MicroRNAs as Modulators of the Platelet Proteome

Patricia Landry and Patrick Provost*

CHUL Research Center/CHUQ and Faculty of Medicine, Université Laval, Quebec, QC, G1K 7P4, Canada

Abstract: MicroRNAs are short 21- to 24-nucleotide (nt) RNA species that act as key regulators of gene expression. Known primarily to modulate mRNA translation through recognition of specific binding sites located in the 3'untranslated region (UTR) of messenger RNA (mRNA) targets, microRNAs may regulate between 30% to 92% of the genes in human, thereby controlling a plethora of biological processes. Although devoid of a nucleus and lacking genomic DNA, platelets may be no exception, as recent experimental evidences indicate that they contain all the protein and RNA components and features required for microRNA-regulated mRNA translation: (i) the platelet transcriptome is astonishingly diverse, representing between 15 and 32% of all human genes, (ii) platelet mRNAs can be translated into proteins, (iii) platelets contain an abundant and diverse array of microRNAs, and (iv) the host Dicer and Argonaute 2 (Ago2) complexes. The latter ones are functional in microRNA biogenesis and function, respectively. In this review article, we will summarize and discuss the experimental evidences as well as the most recent advances supporting a role for microRNAs as modulators of the platelet proteome. Expected to play a central role in health and disease, a dysfunctional microRNA-based regulation of gene expression in platelets may represent an important etiologic factor underlying platelet-related and cardiovascular diseases.

Keywords: Platelet, messenger RNA, gene regulation, RNA silencing, microRNA, cardiovascular diseases.

BIOGENESIS AND FUNCTION OF microRNAs

The microRNA pathway is a gene regulatory process based on the biogenesis and function of small, ~21- to 24-nucleotide (nt) non-coding RNAs, known as microRNAs, as reviewed recently [1, 2]. Encoded by the genome of nucleated cells, microRNA genes are transcribed mainly by RNA polymerase II [3] into primary microRNAs (pri-miRNAs), which are trimmed into microRNA precursors (pre-miRNAs) by the nuclear ribonuclease III (RNase III) Drosha [4], acting within the Microprocessor complex with its cofactor DiGeorge syndrome Critical Region gene 8 (DGCR8) protein [5-7] (see Fig. **1** – Megakaryocyte). Accumulating in the nucleus, the ~60- to 70-nt pre-microRNAs are then exported via Exportin-5 [8, 9] to the cytoplasm, where they are processed by the RNase III Dicer [10-12]. Assisted by TAR RNA-binding protein 2 (TRBP2) [13], Dicer cleaves the stem of pre-microRNA substrates at the base of the loop to generate microRNA:microRNA* duplexes. This ribonucleoprotein (RNP) complex is joined by the Argonaute 2 (Ago2) protein [13] and the mature microRNA strand is selected, based on the relative stability of the duplex extremities, to form and guide a microRNA-containing RNP (miRNP) complex for the regulation of specific messenger RNAs (mRNAs), as reviewed recently [1].

The associated microRNAs confer to the miRNP complexes the ability to recognize cellular mRNAs through specific binding sites generally located in the 3' untranslated region (UTR) [1]. The targeted mRNAs are then translocated to the P-bodies, where they are either degraded or returned to the translational machinery for expression upon a specific

cellular signal [14, 15]. Although microRNAs are known mainly as repressors of gene expression, they have also been shown to enhance mRNA translation under specific cellular conditions [16].

Involving relatively few protein and RNA components, the microRNA-guided RNA silencing pathway is a complex and well-integrated regulatory circuit that plays a key role in modulating a very large number of mRNA targets [17]. Predicted to regulate up to 90% of the genes in humans [18], microRNAs may thus control cellular and biochemical processes in all cells and tissues of the human body!

MicroRNAs IN HEALTH AND DISEASE

We recently reviewed the role and importance of microRNAs in health and disease, more specifically in the context of neurological diseases [2] and viral infections [19]. The same concepts and principles may hold true and be applicable to platelets in the context of the cardiovascular system. Involved in the fine tuning of gene expression, a normal microRNA function is required for a tightly regulated expression of the cellular proteins (see Fig. **2-1**), which is critical for the maintenance of health and prevention of disease, as discussed in Perron *et al*. [20]. In contrast, deregulated protein expression induced by a dysfunctional microRNA-based regulatory system may represent the main etiologic factor underlying genetic diseases (see Fig. **2-2**).

For instance, a deregulated microRNA control of mRNA translation may occur when the function of a core protein component of the microRNA pathway is compromised, i.e. is the subject of a deletion, mutation or misexpression. In this situation, a global negative impact on microRNA biogenesis and/or function is observed, as in the case of the fragile X mental retardation protein (FMRP) in the fragile X syndrome [21] and of the behavioral and neuronal deficits associated with haploinsufficiency of the *Dgcr8* gene [22].

