that become methylated under the influence of methyl-H3K9 are in the same genomic location as those harboring the methylated histone. The effect on DNA modification might be remote, rather than localized to the methylated nucleosome. Extending the relationship between H3K9 methylation and DNA methylation to nonfungal eukaryotes is also premature. DNA methylation is not a fact of life for many eukaryotes, including other fungi (yeasts) and certain animals (the nematode worm *Caenorhabditis elegans*), so rigorous conservation of its function cannot be assumed. Cautionary notes aside, however, there is little doubt that the search for SET domain proteins that influence mammalian DNA methylation will now proceed at a frantic pace. Thanks to the awesome power of *Neurospora* genetics, there has never been a better time to probe the mysterious origins of DNA methylation.

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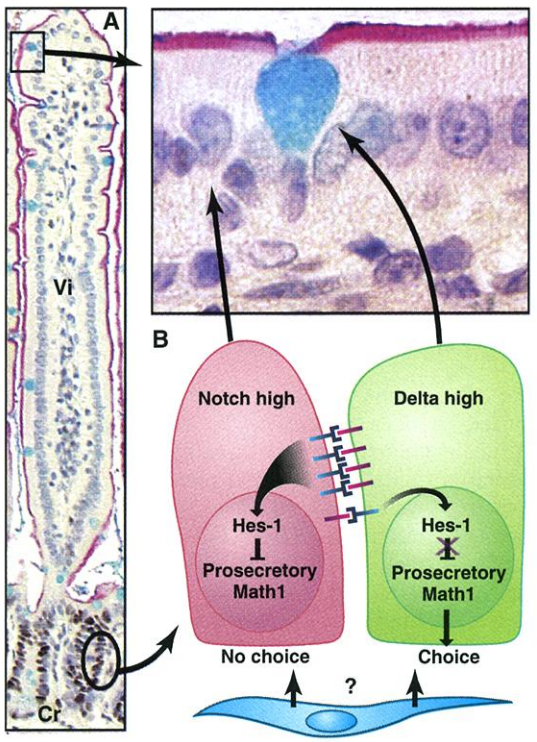
PERSPECTIVES: DEVELOPMENT

Epithelial Cell Differentiation—a Mather of Choice

Gijs R. van den Brink, Pascal de Santa Barbara, Drucilla J. Roberts

The gastrointestinal tract endoderm differentiates into specialized epithelial cells that perform digestive, absorptive, protective, and endocrine tasks. These cells have a relatively short life-span and must be continuously replaced from a pool of progenitor cells. It is still unclear why a progenitor cell makes a "choice" to leave the progenitor pool and to adopt a specific cell fate. An initial decision in epithelial lineage determination involves the Notch signaling pathway, yet subsequent choices and the downstream targets of this pathway have yet to be completely identified. On page 2155 of this issue, Yang *et al.* (*1*) provide evidence that Math1, a basic helix-loop-helix transcription factor, is a "pro-choice" determinant of epithelial cell commitment, and is a downstream target of the Notch pathway.

The intestinal epithelium is thrown into fingerlike folds called villi, which are separated from each other by troughs called crypts (see the figure). The differentiated epithelial cells at the tips of the villi are replaced every few days by progenitor stem cells that dwell in the crypts and move up the villi as they differentiate (*2*). In the crypts, the progenitor stem cells succumb to lineage determination and eventually give rise to four types of differentiated gut epithelial cells—enterocytes, Paneth cells, goblet cells, and enteroendocrine cells. These initial differentiation events are affected by the position of the epithelial cell along the crypt-to-villus axis and by its interactions with neighboring cells (*3*). It is not clear how progenitor stem cells in the crypt become lineage restricted. But, just before they leave the crypt, these lineage-restricted undifferentiated cells undergo a switch, withdraw from the cell cycle, and begin to express specialized proteins. The differentiated cells then migrate up the villi and, after reaching the top, undergo apoptosis, thereby maintaining homeostasis of the intestinal epithelium. Coordination between proliferation, differentiation, and apoptosis requires the well-timed interplay of different signaling pathways.



A gut instinct about cell fate. (**A**) Low-power 4-μm section of adult murine small intestine. Precursor cells (brown) are stained for cyclin PCNA (proliferating cell nuclear antigen); enterocytes (red) express intestinal alkaline phosphatase (IAP); goblet cells (blue) secrete mucins. Inset shows high-power image of small intestine enterocytes and goblet cells. (**B**) Math1, a component of the Notch signaling pathway, influences intestinal epithelial cell fate decisions. In crypt progenitor stem cells that express high levels of Notch, the Hes1 transcription factor is switched on and the expression of Math1 and of other "prosecretory" genes is blocked. The result is that the precursor cells become enterocytes. In cells expressing low amounts of Notch, levels of Delta are high, production of Hes1 is blocked, and Math1 expression is induced. Production of the Math1 helix-loop-helix transcription factor allows precursor cells to make a choice: whether to become goblet cells, Paneth cells, or enteroendocrine cells (Vi, villus; Cr, crypt).

The Notch pathway specifies cell fate through feedback amplification of relative differences in cellular levels of Notch and its ligand Delta (*4*). This results in subsets of cells that produce large amounts of Notch. These cells induce expression of transcription factors, such as *Hes1* (*5*). *Hes1* is a transcriptional repressor, and therefore cells that express the *Hes1* gene remain precursor cells (*6*). Yang *et al.* further dissect this pathway by showing that *Math1* is a downstream target of *Hes1* and controls the initial choice of fate made by crypt progenitor stem cells (see the figure).

