Piwi and Potency: PIWI Proteins in Animal Stem Cells and Regeneration

Josien C. van Wolfswinkel
Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA

1E-mail: josien@wi.mit.edu

Synopsis
PIWI proteins are well known for their roles in the animal germline. They are essential for germline development and maintenance, and together with their binding partners, the piRNAs, they mediate transposon silencing. More recently, PIWI proteins have also been identified in somatic stem cells in diverse animals. The expression of PIWI proteins in these cells could be related to the ability of such cells to contribute to the germline. However, evaluation of stem cell systems across many different animal phyla suggests that PIWI proteins have an ancestral role in somatic stem cells, irrespective of their contribution to the germ cell lineage. Moreover, the data currently available reveal a possible correlation between the differentiation potential of a cell and its PIWI levels.

Germ cells have special status as mediators of the long-term survival of multicellular species, and exhibit several specialized mechanisms for control of gene expression and for protection of their genome. In animals, one of these protective mechanisms is established by PIWI proteins (Khurana and Theurkauf 2010), and loss of PIWI protein expression in animals as diverse as flies and zebrafish results in sterility (Lin and Spradling 1997; Houwing et al. 2007).

Over the last decade, PIWI proteins have also been detected in a growing range of somatic stem cells such as planarian neoblasts and cnidarian interstitial cells (Reddien et al. 2005; Plickert et al. 2012). These pluripotent cells retain the potential to generate germline cells, and therefore the presence of PIWI proteins in these cells could be related to that potential (Juliano et al. 2011), or, alternatively, PIWI proteins could have an ancestral function in somatic stem cells, independent of their involvement in the germline.

Somatic stem cell systems vary considerably in their spatial distribution, their proliferation rate, and most importantly, in their differentiation potential. This review will focus mostly on pluripotent and multipotent adult stem cells. Pluripotent stem cells are able to make cells of all germ layers, and this term is here used for stem cell systems that also contribute to the germline. Stem cell systems that make many cell types, but do not contribute to the germline will be referred to as multipotent. Other lineage-restricted types of stem cells include tissue-specific stem cells, such as epithelial stem cells and intestinal stem cells in vertebrates, which generate several different cell types but only within the tissue in which they reside, and unipotent stem cells, which make only a single cell type.

Different species employ different strategies with regard to their somatic stem cells to maintain tissue homeostasis. The nematode Caenorhabditis elegans, for example, has no adult somatic stem cells, whereas vertebrates typically have many different types (Tanaka and Reddien 2011). In this review, the presence of PIWI proteins in various somatic stem cell systems is evaluated, and possible roles of PIWI proteins in stem cells, and more generally, in potency, will be explored.

A brief introduction to PIWI proteins and piRNAs
Piwi, or “P-element induced wimpy testis” as it is named in full, was first discovered in 1997 as a gene
required for germ cell proliferation in *Drosophila melanogaster* (Lin and Spradling 1997). This gene was soon found to encode a broadly conserved protein (Cox et al. 1998), and became the first-known representative of an animal-specific clade of the Argonaute protein family (Carmell et al. 2002).

**PIWI proteins in the Argonaute protein family**

Argonautes are RNA-binding proteins that represent the core players in silencing mechanisms mediated by small noncoding RNAs (Ghildiyal and Zamore 2009). Argonautes have a bi-lobed structure (Song et al. 2004), containing binding pockets for both ends of the small RNA, and a RNaseH-like domain that establishes endonuclease activity (Tolia and Joshua-Tor 2007). The small RNA acts as a guide that by basepairing directs the Argonaute protein to a specific RNA or DNA target. The downstream implications of target recognition depend on the Argonaute protein and its binding partners, but can involve inhibition of translation, mRNA degradation, or epigenetic modification (Meister 2013).

Phylogenetic analysis has shown that Argonaute proteins fall into two major clades: the Argonaute and the Piwi clade (Carmell et al. 2002). The Argonaute clade has representatives in all eukaryotes and some prokaryotes. They typically are cytoplasmic proteins with ubiquitous expression, and bind 21- to 22-nt long miRNAs and siRNAs. In animals, they play a role in development, mostly by posttranscriptional regulation. The Piwi clade is found exclusively in the animal kingdom (with the exception of some unicellular eukaryotes such as *Tetrahymena*), and expression of PIWI proteins is highly enriched in the germline.