*Address correspondence to this author at the CHUL Research Center/CHUQ, 2705 Blvd Laurier, Room T1-49, Quebec, QC, G1V 4G2 Canada; Tel: 1 418 656 4141; Ext: 48842; Fax: 1 418 654 2765;
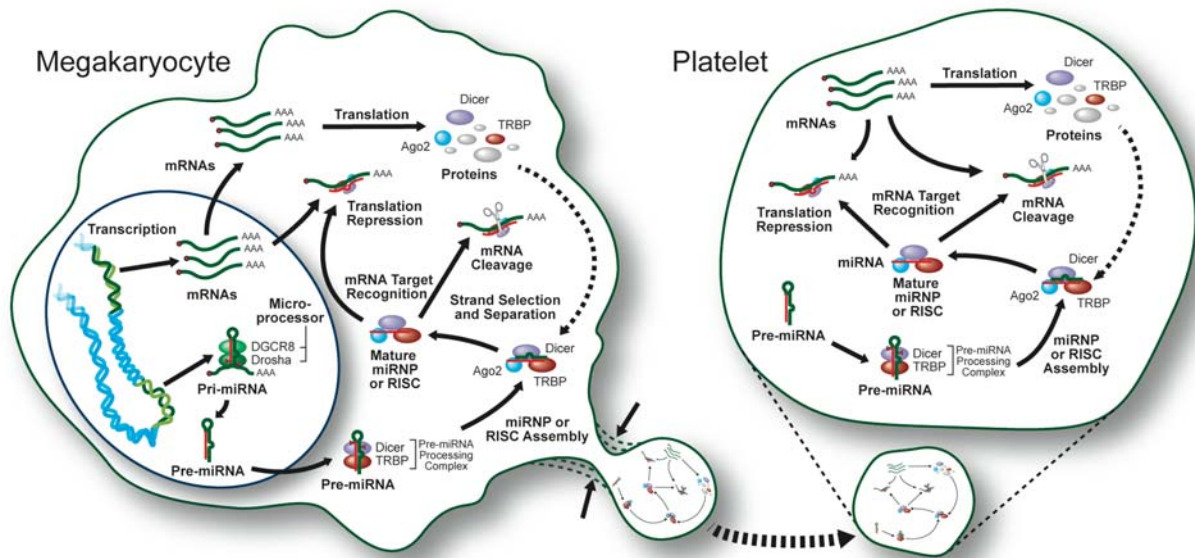E-mail: patrick.provost@crchul.ulaval.ca

Fig. (1). Proposed model for platelet inheritance of a functional microRNA pathway from megakaryocyte precursor cells. Harboring a complete microRNA pathway, megakaryocytes are believed to pass on the cytosolic components of the pathway to the platelets in formation, which end up with a functional microRNA pathway devoid of its habitual nuclear initiation step. From Landry *et al*. [51], with permission from the Nature Publishing Group.

The deletion, mutation or misexpression of a microRNA, or that of its corresponding binding site, may alter the microRNA control of gene expression and lead to microRNA:microRNA binding site pairs becoming dysfunctional or, in some cases, to the emergence of functional microRNA:microRNA binding site combinations. The ensuing deregulation of mRNA translation may then lead to misexpression, i.e. either downregulated or upregulated expression, of a specific protein and provoke the development of a disease [20], as it may be the case of the ß-amyloid precursor protein (APP)-converting enzyme (BACE) in Alzheimer's disease (AD) [23]. On the other hand, microRNAs of foreign origin, such as those released from viruses like HIV-1 [24], may also perturb host gene expression through the recognition and regulation of cellular mRNAs harboring complementary binding sites, as discussed recently [19]. Hence the relevance of using microRNAs as biomarkers and therapeutic targets/drugs in diagnosing and treating human diseases affecting major systems, including the cardiovascular system.

PLATELETS

Platelets, or thrombocytes, are produced in the bone marrow from progenitor cells, known as megakaryocytes, and are central players in processes of hemostasis and thrombosis. Released into the circulation as anucleated cytoplasmic fragments at a daily rate of 10^{11} [25], platelets are discoid blood elements measuring ~0.5 x 3.0 µm in diameter (much smaller than granulocytes; 12-15 µm in diameter) and having a circulating life of 8-10 days [25, 26]. They contain an actin cytoskeleton, microtubules, mitochondria and a canalicular system through which the content of the different types of granules, such as lysosomes (containing acid hydrolases),

dense bodies (containing ADP, ATP serotonin and calcium) and alpha granules (containing platelet factor 4, fibrinogen, fibronectin, thrombospondin, von Willebrand factor, ß-thromboglobulin, transforming growth factor ß1, platelet-derived growth factor, factor V, and factor XIII), is secreted. Playing a critical role in the maintenance of hemostasis, platelets are recruited to sites of damaged vessel walls or atherosclerotic plaques, ruptured or not, and are activated when brought into contact with collagen (exposed upon damage of the endothelial blood vessel lining), thrombin or ADP, or with receptors expressed on white blood cells or vascular endothelial cells. Once activated, platelets release the content of their granules, leading to additional platelet recruitment, activation and aggregation in a sequence of events that may culminate in thrombus formation and vessel occlusion. Platelets thus play a critical role in the mechanisms underlying cardiovascular complications related to atherosclerosis, such as heart attack and stroke, causing significant morbidity and mortality in industrialized societies. A recent study unveiled an inflammatory role for platelets in rhumatoid arthritis [27], thereby expanding their roles into the immune system [28].

