Regulation of the germ stem cell niche as the foundation for adult spermatogenesis

Regulation of the germ stem cell niche as the foundation for adult spermatogenesis: a role for miRNAs?

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Abstract

Within the testis the spermatogonial stem cells reside in a unique microenvironment, or 'niche', which includes the surrounding somatic cells. The regulation of the balance between self-renewal and differentiation of spermatogonial stem cells determines the lifelong supply of spermatozoa by maintaining a population of undifferentiated spermatogonial stem cells and ensuring that adequate numbers of spermatogonia undergo spermatogenesis. Mouse models have been instrumental in determining a large number of factors involved in regulating the spermatogonial stem cell self-renewal and/or differentiation. However, the precise mechanisms controlling regulation of the germ cell niche remain to be elucidated. Recently the discovery of microRNAs, which regulate gene expression at the post-transcriptional level, have provided new insight into testis biology, spermatogenesis and germ stem cell regulation. In this review we summarize the main factors involved in the regulation of the germ stem cell niche and describe the role of microRNA signaling in this regulation.

1. Introduction

Fertility in postpubertal males depends on the continued production of spermatozoa in the testis via the process of spermatogenesis. Spermatogenesis involves a delicate balance between self-renewal and differentiation of spermatogonial stem cells (SSC) to ensure an endless production of mature spermatozoa [1]. In keeping with other stem cell systems, the SSCs reside in a unique microenvironment or 'niche', which is composed of the stem cell and the surrounding somatic cells. The germ stem cell niche is composed of the growth factor environment that is provided by various somatic support cell populations in the testis [2, 3]. In the adult testis, it has been shown that Sertoli cells, peritubular myoid cells, Leydig cells and the surrounding vasculature are all important components of the germ stem cell niche [3-5].

During fetal/early postnatal life the germ cells undergo a crucial period of development from gonocyte to spermatogonium, and it is thought that establishment of a suitable germ cell niche is a prerequisite for this to occur. This is important to ensure the establishment of a supply of SSCs for future fertility, but also because failure of these cells to undergo differentiation in humans results in pre-neoplastic change into carcinoma *in situ* (CIS) cells which will ultimately lead to a testicular germ cell cancer (TGCC) in adulthood [6, 7]. In the postnatal period, active SSC self-renewal takes place to establish the male germline, whereas in adulthood SSC self-renewal only occurs at certain times during specific stages of the seminiferous epithelial cycle when subpopulations of spermatogonia undergo the transition into differentiating spermatogonia [3]. However, increase in SSC self-renewal has also been observed after testicular damage such as after chemotherapy [8].

Most data on the mammalian germ stem cell niche derives from mouse studies and there is still little known about the biology/regulation of the human germ stem cell niche, specifically regarding the maintenance and regulation of SSC self-renewal and differentiation. This ignorance is due partly to the absence of techniques for identifying and isolating pure populations of human SSCs for *in vitro* study [9]. However, transplantation of testis cells from humans into immunodeficient mice results in limited replication and maintenance of spermatogonia in the recipient seminiferous tubules [10, 11], suggesting a degree of conservation of the mechanisms for self-renewal and SSC survival between human and mouse. Furthermore, prepubertal human and mouse spermatogonia have remarkable conservation of their gene expression, especially in the genes involved in SSC self-renewal [9]. Despite these similarities there also exist a number of significant differences. In the human testis there are no mitoses of differentiating spermatogonia, which contrasts with the 6

rounds of mitotic divisions in the rodent testis [12]. In addition, transplantation studies have demonstrated that human SSCs are unable to differentiate following incorporation into the SSC niche in the mouse testis [10]. This suggests that the mechanisms regulating spermatogenic differentiation in the rodent and human are different. Recently, the importance of microRNA (miRNA) signaling for spermatogenesis and testicular function has been demonstrated using Sertoli- or germ cell-specific knockout of key enzymes in the miRNA biosynthesis pathway [13]. Hence, miRNA signaling in testicular somatic and germ cells may comprise another level of regulation of the germ stem cell niche.

