TRANSCRIPTION

Competing forces

Chromatin structure has long been suspected to have a role in regulating cell fate but there has been no convincing evidence for this. However, data published in *Molecular Cell* now support a model whereby transcription factors and epigenetic silencing are integrated quantitatively to control cell differentiation.

Hutchins *et al.* used helper-T-cell (T_H cell) differentiation as a model system, and analysed T_H cells from mice deficient in methyl-CpG-binding-domain protein 2 (Mbd2), one of the molecules that is thought to link DNA methylation and chromatin-based silencing.

In this study, cytokine signalling was manipulated to generate polarized T_{H1} and T_{H2} cells from uncommitted cells. The researchers found that, in Mbd2-deficient cells, the normal pattern of T-cell differentiation was dysregulated. For example, Mbd2-deficient cells produced ectopic interleukin (IL)-4 under T_{H1}-polarizing conditions, and the normally essential transcriptional activator Gata-3 was dispensable for IL-4 induction. Moreover, expression of the signature genes encoding IL-4 and interferon-γ was increased in T_{H2} and T_{H1} cells, respectively, suggesting that Mbd2 might be crucial in silencing these cytokine genes during T_H-cell differentiation.

To investigate the relationships between both Gata-3 and Mbd2, and the *IL-4* gene, the authors determined relative *Gata-3* messenger-RNA levels using the reverse-transcription polymerase chain reaction. *Gata-3* mRNA levels were repressed as normal in cells cultured under T_{H1} conditions regardless of the *Mbd2* genotype, indicating that it is not an excess of Gata-3 that induces *IL-4* in *Mbd2*-mutant T_{H1} cells. In line with this observation, the authors proposed a quantitative competition model for activators (such as Gata-3) and silencers (such as Mbd2) whereby each regulator has an independent quantitative effect.

To test this hypothesis, cells with three different *Mbd2* gene doses ($Mbd2^{+/+}$, $Mbd2^{+/-}$, $Mbd2^{-/-}$) were cultured under T_{H1} conditions (that is, where Gata-3 is not produced) and transduced with a bicistronic retrovirus encoding Gata-3 and green fluorescent protein (GFP). Cells with different levels of Gata-3 were evaluated based on the level of green fluorescence intensity. Increasing levels of Gata-3 rendered cells competent for IL-4 production in a manner that is inversely correlated with *Mbd2* gene dosage.

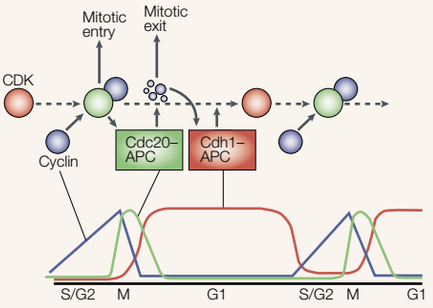
To investigate the basis for the competition between Gata-3 and Mbd2 further, the authors performed

CELL CYCLE

Cyclin' around

Cyclin destruction and the associated drop in cyclin-dependent kinase (CDK) activity are necessary for mitotic exit. In yeast, degradation of the S-phase cyclin Clb5 was thought to be essential, but a study reported in *Nature* now raises doubts about the importance of Clb5 in regulating mitotic exit.

In frog embryos, a single oscillator can manage the cell cycle. This negative-feedback oscillator alternates between S and M phases, and involves the regulation of a ubiquitin–protein ligase called the anaphase-promoting complex (APC). This is activated by a molecule called Cdc20, which then recruits cyclins. CDKs phosphorylate and thereby activate Cdc20–APC, which triggers cyclin destruction and mitotic exit. As a result, the CDK activity decreases, which causes APC inactivation and re-accumulation of cyclin.



The cell cycle in somatic cells and yeast, however, is more complex because it includes a G1 phase that is important for cell growth and differentiation. G1 regulation might be achieved by a second oscillator that differs from the first one in using the APC-regulatory subunit Cdh1 instead of Cdc20. CDKs inhibit Cdh1–APC, which means that, in late mitosis, CDK proteolysis causes activation of Cdh1–APC, which then maintains cyclin destruction throughout G1 (see diagram).

To address the question of whether the Clb5 cyclin must be destroyed, Wäsch and Cross constructed a yeast strain (*CLB5Δdb*) in which the *CLB5* gene lacked the destruction-box sequence that targets Clb5 to Cdc20–APC. However, these cells did not seem to have any defect in mitotic exit, implying that proteolysis of Clb5 is not required. Exit also occurred in *CLB5Δdb* cells that lacked Sic1, a CDK inhibitor responsible for further reducing CDK activity in late mitosis. This suggests that mitotic exit can progress without either Clb5 degradation or Sic1.

Next, Wäsch and Cross analysed the mitotic cyclin Clb2 as a potential Cdc20 target, and showed that *CLB2Δdb* yeast cells were unable to exit mitosis. Clb2 might therefore be the cyclin that needs to be destroyed for mitotic exit to occur.

Previous studies had shown that cells that lacked Cdh1 and Sic1 are not viable, suggesting that both proteins are essential for mitotic exit, and that the Cdh1–Sic1 oscillator might be the more important regulator. By contrast, Wäsch and Cross found that cells lacking these proteins do not have a marked defect in mitotic exit, and indeed survive, although poorly. However, the cells showed some abnormalities associated with an unstable G1 phase. This finding also highlights the important role of the Cdc20 oscillator in destroying Clb2.

