Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5450–5454, September 1982
Biochemistry

(tRNA/rRNA/RNA-RNA crosslink/rRNA function/cyclobutane dimer)

zyme-to-substrate ratio of 1:2 (wt/wt) for 1 hr at 37°C, and dried under reduced pressure. When the oligonucleotides were to be end-labeled, bacterial alkaline phosphatase was also added to the incubation mixture at a final concentration of 5 units/ml and RNA was recovered by extraction with phenol and precipitation with ethanol. The dephosphorylated oligonucleotides were

is U-A-C-A-C-A-C-C-G_{1401} (6). By contrast, a comparable digest of the crosslinked adduct, 1_U, yielded just three products, U and A-C in a molar ratio of 1:3, and a component designated P1 that remained close to the origin (Fig. 2*a*, lane 1). The lack of free C and G in this instance indicates that either C_{1400} or G_{1401} is the site of tRNA attachment. Although the low mobility of spot P1 suggested that it alone contained the crosslinked material, all three products were eluted from the electrophoresis gel and digested with RNase T1. Each of the resulting oligonucleotides was then labeled with [5′-^{32}P]pCp at the 3′ end by using RNA ligase and fractionated by gel electrophoresis. In this case, two crosslinked T1 products, bands 1_E and $1'_E$, were identified by

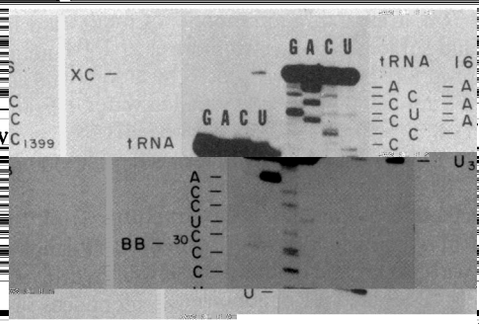
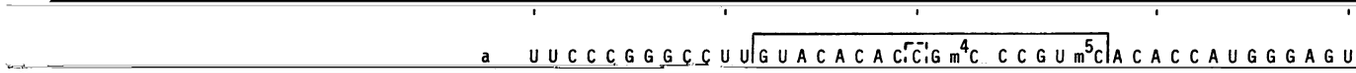


Fig. 1*c*; compare

NA from band 1_E

trophoresis alongside the unirradiated portion (Fig. 1*d*). Here again photoreversal released only one major radioactive component,


a UUCCCGGGCCUUGUACACACCGm4C CCGUm5CACACCAUGGGAGU