knockdown. Notably, 9 of these factors affected erythroid development whereas 8 factors affected myeloid development. More than half of these factors were predicted to regulate the specific lineage based on the sequence or expression-based model. Moreover, two of these factors, HIF3A and AFF1 (encoding the AF9 protein), were not previously implicated in erythropoiesis but emerged from this study based on their expression profile. The scope of the analysis performed in this study on human hematopoietic progenitors provides a rich resource for identifying regulatory networks controlling HSC self-renewal, differentiation, and lineage determination.

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What Your Heart Doth Know

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Combining embryological insight with careful analysis of early stage cardiomyocyte differentiation, Kattman et al. (2011) in this issue of *Cell Stem Cell* have defined minimal culture conditions to efficiently produce cardiomyocytes from hESCs and hiPSCs. The lessons learned are applicable to the derivation of other organotypic cell types.

The promise of stem cell biology as an inexhaustible source of differentiated cells for research applications and disease therapies is predicated on the ability to efficiently produce organotypic cells. A complementary body of knowledge from over a century of experimental embryology offers many insights into fundamental mechanisms of differentiation. The two fields have come together nicely in a paper by Kattman et al. in this issue, describing the efficient and controlled differentiation of pluripotent stem cells (PSCs) into cardiomyocytes (Kattman et al., 2011). The trick to working out the protocol was combining the use of embryological markers with careful titrations of signals that drive cells along discrete steps toward terminally differentiated, functional cardiomyocytes. The novelty resides not in the particular molecules that were used to drive the production of cardiomyocytes but, rather, in the approach they took to establish ideal concentrations and exposure windows. Although embryological development turned out to be a good guide, significant differences between PSC lines, even from the same species, necessitated devising a systematic approach that relied on parsing development into discrete steps and optimizing the use of a small number of embryological signals in each. These findings epitomize the extrapolation of experimental embryology to stem cell biology and reinforce the idea that embryonic development holds many clues for controlled differentiation of stem cells.

The strategy taken by Kattman et al. was enabled by their characterization of the VEGF receptor-2 (called Flk-1 or KDR) and PDGF receptor-α (PDGFRα) together as enriching cardiogenic mesoderm, which can form cardiomyocytes, endothelial cells, and smooth muscle, as distinct from hematopoietic progenitors in the KDR$^+$, PDGFRα^- population. Since the divergent TGFβ molecules Nodal and BMP, along with Wnt, induce cardiac mesoderm in embryos (Mercola et al., 2010), Kattman et al. optimized the concentration and duration of Activin (as a surrogate of Nodal) and BMP treatment (Figure 1). Strikingly, small differences in concentrations affect the yield of KDR$^+$, PDGFRα^+ cells dramatically and, consequently, reduce the yield of cardiomyocytes. Although anticipated by embryologists knowledgeable of threshold effects that subtle gradations in Activin concentration have on inducing different types of mesoderm, originally shown in *Xenopus* (Green et al., 1992) and later generalized to the mouse and zebrafish (Freeman and Gurdon, 2002), the finding is puzzling from a signal transduction perspective because it is not clear how a small concentration difference is reflected in qualitatively different genomic and developmental responses. Timing was as crucial as dose, because greatly reduced Activin and Wnt signaling were subsequently

