Control of asymmetric cell divisions: will cnidarians provide an answer?

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Summary
Cells in the basal metazoan phylum Cnidaria are characterized by remarkable plasticity in their differentiation capacity. The mechanism controlling asymmetric cell divisions is not understood in cnidarians or in any other animal group. PIWI proteins recently have been shown to be involved in maintaining the self-renewal capacity of stem cells in organisms as diverse as ciliates, flies, worms and mammals. Seipel et al.[1] find that, in the cnidarian *Podocoryne carnea*, the *Piwi* homolog *Cniwi* is transcriptionally upregulated when the polyp generates buds, which will develop into medusae. Since transdifferentiation of striated muscle cells to smooth muscle cells also activated *Cniwi* expression, *Cniwi* appears to play a crucial role in differentiation events. The discovery should facilitate elucidation of the poorly understood factors that control asymmetric cell divisions at the beginning of animal evolution. *BioEssays* 26:929–931, 2004. © 2004 Wiley Periodicals, Inc.

Introduction

Stem cells are common to all animals and higher plants. By asymmetric cell divisions, they have the unique ability to undergo self-renewal or to differentiate into cells belonging to multiple lineages.[2,3] The self-renewing ability is regulated both by an intracellular mechanism and by intercellular signalling. Cell-autonomous mechanisms governing asymmetric cell divisions have been elucidated in a few stem cell models such as neuroblasts and germline stem cells (GSC) in *Drosophila*,[4,5] whereas the role of extrinsic signaling in controlling asymmetric cell divisions has been implicated in several systems.[3] External stimuli that alter self-renewal of several classes of stem cells and affect asymmetric divisions include cytokines, matrix proteins, hormones and local interactions between stem cells and their neighboring cells. These extracellular signals may then influence the cell cycle machinery or the cytoskeletal organization of stem cells for their formation and/or divisional asymmetry. Asymmetric cell divisions also involve marked differences in gene expression as well as extraordinary genome modifications. For example, in *C. elegans* and *Drosophila*, transcriptional quiescence in early germ cells is thought to be essential for the establishment of distinct germline and somatic fates.[6,7] Genome modifications associated with asymmetric divisions are of importance in blood stem cells[8] and also play crucial roles in switching of mating types in yeast, programmed chromosome breakage and chromatin elimination in *Ascaris*, and the development of a transcriptionally inert germline micronucleus in spirotrichous ciliates.

The intrinsic cellular processes serving as determinants of asymmetric-segregating cell fates depend in part on the evolutionarily conserved PIWI family of proteins. *Piwi* was identified first in *Drosophila*in embryonic germline stem cells (GSCs) and is a highly basic novel protein with no obvious similarity to other known proteins or functional motifs in the databases.[9,10] Overexpression of *Piwi* in somatic cells causes an increase both in the number of germline stem cells and the rate of their division indicating a role in GSC self-renewal. Although the precise biochemical function of the protein remains to be shown, Piwi has been shown to be essential for germline genetic suppression mechanisms and /or the double-stranded RNA-silencing pathway (for Ref see 11). PIWI is well conserved in worms, flies, mice and humans, and also in plants, in which *piwi*-like genes are required for meristem cell maintenance.[12] Interestingly, *Piwi* homologs are also present in spirotrichous ciliates where they are involved in the genome modifications occurring during macronuclear development.[11] Thus, members of the *Piwi* family are essential stem-cell genes involved in controlling asymmetric cell divisions in various organisms.