In this review we describe the main factors involved in regulation of the germ stem cell niche based on studies performed in rodents. We also summarize how conditional deletions of components of the miRNA biogenesis pathway in either Sertoli or germ cells affect the germ stem cell niche and the consequences for adult spermatogenesis. More importantly, we discuss recent findings of miRNA involvement in the regulation and/or modulation of the germ stem cell niche, suggesting that miRNA may be a new mechanism regulating the correct timing of spermatogonial differentiation and perhaps thus of spermatogenic output in adult life. Because miRNAs work as translational repressors, their expression in the germ stem cell niche could be viewed as part of a 'restraint mechanism', whereby the SSCs are prevented from differentiating into spermatogonia/meiotic germ cells too early. Especially in humans, where there is a long childhood period this is important as germ cells entering meiosis before the pubertal period will undergo apoptosis as there is no sustainable support [14]. Therefore, maintaining SSC and early spermatogonia in a quiescent, non-differentiating state is a crucial necessity and, because of the way that they work, miRNAs could play a big part in controlling differentiation mechanisms, including the niche, to ensure this process occurs correctly.

2. The germ stem cell niche

Sertoli cells are the "nurse" cells in the testis, supporting germ cell survival and spermatogenesis [1]. Sertoli cells control the germ stem cell niche either by direct contact with SCCs through membrane intercellular communications or indirectly by the production of paracrine signals such as growth factors and cytokines [15] (Figure 1). An important factor that Sertoli cells produce is Glial cell line-derived neurotropic factor (GDNF), which signals through the GDNF-family receptor α1 (GFRα1) and the Ret receptor tyrosine-kinase [16-20]. Deletion of GDNF in mice interferes with SSC self-renewal and causes premature differentiation of SCC leading to testes devoid of germ cells, resembling a Sertoli-cell only phenotype [17]. Conversely, overexpression of GDNF in mouse testes results in a block of spermatogonial differentiation as a result of over-stimulated self-renewal of SSCs [17, 20]. GDNF signaling is essential for the maintenance of NANOS2 (an RNA-binding factor) expression in SSCs, which is important for preventing the differentiation of SSCs in the postnatal testis [21]. Conversely, fibroblast growth factor 9 (FGF9) up-regulates NANOS2 and thereby acts as an inhibitor of meiotic differentiation of postnatal germ cells [22], perhaps indicating co-ordination of this with SSC differentiation control. Interestingly, the transcription factor Foxo1 is also required for SSC maintenance, as Foxo1 deficiency results in severe impairment of SSC self-renewal and a complete block of spermatogonial differentiation [23]. One of the identified Foxo1 gene targets in SSC is Ret which, as mentioned above, is part of the GDNF receptor complex, and Foxo1 signaling promotes high levels of Ret protein on the cell surface of SSCs [23].

FSH produced by the pituitary gland acts on the Sertoli cells in the pubertal testis to initiate spermatogenesis [24], and FSH and its second messenger cyclic AMP (cAMP) are able to

induce GDNF expression in Sertoli cells [25, 26], whereas retinoic acid (RA), which is involved in spermatogonial differentiation (see below), down-regulates GDNF in Sertoli cells [25] (Figure 1). Thus, GDNF is essential for SSC proliferation and self-renewal and the control of SSC differentiation, under the control of the hypothalamic-pituitary axis. Whilst GDNF is essential for SSC self-renewal, expression of its receptor GFRα1 is not exclusive to undifferentiated spermatogonia, but is also present in A^{paired} and $A^{aligned}$ spermatogonial subtypes [27-30].

Another factor that is required for SSC self-renewal is the transcription factor Ets-related molecule (ERM or ETV5) which, like GDNF, is expressed solely in Sertoli cells and its deletion results in compromised Sertoli cell function, leading to premature loss of SSCs [31-34]. Interestingly, ERM/ETV5 has been shown to be one of the downstream GDNF targets in the kidney [35].

FGF2 and EGF in combination with GDNF can promote SSC self-renewal *in vitro*, although FGF2 and EGF on their own are not essential for SSC self-renewal as over time the number of SSCs declined in SSC enriched germ cell cultures cultured in the presence of FGF2 or EGF without GDNF, indicating that self-renewal was compromised and spermatogonial differentiation was promoted [36, 37].