THE PLATELET PROTEOME

Most platelet functions are mediated by, or involve, a repertoire of proteins inherited from their megakaryocytic precursor cells. Lacking genomic DNA, and thereby incapable of transcribing nuclear genes into mRNAs, platelets were believed to lack protein synthetic capacity. However, it has been known for some time that platelets contain rough endoplasmic reticulum and ribosomes [29], and that they can synthesize proteins [30-32] (the possible contribution of contaminating leukocytes to these conclusions could hardly be
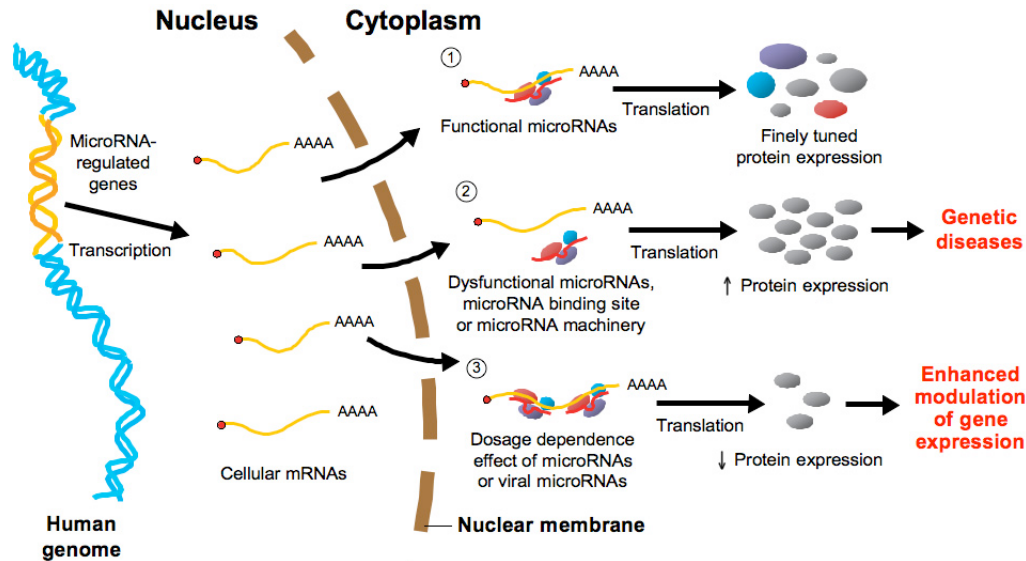
Fig. (2). Importance of microRNA regulation of messenger RNA (mRNA) translation in human cells. (1) For 30 to 90% of the genes, microRNAs control the expression of their encoded proteins through recognition of specific binding sites located mainly in the 3' untranslated region (UTR) of their corresponding mRNAs. (2) Loss of microRNA control, induced either by a dysfunctional microRNA or microRNA binding site, may lead to overexpression of a specific protein and provoke the development of a genetic disease. Deregulation of a protein involved in microRNA biosynthesis and/or function, such as DiGeorge syndrome critical region 8 (DGCR8), transactivating response RNA-binding protein (TRBP) or fragile X mental retardation protein (FMRP), may affect microRNA expression globally and have more serious consequences. (3) An increased number of microRNA binding sites occupied by microRNAs, either of endogenous or foreign (eg, viruses) origin, may enhance the capacity for translational control through a possible dosage dependence effect of microRNAs. Adapted from Perron *et al*. [20].

dismissed, in view of the imperfect platelet purification metholodgies available and used at that time). A study by Roth *et al*. [33] subsequently confirmed that circulating human blood platelets retain a small amount of poly(A)+ RNA from their megakaryocytic precursors, an observation that paralleled their ability for protein biosynthesis [30]. These observations suggested that platelets were capable of making use of the proteins and mRNAs acquired from the megakaryocytes from which they derive in order to synthesize proteins de novo, thereby modulating the nature and content of its proteome.

Later, Weyrich *et al*. [34] showed that thrombin-activated, but not resting, platelets synthesize Bcl-3 along a process blocked by mRNA translation inhibitors. The authors also demonstrated that platelets synthesize numerous proteins after they adhere to fibrinogen, which mediates outside-in signaling by engagement of aIIb/ß3 integrin [34]. Similarly, Evangelista *et al*. [35] documented the occurrence of de novo thromboxane A$_2$ (TxA$_2$) synthesis in platelets. Together, these studies suggest that platelet mRNAs can be translated into proteins and contribute to the platelet proteome along processes that can be induced and regulated by appropriate stimuli.

Determined by (i) the proteins acquired from the megakaryocytic precursor cells, (ii) the relative stability/turnover of the proteins, and (iii) the extent of de novo protein synthesis in platelets, the latter of which depends on the platelet transcriptome, the platelet proteome is relatively dynamic. In a study by the group of Dana Devine [36], the use of complementary proteomic approaches revealed a change in con-

centration for more than 503 individual proteins over a 7-day storage period. Another study by the same group confirmed that platelets are capable of using particularly long-lived mRNAs to synthesize biologically relevant proteins *ex vivo* throughout a 10-day storage period [37], thereby raising interesting issues regarding the molecular mechanisms involved in the translational control of platelet mRNAs.

