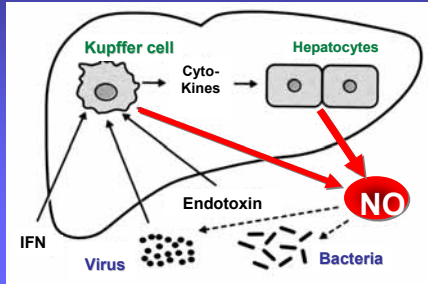


Development of a New Anti-Inflammatory Drug that Reduces Nitric Oxide Production

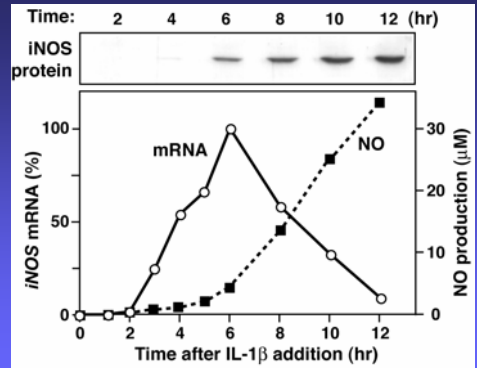
Mikio NISHIZAWA, M.D., Ph.D.

College of Science and Engineering, Ritsumeikan University

Nitric oxide (NO) kills virus and bacteria



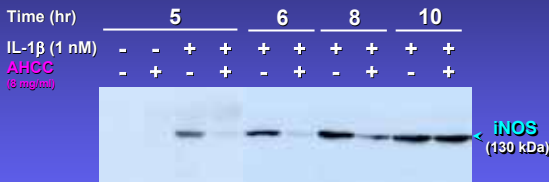
iNOS expression in response to IL-1 β



Rat iNOS mRNA and protein are induced in response to IL-1 β , and NO in the medium increases.

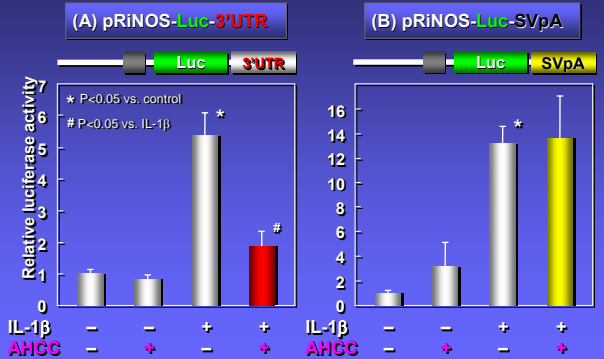
Active hexose correlated compound (AHCC) inhibits iNOS expression

(Western blotting)

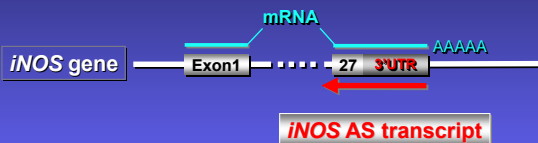


AHCC (Amino Up Chemical, Japan), a functional food extracted from mushrooms *Basidiomycetes*, reduces iNOS protein levels.

AHCC reduces iNOS mRNA stability through the 3'-untranslated region (3'UTR) of iNOS mRNA

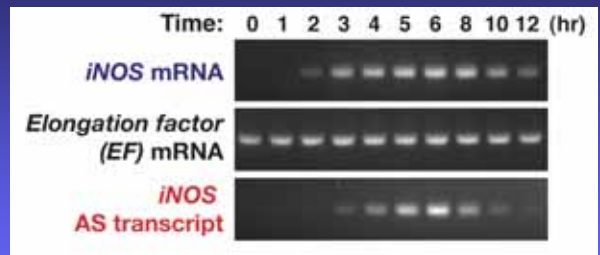


A natural antisense (AS) transcript is transcribed from iNOS gene



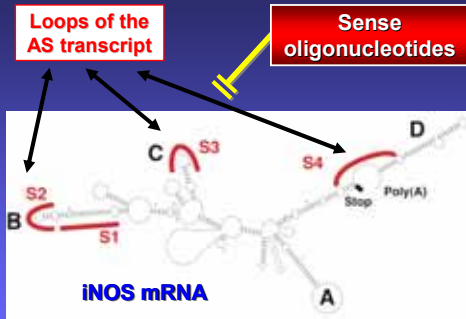
A natural AS transcript is transcribed from rat iNOS gene exon 27, which encodes the 3'-untranslated region (3'UTR) of iNOS mRNA.

