Antisense Experiments Using Morpholinos

The strategy of using anti-sense nucleotides to block gene expression, as discussed on pages 278-279, has taken many different forms. One of the best uses is the application of a synthetic structure similar to an oligonucleotide known as a Morpholino. (Morpholino is the tradename given by Gene Tools and AVI BioPharma to these molecules, which are not found in nature.) The structure of a Morpholino is shown in the Figure below, with the comparison to an RNA sequence on the other strand. Note that on the Morpholino, the sugar ring of the nucleotide (deoxribose or ribose) is replaced by a chemically similar morpholine ring. Rather than the familiar phosphodiester backbone of nucleic acids, the morpholine rings are connected by very stable phosphorodiamidate linkages. The nucleoside bases adenine, guanine, cytosine, and thymine are the same as those for DNA, which allows the possibility of forming a complementary base-paired structure between the Morpholino and a nucleic acid.

Morpholinos are typically synthesized to have about 25 bases, enough to provide a transcript-specific interaction with the complementary sequence in mRNA. Unlike RNAi and microRNA interactions, however, the interaction between mRNA and its complementary Morpholino to form a double-stranded structure does not trigger degradation of the mRNA but instead appears to block translation of the message. They are not charged, and thus do not have ionic interactions with proteins. Because the rings are not nucleotides and the backbone is not a phosphodiester, the Morpholino is not targeted for degradation by endonucleases and does not trigger an innate immune response. Thus, Morpholinos work by the antisense mechanism that many assumed lay behind the initial antisense nucleic acid experiments.

Careful controls have shown that Morpholinos block protein expression extremely well for a wide range of different transcripts, usually with few effects other than those on the target sequence. However, it has been recommended that an independent assay be used to monitor their effectiveness, such as using an antibody to the protein product of the gene of interest.

Morpholinos have been very widely used in vertebrates with large, easily-injected eggs and embryos, such as frogs and fish, and in some invertebrates such as sea urchins. They have also been used in cultured cells, with electroporation as the means for delivery. They have not been widely adopted in other organisms, in part because techniques involving RNAi have been so well-developed in many other organisms.

So, how does the Morpholino have its effect? A Morpholino sequence complementary to the 5’ end of the target mRNA is synthesized commercially. This is then injected into the egg or embryo, which blocks the binding and progression of the ribosomal initiation complex, and thus prevents translation. Other applications have used Morpholinos to block RNA splicing of a specific exon by binding at the splice donor or acceptor sites, resulting in exclusion of an exon or alternative and modified splicing. The effect of the Morpholino lasts as much as 5 to 7 days, long enough that injection into a fertilized frog or fish egg blocks expression until after most of organogenesis has occurred.

As with any technique that relies on base complementarity, off-target effects are a concern. One common control is to co-inject the Morpholino with a modified mRNA for the

target gene. The modified mRNA codes for the same amino acid sequence as the original target gene but with a different nucleotide sequence. Thus, the co-injected mRNA is not blocked by the Morpholino and the wild-type function is restored. Such a control demonstrates that the Morpholino effect is specific for its target.

Morpholinos have also been suggested as promising agents for blocking the pathogenicity of bacteria and viruses, and for blocking the splicing defects that lead to Duchenne's muscular dystrophy. Although their research applications have been most important in organisms for which standard genetics and reverse genetic approaches are limited, Morpholinos may prove to be nearly as important as RNAi for blocking gene expression.