**PIWI-interacting RNAs**

As small RNAs guide Argonaute proteins to their targets, they are central to the understanding of PIWI proteins. Just like PIWI proteins, piRNAs are animal-specific and are highly enriched in the germline (Aravin et al. 2006; Girard et al. 2006; Grivna et al. 2006). Their production, in contrast to miRNAs and siRNAs, does not require the dsRNA-processing enzyme Dicer (Vagin et al. 2006; Houwing et al. 2007). Instead, piRNAs are transcribed as single-stranded precursors from specific loci or clusters in the genome (Brennecke et al. 2007; Malone et al. 2009; Li et al. 2013), and are subsequently amplified through the alternated catalytic activity of two different PIWI proteins in a mechanism known as the ping-pong cycle (Brennecke et al. 2007; Gunawardane et al. 2007). PIWI proteins are thus involved in the function as well as in the biogenesis of piRNAs.

The source of the initial piRNAs that start this amplification cycle is still enigmatic. In some cases the initial piRNAs may be maternally deposited (Brennecke et al. 2008). In other cases they are generated de novo, by the primary pathway of piRNA biosynthesis, which likely involves nucleases other than the PIWI proteins (Siomi et al. 2011; Luteijn and Ketting 2013).

**Subcellular localization of PIWI proteins**

The ping-pong cycle of piRNA production takes place in nuage (Lim and Kai 2007; Aravin et al. 2009), and this is also where most PIWI proteins are found (Fig. 1). Nuage is an RNA-rich, electron-dense structure that can take many forms, from a diffuse cloud to distinct granules. It is found in the perinuclear region of germline cells and is closely associated with the nuclear pores (Eddy1975; Voronina et al. 2011). Nuage contains several other RNA-binding proteins such as the RNA helicase Vasa, the Vasa-like protein PL10, and the RNA-binding protein Bruno (Vorona et al. 2011). Tudor proteins, which bind dimethylated Arginine residues that are present in both Vasa and PIWI proteins, function as the scaffold of nuage (Kirino et al. 2009; Huang et al. 2011; Mathioudakis et al. 2012) (Fig. 1). Nuage-associated Vasa and Tudor proteins are required for efficient biogenesis and function of piRNAs (Malone et al. 2009; Reuter et al. 2009; Vagin et al. 2009; Kuramochi-Miyagawa et al. 2010).

Several PIWI proteins have also been detected in nuclei of germ cells, often during specific developmental stages. *Drosophila* Piwi is nuclear in germ cell precursors as well as in mature germ cells (Cox et al. 2000), mouse Miwi2 (also known as Pwili4) is found in the nuclei of pre-pachytene spermatocytes (Aravin et al. 2008), and zebrafish Zili is nuclear in primordial germ cells (PGCs) as well as in maturing oocytes (Houwing et al. 2007, 2008).

**Molecular function of PIWI proteins**

A prominent and conserved role of PIWI proteins and piRNAs is to regulate transposon activity. The majority of *Drosophila* piRNAs maps to transposon sequences (Vagin et al. 2006; Brennecke et al. 2007; Klenov et al. 2007), and interference with the PIWI-piRNA pathway results in elevated levels of transposon activity in the *Drosophila* germline. In mouse male gonads, roughly half of the piRNAs is related to transposable elements, and PIWI proteins are required to maintain transposon repression (Watanabe
et al. 2006; Aravin et al. 2007; Carmell et al. 2007; Aravin et al. 2008; Kuramochi-Miyagawa et al. 2008). Also in zebrafish (Houwing et al. 2007), *Xenopus* (Lau et al. 2009), and *C. elegans* (Bagijn et al. 2012), piRNAs are enriched for transposon-related sequences.

PIWI proteins suppress transposon activity by two complementary mechanisms (Rozhkov et al. 2013) (Fig. 1). First, as part of the ping-pong cycle they degrade transposase mRNAs and other transposon-derived RNAs that have sequence complementarity to the piRNA. Nuage is closely associated with nuclear pores. Some PIWI proteins are present in the nuage and function in epigenetic modification of target DNA, with the help of proteins such as histone methylase HP1 (*Drosophila*) or DNA methylase Dnmt3 (*mouse*).

![Fig. 1](https://academic.oup.com/icb/article-abstract/54/4/700/2797836)

**Fig. 1** Subcellular localization and molecular functions of PIWI proteins. The primary location of PIWI proteins is the perinuclear nuage, shown as three smaller spheres attached to the nucleus. In nuage, PIWI proteins associate with Tudor proteins and Vasa, are involved in the ping-pong cycle of piRNA amplification, and target transposon RNA (and possibly other mRNAs) for degradation. Nuage is closely associated with nuclear pores. Some PIWI proteins are present in the nucleus and function in epigenetic modification of target DNA, with the help of proteins such as histone methylase HP1 (*Drosophila*) or DNA methylase Dnmt3 (*mouse*).

