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The uncommon roles of common gene regulatory factors in the genomes of differentiating cells

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Viewed through the lens of comparative regulatory mechanisms in developmental processes, the article of Calero-Nieto *et al* (2014, this issue) is of particular interest. This work uncovers the causal combinatorial subtleties of the distinct enhancer occupancy profiles displayed by ten different transcription factors, which are expressed in common in two hematopoietic cell types, a stem cell-like precursor and primary mast cells.

See also: **FJ Calero-Nieto *et al*** (June 2014)

The paper entitled "Key regulators control transcriptional programs in blood progenitor and mast cells" is the latest in a decade-long avalanche of mechanistic experimental analyses from the Berthold Göttgens laboratory, of which the overall objective has been to reveal how encoded gene regulatory processes drive hematopoietic stem cell function and diversification. The conceptual leverage of this work stems from its general focus on genomic information use and regulatory logic, rather than on biochemical particularities. The question addressed in the current study is how to account for the non-overlapping sets of enhancers targeted by hematopoietic transcription factors in two related cell types, even though these factors are expressed more or less equally in both. The cell types compared are primary mast cells and a permanent hematopoietic cell line of ES cell origin, called HPC7, which previous work established as a realistic surrogate for an embryonic, multipotential stem cell precursor (Pinto do O *et al*, 2001; Wilson *et al*, 2010). Ten regulatory genes known to play key roles in hematopoietic differentiation were studied (*scl, lmo2, runx1, pu.1, gata2, fli, erg, e2a, ctcf, and meis*), and the paper begins with the striking contrast between their similar expression but their apparently distinct functions, in mast cells vs. HPC7 cells. While thousands of downstream genes are expressed in primary mast cells and not in HPC7 cells, and vice versa, the ten regulatory genes are almost equally transcribed in both. However, a quantitated, high-resolution, genome-wide ChIP-seq comparison of the target sites at which these 10 factors bind in the two cell types displays largely different, non-overlapping, sets of genomic targets (for every factor but CTCF, which essentially serves as a control). This is seen even in genes expressed in both mast cells and HPC7 cells. The experiments and analyses carried out in order to resolve this apparent paradox produce three essential conclusions. First, the binding site peaks that differ between the cell types are not adventitious, functionless artifacts, since they can be used to predict, at a statistically significant level, the differential patterns of gene expression between the two cell types. Second, many of the presumptive enhancers where these ten factors bind interact with several of the ten factors at once, and this is demonstrated to be functionally important. Thus when combinatorial binding is taken into account, the gene expression predictions become significantly stronger; the higher the number of factors binding at given sites, the better the prediction. Third, the basic mechanism accounting for the differences in binding sites observed in the two cell types could be that each does indeed also express specific regulatory genes which the other does not. Therefore, in the differentially occupied enhancers, the binding of these uniquely expressed transcription factors may be what results in the assembly of the cell type-specific DNA-transcription factor complexes, including the members of the common set of ten. For the mast cells, these uniquely expressed regulatory genes include *mitf* and *fos*, while some *hox* genes are expressed only in the HPC7 precursor cells. Target binding sites for MITF and Fos are accordingly over-represented in the specifically expressed regions of mast cells. However, it is important that the specifically expressed transcription factors are not the only ones essential for occupancy of the differentially displayed enhancers, since knockdown even of members of the set of ten caused disappearance of significant fractions of binding peaks.

The most general and most important conclusion from this work is that it shows that at root which enhancers are active depends, as of course it ultimately must, on the occurrence or absence of transcription factor-DNA interactions, not to say that the differential abundance of the shared factors is unimportant (e.g., there is about 10× more *gata2* mRNA in mast cells than in HPC7 cells). The differential enhancer occupancy measured in this study thus could depend basically on the sequence-specific recognition of encoded target sites by those few factors that are cell type-specific, as well as on quantitative parameters. An exactly similar conclusion was reached in a comparison of macrophage vs. B-cell enhancer binding,

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where again the cell type-specific loci of binding of a common factor, PU.1, were co-occupied with cell type-specific factors (Heinz *et al*, 2010).

The work illuminates the generality and functionality of combinatorial enhancer binding by multiple factors present in each cell type, though, no surprise, certain subsets of the 10 common factors are likely to be found together in enhancers of mast cells and others in enhancers bound in HPC7 cells. For given episodes of differentiation, it is now often noted that cohorts of cell type-specific transcription factors predicatively bind together on cell type-specific enhancers. One of many excellent examples now available, based on characterized enhancers and genome-wide measurements, is provided by studies on *Drosophila* cardiac enhancers (Jin *et al*, 2013; Junion *et al*, 2012), and a similar result has been reported for mammalian cardiac enhancers (He *et al*, 2011). A major implication of such studies is that multiple combinatorial binding of dedicated sets of transcription factors is required for enhancer occupancy as well as for enhancer function. This is to say that AND logic pertains to multiple transcription factor occupancy of enhancers as well as to function. That general conclusion does not imply any specific biochemical mechanism. For example, some factors have the capacity to invade a nucleosome covered enhancer, 'open' or alter the chromatin, and thus perform a function necessary for binding of additional necessary factors, but not per se sufficient for transcriptional activity (Zaret & Carroll, 2011). Or the multiple factors might bind cooperatively *sensu stricto*, and their free energy exchange be required for complex stability. Or they might be jointly required to attract co-factors that stabilize the complex, or to cause DNA torsion that facilitates binding, etc. But one idea that in this pervasive light has outlived its usefulness is the concept of the single 'master regulator'. Obligatory combinatorial AND logic is incompatible with this concept. As the work discussed here shows, both uniquely expressed MITF and commonly expressed Gata2 are required for differential enhancer binding (and for mast cell transcriptional functions). There are no 'masters' here; there are specific combinatorial enhancer occupancies that function as logic gates.

Finally, the contrast with more general developmental regulatory systems is illuminating. As thousands of observations on developing embryos, body parts, and cell types show (Davidson, 2006), the primary level of control in development is the generation in each developmental process of very diverse regulatory states, by expression of very different sets of regulatory genes. Here, in comparing a precursor and a product cell, the regulatory states differ far less, and in the case of the 10 key hematopoietic genes, hardly at all. This is what provides the precious opportunity of seeing in relief the additional level of DNA sequence-specific transcriptional control afforded by combinatoriality, with only minor contributions from the developmental institution of regulatory state diversity.

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Conflict of interest

The author declares that he has no conflict of interest.

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