

Deeper insight into the nature of the proton transfer to water could doubtless be achieved if neutron scattering studies were made on progressively dehydrated (or hydrated) samples of HSAPC-34.

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Mutagenesis in Mammalian Cells Induced by Triple Helix Formation and Transcription-Coupled Repair

Gan Wang, Michael M. Seidman, Peter M. Glazer*

When mammalian cells were treated with triplex-forming oligonucleotides of sufficient binding affinity, mutations were specifically induced in a simian virus 40 vector contained within the cells. Triplex-induced mutagenesis was not detected in xeroderma pigmentosum group A cells nor in Cockayne's syndrome group B cells, indicating a requirement for excision repair and for transcription-coupled repair, respectively, in the process. Triplex formation was also found to stimulate DNA repair synthesis in human cell extracts, in a pattern correlating with the inhibition of transcription in such extracts. These findings may have implications for therapeutic applications of triplex DNA and raise the possibility that naturally occurring triple helices are a source of genetic instability.

Triple helices can be formed when oligonucleotides bind in the major groove of duplex DNA at polypurine-polypyrimidine sequences (1). Triple helix formation has been used to block transcription initiation and elongation (2) and to cleave DNA *in vivo* (3). We have explored the use of triplex-forming oligonucleotides (TFOs) as a mechanism to deliver a tethered mutagen to a selected gene for the site-specific introductions of DNA damage and consequent mutations within cells (4, 5). In the course of this investigation, we observed that TFOs have the potential to induce mutations *in vivo* even in the absence of a tethered mutagen.

Three oligonucleotides (AG10, AG20, and AG30) were designed to bind as third strands in the antiparallel triple helix motif (1) to part or all of a 30-base pair (bp) polypurine-polypyrimidine target site in the

supFGI reporter gene within the simian virus 40 (SV40) vector, pSupFGI (5, 6). These oligonucleotides were tested for their

ability to induce mutations in the vector within monkey COS cells (Table 1). The cells were first transfected with SV40 vector DNA (7). After 12 hours to allow chromatinization of the vector, the oligonucleotides were added to the growth medium at a concentration of 2 μ M (8). Two days later, the vector DNA was harvested from the cells for analysis of supFGI gene mutations (9). Oligonucleotide AG30 generated mutations in the target gene at a frequency of 0.27%, 13 times above the spontaneous background in the assay. In contrast, AG10 and AG20, which show weaker third-strand binding to supFGI, were less effective in producing mutations. As an additional control, an oligonucleotide of 30 nucleotides consisting of a mixture of all four bases (Mix30) (5, 6) was also tested. This oligonucleotide did not form a detectable triple helix with supFGI (5) and it did not generate any mutagenesis above the background. The supFGI mutations generated



Fig. 1. Sequences of supFGI mutations induced by triple helix formation in COS cells. Three classes of mutations were observed, including single point mutations, deletions, and multiple, simultaneous point mutations, as indicated. The base substitutions listed above the corresponding supFGI gene sequence represent changes with respect to the upper strand. The multiple point mutations are underlined, with each set of simultaneous changes presented on a separate line. Point mutations occurring outside of the listed sequence are indicated by position numbers, with the involved base changes given. The deletions are presented below the gene sequence, with the deletion end points indicated. The triple helix target site at base pairs 167 to 196 of the supFGI gene is indicated.

G. Wang and P. M. Glazer, Department of Therapeutic Radiology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040, USA.
M. M. Seidman, Oncopharm, 200 Ferry Parkway, Gaithersburg, MD 20827, USA.

*To whom correspondence should be addressed.

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