Although transposons are the primary targets of piRNAs, a significant fraction of the piRNAs has no apparent relationship to transposons. In certain contexts, such as in pachytene cells in the mouse male germline, even the majority of piRNAs is unrelated to repetitive elements (Grivna et al. 2006; Watanabe et al. 2006). PIWI proteins have been implicated in the regulation of nonrepetitive sequences, mostly at the posttranscriptional level, for example during embryonic development in *Drosophila* and during spermatogenesis in mouse (Yin and Lin 2007; Robine et al. 2009; Unhavaithaya et al. 2009; Rouget et al. 2010; Gou et al. 2014). Although these results await confirmation, the findings suggest that regulation of coding transcripts could be another conserved role of PIWI proteins.

### PIWI proteins in adult pluripotent stem cells

Although it is clear that PIWI proteins have important functions in the germline, it is possible that the prevailing germline-centered view of PIWI proteins is a consequence of the selection of model organisms used in developmental biology. Specifically, the ability to regenerate, although widely conserved in the animal kingdom, is fairly restricted in the primary model species used to study animal development (Bely and Nyberg 2009). Newly developed invertebrate models that possess a capacity for whole-body regeneration (i.e., the ability to reestablish an organism with a mature body plan from small body fragments) are demonstrating conserved features of regenerating systems and show PIWI proteins in a new light (Fig. 2).

### Planarian neoblasts

Adult flatworms have a legendary ability to repair extensive wounds, reorganize complex structures such as the intestinal system, and recreate entire organs such as the brain and the germline (Reddien and Sanchez Alvarado 2004; Newmark et al. 2008). This regenerative capacity is mediated by a population of small, mitotically active cells named “neoblasts” that are dispersed throughout the parenchymal space of the body. Although neoblasts probably are a heterogeneous population, at least some of these cells are pluripotent (Wagner et al. 2011). When neoblasts are eliminated by irradiation or RNAi (Dubois and Wolff 1947; Solana et al. 2012), regeneration and cell turnover are blocked and the animal invariably dies.
Neoblasts have several features that previously had been associated with germ cells. They have an electrondense nuage-related structure called the chromatoïd body (Morita et al. 1969; Coward 1974; Rouhana et al. 2012), and they express transcripts corresponding to three PIWI proteins (Smedwi-1, Smedwi-2, and Smedwi-3) (Reddien et al. 2005; Palakodeti et al. 2008), two Vasa proteins, two Tudor proteins, and the Bruno homolog Bruli (Guo et al. 2006; Onal et al. 2012; Wagner et al. 2012). Whereas Smedwi-1 protein is cytoplasmic (Guo et al. 2006), Smedwi-2 has been detected in the nucleus (Zeng et al. 2013).

Planarian piRNAs with all hallmarks of a ping-pong cycle have been identified (Palakodeti et al. 2008; Friedlander et al. 2009). Inhibition of smedwi-2 or smedwi-3 (but not smedwi-1) caused significant reductions in piRNA levels, and resulted

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**Fig. 2** PIWI proteins in stem cells. Phylogenetic tree indicating the presence of PIWI proteins in various stem cell types in each group. Presence of a cell type is indicated with the corresponding letter. Black letters correspond to cell types in which PIWI proteins have been identified; gray letters indicate cell types without PIWI expression. Demonstrated requirement of a PIWI protein for the cell type is indicated with an asterisk; presence of Vasa or nuage in the same cell type is indicated with a V or N, respectively. Ability for whole-body regeneration or posterior body regeneration is indicated with a W or P.
in regenerative defects and lethality (Reddien et al. 2005; Palakodeti et al. 2008; Friedlander et al. 2009). *Brul* and *vasa-1* were also required for regeneration and animal viability (Guo et al. 2006; Wagner et al. 2012), but their effects on piRNAs are currently unknown.

Neoblasts with similar features have been identified in several other flatworms. *Dugesia japonica* neoblasts have chromatoid bodies and express *DjPiwi* mRNA and two Vasa-related transcripts (Shibata et al. 1999; Rossi et al. 2006). In *Macrostromum big-nano*, transcripts *macpiwi* and *macvasa* were detected in the germline and in a subset of the neoblasts (Pfister et al. 2008; De Mulder et al. 2009b), and nuage-like perinuclear granules were observed in neoblasts by visualization of MacVasa protein (Pfister et al. 2008). Moreover, inhibition of *macpiwi* eliminated adult neoblasts, resulting in regeneration defects and animal lethality (De Mulder et al. 2009b).

