Three transcription factors, Sox2, Oct-3/4 and Nanog, have been identified as master regulators that orchestrate mammalian embryogenesis as well as the self-renewal and pluripotency of ES (embryonic stem) cells. Efforts to understand how these transcription factors function have shown that they have a special property in common. Small changes in the expression of any one of these factors dramatically alter the self-renewal and pluripotency of ES cells. In this way, each functions as a molecular rheostat to control the behaviour of ES cells. Recent studies have begun to examine the molecular mechanisms that regulate the levels of these transcription factors. In this issue of the Biochemical Journal, Mullin and co-workers report that Nanog can self-associate to form dimers. Importantly, they also show that the domain responsible for dimerization is also needed for Nanog to sustain the self-renewal of ES cells in the absence of the cytokine LIF (leukaemia inhibitory factor). On the basis of their studies, they propose a novel mechanism for regulating the interactions between Nanog and other nuclear proteins.

Key words: embryonic stem cell (ES cell), gene regulatory network, Nanog, Oct-3/4, Oct4, pluripotency, Sox2.
unknown. However, this fundamental gap in our knowledge is beginning to be addressed, especially in the case of Nanog. Recent proteomic studies have identified a large list of transcription factors that interact, either directly or indirectly, with Nanog, including Sall4, Sall1, Rif1, Tif1, Dax1, Zfp281, Oct4 and Nac1 [10,11]. Importantly, these findings have already provided new insights into the regulation of genes that play key roles during development. For example, these findings led to the discovery that Nanog and Sall4 bind in close proximity to one another in the putative regulatory regions of their own genes [10]. This suggests that these two essential transcription factors co-operatively regulate their own expression by a positive feedback loop, similar to the one used by Sox2 and Oct-3/4 to regulate their own expression [2,5,12]. If this proves to be the case, one might also expect to find a negative feedback loop that limits the transcription of both the Nanog gene and the Sall4 gene, as well as that of their primary target genes. In the case of Nanog, evidence already exists that its overexpression limits the activity of the Nanog promoter [2]. Proteomic analyses also led directly to the finding that Nanog, Nac1 and Zfp281 can each bind to the GATA6 promoter, where it appears that they repress GATA6 transcription and prevent its expression until ES cells differentiate into endoderm [11]. Perhaps most importantly of all, Dax1, Nac1, Zfp281 and Oct4, like Nanog, have each been found in large protein complexes, which range in size up to several megadaltons [11]. Given that many of these proteins are found associated with one another in a wide distribution of multiprotein complexes, it is possible, if not likely, that these transcription factors work together co-operatively to provide combinatorial control over large sets of critical genes. Thus small increases or small decreases in one or more of these critical transcription factors would be expected to disrupt the overall stoichiometry and complexity of the multiprotein complexes required to maintain the self-renewal and/or pluripotency of ES cells. As attractive and straightforward as this model may be for a molecular rheostat, recent studies argue that there is far more to be learned about the mechanisms that regulate the formation of Nanog multiprotein complexes.

In this issue of the *Biochemical Journal*, Mullin et al. [13] report that Nanog (305 amino acids) can self-associate and form dimers. Working with rNanog (recombinant Nanog), which required an on-column refolding protocol, these workers determined from sedimentation velocity measurements that rNanog can exist as a dimer. Moreover, using size-exclusion chromatography, they determined that rNanog can also form large complexes that migrate with sizes well in excess of 200 kDa. On the basis of their findings, Mullin and co-workers argue that the broad range in the sizes of endogenous Nanog complexes present in extracts isolated from ES cells may not be solely due to the interaction of Nanog with other nuclear proteins. This argument is supported by their discovery that the WR (tryptophan-rich) domain of Nanog, which is located in the C-terminal half of the protein (residues 198–243), is primarily responsible for Nanog self-association. In agreement with earlier studies, Mullin et al. [13] show, using size-exclusion chromatography, that Nanog expressed by ES cells migrates in a wide range of protein complexes, many of which are much larger than 200 kDa. In strong contrast, expression of a mutant form of Nanog in ES cells, in which only the WR domain of Nanog has been deleted (Nanog ΔWR), gives rise to Nanog that migrates predominantly as a monomer. If this surprising finding is not due to the misfolding of Nanog ΔWR, which seems unlikely, it raises the intriguing possibility that the WR domain functions as a regulatory switch to selectively control which nuclear proteins interact with Nanog. For example, monomeric and dimeric forms of Nanog may each interact with different groups of nuclear proteins [13]. Although less likely, the dimerization of Nanog may simply restrict its ability to interact with other nuclear proteins altogether. In either case, controlling Nanog dimerization, by the availability of appropriate partner proteins, and/or by post-translational modification, could provide an important mechanism by which the levels of this molecular rheostat are regulated precisely.

The study by Mullin et al. [13] is remarkable for another reason. Previous studies had reported that the C-terminal tail of Nanog (the CD2 domain; residues 248–305) is required for Nanog, when overexpressed in ES cells, to replace the requirement for LIF [14]. However, Mullin et al. [13] report that deleting the last 49 amino acids of Nanog (virtually the entire CD2 domain) has little effect on the ability of Nanog ΔC49-engineered ES cells to generate colonies of morphologically undifferentiated cells when LIF is removed from these cultures. Moreover, Mullin and co-workers report that ES cells expressing elevated Nanog ΔWR are unable to self-renew and form morphologically undifferentiated cells when LIF is removed. There are several possible explanations for the radically different results reported in these two studies [13,14], including differences in the ES cells used, culture conditions employed, levels of Nanog expression and/or other differences in experimental design. Hopefully, this important issue will be resolved in the not-too-distant future.

In summary, although it is becoming increasingly clear that key transcription factors, such as Sox2, Oct-3/4 and Nanog, behave as master regulators during development, the molecular mechanisms that enable them to function as molecular rheostats are poorly understood. Efforts under way in many laboratories offer the hope that we will soon gain a far better understanding of the mechanisms involved. Towards this goal, Mullin et al. [13] propose a novel mechanism for regulating the interactions between Nanog and other nuclear proteins. Finally, given our evolving view of molecular rheostats, we should bear in mind that small increases may be just as likely as small decreases in the expression of master regulators to direct cell fate decisions during development.

REFERENCES


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