 μmole; Im-$HClO_4$ (pH 8.0), 25 μmole, with or without matrix nucleotide as indicated; $Pb(NO_3)_2$, 7 μmole; in 50 μl H_2O for 6 days at 0°C

[a]The numbers given in parentheses indicate the percentage of $\overset{*}{C}pC$ that is exclusively [3'-5']-linked

Table 9. Polycondensation of Imp$\overset{*}{C}$ on various Pb^{2+}-containing matrices. The yields are based on total radioactivity

Reaction products	–	pA	pC	pG	pU	pI	pdA	pdC	pdG	pT	Poly(I)
ImpC	29.0	50.0	27.3	11.0	24.1	16.5	55.1	43.3	21.7	38.5	27.5
ImpCpC	7.0	8.5	2.4	1.0	3.1	3.5	7.6	10.0	4.1	8.2	7.4
CppC / CppN	6.0	3.2	5.5	5.8	5.6	4.2	3.2	8.9	8.1	9.3	7.4
pC	34.0	24.5	17.9	40.3	23.7	26.4	29.7	27.2	32.1	30.2	34.4
$(pC)_2$[a]	14.0	4.0	40.5	10.5	11.2	2.0	4.3	7.2	22.2	10.4	14.3
			(38)						(74)		(22)
pNpC		7.7		21.2	23.4	38.0					
Trimer	5.5	2.1	6.3	8.8	6.8	7.1	tr.	3.3	7.7	2.8	4.8
Tetramer	2.5		tr.	1.5	2.1	2.4			2.6	0.6	2.1
$Pentamer_+$	2.0					tr.			1.6		2.1

Reaction conditions: Imp$\overset{*}{C}$ (0.26 mCi/mmole), 1.15 μmole; NaCl, 20 μmole; Im-$HClO_4$ (pH 8.0), 25 μmole; ± pN, pdN or poly(N), 8 μmole; $Pb(NO_3)_2$, 8 μmole; in H_2O (50 μl) for 5 d at 0°C

[a]The numbers given in parentheses give the percentage of the dimer which is exclusively [3'-5']-linked

Table 10. Reaction of ImpU with *U on various Pb^{2+}-containing nucleotide matrices. The yields are based on total radioactivity

Reaction products	Pb-nucleotide matrix (μmole)															
				pA				Ap	poly(A)	pC	pG	pU	pdA	pdC	pdG	pT
	0	3	4	5	6	7	8	9	10	8	2.5	7	7	7	8	8	8	8
*U	87.0	78.5	72.9	64.4	59.7	57.0	52.6	64.2	70.2	61.5	80.4	87.9	88.9	85.3	74.7	79.6	86.0	81.8
*UpU[a]	7.4	8.5	10.4	18.3	28.0	30.9	39.6	29.9	24.3	25.8	9.3	6.6	5.9	10.2	11.8	12.4	8.7	12.6
	(38)	(22)	(25)	(19)		(11)	(13)	(13)		(24)	(17)		(31)	(30)	(53)			(25)
*Up	4.6	9.8	11.5	7.3	4.7	6.6	3.9	4.0	4.0	3.2	7.5	3.3	3.5	2.6	7.9	4.2	1.1	2.4
*U(pU)$_2$	1.1	3.2	3.7	7.8	5.7	4.5	2.8	1.8	1.6	6.4	2.8	2.2	1.8	2.0	3.9	4.0	2.2	3.2
*U(pU)$_3$			1.6	2.2	1.9	1.0	1.0			2.1				tr.	1.1	tr.	1.8	tr.
*U(pU)$_4$+							tr.			0.9					0.6			

Reaction conditions: *U (3 mCi/mmole), 0.10 μmole; ImpU, 1.15 μmole, $NaClO_4$, 25 μmole; the matrix nucleotide as indicated; and $Pb(ac)_2$, 10 μmole; in H_2O (50 μl), for 4 d at 0°C

[a]The numbers given in parentheses indicate the percentage of *UpU that is exclusively [3'-5']-linked

Among the deoxynucleotides, the complementary pdA served as a somewhat better matrix than the other deoxynucleotides. Compared to pA, it tends to give longer chain oligomers, though the incorporation of radioactivity is less than that observed with (Pb–pA). pA-matrix reduces the 3'-isomer content of *UpU while Ap and poly(A) lead to somewhat higher contents of the 3'-isomer. The high 3'-isomer content obtained on the Pb-pdA matrix (53%) is surprising.

