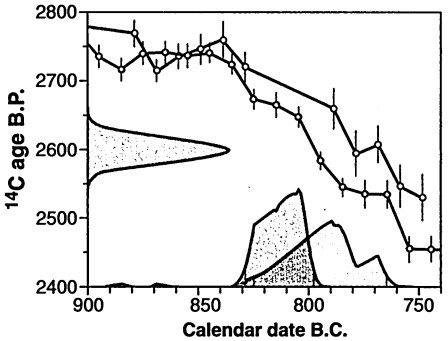

The importance of regional radiocarbon offsets. Calibration of a hypothetical radiocarbon age of 2650 ± 20 ^{14}C years before the present (B.P.) (black) with the Northern Hemisphere calibration data set (blue data points) and the Anatolian tree-ring sequence (red data points). The 2 σ calibrated age ranges (shaded areas) for the resulting probability distributions are 829 to 798 B.C. and 825 to 765 B.C., respectively. Calibration was done with CALIB 4.3 (*11*).

low-Middle Chronology (*5*). Manning *et al.* are also able to add support to the standard chronology of ancient Egypt from the dating of pieces of cargo wood from the Uluburun shipwreck, although this requires confirmation from timbers from the wreck itself. Furthermore, the 3 to 5 year growth anomaly found in the tree rings of the Anatolian sequence (~1650 B.C.), which may be a result of the volcanic eruption of Thera on Santorini, reinforces ice-core evidence for an older eruption date than previously indicated (*6*), with important implications for Aegean Bronze Age chronology.

On page 2529, Kromer *et al.* (*7*) further explore the magnitude and likely mechanisms of ^{14}C offsets. They report evidence that during a more recent interval of high ^{14}C production, radiocarbon ages for trees growing in Turkey are 17 years older than those of German trees with the same calendar age. The difference in the individual measurements is not statistically significant, but the trend in the data set is convincing. The authors conclude that the different growing season of the trees in the Mediterranean compared with those in Germany is responsible for the offset, which is seen only during a solar minimum when ^{14}C production is high.

To understand their reasoning, we must look at the mechanism of ^{14}C production. ^{14}C is primarily produced at high latitudes in the lower stratosphere by the collision of cosmic ray–produced neutrons with nitrogen. During periods of high solar activity, distortion of Earth's geomagnetic field by the solar wind prevents charged particles from entering the atmosphere and little ^{14}C is produced, whereas ^{14}C production peaks during periods of low solar activity (solar minima). The atomic ^{14}C is quickly oxidized to $^{14}CO_2$ and enters the troposphere during the late spring, a period of high stratospheric-tropospheric exchange. By the next spring, the higher ^{14}C concentration in the atmosphere has been well mixed and diluted by exchange with other carbon reservoirs, particularly the surface ocean. The German trees, which grow mostly in the mid to late summer, take up more $^{14}CO_2$ during photosynthesis than do the Mediterranean trees, which grow in the spring and early summer.

Kromer *et al.* suggest that the colder and wetter climate associated with the solar minima may further accentuate the different growth patterns. The regional offset between the Turkish and German trees occurs during the early stages of the cooling associated with "Little Ice Age" glacial advances in Europe (*8*), and the Anatolian offset occurs during a well-documented cold period in the Northern Hemisphere (*9*, *10*).

Regional radiocarbon offsets such as that between Germany and the Mediterranean will not have a noticeable effect on most radiocarbon calibrations, but they do make a difference to high-precision chronologies (see the second figure). It will be important to establish the maximum regional offsets for other regions and for other intervals of high ^{14}C production. Whether regional calibration data sets will become necessary or whether a correction can be made in the calibration process will depend on how well we can understand and predict these offsets.

The confirmation of regional ^{14}C offsets has important implications, not only for high-precision chronologies in archaeological, geophysical, and paleoclimatic studies, but also for our understanding of variations in the exchange between Earth's carbon reservoirs. These offsets will be a challenge for climate modelers to explain.