Asymmetric divisions in Cnidaria

Cells in the basal metazoan phylum Cnidaria are characterized by remarkable plasticity in their differentiation capacity. Cnidarians, similar to tunicates and flatworms, lack a clear distinction between germ and soma, and can form germ cells from somatic cells. In *Hydra*, for example, asymmetric cell division of multipotent interstitial stem cells allows not only differentiation into multiple somatic cell lineages (cnidocytes, nerve cells, gland cells), but also the generation of gametes.[13,14] Other stem cell lineages in *Hydra* are responsible for maintaining the ectodermal and the endodermal epithelial cell lineage. All differentiation decisions are precisely regulated so that, in the polyp, the density of the different cell

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types is maintained constant. How this marvellous homeostasis is achieved, is largely unknown. For the maintenance of nerve cell density, a feedback control mechanism based on diffusible short peptides has been proposed.[15] The life cycle in many cnidarians includes metamorphosis of a larvae into a polyp, asexual proliferation of polyps by budding, differentiation of polyp tissue into medusa and gamete production in adult medusae (see for example Ref. 16). In some cnidarians such as *Turritopsis*, even the reverse seems possible since a medusa can transform into a polyp.[17] In all these life cycles, one genome allows formation of two completely different morphotypes. Although this is an impressive example of the plasticity of the cnidarian genome, nothing is known about the nuclear changes involved in the transition between polyp and medusa. Another striking feature of Cnidaria is their extensive regeneration and transdifferentiation capacity.[18] In *Hydra*, polyps can regenerate from clumps of dissociated cells within a few days. In *Podocoryne*, under certain conditions, even terminally differentiated cells of the medusa such as striated muscle cells can be induced to de-differentiate into proliferating smooth muscle cells; by asymmetric cell divisions, these cells then undergo self-renewal or differentiate into unrelated phenotypes such as nerve cells.[19]

Taken together, although asymmetric cell divisions are common to all multicellular animals, within the phylum Cnidaria there is a remarkable plasticity of somatic cells in their differentiation and decision-making program. But although we know many details about the cellular and morphological events, we know very little about the underlying molecular mechanisms. Some answers may now be found in a recent paper by Seipel et al.[1]

A *Piwi* homolog is expressed when *Podocoryne* cells make asymmetric cell divisions

Seipel and coworker[1] identified a *Piwi* homolog in the hydrozoan *Podocoryne*. The 867 amino acid protein, termed *Cniwi*, has all the features to classify it as a bona fide member of the *Piwi* family including highly conserved PAZ and Piwi domains. While feeding polyps express the gene only at a very low level, *Cniwi* expression is activated in cells in polyps that develop buds to generate medusae. Seipel et al.[1] show by RT PCR and in situ hybridisation that *Cniwi* transcripts accumulate in dedifferentiated and highly proliferative cells from both polyp ectoderm and endoderm in which medusa development in *Podocoryne* starts (Fig. 1). Within adult medusae, the authors could localize *Cniwi* transcripts in gonads as well as in

