Function of miRNAs

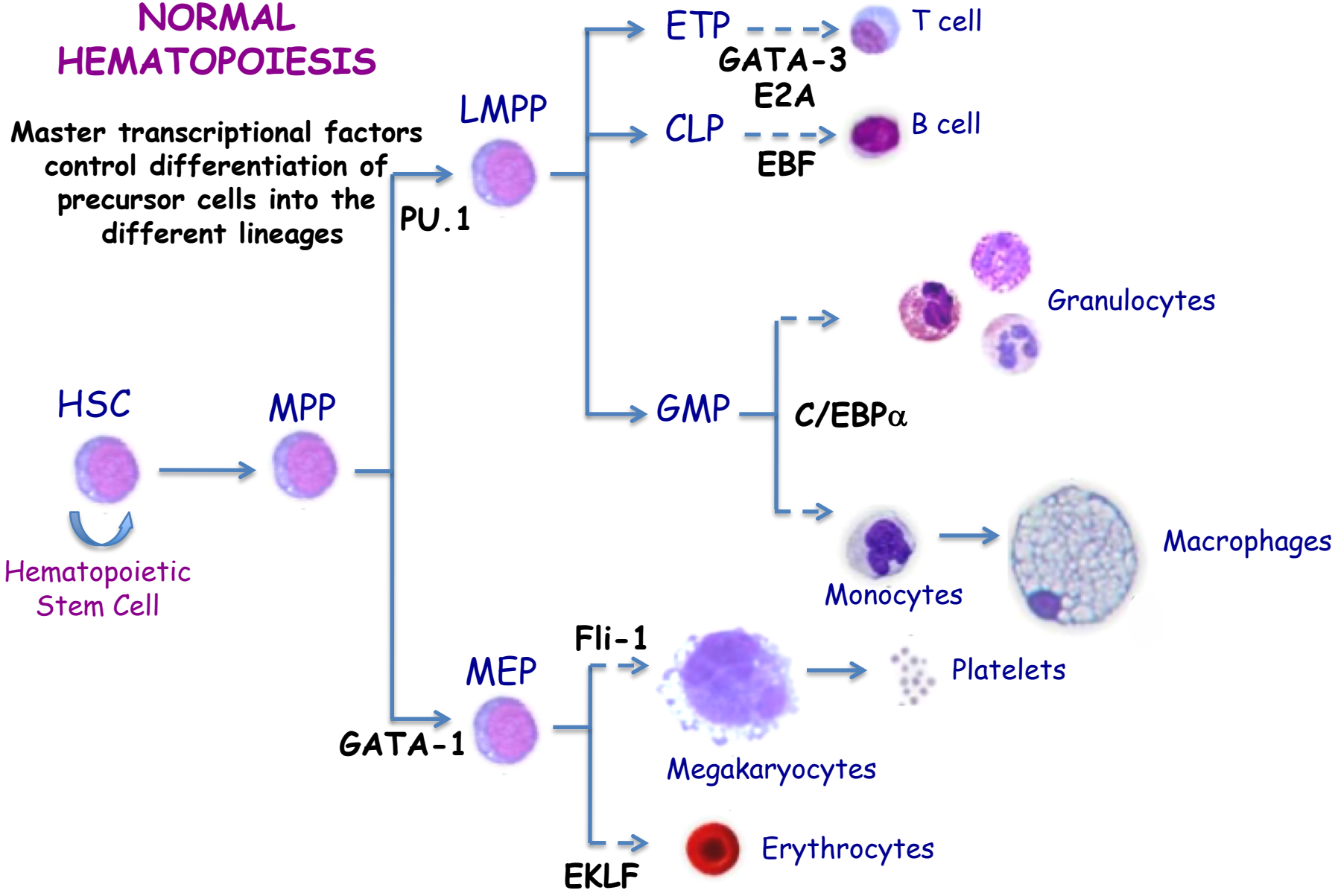
Hematopoietic differentiation

Mammalian microRNAs

Expression pattern	microRNA
Tissue-specific expression patterns of mammalian microRNAs	
ES-cell specific	*miR-296*
Expressed in ES cells, but upreguated on differentiation	*miR-21* and *miR-22*
Expressed in both ES cells and various adult tissues	*miR-15a, miR-16, miR-19,b, miR-92, miR-93, miR-96, miR-130* and *miR-130b*
Enriched during mouse brain development	*miR-128, miR-19b, miR-9, miR-125b, miR-131, miR-178, miR-124a, miR-266* and *miR-103*
Enriched in adult brain	*miR-9*, miR-125a, miR-125b, miR-128, miR-132, miR-137, miR-139, miR-7, miR-9, miR-124a, miR-124b, miR-135, miR-153, miR-149, miR-183, miR-190* and *miR-219*
Enriched in lung	*miR-18, miR-19a, miR-24, miR-32, miR-130, miR-213, miR-20, miR -141, miR-193* and *miR-200b*
Enriched in spleen	*miR-99a, miR-127, miR-142-a, miR-142-s, miR-151, miR-189* and *miR-212*
Haemetopoietic tissues	*miR-181, miR-223* and *miR-142*
Enriched in liver	*miR-122a, miR-152, miR-194, miR-199* and *miR-215*
Enriched in heart	*miR-1b, miR-1d, miR-133, miR-206, miR-206* and *miR-143*
Enriched in kidney	*miR-30b, miR-30c, miR-18, miR-20, miR-24, miR-32, miR-141, miR-193* and *miR-200b*
Ubiquitously expressed	*miR-16, miR-26a, miR-27a, miR143a, miR-21, let-7a, miR-7b, miR-30b* and *miR-30c*
Abnormal microRNA expression during tumorigenesis	
Downregulated in chronic lymphocytic leukaemias	*miR-15* and *miR-16*
Downregulated in lung cancer cell lines	*miR-26a* and *miR-99a*
Downregulated in colon cancers	*miR143/miR-145* cluster
Upregulated in Burkitt lymphoma	*miR-155*

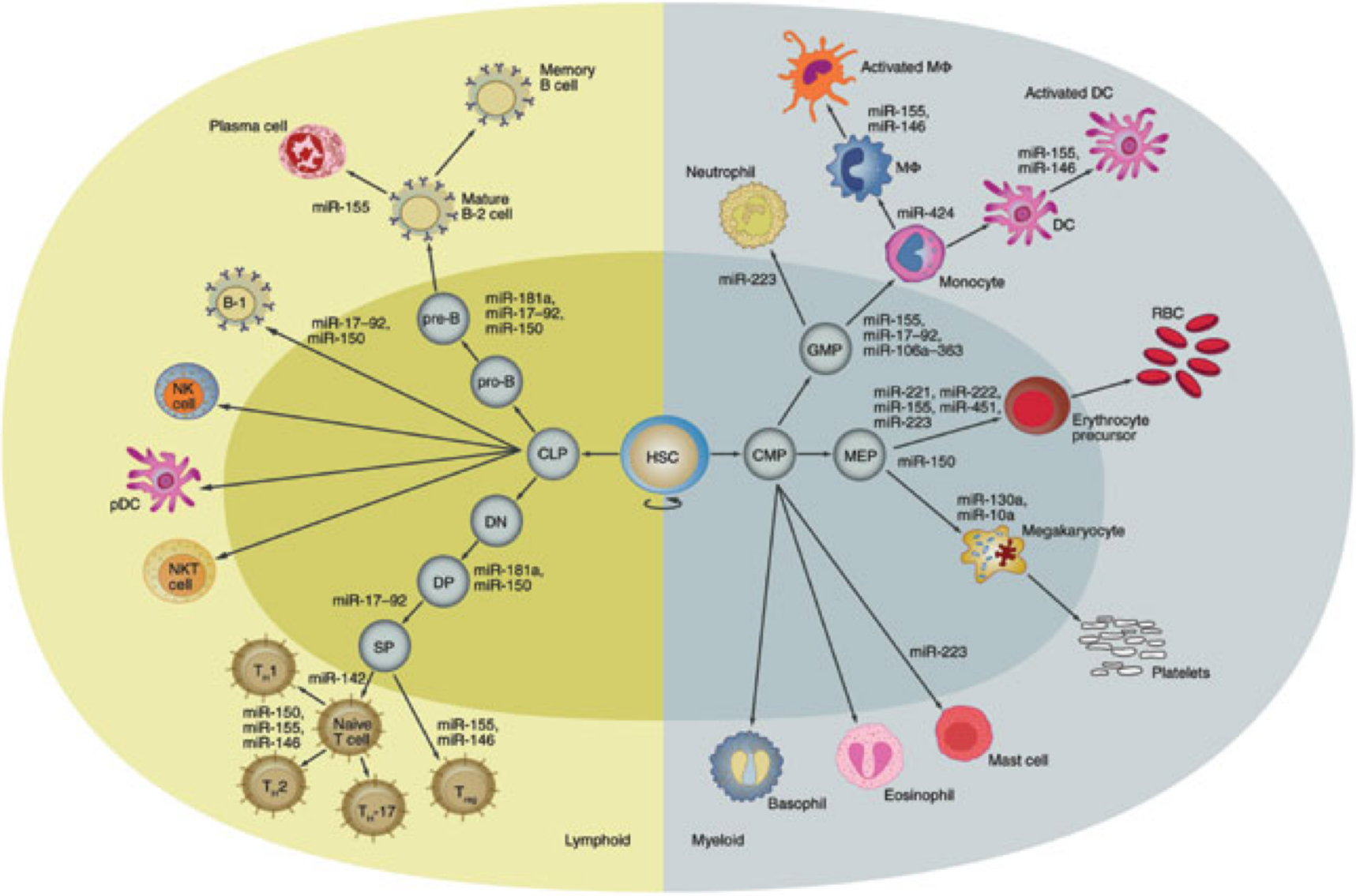
NORMAL HEMATOPOIESIS

Master transcriptional factors control differentiation of precursor cells into the different lineages



Self-renewal

Differentiation



Overview of the involvement of the miRNAs discussed in this review in the differentiation and function of various hematopoietic lineages. The miRNAs near arrows are involved in but do not necessarily enhance the developmental transition indicated by the arrow (full explanation in text). B-1, B-1 type B cell; B-2, B-2 type 'conventional' B cell; pre-B, pre–B cell; pro-B, pro–B cell; CLP, common lymphoid progenitor; DN, double-negative T cell; DP, double-positive T cell; SP, single-positive T cell; NK, natural killer; pDC, plasmacytoid dendritic cell; NKT, natural killer T; TH1, T helper type 1 cell; TH2, T helper type 2 cell; TH-17, interleukin 17–producing helper T cell; Treg, regulatory T cell; Mphi, macrophage; DC, dendritic cell; GMP, granulocyte-macrophage progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythrocyte progenitor; RBC, red blood cell. Created by Cici Koenig.

Hematopoietic tissue specific microRNAs

miR181 (thymus)

miR142 (all hematopoietic tissues)

miR223 (bone marrow)

(Chen et al., 2004. Science)

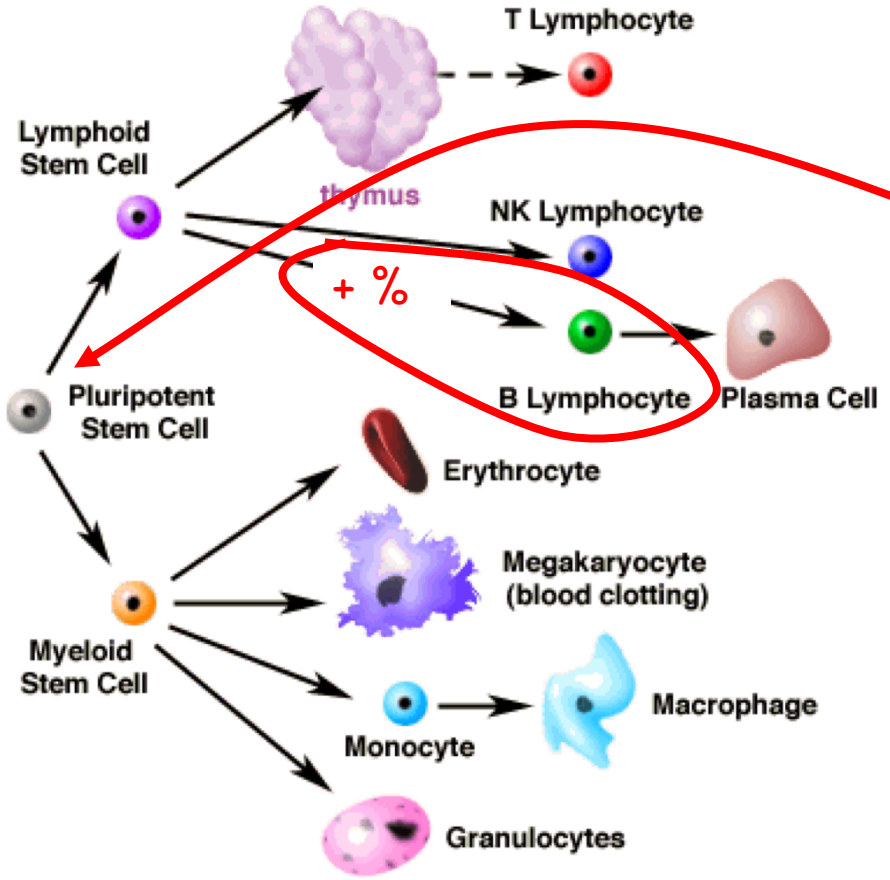
Chen CZ, Li L, Lodish HF, Bartel DP. Science. 2004 303:83-6

MicroRNAs modulate hematopoietic lineage differentiation.

Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA.

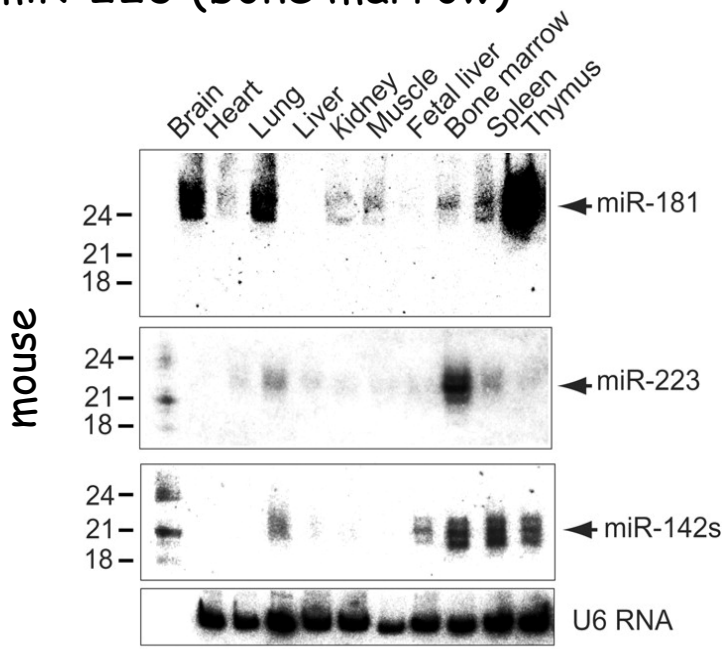
MicroRNAs (miRNAs) are an abundant class of approximately 22-nucleotide regulatory RNAs found in plants and animals. Some miRNAs of plants, Caenorhabditis elegans, and Drosophila play important gene-regulatory roles during development by pairing to target mRNAs to specify posttranscriptional repression of these messages. We identify three miRNAs that are specifically expressed in hematopoietic cells and show that their expression is dynamically regulated during early hematopoiesis and lineage commitment. **One of these miRNAs, miR-181, was preferentially expressed in the B-lymphoid cells of mouse bone marrow, and its ectopic expression in hematopoietic stem/progenitor cells led to an increased fraction of B-lineage cells** in both tissue-culture differentiation assays and adult mice. Our results indicate that microRNAs are components of the molecular circuitry that controls mouse hematopoiesis and suggest that other microRNAs have similar regulatory roles during other facets of vertebrate development.