Another important system for regulation of the germ stem cell niche is the KIT signaling system (Figure 1). C-kit is a receptor present on the cell surface of differentiating spermatogonia that distinguishes them from SSC. C-kit has been shown to mediate proliferation, survival and differentiation of type A spermatogonia in response to stimulation by kit ligand, which is produced by Sertoli cells [25, 38-41]. This stimulates spermatogonia to undergo a number of mitotic divisions, forming the A2-A4, intermediate, and B

spermatogonia in rodents before they enter meiosis [42]. In Sertoli cells, cAMP and retinoic acid (RA) signaling [25, 43] induce the production of a paracrine factor called BMP4, which can induce expression of c-kit in spermatogonia [43-45]. In prepubertal human spermatogonia, higher levels of *C-KIT* mRNA are found than in the prepubertal mouse, which may reflect the signal for some SSCs to differentiate into type B spermatogonia as this is a process known to happen during the prepubertal period in humans [9, 46].

Sertoli cells produce RA, which is another paracrine factor that has been demonstrated to be essential for spermatogonial differentiation [47] (Figure 1). RA promotes differentiation of SSCs to differentiated spermatogonia through several mechanisms. One such mechanism is the down-regulation of Promyelocytic Leukemia Zinc Finger (PLZF) [48], which is specifically expressed in SSCs and upon deletion in mice impairs spermatogonial differentiation by affecting the expression of genes involved in SSC self-renewal and differentiation [49-52]. One function of PLZF is to repress the expression of c-kit in spermatogonia [53] and as RA down-regulates PLZF in SSC this could be one mechanism that controls the up-regulation of c-kit in spermatogonia to steer them down the differentiation path.

Treatment of postnatal spermatogonia with RA results in up-regulation of the b-Helix-Loop-Helix transcription factors, Spermatogenesis and Oogenesis HLH1 (Sohlh1) and Sohlh2 [54]. Both Sohlh1 and Sohlh2 are essential for spermatogonial differentiation as deletion of either leads to disappearance of c-kit-expressing spermatogonia in the prepubertal testis [55-59]. A strong correlation between expression of c-kit and both Sohlh1 and Sohlh2 in postnatal spermatogonia has been shown [54], and a direct interaction between c-kit and Sohlh1 was found *in vivo* in chromatin isolated from spermatogonia [54, 58]. Therefore, up-regulation of

Sohlh1 in spermatogonia by RA could be another mechanism by which RA increases expression of c-kit to promote spermatogonial differentiation. Involvement of Doublesex-related transcription factor (Dmrt1) is also likely in this process as c-kit expression is significantly reduced in Dmrt1 conditional knockout mice [60]. Furthermore, when Dmrt1 is deleted from spermatogonia, loss of SSCs was observed as they entered meiosis precociously [60]. Dmrt1 is important for repressing the RA-responsive, meiosis-inducing genes *Stra8* and *Sohlh1*, thereby preventing meiotic entry of differentiated spermatogonia [60]. Indeed, DMRT1 expression in germ cells is considered as a likely master switch for controlling the mitosis-meiosis transition [60], failure of which appears to be associated with the development of TGCC in humans [61].

3. Biogenesis of miRNAs and conditional Dicer knockout mouse models

Conceptually, it is generally thought that in the male germ cells are pre-programmed to go through their sequential development into spermatozoa, provided that there is a supportive environment (the niche), determined by the surrounding somatic cells. However, in situations when the niche is non-supportive, it might be expected that other mechanisms may operate to literally hold the germ cells under restraint. For example, during childhood in boys, it is essential for germ cells not to enter meiosis before puberty starts as there is no support system in place for sustaining germ cells that enter the spermatogenic pathway. Small non-coding RNAs, such as miRNAs have begun to attract attention as important regulators of cell function, as they provide another level of translational control of gene expression. Since miRNAs work as translational repressors, a logical role for them might be to act as part of a 'restraint mechanism' that stops germ cells from entering meiosis too early and ensuring that the right number of germ cells are ready to enter meiosis when the time is right.

MiRNAs are short single-stranded RNA molecules of 19-23 nucleotides that bind specifically to several mRNA molecules to control their translation [62-65]. In animals, miRNA genes are usually transcribed from the genome by RNA Polymerase II, forming primary miRNA transcripts (pri-miRNA) (Figure 2). The pri-miRNA molecules then fold into stem-loop structures within the nucleus that undergo cleavage by the Microprocessor complex, which contains the RNaseIII endonuclease enzyme Drosha and DGCR8 (DiGeorge syndrome critical region gene 8), to form shorter isolated hairpin loops called precursor miRNA (pre-miRNA). These are transported to the cytoplasm by the nuclear export factor exportin 5 where the stem-loop is cleaved by the endoribonuclease Dicer, thereby forming a double-stranded mature miRNA complex called the miRNA:miRNA* duplex. This duplex is then unwound by a helicase and the miRNA* strand gets degraded whereas the miRNA strand, or the mature strand, gets loaded into the effector miRNA-induced silencer complex (miRISC), which contains members of the Argonaute (AGO) protein family. The miRISC with the AGO proteins mediates the translational repression or degradation of the mRNA targets. Therefore, miRNAs typically have a negative effect on protein expression [62-65].

