Proteomic and genomic approaches reveal critical functions of H3K9 methylation and heterochromatin protein-1gamma in reprogramming to pluripotency.

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Public Summary:
Remarkably, a single cell, the fertilized egg, can give rise to every specialized, mature cell in our bodies through the process of development. Cells with this property in the early embryo are referred to as pluripotent stem cells. It was long thought that once a mature, specialized cell is formed, it is incapable of returning to the pluripotent stem cell state. However, in 2006 it was discovered that mature cells can be experimentally reset into a pluripotent state, known as induced pluripotent stem cell, by the introduction of just a few specific genes. This advance offers exciting new opportunities to study diseases and develop new therapies. However, despite the promise of this approach, reprogramming of mature cells into induced pluripotent stem cells remains a very inefficient procedure. Consequently a major goal in the field is to improve the efficiency of reprogramming to the pluripotent state. Here, we gained new insights into the barriers of the reprogramming process, which may, in the future, lead to improved methods for the derivation of induced pluripotent stem cell lines and facilitate stem cell-based therapeutic applications and disease modeling.

Scientific Abstract:
Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) involves a marked reorganization of chromatin. To identify post-translational histone modifications that change in global abundance during this process, we have applied a quantitative mass-spectrometry-based approach. We found that iPSCs, compared with both the starting fibroblasts and a late reprogramming intermediate (pre-iPSCs), are enriched for histone modifications associated with active chromatin, and depleted for marks of transcriptional elongation and a subset of repressive modifications including H3K9me2/me3. Dissecting the contribution of H3K9 methylation to reprogramming, we show that the H3K9 methyltransferases Ehmt1, Ehmt2 and Setdb1 regulate global H3K9me2/me3 levels and that their depletion increases iPSC formation from both fibroblasts and pre-iPSCs. Similarly, we find that inhibition of heterochromatin protein-1gamma (Cbx3), a protein known to recognize H3K9 methylation, enhances reprogramming. Genome-wide location analysis revealed that Cbx3 predominantly binds active genes in both pre-iPSCs and pluripotent cells but with a strikingly different distribution: in pre-iPSCs, but not in embryonic stem cells, Cbx3 associates with active transcriptional start sites, suggesting a developmentally regulated role for Cbx3 in transcriptional activation. Despite largely non-overlapping functions and the predominant association of Cbx3 with active transcription, the H3K9 methyltransferases and Cbx3 both inhibit reprogramming by repressing the pluripotency factor Nanog. Together, our findings demonstrate that Cbx3 and H3K9 methylation restrict late reprogramming events, and suggest that a marked change in global chromatin character constitutes an epigenetic roadblock for reprogramming.