

tential similarities with that of spirochetes, is likely to differ from the known swimming mechanisms of other helical organisms.

Could a rotary mechanism such as that seen in spirochetes be at play during *Spiroplasma* movement? Initial evidence suggested that deformation of the cytoskeletal ribbon produces traveling kinks in the cell body of *Spiroplasma* that induce movement (Trachtenberg, 2004). In their new work, Shaevitz et al. (2005) show that kinking in *Spiroplasma* is actually composed of two temporally distinct types of cell deformation. The first deformation flips the handedness of the cell helix (a right-handed helix becomes left-handed) at one end of the cell body. The deformation grows in the direction of the opposite end of the cell. After an average of 0.26 s, the initiating end of the cell, in a second deformation, flips back to its original handedness and creates a packet of opposite handedness that travels the length of the cell to the distal end. Propagation of these double kinks produces motility in the direction of the cell body helix axis and also rotation of the cell body around the helix axis.

This type of movement behavior in *Spiroplasma* can best be explained by the presence of an internal contractile apparatus (Kurner et al., 2005; Trachtenberg, 2004; Wolgemuth et al., 2003). A mathematical model describes how periodic changes in helix pitch can produce propulsive force (Wolgemuth et al., 2003). This mathematical model predicts that deformations driven by contractions would lead to swimming velocities that increase with increasing fluid viscosity, if kink velocity is independent of the viscosity. Confirming this prediction, Shaevitz et al. (2005) show that the kink velocity is indeed independent of viscosity. It should be mentioned that no contractile apparatus has yet been definitively shown for any bacterial motility apparatus (although the bacterial tubulin-like protein, FtsZ, does form a contractile ring during cell division [Bi and Lutkenhaus, 1991]). Also, no genes encoding eukaryotic contractile proteins have been detected in the *Spiroplasma* genome.

Although *Spiroplasma* movement mediated by an internal contractile apparatus is the favored explanation, another possibility that cannot be ruled out is the presence of a rotating internal filament. A rotation model would require that the helical ribbons be polymorphic like bacterial flagella, which change handedness upon reversal of the flagellar motor. Torque placed on the ribbons could flip the handedness of the cell shape thereby causing kinks.

Many questions remain to be answered. For instance, observations of *Spiroplasma* do not suggest that this bacterium has polarity: one end of the cell appears no different than the other. Yet, the results presented by Shaevitz et al. (2005) suggest that the same end of the cell always initiates the kinks. This result may have bearing on the motility of many other bacterial species. For example, it remains unclear how spirochetes can simultaneously regulate their flagellar motors at both ends of the cell during chemotaxis. Polarity of the cell may provide an easy answer. However, the most intriguing questions relate to how the kinks are generated.

The answers to these questions will likely come from the establishment of a system that allows kinks to be studied in vitro. We excitedly await this development.

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Chipping away at the Embryonic Stem Cell Network

Critical transcription factors, notably OCT4, SOX2, and NANOG, are necessary to maintain self-renewal and pluripotency, two properties characteristic of embryonic stem (ES) cells. By analyzing the genome-wide localization of these factors at promoter regions in human ES cells, Boyer et al. (2005) demonstrate frequent promoter cooccupancy at numerous target genes. As they discuss in this issue of *Cell*, their findings indicate the presence of a complex network of autoregulatory and feedforward loops in human ES cells.

Embryonic stem (ES) cells, grown from the inner cell mass of early blastocysts, are remarkable cells. They display the two defining properties of true stem cells, that is, self-renewal and pluripotency. Propagated un-

