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Hybridization of oligodeoxynucleotide probes to RNA molecules: specificity and stability of duplexes

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Hybridization of oligonucleotides to DNA can be abolished by a single mismatch; this allows the detection of single base changes in a DNA sequence (1). Oligonucleotide hybridization to detect single-base changes in RNA has not been thoroughly studied. We have used this technique to measure the relative abundance of two mRNA isoforms of the hnRNP protein A1 that are different for only 2 in 1700 nucleotides (in publication). One such substitution (A→U) was chosen for selective hybridization. Two 15n long oligodeoxynucleotides complementary to the two RNAs and centered around the substituted nucleotides were synthesized (I and II) while the RNAs corresponding to the two forms (α and ß) were obtained by in vitro transcription of the corresponding cDNAs. The results of the slot-blot hybridizations carried out as reported in the legend to Fig. 1 are the following: 1) Each of the two oligos hybridizes selectively to the cognate RNA, thus, as in DNA-DNA hybrids, conditions can be found where a single mismatch in 15 bp completely abolishes duplex formation. 2) The stability of perfectly paired DNA-RNA duplexes can be quite different. An increases of 7°C destabilizes oligo II but not oligo I. This is a novel confirmation of theoretical predictions on the effect of sequence on duplex stability (2). In conclusion oligonucleotide hybridization can selectively discriminate RNA molecules. However conditions should be carefully controlled to avoid an erroneous evaluation of results.

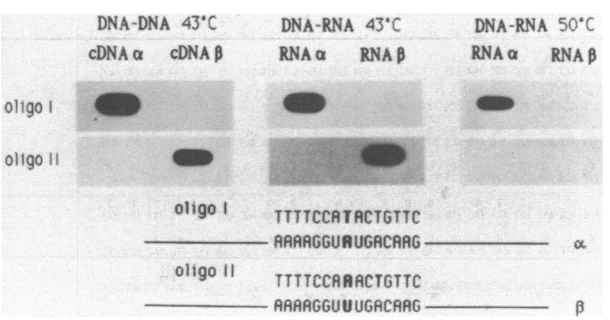

Fig. 1: 100 ng (DNA or RNA) were loaded per slot. Filters were hybridized with end-labeled oligos (10^6 cpm/ml; sp.act.: 10^9 cpm/μg) at 32°C ON in 6xSSC, 10 x Denhardt, 0.1% SDS, 125 μg/ml yeast tRNA, 25 mM Na-phosphate and then rinsed in 6xSSC for 2 min at 43°C or 1 min at 50°C.

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