What can you do with a luciferase Reporter Assay?

Post-Transcription miRNA Control Applications

Presented Fall 2009

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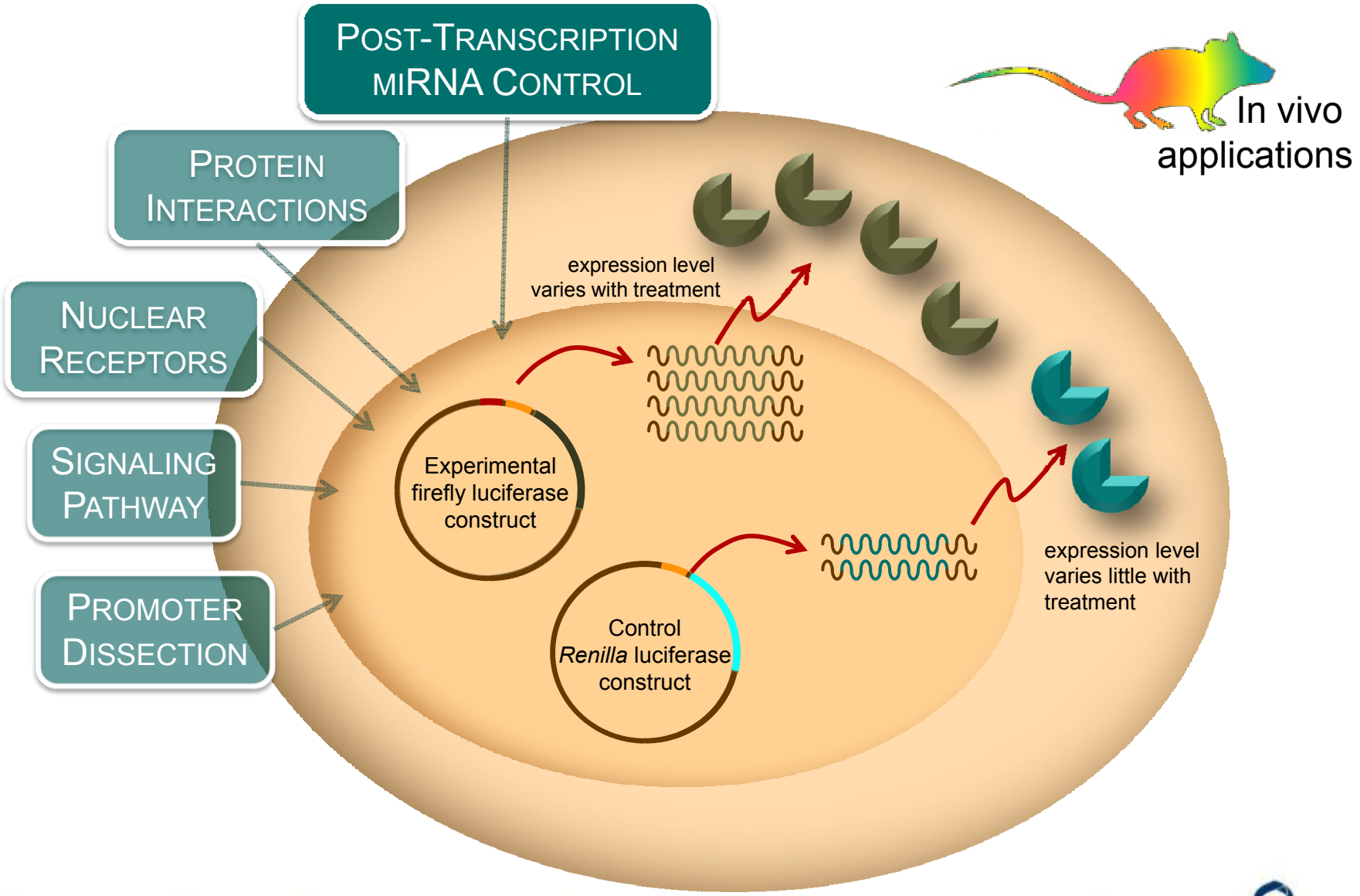
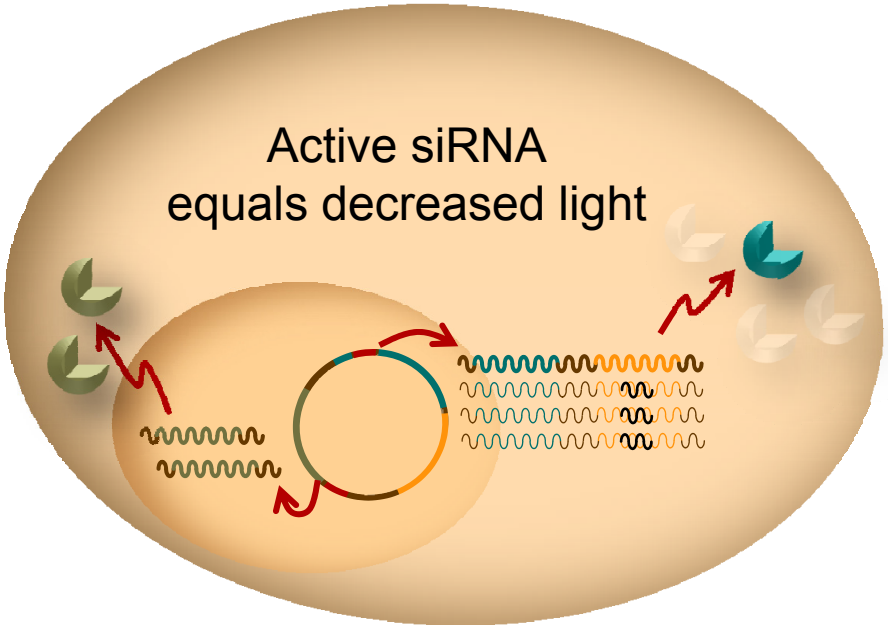
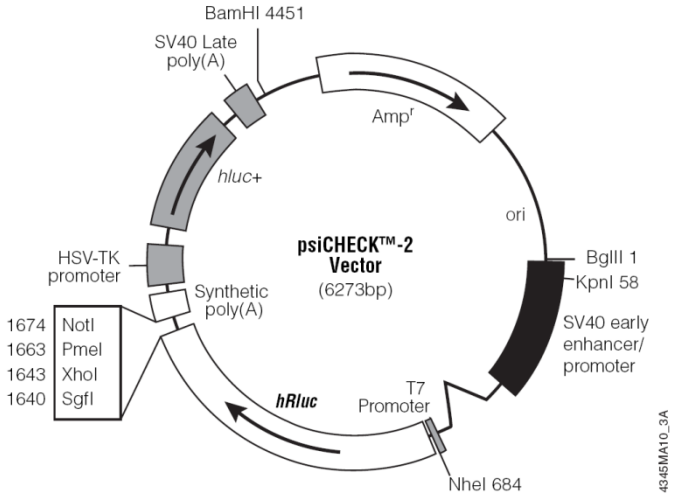
Application Overview



