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ErbB-4 receptor is important for expression of genes controlling growth inhibition. Ni *et al*. also report that the carboxyl-terminal region of ErbB-4 has weak transcriptional activity when fused to the DNA binding domain of GAL4. Interestingly, Lin *et al*. (*8*) recently reported that the EGF receptor, a relative of ErbB-4, may also act directly in the nucleus and may affect gene expression. They found that a fraction of the EGF receptor moved to the nucleus after activation of the receptor by ligand binding. A strong transcriptional activity was observed when the carboxyl-terminal region of the EGF receptor was fused to the DNA binding domain of Gal4. Furthermore, the EGF receptor complex bound to an AT-rich DNA sequence motif. The promoter for the gene encoding cyclin D1 contains two copies of this AT-rich motif, which are required for activation of this gene by EGF. Together, the results of Ni *et al*. (*1*) and Lin *et al*. (*8*) suggest that cytoplasmic fragments of members of the EGF receptor family are important regulators of gene expression.

A crucial question is how receptor cleavage is itself regulated. It appears that, for the cases studied so far, the triggering event is the removal of most of the extracytoplasmic part of the protein. This appears to be a prerequisite for intramembrane cleavage by γ-secretase or related proteases. In the case of ErbB-4, the metalloprotease TACE that removes its ectodomain is activated by protein kinase C (PKC). The physiological activator of PKC is phospholipase, but experimentally, as used by Ni *et al*. (*1*), the phorbol ester TPA also does the job. Interestingly, phospholipase C–γ is one of the SH2 domain proteins that are activated by several tyrosine kinase receptors. Thus, intramembrane proteolysis of ErbB-4 may be induced by its activation of SH2 domain proteins (see the figure).

It is still unclear how the liberated intracellular fragments of the receptors are translocated to the nucleus. Because only a small portion of ErbB-4 undergoes RIP, it is likely that nuclear transport does not occur by random diffusion but rather is carefully regulated. Ni *et al*. report that ErbB-4 has putative nuclear localization and nuclear export sequences. At the very least, the nuclear export sequence is involved in nuclear shuttling of the receptor fragment, because accumulation of the ErbB-4 receptor fragment in the nucleus is enhanced by leptomycin B, which blocks nuclear export of proteins (*1*). Lin *et al*. (*8*) also identified a putative nuclear localization sequence in the juxtamembrane portion of the EGF receptor. This sequence is conserved in all members of the EGF receptor family.

In both ErbB-4 and the EGF receptor, it is the extreme carboxyl-terminal region of the receptor that is the strongest activator of transcription. However, the entire cytoplasmic domain of both receptors appears to be translocated to the nucleus. It is possible that additional specific proteolytic events take place in the nucleus such that the juxtamembrane domain (containing the nuclear localization signal) and the kinase domain are cleaved off, liberating the transcriptionally active carboxyl-terminal tail. Another intriguing possibility is that the cytoplasmic receptor domains in the nucleus also regulate transcription through phosphorylation events.

The discovery that RIP is another way in which the cell conveys signals from the plasma membrane (and endoplasmic reticulum membrane) to the nucleus provides a new example of the multifaceted nature of intracellular signal transduction. The Ni *et al*. work shows that tyrosine kinase receptors are also subject to RIP. One avenue for future investigation will be to elucidate how important RIP is compared with other pathways in ErbB-4 signal transduction, and to see whether RIP is required for signaling through other tyrosine kinase receptors. Different members of this receptor family exhibit substantial overlap in their abilities to activate various SH2-domain signaling molecules. RIP offers one way to induce specific signals from different receptors (see the figure). Finally, it will be imperative to identify the nuclear partners of RIP-derived receptor fragments and their target genes.

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PERSPECTIVES: MOLECULAR BIOLOGY

Methylation Talk Between Histones and DNA

Adrian Bird

Biological phenomena are complex, but biologists, being human, crave simplicity. Hence the frisson of excitement, mixed with relief, with the union of two hitherto separate domains of study—in this case, the methylation of DNA and the methylation of histone proteins. DNA methylation is a mark on genomic DNA made by addition of methyl groups to cytosine bases, whereas histone methylation marks proteins that coat the DNA by addition of methyl groups to certain lysine residues. In their recent *Nature* paper, Tamaru and Selker (*1*) report that DNA methylation and histone methylation share a common pathway in the filamentous fungus *Neurospora crassa*. Their discovery sets the stage for an experimental attack on one of the abiding mysteries of the genome: How do patterns of DNA methylation originate?