References

Corey and Abrams, 2001 Genome Biology 2: 1015.1-1015.3
Eisen and Smith, 2008 Development 135: 1735-1743

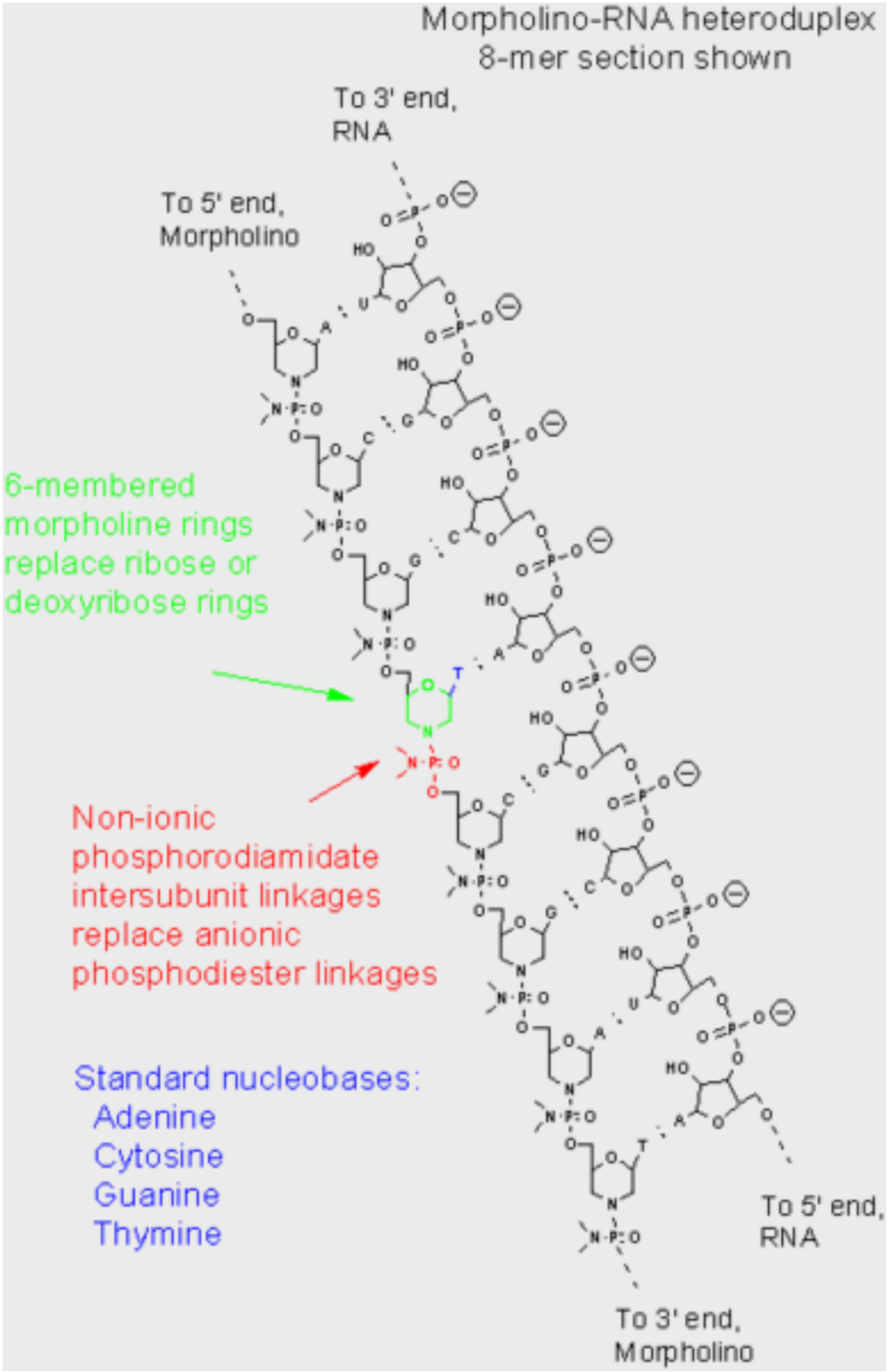

Figure. A comparison of the Morpholino structure with RNA. Note that Morpholinos have the same bases as DNA but a morpholine ring rather than a deoxyribose sugar, and a different, non-ionic backbone. Like nucleic acids, Morpholinos have a 5' and 3' direction.