Hematopoietic lineage differentiation



Hematopoietic tissue specific miRNAs:

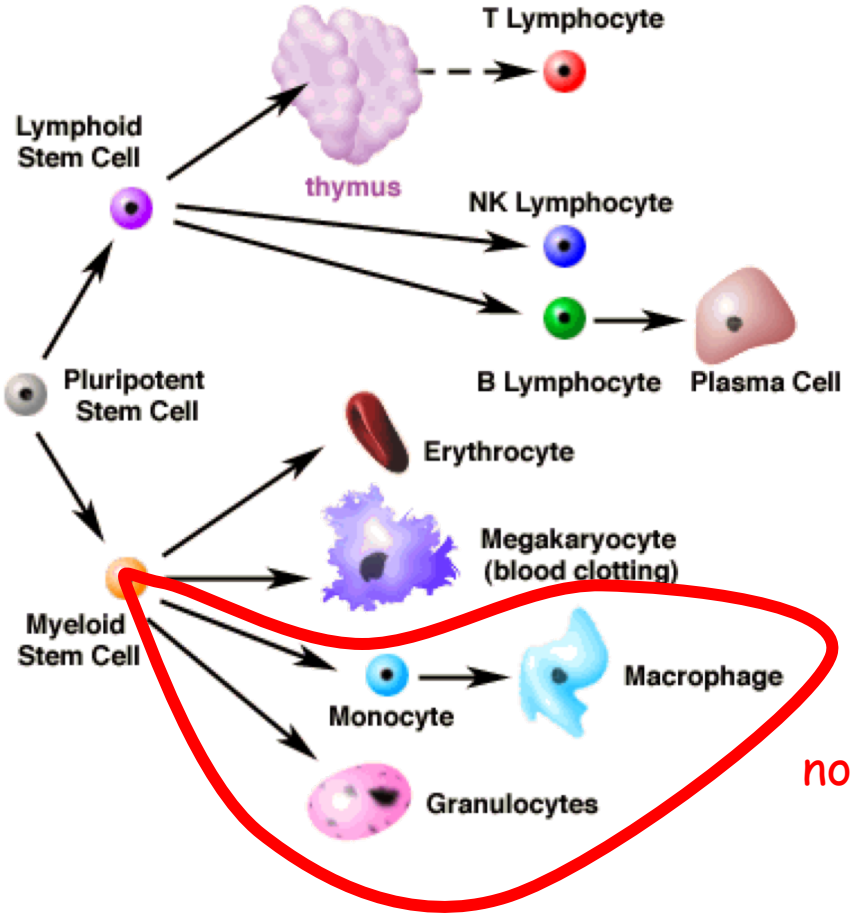
- miR-181a (thymus)
- miR-142 (all hematopoietic tissues)
- miR-223 (bone marrow)



(Chen et al., 2004. Science)

Acute Promyelocytic Leukemia (APL)

•is characterized by an accumulation in the bone marrow and blood of hematopoietic precursor cells blocked at the level of promyelocytic precursors



no differentiation !!

Cell 2005:819-31

A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPalpha regulates human granulopoiesis.

Fazi F, Rosa A, Fatica A, Gelmetti V, De Marchis ML, Nervi C, Bozzoni I.

Department of Histology and Medical Embryology, University of Rome La Sapienza and San Raffaele Biomedical Science Park of Rome, Via di Castel Romano 100, 00128, Rome, Italy.

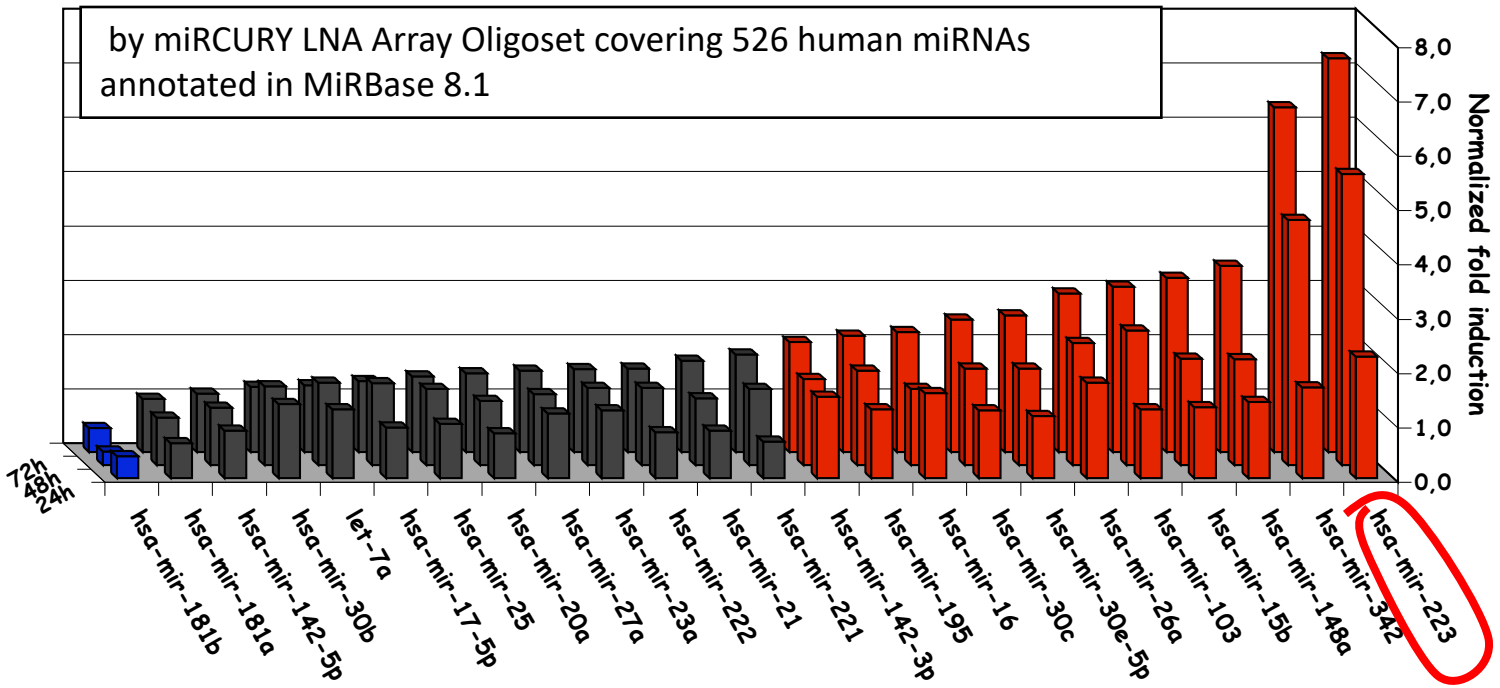
MicroRNAs play important roles in cell differentiation by acting as translational inhibitors of specific target genes. Here we show that human granulocytic differentiation is controlled by a regulatory circuitry involving miR-223 and two transcriptional factors, NFI-A and C/EBPalpha. The two factors compete for binding to the miR-223 promoter: NFI-A maintains miR-223 at low levels, whereas its replacement by C/EBPalpha, following retinoic acid (RA)-induced differentiation, upregulates miR-223 expression. The competition by C/EBPalpha and the granulocytic differentiation are favored by a negative-feedback loop in which miR-223 represses NFI-A translation. In line with this, both RNAi against NFI-A and ectopic expression of miR-223 in acute promyelocytic leukemia (APL) cells enhance differentiation, whereas miR-223 knockdown inhibits the differentiation response to RA. Altogether, our data indicate that miR-223 plays a crucial role during granulopoiesis and point to the NFI-A repression as an important molecular pathway mediating gene reprogramming in this cell lineage.

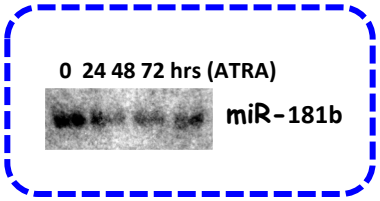
Acute Promyelocytic Leukemia (APL)

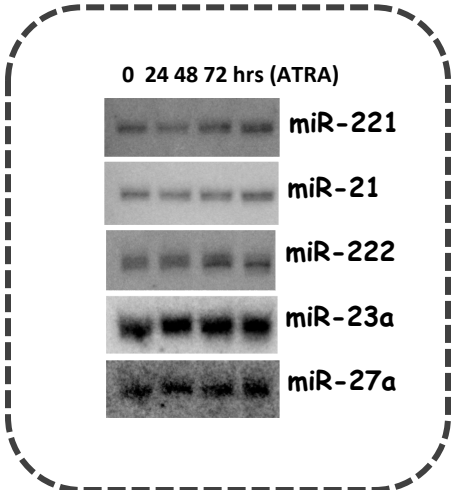
·APL is characterized by traslocations involving Retinoic Acid Receptor α (RARα). The fusion protein does not respond to the physiological level of retinoic acid (RA) and this causes the block of differentiation and neoplastic transformation of myeloid precursor cells

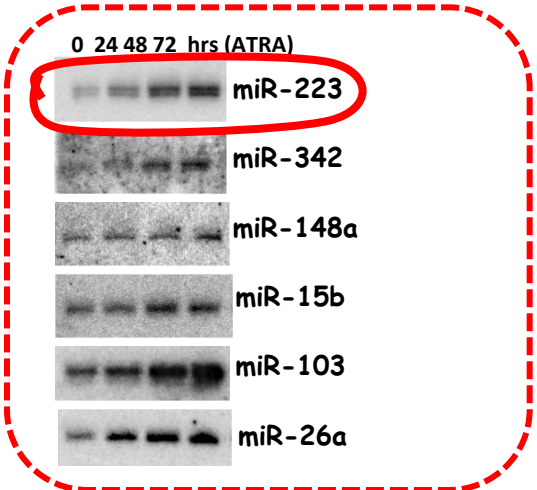
·The differentiation block in this leukemia can be rescued by treatment with *trans* RA

MicroRNAs differentially expressed during RA induced granulocytic differentiation of APL cells

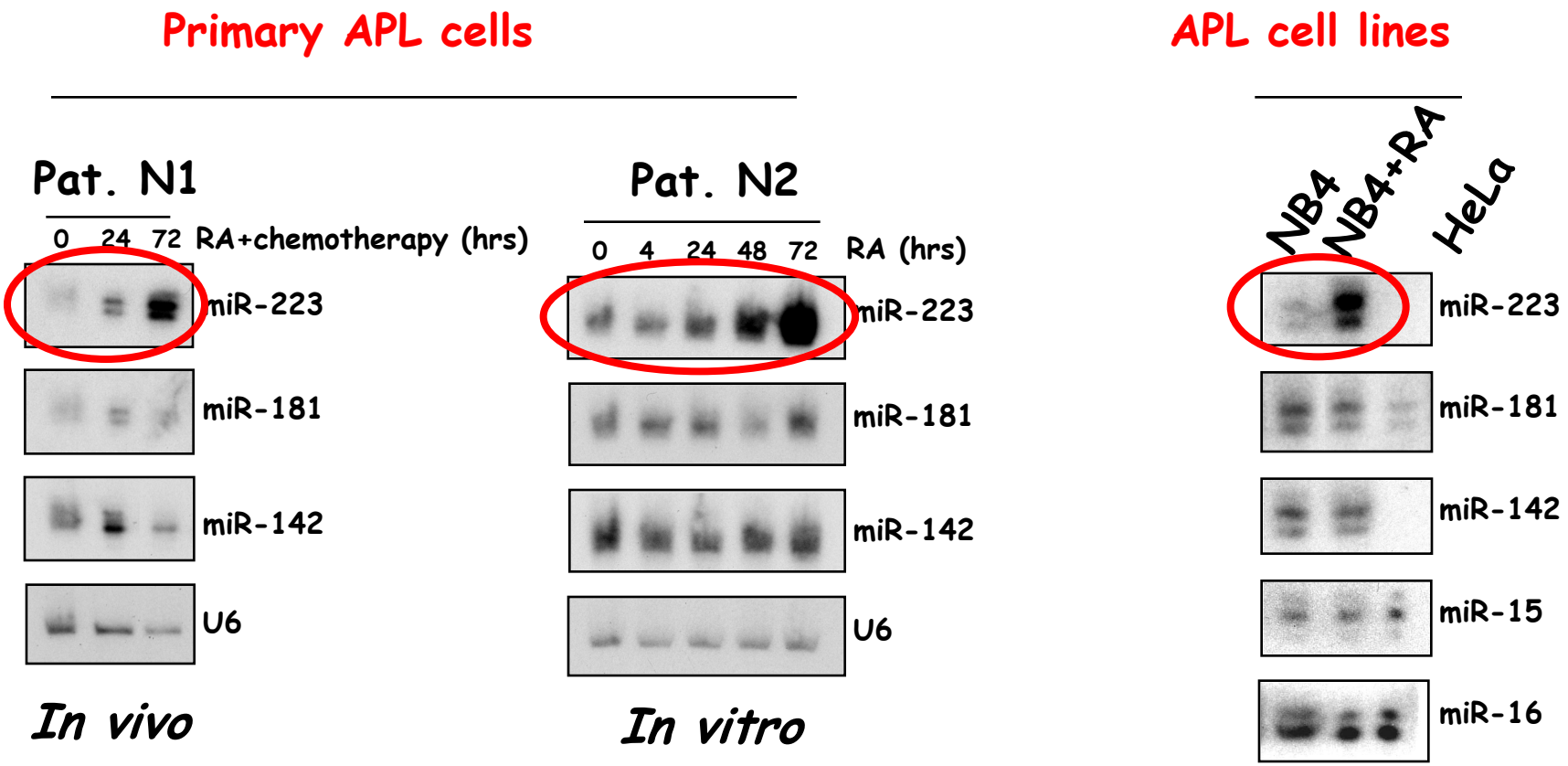




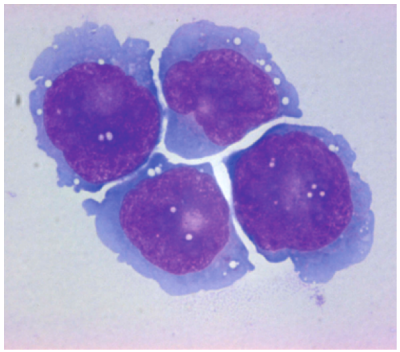




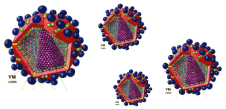
miR-223 expression is induced upon RA mediated granulocytic differentiation of APL cells

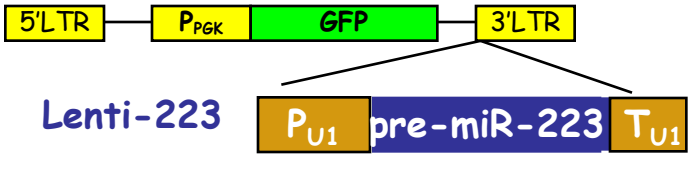


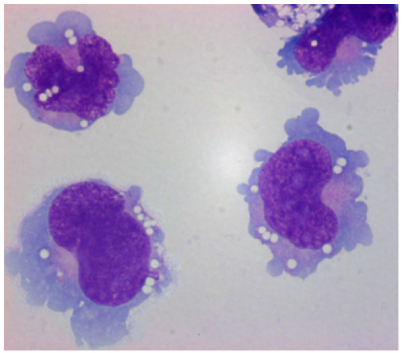
Ectopic expression of miR-223 in NB4 cells is able to induce differentiation in the absence of Retinoic Acid

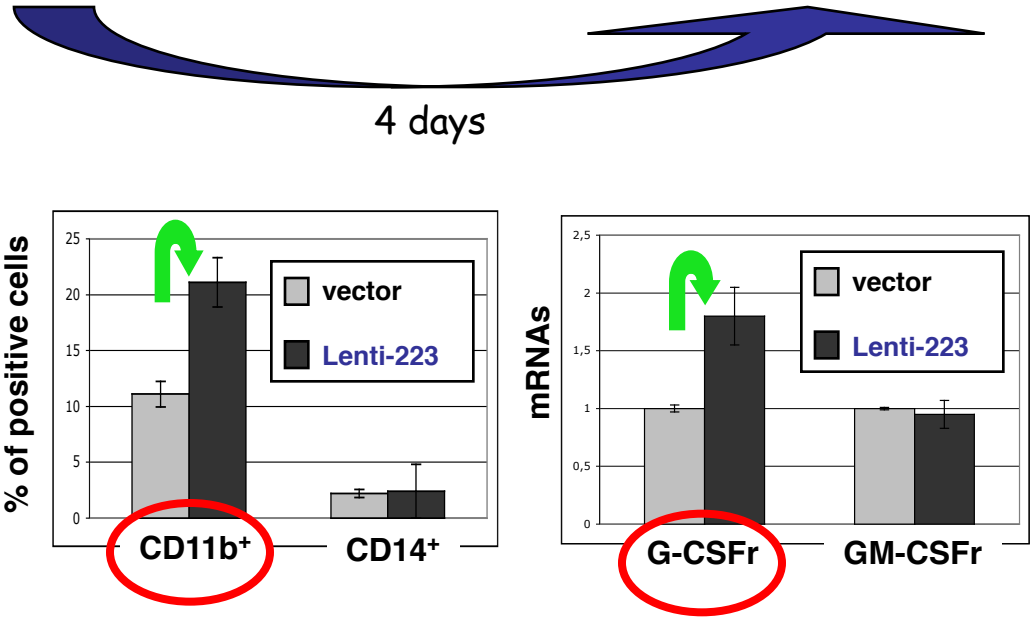


Infection with a lentivirus expressing miR-223 under a constitutive polII promoter



