

and in noncoding exons versus 5' UTRs of intronless genes (Zhang and Chasin, 2004). However, the two datasets overlap only partially due likely to certain levels of false positives and negatives in both analyses. Both the Chasin and Burge groups used the power of statistics to further explore the distribution of ESSs in exons, introns, alternative exons, and pseudoexons. These analyses clearly indicate that ESEs are prevalent in exons whereas ESSs are enriched in pseudoexons, leading to an emerging rule regarding how real exons may be defined based on ESE and ESS frequencies in combination with consensus splicing signals at the intron-exon boundaries. The first generation of such a splicing simulation algorithm was developed in the current work by Burge and coworkers with reasonable success (Wang et al., 2004). Interestingly, previous gene prediction programs also accounted for motif enrichment in real exons and depletion in pseudoexons (for example, see Zhang [1997]), and now it appears that many of those enriched and depleted motifs may correspond to ESEs and ESSs, respectively.

Implications for Regulated Splicing

Since alternative splicing is common in higher eukaryotic cells, the current work on ESSs, in combination with the earlier work on ESEs, also sheds light on how splicing may be regulated. In many reported cases, constitutive exons are not as dramatically different from alternative exons as previously thought. Multiple ESEs experience protein coding constraints in constitutive exons, and the combination of ESEs and ESSs have been found in many regulated splicing events (Black, 2003). Indeed, the analysis of the distribution of ESSs revealed that ESSs appear more frequently in skipped exons as well as in alternative 5' and 3' exons in comparison with constitutive exons (Zhang and Chasin, 2004; Wang et al., 2004). A balance between ESEs and ESSs may serve as a foundation for regulation when different spectra of positive and negative regulators are expressed in a given cell type or in a defined developmental process. Point mutations in exons may affect protein coding or disrupt specific regulatory splicing elements; the "double" codes in exons can now be fully appreciated in understanding some disease mechanisms (Cartegni et al., 2002). It should be pointed out that, in addition to ESEs and ESSs, intronic splicing enhancers (ISEs) and silencers (ISSs) are also an important part of the regulatory program in many alternative splicing events (Black, 2003). ISEs and ISSs may also contribute to the definition of constitutive exons.

Challenges ahead

High throughput technologies are needed for functional validation of computationally derived ESEs and ESSs. Technology development in this area will allow characterization of these *cis*-acting splicing regulatory elements in multiple cell types, under differential conditions, and with different combinations of ESEs and ESSs. This information will allow the rules to be more clearly defined regarding exon definition and splicing regulation. An interesting question is how the "digital" information in terms of ESE and ESS ratio is transformed into the yes or no "analog" decision for exon selection or exclusion by the splicing machinery. It is also widely held that ESEs and ESSs function through their interactions with prospective RNA binding factors (although

a certain ESS may antagonize a nearby ESE without engaging a negative factor). In the human genome, RNA binding proteins are almost as abundant as transcription factors and the majority of them are of unknown function. Assignment of individual ESEs and ESSs to specific mediators will be essential for deciphering regulatory networks. Finally, we know little about the rules for potential co-variation of ESEs and ESSs in exons. When this information becomes available, and by integrating the information with gene expression profiles (for both substrates and regulators), a true splicing regulatory code might be possible. In this postgenomic era, the power of coupled computational and experimental approaches will be used to address some of these fundamental questions.

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Fueling Synapses

The transmission of information across neuronal synapses is an energetically taxing business. Sheng and colleagues monitored the localization of mitochondria following different levels of synaptic activation and discovered that these organelles change their distribution in interesting ways, stalling near synapses when neurons are activated and increasing their movement when neurons are silent (Li et al., 2004 [this issue of *Cell*]).

The neuronal mitochondria that fuel the intense energy demands associated with synaptic transmission are often overlooked. A new study by Li et al. in this issue of *Cell* suggests a role for mitochondria in effecting changes in dendritic and synaptic morphology. Li et al. used a vital marker of mitochondria to study the distribution and movement of the mitochondria in cul-