References and Notes

1. M. Stuiver *et al.*, *Radiocarbon* **40**, 1041 (1998).
2. P. E. Damon, *Radiocarbon* **37**, 829 (1995).
3. F. G. McCormac, M. G. L. Baillie, J. R. Pilcher, R. M. Kalin, *Radiocarbon* **37**, 395 (1995).
4. T. F. Braziunas, I. Y. Fung, M. Stuiver, *Global Biogeochem. Cycles* **9**, 565 (1995).
5. S. W. Manning, B. Kromer, P. I. Kuniholm, M. W. Newton, *Science* **294**, 2532 (2001); published online 6 December 2001 (10.1126/science.1066112).
6. S. W. Manning, *A Test of Time: The Volcano of Thera and the Chronology and History of the Aegean and East Mediterranean in the Mid Second Millennium BC* (Oxbow Books, Oxford, 1999).
7. B. Kromer *et al.*, *Science* **294**, 2529 (2001); published online 6 December 2001 (10.1126/science.1066114).
8. J. M. Grove, *Clim. Change* **48**, 53 (2001).
9. D. A. Meese *et al.*, *Science* **266**, 1680 (1994).
10. B. van Geel *et al.*, *Radiocarbon* **40**, 1163 (1998).
11. M. Stuiver, P. J. Reimer, *Radiocarbon* **35**, 215 (1993).
12. This work was performed in part under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory, under Contract No. W-7405-Eng-48.

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PERSPECTIVES: DEVELOPMENT

Staying a Boy Forever

Steven A. Wasserman and Stephen DiNardo

I won't grow up.
I don't wanna wear a tie,
Or a serious expression
In the middle of July!
–Peter Pan

Central to the homeostasis of many tissues and organs is a remarkable population of cells—the stem cells. These cells are endowed with two unique abilities: They can reproduce themselves (self-renew) and can produce pluripotent daughter cells that differentiate along different lineages to replenish tissues. Of paramount interest are the signals that generate this stem cell Neverland, where mortality and maturation are both kept at bay. In particular, signals emanating from the microenvironment, or niche, where stem cells reside are believed to be important for determining stem cell fate. Studies of stem cell–dependent tissues—such as blood, skin, and sperm—have provided substantial insights into stem cell biology. A landmark achievement has been the propagation of pluripotent bone marrow stem cells (that differentiate into all types of blood cells) under defined culture conditions. But there are plenty of obstacles to studying stem cells, as they make up a vanishingly small fraction of the cell population within tissues. Difficult to unambiguously purify, they are usually only identified retrospectively, through complex serial culture assays that test their capacity for self-renewal and differentiation. Furthermore, many observations made on relatively heterogeneous stem cell popula-

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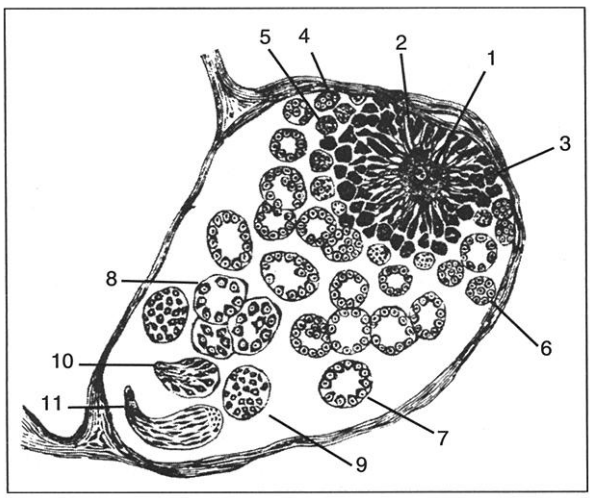
tions in culture are difficult to translate to an individual stem cell in its in vivo niche. Therefore, the advance reported by Kiger *et al.* (*1*) and Tulina and Matunis (*2*) on pages 2542 and 2546 of this issue, is an important one. These investigators define the molecular nature and spatial organization of a signaling pathway that governs stem cell fate in the fruit fly testis.

In flies, the germ line stem cells of the testis encircle a hub of nondividing somatic support cells, clustered at the apex of each testis tubule (*3–5*). Oriented division of these stem cells generates one daughter cell that remains a stem cell and another that becomes a blast cell, poised for the defined series of cell divisions (mitosis and meiosis) that culminate in its differentiation into a cohort of male germ cells (spermatids). Both groups demonstrate that maintenance of these germ line stem cells requires activation of the JAK-STAT signaling pathway. In flies, as in mammals, JAK-mediated phosphorylation of the transcription factor STAT in response to pathway activation drives translocation of STAT to the nucleus where it switches on transcription of target genes (*6–9*). When STAT activity is abrogated in germ line stem cells, these stem cells are directed away from a stem cell fate and toward a blast cell fate. In contrast to experimental manipulations of cultured cells, the beauty here is that signaling is disrupted in an individual stem cell in vivo, while that cell still occupies its niche.



