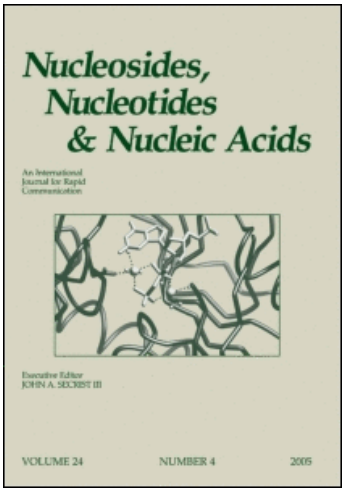

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Modulation of Splicing in the *DMD* Gene by Antisense Oligoribonucleotides

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VII. BIOLOGICAL STUDIES OF OLIGOMERS

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MODULATION OF SPLICING IN THE *DMD* GENE BY ANTISENSE OLIGORIBONUCLEOTIDES.

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ABSTRACT
Splicing of the *DMD* gene pre-mRNA is being examined as a model system to study the skipping of mutant exons, especially where disrupted translational reading frames are restored. Naturally-occurring examples and induced exon skipping by specific synthetic RNA oligonucleotides are under investigation.

Genetic mutations underlying the X-linked myopathy Duchenne muscular dystrophy (DMD) normally result in premature truncation of the large cytoskeletal protein, dystrophin[1]. The C-terminus of dystrophin seems to be the most critical functional domain of the molecule in skeletal muscle and nerve cells, binding a complex group of proteins/glycoproteins at the cell surface to mediate a connection between the extracellular matrix and the intracellular cytoskeleton[2]. Indeed, mutations in the 2.3Mb *DMD* gene which do not disturb the reading frame cause a much milder myopathy, Becker muscular dystrophy (BMD)[3]. While viral vector based approaches toward a gene therapy for DMD have achieved limited success in the *mdx* mouse model of DMD[4-6], problems remaining include (i) packaging of large DNA molecules, such as the dystrophin cDNA, in viral particles, and (ii) immune responses to both vector and recombinant gene product. As an alternative strategy, we are developing a method to restore perturbed reading frames during the splicing process in dystrophin-deficient muscle which circumvents the need for viral vectors or large recombinant genes.

Initial investigations are designed to examine the effect on pre-mRNA splicing of specific intron and exon sequences adjacent to a murine dystrophin mutation (nt 3185,

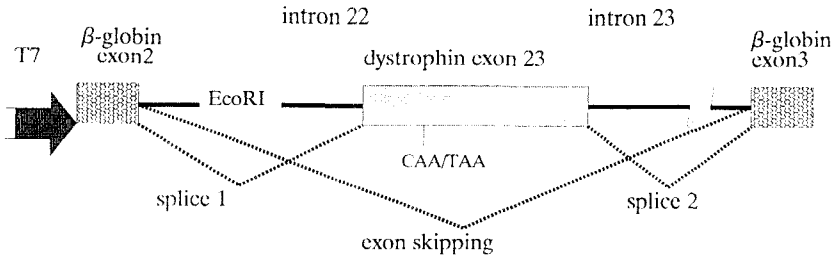

FIG. 1. Map of the pre-mRNA template construct used to analyse transcription and splicing around exon 23 of the mouse dystrophin gene. Transcription is driven by the 5' bacterial T7 promoter.

exon 23) in the *mdx* mouse[7]. We are studying whether interference with these sequences can induce skipping of the mutant exon(s), restoring the reading frame. Indeed, skipping of mutant exons has been demonstrated in some DMD patients[8,9] and may be a cause of the rare dystrophin-positive muscle fibers in dystrophic muscle[10,11].

As internally-deleted, in-frame dystrophin molecules generally cause mild BMD phenotypes, the products of exon skipping may prevent muscle damage. The ideal molecule required should be sequence-specific, of high affinity, nuclease resistant and, as stability of the target pre-mRNA is required (unlike antisense DNA strategies), should not induce RNase H activity. 2'-modified RNA oligonucleotides satisfy these criteria and have previously been used *in vitro* to modify splicing activity in the β-globin gene[12] and correct the effects of an insertion in the DMD gene[13].

Dystrophin transcripts comprise 0.001-0.01% of total mRNA in skeletal muscle and are approximately 100 times less abundant in *mdx* muscle, restricting *in vivo* analysis to optimised RT-PCR. Therefore, in order to test the concept of using antisense oligoribonucleotides to modify the splicing process in the DMD pre-mRNA, a template incorporating mouse dystrophin exon 23 was constructed by a recombinant PCR approach for analysis in cell free transcription/splicing systems (Fig. 1).

