Discuss the experimental evidence that neoblasts in planaria flatworms are pluripotent and how this property is relevant (or not) to their ability to regenerate missing tissues.

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While there are many nature tv programs focused on apex predators, I would argue that they should be making more on apex regenerators, not only is it where Hollywood is going with movies like Deadpool, it is more importantly where research is going, as it human tissue regeneration is becoming more of an achievable goal, as opposed to science fiction. Planaria I would argue are a candidate for being an apex regenerator, and their cells behind their ability to regenerate are called neoblasts. Gimmicks aside, the better we understand these neoblasts the closer we get to regenerating human tissues. This essay will describe planaria and discuss the experimental evidence that their neoblasts are what make them pluripotent and how this property is relevant to their ability to regenerate missing tissues.

Planaria are non-parasitic aquatic flatworms with bilateral symmetry and 3 germ layers. There are two main types used in research in labs all around the world these are; Dugesia Japonica, and Schmidtea mediterranea and all of them are clonally related as they can reproduce asexually, this coupled with the fact that they can also reproduce sexually making them useful tools. Other aspects that make them useful model organism is that they similarly have a brain, spinal cord (sort of), a pharynx, a gut and they express homologs of important vertebrate genes, they retain 13 Hox genes though they aren't clustered, and only 5/13 contribute to axial patterning. But most importantly they have the ability to regenerate and are considered immortal. They have been studied since the 1800s, one of the most epic observations was done by H. Randolph in 1897, that showed that she could cut a planaria into pieces as small as 1/279th of a worm and they would regrow. This regenerative capacity comes from the neoblast, these are stem cells that make up 20-30 percent of cells are located throughout the organism, apart from at the tip of the head (antiorior to the eyes) or in the pharynx, this suggests that they are very important for the organism. Neoblasts have been observed to regulate the organism's size as after eating, neoblasts become mitotic and cause growth, and in starvation neoblasts die back, reducing the size of the organism "de grow". This implication with the fact that neoblasts have been shown to have very little cytoplasm with the majority of the cell being nucleus, points to the fact that this cell has not much other functions like moving around or producing functional proteins etc, implicating their sole job could be to mitotically divide. Neoblasts were shown to be the only mitotic cells in planaria using Smedwi-1 and phospho-histone H3 (H3P), so if they are the only mitotic cell and an organism can regenerate all the tissues after being cut down to 279th of its original size this strongly suggests that neoblasts are pluripotent.

This was studied using radiation as in response to radiation, worms de-grow as they are unable to replace losing cells. At lethal doses (6000 rads) neoblasts cannot repopulate the cells, indicating that neoblasts have a homeostatic function to maintain worm size and function. Interestingly this is dose dependent so the more radiation the less neoblasts are able to replace dead and missing cells. At 1750 rads neoblasts begin to reappear mainly between the eyes and the pharynx. The cluster size increases while frequency of the clusters decreases indicating that these cells are dividing. And this mitosis recovers over time then plateaus after 2 weeks showing that it is under control and not uncontrolled cancer. Then in 2011 to prove neoblast pluripotency they inserted of single neoblasts (from an asexual colony) into fatally irradiated worms (sexual colony) to show that it can cause survival. Interestingly, using a single neoblast from asexual worms into lethally irradiated sexual worms can prompt not only regeneration but loss of sexual characteristics. Given that germ line neoblasts express nanos, perhaps asexual neoblasts cannot express this. Importantly only 20 percent of single neoblasts injected worms

had colonies form. In the best conditions, which included using neoblasts with protrusions, 75 percent of worms could be rescued with cluster formation. So, with unknown specific type of protruding neoblast a larger percentage survive compared to neoblasts without this protrusion (which have shown to express different markers through FACs indicating they are fundamentally different cells), showing that some cells neoblasts are better than others, and hence showed that neoblasts are pluripotent but not all of them are, suggesting that these protruding neoblasts are the pluripotent ones and others are not.

The evidence that neoblasts control regeneration is shown that in "cut up" and fatally irradiated injected neoblasts can save the planaria. Also damage at the very tip where there are no neoblasts normally show that neoblasts can actually migrating across to (possibly being pushed there by other cells), colonise and regenerate the site of the injury. There, cells have been shown to divide to produce nerve cells or differentiate for tissue repair. Wound response has been characterised by 2 phases and neoblasts are central to this, where neoblasts migrate to the damaged region. Phase 1 is a generic response and mitosis of neoblasts throughout the planaria, and phase 2 is where they actually detect missing tissue, a signal that causes neoblasts to accumulate in the damaged area, and cells divide while some neoblasts fall out of their stem cell state to initiate blastema formation. So, some neoblasts act in different ways and neoblasts have been shown to come in three categories though no direct homology to transit amplifying cells has yet to be found. Category 1 neoblast are formed at the middle of the worm and have tentative pluripotency marker piwi/smedwi. Category 2 neoblasts are early division progeny in the middle of the worm and express nb21.113 and inx11 while category 3 are late progeny which are towards the periphery that express Agat. Unique populations of neoblasts may exist, like FoxA at phaynx and Ovo+ at eyes. This shows that not all neoblasts are created equal but depending on what structure they are regenerating will have different roles, whether that be just creating more cells or creating specific cells to that new repaired tissue.

In conclusion neoblasts in planaria are varied in their pluripotency as there are different subdivisions of neoblasts, some can be considered totally pluripotent some other categories are not. This pluripotency is essential when considering the scale, it can regenerate from (1/279) but the full extent of pluripotency might not always be necessary when dealing with minor damage to the periphery in which case lessor neoblasts could repair the structures as it might not need as wide array of cell types. Through understanding these varied neoblasts we can use this knowledge to make breakthroughs in human regeneration, and just as we have become apex predators through weapon engineering, we can become apex regenerators through genetic engineering (ethics permitting).