Action at the hub. 1889 woodcut showing a portion of the testis of the silk moth, *Bombyx mori*, where the hub hypothesis was first presented. In the testis, germ line stem cells surround a cluster of support cells that constitute the hub (1). These stem cells divide, giving rise to some daughter cells that remain stem cells (self-renewal), and others that divide further and begin to differentiate as they move further away from the hub (2 to 11). [Reprinted with permission from (*22*)].

How is the JAK-STAT pathway activated in fly germ line stem cells? Both groups find that the putative ligand for this pathway, Unpaired (*10*), is specifically expressed by hub cells that inhabit the same niche as the stem cells. Although the authors were not able to inactivate Unpaired, they did show that misexpression of Unpaired was sufficient to cause expansion of the germ line stem cell population. The simplest interpretation is that a local signal from hub cells defines this stem cell niche and, as first postulated in 1911 (*11*), it is those stem cell daughters displaced from the hub that embark on the program of differentiation (spermatogenesis) (see the figure).

In insects and mammals alike, stem cell populations share many features, including a regulated decision between self-renewal and differentiation, stem cell perdurance with little aging, the opportunity for stem cell replacement, and the existence of niche cohabitation. That there exists a molecularly defined and tractable stem cell niche in the *Drosophila* testis holds considerable attraction for studying the properties of the stem cell microenvironment.

Maintenance of stem cell fate by the Unpaired ligand may require contact between stem cells and other cells in the niche or between stem cells and the extracellular matrix secreted by hub cells. Alternatively, the effective signaling range of the secreted Unpaired ligand may be relatively broad and may dissipate with distance from the hub. Both the mammalian and fly systems provide clues. In some circumstances, JAK-STAT activation is restricted locally, consistent with a contact-dependent mechanism of signaling. In the fly eye, however, JAK-STAT is activated at a distance from the source of Unpaired (*12*). Does this suggest that in the testis, many tiers of germ line cells experience JAK-STAT activation, but that other signals trump this stem cell signal as cells move further away from the hub? In fact, just such a competing signal—one that promotes the differentiation of germ line stem cells—appears to be delivered by another group of somatic support cells near the hub (*13*, *14*). What remains is to define that second signal and ask whether it directly influences responses to JAK-STAT signaling. Another possibility is that the influence of JAK-STAT activation is dampened intrinsically in the germ cells. For example, analysis of mammalian systems suggests that JAK-STAT activation is only transiently effective, owing to the rapid induction of antagonists of this pathway (*15*). Thus, perhaps intrinsic events in the germ cells restrict self-renewal to those cells nearest the hub, those further away differentiating instead. An attractive possibility is that the induced antagonists are segregated asymmetrically and only affect the blast cell daughters.

Although stem cells are long-lived, they are mortal. For example, stem cells in the fly's female germ line have a half-life of 4.5 weeks (*16*). In the male, there are more germ line stem cells during larval than adult stages, and evidence suggests an age-induced quiescence of the germ line (*3*, *14*). It will be important to determine if the level of Unpaired expression influences stem cell half-life in the fly testis.

Is there room for expansion of the stem cell niche? Upon loss of one or more stem cells, does repopulation of the niche occur? For example, if a stem cell vacates its position near a hub cell, will nearby blast cells move in and adopt a stem cell fate, as appears to happen with stem cells during oogenesis in the fly (*17*, *18*)? If repopulation does take place, does this result from alterations in the orientation of the division plane of the stem cell, as occurs during fly neural development (*19*, *20*)?