Table 11. Polycondensation of *ImpU on various Pb-containing matrices. The yields are based on total radioactivity

Reaction products	–	pA	pC	pG	pU
ImpU	49.1	8.9	37.3	19.6	33.8
ImpUpU	34.3	42.0	24.5	44.4	24.2
pU	6.0	6.7	7.5	7.9	8.6
UppU	8.1	14.1	5.6	6.5	28.2
NppU	(19)	(7)	(33)		(25)
$(pU)_2$[a]		9.6	14.5	16.8	
pNpU[a]		(9)	(39)	(24)	
trimer	0.9	9.7	4.2	2.8	5.1
UppUpU	1.6	4.0	6.4	1.9	tr.
NppUpU		3.5			
tetramer		1.6			
pentamer+					

Reaction conditions: *ImpU (0.24 mCi/mmole) 1.25 μmole; NaCl, 25 μmole; Im-$HClO_4$ (pH 8.0), 10 μmole; ± pN, 8 μmole; $Pb(ac)_2$ (pH 7.3), 10 μmole; in H_2O, 50 μl, kept for 5 d at 0°C

[a]The number in parentheses indicate the percentage of dimer that is exclusively [3'-5']-linked

Finally, Table 11 gives the results obtained by the self-condensation of *ImpU in presence of the Pb^{2+} ion. Without a matrix nearly 50% of the *ImpU remained unreacted while only about 10% was incorporated into oligomers. The presence of a nucleotide matrix increased both the rate of reaction and the yield of oligomer. (Pb–pA), the matrix with the complementary base, gave the best overall yield. It must be noted that matrices derived from other nucleotides, e.g. from pC, also led to considerable oligomerization. As with *ImpC the complementarity principle in the *ImpU reactions is not well expressed. However, the fact that the dimer formed on (Pb–pA) contained 93% of 2'-isomer while in all the other reactions the content of this isomer was between 66 and 81% suggests that base-pairing with the matrix had an influence on the reaction.

General Discussion and Prebiotic Significance

In this paper we confirm that Pb^{2+} ion is an efficient catalyst for the self-condensation of activated nucleotides (Sawai and Orgel 1975; Sawai 1976; Sleeper and Orgel 1979). More importantly, the results suggest that the same base-complementarity rules that govern template-directed reactions, in vitro, and nucleic acid replication, in vivo, to some extent control matrix-directed reactions. The results are most clear-cut in the reactions between a radioactively-labeled nucleoside and the corresponding 5'-phosphorimidazolide. In the reaction between *A and ImpA for example up to 95%

of the radioactivity is incorporated into oligomers in the presence of certain uracil- or thymine-containing matrices (Tables 2–4). Similar results are obtained when $\overset{*}{G}$ and ImpG react in the presence of some C-containing matrices (Table 6).

The 5'-phosphorimidazolides of U and C do not undergo template-directed reactions because organized helical structures are not formed between purine homopolynucleotides and monomeric derivatives of the complementary pyrimidines. The finding that derivatives of U and C undergo matrix-directed condensation is, therefore, surprising. It is important, because it suggests that template-directed incorporation of pyrimidines on purine-containing polymers may be possible under appropriate conditions, even if organized helical structures are not stable in aqueous solution.

