JOURNALS IN SCIENCE

Cell

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A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells

B. E. Bernstein et al.

The most highly conserved non-coding elements (HCNEs) in mammalian genomes cluster within regions enriched for genes encoding developmentally important transcription factors (TFs). This suggests that HCNE-rich regions may contain key regulatory controls involved in development. The authors explored this by examining histone methylation in mouse embryonic stem (ES) cells across fifty-six large HCNE-rich loci. They identified a specific modification pattern, termed "bivalent domains," consisting of large regions of H3 lysine 27 methylation harboring smaller regions of H3 lysine 4 methylation. Bivalent domains tend to coincide with TF genes expressed at low levels. They propose that bivalent domains silence developmental genes in ES cells while keeping them poised for activation. They also found striking correspondences between genome sequence and histone methylation in ES cells, which become notably weaker in differentiated cells. These results highlight the importance of DNA sequence in defining the initial epigenetic landscape and suggest a novel chromatin-based mechanism for maintaining pluripotency.

Control of Developmental Regulators by Polycomb in Human Embryonic Stem Cells

T. I. Lee et al.

Polycomb group proteins are essential for early development in metazoans, but their contributions to human development are not well understood. The authors have mapped the Polycomb Repressive Complex 2 (PRC2) subunit SUZ12 across the entire non-repeat portion of the genome in human embryonic stem (ES) cells. They found that SUZ12 is distributed across large portions of over two hundred genes encoding key developmental regulators. These genes are occupied by nucleosomes trimethylated at histone H3K27, are transcriptionally repressed, and contain some of the most highly conserved non-coding elements in the genome. They found that PRC2 target genes are preferentially activated during ES cell differentiation and that the ES cell regulators OCT4, SOX2, and NANOG co-occupy a significant subset of these genes. These results indicate that PRC2 occupies a special set of developmental genes in ES cells that must be repressed to maintain pluripotency and that are poised for activation during ES cell differentiation.

Cell Research

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Skeletal Myogenesis by Human Embryonic Stem Cells

Jun Ke Zheng et al.

The authors have examined the myogenic potential of human embryonic stem (hES) cells in a xenotransplantation animal model. Here they show that precursors differentiated from hES cells can undergo myogenesis in an adult environment and give rise to a range of cell types in the myogenic lineage. This study provides direct evidences that hES cells can regenerate both muscle and satellite cells in vivo and are another promising cell type for treating muscle degenerative disorders in addition to other myogenic cell types.

Developmental Biology

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Genome Restructuring in Mouse Embryos during Reprogramming and Early Development

C. Martin et al.

Although a growing number of studies investigate functional genome organization in somatic cell nuclei, it is largely unknown how mammalian genome organization is established during embryogenesis. To address this question, the authors investigated chromo center formation and the peculiar arrangements of chromosome domains in early mouse embryos. At the one-cell stage, they observed characteristic arrangements of chromosomes and chromo center components. Subsequently, starting with the burst of zygotic genome transcription, major rearrangements led to the establishment of somatic type chromo centers with a defined spatio-temporal organization. These processes appeared to be completed at the blastocyst stage with the onset of cell differentiation. During the same developmental period, a fraction of pericentric heterochromatin that was late replicating in the first cycle underwent switches in replication timing, spatial organization, and epigenetic marks. Cloning experiments revealed that the genome organization typical for more advanced stages was quickly reverted into the one-cell stage-specific form after nuclear transfer, supporting the idea that reprogramming associated genome remodeling in normal and cloned embryos is determined by cytoplasmic factors. Together, the results suggest that distinct but characteristic forms of nuclear genome organization are required for genome reprogramming in early embryos and for proper regulation of differential gene expression patterns at later stages.

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Contribution of Human Embryonic Stem Cells to Mouse Blastocysts

D. James et al.

In addition to their potential for cell-based therapies in the treatment of disease and injury, the broad developmental capacity of human embryonic stem cells (hESCs) offers potential for studying the origins of all human cell types. To date, the emergence of specialized cells from hESCs has commonly been studied in tissue culture or in teratomas, yet these methods have stopped short of demonstrating the ESC potential exhibited in the mouse (mESCs), which can give rise to every cell type when combined with blastocysts. Because of obvious barriers precluding the use of human embryos in similar cell mixing experiments with hESCs, human–nonhuman chimeras may need to be generated for this purpose. The results show that hESCs can engraft into mouse blastocysts, where they proliferate and differentiate in vitro and persist in mouse–human embryonic chimeras that implant and develop in the uterus of pseudopregnant foster mice. Embryonic chimeras generated in this way offer the opportunity to study the behavior of specialized human cell types in a nonhuman animal model. Our data demonstrate the feasibility of this approach, using mouse embryos as a surrogate for hESC differentiation.