Using a reporter to find the best siRNA

stop codon





Easy, quantitative assay to find which siRNA to use in knockdown work

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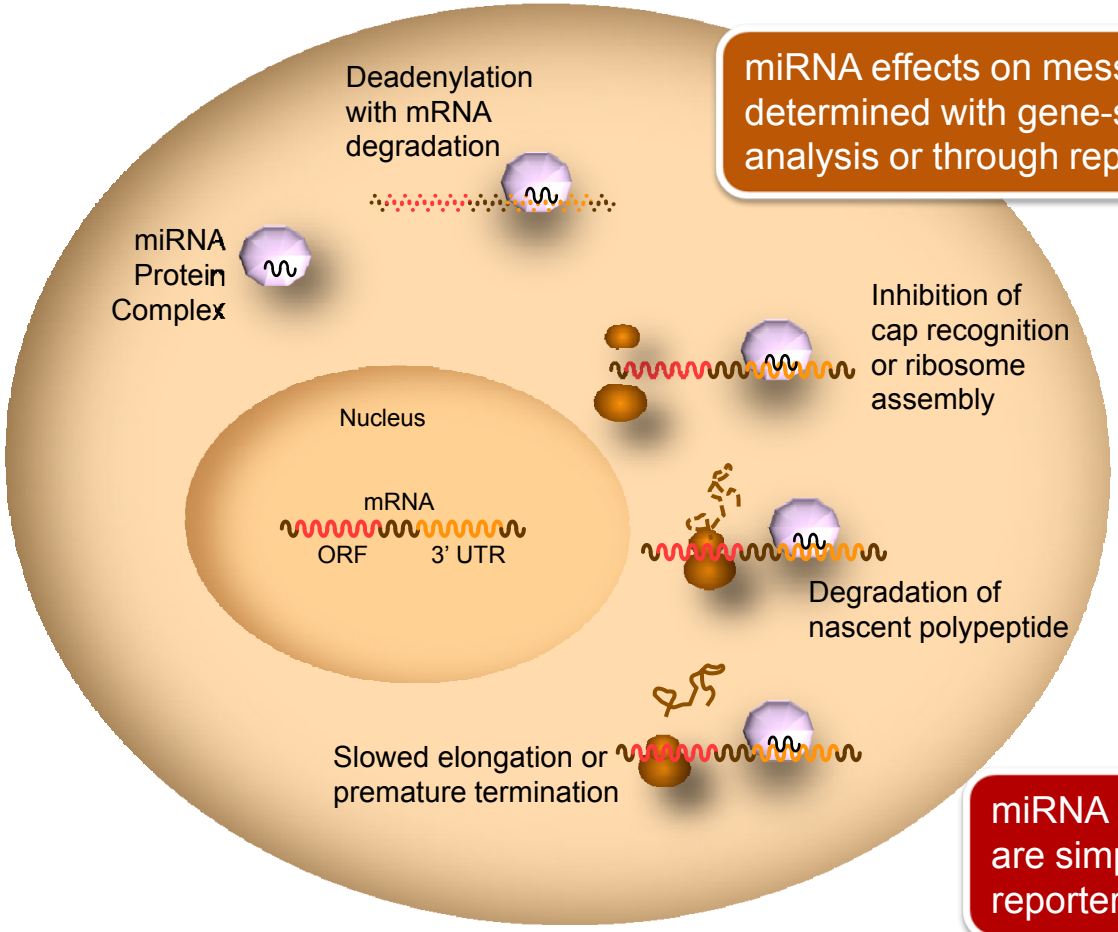
Post-Transcriptional Control through miRNA

Possible mechanisms of miRNA-mediated post-transcriptional control



miRNA effects on message stability can be determined with gene-specific transcript analysis or through reporter gene assays.

miRNA effects on protein translation are simplified through use of a reporter enzyme like luciferase

Adapted from: Chekulaeva, M. and Filipowicz, W. (2009) *Curr. Opin. Cell Biol.* 21, 452-60.

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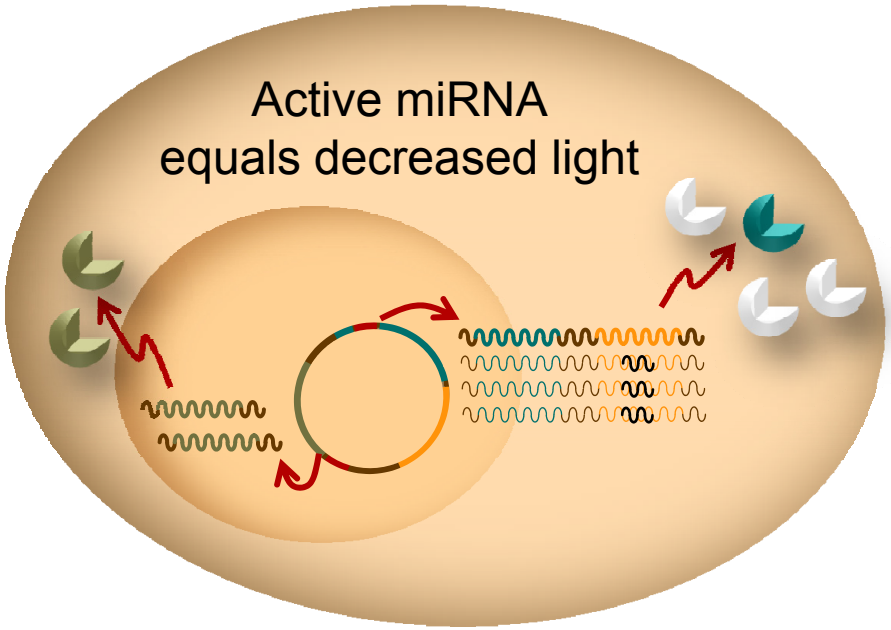
psiCHECK™-2 Vector Employed to study miRNA



Obvious application of the vector, however, not ideal.

