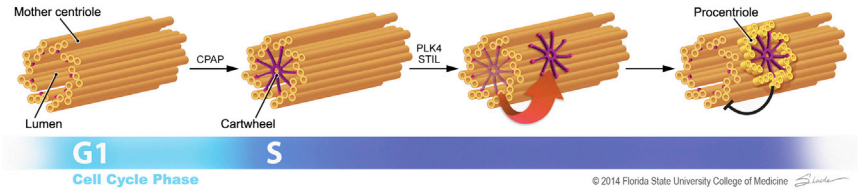

Figure 1. New Model for Centriole Duplication
In this model, the cartwheel (purple) is preassembled in the lumen of the mother centriole (gold) in a CPAP/SAS-4-dependent manner during S phase. This scaffold is then transferred to the outside surface of the mother through PLK4 and STIL/SAS-5 to initiate daughter centriole assembly. The nascent, engaged daughter centriole blocks additional cartwheel preassembly in the mother, preventing further duplication. Illustration by Jodi Slade.

on the outer wall. They also reveal that centriole engagement regulates the recruitment of SAS-6 to the centriole lumen. Thus, when a daughter procentriole assembles, further SAS-6 recruitment to the lumen of the mother centriole is blocked. This regulation ensures that cartwheels preassemble in disengaged centrioles in early S phase to promote efficient centriole duplication while also prohibiting any reduplication of engaged centrioles before mitosis.

The work by Fong et al. (2014) provides an attractive new model to explain how centrioles duplicate only once during each centriole replication cycle. By using the mother's lumen as a "womb" to incubate cartwheel assembly and then transplanting it to her outside surface, this can conceptually explain how only one daughter procentriole is templated by a mother centriole, although the mechanism by which the procentriole blocks the formation of a new precursor in the lumen of the mother remains unknown. It is notable that during de novo centriole assembly, when there is no mother to template cartwheel assembly, the number of centrioles assembled is highly variable (Loncarek and Khodjakov, 2009). The next challenge for centrosome scientists is to directly show that the cartwheel formed in the mother centriole lumen is the same one that later assembles the daughter centriole on the side of the mother centriole. Furthermore, while the essential role for PLK4 might be cartwheel release from the mother's lumen, as revealed by Fong et al. (2014), it is also required for de novo centriole assembly (Nigg and Raff, 2009), so PLK4 must have additional functions in this context. Nevertheless, the provocative new model that emerges from the work by Fong et al. can be tested further and may finally begin to explain the fidelity of centriole duplication.

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Sizing Up Lung Stem Cells

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Mammalian lungs are comprised of conducting airways and alveoli. How the distinct epithelial linings of these two zones are differentially specified and maintained is not fully understood. In this issue of *Developmental Cell*, two groups find critical roles for the Hippo pathway in regulation of lung progenitor cell differentiation.

Mammalian lungs can be divided into at least two functional zones: (1) the branched system of tubes (also known as conducting airways) that transport air and (2) the blind-ended sacs called alveoli, where gas exchange occurs. The epithelia that line these regions have distinct cellular compositions, allowing them to perform their specialized functions. In recent years, great strides have been made toward identifying the molecular signals that allow embryonic and adult progenitor cells to generate these epithelial linings (reviewed in Morrisey and Hogan, 2010). Despite these advances, important questions remain. For example, what signals dictate whether embryonic progenitors will give rise to

alveolar or airway lineages? Once generated, how are pools of stem cells maintained so that they can repair cell loss due to wear and tear or injury? In this issue of *Developmental Cell*, two groups (Mahoney et al., 2014; Zhao et al., 2014) report critical roles for the Hippo pathway in the regulation of embryonic and adult lung progenitor cell differentiation.