**Acoel stem cells**

Although acoels and flatworms are not closely related, their stem cell systems show remarkable similarities. Acoels have extensive regenerative capacities and possess small parenchymal cells that are the only mitotically active cells in the body (De Mulder et al. 2009a). In the acoel *Hofstenia miamia*, dividing cells expressed transcripts of two PIWI homologues (Srivastava et al. 2014), and *piwi-1* was found to be required for regeneration. In another acoel, *Isodiametra pulchra*, a subpopulation of the neoblasts expressed *ipiw-1* and *ivasa-1* (De Mulder et al. 2009a). Inhibition of *ipiw-1* by RNAi did not interfere with regeneration (De Mulder et al. 2009a), although this could be due to redundancy or incomplete inhibition.

**Sponge archaeocytes**

Sponges belong to an early-diverging animal phylum. They lack body symmetry and true tissues or organs, but have the ability to recover from any type of injury (Simpson 1984; Leys et al. 2007). Even upon complete cell dissociation, an amoeboid cell type, named the archaeocyte, was observed to reaggregate and through mitosis and well-defined differentiation paths recreate all the characteristic structures of a mature sponge (De Sutter and Van de Vyver 1979; Buscema et al. 1980).

*Ephydatia fluviatilis* archaeocytes expressed mRNAs *EfPiwiA* and *EfPiwiB*, and this expression was lost upon differentiation into all other cell types except for the choanocytes (Funayama et al. 2010). The flagellated choanocytes are involved in nutrient entrapment, but maintain mitotic ability and can produce sperm (Simpson 1984). Moreover, under specific circumstances, choanocytes have been proposed to dedifferentiate into archaeocytes (Simpson 1984). Therefore, although choanocytes have a clear differentiated morphology, they appear to maintain certain features and capabilities of the pluripotent archaeocytes.

**Stem cells in Botryllus**

The urochordate *Botryllus* is a colonial tunicate, composed of up to thousands of genetically identical zooids, which are connected by “blood vessels” and are embedded in a gelatinous matrix called the tunic. Each zooid is only a few millimeters large, but has a variety of organs among which a two-chambered heart, a neural network, and a gastrointestinal system (Manni et al. 2007). Tiny fragments of vasculature can regenerate an entire mature organism (Rinkevich et al. 1995; Brown et al. 2009), leading to the view that pluripotent stem cells circulate through the blood, although their identity has remained elusive.

In *Botryllus leachi*, induction of regeneration revealed a population of *BlPiwi* positive cells on the lumbar side of the vasculature (Rinkevich et al. 2010). These cells subsequently entered the blood vessel lumen, proliferated, and moved through the vasculature, consistent with the notion of circulating stem cells. Inhibition of *BlPiwi* expression resulted in a complete halt of whole body regeneration (Rinkevich et al. 2010). Surprisingly, these cells appeared to upregulate *BlPiwi* specifically when activated by injury, suggesting that PIWI proteins might be absent from these cells for prolonged time spans.

In the related species *Botryllus schlosseri*, *BsPiwi* positive cells were detected in so called “Cell Islands”, which also expressed mRNA for Vasa and PI10 (Rosner et al. 2009; Rinkevich et al. 2012). Transplantation of these CIs resulted in chimerism, indicating that they contain self-renewing cells that can give rise to differentiated cell types. Similar to the situation in *Botryllus leachi*, RNAi against *BsPiwi* abrogated regeneration completely.

**Cnidarian i-cells**

Cnidarians are aquatic animals with a sac-like body plan composed of two germ layers (endoderm and ectoderm). The adult *Hydra* polyp is radially symmetric and consists of a foot, a cylindrical body column, and a head with tentacles. Any part of the body column, when dissected, is able to regenerate a
new head and a foot (Holstein et al. 2003). Even upon complete dissociation, Hydra cells can reaggregate and form a new animal de novo (Grier et al. 1972).

The Hydra endoderm and ectoderm each make a single-layer epithelium and together form the polyp body. Each epithelium is constantly replenished by mitosis of its own lineage-committed stem cells, which are located in the body column (Holstein et al. 1991). Found between these two epithelial layers are migratory interstitial cells (i-cells). I-cells are mitotically active and give rise to a constant supply of neurons, secretory cells, nematocytes, and germ cells (David and Murphy 1977; David 2012).