- SV40 promoter is extremely active.
- Subtle changes in luciferase levels could be missed.

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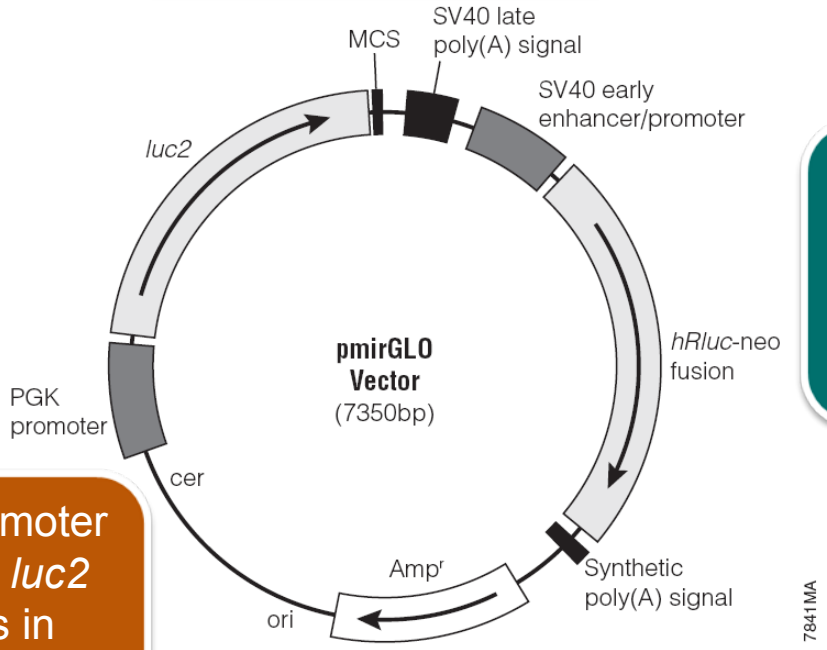
pmirGlo Vector for miRNA targeting

Multiple Cloning Site downstream of *luc2* for insertion of 3' UTR.



hRluc-neo fusion provides a control reporter and the ability to produce stable transfectants.

PGK non-viral promoter provides low-level *luc2* expression. Works in human, mouse, rat, yeast, etc.

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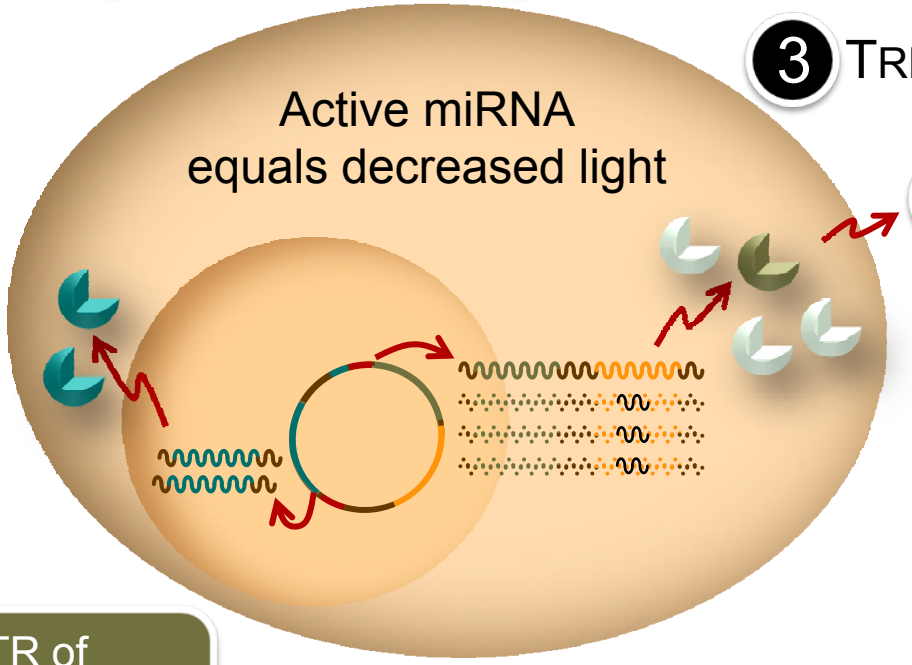
pmirGlo Assay Principle