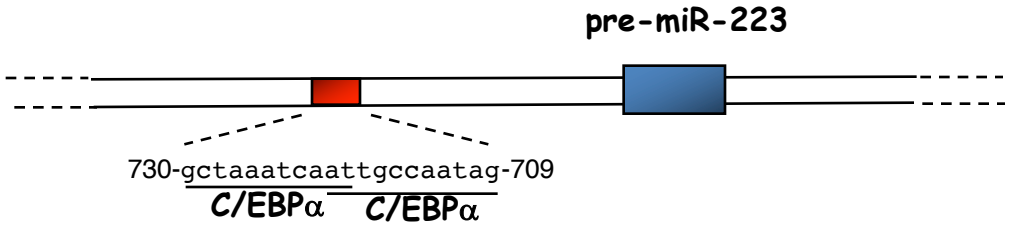




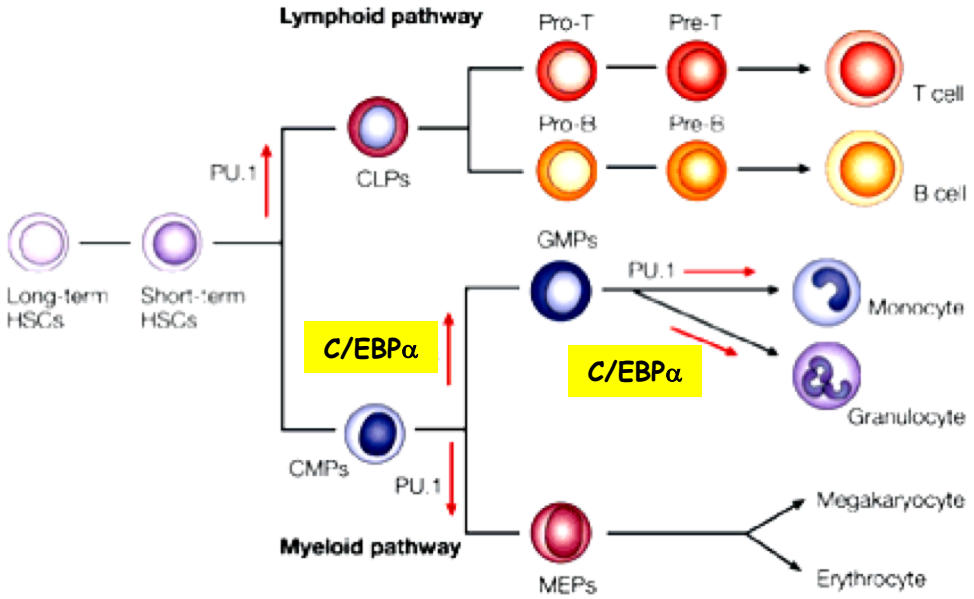


4 days

C/EBPα binding sites are present in the miR-223 promoter region



C/EBPα: lineage specific CCAAT-box binding transcription factor essential for granulocytic differentiation that is activated in early myeloid precursor



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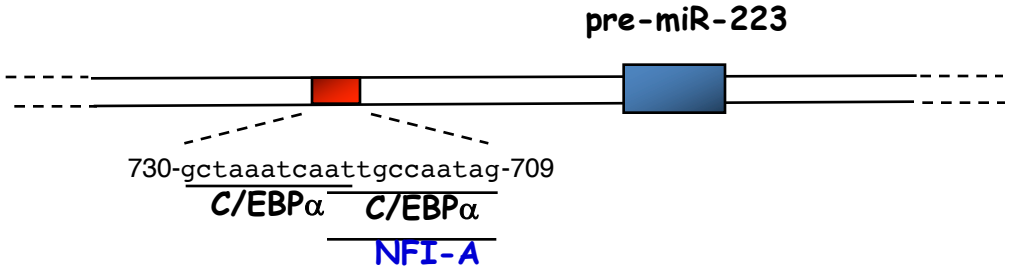


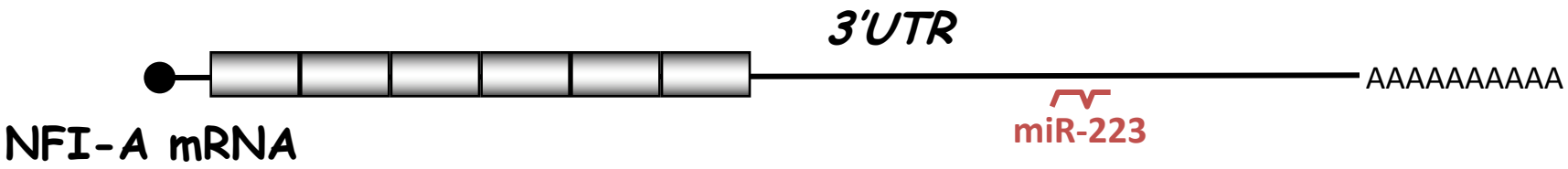
C/EBPα: lineage specific CCAAT-box binding transcription factor essential for granulocytic differentiation that is activated in early myeloid precursor

and……

NFI-A: CCAAT-box binding transcription factor capable of controlling transcription and activating replication.

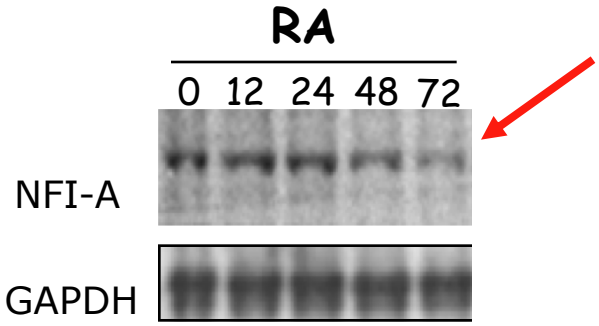
It is a target of miR-223 !!!!!!

NFI-A undergoes translational repression
in RA-induced differentiation of NB4 cells



The NFI-A protein levels decrease during RA-induced differentiation

Western Blot Analysis

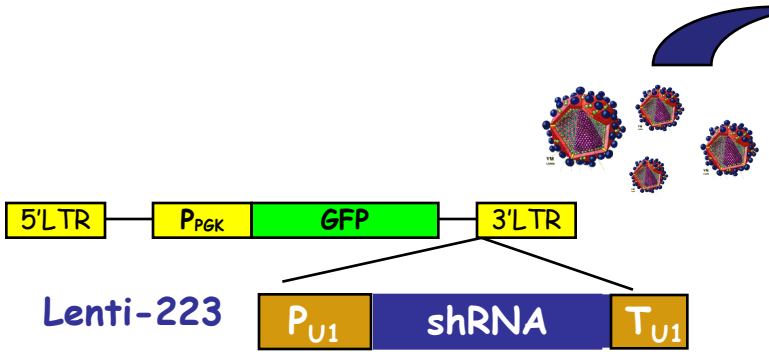


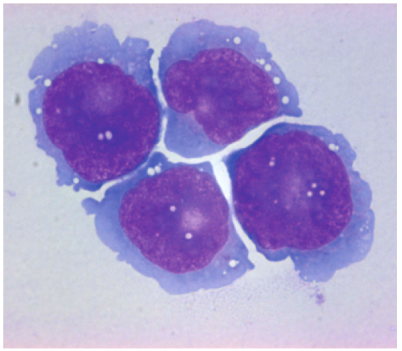
LUCIFERASE ASSAY:

miR-223 represses NFI-A at the translational level

RNA interference against NFI-A and C/EBPα in order to demonstrate their role in miR-223 activation and in granulocytic differentiation

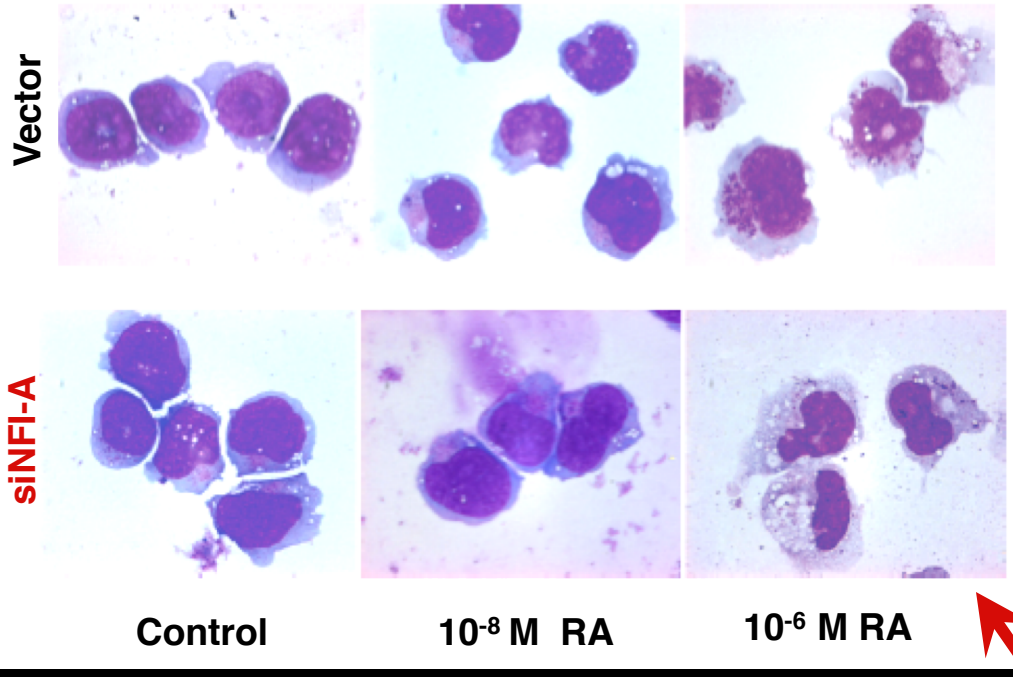
Infection of NB4 cells with lentiviruses expressing **siRNAs** against the **NFI-A** or **C/EBPα** mRNAs under a constitutive polII promoter



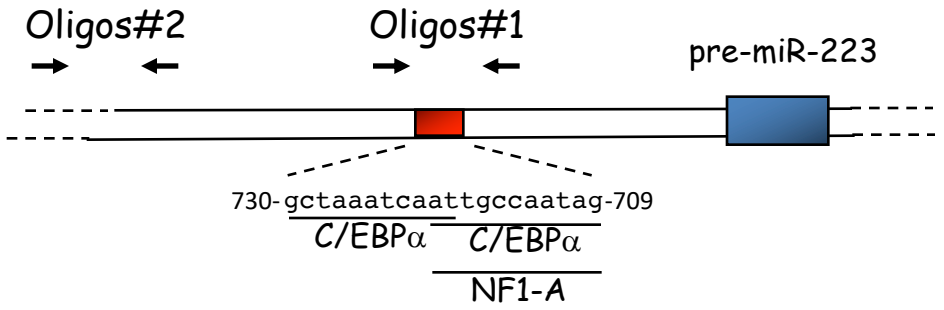


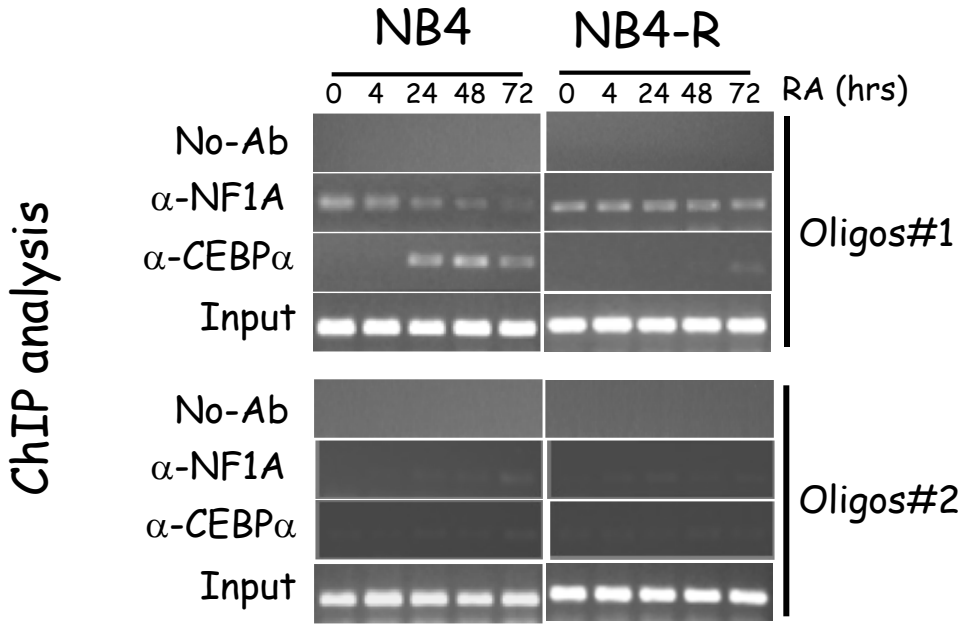
RNAi against NFI-A
enhances differentiation

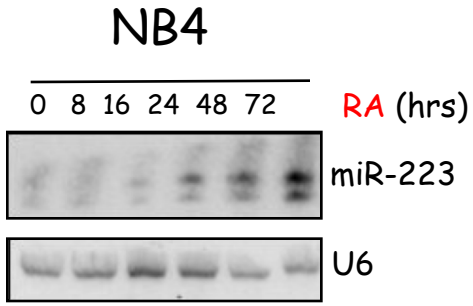
!! NFI-A is counteracting differentiation



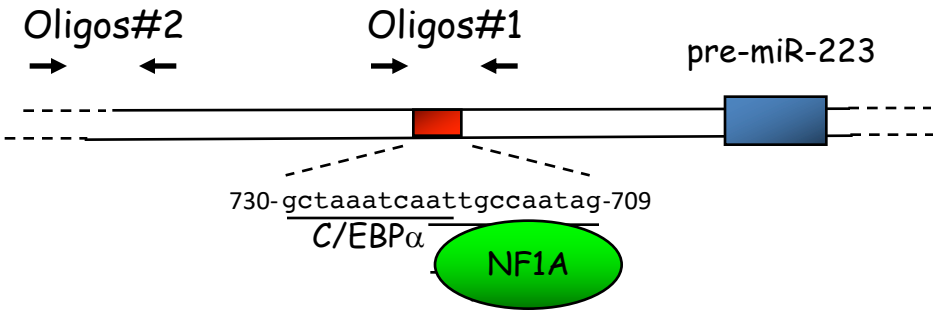
NF1-A competes with C/EBPα for miR-223 promoter binding

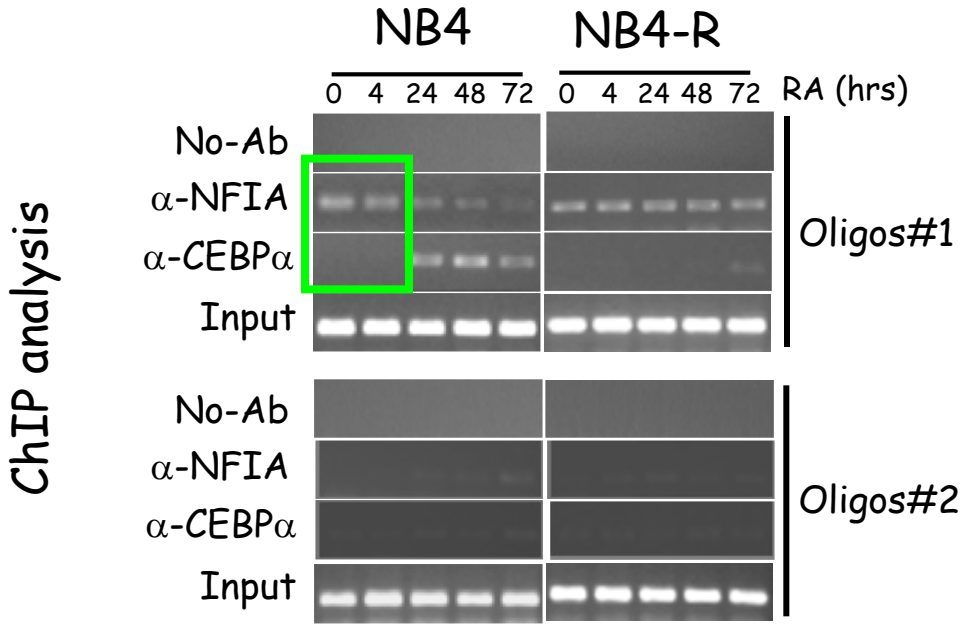


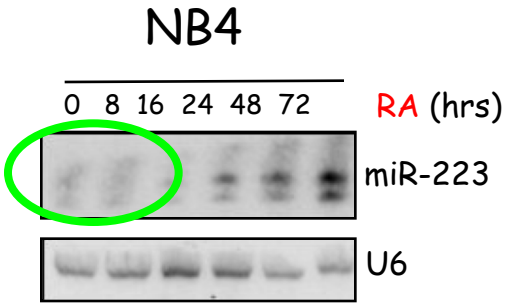




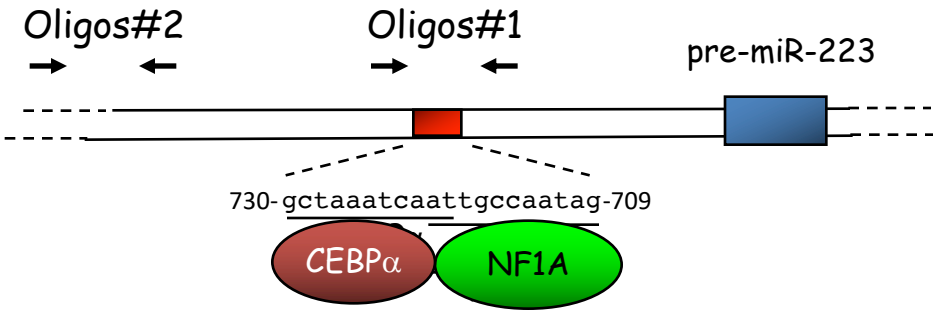
NFI-A competes with C/EBPα for miR-223 promoter binding

