PERSPECTIVES: TRANSCRIPTION

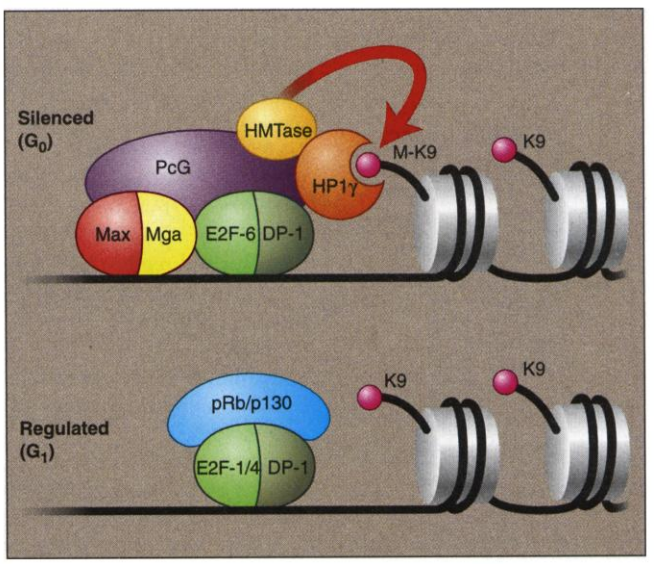
Chromatin Control—a Place for E2F and Myc to Meet

Nicholas B. La Thangue

Most of the genes switched on by the transcription factor E2F are involved in control of early events in the cell cycle (*1*). Some of the same genes are also regulated by the transcription factor Myc, and by other Myc family members (*1*, *2*). Together, these two transcription factors coordinate the expression of banks of target genes implicated in cell cycle control, DNA replication, and apoptosis. A provocative study by Ogawa *et al.* (*3*) on page 1132 of this issue provides new insights into the regulation of E2F- and Myc-responsive genes during the earliest phases (G_0 and G_1) of the cell cycle in mammalian cells. Although E2F and Myc are firmly established as key regulators of cellular proliferation, the new work points to an additional, perhaps crucial, role for E2F in maintaining cells in the quiescent G_0 phase of the cell cycle.

It is well established that E2F regulates early cell cycle transitions, from G_0 into G_1 and S phase (*4*). But what E2F does in quiescent cells is not clear. The transcriptional activity of E2F in G_1 cells is regulated by the retinoblastoma tumor suppressor protein pRb (see the figure). This protein, a member of the pocket protein family, is pivotal in the G_1- to S-phase transition. By binding to the transcriptional activation domains of E2F, pRb maintains E2F target genes in a transcriptionally inactive state. Cyclin-dependent kinases release active E2F by phosphorylating pRb, resulting in transcription of E2F-responsive genes and cell cycle progression. Thus, through E2F, the cell cycle machinery is connected to the transcription apparatus, allowing the timely expression of target genes required for cells to move into S phase (*5*).

The pRb tumor suppressor protein regulates gene expression by directing assembly of a protein complex that modifies the chromatin environment of target genes. Altering the chromatin environment depends in part on the enzymatic modification of the amino-terminal tail regions of chromatin's core histone proteins. This modification provides a "histone code" in which the pattern of posttranslational modifications dictates whether chromatin is in an open (accessible) or closed (inaccessible) state (*6*). Both histone deacetylase (HDAC) and histone methyltransferase (HMTase)—enzymes responsible for modifying histones—are present in pRb complexes. In these complexes, HDAC maintains the hypoacetylated state of critical lysine residues, which subsequently may become methylated by HMTase. In turn, methylated lysine residues bind to the HP1 family of repressor proteins that are involved in long-term transcriptional silencing (*7*, *8*).



The dark horse of the E2F family. Transcriptional silencing by E2F-6. In quiescent cells in G_0 of the cell cycle, E2F-6 nucleates the assembly of a chromatin-modifying complex. This complex contains HMTase, PcG proteins, and HP1γ, together with other sequence-specific transcription factors like Max and its partner Mga. By modifying the chromatin environment of target genes, this complex coordinates the long-term silencing of cell cycle–regulated genes. As the cell cycle ensues, the promoter binding sites occupied by E2F-6 become occupied by other E2F family proteins, such as E2F-1 and E2F-4, and by members of the pocket protein family, such as pRb and p130. This allows the regulated transcription of target genes that are involved in cell cycle progression. (K9, lysine 9; M-K9, methylated lysine 9).