1 TRANSFECT 2 CULTURE 2-3 DAYS

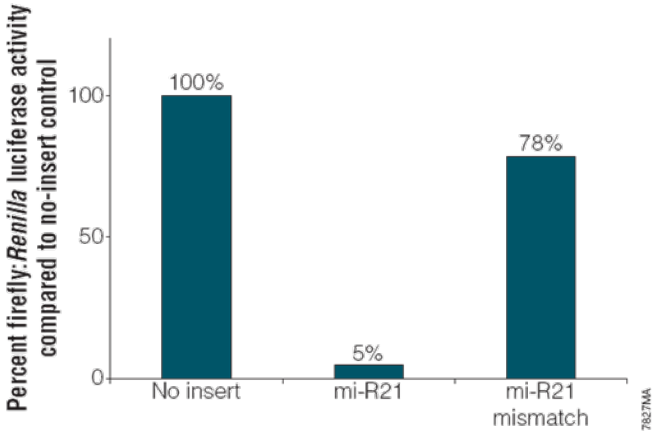
3 TREAT WITH MIRNA

4 DUAL-LUCIFERASE® ASSAY



Insert 3'UTR of gene of interest into pmirGlo vector

example with miR-21 seed sequence in pmirGlo Vector



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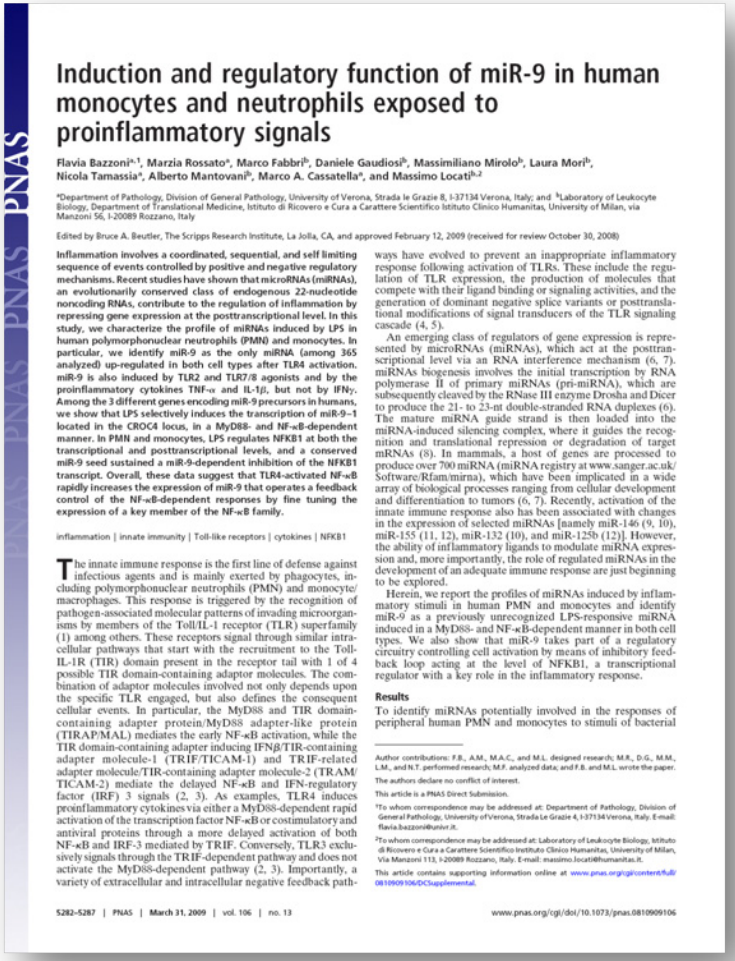
Case Study: miR-9 and NFKB1 control



PNAS

Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals

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Edited by Bruce A. Beutler, The Scripps Research Institute, La Jolla, CA, and approved February 12, 2009 (received for review October 30, 2008)

