able and the results will surely provide further intriguing insights.

Already, it is clear that the *Dscam* exon 6 array uses a new mechanism to achieve ME splicing. Rather than resulting from an absolute physical impediment to splicing, ME behavior appears to arise as an intrinsic consequence of the regulatory mechanism used to select individual exons. How the docker:selector structure might lead to derepression is one of many open questions. The docker:selector duplex might bind to an activator that antagonizes the repressor. Alternatively, the single-stranded selectors might be intron-splicing silencers to which the repressor binds. These possibilities would be distinguished by the effects of selector mutation. A particularly puzzling feature of the model is how docker-selector pairing is regulated. The exon 6.1 selector is only 120 bases downstream of the docker, whereas that of 6.48 is over 11 kb distant. If docker-selector pairing were dictated on a cotranscriptional "first-come, first-served" basis (Eperon et al., 1988), there would be an overwhelming preference for selection of the 5′ proximal exon 6 variants, but this is not observed. Neither does the predicted thermodynamic stability of selector:docker pairs correlate with the frequency of selection of the associated exons. Both observations strongly suggest that selector:docker pairing must be regulated, although the manner of such regulation remains to be elucidated. Open questions notwithstanding, the docker-selector model is so immediately attractive that it seems surprising that it does not obviously apply to any of the other arrays of *Drosophila* ME exons, not even in *Dscam*. Perhaps the power of persistent staring and luck (see Experimental Procedures in Graveley, 2005) will unlock their secrets and possibly reveal some general mechanistic principles underlying this complex form of alternative splicing.

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A Cellular Response to an Internal Energy Crisis

Lack of an appropriate energy supply has been thought to induce cell death in a nonspecific manner by causing a decline in metabolism and a gradual cessation of cellular function. In this issue of *Cell*, Nutt et al. (2005) describe a new mechanism that directly links nutrient availability to apoptosis in *Xenopus* oocytes and show that age-dependent changes in the nutritional state of a cell might lead to caspase activation and apoptotic cell death.

Under normal physiological conditions, most cell types in our body obtain their energy from nutrients that are present in abundance in the extracellular environment. The availability of these nutrients to each cell is "rationed" by the limited amount of trophic factors that control nutrient uptake. Only a few select cell types, such as oocytes, are "self-sufficient" and rely entirely on internal energy stores for survival. Thus, oocytes are an interesting model in which to study the events that occur during a cellular energy crisis. In this issue of *Cell*, Nutt et al. (2005) explore the biochemical events that take place when oocytes exhaust their internal energy stores and define a new pathway regulating cell survival in response to nutrient depletion.

Previous studies have demonstrated that oocytes can be induced to undergo apoptosis regulated by caspases, a family of aspartic proteases necessary for the execution of apoptotic cell death, as well as by both the pro- and antiapoptotic members of the Bcl-2 family of apoptotic regulators. To address how the availability of nutrients may regulate oocyte apoptosis, the authors used oocyte extracts from the frog *Xenopus laevis*. They demonstrated that depletion of stores of glucose-6-phosphate (G6P), an intermediate of glucose metabolism, caused the loss of an inhibitory phosphorylation of caspase-2 by the Ca^{2+}/Calmodulin dependent kinase II (CaMKII), thereby activating caspase-2 and resulting in apoptosis. They also showed that caspase-2 activation occurs upstream of apoptotic events in the mitochondria. This suggests that activation of this protease is an initiating event linking glucose depletion to the induction of apoptosis in oocytes. Caspase-2 is an upstream caspase involved in the initiation of apoptosis induced by cellular stress caused by factors such as DNA damage (Tinel and Tschopp, 2004). However, the phenotype of mice deficient in caspase-2 revealed surprisingly little other than the resistance of their oocytes to DNA damage and naturally occurring cell death (Bergeron et al., 1998). The data in the Nutt et al. paper

may finally explain the phenotype of these mice. If oocytes undergo apoptosis in response to insufficient nutrients, then the loss of caspase-2, a proposed mediator of cell death induced by nutrient depletion, would result in resistance to apoptosis.

Although it is apparent from this study that, during oocyte apoptosis, caspase-2 operates upstream of mitochondrial events and may exert its effects through regulation of the Bcl-2 family, it is not clear which members might be involved. Although the Bcl-2 family member Bad may mediate the apoptotic response to glucose deprivation, Bad is regulated through phosphorylation rather than by cleavage by caspases (Danial et al., 2003). Other proapoptotic Bcl-2 family proteins known to be regulated by caspase-dependent cleavage, such as Bid, are possible candidates. The substrate specificity of caspase-2 is unusual, and very few substrates of caspase-2 have been identified. In the presence of active caspase-2, there is still a lag time until induction of apoptosis in oocyte extracts, indicating that additional signaling steps might also be involved in the suppression of apoptosis by glucose in this system.