THE PLATELET TRANSCRIPTOME

The importance and diversity of the platelet transcriptome has only been appreciated recently, in coincidence with the advent of more advanced technologies such as PCR, serial analysis of gene expression (SAGE) and microarrays [38]. A study by Bugert and Klüter [39] reported a positive hybridization signal for ~21% of the 34,039 genes examined by microarray profiling, surpassing previous estimates of 3,000-6,000 transcripts present in platelets [40]. Published microarray studies estimate that platelets may contain 15 to 32% of the human genome in the form of messenger RNAs, and this, despite lacking genomic DNA [41-43]! These numbers are surprisingly high considering the limited amount of mRNA in these anucleate elements; each platelet is estimated to contain just 0.002 fg mRNA, a level ~12,500-fold less than a nucleated cell [44]. Among the top transcripts that are most relevant to platelet function, those encoding GPIIIa, platelet factor 4, vWF receptor GPIb ß, GPIIb, ß-thromboglobulin, actin and α-tubulin are found [43]. In two of these studies, a strong correlation between transcript abundance and protein expression was established [42, 43], further supporting the functionality of these transcripts in platelets.

This raised the following question: How are these mRNA transcripts regulated? In a breakthrough paper, Denis *et al.* [45] reported the existence in human platelets of a major post-transcriptional regulatory mechanism of gene expression, i.e. mRNA splicing, which is usually encountered in nucleated cells. In response to integrin engagement and surface receptor activation, platelets excise introns from IL-1ß pre-mRNA, yielding a mature mRNA that is translated into protein. Suggesting that the regulation of mRNA translation in this relatively "simple", anucleate element of the blood is more complex than previously thought, these findings supported the existence of post-transcriptional control of gene expression in platelets.

PLATELET mRNA 3'UTRs

Another key aspect of the post-transcriptional control of gene expression involves regulatory elements present in mRNA 3'UTRs, which are playing an important role in the modulation of translation. Here, it is relevant to note that SAGE analyses revealed that, whereas the average length of platelet mRNA 5'UTRs does not differ significantly from nucleated cells (platelets: 151 nt; nucleated cells: 120 nt), that of the 3'UTRs is significantly longer (1,047 nt) than nucleated cells (492 nt). In fact, platelet mRNAs have the longest 3'UTRs of all examined tissues, with a large margin to the second-ranking fibroblasts (681 nt) [46]. Harboring longer 3'UTRs, platelet mRNAs may thus contain more regulatory elements than their nucleated cell counterparts, including binding sites for specific microRNAs, which are presumed to exert dosage dependence effects on translation, i.e. proportional to the number of occupied binding sites (see Fig. **2-3**).

Interestingly, the regulatory element Brd box (bearded box) [47] has been found to be enriched in platelet transcripts [46]. It is of particular interest to note that regulation of the Brd box likely involves complementary sequences found at the 5' end of certain microRNAs [47]. Together, these observations strengthen the possibility that microRNAs provide an additional layer of regulation for the fine tuning of gene expression in human platelets.

THE microRNA PATHWAY OF PLATELETS

Following initial reports by independent groups of the possible presence of microRNAs in platelets [48-50], we recently reported the existence of a microRNA pathway in these anucleate elements of the blood (see Fig. **1** – Platelet) [51]. These findings have expanded the platelet transcriptome to include small non-coding RNA species. Previously found to be differentially expressed in polycythemia vera [48], platelet microRNAs have, since then, been the subject of further studies in the context of platelet hyperreactivity [52] and BCR-ABL negative myeloproliferative neoplasms [53]. Not only do platelets harbor an abundant and diverse array of microRNAs – they may even represent the richest and most abundant source of microRNAs in the human body –, but they host Dicer and Argonaute 2 (Ago2) complexes functional in microRNA biogenesis and the control of specific RNA transcripts, respectively (see Fig. **1** – Platelet) [51]. The relative abundance of pre-miRNA versus mature microRNA species in human platelets, however, suggest that de novo biogenesis of microRNAs within platelets may not be significant and that most of the mature microRNAs detected in circulating platelets may derive from megakaryocytes.

MicroRNAs AS REGULATORS OF mRNA TRANSLATION IN PLATELETS

Although human platelets harbor an abundant and diverse array of microRNAs [51], the question of their biological role and significance remains. With their primary function in nucleated cells assumed to be the control of mRNA translation (see Fig. **2-1**), microRNAs are also very likely involved in regulating translation of platelet mRNAs. In this case, microRNAs would need to be considered as key modulators of the platelet proteome and important modifiers of the ability of platelets to respond to physiological stimuli.

The experimental evidences suggesting that microRNAs may regulate mRNA translation in platelets come mainly from the detection of the purinergic receptor P2Y$_{12}$ mRNA in Ago2 immunoprecipitates [51]. Relevant to the identification of microRNA-targeted mRNAs [54-56], this biochemical approach allowed to address the major limitation associated with working with primary human platelets, i.e. their relative refractoriness to transfection. Additional platelet mRNAs are very likely to be found in association with Ago2, which would further strengthen the concept that microRNAs contribute to the fine tuning and more tightly regulated expression of specific gene products in platelets.