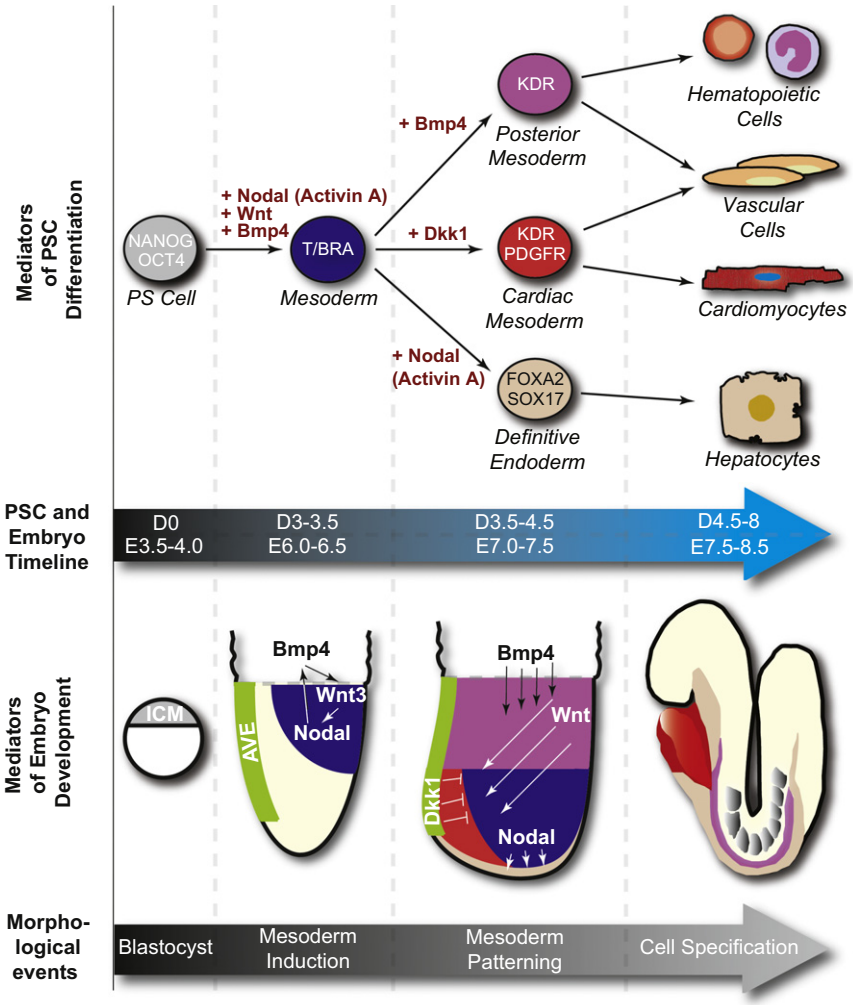

Figure 1. Directing Cardiomyocyte Differentiation
A small number of factors can efficiently drive cardiomyogenesis in PSCs (upper panel) in an apparent recapitulation of the processes that direct heart development, as depicted for mouse between days 3–8 of gestation (E3-8; lower panel). Nodal, Wnt, and BMP induce cardiogenic mesoderm (blue), but then need to be blocked in order for cardiac development to proceed, constraining heart development to a region of anterior endoderm in the late gastrula-stage embryo (red region of the E7-7.5 embryo, lower panel). As a result of this work, the emphasis of future research will shift to decipher how diverse types of mature cardiomyocytes can be generated, and other lineages can be similarly derived, such as hepatocytes from endoderm (brown) and hematopoetic cells from posteriorized mesoderm (purple).

required for commitment to the cardiac lineage and differentiation, evident by transcripts for *NKX2.5*, *TBX5*, *ISL1*, and *MEF2C* encoding cardiac transcription factors. This pattern also reflects embryology because, after transiently inducing and patterning primitive streak mesendoderm, Nodal, BMP, and Wnt signaling must be inhibited by secreted antagonists from the anterior visceral endoderm (AVE), including Dickkopf-1 (Dkk1), to induce heart tissue, effectively constraining cardiogenesis to a small region of anterior mesoderm classically called the heart field (Figure 1) (Mercola et al., 2010).

Attempting to decipher and recapitulate development in a dish of PSCs has its limits. First, cell-surface markers often lack specificity for the desired tissue, or their patterns of expression cannot parse a developmental program as finely as desired. Second, intrinsic differences between PSC cell lines make them respond differently to developmental signals. The first imposes a technical limit on the ability to isolate progenitors at each developmental stage and, hence, the ability to unravel the signals that drive them to the next. Moreover, earlier stage cells are less capable than later stage progenitors or precursors of producing pure populations of organotypic derivatives. This problem is beautifully illustrated by the Kattman et al. finding that FACS-isolated KDR$^+$, PDGFRα^+ cells do not generate a higher percentage of cardiomyocytes than does the unsorted parent population (see Figure 2B of Kattman et al., 2011). This failure could simply reflect that early stage cells are poised to generate geometrically more cell types. For instance, *Nkx2.5*$^+$, *Isl1*$^+$ cardiac precursors more efficiently yield myocardial cells than do the early KDR$^+$, PDGFRα^+ cardiogenic mesoderm (Domian et al., 2009). Alternatively, it could also mean that the marker-based isolation of progenitors eliminates cells that secrete inducing signals or does not capture all the cardiomyogenic progenitors. The latter possibility is consistent with multiple potential lineages giving rise to cardiomyocytes. For instance, it is unclear whether all cardiomyocytes in the embryo arise from KDR$^+$ progenitors or whether a KDR$^-$ source also exists in embryos and ESC cultures (Blin et al., 2010). Moreover, the recognition that different PSC lines require individually tailored differentiation protocols demonstrates how much remains to be learned about line-to-line differences, in particular, vestiges of diverse origins and methods of reprogramming in induced pluripotent stem cells (Stadtfeld and Hochedlinger, 2010). The clinical implication of this finding for hiPSC-based therapy means that protocols must be customized for each patient.