A niche was originally defined as the microenvironment that regulates the self-renewal and output of a stem cell population. An emerging complexity is the fact that more than one stem cell type may populate a niche. For example, bone marrow contains both hematopoietic and mesenchymal stem cells. Do distinct signals govern each stem cell type in the same niche? In the fly testis, a second set of stem cells also surrounds the hub (*21*). These somatic stem cells generate a pair of cells that encyst each germ line blast cell and its progeny. The new work reveals that the hub-derived Unpaired signal governs the self-renewal of both germ line and somatic stem cells, although only for the germ line cells could the authors address and demonstrate direct signaling from hub cells to the target cells. Further work should definitively establish whether one niche signal independently governs two stem cell types or, alternatively, whether functional cross talk between stem cells in a common niche coordinates their behavior.

References

1. A. A. Kiger *et al.*, *Science* **294**, 2542 (2001).
2. N. Tulina, E. Matunis, *Science* **294**, 2546 (2001).
3. R. W. Hardy, K. T. Tokuyasu, D. L. Lindsley, M. Garavito, *J. Ultrastruct. Res.* **69**, 180 (1979).
4. Reviewed in D. L. Lindsley, K. T. Tokuyasu, Eds., *Spermatogenesis* (Academic Press, London, ed. 2, 1980).

5. Reviewed in M. T. Fuller, in *The Development of* Drosophila melanogaster, M. Bate, A. Martinez-Arias, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), vol. 1, pp. 71–147.
6. J. E. Darnell Jr., *Science* **277**, 1630 (1997).
7. R. Yan, S. Small, C. Desplan, C. R. Dearolf, J. E. Darnell Jr., *Cell* **84**, 421 (1996).
8. R. Binari, N. Perrimon, *Genes Dev.* **8**, 300 (1994).
9. X. S. Hou, M. B. Melnick, N. Perrimon, *Cell* **84**, 411 (1996).
10. D. A. Harrison, P. E. McCoon, R. Binari, M. Gilman, N. Perrimon, *Genes Dev.* **12**, 3252 (1998).
11. K. Zick, *Z. Wiss. Zool.* **98**, 430 (1911).
12. M. P. Zeidler, N. Perrimon, D. I. Strutt, *Genes Dev.* **13**, 1342 (1999).
13. A. A. Kiger, H. White-Cooper, M. T. Fuller, *Nature* **407**, 750 (2000).
14. J. Tran, T. J. Brenner, S. DiNardo, *Nature* **407**, 754 (2000).
15. Reviewed in N. A. Nicola, C. J. Greenhalgh, *Exp. Hematol.* **28**, 1105 (2000).
16. T. Xie, A. C. Spradling, *Cell* **94**, 251 (1998).
17. _____, *Science* **290**, 328 (2000).
18. Y. Zhang, D. Kalderon, *Nature* **410**, 599 (2001).
19. J. A. Kaltschmidt, C. M. Davidson, N. H. Brown, A. H. Brand, *Nature Cell Biol.* **2**, 7 (2000).
20. Reviewed in C. Q. Doe, B. Bowerman, *Curr. Opin. Cell Biol.* **13**, 68 (2001).
21. P. Gönczy, S. DiNardo, *Development* **122**, 2437 (1996).
22. E. C. Roosen-Runge, *The Process of Spermatogenesis in Animals* (Cambridge Univ. Press, Cambridge, 1977), p. 78.

PERSPECTIVES: TRANSCRIPTION

Switching Partners in a Regulatory Tango

Kenichi Nishioka and Danny Reinberg

An intricate network of protein-modifying enzymes operates in the transmission of signals from the cell surface to the nucleus. Proteins are most commonly modified by the addition or removal of phosphate groups (phosphorylation/dephosphorylation) or acetyl groups (acetylation/deacetylation) (*1*). Recently, addition of methyl groups (methylation) to proteins has been discovered, although this process may not be reversible (*2, 3*). One of the targets of these three types of modification are the histone proteins that form the beads (nucleosomes) around which the DNA is wrapped. On page 2507 of this issue, Xu *et al.* (*4*) shed new light on how protein methylation regulates gene expression driven by the nuclear hormone receptor (NR) class of transcription factors.