Hydra PIWI proteins Hywi and Hyli were detected in the i-cells, as well as in the germline (Juliano et al. 2013; Lim et al. 2013), but were absent from postmitotic differentiated cells. I-cells also contained transposon-related piRNAs with hallmarks of the ping-pong cycle (Juliano et al. 2013; Lim et al. 2013), and expressed Vasa, PL10, and the Tudor protein TDRD9, all of which were enriched in perinuclear granules that resemble nuage (Lim et al. 2013). Interestingly, variations exist between different cnidarian stem cell systems. Morphology of the Hydractinia polyp is very similar to that of Hydra, but Hydractinia i-cells, which expressed piwi, vasa, and nanos2 (Rebscher et al. 2008; Plickert et al. 2012), were also able to generate epithelial cells, and therefore are truly pluripotent (Muller et al. 2004).

In another cnidarian, Podocoryna carnea, the transcript for the PIWI protein Cniwi was most highly expressed in the germline, but lower levels were detected in differentiated epithelio-muscular cells (Seipel et al. 2004). Upon stimulation, these cells have been proposed to dedifferentiate and differentiate into various other cell types (Schmid and Alder 1984), which at least in vitro, included gametes (Schmid et al. 1982).

In summary, pluripotent stem cells are found in diverse animal species, and in each of the cases studied so far these stem cells express PIWI proteins (or at least mRNA), often combined with the presence of other typical nuage constituents and a nuage-like structure. Moreover, PIWI expression tends to decrease upon cell differentiation, and to be required for stem cell function. One could argue that it is expected that pluripotent stem cells, which can generate germ cells, maintain the same degree of genome protection that germ cells have, and therefore maintain nuage and PIWI proteins. An alternative possibility, however, is that these proteins have a function in stem cells that is unrelated to the germ cell lineage. An important question therefore is what the status of PIWI proteins is in lineage-committed stem cells that do not generate germ cells.

**PIWI proteins in adult lineage-restricted stem cells**

Cnidarians revisited

Although Hydra i-cells can be considered pluripotent, Hydra endoderm and ectoderm are formed from the lineage-restricted stem cells (Holstein et al. 1991). These stem cells were found to express PIWI proteins, albeit at lower levels than the i-cells (Juliano et al. 2013). Interestingly, elimination of Hywi specifically from the epithelial lineages resulted in a loss of epithelial integrity followed by death within days after hatching (Juliano et al. 2013), suggesting that Hywi has an essential function in these lineage-committed cells. Similarly, in Clytia hemisphaerica medusas, expression of pivi, vasa, pl10, and nanos1 transcripts was detected in mitotic cells of the tentacle bulb, which are thought to be lineage-committed cells that give rise to only nematocytes (Denker et al. 2008).

Localized stem cell populations in ctenophores

The tentacle root of the ctenophore Pleurobrachia pileus shows similarities to the Clytia tentacle bulb. It contains undifferentiated cells that express high levels of ppiPiwi1, ppiVasa, ppiPL10, and ppiBruno transcripts (Alie et al. 2010), and are able to regenerate the tentacle structure consisting of muscle cells and colloblasts throughout the animal’s lifespan, but are not known to contribute to any other cell types. Pleurobrachia has several additional lineage-restricted stem cell types, including a spatially confined proliferative population that replenishes the ciliated polster cells in the combs (which are required for ctenophore locomotion), and a population of stem cells that specifically renews sensory or neuronal cells at the aboral pole. Both of these stem cell populations were found to express ppiPiwi1, ppiVasa, ppiPL10, and ppiBruno (Alie et al. 2010). The same set of genes, together with ppiPiwi2, was also expressed in the germline. The germline however is spatially separated from the other lineages, indicating that the expression of nuage-related genes in the other stem cell types is not related to the germline lineage.

**Posterior growth zone in annelids**

Juveniles of segmented annelids typically have a limited number of segments, which is expanded over the
course of their life in a process known as posterior elongation, by a region called the Posterior Growth Zone (PGZ) (de Rosa et al. 2005). In addition to its developmental function, the PGZ also mediates regeneration of posterior segments upon amputation or injury. In *Platynereis dumerilii*, transcripts *pdu-Piwi*, *pdu-Vasa*, *pdu-PL10*, *pdu-nanos*, and transcripts for three Tudor proteins were detected in undifferentiated cells of the PGZ (Rebscher et al. 2007; Gazave et al. 2013). These same genes were also expressed in germ cell precursors, which during embryogenesis are located in the same region that gives rise to the PGZ, and only later migrate anteriorly. For a long time it was believed that PGCs differentiate from the PGZ cells, however, recent EdU-labeling experiments showed that although these populations of cells share gene expression profiles and an anatomical position during embryogenesis, they are separate cell populations formed during different stages of embryogenesis (Rebscher et al. 2012; Gazave et al. 2013).