Normally, E2F exists as a heterodimer bound to DP proteins (see the figure) (*4*). Similarly, Myc exists as a heterodimer in which activity is dictated by the nature of its partner: The Myc-Max dimer activates transcription, whereas the Mad-Max dimer blocks transcription (*3*). Like E2F, Myc-Max and Mad-Max are found in protein complexes with chromatin-modifying activity that alter the chromatin environment of target genes. Other members of the Myc family, such as Mga, bind to Max, blocking the oncogenic activity of Myc by preventing its association with Max (*9*).

There are six members of the E2F family. E2F-1 to E2F-5 bind to members of the pocket protein family in a manner that is dependent on the phase of the cell cycle, leading to the periodic induction of active E2F. The sixth member of the family, E2F-6, has an unusual organization: It has a truncated carboxyl-terminal region that cannot bind to pocket proteins, and also lacks transcription activation domains. Although it is clear that E2F-1 through E2F-5 are involved in cell cycle progression, differentiation, and apoptosis, it is not clear what E2F-6 does (*4*).

This situation has now changed with the work of Ogawa and colleagues (*3*), who reveal that E2F-6 promotes gene silencing during G_0 of the cell cycle. They show that E2F-6 recruits a multimeric chromatin-modifying complex (analogous to the pRb complex) to regions of chromatin in quiescent cells. The authors characterize the components of this E2F-6 complex and demonstrate that two histone methyltransferases—including a new enzyme called Eu-HMTase1 (euchromatic histone methyltransferase 1)—modify amino acid lysine 9 in the tail region of histone H3. The presence of these HMTases together with the transcriptional repressor protein HP1γ is highly significant because HP1γ binds to methylated lysine 9 to facilitate long-term transcriptional silencing (see the figure). Of the three human HP1 proteins, HP1β and HP1α influence silencing of heterochromatin. In contrast, HP1γ has been implicated in the silencing of euchromatin where most genes encoding proteins are found, including many of the genes under E2F and Myc control (*10*). Further evidence that E2F-6 is involved in transcriptional silencing comes from the discovery

The author is in the Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, Davidson Building, University of Glasgow, Glasgow G12 8QQ, UK. E-mail: n.lathangue@bio.gla.ac.uk

of several Polycomb group (PcG)–related proteins in the E2F-6 complex. This group of proteins interacts with E2F-6 and has been implicated in the maintenance of inactive chromatin (*11*).

Perhaps one of the most tantalizing observations has been the identification of Max together with its protein partner Mga in the E2F-6 complex (*9*). Consistent with the presence of the Max-Mga heterodimer is the finding that the E2F-6 complex can bind not only to E2F sites but also to Myc sites in the DNA of target gene promoters. Moreover, Mga has a separate DNA binding domain, the T-box, which also dictates binding of the E2F-6 complex to unrelated T-box DNA binding sites. The E2F-6 chromatin-modifying complex therefore contains several distinct DNA binding activities that may allow its recruitment to the promoters of many different genes.

We are left with the overriding view that E2F-6 directs transcriptional silencing by modifying chromatin. It is likely that E2F-6 silences gene expression in quiescent cells in vivo because E2F-6, Max, and HP1γ preferentially occupy sites in the promoters of cell cycle–regulated genes during G_0. E2F-6 is replaced by other E2F family members including E2F-1 and E2F-4 as the cells move into G_1 (see the figure). Moreover, the presence of distinct DNA binding activities in the E2F-6 complex may allow for the coordinated regulation of diverse target genes through a common long-term gene-silencing mechanism that depends on chromatin modification.

The Ogawa *et al.* study represents a milestone in cell cycle research, with its revelation that E2F-6 is a key regulator of gene activity in quiescent cells. The new work has implications for tumor biology because cancer cells frequently harbor mutations in E2F and Myc. Intriguingly, as Ogawa and colleagues note, the E2F-6 expressed in HeLa cells and other tumor cells seems to be inactive. It is possible that abnormal E2F-6 is causally related to the reluctance of tumor cells to enter a quiescent state, and their continued drive toward proliferation. As the E2F-6 story continues to unfold with the characterization of other E2F-6 complexes and the identity of regulatory cues for E2F-6 control, we confidently anticipate a better understanding of the connection between chromatin modification, cell cycle progression, and tumorigenesis.