Binding of a gene-specific transcription factor to a target DNA sequence (promoter) initiates recruitment of a plethora of coactivators that are necessary for the gene to be transcribed. Some coactivators interact with only one class of gene-specific transcription factor. For example, members of the p160 family of coactivators collaborate with NRs to initiate transcription. Other coactivators have looser specificities and interact with multiple types of transcription factors as a way of integrating several signal transduction pathways. Examples of such coactivators include the CREB (cyclic adenosine monophosphate response element-binding protein) binding protein (CBP) and its paralog p300, which possess histone acetyltransferase activity. CBP/p300 is recruited to many distinct target genes as a coactivator and initiates gene activation by acetylating histone proteins and associating with the enzyme responsible for transcription, RNA polymerase II (*5*).

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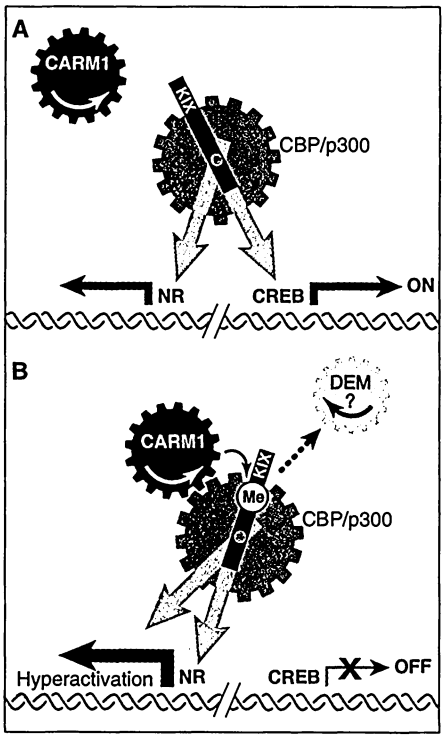
The fact that CBP/p300 has such a general effect raises the question of whether the cell contains enough CBP/p300 to translate signal transduction events into gene transcription at so many promoter sites. Several lines of evidence suggest that both CBP and p300 are present in small amounts and thus are limiting in most cells. For example, patients with Rubinstein-Taybi syndrome, who lack one CBP allele, suffer severe developmental defects (*6*). In another example, HIV gene expression is induced initially by binding of tumor necrosis factor–α (TNF-α) to its receptor on the cell surface. TNF-α stimulates the p65 subunit of the transcription factor NF-κB, which then translocates to the nucleus to activate transcription of the HIV genome. When the Stat2 protein is activated in concert with NF-κB, Stat2 competes with TNF-α–stimulated p65 for binding to CBP/p300, which results in the inhibition of HIV gene expression (*7*). Finally, an analysis of protein partner switching during nerve cell differentiation disclosed that neurogenin interacts with CBP and promotes neuronal differentiation. When the amount of neurogenin in the nerve cell drops, CBP is then free to interact with the Stat3 protein, resulting in the induction of glial cell differentiation (*8*). This suggests that CBP is at the center of a differentiation "switch" that dictates whether progenitor nerve cells will become neurons or glia. Taken together, the results of these three studies suggest that the cellular pool of CBP/p300 is so small that any competition for these proteins has a demonstrable effect on cellular phenotype. But how CBP/p300 switches its partners so smoothly is not clear.



The CARM1 molecular switch. (**A**) Usually CBP/p300 is not methylated and this coactivator complex is able to initiate transcription of both NR- and CREB-dependent genes (green arrows). (**B**) CARM1 methylates (dark blue circle) the KIX domain (red) of CBP/p300, such that CBP/p300 is no longer able to activate CREB-dependent genes. In this case, CBP/p300 is now available to direct NR-dependent gene activation exclusively. A hypothetical demethylase (DEM) might be able to reverse the consequences of CARM1 methylation.

Previous studies showed that the enzyme CARM1 (coactivator-associated arginine methyltransferase 1) binds to the carboxyl-terminal region of members of the p160 family of coactivators. Upon binding, the histone methyltransferase activity of CARM1 enhances NR-dependent gene transcription (*9*). Subsequent work established that CARM1 and CBP/p300 synergistically enhance NR-dependent gene expression (*10*). Now, Xu *et al.* illustrate the molecular basis of the observed synergy between these two transcriptional coactivators, and describe how CARM1 confers gene specificity upon CBP/p300 (*4*). First, the authors show that CARM1 binds directly to CBP/p300 (see the figure). This work suggests that CBP/p300 and CARM1 exist as a coactivator complex in which the histone acetyltransferase activity of CBP/p300 potentiates the his-