Inflammation involves a coordinated, sequential, and self limiting sequence of events controlled by positive and negative regulatory mechanisms. Recent studies have shown that microRNAs (miRNAs), an evolutionarily conserved class of endogenous 22-nucleotide noncoding RNAs, contribute to the regulation of inflammation by repressing gene expression at the posttranscriptional level. In this study, we characterize the profile of miRNAs induced by LPS in human polymorphonuclear neutrophils (PMN) and monocytes. In particular, we identify miR-9 as the only miRNA (among 365 analyzed) up-regulated in both cell types after TLR4 activation. miR-9 is also induced by TLR2 and TLR7/8 agonists and by the proinflammatory cytokines TNF-α and IL-1β, but not by IFNγ. Among the 3 different genes encoding miR-9 precursors in humans, we show that LPS selectively induces the transcription of miR-9–1 located in the CROC4 locus, in a MyD88- and NF-κB-dependent manner. In PMN and monocytes, LPS regulates NFKB1 at both the transcriptional and posttranscriptional levels, and a conserved miR-9 seed sustained a miR-9-dependent inhibition of the NFKB1 transcript. Overall, these data suggest that TLR4-activated NF-κB rapidly increases the expression of miR-9 that operates a feedback control of the NF-κB-dependent responses by fine tuning the expression of a key member of the NF-κB family.

inflammation | innate immunity | Toll-like receptors | cytokines | NFKB1

The innate immune response is the first line of defense against infectious agents and is mainly exerted by phagocytes, including polymorphonuclear neutrophils (PMN) and monocyte/macrophages. This response is triggered by the recognition of pathogen-associated molecular patterns of invading microorganisms by members of the Toll/IL-1 receptor (TLR) superfamily (1) among others. These receptors signal through similar intracellular pathways that start with the recruitment to the Toll-IL-1R (TIR) domain present in the receptor tail with 1 of 4 possible TIR domain-containing adaptor molecules. The combination of adaptor molecules involved not only depends upon the specific TLR engaged, but also defines the consequent cellular events. In particular, the MyD88 and TIR domain-containing adapter protein/MyD88 adapter-like protein (TIRAP/MAL) mediates the early NF-κB activation, while the TIR domain-containing adapter inducing IFNβ/TIR-containing adapter molecule-1 (TRIF/TICAM-1) and TRIF-related adapter molecule/TIR-containing adapter molecule-2 (TRAM/TICAM-2) mediate the delayed NF-κB and IFN-regulatory factor (IRF) 3 signals (2, 3). As examples, TLR4 induces proinflammatory cytokines via either a MyD88-dependent rapid activation of the transcription factor NF-κB or costimulatory and antiviral proteins through a more delayed activation of both NF-κB and IRF-3 mediated by TRIF. Conversely, TLR3 exclusively signals through the TRIF-dependent pathway and does not activate the MyD88-dependent pathway (2, 3). Importantly, a variety of extracellular and intracellular negative feedback pathways have evolved to prevent an inappropriate inflammatory response following activation of TLRs. These include the regulation of TLR expression, the production of molecules that compete with their ligand binding or signaling activities, and the generation of dominant negative splice variants or posttranslational modifications of signal transducers of the TLR signaling cascade (4, 5).

An emerging class of regulators of gene expression is represented by microRNAs (miRNAs), which act at the posttranscriptional level via an RNA interference mechanism (6, 7). miRNAs biogenesis involves the initial transcription by RNA polymerase II of primary miRNAs (pri-miRNA), which are subsequently cleaved by the RNase III enzyme Drosha and Dicer to produce the 21- to 23-nt double-stranded RNA duplexes (6). The mature miRNA guide strand is then loaded into the miRNA-induced silencing complex, where it guides the recognition and translational repression or degradation of target mRNAs (8). In mammals, a host of genes are processed to produce over 700 miRNA (miRNA registry at www.sanger.ac.uk/Software/Rfam/mirna), which have been implicated in a wide array of biological processes ranging from cellular development and differentiation to tumors (6, 7). Recently, activation of the innate immune response also has been associated with changes in the expression of selected miRNAs [namely miR-146 (9, 10), miR-155 (11, 12), miR-132 (10), and miR-125b (12)]. However, the ability of inflammatory ligands to modulate miRNA expression and, more importantly, the role of regulated miRNAs in the development of an adequate immune response are just beginning to be explored.