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A Negative Feedback Loop of Transcription Factors That Controls Stem Cell Pluripotency and Self-Renewal

G. Pan et al.

Embryonic stem (ES) cells possess the ability to renew themselves while maintaining the capacity to differentiate into virtually all cell types of the body. Current evidence sug-

gests that ES cells maintain their pluripotent state by expressing a battery of transcription factors, including Oct4 and Nanog. However, little is known about how ES cells maintain the expression of these pluripotent factors in ES cells. Here the authors present evidence that Oct4, Nanog, and FoxD3 form a negative feedback loop to maintain their expression in pluripotent ES cells. First, Oct4 maintains Nanog activity by directly activating its promoter at sub-steady-state concentration but repressing it at or above steady-state levels. On the other hand, FoxD3 behaves as a positive activator of Nanog to counter the repressive effect of Oct4. The expression of Oct4 is activated by FoxD3 and Nanog but repressed by Oct4 itself, thus exerting an important negative feedback loop to limit its own activity. Indeed, overexpression of either FoxD3 or Nanog in ES cells failed to increase the concentration of Oct4 beyond the steady-state concentration, whereas knocking down either FoxD3 or Nanog reduces the expression of Oct4 in ES cells. Finally, overexpression of Oct4 or Nanog failed to compensate the loss of Nanog or Oct4, respectively, suggesting that both are required for ES self-renewal and pluripotency. These results suggest the FoxD3-Nanog-Oct4 loop anchors an interdependent network of transcription factors that regulate stem cell pluripotency.

Human Reproduction

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Single versus Double Embryo Transfer: Cost-Effectiveness Analysis alongside a Randomized Clinical Trial

A. A. A. Fiddelers et al.

Background: Twin pregnancies after in vitro fertilization are still frequent and are considered high-risk pregnancies leading to high costs. Transferring one embryo can reduce the twin pregnancy rate. We compared cost-effectiveness of one fresh cycle elective single embryo transfer (eSET) versus one fresh cycle double embryo transfer (DET) in an unselected patient population. *Methods*: Patients starting their first IVF cycle were randomized between eSET and DET. Societal costs per couple were determined empirically, from hormonal stimulation up to forty-two weeks after embryo transfer. An incremental cost-effectiveness ratio (ICER) was calculated, representing additional costs per successful pregnancy. *Results*: Successful pregnancy rates were 20.8 percent for eSET and 39.6 percent for DET. Societal costs per couple were significantly lower after eSET (7,334 euro) compared with DET (10, 924 euro). The ICER of DET compared with eSET was 19,096 euro, meaning that each additional successful pregnancy in the DET group will cost 19,096 euro extra. *Conclusions*: One cycle eSET was less expensive, but also less effective compared to one cycle DET. It depends on the society's willingness to pay for one extra successful pregnancy, whether one cycle DET is preferred from a cost-effectiveness point of view.

Nature

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Dissecting Self-Renewal in Stem Cells with RNA Interference

N. Ivanova et al.

The authors present an integrated approach to identify genetic mechanisms that control self-renewal in mouse embryonic stem cells. They use short hairpin RNA (shRNA) loss-of-function techniques to downregulate a set of gene products whose expression patterns suggest self-renewal regulatory functions. They focus on transcriptional regulators and identify seven genes for which shRNA-mediated depletion negatively affects self-renewal, including four genes with previously unrecognized roles in self-renewal. Perturbations of these gene products are combined with dynamic, global analyses of gene expression. Their studies suggest

specific biological roles for these molecules and reveal the complexity of cell fate regulation in embryonic stem cells.

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Nanog Promotes Transfer of Pluripotency after Cell Fusion.