Several studies have documented the preferential or exclusive expression of specific miRNAs in the immature and adult testis [66-79]. The overall importance of miRNA signaling for regulation of spermatogenesis has been demonstrated using cell type-specific conditional knockout studies of Dicer and other miRNA biogenesis genes (Figure 2), which show that miRNA-mediated post-transcriptional control is an important regulator of spermatogenesis [69, 80]. For example, conditional deletion of *Dicer1* from Sertoli cells in mice results in infertility due to complete absence of spermatogenesis and progressive degeneration of the testis [81, 82]. Both studies demonstrated defects in early postnatal testis development and

Sertoli cell proliferation, resulting in massive Sertoli cell and germ cell apoptosis in the prepubertal testis [81, 82].

Besides conditionally ablating Dicer from Sertoli cells, various groups have reported studies in which they deleted Dicer from germ cells [83-86]. This resulted in adult infertility or subfertility due to spermatogenic arrest. The study by Hayashi et al. showed that deletion of *Dicer1* in germ cells caused a defect in the proliferation of male germ cells [83], whereas Maatouk et al. reported defects in both sperm motility and the transition from round to elongated spermatids [85]. Both of these studies used the *TNAP-Cre* (Tissue Non-Specific Alkaline Phosphatase) mouse line to generate the ablation of Dicer in germ cells, but since the *TNAP-Cre* transgenic mouse expresses Cre in only ~50% of the germ cells, and is not totally specific to germ cells [86], the effects are difficult to interpret. Furthermore, *TNAP-Cre* expression begins as early as embryonic day (E) 10 [87], making it very difficult to pinpoint the exact mechanism via which the spermatogenic defects arise in these *Dicer1* mutant mice, since Dicer ablation already takes place in (a proportion of) the early primordial germ cell population in the early mouse embryo, thereby interfering with the development of embryonic germ cells. The studies by Romero et al. and Liu et al. used a more specific germ cell expressing Cre mouse line, namely *Ddx4-Cre*, which deleted Dicer just before birth at the stage when spermatogonia are just beginning to appear. Using this transgenic line they concluded that *Dicer1* expression in mouse germ cells is not needed for SSC renewal and mitotic proliferation, but is necessary for the differentiation of germ cells through meiotic and haploid phases of spermatogenesis [84, 86].

Several groups reported studies in which *Dicer1* was deleted from spermatogonia after birth using transgenic mouse lines expressing the Cre recombinase under the control of a

Neurogenin3 (*Ngn3*) [88] or *Stra8* [89, 90] promoter, which resulted in less severe phenotypes when compared to the Romero [86] and Liu [84] studies. Ngn3 is expressed endogenously in male germ cells starting from postnatal day 5, and its expression has been shown in type A^{single}, A^{paired} and $A^{aligned}$ spermatogonia that gives rise to all differentiating germ cells [91]. This *Ngn3Cre-Dicer1* transgenic mouse line did not show an effect on meiotic progression, but a reduction in the number of haploid germ cells and an increase in the number of apoptotic spermatocytes [88], whilst postnatal germ cell-specific deletion of Dicer was demonstrated to be critical for the normal organization of chromatin and nuclear shaping of elongated spermatids [88]. Interestingly, in the *Ddx4Cre-Dicer1* mouse model, upregulation of transposon expression was observed [86], whereas transposon expression was unaffected in the *Ngn3Cre-Dicer1* knockout testis [88]. This indicates that, as well as miRNA biogenesis, Dicer is also involved in transposon control in germ cells in the perinatal phase of testis development, which takes place before *Ngn3* expression. Deleting *Dicer1* from early spermatogonia using the *Stra8-Cre* transgenic mouse lines resulted in similar phenotypes to the *Nrg3Cre-Dicer1* model [89, 90]. Another study using the *protamine 1 (Prm1)-Cre* transgene to delete *Dicer1* specifically in post-meiotic haploid male germ cells [92], demonstrated a less severe phenotype compared with those in which *Dicer1* was deleted from pre-meiotic spermatogonia [88-90]. However, Chang et al. did observe abnormal morphology in the elongated spermatids indicating that post-meiotic differentiation was disrupted in the *Prm1Cre-Dicer1* mice [92]. Taken together, these studies show a clear requirement for expression of *Dicer1*, and therefore the small non-coding RNA machinery, in both Sertoli and germ cells for the onset of spermatogenesis and for male fertility. The earlier the deletion of Dicer takes place, the more severe effects on spermatogenesis are found, perhaps due to an accumulation of defects.

