Finding the potency in planarians

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Planarian flatworms are well-known for their regenerative ability, which is dependent on a large population of adult stem cells, called neoblasts, at least some of which are pluripotent. Here, two recent studies are compared that have begun to address this fundamental question of whether all or only some neoblasts are pluripotent.

Over the past two decades, freshwater planarians have found a resurgence in the laboratory in North America due to their incredible ability to regenerate any body part. The regenerative ability of planarians is dependent on a large population of adult stem cells called neoblasts, which have made planarians an attractive model system for understanding fundamental stem cell biology. In principle, because planarians can regenerate any missing body part from virtually any amputation fragment along the anterior–posterior axis, it has been long-established that neoblasts are collectively totipotent—that is, neoblasts together can remake all missing tissues, including the germline. A key study in 2011 showed that a single neoblast could be totipotent when transplanted into an animal with no stem cells. Since then, there has been a flurry of research investigating how specific cellular lineages are regulated and what heterogeneity, if any, exists within the stem cell population. Recently, two studies have further elucidated the potency of neoblasts, which begins to answer whether totipotent stem cells are a unique subpopulation or whether most/all neoblasts retain high levels of potency and plasticity. Here, I will compare and contrast these studies.

Although the planarian field is relatively small, it has powerful tools and genomic resources at its disposal. Single-cell RNA-seq has opened up the field, and multiple, high-quality cellular atlases exist to directly address the molecular heterogeneity in the planarian stem cell population at the RNA level. Over the past decade, many studies have shown in situ molecular heterogeneity of neoblasts where the pan-neoblast marker piwi-1 can be co-expressed with differentiated cellular markers for multiple cell types. These data assume that a given piwi-1+ cell marker + neoblast is committed to differentiation into the cell marker+ cell type and has withdrawn from the cell cycle. Further functional data have shown that key transcription factors are co-expressed in subsets of piwi-1+ + neoblasts, and when removed by RNAi, the given cell lineage is lost. This has been demonstrated for the factors: zfp-1, which is required for differentiation into the epithelial lineage; myoD for the muscle lineage; and hnf4 for an endodermal lineage, for a few examples. From these studies, the working model is a classical top-down cellular hierarchy, which has a pluripotent, clonogenic neoblast (cNeoblast) at the top and progressive restriction through other lineages (Fig. 1). What is unknown from these studies is whether a piwi-1+ cell marker + neoblast is truly committed (irreversibly) to a given lineage or whether it can retain plasticity for multiple lineages (or even pluripotency)? Alternatively, if a totipotent neoblast sits at the apex of the hierarchy and has a distinct transcriptional cell state, then it may be detectable by single-cell sequencing. The two studies here take different approaches to investigate the molecular signature, if any, of pluripotent neoblasts and whether their potency becomes restricted.
Recent findings
In the first study by Zeng et al., the authors specifically isolated and sequenced ~7000 neoblasts with the goal of detecting a gene signature of a pluripotent neoblast, the potency of which was then functionally validated by gold-standard single-cell transplantations. Based on sequencing, the authors detected 12 distinct neoblast subtypes in silico based on the similarity of gene expression. Importantly, the authors detected all previously identified neoblast subtypes. The authors then focused on a large subclass of neoblasts that they could not classify and found that this subtype, called Nb2, expressed high levels of a cell surface protein homolog of tetraspanin (tspan-1). Importantly, the authors made an antibody to TSPAN-1 and could, for the first time, prospectively isolate a TSPAN-1+ neoblast subpopulation by flow cytometry. To test the potency of TSPAN-1+ neoblasts, single cells were transplanted into host amoebozoa of stem cells to test multilineage potential. The authors conclusively demonstrated that some of the TSPAN-1+ stem cells could restore the stem cell compartment, and thus, were functionally pluripotent.

While the Zeng et al., study found a method and gene signature to enrich pluripotent stem cells, there are some other interesting observations. First, while TSPAN-1- cells could not rescue the stem cell compartment, the TSPAN-1+piwi-1+ stem cells could only rescue the stem cell compartment of ~25% of animals following transplant. In the 2011 study, the rescue efficiency of single-cell transplants isolated only by morphology and without a cell surface marker was ~5%, so this study was a marked improvement in enriching for pluripotent neoblasts (or simply enriching for piwi-1+ cells). However, it remains unknown whether the 25% rescue in the Zeng study reflects the difficulty of the method (i.e., accidental killing of the transplanted stem cell), or whether this reflects true biological differences in potency. If it accurately reflects biology and only 25% of TSPAN-1+ stem cells are pluripotent, then there is much more room to home in on the exact pluripotent stem cell population.