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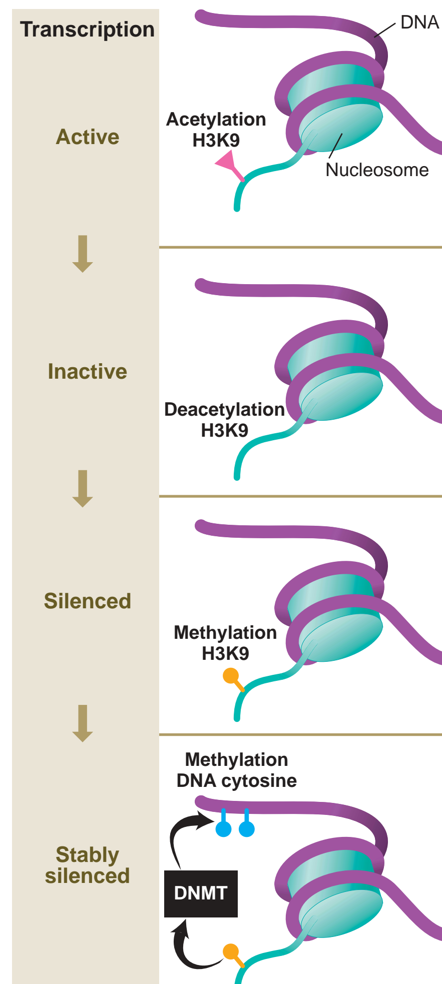
The problems inherent in managing large eukaryotic genomes can be eased by marking regions of DNA. Such marked DNA becomes structurally adapted so that it can perform certain activities. The most direct mark is one applied to the DNA itself, but this addition presumably must avoid adverse effects on the genome's stability and coding properties. The only marking system that is widespread among eukaryotes is methylation of DNA cytosine rings at position 5 to give the modified base, 5-methylcytosine (m^5C). More elaborate marking can be achieved by coating the genome with immobile proteins that are specifically designed to carry covalent messages. Core histones—the proteins assembled into the beadlike nucleosomes around which the DNA is wrapped—can be thought of as information modules that acquire coded information based on addition or removal of chemical groups. Their amino-terminal tails protrude from the nucleosome beads and can be modified through the attachment (or removal) of acetyl, phosphate, or methyl groups (*2*).

The resulting "histone code" affects the accessibility of the packaged DNA to transcriptional activator proteins, and hence the ability to switch on gene expression. For example, the lysine residue K9 in histone H3—the ninth amino acid from the

amino-terminal end of H3—is acetylated in active chromatin, but methylated in regions of the genome that are silenced. When methylated, this lysine (called H3K9) attracts HP1, a protein associated with condensed (silenced) "heterochromatic" regions of the genome. Thus, methylation of H3K9 appears to be read by HP1 as a signal to condense that region of the DNA (*3–5*). Like H3K9 methylation, DNA methylation too is associated with transcriptional silencing and can lead in animals to the recruitment of m^5C binding proteins (*6*). The resemblances have led some to wonder if the two methyltransfer reactions might turn out to be connected. There would be a precedent of sorts, because mammalian proteins that bind to genomic m^5C sites recruit proteins that deacetylate histone tails, leading to transcriptional shutdown (*7*, *8*). In that case, though, the flow of "epigenetic" information is from DNA to histones. The Tamaru and Selker report is exciting because it suggests the reverse: that histone modification can influence the methylation of DNA.

In *Neurospora*, DNA methylation affects duplicated DNA sequences that were detected early in the organism's sexual cycle and subjected to active mutation (*9*, *10*). As a result of this process, known as repeat-induced point mutation (RIP), the wild-type *Neurospora* genome contains a small fraction of methylated DNA, the majority of the DNA remaining nonmethylated. The victims of RIP are predominantly mobile genetic elements that normally move from place to place within the genome, unless eliminated by a defense mechanism of this kind. Our story begins with a search for mutant forms of *Neurospora* that lack DNA methylation. Several *dim* (defective in methylation) genes that encode DNA methyltransferase enzymes have already been identified. Tamaru and Selker (*1*) now show that a mutation in a newly discovered *Neurospora* gene, *dim-5*, abolishes methylation of all tested DNA sequences. Through genetic mapping and DNA sequence analysis, these investigators show that this gene encodes a protein containing a so-called SET domain. Intriguingly, the SET domain of chromosomal proteins found in mammals, insects, and yeast can be an enzyme that transfers methyl groups to H3K9 (*11*). By providing the *Neurospora* mutant with the wild-type *dim-5* gene, the authors were able to correct the mutation and to restore methylation.