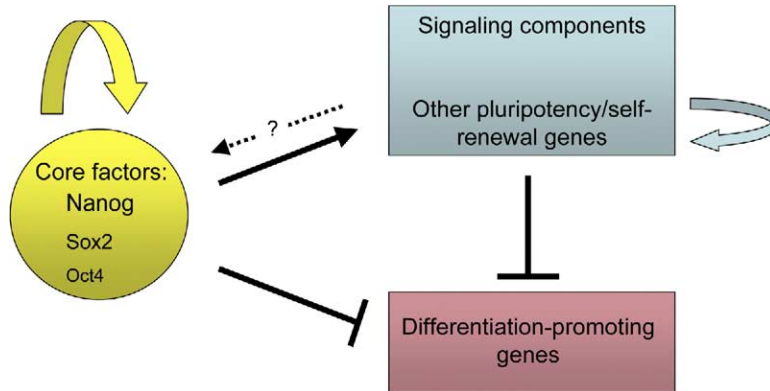


Figure 1. Regulatory Circuitry in ES Cells

The core transcription factors, NANOG, SOX2, and OCT4, activate target genes in ES cells that encode signaling components and other pluripotency/self-renewal factors. They also repress the expression of genes encoding components of pathways that promote ES cell differentiation. Pluripotency/self-renewal factors “downstream” of the core factors are also likely to contribute to repression of genes that induce ES cell differentiation, thereby reinforcing the off state. The core factors and downstream pluripotency/self-renewal genes are involved in auto- and crossregulatory loops. It is likely that downstream pluripotency/self-renewal factors contribute to transcriptional control of the core factors.

der appropriate conditions, ES cells continue to produce more stem cells (the process of self-renewal). Simultaneously, they retain the capacity to generate all types of differentiated progeny, either in vitro or in vivo (the phenomenon of pluripotency). As such, ES cells of human origin (hES cells) are envisioned as an excellent source for cellular approaches to the therapy of various diseases, which is the emerging area of regenerative medicine (Pera and Trounson, 2004).

For all of their promise, hES cells are not without controversy. Their derivation from early human embryos presents ethical issues for many that trump any potential health benefits. If we could coax somatic cells to become “ES cell like” by manipulations in the laboratory, cells for regenerative medicine might be obtained without the destruction of embryos. Seen in this context, understanding how the ES cell state is established and maintained is fundamental to intelligent and acceptable use of hES cells in the future.

In practice, deconstructing the ES cell phenotype can be reduced to elucidating the transcriptional circuitry that controls self-renewal and pluripotency. If a few key transcription factors dominate, one might envision introducing these in a controlled fashion into somatic cells or activating expression of their resident genes to elicit an ES cell fate. If a larger number of factors are involved, a similar conversion might require a series of successive, directed steps. The recent demonstration that the ES cell phenotype is dominant to somatic cell fate in cell hybrids provides evidence that the cellular components that maintain ES cell properties override more differentiated phenotypes (Cowan et al., 2005).

Currently, the repertoire of transcription factors required for ES cell identity is deceptively small (Chambers, 2004). Indeed, two variant homeodomain proteins, OCT4 and NANOG, are principal players, along with an HMG factor, SOX2, that acts with OCT4 at specific promoter elements. Boyer et al. (2005) in this issue of *Cell* provide a first glimpse into the complexity of gene targets regulated by these factors in hES cells. They couple chromatin immunoprecipitation (ChIP) with DNA microarrays (Chips), the so-called ChIP-Chip analysis (Horak and Snyder, 2002), to identify DNA bound individually by OCT4, NANOG, and SOX2. Their major experimental finding is that these factors co-

occupy the promoters of many genes in hES cells, implicating an intertwined network in the control of ES cell fate by a relatively small number of transcription factors.

Boyer et al. (2005) use state-of-the-art ChIP-Chip methodology in their study. Although the DNA microarrays encompass only the -8 kb to +2 kb region (relative to the transcript start sites) and transcriptional control may take place over far greater distances in vivo, they provide evidence that the vast majority of detected binding sites occur within this interval. As the Chips assay includes promoters of nearly 18,000 human genes, their analysis is truly genome-scale in scope and will provide much fodder for subsequent analysis. An immediate surprise is the high number of promoter regions bound by the three transcription factors tested. NANOG occupied 9% of the promoter regions, SOX2 7%, and OCT4 3%. Taken at face value, these data point to a pervasive contribution of these transcription factors to the cellular phenotype, rather than a model in which each regulates a very limited number of target genes in a cascade. The proportion of genes bound by these factors approximates that observed previously for dominant lineage-specific differentiation factors, such as the hepatocyte nuclear factors, in pancreatic and liver cells (Odom et al., 2004). The most provocative finding in this work is the frequent co-occupancy of the gene promoters bound by OCT4, NANOG, and SOX2. Indeed, 353 genes were bound by all three transcription factors. At least as many (492) genes were bound by SOX2 and NANOG, whereas a smaller number (80 genes) were bound by OCT4 and NANOG. Indeed, the vast majority of genes bound by OCT4 and SOX2 were also bound by NANOG. The inescapable conclusion is that these three factors work together, rather than separately, to control whole sets of target genes in ES cells.