tured hippocampal neuron dendrites during development and following large-scale manipulations of neuronal activity. Under normal conditions, mitochondria of various shapes and sizes occupied about 50% of the main dendritic shaft. Only a modest fraction of protrusions (including filopodia and spines) possessed mitochondria (~10%–15%), and this number decreased as neurons matured *in vitro*. Assuming that most synapses are active, these numbers, along with previous observations, would seem to indicate that the majority of the energetic demands can be met by mitochondria located proximal to the synapses in the nearby dendritic shaft. Li et al. also examined what happened to the distribution of mitochondria following repeated depolarization (with KCl) of the culture dish. As shown by others, depolarization caused a large increase in the number of filopodia and dendritic spines. The fraction of protrusions that possessed mitochondria also went up significantly following depolarization, from about 4% to about 13%. In a really neat experiment, Li et al. also examined the mitochondrial distribution in an individual neuron in a hippocampal slice before and after electrical stimulation. Regions of the dendrite that were stimulated first showed accumulation of mitochondria in the dendrite followed by a movement of mitochondria into the spines. These changes were not observed in an adjacent region of the dendrite that was not stimulated. Mitochondria can exist in many shapes and sizes; their fission is driven by a GTPase of the dynamin family, Drp1. Li et al. found that dominant-negative Drp1 reduced the mitochondrial content in dendrites whereas overexpression of Drp1 increased the mitochondrial density.

What are the potential functions of the mitochondrial movements that Li et al. have carefully documented? Clearly the large ionic load associated with the opening of neurotransmitter and voltage-gated channels will require the activity of ATP-driven pumps. In addition, the mitochondria resident in protrusions may be important for the buffering of Ca^{2+} associated with NMDA receptor activity and voltage-gated Ca^{2+} channels. Although the changes in mitochondrial movement following depolarization were significant, the fraction of protrusions possessing mitochondria was rather small when compared to the rather dramatic effects of KCl on neuronal morphology. This raises the possibility that the movement of mitochondria into a small number of protrusions marks these synapses for a process as yet undiscovered. What if, for example, the mitochondria are mobilized to fuel the local protein translation or degradation that is important for some forms of synaptic plasticity (Steward and Schuman, 2003)? Related to this point, the apparent trafficking of polyribosomes into a subset of spines has been observed following long-term potentiation (Ostroff et al., 2002). Alternatively, the slow release of Ca^{2+} from mitochondria has been observed (Yang et al., 2003), raising the possibility that a select population of spines could experience sustained Ca^{2+} elevations, and hence sustained activation of Ca^{2+} -dependent enzymes, as a result of the local import of mitochondria. Lastly, the ATPase NSF has been implicated in glutamate receptor (GluR) trafficking (Song et al., 1998), raising the possibility that recruitment of mitochondria to spines is used to fuel GluR exocytosis or endocytosis.

Another issue raised by this study concerns the mech-

anisms by which these intriguing mitochondrial movements into dendritic protrusions are accomplished and regulated. Previous studies on cultured neurons have indicated a role for both microtubules and actin filaments in supporting the back and forth movement of mitochondria along axons and dendrites (Ligon and Steward, 2000; Morris and Hollenbeck, 1995). It should be noted that the average velocity of dendritic mitochondria in the current study (0.4 $\mu\text{m}/\text{min}$) is much, much slower than in the earlier studies (0.1 to 1 $\mu\text{m}/\text{s}$). Nevertheless, it is likely that both types of cytoskeletal elements will regulate the dynamic localization of mitochondria into dendritic protrusions because whereas microtubules are prominent in dendritic shafts, actin filaments play a prominent role in dendritic spine formation and dynamics. If excursion of mitochondria into dendritic protrusions indeed requires a transition to a dynamic actin-based cytoskeleton, it is possible that such transitions are regulated by local synaptic activity or metabolic demand. To understand these issues, we will need to learn more about the complex set of molecular motors that must guide the movement of mitochondria along the cytoskeleton. Likewise, the mechanisms regulating mitochondrial velocity are also unknown. Li et al. find that mitochondrial motility slows upon neuron depolarization, supporting previous suggestions that mitochondria are retained in active regions of the neuron by stalling of mitochondrial movement (Morris and Hollenbeck, 1995).

Finally, this study may be relevant for an understanding of human diseases resulting from mitochondrial dysfunction. *OPA1* and *Mfn2*, genes involved in mitochondrial fusion, are mutated in two neuropathies, Dominant Optic Atrophy (Delettre et al., 2002) and a subtype of Charcot-Marie-Tooth disease (Zuchner et al., 2004), respectively. In addition, a large set of disorders classified as mitochondrial diseases involve either mutations in mitochondrial DNA or in nuclear genes that function in the mitochondria (Wallace, 1999). These diseases have diverse presentations, but many involve neuronal dysfunction, characterized by symptoms as varied as blindness, mental retardation, hearing loss, or epilepsy. These manifestations of specific neuronal dysfunction in the context of a generalized mitochondrial defect have usually been interpreted as evidence for an unusually high metabolic demand in these cells. In light of this new study, it will be interesting to determine whether neurons, because of their unique cellular architecture and signaling functions, have particularly high local demand for mitochondria at sites far from the cell body. If so, some mitochondrial diseases may disrupt neuronal function by interfering with the ability to deliver mitochondrial function at neuronal extensions.

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