As for the nature of the microRNA control of platelet mRNAs, it is expected to proceed mainly via translational repression, as opposed to activation. MicroRNAs have been shown to enhance mRNA translation in HEK293 and HeLa cells under specific conditions (i.e. cell cycle arrest) [16], but the possibility that they may favor translation of platelet mRNAs can hardly be reconciled with the very low rate of protein synthesis observed in these anucleate elements under resting conditions. Although the recent findings that platelets can generate progeny [57], through some sort of a "cell cycle", raises interesting and intriguing issues.

CLINICAL APPLICATIONS

Likely involved in finely tuning expression of specific gene products that may govern platelet reactivity, a dysfunctional microRNA-based regulatory system may lead to deregulated platelet function and favor the development of serious, platelet-related cardiovascular diseases, including atherothrombosis [58]. It is tempting to envision and speculate about the eventual use of platelet microRNAs as biomarkers of specific platelet disorders, whereby platelet microRNA profiling would be indicative of underlying diseases and have diagnostic and/or therapeutic values to physicians, as in the field of cancer [59, 60]. In that context, the possibility of subtle differences between the platelet transcriptomes of individuals, regarding the expression level or the absence, presence or sequence variants of a microRNA or those of a microRNA binding site, as observed in the case of the glycoprotein 6 haplotype [61] or the naturally occurring Leu33Val mutation in beta3-integrin [62], will need to be taken into account. Such differences may explain some of the reported inter-individual variations in platelet reactivity,

an assertion supported by a recent study by the group of Paul Bray demonstrating a role for the miR-96-regulated VAMP8/endobrevin gene in the heterogeneity of platelet reactivity. The authors of this study also also reported relatively lower levels of miR-96 in subjects with differing platelet reactivity [52], suggesting that microRNAs may govern platelet reactivity through the regulation of specific mRNAs. Such clinically-oriented studies investigating the possible implication of microRNAs in specific platelet phenotypes appear to be promising and is expected to accelerate the transfer of knowledge from the bench to the bedside of patients.

THERAPEUTIC APPLICATIONS

The existence of a gene regulatory process based on microRNAs in primary human platelets also opens up the possibility of modulating platelet gene expression through the use of therapeutic RNAs. Some synthetic RNA species, like the microRNA duplex mimetic small interfering RNA (siRNA) species, can enter the microRNA pathway and exert potent gene regulatory effects through their ability to mediate cleavage and degradation of specific mRNAs. Similarly, microRNA inhibitors, such as 2'OMe antisense oligonucleotides (AS), may be used to neutralize specific microRNA(s) and thus modulate expression of specific mRNAs under their control. Representing a promising approach for the treatment of platelet-related diseases, siRNA-mediated targeting of specific platelet mRNAs or 2'OMe AS-mediated neutralization of specific microRNAs may prove to be effective in modulating platelet function and achieving desirable therapeutic benefits. In view of the relative refractoriness of platelets to transfection, significant delivery issues may be anticipated, which could limit the applicability of therapeutic RNAs to platelet biology, although recent advances in that area tend to suggest that this hurdle may not be unsurmountable.

CONCLUSION AND PERSPECTIVES

Further investigations are required in order to improve our understanding of the platelet microRNA pathway and of biological role and importance of microRNAs in regulating the proteome and function of platelets in health and disease [40]. As for neurological [2] and infectious [19] diseases, research advances in the field of platelet microRNAs may not only provide new perspectives to the etiology of platelet-related disorders (thrombotic or bleeding), but are key to ensure the development of efficient therapeutic tools and strategies aimed to modulate platelet function by preserving, restoring or neutralizing global and/or specific microRNA function in the platelets of patients at risk of developing, or suffering from, cardiovascular and platelet-related diseases.

ACKNOWLEDGEMENTS

ABBREVIATIONS

Ago2	=	Argonaute 2
DGCR8	=	DiGeorge syndrome critical region 8
FMRP	=	Fragile X mental retardation protein
mRNA	=	Messenger RNA
miRNP	=	microRNA-containing RNP
PCR	=	Polymerase chain reaction
pre-miRNA	=	microRNA precursor
pri-miRNA	=	Primary microRNA
qRT-PCR	=	Quantitative real-time PCR
RNP	=	Ribonucleoprotein
RT	=	Reverse transcription
siRNA	=	Small interfering RNA
TRBP	=	Transactivating response RNA-binding protein
UTR	=	Untranslated region

REFERENCES

[1] Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell,* **2009,** *136,* 215-233.

[2] Provost, P. MicroRNAs as a molecular basis for mental retardation, Alzheimer's and prion diseases. *Brain Res.,* **2010,** *1338,* 58-66.

[3] Lee, Y.; Kim, M.; Han, J.; Yeom, K. H.; Lee, S.; Baek, S. H. and Kim, V. N. MicroRNA genes are transcribed by RNA polymerase II. *Embo. J.,* **2004,** *23,* 4051-4060.

