Chimeric cDNA clones: a novel PCR artifact

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During the cloning of a transcript of one member of a closely related gene family, the human γ-crystallin gene family (1), we encountered a novel artifact of the polymerase chain reaction: the formation of chimeric cDNA molecules. Our experimental strategy in cloning the human γE-crystallin transcript was a common one (2): first strand cDNA synthesis on human lens RNA using AMV reverse transcriptase and a γE specific primer followed by PCR with the same γE specific primer as reverse primer and a common γ-crystallin forward primer (see Fig. 1; the sequence similarity between the γ-crystallin genes precludes the synthesis of a gene specific 1st exon primer). The resulting fragment was purified and cloned into M13 mp vectors. Sequencing of three of these clones, however, revealed that two were chimeric, switching from either the γC or γD sequence to the γE sequence in the 3d exon. The third one contained a correct γE transcript (Fig. 1). These chimeric sequences could have resulted from somatic recombination or trans-splicing but are more likely an experimental artifact. Since the chimeric clones end with γE sequence, the initial reverse transcription reaction must have been specific for the γE transcript. However, we had noted that reverse transcription often yielded prematurely terminated γE cDNAs. We reasoned that such partial γE cDNAs could have hybridized to the γC or γD transcripts (which are 10 or 25 fold, respectively, more abundant than the γE transcript; unpubl. data, 1) and served as primer for reverse transcription by Taq polymerase (3). As the 5′ PCR primer fits the γC and γD sequences as well, such chimeric molecules would have been amplified in the PCR reaction. To test this hypothesis we repeated the experiment but included a RNase A treatment after first strand synthesis. Five out of five recombinant clones contained the correct γE transcript and no chimeric clones were found. We thus conclude that the synthesis of the chimeric cDNA clones is a PCR artifact caused by the reverse transcriptase activity of Taq polymerase. Hence, this reverse transcriptase activity is actually a drawback rather than an advantage during cDNA cloning.

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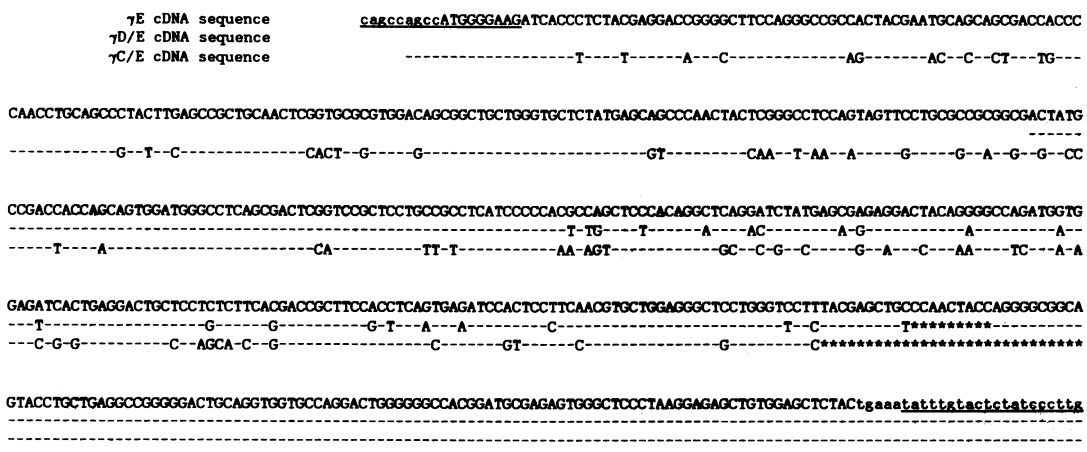

Figure 1. The sequence of the chimeric γE cDNA clones. The γE cDNA sequence is shown in full. For the γD/E or γC/E sequences only differences are specified. The sequence of the γD/E cDNA was determined only in part. The regions where the sequences of the γD/E or γC/E cDNA clones switch from those of the γD or γC genes to that of the γE gene are indicated with asterisks (note that due to the sequence identity the exact 'cross-over' point cannot be determined). Coding sequences are capitalized. The primers used for cDNA synthesis and PCR are underlined.

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