As no sequence data was previously known for mouse intron 23, a YAC contig covering mouse dystrophin exon 23 (gift from Dr Tony Monaco, Oxford, UK) was analysed by "vectorette PCR"[14]. The YAC was digested with *Hinf1* and vectorette oligonucleotides ligated into the digest prior to PCR using exon 23 (forward) and

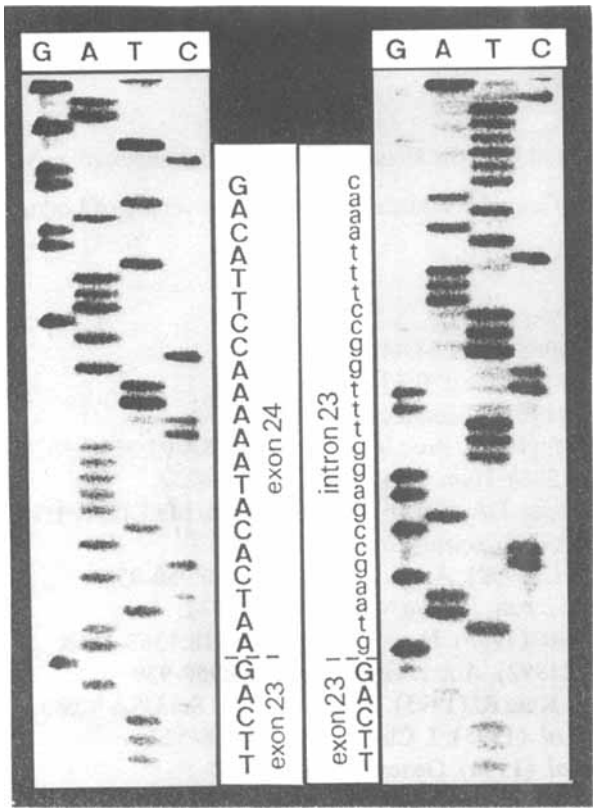

FIG.2. Sequence of mouse dystrophin exon 23/intron 23 junction established by vectorette PCR and direct sequencing.

vectorette-specific (reverse) primers (gift from Dr R. Roberts, Cambridge, UK). A 320bp product was directly sequenced to characterise the exon 23/intron 23 junction and design primers for template construction (Fig.2).

The recombinant template is currently under analysis in cell free *in vitro* transcription systems and HeLa cell *in vitro* splicing assays to which 2'-modified oligoribonucleotides will be added. These have been designed to target a series of splice sites within and adjacent to mouse dystrophin exon 23 in order to induce maximum exon skipping *in vitro*. In addition, a variety of constructs containing human dystrophin exons/introns relating to mutations in DMD patients are in preparation. Parallel studies are focussing on the optimised delivery of oligoribonucleotides to the nuclei of myoblasts and myotubes in

culture using various commercial cationic lipid formulations and nuclear-targeting peptide conjugates[15,16].

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REFERENCES.

1. Bonilla E. *et al.* (1988). Cell 54:447-452.
2. Worton R. (1995). Science 270:755-756.
3. Monaco A. *et al.* (1988). Genomics 2:90-95.
4. Deconinck N. *et al.* (1996). Proc.Natl.Acad.Sci.USA 93:3570-3574.
5. Fassati A. *et al.* (1996). Hum. Gene Ther. 7:595-602.
6. Dunckley M.G., Piper T.A. and Dickson G. (1996). MRDDRR 1:71-78.
7. Sicinski P. *et al.* (1989). Science 244:1578-1580.
8. Sherratt T.M. *et al.* (1992). Am. J. Hum. Genet. 50:950-959.
9. Thanh *et al.* (1995). Am. J. Hum. Genet. 56:725-731.
10. Hoffman E.P. *et al.* (1988). New Engl. J. Med. 318:1363-1368.
11. Klein C.J. *et al.* (1992). Am. J. Hum. Genet. 50:950-959.
12. Dominski Z. and Kole R. (1995). Proc.Natl.Acad.Sci.USA 90:8673-8677.
13. Takeshima Y. *et al.* (1995). J. Clin. Invest. 95:515-520.
14. Roberts R.G. *et al.* (1994). Genomics 16:536-538.
15. Trivedi R.A. and Dickson G. (1995). J. Neurochem. 64:2230-2238.
16. Derossi D. *et al.* (1994). J. Biol. Chem. 269:10444-10450.