Nutt et al. (2005) suggest that a sensor of intracellular energy levels may be an important direct regulator of the apoptotic cascade in oocytes. It is not clear what might serve as the upstream molecular sensor of glucose levels signaling to caspase-2. Although the ratio of ATP to ADP and cAMP levels have been previously reported to mediate cellular responses to energy and nutrients, they are unlikely to play a direct role in this case. The authors demonstrate that suppression of caspase-2-mediated apoptosis by G6P depends on the continued operation of the pentose cycle and production of NADPH. In fact, NADPH can suppress caspase-2 activation in oocyte extracts in the absence of G6P. Therefore, the energy sensor operating in this system may be measuring NADPH or the ratio of NADPH to NADP+.

The findings described by Nutt et al. (2005) may be applicable to other cellular systems. Caspase-2 is involved in the programmed cell death of mouse sympathetic neurons deprived of nerve growth factor (NGF). Given that NGF also regulates glucose uptake in neurons, it is conceivable that caspase-2 could be activated in sympathetic neurons deprived of NGF due to the reduction in intracellular glucose levels. Other caspases may also be involved in mediating apoptosis induced by energy crisis. For example, caspase-8 has been implicated in the apoptosis of human hepatoma cells induced by glucose starvation (Suzuki et al., 2003). In this system, caspase-8 is activated upstream of apoptotic events in mitochondria. Caspase-8 activation is suppressed by a new member of the AMP-dependent protein kinase family, ARK5. Although the mechanism of inhibition of caspase-8 by ARK5 in this system is not clear, this kinase is known to block the activity of caspase-6 through phosphorylation (Suzuki et al., 2004). Therefore, altering the phosphorylation state of caspases may represent a common mechanism to control the induction or repression of apoptosis in response to cellular energy levels.

Direct coupling of cellular glucose metabolism to caspase activation has a number of important implications. First, it suggests that glucose metabolism may play a larger part in tissue homeostasis than previously appreciated. Although glucose is generally in abundant supply in the extracellular environment of a multicellular organism, the uptake of glucose is regulated by extracellular trophic factors and their intracellular signaling mediators (recently reviewed in Hammerman et al., 2004). The best characterized of these mediators is the proto-oncogene Akt. Akt can directly regulate cellular glucose uptake by inducing expression of the glucose transporter, Glut1, at the plasma membrane and targeting hexokinase activity to the mitochondria, possibly in part through its ability to influence the inhibitory phosphorylation of Bad. The ability of activated Akt to promote cell survival has been recently demonstrated to depend on glucose availability because, in glucose-free media, constitutively active Akt is unable to support sustained cell viability despite the availability of an alternative energy source. The importance of sustained glucose metabolism for the effects of Akt is further illustrated by the fact that enhancement of glucose uptake and utilization can partially restore cell viability in the absence of Akt function (Rathmell et al., 2003). Thus, we can consider glucose as an essential cofactor of Akt signaling. It is interesting to speculate that perhaps the reason that the prosurvival effects of Akt depend on the presence of glucose is because glucose is required to suppress the caspase-2-mediated apoptotic pathway. This hypothesis would further predict that, perhaps in caspase-2-deficient cells, Akt activity would no longer require the presence of glucose.

Furthermore, coupling metabolic state and cell survival may provide a mechanism for regulating cell numbers during aging at both the cellular and organismal level. Nutt et al. (2005) noted an age-related decrease in the activity of G6P dehydrogenase in murine oocytes, which could contribute to their decreased viability. It is attractive to speculate that a similar mechanism may operate in other tissue types such as neurons or hepatocytes, which are particularly sensitive to glucose levels and are affected by deleterious age-related changes. Proteins involved in Ca^{2+} signaling (including an isoform of CaMKII), cAMP signaling, MAP kinase signaling, cell survival, and mitochondrial metabolism are among those downregulated in response to DNA damage during aging of the human brain (Lu et al., 2004). These changes could directly contribute to the slowing of cellular metabolism observed during normal aging, leading to lower internal levels of glucose and, in sensitive tissues, contributing to the decline in cell resistance to proapoptotic stimuli and eventual cell loss. Additionally, age-related changes have been observed in hormone and growth-factor expression, which could indirectly affect both survival and cellular metabolism through their effects on Akt and other survival pathways. In either case, the gradual decline in metabolism and intracellular glucose could directly lead to cell death through either activation of caspase-2 or an alternative proapoptotic pathway.

It has long been appreciated that nutrient deprivation due to either growth-factor withdrawal, embolism-induced loss of blood supply, or age- and disease-related changes in metabolism can lead to cell death. However, it has been assumed that lack of nutrients is likely to induce cell death due to the gradual shutdown of cellular metabolism and consequent cessation of all

cellular functions. The data presented by Nutt et al. (2005) demonstrate that nutrient deprivation is, instead, directly linked through caspase-2 to the apoptotic machinery and that active suppression of this pathway by continuous glucose metabolism is required for survival. The fact that this pathway can be activated due to age-related changes also suggests that metabolic decline may contribute directly to cellular and organismal aging by inducing caspase activation.

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