Is mRNA transcribed from the strand complementary to it in a DNA duplex?

We have tested the assumption that the messenger RNA sequence is uniquely determined by the DNA sequence complementary to it. Because RNA is usually transcribed from a DNA duplex, there is the possibility that the DNA strand that is not complementary to the mRNA influences the RNA base selection. This possibility exists in models that posulate linear-with-like base pairs, triple helix models of DNA-DNA-RNA, triple base interactions such as those described in RNA, and models in which RNA polymerase may confer a special type of specificity (non-Watson-Crick) to RNA base-DNA duplex interactions, as well as in experiments in which separated were compared with non-separated strands.

The logic of our experiment is as follows. If one makes DNA heteroduplexes in which one strand has the coding for the premature termination of a given protein, while the other strand has the coding for the complete protein, and then uses these heteroduplexes as templates for transcription-translation in a coupled cell-free system, the product should be the complete protein, a fragment or neither? If coding for RNA synthesis was solely a function of the nucleotide sequence of only one strand of a DNA duplex, then no matter...

Fig. 1 Autoradiography of urea SDS-Tris-glycine acrylamide gels showing the in vitro protein products stimulated by various homo- and hetero-duplex DNAs. Gene products were identified as described before and are denoted by Roman numerals. DNAs used as template for incorporation shown in (a) and (b) were homoduplex (had not been subjected to cleavage, denaturation and renaturation). DNAs used were: (a) WT homoduplex; (b) amber IV, and VIII homoduplex; (c) amber IV, and VIII; WT; (d) amber IV, and VIII; WT; amber IV, and VIII; GTK and amber IV, and VIII; WT; amber IV, and VIII; WT...
which or how many termination signals were on the other strand. A complete protein would be made from at least one of the two possible heteroduplexes. Furthermore, if the results indicate that only one strand actively codes for RNA and the resultant protein, one can determine which strand is active.

To test these hypotheses, we constructed heteroduplex DNA molecules using coliphage f1 DNA. RNA transcribed from this DNA, both in vivo and in vitro, is complementary to only one of the DNA strands, the non-phase or (−-strand)
. The heteroduplexes were constructed by annealing plus strands obtained from phage with minus strands obtained from intercellular double-stranded-replicative form DNA (RFI) as described by Vovis et al. Six types of heteroduplexes were constructed using two distinct gene IV amber mutants; these heteroduplexes included all permutations of amber and wild type genes in both plus and minus strands.

As Figs 1 and 2 show, complete proteins are synthesised by the coupled transcription-translation system whenever the minus strand of the heteroduplex is in the wild type with regard to gene IV. When the minus strand contains a gene IV amber mutation, the gene IV product is not made. Because of intrinsic sensitivity limitations of the assay system, we would not have been able to detect effects on protein synthesis comprising less than 10% of the wild type control.

These results thus support the hypothesis that the information for RNA transcription of structural genes is not strongly affected by a single base change in the duplex strand not complementary to the mRNA synthesized. We do not know what the effect of other sorts of changes would be.

All of the heteroduplex DNA molecules were constructed by a method which includes the use of endonuclease R-Hind to cleave f1 RFI. This endonuclease makes a single, unique double-strand break in this DNA, which is located within gene II on the template strand. It is interesting to note that some gene II product was synthesised in reactions primed by the circular heteroduplex DNA molecules which initially contained a single-stranded break within gene II. This suggests that transcription may not be affected by the presence of a nick in the template strand, although further experiments are needed to establish the point.

This experiment was designed to test the transcription rules for structural genes coding for proteins, and showed that only the template strand seems to be significant. DNA which is

heteroduplex in regions corresponding to promoters, operators, or transcription termination regions might not function normally, since in these instances DNA-protein recognition is quite likely to involve both strands of a duplex, and there is no a priori reason to think that function is limited to one strand.

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The Rockefeller University
New York, New York 10021

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Template activity of f1 RFI cleaved with endonucleases R-Hind, R-EcoP1 or R-EcoB

ENDONUCLEASE R-Hind makes a single, unique, double-stranded break in the double-stranded circular DNA (RFI) which can be isolated from bacteria infected with bacteriophage M13 (ref. 1); fI (refs 2 and 3). or fI (Horiiuchi and Zinder, unpublished observations). We wish to report that fI RFI cleaved by endo R-Hind acts as a template for protein synthesis in a coupled in vitro transcription-translation system, and that with the exception of the product of gene II of fI the normal complement of in vitro phage-specific proteins is made. No prominent new polypeptide is observed (Fig. 1); this suggests that a substantial fragment of the gene II product is not coded for by RFI cleaved with endo R-Hind. In addition, neither the size nor the relative yield of the product of the proximal gene IV, gene IV, is affected. This implies that the break is likely to be in a region extending from the C-terminus of gene IV to the N-terminal portion of gene II. A similar location has been assigned to this break with the use of entirely different methods by Seeburg and Schaller.

Endo R-Hind is one of a class of endonucleases which cleave DNA at specific sites and do not require a specific cofactor. The full length, linear, double-stranded DNA