4. MiRNAs and the germ stem cell niche

Interestingly, a series of publications implicate miRNA involvement in regulation of the germ stem cell niche. Two particular miRNAs, *miR-221* and *miR-222* negatively regulate expression of c-kit at the post-transcriptional level [93]. Over-expression of *miR221/222* renders RA ineffective at inducing the transition of undifferentiated spermatogonia into c-kit-positive differentiated spermatogonia [93]. Furthermore, GDNF can up-regulate expression of *miR-221/222*, whereas RA has the opposite effect [93]. Moreover, *miR-146* interferes with the expression of *c-kit*, *Stra8* and *Sohlh2* in RA-treated spermatogonia, suggesting that it modulates the effects of RA on spermatogonial differentiation [94]. RA significantly induced expression of the *Mirlet7* family miRNAs in spermatogonia, through suppression of *Lin28*, suggesting a role in RA-induced spermatogonial differentiation [95]. The same group showed that RA down-regulates expression of members of the *Mir-17-92 (Mirc1)* and *Mir-106b-25 (Mirc3)* clusters in undifferentiated spermatogonia, both *in vitro* and *in vivo*, suggesting that the *Mirlet7/Mir-17-92 (Mirc1)/Mir-106b-25 (Mirc3)* clusters could play a role in SSC self-renewal and the proliferation of undifferentiated spermatogonia [96]. Another study showed expression of *miRNA-20* and *miRNA-106a* in mouse SSCs and their involvement in SSC self-renewal by targeting Stat3 and Cyclin D1, both involved in spermatogonial differentiation, at the post-transcriptional level [97]. Moreover, *miR-21* is regulated by ERM/ETV5 in SSC-enriched germ cell cultures, and *miR-21* was shown to be important in maintaining the SSC population *in vitro*, demonstrating for the first time the involvement of a specific miRNA in SSC self-renewal [98]. In neuronal cells *miR-21* is induced by GDNF [99], raising the possibility of a similar role in Sertoli cells within the germ stem cell niche.

5. Hormonal regulation of miRNA expression

Androgen regulation of spermatogenesis is fundamentally important, but how it does so is

still unclear. Modulation of miRNAs, and perhaps thus of the niche, is a potentially novel mechanism that has only been superficially addressed. Interestingly, androgens are major regulators of miRNA expression in the prostate [100-103], muscle [101, 104] and liver [105]. Moreover, prenatal testosterone exposure alters fetal ovarian miRNA expression in the sheep, and may lead to adult ovarian pathologies [106]; similar studies for the testis have not been reported. Using primary Sertoli cell cultures and an *in vivo* rat model, upregulation of a subset of miRNAs in Sertoli cells was reported after FSH or androgen suppression [107]. This included *miR-23b*, *miR-30c*, *miR-30d* and *miR-690*, whose predicted targets include genes important for focal adhesion and regulation of the actin cytoskeleton, such as *Pten* and *Eps15*. Another study looked at adult Sertoli cells in mice and identified a group of testosterone-dependent miRNAs playing a crucial role in androgen-mediated events during spermatogenesis [108]. Some of the *miR-471* targets found in this study included *Foxd1*, important in Sertoli cell metabolism, and desmocollin-1 (*Dsc1*), that plays a crucial role in cell-cell adhesion in epithelial cells [108]. Even though the miRNAs identified in this study differed from those identified in the Nicholls et al. study, both proposed that testosterone-mediated inhibition of miRNAs in Sertoli cells could facilitate essential gene expression necessary for progression of spermatogenesis [107, 108].