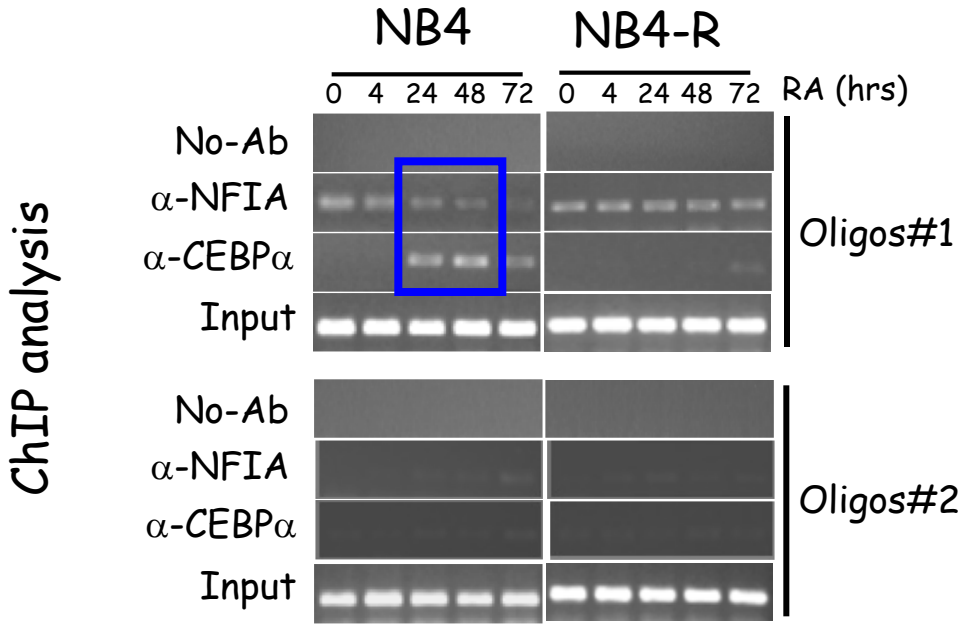


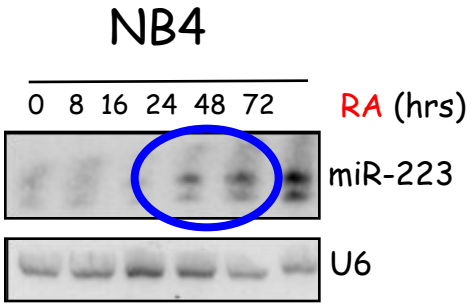




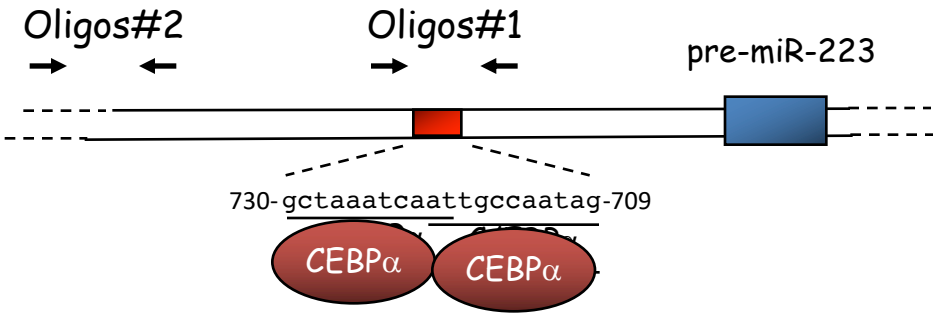
NFI-A competes with C/EBPα for miR-223 promoter binding

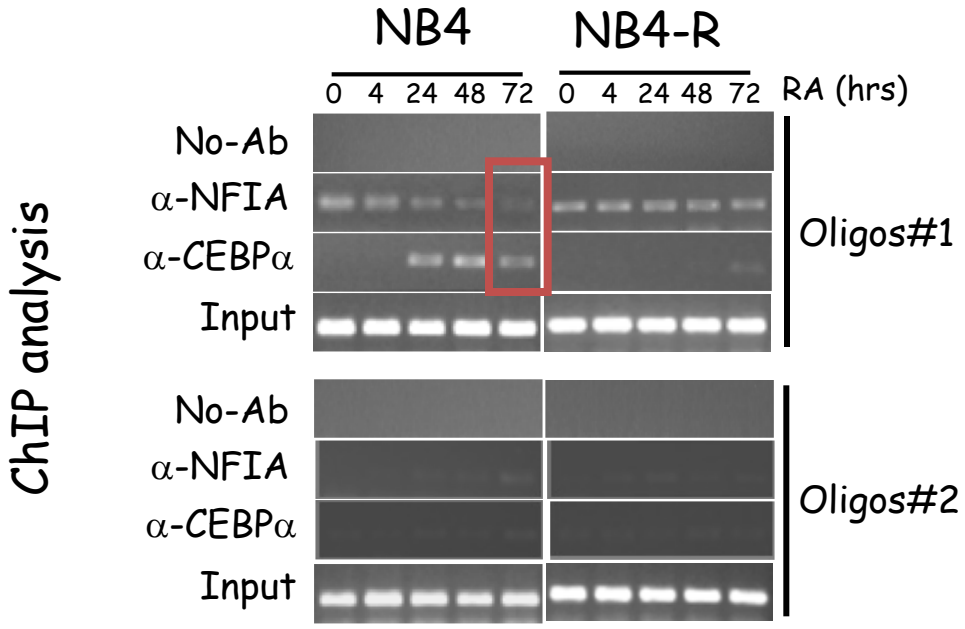


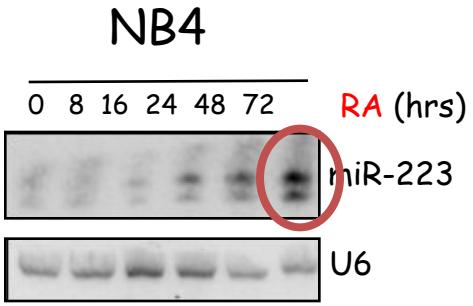




NFI-A competes with C/EBPα for miR-223 promoter binding







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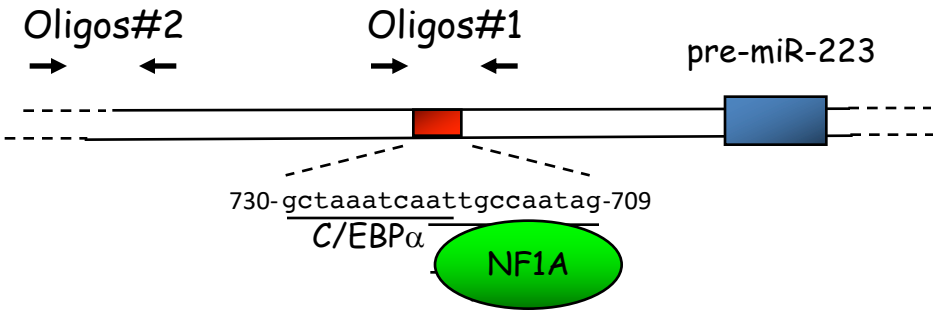
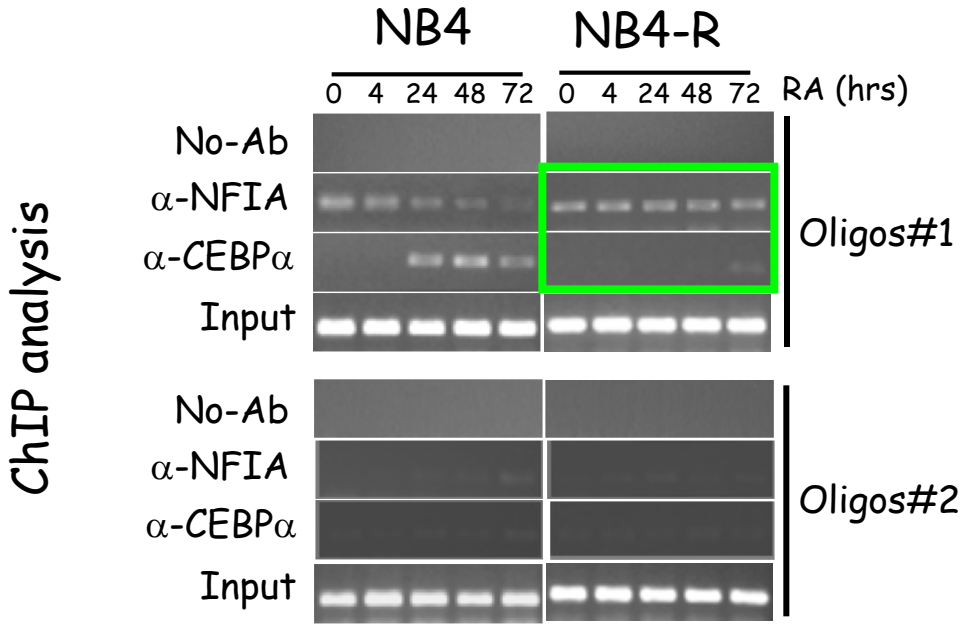
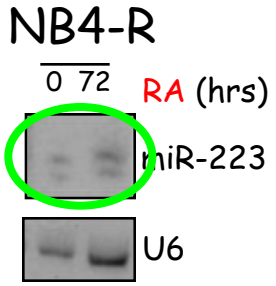
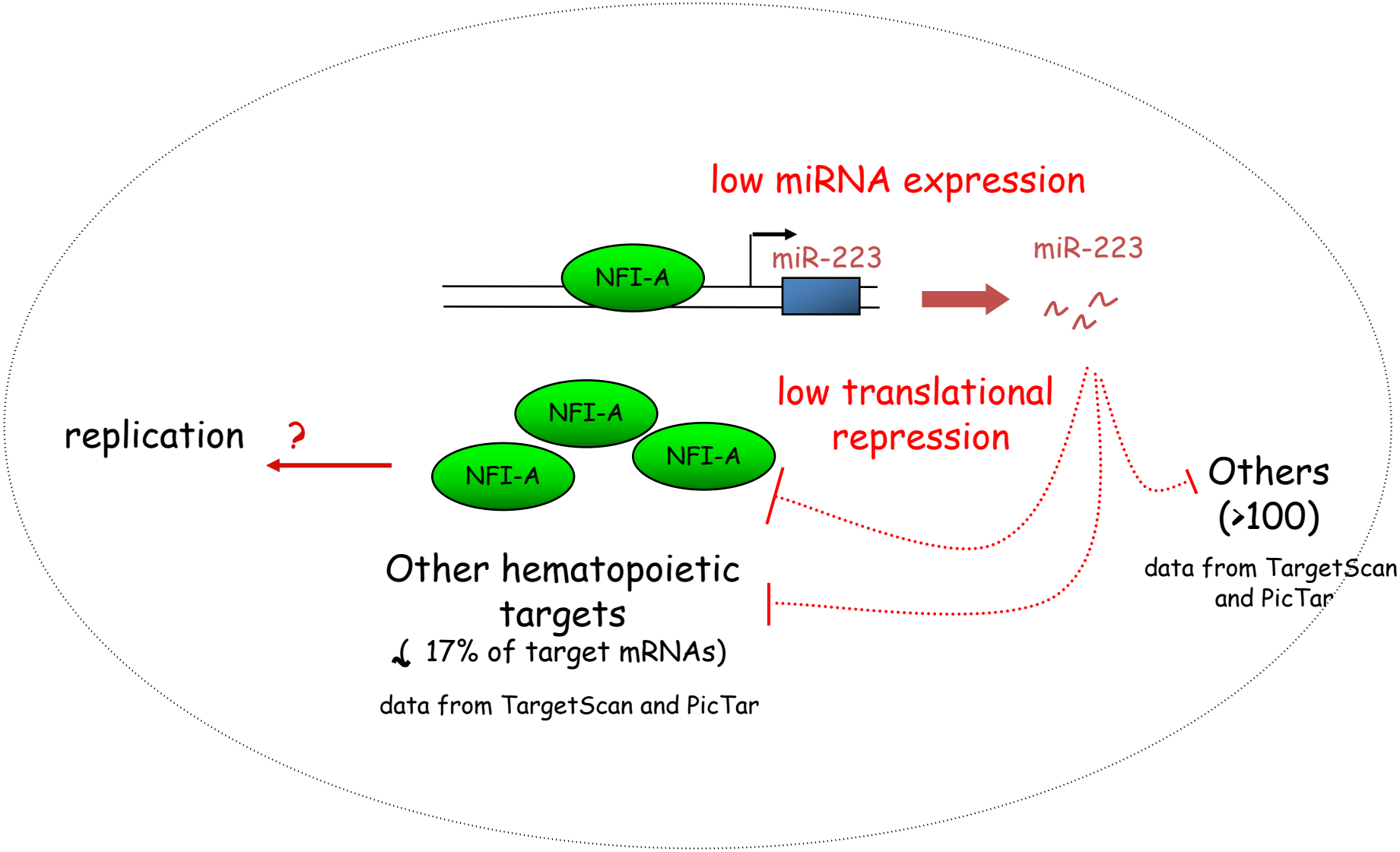
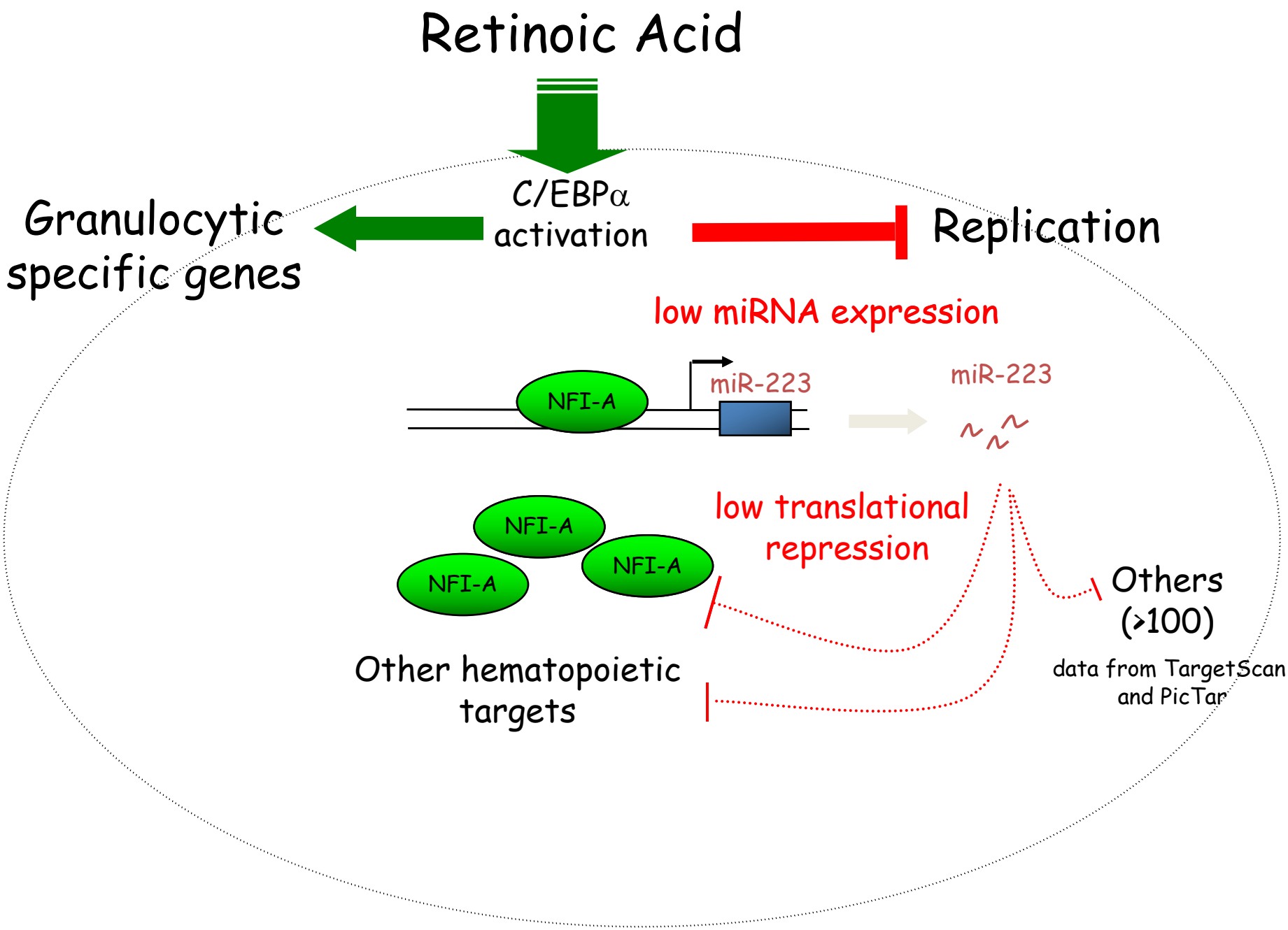


