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NEWS AND VIEWS

# Alternative splicing variability: exactly how similar are two identical cells?

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Can any two things be identical? The question has puzzled metaphysicists since Leibniz proposed his 'Identity of Indiscernibles' law four centuries ago. Average measurements performed on large populations of cells are routine in biochemistry and rest on the assumption—often made implicitly—that genetically identical cells behave identically. Since this approach prevents detecting any difference between single cells, the validity of this assumption remains unchallenged. In cell biology, on the other hand, direct observation of individual cells naturally raises the issue of how and why isogenic cells look and behave differently from one another. What are the biochemical explanations for the differences between individual cells?

As nucleic acid detection methods have become more sensitive, our ability to observe cell-to-cell variability in gene expression has improved dramatically. We can now ask: what are the sources, controlling factors, and biological roles of stochastic variability in gene expression? Until now virtually all of this work has been focused on transcription, with other key steps in the gene expression pathway—splicing, RNA decay, translation, protein turnover—left yet to be studied. In a study just published in *Molecular Systems Biology* (Waks *et al*, 2011), Pamela Silver and colleagues use single molecule fluorescent *in situ* hybridization (smFISH) to fill this gap and provide the first direct view of alternative splicing at the single cell level. Early hints that alternative splicing might vary within an isogenic cell population came from studies using clever dual fluorescent protein-based reporters (Orengo *et al*, 2006, Newman *et al*, 2006, Stoilov *et al*, 2008), but part of the cell-to-cell variation in reporter protein expression could have been contributed by steps other than alternative splicing. By using smFISH to count alternatively spliced mRNA transcripts at the single molecule level, Waks and colleagues directly sampled the cell-to-cell variation in spliced isoforms.

Why should we care about variation in alternative splicing, or transcription—isn't it just noise? Apparently evolution cares, leading to situations in which critical developmental decisions are left to a roll of the dice. A familiar example is the lytic-lysogeny decision of the temperate bacteriophages like lambda, beautifully deconstructed by Arkin *et al* (1998), who modeled the contribution of stochastic events to this well-described developmental decision.

Eukaryotic mRNAs from genetically identical cells show surprisingly high levels of cell-to-cell variability in their abundance. Importantly, these fluctuations have impact on developmental decisions in stem cells (Chang *et al*, 2008), cancer cells (Spencer *et al*, 2009), and HIV-1-infected cells (Weinberger *et al*, 2008). Along with differences in expression levels, mRNAs generated by alternative splicing can also differ in their coding sequence, which leads to differently functioning proteins or alternate regulatory control through non-sense mediated mRNA decay (NMD). Since >90% of human genes generate multiple distinct mRNAs, it simply won't do to count the total number of transcripts from each gene—we must take into account each mRNA isoform separately. This complexity is embraced by Waks *et al* and resolved for alternatively spliced mRNAs from two human genes, CAPRIN1 and MKNK2, in two human cell lines, Rpe1 and HeLa.

Inevitably, the application of smFISH to the question of cell–cell variability in alternative splicing comes with substantial technical constraints. A large (>800 nt) target sequence must be unique to at least one isoform because multiple (ideally >50) specific, fluorescently labeled oligonucleotides must be hybridized (to fixed cells) in order to detect a fluorescent dot representing the transcript. This restriction leaves many isoforms that differ critically by the size of a typical alternative exon for future investigations. Even so, the CAPRIN1 and MKNK2 genes in this study produce isoforms with distinct biological functions, and their variability has been captured for two distinct clonal cell lines, Rpe1—a diploid cell line derived from retinal pigment epithelial cells and HeLa—a cervical epithelial cancer cell line. Intriguingly, isoform ratio variability is less in Rpe1 cells than in HeLa cells, where it is considerable. For Rpe1, the data are a close fit to a binomial model of distribution without invoking feedback. Thus, different cells have different mRNA isoform variability. What causes this and how is it regulated?

The authors address several possible sources of variability beyond the intrinsic stochasticity of alternative splicing choice, including (1) fluctuation in mRNA synthesis, (2) fluctuation in splicing factors, (3) fluctuation in relative decay times between isoforms, and (4) variability due to cell-cycle stage. Examination of these possible sources using modeling and other measurements leads them to argue that cell-to-cell variation in isoform ratio is likely due in large part to cell-to-cell variation in the level of splicing regulators. They support this idea by RNAi knockdown of SFSR1 (a.k.a. ASF/SF2—a splicing factor

known to regulate MKNK2 splicing), and observing an increase in MKNK2 isoform ratio cell-to-cell variability. This offers tantalizing evidence that tight regulation of the splicing machinery, in part through autogenous regulation of splicing factors (Ni *et al*, 2007; Lareau *et al*, 2007) might be an important general mechanism by which cells temper fluctuations in mRNA isoform ratios.

The work by Waks and colleagues provides a platform for future studies of the mechanisms underlying single cell expression heterogeneity. The broader application of these techniques holds great promises for the exploration of stochastic fluctuations in alternative splicing and their role in complex developmental decisions or in clinical relevant processes affecting, for example, disease progression and efficacy of therapeutic treatments. Leibniz's law of identity is determined by the discernability of two objects' characteristics. With the study by Waks *et al*, single cell isoform ratios can now be discerned uncovering yet another way in which genetically identical cells are not identical, and thus may diverge in their biological trajectories.

## Conflict of interest

The authors declare that they have no conflict of interest.

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