Expression of iNOS AS transcript in response to IL-1 β



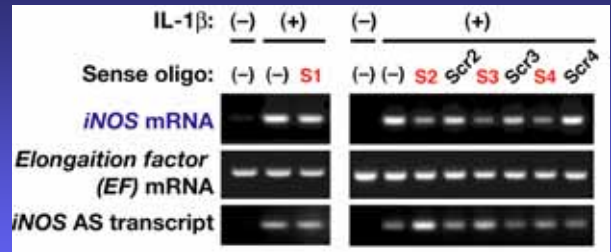
The expression pattern of the iNOS AS transcript correlates with that of iNOS mRNA in the strand-specific RT-PCR analysis.

Interactions between iNOS mRNA and AS transcript



The RNA-RNA interaction may occur on the loops of iNOS mRNA and AS transcript. Sense oligonucleotides to iNOS mRNA may interrupt the iNOS mRNA-AS transcript interactions.

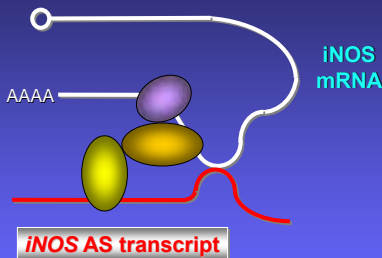
Sense oligonucleotides degrade iNOS mRNA



S = Sense oligos; Scr = scramble oligos (control)

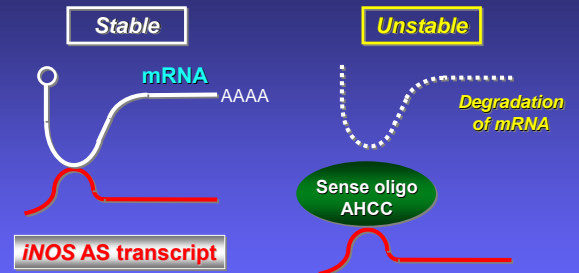
Sense oligonucleotides (S2-S4) reduced the iNOS mRNA levels by blocking the iNOS mRNA-AS transcript interaction.

Natural AS transcript stabilizes iNOS mRNA (Post-transcriptional regulation)



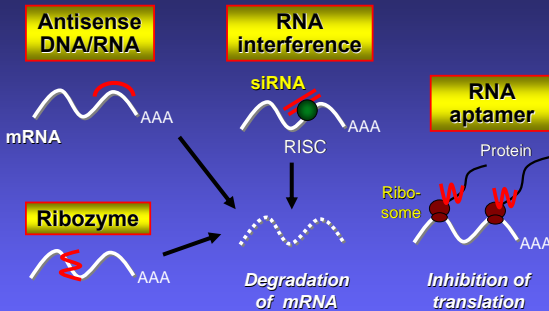
A natural AS transcript stabilizes iNOS mRNA by interacting with iNOS mRNA, as well as RNA-binding proteins (HuR, hnRNP L, etc.).

Sense oligos and AHCC degrade iNOS mRNA (Post-transcriptional regulation)



Sense oligonucleotides and AHCC degrade iNOS mRNA through the post-transcriptional regulation system.

Other nucleic acid-based phenomena



The mRNA stabilization by an AS transcript is a new mechanism that is different from other systems.

Conclusions

A natural iNOS AS transcript stabilizes iNOS mRNA by interacting with the iNOS mRNA 3'UTR and RNA-binding proteins.

AHCC, a functional food, is also involved in the degradation of iNOS mRNA through the iNOS mRNA 3'UTR.

The iNOS sense oligonucleotides and AHCC may be used to treat inflammatory diseases in which NO mediates.