The second study, performed by Raz et al., stratified the >12,000 piwi-1+ cells previously sequenced into the cell cycle stage based on gene expression. Further, they sequenced several thousand new piwi-1+ cells taken from the 2C flow cytometry gate (representing G1/G0 stem cells) and the 4C gate (representing G2/M stem cells). The authors then examined the expression of known fate-specifying transcription factors (FSTFs) and observed an increase in FSTF expression as stem cells proceeded through the cell cycle. The authors showed that the 2 cells produced by a division often have an asymmetric expression of an FSTF in the two daughter cells: one that remains piwi-1+[hi] and FSTF+[low] and the other that is piwi-1+[hi] FSTF+[hi]. Through careful analyses, the authors show, surprisingly, that FSTF+ stem cells can give rise to FSTF− stem cells, implying that fate specification may either be reversible or simply adopted by a daughter cell at G2/M and that many or most piwi-1+[hi] stem cells are pluripotent (Fig. 2b). The Raz model is attractive because pluripotency can be accessed by most stem cells, and thus, these would be present in any given amputation fragment.

Interestingly, Raz et al. find that tspan-1+ (assayed using the additional co-expressed transcript tgs-1) stem cells largely express FSTFs toward neural fates and are not simply an FSTF− cell and the permanent expression is retained only in the differentiated daughter cell. For example, in Drosophila embryonic neuroblasts, FSTF expression is often seen in the mother neuroblast, but the expression is only retained in the daughter cell.

Outlook
Despite some key insights into planarian stem cell pluripotency and fate specification, it remains unknown when a reversible specification becomes an irreversible commitment. It also remains unknown what expression of an FSTF in a G1 stem cell means. There is precedent in the literature from other organisms that cell fate transcription factors can turn on in the mother stem cell and the permanent expression is retained only in the differentiated daughter cell. For example, in Drosophila embryonic neuroblasts, FSTF expression is often seen in the mother neuroblast, but the expression is only retained in the daughter cell.
during an asymmetric division\textsuperscript{15}. Similarly, in the vertebrate retina, neural progenitors cycle through different expression states of the cell type they are making, yet will retain potency while the daughter differentiates\textsuperscript{16}. So, while Raz et al. conclude that specified stem cells can change FSTF marker expression following a division, this is not unprecedented.

Although both of these studies bring us closer to understanding pluripotency and fate decisions in planarian stem cell lineages, both have limitations that can only be addressed with additional methods to either indelibly mark specific stem cell populations, and/or prospectively isolate them through more cell surface markers. For example, if a zfp-1+ stem cell could be isolated confidently by a specific cell surface marker, it could be transplanted and a cellular clone analyzed. If it were to give rise to the expected result of only making epithelial clones, this would give high confidence that zfp-1+ zeta neoblasts are indeed irreversibly committed to the epidermal lineage. Other subtypes of planarian stem cells in Fig. 1 could similarly be tested in this way. The converse result would also be highly informative if zfp-1+ stem cells could go back and produce cell types of any lineage and rescue animals devoid of stem cells, demonstrating the pluripotency of any stem cell subtype. Unfortunately, these tools do not yet exist and without them, it is unclear how much further single-cell genomics can take us towards answers.

Despite the recent studies, the biggest unknown in planarian stem cell biology remains trying to understand how a single stem cell can give rise to different cell types of multiple lineages. Is this process controlled extrinsically to the stem cell, or do all pluripotent stem cells cycle through making a specific order or ratio of differentiated cell types? To ask this another way, can the pluripotent stem cell sense what differentiated cell needs to be made in a particular location and respond accordingly (i.e., a stem cell near the gut will be biased to make gut), or will it always make a set order of fates (i.e., regardless of location, a stem cell will make a gut cell, then an epidermal cell, then a neuron)? There is much work to be done to answer these fundamental questions to resolve where potency lies in planarian stem cell lineages.

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**Competing interests**
The author declares no competing interests.

**Additional information**
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