conundrum (Liang and Biggin, 1998). In the embryo, most randomly selected genes show unique, quantitative changes in expression between cells, even between neighbors of the same cell type. Such within-cell-type gene expression-regulation is controlled by factors, such as HOX proteins, that specify positional information within an organism (Wolpert, 1994). These transcription factors have exceptionally broad regulatory effects, each directly or indirectly coordinating the expression of ~60% of genes by 2-fold or more (Liang and Biggin, 1998). Cells within a muscle or any other animal tissue are not identical to each other, but differ in qualities such as shape, size, and cell contacts in order to create the required tissue structure. The control of such subtle differences between cells of the same type may require modest quantitative changes in the expression ratios of a large percent of genes (Liang and Biggin, 1998). Thus, it is plausible that MyoD may control many more genes in the animal than suggested by expression analysis of cultured cells through interactions with transcription factors that specify positional value and that are active in differing parts of the developing muscle.

Nevertheless, not all DNA binding and acetylation events in the genome are likely functional. It will be the next major challenge to discover, in the context of the organism, what fraction of factor DNA binding and chromatin modification events lead to detectable transcriptional responses and what fraction of these responses offer an evolutionary selective advantage.

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Realizing the Promise of RNAi High Throughput Screening

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Recently reporting in *Nature*, Collinet et al. describes the application of quantitative multiparametric methods to a genome-wide RNAi screen for regulators of endocytosis. The study illustrates the power of this approach beyond the identification of new endocytic components to providing insights into the design principles of the endocytic system.

One of the goals of systems biology is to understand how specific cellular processes operate and are integrated in the overall higher order unit—the cell. In recent years, genome-wide RNAi high-throughput screens (HTS) have provided the means to functionally interrogate the entire genome. Arguably, the most exciting application of HTS RNAi screening is not simply to use the methodology to identify new components of a biological process, but to gain a broader understanding of how the regulation of a single process is dynamically coordinated with others (Mathey-Prevot and Perrimon, 2006). However, the promise of using HTS RNAi technology to gain systems level understanding and to describe emergent cellular behaviors has been slow to be realized, largely because of setbacks due to the high rate of false positives created by initially unexpected off-target effects (OTEs) of RNAi reagents. In a new study recently published in *Nature*, Marino Zerial and colleagues make use of extensive resources and elegant data analysis methods to drastically limit OTEs, thus providing a striking example of how a genome-wide HTS screen performed in a rigorous and quantitative manner can lead to systems-wide insights into a fundamental cellular process—in this case, that of endocytosis (Collinet et al., 2010).

Endocytosis is a means by which cells absorb molecules and particles (such as nutrients, signaling molecules, and

pathogens) and can occur through two major routes: clathrin mediated and non-clathrin mediated (Scita and Di Fiore, 2010; Sorkin and von Zastrow, 2009). Almost all activated signaling receptors are internalized by endocytosis, but once internalized, they can have very different destinations. Activated receptors can be targeted for degradation and signal attenuation following endocytosis. Alternatively, endocytosis can result in receptor recycling, thus maintaining signaling activity, in some cases by ''refreshing'' activated receptors through promoting the disassociation of their activating ligands. The endosome can also act as a platform for the assembly of signaling complexes that could not be assembled at the membrane, thus acting to modulate and alter signals originating from the membrane. These and other examples demonstrate that endocytosis plays a key role in regulating signaling dynamics. In addition to providing mechanistic insight into the endocytic process itself, Collinet et al. (2010) find that endocytosis is under the control of multiple cellular signals such as Wnt, TGF-β, Notch, and adhesion, implying that endocytosis serves as a central point where the activity of distinct signaling subnetworks converges and engage in cross-talk.

To investigate how endocytosis is regulated, Collinet et al. (2010) quantified in cultured mammalian cells the contribution of all genes to two different types of endocytosis in a multiplexed fashion by monitoring the endocytosis of the transferrin receptor (TF), which is recycled back to the plasma membrane, and the endocytosis of epidermal growth factor (EGF), which is transported to the lysosomes for degradation. Following inhibition of individual genes by RNAi, the authors imaged cells using high-throughput confocal microscopy. To limit OTEs, the authors screened two siRNA libraries and a third endoribonuclease-prepared siRNA (esiRNA). In total, 161,492 si/esiRNAs were screened corresponding to 7–8 si/esiRNAs per gene. To date, no other RNAi screen in any organism has been performed at such a scale.

As there are a diverse number of potential phenotypes that result following gene inhibition (e.g., changes in endosome size, number, or distribution in cellular space), image analysis algorithms were used by the authors to describe multiple parameters of endosomal morphology and organization and to derive a ''phenoscore.'' Such scores are vectors composed of multiple features that uniquely describe the effects of a specific si/esiRNA treatment on endocytosis. Due to the fact that multiple si/esiRNAs were used to target the same gene, Collinet et al. (2010) implemented a method to calculate the most probable profile (the mode of the posteriori joint probability distribution) for each gene considering all individual si/esiRNA profiles. Combining RNAi score in such a way has the beneficial effect of eliminating the potential contribution of OTEs to a gene profile.

The pheno-scores and gene profiles described by Collinet et al. (2010) are in fact similar to the Phenoprint (Piano et al., 2002) and Quantitative Morphological Signatures (QMSes) (Bakal et al., 2007) that have been used previously to describe the phenotypes in RNAi screens for early *C. elegans* development and cell morphology, respectively. Multiparametric phenotypic signatures provide insight regarding the architecture of signaling networks and how different aspects or morphological regulation are controlled in space and time (Bakal et al., 2007; Gunsalus et al., 2005; Sönnichsen et al., 2005). For example, the protein products of two genes with highly correlated phenotypic signatures are more likely to exist in the same signaling complex (Gunsalus et al., 2005). Collinet et al. (2010) observe through grouping of gene profiles that the activity of numerous signaling networks is coupled to the regulation of the endocytic process. Moreover, in a significant conceptual leap forward in the analysis of image-based data, the authors use phenotypic signatures in a completely novel fashion to describe the design principles of the endocytic system. Using Bayesian analysis, they describe statistical relationship between endosome size, number, the distance of endosome from the nuclei, cargo content, and cargo concentration, and how such relationships differ between TF and EGF endocytosis.

The work by Collinet et al. (2010) is important not only because it provides new understanding of endocytosis, but because it illustrates that when quantitative multiparametric methods are deployed to screen multiple RNAi libraries, such screens can provide new insights into complex cellular processes. Thus, this study is truly an example of RNAi screening technology delivering on its early promise. However, as emphasized by the authors, ''this exploratory endeavor required uncommon resources for a primary screen,'' and it is important to realize that RNAi HTS will deliver systems-wide information only if done at a level that permits high quality data to be generated. A lesson for laboratories interested in deploying HTS RNAi screens