J. Silva et al.

Through cell fusion, embryonic stem (ES) cells can erase the developmental programming of differentiated cell nuclei and impose pluripotency. Molecules that mediate this conversion should be identifiable in ES cells. One candidate is the variant homeodomain protein Nanog, which has the capacity to entrain undifferentiated ES cell propagation. Here the authors report that in fusions between ES cells and neural stem (NS) cells, increased levels of Nanog stimulate pluripotent gene activation from the somatic cell genome and enable an up to two-hundred-fold increase in the recovery of hybrid colonies, all of which show ES cell characteristics. Nanog also improves hybrid yield when thymocytes or fibroblasts are fused to ES cells; however, fewer colonies are obtained than from ES x NS cell fusions, consistent with a hierarchical susceptibility to reprogramming among somatic cell types. Notably, for NS x ES cell fusions elevated Nanog enables primary hybrids to develop into ES cell colonies with identical frequency to homotypic ES x ES fusion products. This means that in hybrids, increased Nanog is sufficient for the NS cell epigenome to be reset completely to a state of pluripotency. The authors conclude that Nanog can orchestrate ES cell machinery to instate pluripotency with an efficiency of up to 100 percent, depending on the differentiation status of the somatic cell.

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Notch Signalling Regulates Stem Cell Numbers In Vitro and In Vivo

A. Androutsellis-Theotokis et al.

The hope of developing new transplantation therapies for degenerative diseases is limited by inefficient stem cell growth and immunological incompatibility with the host. Here the authors show that Notch receptor activation induces the expression of the specific target genes hairy and enhancer of split 3 (Hes3) and Sonic hedgehog (Shh) through rapid activation of cytoplasmic signals, including the serine/threonine kinase Akt, the transcription factor STAT3, and mammalian target of rapamycin, and thereby promotes the survival of neural stem cells. In both murine somatic and human embryonic stem cells, these positive signals are opposed by a control mechanism that involves the p38 mitogen-activated protein kinase. Transient administration of Notch ligands to the brain of adult rats increases the numbers of newly generated precursor cells and improves motor skills after ischemic injury. These data indicate that stem cell expansion in vitro and in vivo, two central goals of regenerative medicine, may be achieved by Notch ligands through a pathway that is fundamental to development and cancer.

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Ovulated Oocytes in Adult Mice Derive from Non-circulating Germ Cells

K. Eggan et al.

Decades of research in reproductive biology have led to the generally accepted belief that in female mammals, all surviving germ cells enter meiosis at the end of fetal development and, as a result, the postnatal ovary harbors a limited supply of oocytes that cannot be replenished or regenerated if lost to injury or disease. However, recent reports have challenged this view, suggesting instead that oocyte production is maintained through continual seeding of the ovary by circulating bone-marrow-derived germ cells. To test directly the physiological relevance of circulating cells for female fertility, the authors established transplantation and parabiotic mouse models to assess the capacity of circulating bone marrow cells to generate ovulated oocytes, both in the steady state and after induced damage. Their studies showed no

evidence that bone marrow cells, or any other normally circulating cells, contribute to the formation of mature, ovulated oocytes. Instead, cells that traveled to the ovary through the bloodstream exhibited properties characteristic of committed blood leukocytes.

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Brain Response to Putative Pheromones in Lesbian Women

H. Berglund, P. Lindström, and I. Savic

The progesterone derivative 4,16-androstadien-3-one (AND) and the estrogen-like steroid estra-1,3,5,16-tetraen-3-ol (EST) are candidate compounds for human pheromones. In previous positron emission tomography studies, the authors found that smelling AND and EST activated regions primarily incorporating the sexually dimorphic nuclei of the anterior hypothalamus, that this activation was differentiated with respect to sex and compound, and that homosexual men processed AND congruently with heterosexual women rather than heterosexual men. These observations indicate involvement of the anterior hypothalamus in physiological processes related to sexual orientation in humans. The authors expand the information on this issue in the present study by performing identical positron emission tomography experiments on twelve lesbian women. In contrast to heterosexual women, lesbian women processed AND stimuli by the olfactory networks and not the anterior hypothalamus. Furthermore, when smelling EST, they partly shared activation of the anterior hypothalamus with heterosexual men. These data support the authors previous results about differentiated processing of pheromone-like stimuli in humans and further strengthen the notion of a coupling between hypothalamic neuronal circuits and sexual preferences.

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Biological versus Nonbiological Older Brothers and Men's Sexual Orientation

A. F. Bogaert

The most consistent biodemographic correlate of sexual orientation in men is the number of older brothers (fraternal birth order). The mechanism underlying this effect remains unknown. In this article, the author provides a direct test pitting prenatal against postnatal (e.g., social/rearing) mechanisms. Four samples of homosexual and heterosexual men (total n=944), including one sample of men raised in nonbiological and blended families (e.g., raised with half- or step-siblings or as adoptees) were studied. Only biological older brothers, and not any other sibling characteristic, including nonbiological older brothers, predicted men's sexual orientation, regardless of the amount of time reared with these siblings. These results strongly suggest a prenatal origin to the fraternal birth-order effect.