A similar situation has been described for the annelid *Capitella teleta*, where transcripts *ct-piwi1*, *ct-piwi2*, *ct-Vasa*, and *ct-nanos* were expressed both in germ cells and the PGZ, but these lineages were generated at different times during embryogenesis and remained separate during the remainder of the animal’s life (Dill and Seaver 2008; Giani et al. 2011).

Tail bud in cephalochordates

Expression of PIWI protein in cephalochordates shows remarkable resemblance to that in annelids. During embryonic development of the amphioxus *Branchiostoma floridae*, two populations of cells expressed transcripts *bf-Piwi1*, *bf-Vasa*, and *bf-Nanos*: the PGCs and the cells of the tail bud, which is the site of posterior elongation where new somites progressively develop (Wu et al. 2011; Zhang et al. 2013). After gastrulation both of these cell types localize to the tail bud, resembling a single population, from where the germ cell precursors migrate to their final ventral position later during larval development. The localization of PGCs to the tail bud has, again, led to the view that the PGCs develop from the stem cells in the tail bud. However, closer observations demonstrated that during the first embryonic divisions germ plasm containing maternal transcript of *Bf-Piwi1*, *Bf-Vasa*, *Bf-Nanos*, and *Bf-TDRD7* was segregated asymmetrically to one cell, with asymmetric segregation of these components continuing all the way to the 64-cell stage. The single cell that received maternal transcripts for nuage proteins, was the only cell that gave rise to the PGCs. The stem cells that form the tail bud expressed similar transcripts zygotically, but were not able to generate PGCs. An experiment separating the first two embryonic blastomeres showed that the larva developing from the blastomere lacking the germ plasm still generated a tail bud, but never generated PGCs (Wu et al. 2011), confirming the independence of the two lineages.

Together, these studies show that in nonbilaterians, protostomes, and deuterostomes, multipotent stem cells that do not appear to give rise to germ cells do express PIWI proteins, together with several other nuage-constituents. In fact, most-to-all multipotent adult stem cells involved in wound-induced regeneration studied thus far show expression of nuage-associated factors, indicating that PIWI proteins and nuage components likely have some somatic function in addition to their germline roles.

**PIWI proteins in vertebrate stem cells**

In vertebrates, no cases of whole-body regeneration are known. Some animals have prominent wound-induced regeneration, such as newts and amphibians, which can regenerate amputated limbs, or zebrafish which are able to regenerate their tail fin, heart, and liver (Tanaka and Reddien 2011). Although such regenerated structures can consist of many cell types, the stem cells involved in these processes are typically restricted in their potential and no expression of PIWI proteins has been reported in dividing cells of these systems (Gargioli and Slack 2004; Schebesta et al. 2006; Kragl et al. 2009; Yoshinari et al. 2009; Jopling et al. 2010; Sleep et al. 2010; Knopf et al. 2011; Sousa et al. 2011; Tu and Johnson 2011; Monaghan et al. 2012; Stewart and Stankunas 2012). Transcriptional profiling showed that PIWI proteins were also not significantly enriched in two well-studied tissue-specific stem cell systems: the mammalian intestinal stem cells (Munoz et al. 2012) and epithelial stem cells (Kocer et al. 2008).

Two other mammalian stem cell systems, however, have been reported to express PIWI proteins. Human hematopoietic stem cells, but not the more lineage-restricted progenitors, were found to express *Hiwi* mRNA (Sharma et al. 2001), which rapidly decreased upon induction of differentiation. Ectopic expression of *Hiwi*, in the acute leukemic cell line KG1 resulted in inhibition of proliferation activity and induction of apoptosis, suggesting that this PIWI protein might function to keep hematopoietic cell division under control. Somewhat conflicting results however were obtained in mouse. First, murine hematopoietic stem
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...cells did not express Miwi or Mili, but instead expressed mRNA for the PIWI protein Miwi2 (also known as Piwil4) (Nolde et al. 2013). Second, in vitro overexpression of Mili in murine bone marrow cells resulted in increased cell proliferation (Chen et al. 2007). Finally, a triple PIWI knockout mouse showed no major defects in hematopoiesis (Nolde et al. 2013) casting doubt on the physiological relevance of PIWI protein expression in the hematopoietic system.