Math1 is expressed in developing and mature mouse intestinal epithelium. Yang and colleagues used reporter constructs to show that *Math1*-deficient mice have increased expression of the reporter gene in crypt cells. However, they lack goblet, Paneth, and enteroendocrine cells, and show no increase in the programmed cell death of cells at the tips of the villi. These findings suggest that Math1 is involved in epithelial cell fate decisions. The authors propose that *Math1* expression is needed for cells to make the first lineage-specifying choice, that is, to adopt one of the following three fates: Paneth,

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goblet, or enteroendocrine. Without *Math1*, cells remain in the progenitor stem cell pool and can only become enterocytes.

To fully understand epithelial cell lineage determination in the gut, more questions need to be answered. For example, *Math1* is not expressed in the stomach or pancreas, locations where Hes1-deficient mice show considerable enteroendocrine cell loss (*7*). This implies that there might be other factors similar to Math1 at these sites. The hedgehog family of morphogens are candidate Math1-like factors. The hedgehog signaling pathway is critical in gastric (*8*), pancreatic (*9*), and intestinal (*10*) epithelial cell differentiation. Although interactions between the Notch and hedgehog pathways have been described in other systems (*11*, *12*), there is much to be learned about how these pathways interact in gut epithelium.

Yang *et al.*'s findings do not solve the puzzle of how epithelial cell differentiation is influenced by the cell's position along the crypt-to-villus axis. Cell fate decisions modulated by the crypt-to-villus axis are likely to involve morphogens expressed in the mesoderm and endoderm. A morphogen gradient of Bmp4, for example, may extrinsically regulate the outcome of Notch signaling in progenitor stem cells. Bmp4, a secreted morphogen expressed by the mesoderm (*13*), may be important in development of the intestinal epithelium (*14*) and is known to affect Math1-regulated cell fate decisions in other systems (*15*, *16*). Elucidating different molecular regulators of epithelial cell differentiation along the crypt-to-villus axis will be the focus of future work.

With the Yang *et al.* results, we can now begin to understand the signals needed for intestinal epithelial cell fate decisions. It is interesting that one molecule, Math1, provides the signal for cells to pick one of three fate choices, leaving the enterocyte as the default state. Whether a different signal would be required for gut epithelial precursor cells to become enterocytes in Math1-deficient animals has yet to be investigated. Doubtless, many interesting discoveries about intestinal cell fate decisions will be revealed in the near future.

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PERSPECTIVES: STEM CELLS

PTEN—Coupling Tumor Suppression to Stem Cells?

Josef M. Penninger and James Woodgett

In medieval times people dreamed of finding the Holy Grail, which promised them eternal youth and renewal of life. Stem cells have arguably achieved similar mythological status in our world with their potential to self-renew and to regenerate heart muscle, neurons, and blood cells. However, to realize the full potential of both embryonic and adult stem cells, we need to fully understand their biology. Taking a big step in this direction, Groszer and co-workers (*1*) report on page 2186 of this issue that a tumor suppressor protein called PTEN controls the proliferation and possibly also the self-renewal of neuronal stem cells.

PTEN (phosphatase and tensin homolog deleted from chromosome 10)—also known as MMAC1 (mutated in multiple advanced cancers) or TEP1 (TGF-regulated and epithelial cell–enriched phosphatase)—was identified as a candidate tumor suppressor on chromosome 10q22-24 (*2–4*). Loss of heterozygosity (inactivation of the second *PTEN* allele) at this locus is observed in several spontaneous human malignancies including glioblastomas, endometrial tumors, and breast as well as prostate cancers. Germ line PTEN mutations have been detected in more than 80% of patients with Bannayan-Zonana syndrome, Cowden disease, and Lhermitte-Duclos disease (*5*). Although these autosomal dominant disorders have distinctive phenotypic features (visible characteristics), they also share common clinical features such as benign growths and a high incidence of systemic malignancies. Identical PTEN mutations have been found in these three diseases, indicating that the same PTEN mutation induces distinct clinical manifestations depending on the type of tissue and the involvement of additional gene loci.

Mice carrying germ line mutations of the *PTEN* gene show that PTEN is indeed a critical tumor suppressor protein. Mouse embryos homozygous for the *PTEN*-inactivating mutation die between day 6.5 and 9.5 of development. They show abnormal patterning of ectodermal and mesodermal germ layers and overgrowth of the cephalic and caudal regions, probably due to enhanced proliferation of the neuroepithelium (*6*, *7*). Mice heterozygous for the *PTEN* mutation are viable but display hyperplastic-dysplastic changes in the prostate, skin, and colon and a high incidence of spontaneous tumors of various histological origins (*6*, *8*). In general, tumors from heterozygous *PTEN*-mutant mice display loss of heterozygosity at the second allele, demonstrating that loss of PTEN contributes to tumor formation (*9*).

The PTEN tumor suppressor is a lipid phosphatase that dephosphorylates the D3 position of phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a product of phosphatidylinositol 3-kinase (PI3K) (*10*). Thus, PTEN lowers the amount of the PI3K product, PIP_3, within cells and antagonizes PI3K-mediated cellular signaling pathways (see the figure, top of the next page). There also may be additional PTEN substrates because PTEN can also dephosphorylate (remove phosphate groups from) both phosphotyrosine- and phosphoserine/threonine-containing substrates in vitro (*11*). However, negative regulation of PIP_3 is the critical determinant for control of tumor growth (*12*). PI3K and its product PIP_3 regulate many cellular processes including proliferation, transcriptional regulation, glucose metabolism, cell migration, and protein synthesis, and they also protect against apoptosis. Groszer *et al.* (*1*) now add another important cellular process to this list: the negative regulation of neural stem cell proliferation.

Given that inactivation of PTEN in all cells results in death of the embryo, Groszer and colleagues (*1*), in parallel

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