To look for a connection between *dim-5*, DNA methylation, and gene silencing, Tamaru and Selker exposed different strains of *Neurospora* to the toxic drug hygromycin. Strains engineered to carry a nonmethylated hygromycin-resistance gene (*hph*) grow in the presence of the drug, but strains with a methylated *hph* gene cannot grow because the gene is silenced. Interfering with *dim-5* gene expression in strains with a methylated *hph* gene caused demethylation and reactivation of *hph*, thereby allowing the fungal hyphae to grow in the presence of hygromycin. Methylation and silencing clearly were undermined in the absence of the *dim-5* gene, but still the question remained: Was methylation of histones in the region of *dim-5* responsible for this effect? Early results already favored the involvement of histone methylation, because the original *dim-5* mutation was located at a highly conserved residue of the catalytic SET domain. Following up on this lead, Tamaru and Selker expressed the DIM-5 protein and exposed it to core histones in the presence of labeled *S*-adenosyl methionine. Only histone H3 became methylated, although on which residue was not clear. It is important to know the particular residue, because methylation of H3K9 correlates with gene silencing, but methylation of a different lysine H3K4 in the same histone signifies transcriptionally active chromatin (*12*). To answer this question, the investigators created strains of *Neurospora* in which K9 in H3 was replaced with amino acids (arginine and leucine) that cannot be methylated. Although these strains retained their natural H3 genes, they showed marked reactivation and demethylation of the *hph* gene in the presence of mutant histone H3. Therefore, the presence of H3 lacking a K9 residue that could be methylated mimics the effect of losing the H3 methyltransferase DIM-5 through mutation. The obvious deduction is that methylation of cytosine in *Neurospora* DNA depends upon prior methylation of K9 in histone H3.



Methyl groups stick together. Modifications of the lysine residue K9 in histone protein H3. Depicted are the effects of deacetylation, histone methylation, and DNA methylation of H3K9 on gene transcription. The H3 amino-terminal tail protrudes from a nucleosome (green). H3K9 can be acetylated (pink) in transcribed chromatin, but is deacetylated under the influence of repressors of transcription. This permits methylation (orange) of H3K9 by a SET domain protein. Methylated H3K9 may directly or indirectly affect enzymes called DNA methyltransferases (DNMT), leading to methylation of cytosine on nearby DNA (blue). DNA methylation prompted by methylation of H3K9 on a local nucleosome has not yet been demonstrated. Interaction of methylated H3K9 with chromodomain proteins such as HP1 and interactions of methylated cytosine residues with methylated DNA binding proteins are not shown.

Eukaryotic DNA methylation does not cover the genome like a featureless blanket, but conforms to a pattern of methylated and nonmethylated DNA sequence domains. An attractive feature of the new data is its potential to shed light on the origin of these DNA-methylation patterns, a subject about which we currently know very little. It is possible that DNA methyltransferases can only productively access DNA that is wrapped around nucleosomal histones carrying an H3K9 methylation signal (see the figure). This scenario is compatible with other known features of H3K9 behavior: its acetylation in transcriptionally active chromatin, its deacetylation under the influence of many gene-expression silencers (transcriptional repressors), and its methylation by SET domain proteins such as SU(VAR)3-9 and DIM-5. The notional "descent" from active to profoundly inactive chromatin culminates in DNA methylation. This would also agree with the prevailing evidence (at least in animals) that cytosine methylation is not a primary agent of gene silencing, but affects genes that have already been shut down in other ways.

Linear progressions of this kind (see the figure) are seductive, but should be viewed with suspicion. There is as yet no evidence in *Neurospora* that the sequences

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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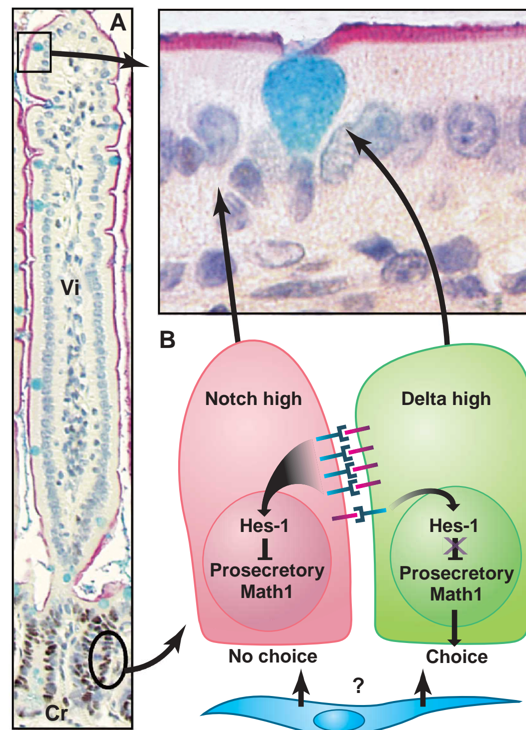
PERSPECTIVES: DEVELOPMENT

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 "prochoice" 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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