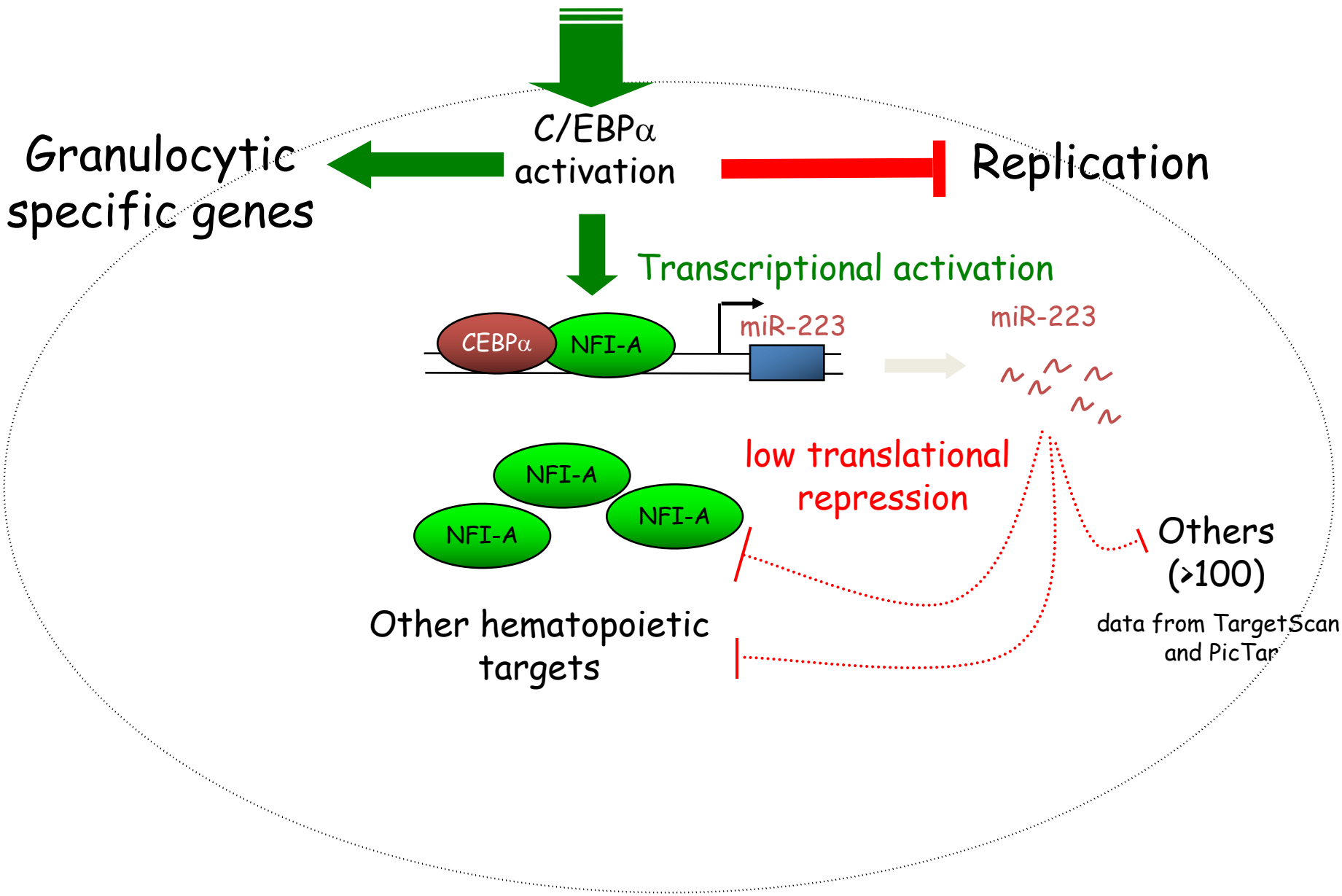


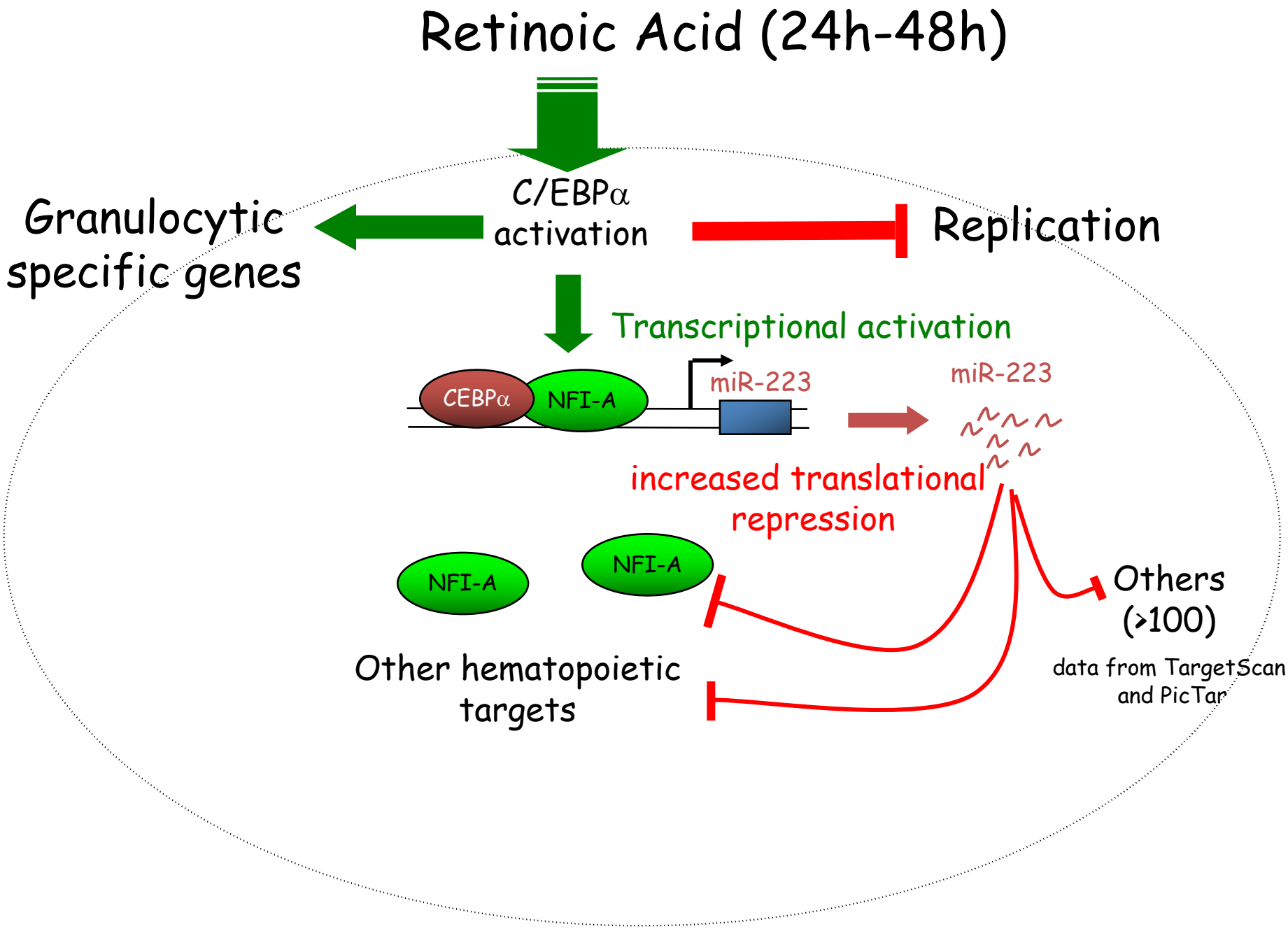


Myeloid precursor cell




Retinoic Acid
C/EBPα activation
Granulocytic specific genes
Replication
low miRNA expression
NFI-A
miR-223
miR-223
NFI-A
NFI-A
NFI-A
low translational repression
Others (>100)
Other hematopoietic targets
data from TargetScan and PicTar


Retinoic Acid (24h-48h)
C/EBPα activation
Granulocytic specific genes
Replication
Transcriptional activation
CEBPα
NFI-A
miR-223
miR-223
NFI-A
NFI-A
NFI-A
low translational repression
Others (>100)
Other hematopoietic targets
data from TargetScan and PicTar


Retinoic Acid (24h-48h)
C/EBPα activation
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Replication
Transcriptional activation
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miR-223
increased translational repression
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NFI-A
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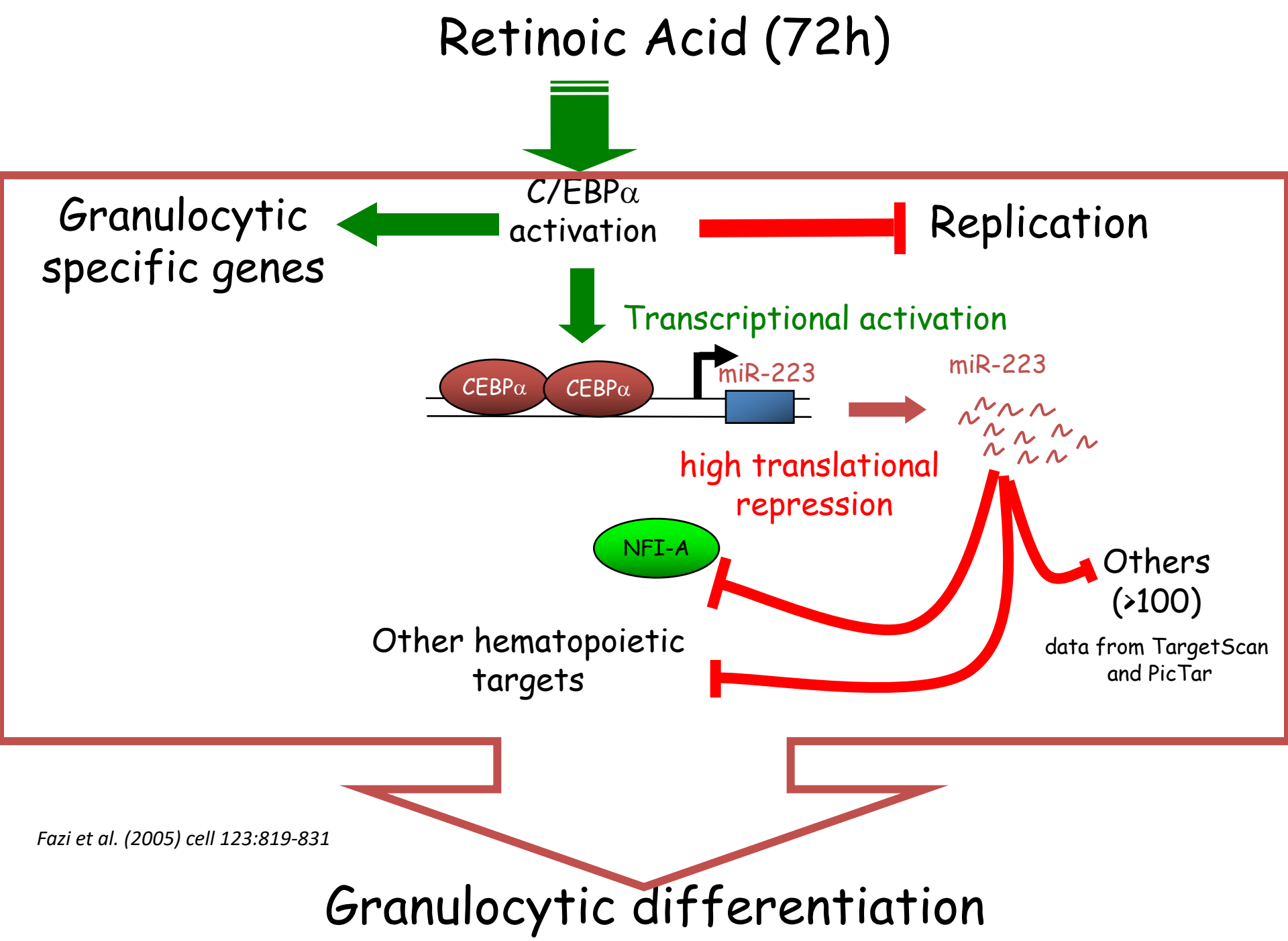
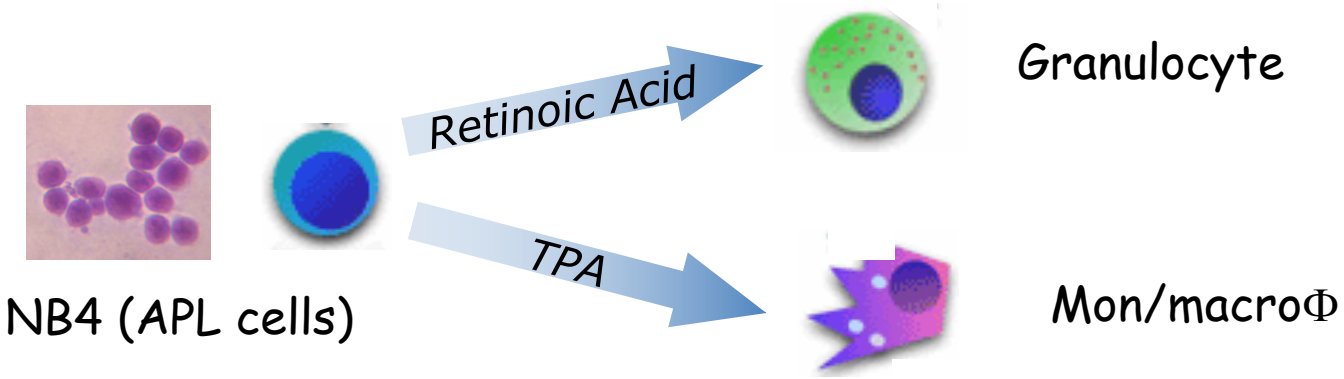
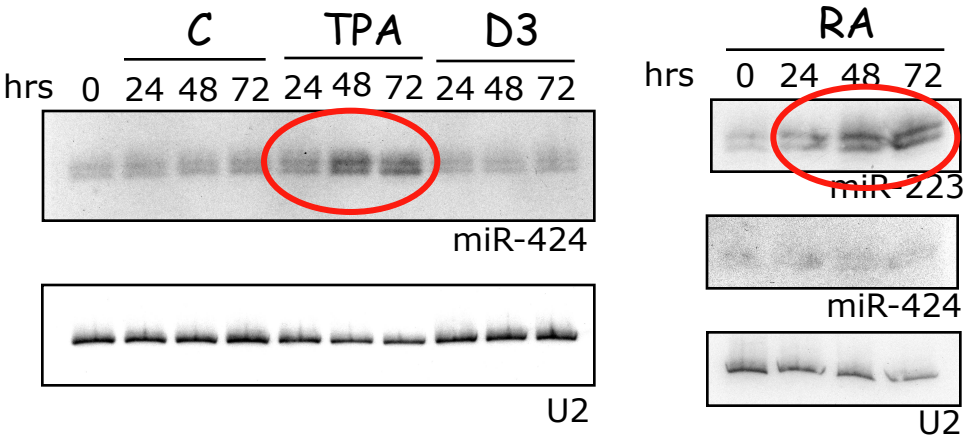
Other hematopoietic targets
data from TargetScan and PicTar