One of the most striking features of our results is the effect of small changes in the structure of the matrix nucleotide on the outcome of the reaction. A pG-matrix, for example, has very little effect on the condensation reaction of ImpC, while a pdG-matrix directs an efficient condensation reaction (Tables 8, 9). We do not understand these and other related examples of matrix specificity in detail, but we believe that the differences between the effects of related matrices must have their origin in the nature of the exposed surfaces of the microcrystals making up the precipitate. Perhaps the bases are exposed in the case of (Pb–pdG), but buried in the case of (Pb–pG). Alternately, it may be that they are exposed in both cases, but only in the case of pdG are they in a relative orientation that permits reactions between ImpC molecules attached to them by Watson-Crick base-pairing. Similar arguments may explain the difference between poly(U) and poly(T); the former is an efficient template, while the latter has little effect on the self-condensation of ImpA in presence of Pb^{2+} ions (Lohrmann, unpublished).

The importance of the detailed structure of the template or matrix is further emphasized by the great variation that is observed in the ratio of the yields of [2'–5']- to [3'–5']-linked oligomers. The 2'-OH group is intrinsically more reactive than the 3'-OH group in nucleosides (Lohrmann and Orgel 1978). Consequently [2'–5']-linked oligomers are to be expected as the predominant product in most reactions. However, the structure of the template and the nature of the metal-ion catalyst can reverse this trend. The Pb^{2+} ion catalyzes the formation of [3'–5']-linkages in the poly(U)-directed self-condensation of ImpA (Sleeper et al 1979) while the Zn^{2+} ion is a remarkably regio-specific catalyst for the formation of [3'–5']-linked oligomers in the poly(C)-directed oligomerization of ImpG(Lohrmann et al. 1980; Bridson and Orgel 1980). The reactions reported in this paper include a striking example of regiospecific catalysis. While ImpA, ImpG and ImpU give predominantly [2'–5']-linked products on complementary matrices, ImpC condenses with C on (Pb–pdG) to give a dimeric product that is 89% [3'–5']-linked.

The results in Tables 2–11, taken together, reemphasize the importance of Watson-Crick base-pairing. There are a few cases in which a non-complementary matrix catalyzes oligonucleotide formation, but the effects are always small. Perhaps they can be explained in terms of non-classical hydrogen-bonding interactions. However, the most impressive results are always obtained with Watson-Crick base pairs. The importance of the Watson-Crick base pairs even when the geometrical constraints are different from those in a double helix could not have been predicted.

There has been much speculation about the origin of polynucleotides on the primitive Earth, and about the nature of catalysts involved in this formation. Many model reactions have been proposed. Since condensation of nucleotides in aqueous solution are inefficient, it has often been suggested that certain minerals present on the primitive Earth adsorbed activated nucleotides and facilitated their condensation, in situ, into polynucleotides. The importance of metal ion catalysis in this context has been stressed repeatedly. Some lead minerals, for example, have been shown to act as catalysts for the self-condensation of nucleotides (Sleeper and Orgel 1979). Our finding that precipitates formed from a nucleotide with Pb^{2+} ions have catalytic activity in the formation of internucleotide linkages and that the reactions are base-specific, is important in this prebiotic context. Wherever nucleotide chemistry evolved on the primitive Earth, nucleotides could be precipitated by metal ions or could be absorbed to mineral surfaces. Our experiments suggest that certain nucleotide precipitates could then have acted as matrices, directing oligonucleotide formation, principally by classical Watson-Crick base-pairing.

Though our experiments should only be understood as model reactions for the evolution of RNA molecules, they suggest that the principle of base-specificity via H-bonding came already into play at a very early stage of molecular evolution, when preactivated monomers were able to condense in presence of catalytically active nucleotide matrices. Due to the increasing polyanionic character of the oligomers formed under these conditions, they would have gradually displaced the inactive monomers in the matrices and started to act as primitive templates for the condensation of activated nucleotide monomers and/or oligomers. Matrix-directed reactions would so have been gradually replaced by template-directed condensations.

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