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Review article

An 'alpha-beta' of pancreatic islet microribonucleotides

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ABSTRACT

MicroRNAs (miRNAs) are cellular, short, non-coding ribonucleotides acting as endogenous posttranscriptional repressors following incorporation in the RNA-induced silencing complex. Despite being chemically and mechanistically very similar, miRNAs exert a multitude of different cellular effects by acting on mRNA species, whose gene-products partake in a wide array of processes.

Here, the aim was to review the knowledge of miRNA expression and action in the islet of Langerhans. We have focused on: 1) physiological consequences of islet or beta cell specific inhibition of miRNA processing, 2) mechanisms regulating processing of miRNAs in islet cells, 3) presence and function of miRNAs in alpha versus beta cells – the two main cell types of islets, and 4) miRNA mediators of beta cell decompensation.

It is clear that miRNAs regulate pancreatic islet development, maturation, and function in vivo. Moreover, processing of miRNAs appears to be altered by obesity, diabetes, and aging. A number of miRNAs (such as miR-7, miR-21, miR-29, miR-34a, miR-212/miR-132, miR-184, miR-200 and miR-375) are involved in mediating beta cell dysfunction and/or compensation induced by hyperglycemia, oxidative stress, cytotoxic cytokines, and in rodent models of fetal metabolic programming prediabetes and overt diabetes. Studies of human type 2 diabetic islets underline that these miRNA families could have important roles also in human type 2 diabetes.

Furthermore, there is a genuine gap of knowledge regarding miRNA expression and function in pancreatic alpha cells. Progress in this area would be enhanced by improved *in vitro* alpha cell models and better tools for islet cell sorting.

1. Introduction to miRNA generation and action

MiRNAs belong to the diverse class of small non-coding RNA species also encompassing piwi-interacting RNAs (piRNAs), small nucleolar RNA (snRNAs), transfer RNAs (tRNAs) and mirtrons (Hansen et al., 2013; Hansen et al., 2016; Denzler and Stoffel, 2015). They are characterized by their small size of about 20 nucleotides (nt) and characteristic pathway of generation and action involving several enzymatic processing steps of a hairpin transcript. MiRNAs are individually set apart by their nt sequence and are systematically named by number following their registration in miR-base (miR-1, miR-2 etc.) (Kozomara and Griffiths-Jones, 2014). MiRNAs are in general exceptionally well conserved and many miRNA species are almost identical from nematodes to humans, although primate or human specific miRNAs have been reported (Kozomara and Griffiths-Jones, 2014). In model organisms such as c. elegans miRNAs are important for cell fate decisions, stress response, and can act in negative feed-back loops, and miRNAs may play similar roles in mammals (Ren and Ambros, 2015).

The generation and maturation of miRNAs has been extensively reviewed previously (Vienberg et al., 2016; Filipowicz and Sonenberg, 2015) and thus, will only be covered briefly here. The mechanisms by which miRNAs are generated appear to be diverse, but most miRNAs are transcribed by RNA polymerase 2 (Xie and Steitz, 2014). In the general pathway for miRNA maturation, the primary hairpin (stem-loop-stem) transcript is processed by the RNAse Drosha with Dgcr8 (DiGeorge Critical Region 8) to generate precursor miRNA (Fig. 1A).

Precursor miRNA are exported to the cytoplasm through Exportin 5 (Exp 5) (Xie and Steitz, 2014) and further cleaved by Dicer and the double-stranded mature miRNA is incorporated in the RNA induced silencing complex (RISC) (Fig. 1A). Thus, multiple steps are involved in generating the mature miRNA each of which may be regulated.

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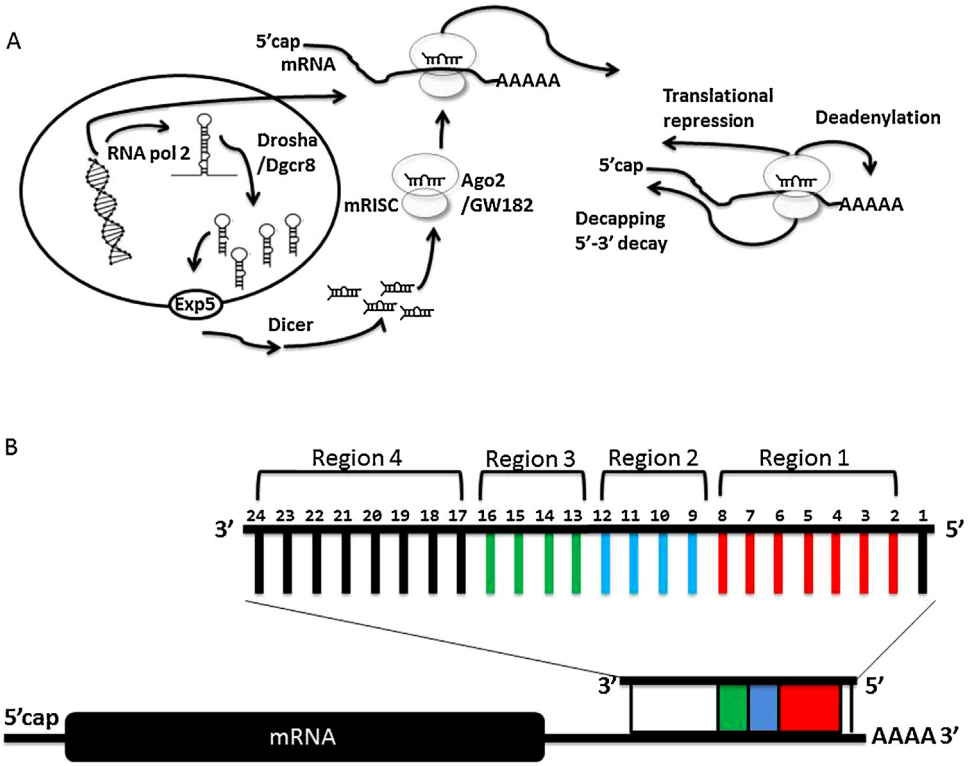

