Distributed and centralised control during differentiation

Jonathan R. Chubb*, Hugh Z. Ford and Vlatka Antolović

MRC Laboratory for Molecular Cell Biology and Department of Cell and Developmental Biology
University College London
Gower Street
London
WC1E 6BT
*j.chubb@ucl.ac.uk

A persistent view of cell fate choices during development entails centralised control by so-called master regulators. A recent single cell study of the large-scale fate specification during mammalian gastrulation (Mittnenzweig et al., 2021) implies the prevalence of more distributed forms of control.

Decision-making in biology may be either centralised or distributed (Figure 1). These control strategies occur across a broad range of biological scales, from molecular interactions and cellular behaviors to coordinated decision-making in animal groups. In centralised decision-making, the population responds to a small number of empowered agents (molecules, cells, alpha individuals). In distributed decision-making, behaviours emerge from the set of pairwise interactions between individuals in the group. Control is often neither wholly centralised nor distributed, yet in animal developmental biology, the view that differentiation decisions are centralised, with regulation converging on master regulators, is entrenched. Indeed, master regulators have remarkably potent and specific biological effects: loss of their expression causes the loss of a specific cell type or structure, while overexpression is sufficient to induce formation of the cell type or structure where it would not normally be. However, master control may not be appropriate for all decision-making in development. A recent high temporal resolution study of transcript dynamics during mouse gastrulation implies that distributed forms of molecular control can be surprisingly widespread (Mittnenzweig et al., 2021).

During gastrulation in vertebrates, the relatively unpatterned cell population in the early embryo is converted, via extensive morphogenetic changes, rapid cell proliferation and large-scale cell fate diversification, into the archetypal vertebrate body plan. In the mouse, this process takes around 36 hours and generates some 30 different cell types alongside a 10-fold increase in the number of cells. Understanding this process requires a descriptive survey of the acquisition of cell identity. To this end, single cell transcriptomics (scRNAseq) has been used to define possible cell states, and to infer how these states map onto each other during developmental progression. ScRNAseq cannot be used to follow single cells over time; instead, the population is sampled at intervals, with a variety of inference methods used to "join the dots" between cell states. This approach is limited by sampling frequency and variability in developmental progression between embryos in a litter. To move past these sampling issues, Mittnenzweig and colleagues (2021) superimposed precise embryo staging onto transcriptome staging, generating a high temporal resolution spectrum of the gene expression transitions of gastrulation. These different approaches to staging showed very strong concordance with each other and with data from a previous scRNAseq study on the gastrulating mouse embryo (Pijuan-Sala et al., 2019). The new analysis confirmed and extended several aspects of transcriptome dynamics during gastrulation and refined earlier cell type annotations. In addition, the data highlighted that development is not precisely synchronous across the embryo, with similar cell states identified in embryos of different ages. However, the most striking inference is that most fate choices during gastrulation respond to distributed rather than master control.

This inference emerged from a network flow model used to map how cell states project onto each other during developmental time. The model identified different types of transition: i) rapid establishment of specialised transcript states, ii) a gradual fate change over time in cells leaving a progenitor population, iii) rapid bifurcations and iv) more complex multifurcations, where equivalent cells adopt several possible fates. A few fate transitions (to blood, node and heart) were associated with highly specific transcript

compositions- possibly consistent with hierarchical control of a differentiation programme by a master regulator. However, most transitions were not dominated by enrichment for specific regulators, and were instead more consistent with combinatorial control from many inputs. Mittnenzweig et al. then focussed on a set of 63 transcription factors (TFs) with variable expression in the mesoderm. Although there were differences in TF expression between higher level mesodermal states, none of the TFs were specific to a precise fate decision, implying combinatorial, or distributed control.

Why would gastrulation suit distributed control? From an engineering perspective, the greater connectivity of distributed networks allows: i) potential to generate more stable states- ideal for the rapid establishment of around 30 different cell fates during gastrulation, and ii) robustness to perturbation (Albert et al., 2000). Robustness is presumably at a premium during gastrulation, with so many molecular and cellular events that need to be coordinated during a very short window of embryonic time. Cells and their environment are noisy- control by cell autonomous masters would prohibit feedback when decisions occur at the wrong time and place. It seems unlikely that cell death or dedifferentiation could be effectively deployed to tidy up after mis-specification. There is little cell death during gastrulation in the mouse, and dedifferentiation events, even in comparatively simple cell types, are too slow to be useful in a developmental process with such rapid change. Other mammalian differentiation processes may also be well-suited to distributed control. In a recent single cell study of haematopoeisis, the ability of differentially expressed genes to predict specific fates was limited, with TFs providing no strong increase in predictive power (Weinreb et al., 2020). As with gastrulation, haematopoiesis generates many distinct fates, in a complex niche, with cells exposed to a continual cacophony of signalling. A relatively non-specialised transcriptional complement may be necessary to respond appropriately to this diversity of inputs, and to permit the required level of connectivity.

How will the future play out for the masters? Will our concepts follow those of metabolism, where ideas of pathway control by a single rate-limiting protein were replaced by theories of control at multiple steps (Westerhoff et al., 1984)? It is noteworthy that some of the more illustrious masters, notably MyoD (Rudnicki et al., 1993) and Eyeless (Jang et al., 2003), operate in networks with other proteins of similar potency. Masters are often themselves heavily regulated, from the genomic organisation of their transcription units through to dense post-translational modifications. This does not seem especially masterful. An alternative future for the masters may instead be more analogous to the inclusive view emerging in the study of tissue morphogenesis, where control (at the level of cells, not molecules) can be leader-driven, distributed, or some combination of both (Clarke and Martin, 2021).

In what contexts might we expect to find a master operating? Contexts with a lone agent sitting above a regulatory hierarchy are likely limited. How could unleashing such an agent be compatible with establishing functional tissue organisation? Are cells not too noisy to stay “on message” for the coordinated specialisation required to build a tissue? Perhaps the answer is that masters are deployed in situations where stochastic outcomes are tolerable (for example in sex determination) or beneficial (for example in contexts with scattered differentiation).

The distinction between master and distributed control depends in part on the experimental approach. One-lab-one-gene, knockdown and overexpression approaches are more likely to generate single-gene answers. Conversely, complex transcriptomic data is naturally more likely to allow inferences of distributed regulation. However, it is

encouraging that the transcriptome data of Mittenzweig et al. (2021) support both master and distributed control. Ideally, studies of decision-making during differentiation should also incorporate dynamic imaging of the implicated agents, combined with perturbations of molecular control tuned to the dynamic range of cell signalling. Still, the aura of the master will no doubt persist. The ideas are simple and the phenomenology striking. Yet how often will the aura be matched by any clear-cut centralised control? Is a master simply a useful waypoint to anchor our fledgling understanding of cell choices?

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Figure 1: **Regulatory networks associated with centralised and distributed control**
Different schemes of networked control, from a more centralized configuration (lower connectivity) on the left, to more distributed (higher connectivity) on the right. Nodes represent individual agents in the population (e.g. molecules, cells, organisms). Edges (connectors) represent interactions between individuals.