Lungs develop through a program called branching morphogenesis. Multipotent cells in the distal tips, characterized by the expression of the transcription factors Id2 and Sox9, self-renew and give rise to the full complement of epithelial cells (Rawlins et al., 2009a). Genetic experiments in mice have identified several signals, including Notch and glucocorticoid signaling, that balance branching with differentiation along airway or alveolar lineages (Alanis et al., 2014; Guseh et al., 2009). Mahoney et al. (2014) now demonstrate a role for the evolutionarily conserved Hippo pathway in specifying the border between Sox2+ progenitors that will generate airways and those expressing Sox9, which generate alveoli. The authors first show that the downstream transcription factor Yap is localized to the nucleus in alveolar progenitor cells, whereas in airway epithelium progenitors, Yap is phosphorylated and localized in the cytoplasm. Using a conditional genetic loss-of-function approach in mice, the authors demonstrate that when Yap is deleted from the respiratory epithelium, distal sacs lined with an abnormal cuboid epithelium expand at the expense of airways lined with columnar epithelium. These data support a model in which Yap-deficient cells are unable to respond to signals from the mesenchyme to induce expression of Sox2.

Because Sox2 is known to regulate the differentiation of airway epithelial cells (Que et al., 2009), Mahoney et al. (2014) go on to characterize epithelial differentiation in Yap mutant airways. Yap conditional mutants had decreased numbers of ciliated and secretory cells in the cuboid epithelium, suggestive of impaired differentiation. Based on these data, the authors test the hypothesis that Yap also modulates the differentiation of adult airway stem cells. Indeed, deletion of Yap (or expression of phosphodeficient Yap that is not maintained in the cytoplasm) impaired the generation of ciliated and secretory cells in vitro, further supporting the hypothesis that both the abundance and localization of Yap are critical for its regulation of airway stem cells.

Zhao et al. (2014) further define the mechanisms by which Yap regulates airway stem cell behavior in the adult. Krt5+p63+ basal cells have been well established as stem cells of the pseudostratified airway epithelia in both mice and humans (reviewed in Rock et al., 2010). Zhao et al. (2014) show that deleting Yap specifically from adult basal cells using a Krt5-CreER allele caused a significant reduction in the number of K5+p63+ basal cells. Because there was no change in basal cell proliferation or apoptosis, the authors tested the hypothesis that basal cells are lost due to aberrant differentiation. Indeed, genetic lineage tracing analysis showed a dramatic increase in the generation of ciliated and secretory cells from Yap-deficient basal cells compared to controls. In contrast, overexpression of a constitutively active Yap mutant in adult basal cells promoted an expansion of this population and impaired their differentiation, resulting in stratification of the airway epithelium. Interestingly, Yap overexpression led to the generation of p63−K5+K8+ cells that are not seen with great frequency in control lungs but that have been reported in the context of airway repair (Rock et al., 2011). These cells may represent a transient intermediate between basal cells and their terminally differentiated daughters. Importantly, the effect of Yap overexpression on basal cells is not irreversible; if overexpression is stopped, the normal epithelial architecture, including an appropriate number of basal stem cells, is restored.

Multiple lines of evidence suggest that secretory club cells (previously known as Clara cells) of the airway are capable of dedifferentiating into basal cells following injury (Rawlins et al., 2009b; Tata et al., 2013). To test the hypothesis that Yap promotes dedifferentiation of secretory cells, Zhao et al. (2014) specifically overexpressed Yap in these cells in adult mice. Many of these Yap-expressing cells lost expression of secretory markers, and some of them began to express p63, suggesting that Yap is sufficient to induce the dedifferentiation of secretory cells in vivo. Conversely, knockdown of Yap in secretory cells impaired their dedifferentiation in vitro. Together, these data suggest that Yap, in conjunction with other signals, regulates the pool of adult airway stem cells.

As a first step toward identifying some of these additional signals, Zhao et al. demonstrated that Yap physically interacts with the basal cell transcription factor p63 and that knockdown of Yap decreased the transcription of p63 target genes. Similar to deletion of Yap, deleting the ΔNp63 splice variant of p63 led to a loss of basal cells, suggestive of a conserved mechanism. Finally, to corroborate the physical interaction of p63 and Yap, the authors deleted one copy of p63 from basal cells that were overexpressing Yap. This rescued the epithelial stratification and proliferation that was seen with Yap overexpression alone.

