Replication Protein-A (RPA)-dependent Melting of Triplex DNA at NOS2a Gene Promoter is Indispensable for p53-mediated NOS2a Synthesis and Cardioprotection

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Nitric oxide (NO) is a critical signal-transduction molecule involved in the protection and survival of the ischemic myocardium (1). NO is generated by a family of enzymes, nitric oxide synthases (NOS), of which the inducible nitric oxide (iNOS or NOS2) isoform plays crucial role in reducing infarct size post-myocardial infarction (2). Recently, we have identified a p53-dependent pathway of cardioprotection via upregulation of p53-induced transcriptional activation of the NOS family members (NOS3) (3). In this study, we observed that p53 has a response element (RE) in the NOS2a promoter 3451 base-pairs upstream of the +1 transcription start site (TSS). However, the in vitro transcription assay at the 4 kb NOS2a promoter resulted in the absence of p53-mediated transcription. Analysis of the NOS2a promoter region showed the presence of a triplex-forming pentanucleotide sequence (CCTT)₇, 2753 base-pairs upstream of +1 TSS and downstream of p53 RE (Fig. 1). We hypothesized that the melting of this triplex DNA structure by replication protein-A (RPA) (4) results in p53-dependent transcription at the NOS2a promoter. We have presented evidence using p53 and RPA knockout models of human cardiomyocytes that both p53 and RPA are required for transcription at the NOS2a promoter (Fig. 2). Essentially, the RPA protein melts the triplex DNA structure (Fig. 3) and facilitates p53-mediated transcription at the NOS2a gene promoter and NOS2a expression in human cardiomyocytes. The under-standing of this novel molecular mechanism of p53- and RPA-mediated transcription at NOS2a gene promoter will result in designing new strategies for cardiac therapy.

**References**


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**Fig. 1. Bioinformatics analysis of the NOS2a promoter using MatInspector database.** A putative p53RE 3428 base-pairs upstream +1 TSS, triplex forming region was identified.

**Fig. 2. The role of p53 and RPA in the expression of NOS2a gene is observed in p53-knocked out cardiomyocytes.** LPS was used to induce NOS2a expression. (A) p53 WT cDNA or RPA cDNA significantly increased the NOS2a protein expression. Silencing of either p53 or RPA abolished the LPS-induced NOS2a protein synthesis. (B) In p53 (-/-) human cardiomyocytes LPS induced NOS2a gene synthesis only upon exogenous addition of p53 cDNA. Addition of both p53 and RPA also induced NOS2a protein synthesis. Addition of RPA cDNA in the absence of p53 and p53 addition to RPA-silenced cardiomyocytes resulted in inhibition of NOS2a expression.

**Fig. 3. EMSA analysis of the triplex forming DNA sequence of NOS2a promoter.** NOS2 triplex DNA was incubated with the anti-triplex Ab and run on the EMSA gel. The data established the presence of triplex DNA in the NOS2a promoter. Next the triplex DNA sequence was incubated with 100 ng of the RPA protein for 24 h before incubation with anti-triplex Ab. The complex between the DNA and anti-triplex Ab was abolished upon incubating triplex DNA with RPA protein suggesting that RPA melts triplex DNA structure present on the NOS2a promoter.