The efficient production of cardiomyocytes has many applications, including use in regenerative medicine and drug safety evaluation (Segers and Lee, 2008). However, this and other protocols generate electrically and mechanically immature cells that resemble fetal, rather than adult, cardiomyocytes. Immature cardiomyocytes do not generate the contractile force of mature myocytes, and their ion channel properties diminish the predictive value of in vitro analyses, such as for cardiotoxicity, and present potential risk in transplantation. Moreover, myocytes in the heart are naturally diverse, with highly specialized physiological attributes (e.g., pacing, conducting and working myocytes) and regional heterogeneity—even within the ventricular wall—that are critical for normal

function and heart pathologies. Thus, there is an obvious need to understand the signals and cues that direct cardiomyocyte diversity and promote electrical and mechanical maturation. This goal is challenging using embryos generated with conventional transgenic technologies since many genes have multiple essential roles and their inactivation early on can mask later functions. As a result, PSCs represent a straightforward means to dissect the molecular basis for late-stage developmental or maturational events by bringing in novel technologies such as siRNAs, miRNAs, or small molecules, especially using high-throughput technology.

In summary, embryology redux can lead to efficient, directed differentiation of PSCs, enhancing knowledge of embryogenesis and increasing the relevance of PSC-derived cells for practical applications and research. This and other recent examples of efficient differentiation [e.g., pancreatic endocrine cells and motor neurons (Kroon et al., 2008; Wichterle et al., 2002) bring us one step closer to being able to scrutinize highly complex normal and pathological behaviors of terminally differentiated cells in culture dishes.

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A Twist of Cell Fate

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Individuals carrying deleterious BRCA1 mutations typically develop basal-like rather than luminal breast cancers. In this issue of *Cell Stem Cell*, Proia et al. (2011) study breast tissue from women with heterozygous BRCA1 mutations and identify molecular mechanisms that regulate mammary progenitor cell differentiation and bias toward subsequent basal-like tumor formation.

Since the early 1990s, investigators have known that certain families contain women who exhibit a significant increased lifetime risk for breast and ovarian cancer. In this population, the genetic locus responsible for this risk, BRCA1, is heterozygous for deleterious mutations where one copy of the gene is truncated, mutated, deleted, or silenced by DNA hypermethylation (Ostermeyer et al., 1994). Breast tumors that form in these women are unusual on several levels. First, the tumors develop at an early age and are a manifestation of an increased lifetime risk of developing breast cancer. Although several BRCA1 functions may be altered in individuals who bear a mutated copy of the gene, the actual molecular basis for this increased risk is unknown. Second, the tissue types that exhibit an increased likelihood of developing tumors are restricted to a small number, mostly breast and ovarian tissues. Why the increased risk for cancer does not extend to other organs or lineages is also a mystery. Third, and the topic of this Preview, the type of breast cancer that is most typically diagnosed in BRCA1 mutation carriers is of a particular subtype, the highly lethal basal-like subtype. Understanding how the BRCA1 protein participates in any of these three malignant patterns (Venkitaraman, 2002) would be a major step forward in the effort to treat and eventually eradicate the disease. In this issue of *Cell Stem Cell*, Proia and colleagues in the Kuperwasser laboratory make use of primary breast tissue samples isolated from disease-free BRCA1 mutation carriers (Proia et al., 2011). The authors present a carefully crafted study that addresses the origin of the basal-like phenotype that predominates in the tumors that develop in these women. This study constitutes a cornerstone in the field as the use of tissues obtained from women that carry these mutations provides unparalleled opportunities to gain insights into the molecular events that underlie the puzzles noted above. Through a compelling set of supporting data, the authors reveal that the propensity to generate basal-like tumors in breast tissue that