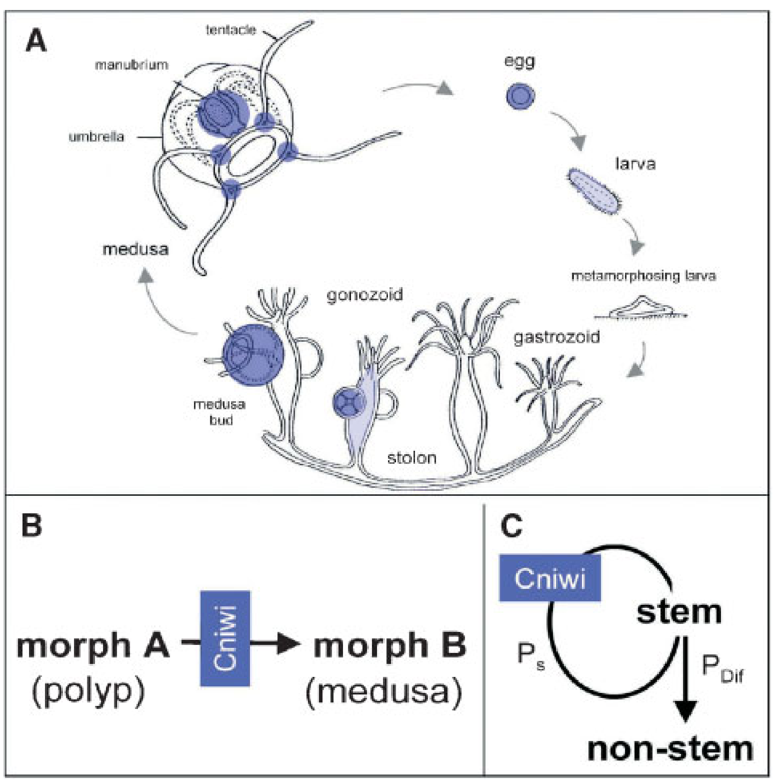

Figure 1. Proposed dual function of *Cniwi* in Cnidaria. **(A)** The life cycle of *Podocoryne carnea*. Stages found in the transcriptional analyses by Seipel et al[1] to express *Cniwi* are coloured in blue. Modified from Aerne et al.[21] **(B)** In Cnidaria with a complete life cycle, *Cniwi* may be involved in reprograming the genome and mediate the transition from polyp to medusa. **(C)** In Cnidaria with multipotent stem cells, *Cniwi* may function as essential stem cell gene controlling asymmetric cell divisions.

the manubrium and the tentacle bulbs. The latter two tissues contain highly proliferative cells, which make asymmetric divisions to undergo self-renewal or differentiation. *Cniwi* transcripts can also be found in the egg and at a reduced level in the developing embryo and larvae indicating that it is a maternal message. Interestingly, induction of transdifferentiation of striated muscle cells into proliferating smooth muscle also causes activation of *Cniwi* expression. These smooth muscle cells are characterized by their dual abilities to undergo self-renewal or to differentiate into non-proliferating cells such as nerve cells. The finding that a *Piwi* homolog in *Podocoryne* is exclusively expressed during medusa formation from gonozoid polyps, germline differentiation and transdifferentiation suggest that *Cniwi* plays a crucial role in these processes. In sum, *Podocoryne* cells expressing *Cniwi* are proliferating cells, which undergo asymmetric divisions with daughter cells dedicated to self-renewal or differentiation into multiple cell types including germ cells. In addition, *Cniwi*-expressing cells also take part in the transformation of a polyp into a medusa morph. Since all these differentiation events appear to require some reprogramming of genomic activity, it will be important to find out whether *Cniwi* is mediating this reprogramming and, therefore, is directly involved in the transformation of polyps into medusae.

Perspectives

This study provides an fascinating glimpse of the complexities of asymmetric cell divisions in a basal metazoan animal. As is often the case, however, it raises more questions than it answers. What is the biological function of *Cniwi*? With which genes is *Cniwi* interacting and how is it regulated? Do non-cell-autonomous factors such as the extracellular matrix influence *Cniwi* expression? Which other genes are involved in controlling asymmetric cell divisions in *Podocoryne*? To get at least some answers, the study could be expanded by taking advantage of the RNA interference (RNAi) technique, which allows individual genes to be knocked out in a simple and controlled fashion in various animals including *Hydra*.[20] Will *Podocoryne* polyps depleted of *Cniwi* transcripts develop medusae buds and complete their life cycle? Are *Cniwi*-depleted *Podocoryne* medusae capable of developing gametes? Moreover, whether *Cniwi* indeed is playing a role in reprogramming the genome when animals transform from one morphotype into another, *Cniwi*-related genes should be differentially activated in *Aurelia aurita*, a Cnidarian in which transformation of polyp into medusa takes place not by budding but by a segmentation process called strobilation.[16] Finally, since multipotent interstitial stem cells have not yet been identified in *Podocoryne*, do *Cniwi*-related genes play a role in stem cell decisions in Cnidarians such as *Hydra*, which has a reduced life cycle lacking a medusa stage but contains a well-characterized stem cell lineage?

Stem cells have been in the spotlight for years, but public interest so far has focused mainly on bone-marrow and embryonic stem cells from humans. The extraordinary plasticity of differentiation capacities of somatic cells in Cnidaria together with the advent of molecular approaches and genomic resources may turn attention now towards these presumptive simple organisms to elucidate the evolutionary history of stem cell control.

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