Herein, we report the profiles of miRNAs induced by inflammatory stimuli in human PMN and monocytes and identify miR-9 as a previously unrecognized LPS-responsive miRNA induced in a MyD88- and NF-κB-dependent manner in both cell types. We also show that miR-9 takes part of a regulatory circuitry controlling cell activation by means of inhibitory feedback loop acting at the level of NFKB1, a transcriptional regulator with a key role in the inflammatory response.

Results

To identify miRNAs potentially involved in the responses of peripheral human PMN and monocytes to stimuli of bacterial

Author contributions: F.B., A.M., M.A.C., and M.L. designed research; M.R., D.G., M.M., L.M., and N.T. performed research; M.F. analyzed data; and F.B. and M.L. wrote the paper.

The authors declare no conflict of interest.

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[2]To whom correspondence may be addressed at: Laboratory of Leukocyte Biology, Istituto di Ricovero e Cura a Carattere Scientifico Istituto Clinico Humanitas, University of Milan, Via Manzoni 113, I-20089 Rozzano, Italy. E-mail: massimo.locati@humanitas.it.

This article contains supporting information online at www.pnas.org/cgi/content/full/0810909106/DCSupplemental.

- Looking for potential miRNA involvement in the LPS response
- Screen 365 miRNA expression patterns
- Only miR-9 induced in both neutrophils and monocytes in response to LPS
- psiCHECK-2 Vector used in analysis of miR-9 control of NFKB1 through the 3’ UTR

Bazzoni, F., *et al.* (2009) *PNAS* **106**, 5282-7.

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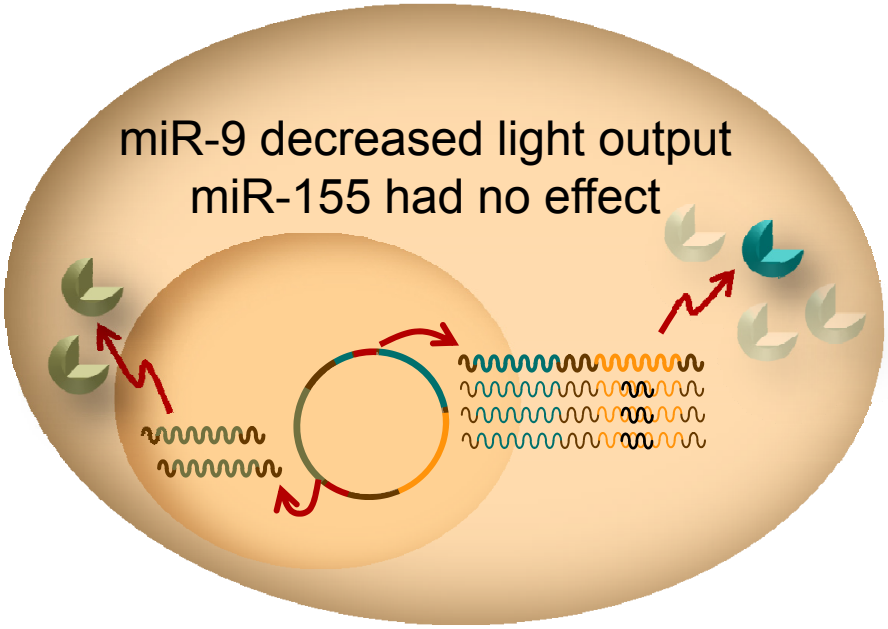
Case Study:
Bazzoni, F., et al.

miR-9 acts on seed in NFKB1 3'UTR

psiCHECK-2 constructs

c-fos/miR-155 positive control

4 point mutations in the miR-9 seed
no influence by miR-9



Authors found that NFKB1 expression increases with LPS treatment but protein levels remain constant.

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More information

pmirGLO Dual-Luciferase miRNA Target Expression Vector Product Information

Schagat, T. and Vidugiriene, J. (2008) MicroRNA biosensors: Application for the psiCHECK™-2 Vector. *Promega Notes* **99**, 16-18.

psiCHECK™-2 Vector citations HighWire Press®

psiCHECK™-2 Vector citations Nature.com

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