In a second level of analysis, Boyer et al. (2005) show that roughly half of the genes bound by OCT4, NANOG, and SOX2 are expressed in ES cells. Among these are the promoters of the genes themselves, as well as components of signaling pathways such as the TGF- β and Wnt pathways that have been implicated in ES cell self-renewal. On the other hand, genes whose promoters are co-occupied by OCT4, NANOG, and SOX2 and are not expressed include many transcription factors be-

lieved to be critical for germ-layer-specific differentiation. Furthermore, they note that among this class, homeodomain proteins appear to be enriched. Though the *in silico* analysis does not reveal whether protein occupancy translates into control of the individual targets identified, experimental evidence from other studies indicates that OCT4 and SOX2 bind to and function at the OCT4 and NANOG gene promoters in more conventional assays (Kuroda *et al.*, 2005; Okumura-Nakanishi *et al.*, 2005). On the other hand, it should be kept in mind that protein occupancy is not necessarily a predictor of action at a presumptive target gene (Zhang *et al.*, 2005). Nonetheless, despite the adage that transcription factor binding is not necessarily equal to activity, it is probable that Boyer and colleagues (2005) have identified many of the biologically relevant targets of OCT4, SOX2, and NANOG. Refined experimental approaches are required to sift through the candidates to assess whether gene activation or repression is truly dependent on these factors.

Two simple regulatory network motifs are proposed to account for the new data. A feedforward loop suggests that OCT4 and SOX2 converge on the regulation of NANOG, which, in turn, acts with these proteins to control a vast array of downstream targets. This arrangement allows for both stability and developmental switching, depending on the activities and concentrations of the individual factors themselves, and also suggests that NANOG maintains a pivotal position in the regulatory hierarchy. Consistent with the co-occupancy of their promoters by the factors themselves, Boyer *et al.* (2005) propose that OCT4, SOX2, and NANOG conform to an autoregulatory loop, which serves again to maintain options of both stability and switching. In the differentiation of tissue-specific lineages, regulatory factors often operate in both positive and negative fashions to refine the ultimate developmental decision (Orkin, 2000). Here, too, it appears that the central factors OCT4, SOX2, and NANOG are likely to promote expression of other pluripotency/self-renewal genes as well as themselves, while simultaneously preventing expression of differentiation-promoting genes. Their actions are reinforced by the inhibitory effects of their target pluripotency genes on differentiation (see Figure 1).

The elegant study of Boyer *et al.* (2005) is an initial step in deciphering the network of transcription factors that regulate ES cells. However, many important questions remain unanswered. Given the intricate network apparent from the consideration of just three components, we must ask how many other key pluripotency factors with properties overlapping those of OCT4, SOX2, and NANOG remain to be identified. If, as seems likely, OCT4, SOX2, and NANOG can have either positive or negative effects on their target genes, how do extracellular signals or other regulatory factors control their transcriptional activities? Do cofactors for these transcription factors further integrate aspects of the regulatory network? What distinguishes those targets that might be acted upon by one of these key regulators (such as NANOG) in the absence of the others? Looking ahead, the success of network dissection may be judged by how well the insights gained allow manipulation of somatic cells to adopt an ES cell identity. We

are just at the beginning of this journey. Given the complexity of the preliminary roadmap there are likely to be many detours along the way.

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