Fazi et al. (2005) cell 123:819-831

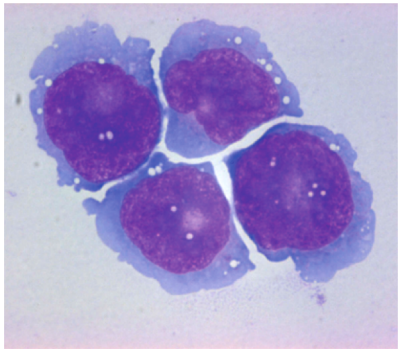
NB4 cells are a useful tool to study myelopoiesis

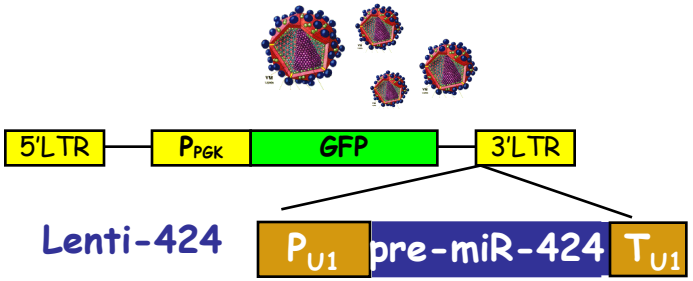


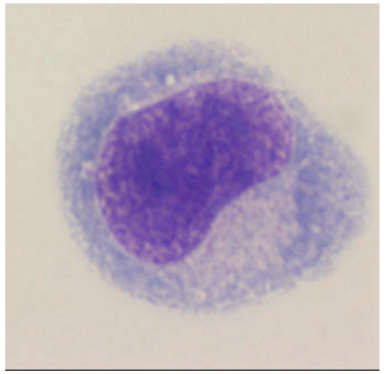


2.5 - 4 fold induction

Ectopic expression of miR-424 in NB4 cells induces the cells towards monocytic differentiation in the absence of TPA



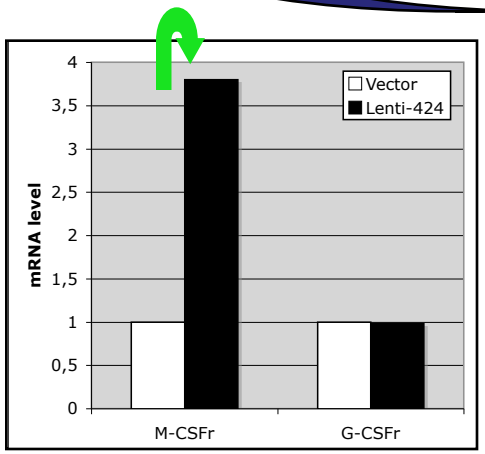


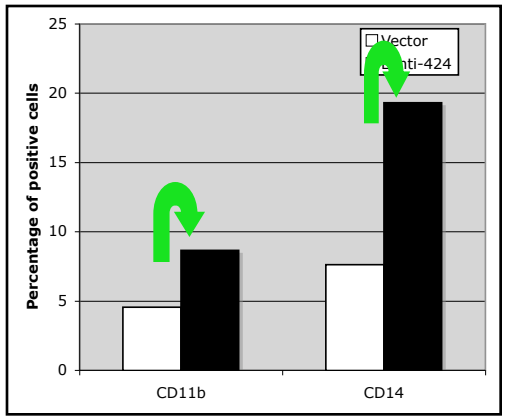


Vector

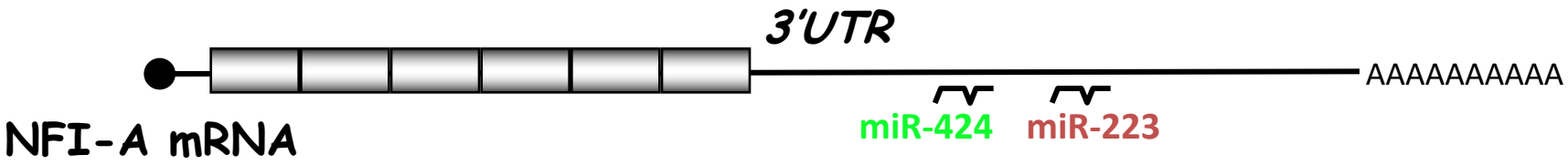
lenti-424

Overexpression of miR-424



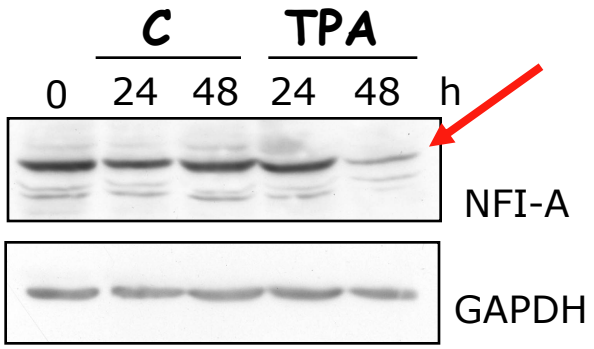


NFI-A is translationally repressed by miR-424



The NFI-A protein levels decrease also in mon/macroϕ differentiation

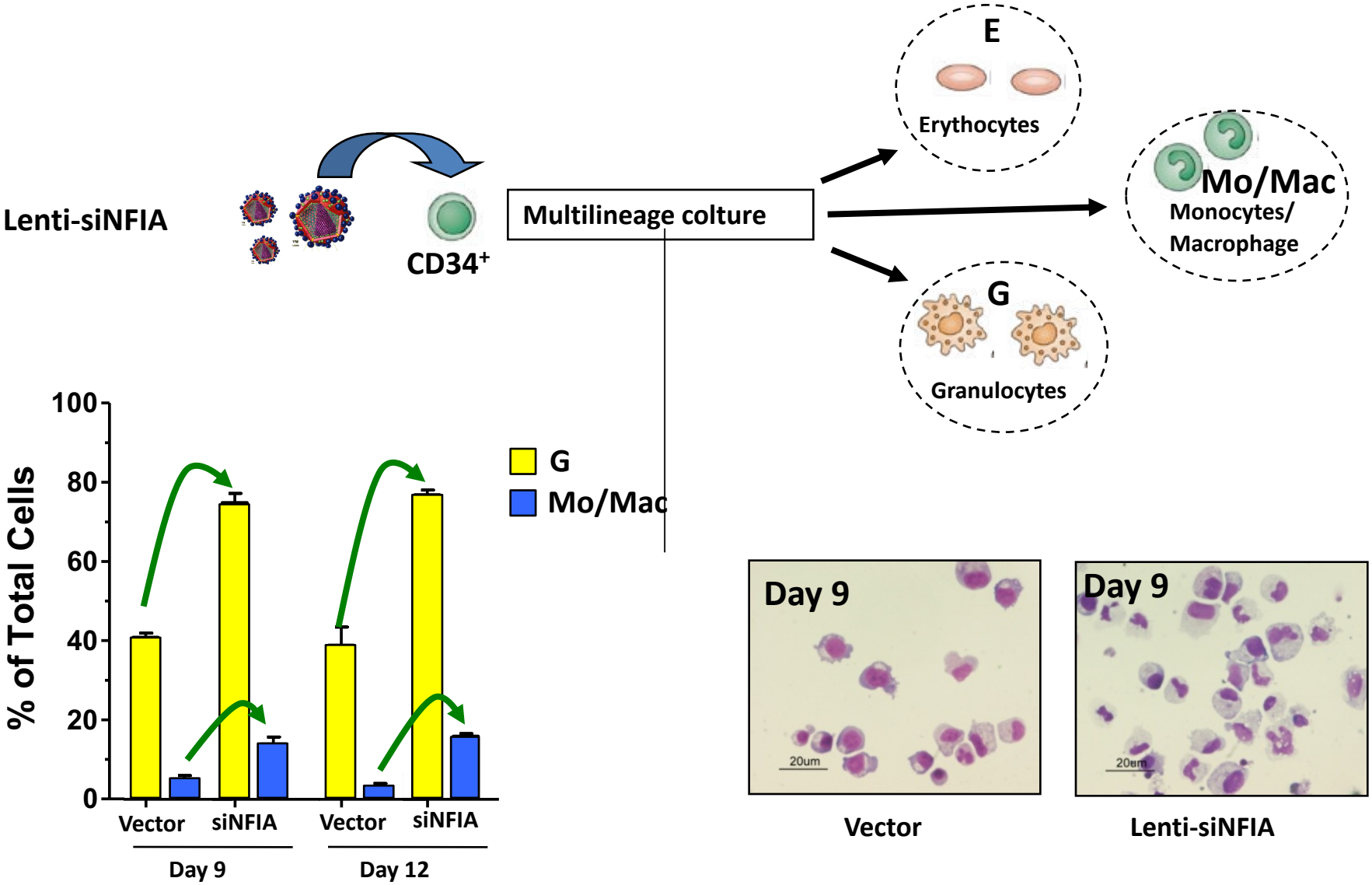
Western Blot Analysis



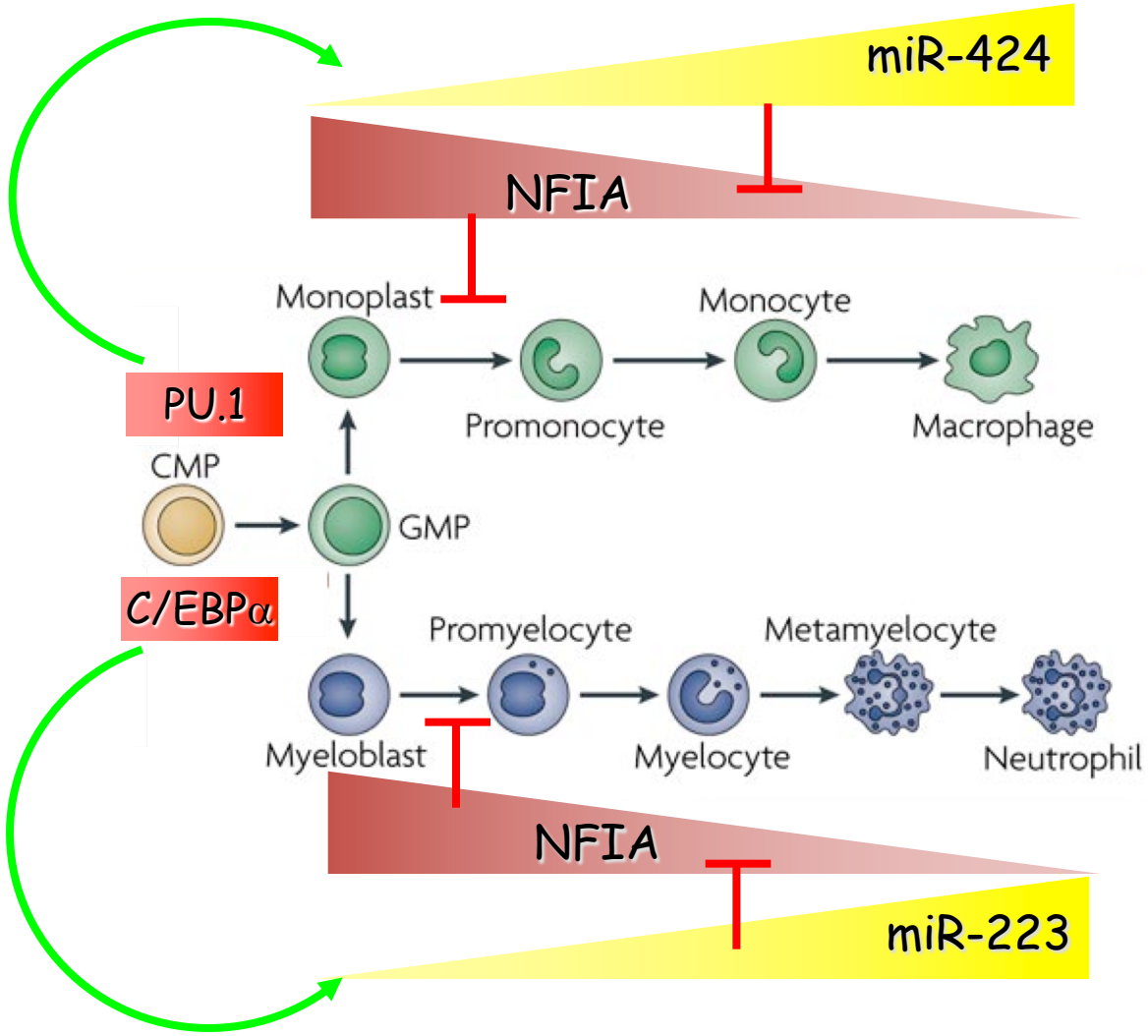
LUCIFERASE ASSAY:

miR-223 and miR-424 repress NFI-A at the translational level

Knock-down of NFIA unbalances myeloid differentiation of CD34+ HPCs



NFIA counteracts Gr/Mo differentiation



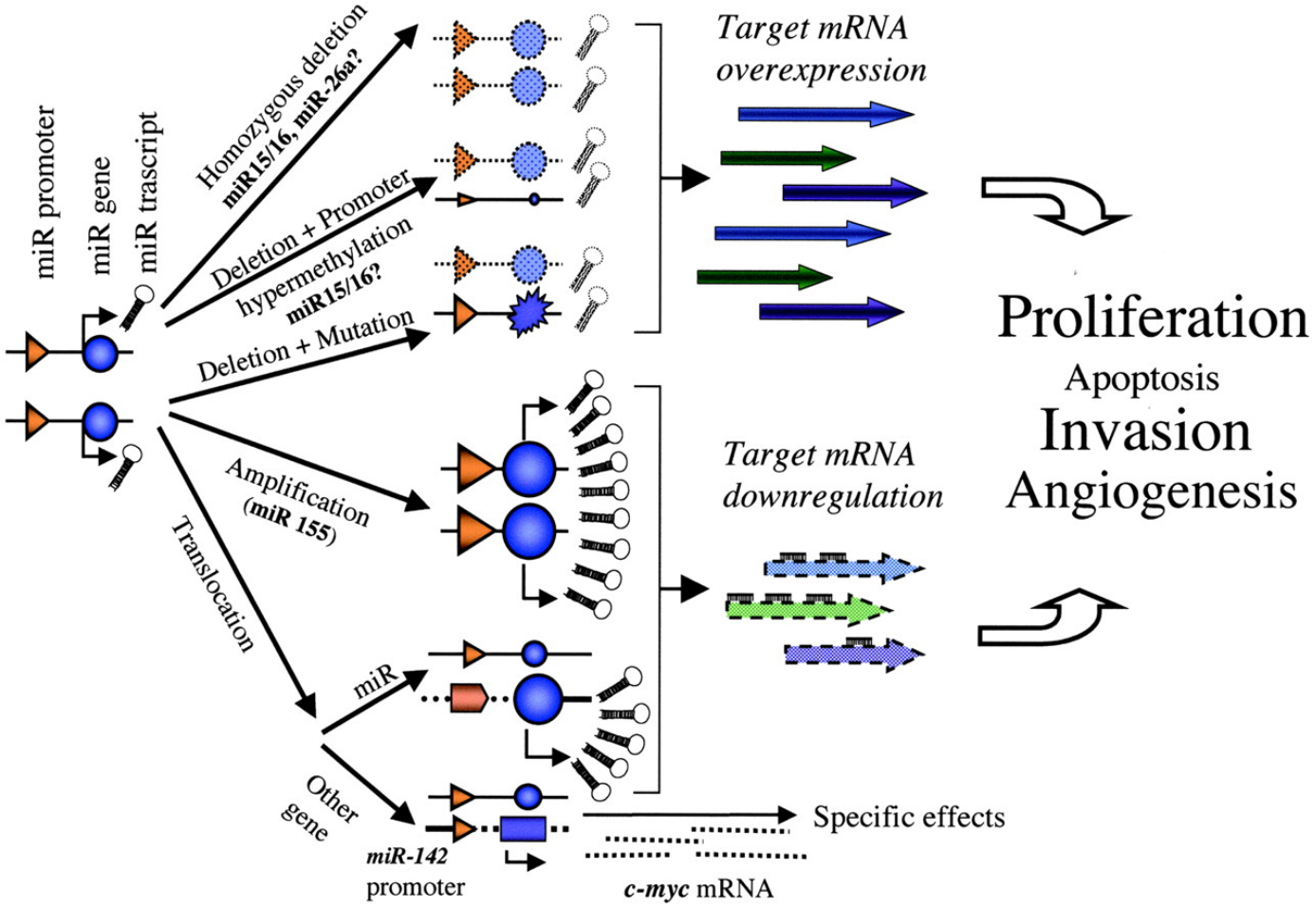
Proc Natl Acad Sci U S A. 2004 Mar 2;101(9):2999-3004.

Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers.

Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM.

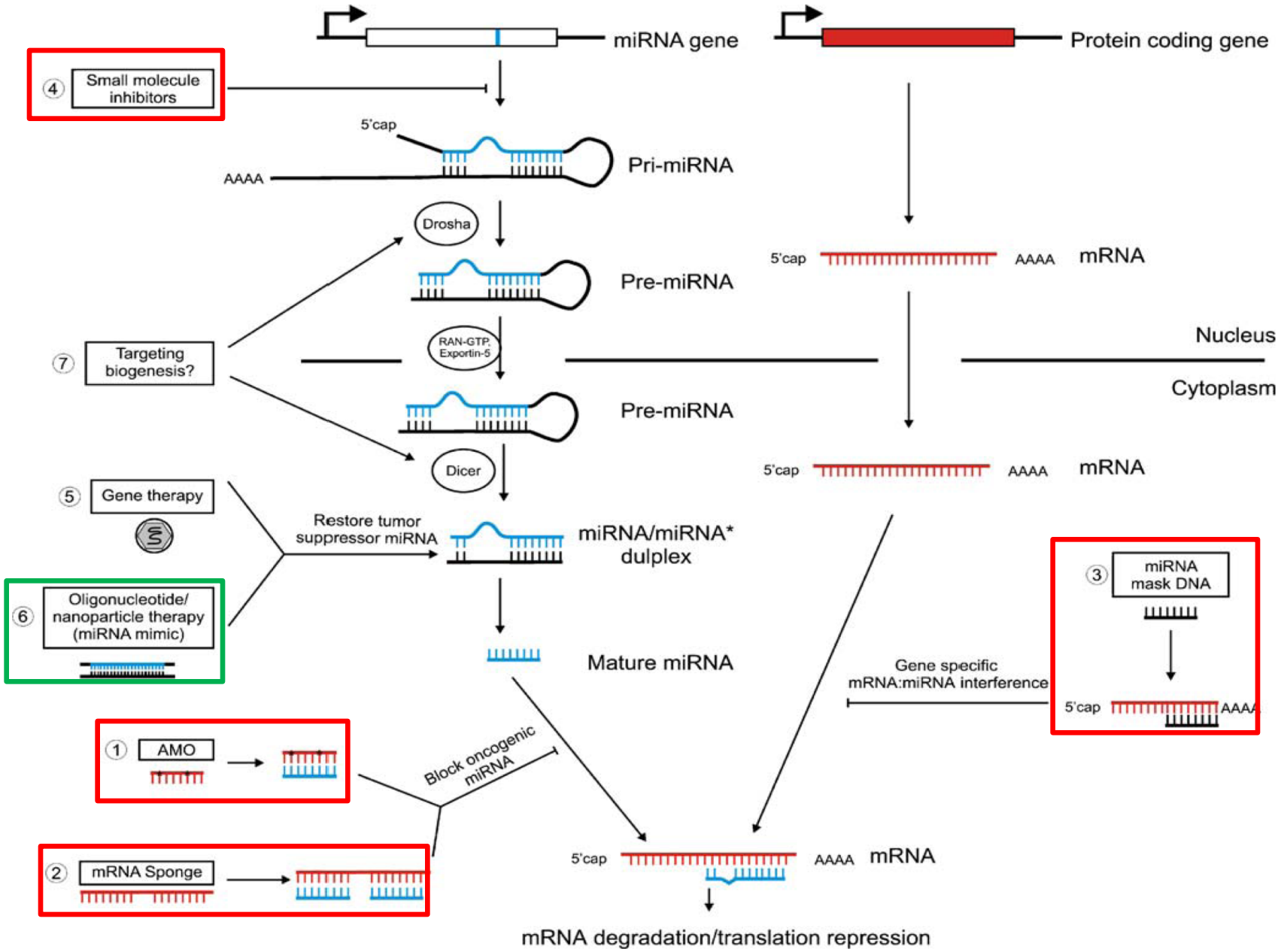
Department of Microbiology and Immunology, Division of Clinical Pharmacology, Biostatistics Section, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA.

A large number of tiny noncoding RNAs have been cloned and named microRNAs (miRs). Recently, we have reported that miR-15a and miR-16a, located at 13q14, are frequently deleted and/or down-regulated in patients with B cell chronic lymphocytic leukemia (B-CLL), a disorder characterized by increased survival. To further investigate the possible involvement of miRs in human cancers on a genome-wide basis, we have mapped 186 miRs and compared their location to the location of previous reported nonrandom genetic alterations. Here, we show that miR genes are frequently located at fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification (minimal amplicons), or common breakpoint regions. Overall, 98 of 186 (52.5%) of miR genes are in cancer-associated genomic regions or in fragile sites. Moreover, by Northern blotting, we have shown that several miRs located in deleted regions have low levels of expression in cancer samples. These data provide a catalog of miR genes that may have roles in cancer and argue that the full complement of miRs in a genome may be extensively involved in cancers.



MiRs as cancer players. Some of these proposed mechanisms are experimentally proven, like the HD of *miR-15a*/*miR-16a* cluster in B-CLL (9), the c-myc overexpression by the reposition near a putative miR promoter, or *miR143*/*miR-145* cluster down-regulation in colon cancers (39).

microRNA e strategie terapeutiche



Chunsheng Li, Yi Feng, George Coukos and Lin Zhang. The AAPS Journal, Vol. 11, No. 4, December 2009

microRNAs as potential human oncogenes

Expression studies of various tumour types have revealed specific alterations in miRNA profiles.

-mir-15 and mir-16 are frequently deleted and/or downregulated in B-cell chronic lymphocytic leukaemia,

-miR-143 and miR-145 show decreased abundance in colorectal neoplasia,

- miR-155 and its non-coding RNA host gene, BIC, are upregulated 100-fold in Burkitt's lymphoma patients..

- Decrease in let-7 levels occur in 60% of cell lines and in 44% of lung carcinoma

-miR-21 is overexpressed in the majority of mammary and colon tumors

-miRNA can act as oncogenes in vivo as integral parts of the molecular architecture of oncogene and tumour suppressor networks. Such oncogenic microRNAs might be designated 'oncomiRs"

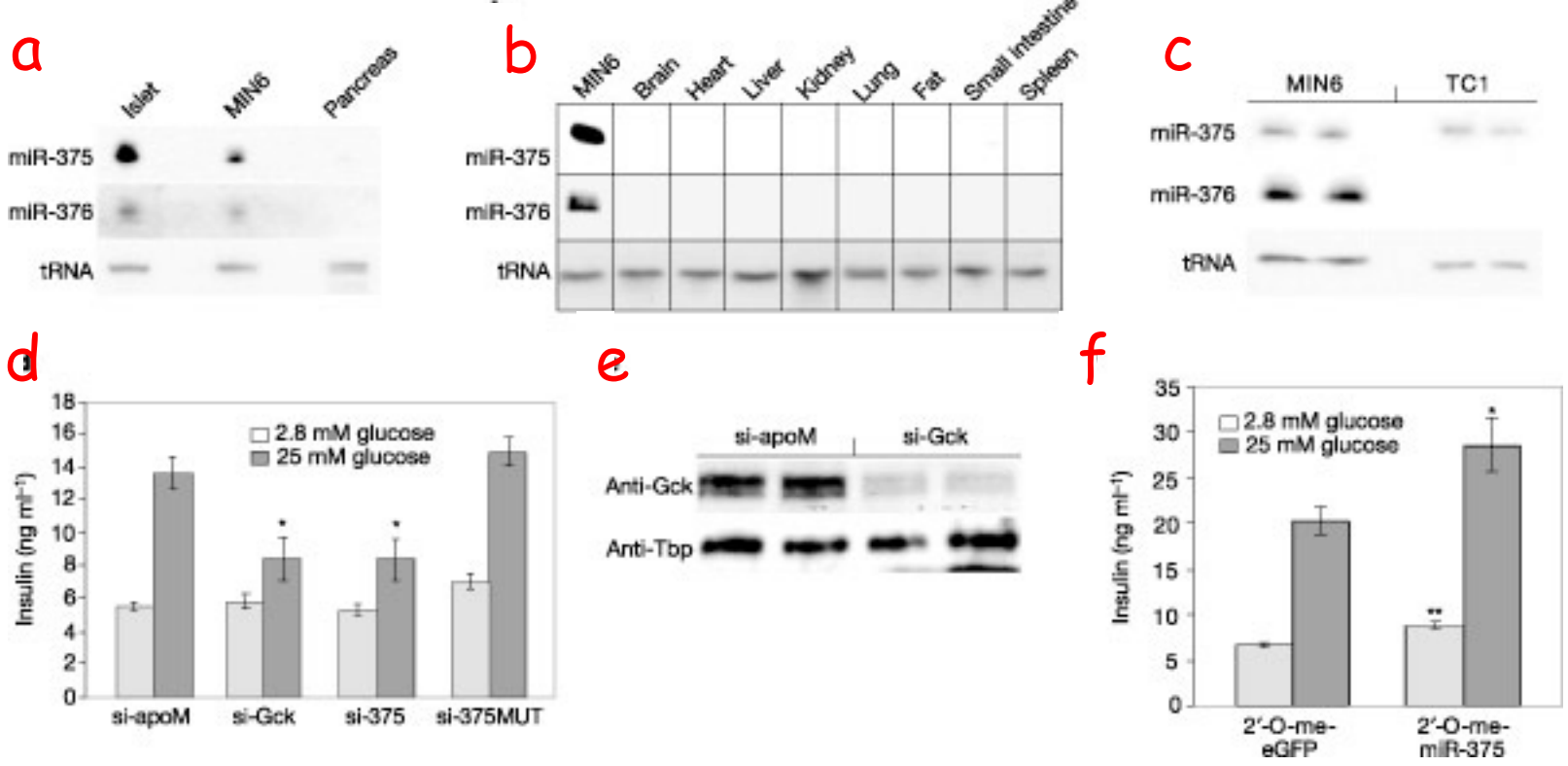
Nature 432, 226 - 230 (11 November 2004)

A pancreatic islet-specific microRNA regulates insulin secretion

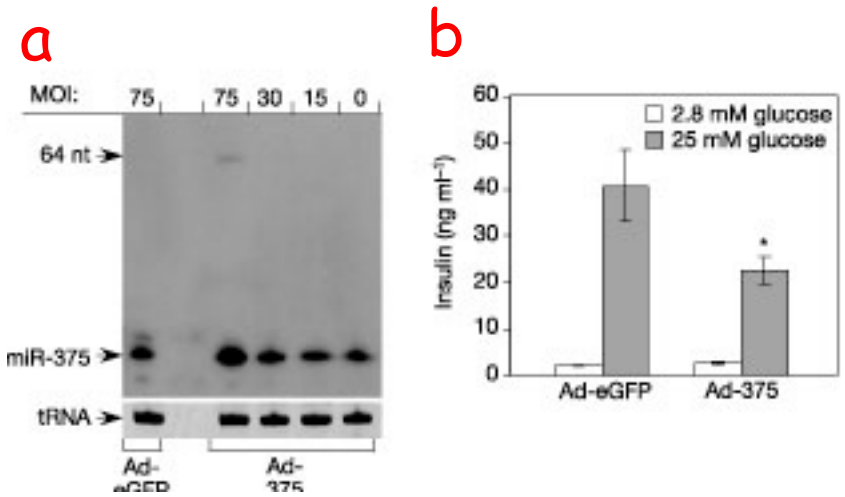
MATTHEW N. POY1, LENA ELIASSON3, JAN KRUTZFELDT1, SATORU KUWAJIMA1, XIAOSONG MA3, PATRICK E. MACDONALD3, SÉBASTIEN PFEFFER1, THOMAS TUSCHL1, NIKOLAUS RAJEWSKY4, PATRIK RORSMAN3,5 & MARKUS STOFFEL1

MicroRNAs (miRNAs) constitute a growing class of non-coding RNAs that are thought to regulate gene expression by translational repression. Several miRNAs in animals exhibit tissue-specific or developmental-stage-specific expression, indicating that they could play important roles in many biological processes. To study the role of miRNAs in pancreatic endocrine cells we cloned and identified a novel, evolutionarily conserved and islet-specific miRNA (*miR-375*). Here we show that overexpression of *miR-375* suppressed glucose-induced insulin secretion, and conversely, inhibition of endogenous *miR-375* function enhanced insulin secretion. The mechanism by which secretion is modified by *miR-375* is independent of changes in glucose metabolism or intracellular Ca2+-signalling but correlated with a direct effect on insulin exocytosis. *Myotrophin* (*Mtpn*) was predicted to be and validated as a target of *miR-375*. Inhibition of *Mtpn* by small interfering (si)RNA mimicked the effects of *miR-375* on glucose-stimulated insulin secretion and exocytosis.

Thus, *miR-375* is a regulator of insulin secretion and may thereby constitute a novel pharmacological target for the treatment of diabetes.

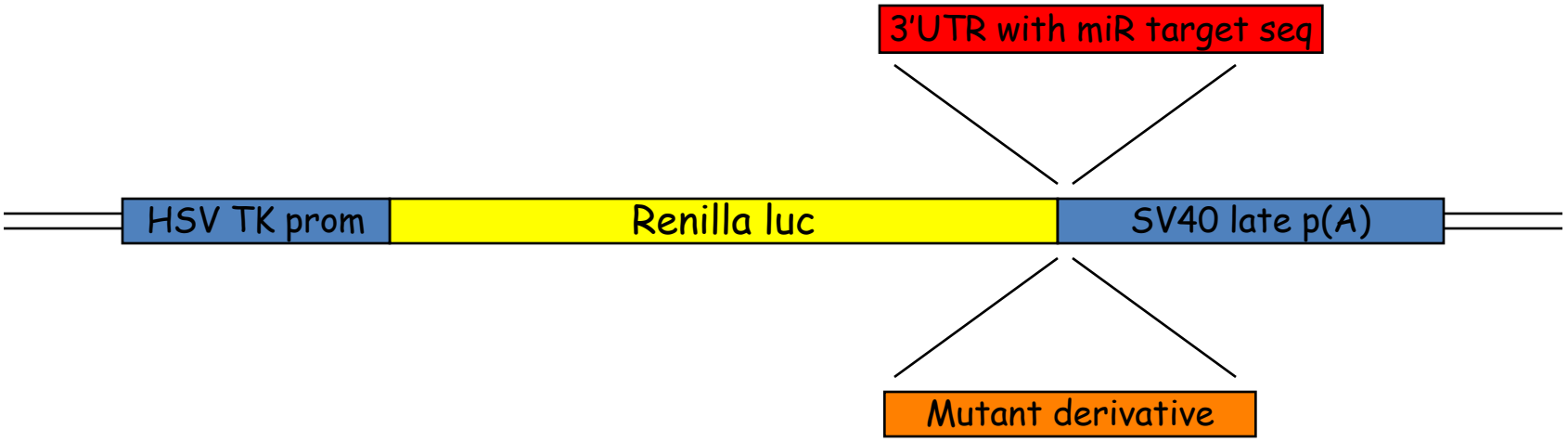


miR-375 is expressed in pancreatic cells and regulates insulin secretion. a, Northern blots of total RNA (10 μg) isolated from purified pancreatic islets, MIN6 cells and total pancreas. High expression levels were detected in mouse pancreatic islets. b, Tissue expression of *miR-375* and *miR-376*. Total RNA (30 μg) was isolated from mouse tissues for northern blots and probed for the indicated miRNAs or transfer RNA (tRNA) as a loading control. c, Northern blots of total RNA (10 μg) isolated from purified MIN6 and TC1 cells. d, In order to increase the cellular miRNA concentration MIN6 cells were transiently transfected with synthetic miRNAs corresponding to *miR-375* (si-375) or a mutated *miR-375* (si-375MUT), or siRNAs targeting *glucokinase* (si-Gck) or *apoM* (si-apoM). After 48 h, the cells were incubated under low (2.8 mM) and stimulatory concentrations of glucose (25 mM) and insulin was measured by RIA (Linco). e, Immunoblot analysis of Gck in MIN6 cells that were transfected with either si-apoM (control) or si-Gck. A 70% reduction in glucokinase protein expression was observed. f, MIN6 cells were transfected with 2'-*O*-methyl oligoribonucleotides complementary to *miR-375* (2'-*O*-me-375), or a control 2'-*O*-methyl oligoribonucleotide (2'-*O*-me-eGFP). Similarly, after 48 h, the cells were incubated at either 2.8 or 25 mM glucose and insulin was measured.