[4] Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Kim, J.; Yim, J.; Lee, J.; Provost, P.; Radmark, O.; Kim, S. and Kim, V. N. The nuclear RNase III Drosha initiates microRNA processing. *Nature,* **2003,** *425,* 415-419.

[5] Denli, A. M.; Tops, B. B.; Plasterk, R. H.; Ketting, R. F. and Hannon, G. J. Processing of primary microRNAs by the Microprocessor complex. *Nature,* **2004,** *432,* 231-235.

[6] Gregory, R. I.; Yan, K. P.; Amuthan, G.; Chendrimada, T.; Doratotaj, B.; Cooch, N. and Shiekhattar, R. The Microprocessor complex mediates the genesis of microRNAs. *Nature,* **2004,** *432,* 235-240.

[7] Han, J.; Lee, Y.; Yeom, K. H.; Kim, Y. K.; Jin, H. and Kim, V. N. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.,* **2004,** *18,* 3016-3027.

[8] Lund, E.; Guttinger, S.; Calado, A.; Dahlberg, J. E. and Kutay, U. Nuclear export of microRNA precursors. *Science,* **2004,** *303,* 95-98.

[9] Yi, R.; Qin, Y.; Macara, I. G. and Cullen, B. R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.,* **2003,** *17,* 3011-3016.

[10] Bernstein, E.; Caudy, A. A.; Hammond, S. M. and Hannon, G. J. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature,* **2001,** *409,* 363-366.

[11] Provost, P.; Dishart, D.; Doucet, J.; Frendewey, D.; Samuelsson, B. and Radmark, O. Ribonuclease activity and RNA binding of recombinant human Dicer. *Embo. J.,* **2002,** *21,* 5864-5874.

[12] Zhang, H.; Kolb, F. A.; Jaskiewicz, L.; Westhof, E. and Filipowicz, W. Single processing center models for human Dicer and bacterial RNase III. *Cell,* **2004,** *118,* 57-68.

[13] Chendrimada, T. P.; Gregory, R. I.; Kumaraswamy, E.; Norman, J.; Cooch, N.; Nishikura, K. and Shiekhattar, R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature,* **2005,** *436,* 740-744.

[14] Bhattacharyya, S. N.; Habermacher, R.; Martine, U.; Closs, E. I. and Filipowicz, W. Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell,* **2006,** *125,* 1111-1124.

[15]   Liu, J.; Rivas, F. V.; Wohlschlegel, J.; Yates, J. R. 3rd; Parker, R. and Hannon, G. J. A role for the P-body component GW182 in microRNA function. *Nat. Cell Biol.,* **2005***, 7*, 1261-1266.

[16]   Vasudevan, S.; Tong, Y. and Steitz, J. A. Switching from repression to activation: microRNAs can up-regulate translation. *Science,* **2007***, 318*, 1931-1934.

[17]   Perron, M. P. and Provost, P. Protein interactions and complexes in human microRNA biogenesis and function. *Front. Biosci.,* **2008***, 13*, 2537-2547.

[18]   Miranda, K. C.; Huynh, T.; Tay, Y.; Ang, Y. S.; Tam, W. L.; Thomson, A. M.; Lim, B. and Rigoutsos, I. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell,* **2006***, 126*, 1203-1217.

[19]   Ouellet, D. L. and Provost, P. Current knowledge of MicroRNAs and noncoding RNAs in virus-infected cells. *Methods Mol. Biol.,* **2010***, 623*, 35-65.

[20]   Perron, M. P.; Boissonneault, V.; Gobeil, L. A.; Ouellet, D. L. and Provost, P. Regulatory RNAs: future perspectives in diagnosis, prognosis, and individualized therapy. *Methods Mol. Biol.,* **2007***, 361*, 311-326.

[21]   Plante, I. and Provost, P. Hypothesis: A Role for Fragile X Mental Retardation Protein in Mediating and Relieving MicroRNA-Guided Translational Repression? *J. Biomed. Biotechnol.,* **2006***, 2006*, 16806.

[22]   Stark, K. L.; Xu, B.; Bagchi, A.; Lai, W. S.; Liu, H.; Hsu, R.; Wan, X.; Pavlidis, P.; Mills, A. A.; Karayiorgou, M. and Gogos, J. A. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat. Genet.,* **2008***, 40*, 751-760.

[23]   Boissonneault, V.; Plante, I.; Rivest, S. and Provost, P. MicroRNA-298 and MicroRNA-328 Regulate Expression of Mouse {beta}-Amyloid Precursor Protein-converting Enzyme 1. *J. Biol. Chem.,* **2009***, 284*, 1971-1981.

[24]   Ouellet, D. L.; Plante, I.; Landry, P.; Barat, C.; Janelle, M. E.; Flamand, L.; Tremblay, M. J. and Provost, P. Identification of functional microRNAs released through asymmetrical processing of HIV-1 TAR element. *Nucleic Acids Res.,* **2008***, 36*, 2353-2365.

[25]   Harker, L. A. and Finch, C. A. Thrombokinetics in man. *J. Clin. Invest.,* **1969***, 48*, 963-974.