6. MiRNA expression in human TGCC

MiRNA expression has also been implicated in TGCC. In humans these tumors present in young adulthood following transformation of pre-malignant carcinoma *in situ* (CIS) cells, which are believed to result from failure of differentiation of fetal germ cells. Currently, CIS cells are considered to result from immaturity of the somatic cell component of the germ stem cell niche [109]. In a genetic screen for miRNAs that work together with oncogenes in cellular transformation, *miR-372* and *miR-373* were identified as potential oncogenes

involved in the development of human TGCC [110], whilst several miRNAs are specifically expressed in TGCC [111, 112] and carcinoma *in situ* (CIS) cells [112]. Similarly, a subset of miRNAs was shown to be differentially expressed in TGCC compared to normal testicular tissue [113]. In human TGCC cell lines, repression of the candidate tumor suppressor protein-tyrosine phosphatase non-receptor type 23 (*PTPN23*) by *miR-142-3p* plays an important role in the pathogenesis of TGCCs by repressing expression of *PTPN23* [114]. TGCC is hypothesized to be part of a group of male reproductive health disorders, that include cryptorchidism and hypospadias, and comprises a testicular dysgenesis syndrome with a common origin in fetal life [115]. In the cryptorchid rat testis it was shown that the miRNA *miR-135a* was expressed at lower levels in the undescended testis and a decreased number of SSCs with Foxo1 activation was observed, suggesting a contribution of *miR-135a* to SSC maintenance by modulating Foxo1 activity [116]. Since miRNAs are quick and easy to measure in blood or tissues, and as their levels can be altered by human diseases, they also have unexplored potential as diagnostic tools and therapeutic targets for these diseases, as demonstrated in the case of (alcoholic) liver disease [117-119]. It will be interesting to determine if there are miRNAs measurable in the blood of testis cancer patients which could provide either a diagnosis and/or prognosis on their disease state.

7. Future directions

Specific miRNAs are expressed in the testis and appear to play a role in SSC self-renewal and spermatogonial differentiation, although detailed understanding of the roles and importance of miRNAs in this context are lacking. For example, it is unclear if Sertoli cell expressed miRNAs act as secreted paracrine factors in the germ stem cell niche, or whether they indirectly modulate the secretion of other Sertoli cell factors that then affect germ cells. Similarly, miRNAs produced in the germ cells themselves could act directly upon target

mRNAs within those germ cells or may act on mRNA targets in neighboring somatic cells. Another important area to explore is to determine the role of other somatic cell components (and their associated miRNAs) e.g. the peritubular myoid cells, the vasculature and Leydig cells in regulation of the germ stem cell niche. Conceptually, it seems clear already that regulation of the miRNA networks in the germ stem cell niche and in the germ cells themselves is likely to be critical for SSC/spermatogonial development and output.

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Figure legends

Figure 1

Schematic diagram of the germ stem cell niche in the mammalian testis. The spermatogonial stem cell (SSC) is supported by the Sertoli cell within the seminiferous tubule. Sertoli cell produced factors such as GDNF in combination with FGF2 and EGF promote SSC self-renewal, whereas retinoic acid (RA) induces spermatogonial differentiation. Upon differentiation, spermatogonia start expressing c-kit, which is the receptor for Sertoli cell derived Kit ligand. RA also induces BMP4 production which is also implicated in spermatogonial differentiation. It has been suggested that peritubular myoid cells, Leydig cells and the vasculature all somehow regulate the germ stem cell niche, but the exact mechanisms are still unknown. Follicle stimulating hormone (FSH) enters the testis through the bloodstream and induces Sertoli cell expressed GDNF, which may be one mechanism via which pubertal onset of spermatogenesis can be regulated centrally. For more detailed reviews on the germ stem cell see [2, 3].

Figure 2

MiRNA biogenesis pathway. The primary miRNA (Pri-miRNA) is transcribed from DNA by RNA polymerase II and gets processed into pre-miRNA structures of roughly 70 nucleotides by the nuclear microprocessor complex, which is comprised of the endonucleases Drosha and DGRC8 (DiGeorge syndrome critical region 8). The pre-miRNA gets transported into the cytoplasm by exportin 5 where the RNase III endonuclease Dicer cleaves the stem loop structure to form 19-23 nucleotide small RNAs. The miRNA strand of the small RNAs gets loaded into the miRNA-induced silencing complex (miRISC), which includes the Argonaute (AGO) proteins and this complex mediates degradation of target mRNAs.