So, the destruction of mitotic cyclins such as Clb2 is crucial to mitotic exit and the Cdc20 and Cdh1–Sic1 oscillators seem to work in a complementary manner, with the latter being important for a stable G1 phase.

Arianne Heinrichs

References and links

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chromatin immunoprecipitation experiments. They found that Gata-3 seems to antagonize the binding of Mbd2 before demethylation of the *IL-4* gene. This suggests that one of the key functions of a developmental transactivator might be to compete with silencing forces to reconfigure the activity of a target locus.

There are some indications that Gata-3 might have other, Mbd2-independent, roles in activating *IL-4* expression. So, future investigation might uncover further complexities in transcriptional programming in development.

Arianne Heinrichs

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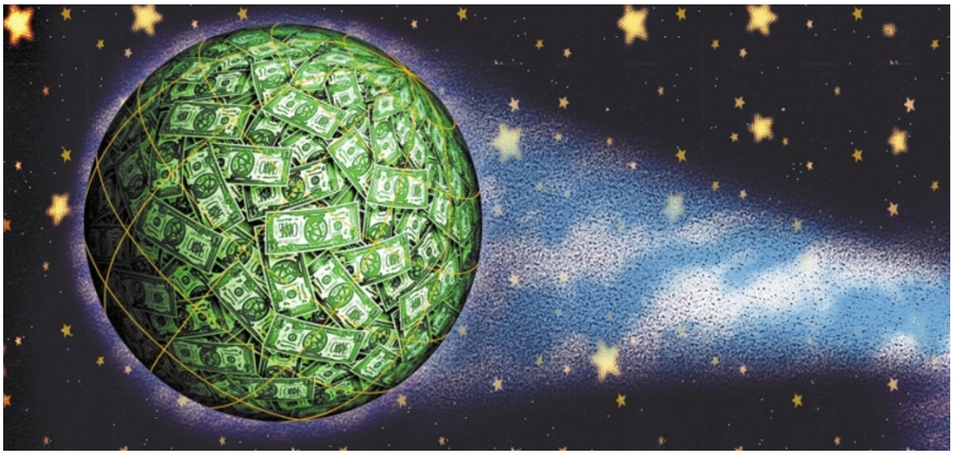
WEB SITE

Steven Reiner's laboratory: http://www.uphs.upenn.edu/abramson/reiner.html



PLANT DEVELOPMENT

Minimizing DET problems

In *Arabidopsis*, the DET1, COP and FUS proteins are thought to be global repressors of photomorphogenesis — changes in growth and development in response to light. Although *DET1* is known to encode a nuclear protein that regulates gene expression, its precise function has remained unclear. Now, however, reporting in *Science*, Joanne Chory and colleagues provide insights into the function of DET1 by isolating suppressors of the *det1* phenotype.

The authors called these suppressors *ted* mutants, and found that the *ted3* mutant is a dominant suppressor of the *det1* phenotype. They mapped and sequenced the *TED3* gene and used database searches to show that *TED3* is homologous to *PEX2* in yeast and mammals. PEX2 is involved in peroxisome assembly and matrix-enzyme import, and peroxisomes are single-membrane-bound organelles that perform many metabolic functions. Using a TED3–green fluorescent protein fusion construct, the authors confirmed the peroxisomal localization of TED3.

Chory and co-workers showed that the phenotype of the homozygous *TED3* knockout is embryonic lethal, and also that transgenic plants containing the antisense *TED3* transcript are dwarfed, pale and sterile. Because they found that *TED3* is ubiquitously expressed throughout development and showed that it is expressed at high levels in seeds, pollen, ovules and cotyledons, the authors propose that it is essential for *Arabidopsis* reproduction and development.

Because ted3 is localized to peroxisomes, the authors investigated whether peroxisomal activities are disrupted in *det1* plants and restored in *det1 ted3* plants. Germination on a sugar-free medium requires active peroxisomes, and they found that *det1* seeds could not fully develop on such a medium unless *ted3* came to the rescue. In addition, glyoxysomes (specialized peroxisomes) can convert indole-3-butyric acid (IBA) to indole-3-acetic acid, an auxin that inhibits root elongation, and they showed that IBA affected root elongation to a lesser extent in *det1* plants than in wild-type or *det1 ted3* plants.

When the authors compared the levels of glyoxysomal enzymes in *det1* and *det1 ted3* plants, they found that the levels were much higher in the latter. They also compared wild-type and *ted3* seedlings, and found that, although RNA levels were similar, protein levels were higher in *ted3* plants. The data therefore indicate that *det1* seedlings have defective peroxisomes that can be rescued by the *ted3* gain-of-function mutation, and that *ted3* might act by stabilizing peroxisomal proteins.

Finally, Chory and colleagues used an *Arabidopsis* oligonucleotide array containing ~8,300 genes to compare gene-expression profiles in wild-type, *ted3* and *det1 ted3* plants. They showed that, in both light- and dark-grown seedlings, a large proportion of the genes that are misregulated in *det1* plants are restored, or partially restored, by *ted3*. In addition, they found that several peroxisome-related genes were underexpressed in *det1* plants.

The authors have therefore found that "increased peroxisomal function can suppress the numerous … defects caused by mutations in *DET1*". Because they also found that *ted3* partially suppresses the effects of *cop1*, they propose that peroxisomes, whose roles still remain largely unknown, have an important function in a photomorphogenetic pathway that is negatively regulated by DET1 and COP. This newly discovered link between peroxisomes and the response to light should open up new avenues in understanding how plants respond to environmental variation.

Rachel Smallridge

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