[26]   Hartwig, J. H. The Platelet: Form and Function. *Semin. Hematol.,* **2006***, 43S1*, S94-S100.

[27]   Boilard, E.; Nigrovic, P. A.; Larabee, K.; Watts, G. F.; Coblyn, J. S.; Weinblatt, M. E.; Massarotti, E. M.; Remold-O'Donnell, E.; Farndale, R. W.; Ware, J. and Lee, D. M. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science,* **2010***, 327*, 580-583.

[28]   Zimmerman, G. A. and Weyrich, A. S. Immunology. Arsonists in rheumatoid arthritis. *Science,* **2010***, 327*, 528-529.

[29]   Ts'ao, C. H. Rough endoplasmic reticulum and ribosomes in blood platelets. *Scand. J. Haematol.,* **1971***, 8*, 134-140.

[30]   Kieffer, N.; Guichard, J.; Farcet, J. P.; Vainchenker, W. and Breton-Gorius, J. Biosynthesis of major platelet proteins in human blood platelets. *Eur. J. Biochem.,* **1987***, 164*, 189-195.

[31]   Booyse, F. M. and Rafelson, M. E., Jr. Studies on human platelets. I. synthesis of platelet protein in a cell-free system. *Biochim. Biophys. Acta,* **1968***, 166*, 689-697.

[32]   Warshaw, A. L.; Laster, L. and Shulman, N. R. Protein synthesis by human platelets. *J. Biol. Chem.,* **1967***, 242*, 2094-2097.

[33]   Roth, G. J.; Hickey, M. J.; Chung, D. W. and Hickstein, D. D. Circulating human blood platelets retain appreciable amounts of poly (A)+ RNA. *Biochem. Biophys. Res. Commun.,* **1989***, 160*, 705-710.

[34]   Weyrich, A. S.; Dixon, D. A.; Pabla, R.; Elstad, M. R.; McIntyre, T. M.; Prescott, S. M. and Zimmerman, G. A. Signal-dependent translation of a regulatory protein, Bcl-3, in activated human platelets. *Proc. Natl Acad. Sci. USA,* **1998***, 95*, 5556-5561.

[35]   Evangelista, V.; Manarini, S.; Di Santo, A.; Capone, M. L.; Ricciotti, E.; Di Francesco, L.; Tacconelli, S.; Sacchetti, A.; D'Angelo, A.; Scilimati, A.; Sciulli, M. G. and Patrignani, P. De novo synthesis of cyclooxygenase-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin. *Circ. Res.,* **2006***, 98*, 593-595.

[36]   Thon, J. N.; Schubert, P.; Duguay, M.; Serrano, K.; Lin, S.; Kast, J. and Devine, D. V. Comprehensive proteomic analysis of protein changes during platelet storage requires complementary proteomic approaches. *Transfusion,* **2008***, 48*, 425-435.

[37]   Thon, J. N. and Devine, D. V. Translation of glycoprotein IIIa in stored blood platelets. *Transfusion,* **2007***, 47*, 2260-2270.

[38]   Dittrich, M.; Birschmann, I.; Stuhlfelder, C.; Sickmann, A.; Herterich, S.; Nieswandt, B.; Walter, U. and Dandekar, T. Understanding platelets. Lessons from proteomics, genomics and promises from network analysis. *Thromb. Haemost.,* **2005***, 94*, 916-925.

[39]   Bugert, P. and Kluter, H. Profiling of gene transcripts in human platelets: An update of the platelet transcriptome. *Platelets,* **2006***, 17*, 503-504.

[40]   Macaulay, I. C.; Carr, P.; Gusnanto, A.; Ouwehand, W. H.; Fitzgerald, D. and Watkins, N. A. Platelet genomics and proteomics in human health and disease. *J. Clin. Invest.,* **2005***, 115*, 3370-3377.

[41]   Bugert, P.; Dugrillon, A.; Gunaydin, A.; Eichler, H. and Kluter, H. Messenger RNA profiling of human platelets by microarray hybridization. *Thromb. Haemost.,* **2003***, 90*, 738-748.

[42]   Gnatenko, D. V.; Dunn, J. J.; McCorkle, S. R.; Weissmann, D.; Perrotta, P. L. and Bahou, W. F. Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood,* **2003***, 101*, 2285-2293.

[43]   McRedmond, J. P.; Park, S. D.; Reilly, D. F.; Coppinger, J. A.; Maguire, P. B.; Shields, D. C. and Fitzgerald, D. J. Integration of proteomics and genomics in platelets: a profile of platelet proteins and platelet-specific genes. *Mol. Cell. Proteomics,* **2004***, 3*, 133-144.

[44]   Fink, L.; Holschermann, H.; Kwapiszewska, G.; Muyal, J. P.; Lengemann, B.; Bohle, R. M. and Santoso, S. Characterization of platelet-specific mRNA by real-time PCR after laser-assisted microdissection. *Thromb. Haemost.,* **2003***, 90*, 749-756.