Murine mesenchymal stem cells, which are multipotent cells that can give rise to cell types as diverse as osteoblasts and fat cells, were also reported to express Mili (or Piwil2) (Wu et al. 2010). In this context, siRNA-mediated inhibition of mili resulted in increased cell proliferation.

Although these findings are intriguing, and suggest the presence of PIWI proteins in some vertebrate stem cells, the relevance of PIWI proteins to these systems remains to be clarified. Meanwhile, some further interesting findings along these lines come from the study of induced stem cell populations in vertebrates.

iPS cells

Induced pluripotent stem (iPS) cells are formed when adult differentiated cells (usually fibroblasts) are reprogrammed to a pluripotent state by stimulation with exogenous factors (Takahashi and Yamanaka 2006). In a study analyzing gene expression in successfully reprogrammed mouse iPS cells originating from various cell types, mili (piwil2) and vasa were consistently upregulated (Mikkelsen et al. 2008). Another study found that mili (piwil2) expression increased early in the reprogramming process, in the same time frame as nanos and the Tudor protein tdrd7 (Samavarchi-Tehrani et al. 2010), suggesting that this is not a late indirect effect. The level of piwil2 in iPS cells may differ between species though (Pashai et al. 2012; Marchetto et al. 2013). Interestingly, the lower piwil2 levels found in nonhuman primate iPS cells compared with human iPS cells, corresponded with increased levels of L1 retrotransposition, which could be suppressed by overexpression of piwil2 in these cells (Marchetto et al. 2013), suggesting that PIWI proteins in iPS cells could function in transposon silencing.

Cancer stem cells

Many studies have shown expression of Hiwi (Piwil1) or Hili (Piwil2) in human cancers, and expression of these PIWI proteins is often associated with adverse long-term prognosis (Sun et al. 2010; Zhao et al. 2011; Suzuki et al. 2012; Wang et al. 2012; Greither et al. 2012; Liang et al. 2013). The human PIWI protein Hiwi was even found to function as an oncogene, increasing the tumorigenic potential of a cell transplant, whereas inhibition of hiwi expression in a sarcoma cell line reduced the efficiency of colony formation and induced differentiation (Siddiqi et al. 2012), suggesting that Hiwi was required to maintain expansion capabilities.

Interestingly, increased expression of PIWI protein in the context of tumor formation is not unique to humans. In the cnidarian Hydractinia echinata, ectopic expression of a POU-domain transcription factor in epithelial cells resulted in tumor-like growths, which exhibited high expression of piwi, vasa, and nanos (Millane et al. 2011). Similarly in Drosophila, mutation of the transcriptional repressor l(3)mbt resulted in brain tumors that expressed elevated levels of aub, piwi, and vasa, and each of these genes was required for tumor growth (Janic et al. 2010).

Together, these results indicate that expression of PIWI proteins tends to be elevated in vertebrate cells that acquired an increased differentiation potential. Although the causal relationship between these events is currently unclear, it raises the possibility that PIWI proteins have a connection to pluripotency in this subphylum.

Conclusions and speculations: the role of PIWI proteins in potency

In summary, PIWI proteins are present in a wide range of somatic stem cells, and in many cases are required for stem cell function. PIWI proteins are found in all stem cell systems that are capable of generating germ cells, but also in many lineage-restricted systems, indicating that their presence in stem cells is not directly related to the ability to contribute to the germline lineage. Rather, expression of PIWI proteins in somatic stem cells seems to be the rule throughout most of the animal kingdom (Fig. 2), involving nonbilaterian phyla, and several protostomes as well as deuterostomes, suggesting that this likely reflects the ancestral metazoan state.

Two major clades tend to lack somatic stem cells with PIWI proteins: ecdysozoans and vertebrates (Fig. 2). PIWI proteins still function in the germline in these clades, where they have well-described and essential roles. Moreover, most members of these groups have somatic stem cells, although these tend to be lineage-restricted. Unlike lineage-restricted somatic stem cells in other phyla however, they have...
not been found to express PIWI protein, or the PIWI proteins appear not essential for their function. Interestingly, ecdysozoans and vertebrates are also two main groups that lack the ability for whole-body regeneration (Bely and Nyberg 2009), raising the possibility of a relation between PIWI-expressing stem cells and regenerative plasticity. Further study of stem cell systems and regenerative potential in nonecdyszoan protostome species such as molluscs and nemerteans, and nonvertebrate deuterostomes will strengthen or weaken this correlation.