Expression of *miR-375* using recombinant adenovirus (Ad-375) leads to impaired glucose-, KCl- and tolbutamide-induced insulin secretion in MIN6 cells. a, Northern blot analysis and dose-dependent expression of *miR-375* following infection of MIN6 cells for 48 h with Ad-eGFP (control, lane 1) or Ad-375. The multiplicity of infection (MOI) is indicated. The precursor and mature *miR-375* can be visualized at 64 and 22 nt, respectively. b, Insulin secretion of MIN6 cells following infection with Ad-eGFP and Ad-375 in response to 25 mM glucose

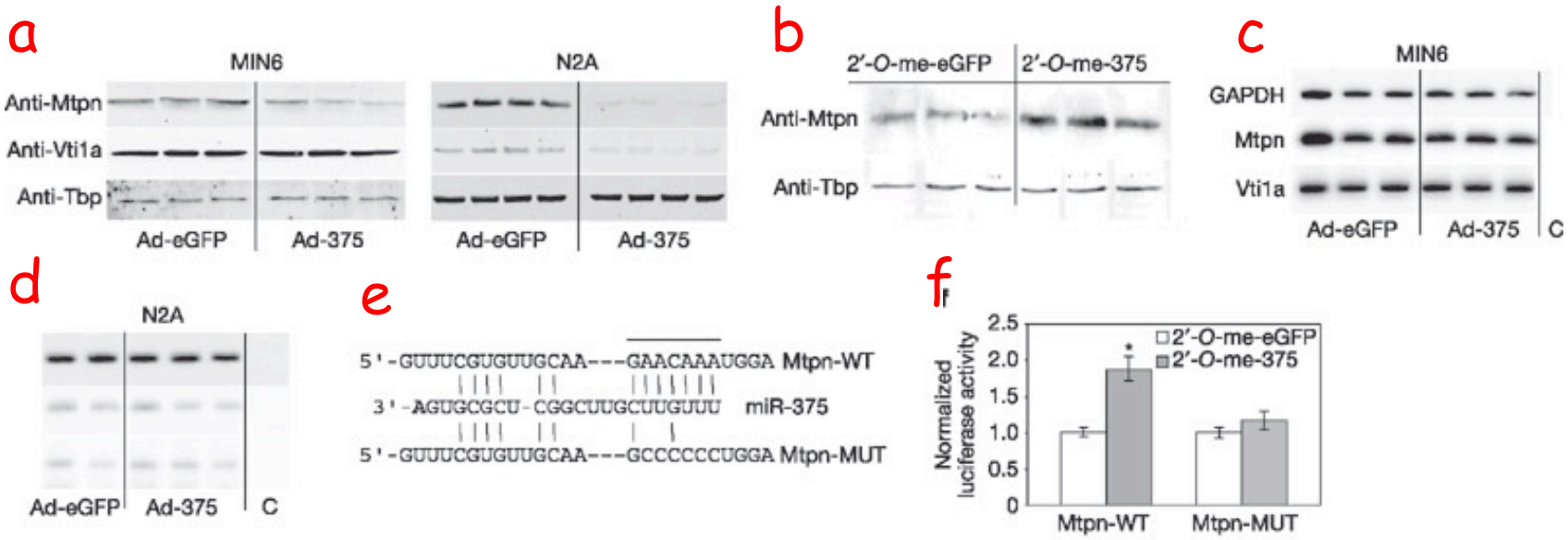
1. Experimental plasmids (pRL-TK derivative)



2. miRNA expressing plasmid

Experimental protocol

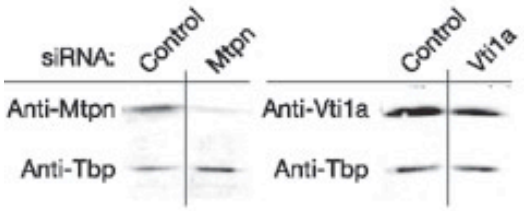
- Transfection in HeLa cells
- Preparation of cell lysate with PLB (passive lysis buffer)
- Measure of Renilla luc activity



Identification of target genes of *miR-375*. a, Western blot analysis of cells infected with Ad-eGFP or Ad-375 (MIN6 cultured for 5 days post-infection; N2A, 2 days) and probed for the expression of myotrophin (anti-Mtpn), Vti1a (anti-Vti1a) or TATA binding protein (anti-Tbp) as a loading control, using specific antisera. b, Immunoblot analysis of Mtpn in MIN6 cells that were transfected with either 2'-*O*-me-eGFP (control) or 2'-*O*-me-375. Expression levels of TATA binding protein (Tbp) were used as a loading control; c, d, RT-PCR analysis of Mtpn, Vti1a and GAPDH (loading control) in MIN6 and N2A cells. e, Sequence of the target site in the 3' UTR of *Mtpn*. The mutant sequence (*Mtpn*-MUT) is identical to the *Mtpn*-WT construct except for five point mutations disrupting base-pairing at the 5' end of *miR-375* (indicated with a bar). f, Mutating the *miR-375* target site in the 3' UTR of *Mtpn* abolishes inhibition of luciferase activity by endogenous *miR-375* in MIN6 cells. MIN6 cells were transiently transfected with either reporter construct in addition to 2'-*O*-methyl-oligoribonucleotides complementary to *miR-375* (2'-*O*-me-375) or a control 2'-oligoribonucleotide (2'-*O*-me-eGFP).

Silencing of *Mtpn* by siRNA impairs insulin secretion. g, MIN6 cells transiently transfected with siRNAs designed against *Mtpn* (si-Mtpn) or *Vti1a* (si-Vti1a) for 48 h and lysed. After separation of proteins by SDS-polyacrylamide gel electrophoresis (PAGE), samples were immunoblotted for either Mtpn or Vti1a expression. The expression of TATA binding protein (Tbp) was analysed for a loading control. h, MIN6 cells were transiently transfected with si-apoM (control), si-Mtpn or si-Vti1a. After 48 h, the cells were incubated under low (2.8 mM) and stimulatory concentrations of glucose (25 mM).

g



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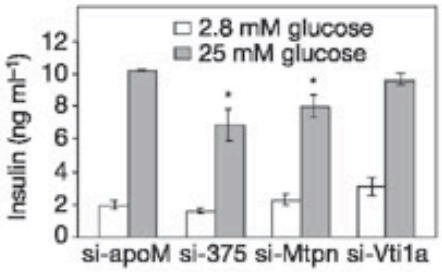
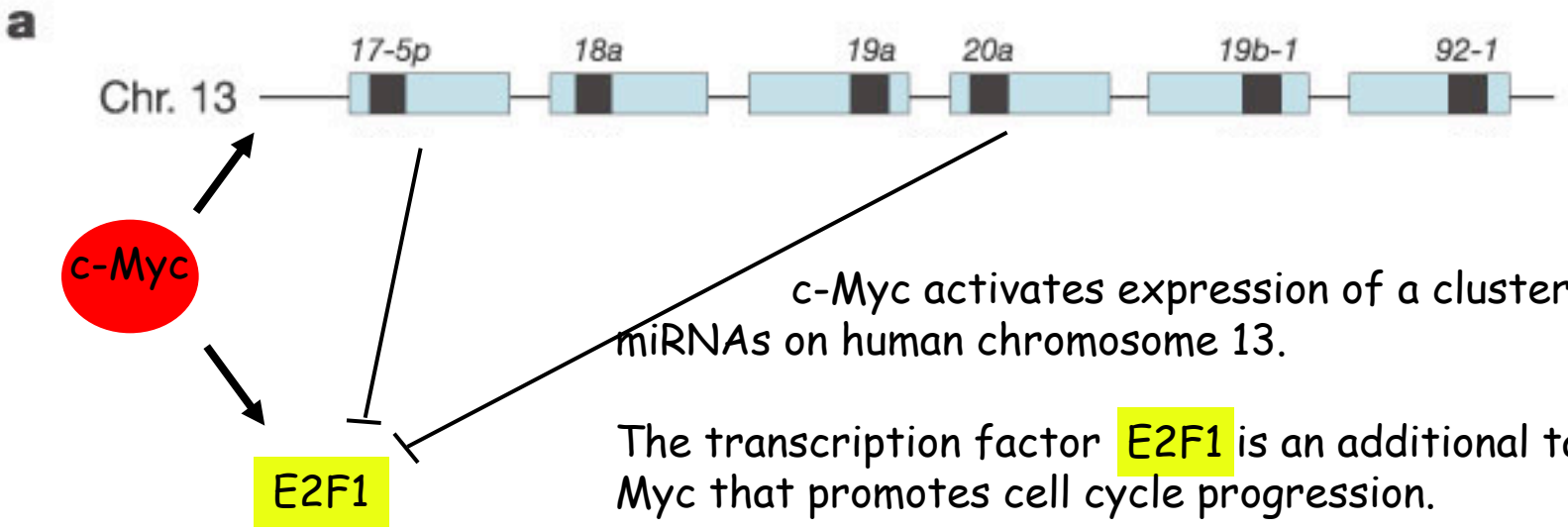


c-Myc-regulated microRNAs modulate E2F1 expression

Kathryn Donnell, Erik Wentze, Karen Zeller, Chi Dang & Joshua Mendell

Nature. 2005 435:839-43



c-Myc activates expression of a cluster of six miRNAs on human chromosome 13.

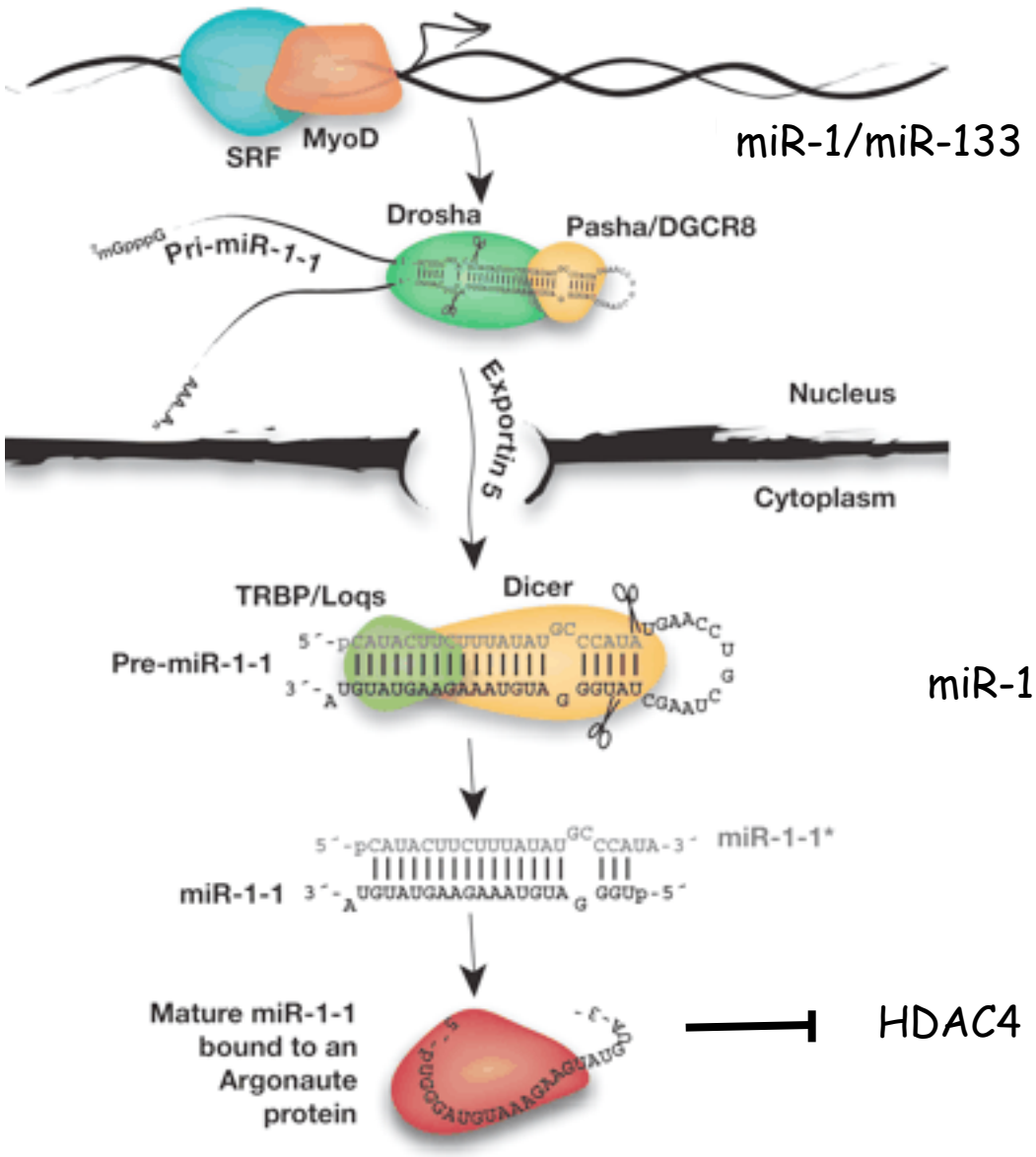
The transcription factor E2F1 is an additional target of c-Myc that promotes cell cycle progression.

E2F1 is negatively regulated by two miRNAs in this cluster, miR-17-5p and miR-20a.

These findings expand the known classes of transcripts within the c-Myc target gene network, and reveal a mechanism through which c-Myc simultaneously activates E2F1 transcription and limits its translation, allowing a tightly controlled proliferative

- mir-17-92 are often substantially increased in B cell lymphoma.

miR-1 genes titrate the effects of critical cardiac regulatory proteins to control the balance between differentiation and proliferation during cardiogenesis.



SRF has a dual role in regulating the balance between proliferation and differentiation during cardiogenesis. Failure to maintain an adequate pool of undifferentiated myocyte precursors could result in organ hypoplasia

miR-133 ⊣ SRF ⊣ proliferation

Mature miR-1-1 bound to an Argonaute protein ⊣ HDAC4 ⊣ Mef2, MyoD → Myoblast → Myotube

Cell. 2009;137:1005-17.

Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model.

Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT.

Therapeutic strategies based on modulation of microRNA (miRNA) activity hold great promise due to the ability of these small RNAs to potently influence cellular behavior. In this study, we investigated the efficacy of a miRNA replacement therapy for liver cancer. We demonstrate that hepatocellular carcinoma (HCC) cells exhibit reduced expression of miR-26a, a miRNA that is normally expressed at high levels in diverse tissues. Expression of this miRNA in liver cancer cells in vitro induces cell-cycle arrest associated with direct targeting of cyclins D2 and E2. Systemic administration of this miRNA in a mouse model of HCC using adeno-associated virus (AAV) results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and dramatic protection from disease progression without toxicity. These findings suggest that delivery of miRNAs that are highly expressed and therefore tolerated in normal tissues but lost in disease cells may provide a general strategy for miRNA replacement therapies.

Nature. 2005 ;438:685-9.

Silencing of microRNAs in vivo with 'antagomirs'.

Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M.

MicroRNAs (miRNAs) are an abundant class of non-coding RNAs that are believed to be important in many biological processes through regulation of gene expression. The precise molecular function of miRNAs in mammals is largely unknown and a better understanding will require loss-of-function studies in vivo. Here we show that a novel class of chemically engineered oligonucleotides, termed 'antagomirs', are efficient and specific silencers of endogenous miRNAs in mice. Intravenous administration of antagomirs against miR-16, miR-122, miR-192 and miR-194 resulted in a marked reduction of corresponding miRNA levels in liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenals. The silencing of endogenous miRNAs by this novel method is specific, efficient and long-lasting. The biological significance of silencing miRNAs with the use of antagomirs was studied for miR-122, an abundant liver-specific miRNA. Gene expression and bioinformatic analysis of messenger RNA from antagomir-treated animals revealed that the 3' untranslated regions of upregulated genes are strongly enriched in miR-122 recognition motifs, whereas downregulated genes are depleted in these motifs. Analysis of the functional annotation of downregulated **genes specifically predicted that cholesterol biosynthesis genes would be affected by miR-122,** and plasma cholesterol measurements showed reduced levels in antagomir-122-treated mice. Our findings show that antagomirs are powerful tools to silence specific miRNAs in vivo and may represent a therapeutic strategy for silencing miRNAs in disease.