References

1. H. Ogawa, K.-i. Ishiguro, S. Gaubatz, D. M. Livingston, Y. Nakatani, *Science* **296**, 1132 (2002).
2. N. Dyson, *Genes Dev.* **12**, 2245 (1998).
3. R. N. Eisenman, *Genes Dev.* **15**, 2023. (2001).
4. J. M. Trimarchi, J. A. Lees, *Nature Rev. Mol. Cell. Biol.* **3**, 11 (2002).
5. R. A. Weinberg, *Cell* **81**, 323 (1995).
6. T. Jenuwein, C. D. Allis, *Science* **293**, 1074 (2001).
7. M. Lachner *et al.*, *Nature* **410** 116 (2001).
8. A. J. Bannister *et al.*, *Nature* **410** 120 (2001).
9. P. J. Hurlin *et al.*, *EMBO J.* **18**, 7019 (1999).
10. D. O. Jones, I. G. Cowell, P. B. Singh, *Bioessays* **22**, 124 (2002).
11. J. M. Trimarchi, B. Fairchild, J. Wen, J. A. Lees, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1519 (2001).

PERSPECTIVES: ECOLOGY

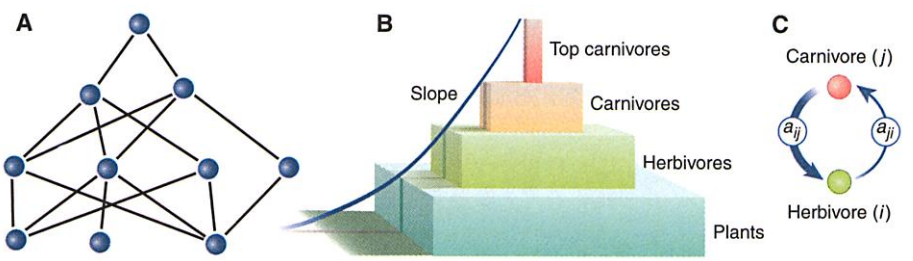
From Elton to Mathematics and Back Again

Dave Raffaelli

We live in a time when biodiversity is being lost at an alarming rate. There is growing pressure for community ecologists to deliver on basic questions about the consequences of biodiversity loss for ecological stability. But the true complexity of natural systems is overwhelming. Representing their dynamics and understanding the underlying processes is extraordinarily difficult; predicting their future states is even harder. Ecologists try to cope with this complexity by thinking in terms of food webs. Food webs can be represented as impressively complicated spider-web pictures (see the figure, A), but such diagrams conceal more than they reveal: Interesting patterns are obscured by the detail. However, by grouping species into feeding types (or trophic levels) and estimating the numbers or biomass at each level, early ecologists like Elton (*1*) and Odum (*2*) were able to show that pyramids are a consistent feature of natural systems (see the figure, B).

A quite different approach is to make abstract representations of food webs as mathematical models. Such models have revealed new and unsuspected behaviors in natural systems that would have been impossible to predict from simple observations (*3, 4*). Blending these different approaches has been a major challenge for food web ecologists, but Neutel *et al.* (*5*), reporting on page 1120 of this issue, demonstrate how this is possible. Their analysis brings us one step nearer to understanding how highly complex ecological systems retain their stability, contrary to theoretical expectations (*3, 4*).

Neutel *et al.* explore a concept called the trophic loop that was first described by Yodzis (*6*). Loops are closed chains representing interaction strengths between adjacent species in a food chain (see the figure, C). For each predator-prey link in a food web, two interaction strengths can be measured as simple coefficients: the effect of the predator on the prey, and the effect of the prey on the predator. Calculating these coefficients for all predator-prey links in a real food web involves highly detailed and painstaking estimates of population sizes, mortality rates, and energy conversion efficiencies (*7*). Un-



Staying connected. Food webs can be represented as species (blue dots) connected by trophic links (lines). Diagrams like these can be extremely complicated, and it is usually impossible to discern interesting patterns within such pictures. By grouping the species in a food web (**A**) into functionally similar types (trophic levels) and measuring the biomass of each of these levels, a pyramid of biomass can be constructed (**B**). The decrease in biomass between successively higher trophic levels is the slope of the pyramid, which may reflect system stability. Every trophic interaction between species in a food web can be described by two coefficients (**C**): The effects of the predator *j* on the prey *i* (a_{ij}) and the effects of the prey on the predator (a_{ji}). Usually, the former is larger than the latter. In the example shown of a two-species loop, the loop weight is the mean of the two coefficients. This can easily be extended to loops that include more than two species. Lower loop weights (that is, weaker species interactions), especially for longer loops, help to stabilize food webs.

The author is in the Environmental Department, University of York, York YO10 5DD, UK. E-mail: dr13@york.ac.uk