Aside from the phylogenetic trends, there appears to be a correlation between cell plasticity and PIWI protein expression. All pluripotent stem cell systems studied to date express PIWI protein. In addition, even in ecdysozoans and vertebrates, when plasticity of a cell is artificially increased (e.g. in iPS cell induction or tumorigenesis) levels of PIWI proteins tend to increase. It remains to be determined whether this increase in PIWI expression is a prerequisite or a side-effect of reprogramming, but it nevertheless suggests that PIWI proteins could be part of a conserved transcriptional program that is activated when the differentiation potential, or potency, of a cell is increased.

What could the role of PIWI proteins in stem cells be? The main known molecular functions of PIWI proteins involve piRNAs, and the structural features of PIWI proteins support piRNAs as their primary interactors. Indeed, piRNAs, typical nuage-components such as Vasa, and structures that resemble nuage have been detected alongside PIWI proteins in many pluripotent cells (Fig. 2). PIWI proteins and piRNAs could be involved in genome protection in somatic stem cells. As stem cells are self-renewing and constantly give rise to new descendants, they may require more protection than terminally differentiated cells, including tighter protection against transposon activity. In addition, the high plasticity of pluripotent cells may involve a more “open” chromatin structure, which could make the cells vulnerable to transposon activation. Pluripotent embryonic stem cells are thought to have such an open chromatin state which is lost upon differentiation (Gaspar-Maia et al. 2011) (although the role of PIWI proteins in these stem cells is currently unknown). Lineage-restricted intestinal stem cells on the other hand were found to undergo little change in overall DNA methylation upon differentiation (Kaaaij et al. 2013), suggesting that their chromatin was already mostly in the “closed” differentiated state. In planarians, chromatin remodeling is thought to play a role during differentiation of stem cell progeny (Scimone et al. 2010; Hubert et al. 2013; Jaber-Hijazi et al. 2013), but it is currently unclear whether differentiation coincides with a global increase in heterochromatin, and whether neoblast chromatin is in an “open” state.

Speculating further along these lines, it is possible that the role of PIWI proteins in the stabilization of the stem cell genome extends beyond the control of repetitive sequences, and includes the maintenance of this “open” chromatin structure. The role of RNA in chromatin structure is gaining appreciation (Caudron-Herger and Rippe 2012), and mostly long noncoding RNAs have been proposed to function in the formation of nuclear domains. Similarly, the interaction of PIWI-piRNA complexes with nascent transcripts or genomic regions could assist in shaping and maintaining the three-dimensional architecture of the chromatin.

PIWI proteins could also play a role in the regulation of nonrepetitive transcripts in stem cells. PIWI proteins have been reported to regulate specific genes or gene groups during specific developmental phases (Yin and Lin 2007; Robine et al. 2009; Unhavaithaya et al. 2009; Rouget et al. 2010; Gou et al. 2014). The fact that modification of PIWI protein levels can affect expansion efficiency in some vertebrate cell systems is consistent with a function in regulation of gene expression beyond repetitive transcripts. PIWI proteins could play a role in inhibition of differentiation genes, thereby maintaining stem cells in an undifferentiated state, or they could be required to regulate genes involved in mitotic activity.

Much remains to be discovered on the presence and function of PIWI proteins in somatic stem cells. First, many stem cell systems have not been probed, and the data indicating absence of PIWI proteins from several stem cell systems is not conclusive. Some stem cell systems have large numbers of amplifying progenitor cells, which may mask the expression signature of the rare stem cells if the analysis is done on a population basis. Studies of expression with single cell resolution, such as in situ hybridization or single cell RNA sequencing, may therefore help to further clarify the presence or absence of PIWI expression. Second, functional studies are essential to determine whether the presence of PIWI in any stem cell system has physiological relevance for stem cell function. And third, closer analysis of the effects of the loss of PIWI proteins in pluripotent stem cells will be required to determine the molecular underpinnings of their requirement. If their main role is in transposon silencing, the loss of stem cells upon inhibition of PIWI protein
expression should correlate with increased transposon activity and genomic damage, possibly accompanied by apoptosis. Alternatively, if their role lies in inhibition of differentiation, loss of PIWI protein should result in activation of differentiation markers. Many other cellular outcomes are possible however. As stem cells provide an opportunity to study PIWI proteins without the complicated cell biology of germ cells, further investigations along these lines could increase our understanding of both stem cells and the functions of PIWI proteins.

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