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LOOKING FOR CATALYSIS IN THE TEMPLATE-DIRECTED NON-ENZYMATIC POLYMERIZATION OF RNA: ACRIDINE-LABELLED OLIGONUCLEOTIDES AS A TOOL FOR THE RAPID AND PRECISE DETERMINATION OF CHAIN ELONGATION KINETICS.

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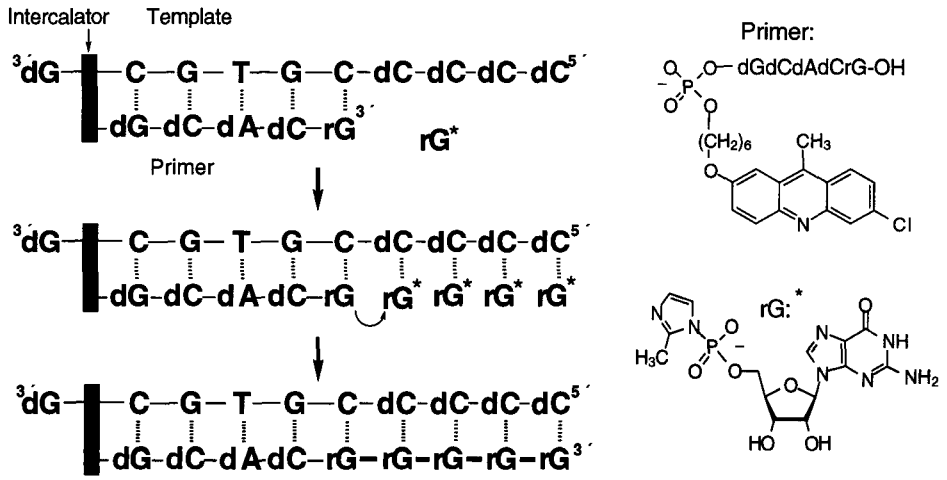
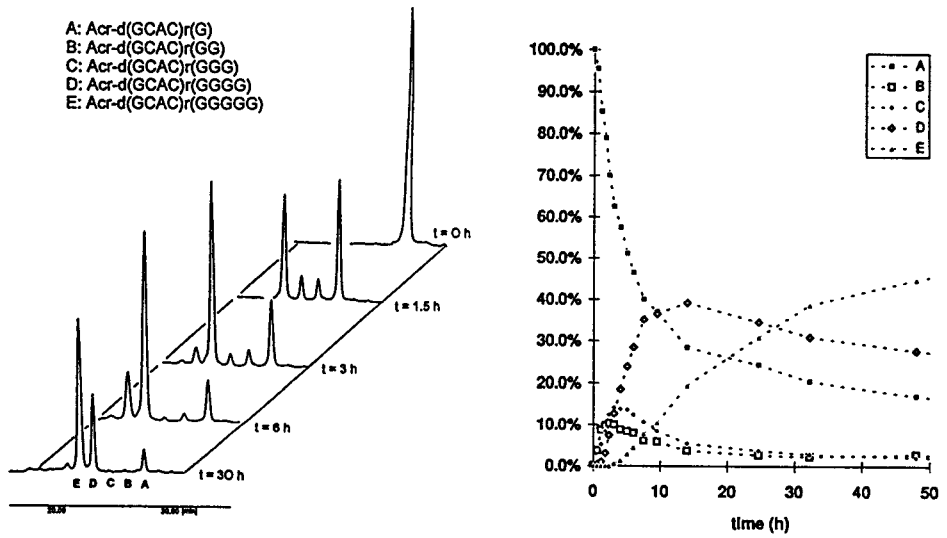
The template controlled polymerization of ribonucleotides can be amazingly efficient in certain cases (Wu and Orgel 1992). However, the nonenzymatic self-replication of RNA (using mononucleotides) is hampered by severe problems. It seems unlikely that this process is possible without the aid of catalysts. While ribozymes are plausible candidates much more simple compounds like organic polycations (Barbier et al. 1993) should be considered as catalysts too. In order to test this idea we had to develop an experimental setup for the detection of even minor influences on the chain elongation kinetics. Furthermore the experiment should be rapid and flexible to allow a broad screening program.



Towards this end we synthesized a new acridine dye with enhanced chemical stability. This dye (6-chloro-2-alkoxy-9-methylacridine) could be

efficiently coupled to short oligodeoxynucleotides bearing a ribonucleotide at their 3' position. In the presence of complementary DNA or RNA templates, activated mononucleotides and Mg^{2+} chain elongation of the primer oligonucleotide occurred. In the experiment shown here four guanosine nucleotides were attached to the primer Acr-d(GCAC)rG in high yield. Due to the lipophilic nature of the dye and its fluorescence a rapid separation and sensitive detection of primer and elongation products was possible by HPLC (Schütz et al. 1995, see below).



HPLC-analysis of primer elongation (10°C) and time dependent species distribution

It turned out that the HPLC pattern produced in these reactions after constant time is highly reproducible and it responds to the addition of organic cations, some of them causing pronounced inhibition. The recent results from our screening will be presented as well as improved versions of the polymerisation experiment shown in scheme 1.

Barbier, B., Visscher, J. and Schwartz, A.W. : 1993, J. Mol. Evol., 554 - 558.

Schütz, K., Kurz, M. and Göbel, M. : 1995, Tetrahedron Lett., 8407 - 8410.

Wu, T. and Orgel, L.E. : 1992, J. Am. Chem. Soc. *114*, 317 - 322, 5496 - 5501 and 7963 - 7969.