Fig. 1. (A) Outline of the canonical miRNA biogenesis pathway. Primary miRNAs (pri-miRNA) are transcribed by RNA polymerase II either as separate, or sometimes polycistronic, transcripts or harbored in protein-coding transcripts. The microprocessor complex Drosha-Dgcr8 cleaves the pri-miRNA and forms precursor miRNAs (pre-miRNA) containing a stem-loop structure. After being exported to the cytoplasm by Exportin 5 (Exp5), the nuclease Dicer further cleaves the pre-miRNA at the loop structure. The resulting miRNA duplex is unwound and the mature strand becomes incorporated in to the RNA induced silencing complex containing Argonaute (Ago2) and GW182 proteins. The assembled RISC complex further binds mRNA target proteins via the imperfect base-pairing of the RISC-incorporated miRNA. This results in translational repression, de-adenylation and de-capping of the mRNA. (B) Nucleotide (nt) 1 does not bind the target mRNA. Nt 2–8 of the miRNA (red) constitutes the seed region, and perfect match in nt 9–12 (blue) is required for Ago dependent RNA cleavage. Binding of nt 13–16 (green) increase repression. Binding in the 5′ end (black) further increases the thermodynamic stability of the complex (Hafner et al., 2012).

Most precursor miRNAs give rise to only one mature miRNA species, but in some cases where the thermodynamic stability of either end of the miRNA duplex is similar, either the 5′ or the 3′ strands of the precursor can become mature, active miRNAs (Khvorova et al., 2003). The active miRNA incorporated RISC (mRISC) binds target mRNAs in their 3′ untranslated regions (UTR) by imperfect complementary base-pairing (Fig. 1B) (Hafner et al., 2010). The miRNA may target a given mRNA towards mRNA degradation, inhibition of translation or the mRNA can be moved into P-bodies for later release (Filipowicz and Sonenberg, 2015). Although miRNAs themselves are well conserved there are examples showing species differences in their targets (see e.g (Salunkhe et al., 2015a)).

The mRISC consists of AGO and GW182 proteins, called TNRC6a/b/c in vertebrates and they have the role of recruiting the deadenylation and decapping complexes necessary for mRNA degradation. Deadenylation complexes comprise poly(A) specific ribonuclease (PARN/PAN2/PAN3) and Poly(A) Binding Protein (PABPC1) and CCR4–NOT proteins (encoded by CNOT1/2/3/9 and CCR4 (C-C motif chemokine receptor 4)) (Mathys et al., 2014). Following deadenylation, decapping enzymes (DCP1/2) with DEAD box protein 6 (DDX6) and several other protein partners removes the 5′mRNA cap, which creates a substrate for complete degradation by exoribonuclease 1 (XRN1) (Braun et al., 2012, 2011). MRISC also actively represses translation, but the molecular mechanism for this is less characterized (Jonas and Izaurralde, 2015).

The majority of miRNAs have the propensity to regulate a large number of cellular mRNAs, often several hundreds. A number of prediction algorithms have been developed to predict target mRNAs of a given miRNA, or vice versa predict which miRNAs are likely to bind to a given 3′UTR., using Examples of inputs in these algorithms are number of seed nucleotides, possibilities for further base pairing, and melting temperature of interacting base pairs. The overlap in predicted target mRNA:miRNA interactions by different databases (Ekimler et al., 2014). is not very impressive, which could reflect that the molecular details governing mRNA:miRNA interactions are still not completely understood (Fang and Bartel, 2015). Moreover, many miRNAs have slight variations to their sequence; having added or deleted nucleotides at the 5′ or 3′ end. These variant isomiRs slightly alter or extend the pool of possible target mRNAs, adding to the complexity of mRNA:miRNA interaction.

2. The pancreatic islet cells, beta cell disallowed genes and miRNAs

Although small, the pancreatic islet of Langerhans constitutes an entire micro-organ focused on homeostatic regulation of physiological blood glucose levels, which is maintained by hormone secreting cells. Insulin-secreting beta cells are the most abundant cell type followed by alpha (glucagon) and delta (somatostatin) cells and even rarer cells positive for either ghrelin (epsilon cells) or pancreatic polypeptide (gamma cells). Islets are highly vascularized enabling fast systemic release of hormones into the vena porta.

The global mRNA expression pattern of whole islets and purified beta cells is well characterized by both RNA-sequencing and gene