Like all good experiments, these studies raise additional questions about the specification and maintenance of embryonic and adult lung progenitor cells. For example, it is noteworthy that Mahoney et al. (2014) did not report that Yap deficiency promoted the differentiation of airway basal cells, as reported by Zhao et al. (2014). Perhaps the culture conditions employed by Mahoney et al. (2014) lacked some factor or cellular component of the basal cell niche that promotes luminal differentiation. Further studies are required to determine how the Hippo pathway interacts with other pathways known to regulate the differentiation of epithelial lineages in the lung. Another interesting question is whether species-specific differences in Hippo pathway activation contribute to the differences in the numbers of airway generations across evolution; the current studies do not address the conservation of these mechanisms in human lungs, which contain about twice as many branches as mice, many of which are lined with a pseudostratified epithelium containing basal cells (reviewed in Rock et al., 2010). Finally, the stratification reported by Zhao et al. (2014) after overexpression of Yap is histologically similar to squamous metaplasia, a condition frequently observed in patients with chronic obstructive pulmonary disease and generally regarded as a precancerous lesion. Exploring whether alterations in the Hippo pathway contribute to this pathology in humans could pave the way for novel molecular therapies to prevent or reverse epithelial remodeling in lung disease.

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A Time and Place for Understanding Neural Stem Cell Specification

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The regulation of neural stem cells is key to their use for repair. Reporting in this issue of *Developmental Cell*, Dirian et al. (2014) identify an adult neural stem cell population surprisingly distinct in Notch independence, lack of radial glia hallmarks, and late contribution to neurogenesis in a strikingly region-specific manner.

Organs differ profoundly in their rates of cell addition and turnover, and the brain has been seen for the longest time as an organ with little to no turnover. However, some vertebrate brains, such as that of zebrafish, continue to grow in adulthood; even in mammalian brains, some regions generate thousands of new neurons daily (Grandel and Brand, 2013). Importantly, however, the continuation of neurogenesis is highly region specific and species specific, with different brain regions in different species continuing neurogenesis (Grandel and Brand, 2013). Thus, key questions in the field are related to the regulation of region-specific continuation of neurogenesis and the developmental mechanisms that determine whether cells continue to generate neurons throughout the organism's lifetime or instead stop at some point. To answer these crucial issues, it is essential to understand the origin of long-term neural stem cells (NSCs) and identify the mechanisms regulating their behavior from development into adulthood—an important task that Dirian et al. (2014) has solved in this issue of *Developmental Cell*.

Dirian and colleagues (2014) addressed these key issues in the zebrafish forebrain, using the dorsal division (pallium) as a model system. In the zebrafish, as well as in different mammalian species, NSCs typically divide fast in development but are largely quiescent in the adult (Doetsch et al., 1999; Adolf et al., 2006). It is therefore a key question whether some of these fast proliferating and actively neurogenic cells in the developing brain indeed have the capacity to contribute to the more laid-back adult NSCs (aNSCs). Dirian and colleagues (2014) answered this by genetic fate mapping the fast dividing and active neurogenic cell population using her4 (a Notch target transcription factor)-driven inducible forms of the Cre recombinase at different developmental stages. They observe that even when they label these cells at very early stages (2 days postfertilization [dpf]), the progeny of these cells continue as NSCs in neurogenesis 3 months later. The surprise came when Dirian and colleagues (2014) found this to be the case only in one region (the dorsomedial portion) of the pallium, whereas the lateral portion of the pallium clearly originated from an unlabeled pool of NSCs. So where do the NSCs of the lateral pallium, which also continues to undergo neurogenesis into adulthood, originate?

Given the strict region-specific organization of the brain early during development and the evidence from mouse showing that aNSCs maintain their regional identity at least from early postnatal stages (Merkle et al., 2007), the authors reasoned that the cells in the lateral pallium could come from a particular domain lateral to the roof of the neural plate, a region equivalent in the embryo to where these NSC clones were found in the adult. In order to shed light on this, they elegantly used focal uncaging of caged cyclofen by laser light at 1.5 dpf at various positions and directly demonstrated that cells close to the roof plate give rise to cells in the lateral pallium, including radial glial-like aNSCs. Strikingly,