[45]   Denis, M. M.; Tolley, N. D.; Bunting, M.; Schwertz, H.; Jiang, H.; Lindemann, S.; Yost, C. C.; Rubner, F. J.; Albertine, K. H.; Swoboda, K. J.; Fratto, C. M.; Tolley, E.; Kraiss, L. W.; McIntyre, T. M.; Zimmerman, G. A. and Weyrich, A. S. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell,* **2005***, 122*, 379-391.

[46]   Dittrich, M.; Birschmann, I.; Pfrang, J.; Herterich, S.; Smolenski, A.; Walter, U. and Dandekar, T. Analysis of SAGE data in human platelets: features of the transcriptome in an anucleate cell. *Thromb. Haemost.,* **2006***, 95*, 643-651.

[47]   Lai, E. C. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat. Genet.,* **2002***, 30*, 363-364.

[48]   Bruchova, H.; Merkerova, M. and Prchal, J. T. Aberrant expression of microRNA in polycythemia vera. *Haematologica,* **2008***, 93*, 1009-1016.

[49]   Merkerova, M.; Belickova, M. and Bruchova, H. Differential expression of microRNAs in hematopoietic cell lineages. *Eur. J, Haematol.,* **2008***, 81*, 304-310.

[50]   Kannan, M.; Mohan, K. V.; Kulkarni, S. and Atreya, C. Membrane array-based differential profiling of platelets during storage for 52 miRNAs associated with apoptosis. *Transfusion,* **2009***, 49*, 1443-1450.

[51]   Landry, P.; Plante, I.; Ouellet, D. L.; Perron, M. P.; Rousseau, G. and Provost, P. Existence of a microRNA pathway in anucleate platelets. *Nat. Struct. Mol. Biol.,* **2009***, 16*, 961-966.

[52]   Kondkar, A. A.; Bray, M. S.; Leal, S. M.; Nagalla, S.; Liu, D. J.; Jin, Y.; Dong, J. F.; Ren, Q.; Whiteheart, S. W.; Shaw, C. and Bray, P. F. VAMP8/endobrevin is overexpressed in hyperreactive human platelets: suggested role for platelet microRNA. *J. Thromb. Haemost.,* **2010***, 8*, 369-378.

[53]   Girardot, M.; Pecquet, C.; Boukour, S.; Knoops, L.; Ferrant, A.; Vainchenker, W.; Giraudier, S. and Constantinescu, S. N. miR-28 is a thrombopoietin receptor targeting microRNA detected in a fraction of myeloproliferative neoplasm patient platelets. *Blood,* **2010***, 116*, 437-445.

[54]   Beitzinger, M.; Peters, L.; Zhu, J. Y.; Kremmer, E. and Meister, G. Identification of human microRNA targets from isolated argonaute protein complexes. *RNA Biol.,* **2007***, 4*, 76-84.

[55]   Easow, G.; Teleman, A. A. and Cohen, S. M. Isolation of microRNA targets by miRNP immunopurification. *Rna,* **2007***, 13*, 1198-1204.

[56]   Karginov, F. V.; Conaco, C.; Xuan, Z.; Schmidt, B. H.; Parker, J. S.; Mandel, G. and Hannon, G. J. A biochemical approach to identifying microRNA targets *Proc. Natl Acad. Sci. USA,* **2007***, 104*, 19291-19296.

[57]   Schwertz, H.; Koster, S.; Kahr, W. H.; Michetti, N.; Kraemer, B. F.; Weitz, D. A.; Blaylock, R. C.; Kraiss, L. W.; Greinacher, A.; Zimmerman, G. A. and Weyrich, A. S. Anucleate platelets generate progeny. *Blood,* **2010,** *115,* 3801-3809.

[58]   Ouwehand, W. H. Platelet genomics and the risk of atherothrombosis. *J. Thromb. Haemost.,* **2007,** *5 Suppl 1,* 188-195.

[59]   Thomson, J. M.; Parker, J.; Perou, C. M. and Hammond, S. M. A custom microarray platform for analysis of microRNA gene expression. *Nat. Methods,* **2004,** *1,* 47-53.

[60]   He, L.; Thomson, J. M.; Hemann, M. T.; Hernando-Monge, E.; Mu, D.; Goodson, S.; Powers, S.; Cordon-Cardo, C.; Lowe, S. W.; Hannon, G. J. and Hammond, S. M. A microRNA polycistron as a potential human oncogene. *Nature,* **2005,** *435,* 828-833.

[61]   Burns, P.; Gusnanto, A.; Macaulay, I. C.; Rankin, A.; Tom, B.; Langford, C. F.; Dudbridge, F.; Ouwehand, W. H. and Watkins, N. A. Identification of variation in the platelet transcriptome associated with glycoprotein 6 haplotype. *Platelets,* **2008,** *19,* 258-267.

[62]   Santoso, S.; Kroll, H.; Andrei-Selmer, C. L.; Socher, I.; Rankin, A.; Kretzschmar, E.; Watkins, N. A. and Ouwehand, W. H. A naturally occurring LeuVal mutation in beta3-integrin impairs the HPA-1a epitope: the third allele of HPA-1. *Transfusion,* **2006,** *46,* 790-799.