is mediated by increased kinase or decreased phosphatase activity. Perhaps low nuclear Ca^{2+} may preferentially prevent dephosphorylation of NFAT4 through calcineurin, which also moves into nuclei during receptor activation. Alternatively, although nuclear export kinases are not Ca^{2+} sensitive, the rate of rephosphorylation of NFAT4 may be faster than NFAT1 with lower nuclear Ca^{2+}.

The studies of Kar and Parekh (2015) provide a glimpse of how global Ca^{2+} spikes and local Ca^{2+} influx can dually impinge upon the activation Ca^{2+}-dependent nuclear regulators. Together, these distinct Ca^{2+} signals tune the fidelity of transcriptional responses mediated by two closely related transcription factors. For immune cells such as T cells, Ca^{2+} is therefore not just a triggering signal that sets things rolling—Ca^{2+} signals continue to spatially and temporally coordinate and refine the ultimate response to graded receptor activation. Despite intense investigation, the nature of nuclear Ca^{2+} signaling remains a contentious issue. The nuclear envelope sequesters Ca^{2+} and the outer membrane has the same Ca^{2+} signaling machinery as normal ER. Although the inner membrane is entirely separated from the outer membrane by nuclear pores and contains very different protein machinery, Ca^{2+} release channels and other signaling proteins do appear to be present in the inner membrane (Resende et al., 2013). However, given the complete permeability of nuclear pores to Ca^{2+}, the case for considering any autonomy in nucleoplasmic Ca^{2+} signaling is a difficult one (Bading, 2013). Indeed, Kar and Parekh (2015) nicely reveal the coincidence of nuclear and cytoplasmic Ca^{2+} signals, and their results militate against local nuclear Ca^{2+} changes as mediating NFAT activation. Nevertheless, just as the authors show that the subtle local Ca^{2+} signals at the PM have such a profound role in initiating transcription factor responses, it would be interesting to investigate whether local nuclear Ca^{2+} signals may play an important role in refining and coordinating other transcriptional and chromosomal regulatory events within the nucleus.

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Life after MOMP

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In this and a recent issue of *Molecular Cell*, Liu et al. (2015) and Ichim et al. (2015) report that low levels of caspase activity triggered by limited mitochondrial outer membrane permeabilization (MOMP) promote genomic instability that drives tumorigenesis, providing a novel and unexpected link between these effectors of apoptosis and cancer initiation.

Compelling functional studies over the last two decades have established the concept that programmed cell death by apoptosis is a natural barrier to the development of cancer. Indeed, resistance to apoptosis is an established hallmark of cancer (Hanahan and Weinberg, 2011). However, as is often the case in science, new and unexpected complexities to this axiom are starting to emerge. An earlier report had shown that not only could cells transiently exposed to an apoptotic stimulus (ethanol) recover, but they could undergo oncogenic transformation (Tang et al., 2012). Now, two studies published in *Molecular Cell* further reinforce this paradoxical phenomenon with new mechanistic insight. Ichim et al. (2015) as well as Liu et al. (2015) report that limited mitochondrial permeabilization and low caspase-3 activity induced by sub-lethal doses of apoptotic stimuli *promote* DNA damage, genomic instability, and carcinogenesis. So how does limited caspase activation have such a markedly different outcome than the high caspase activation seen during cell death?

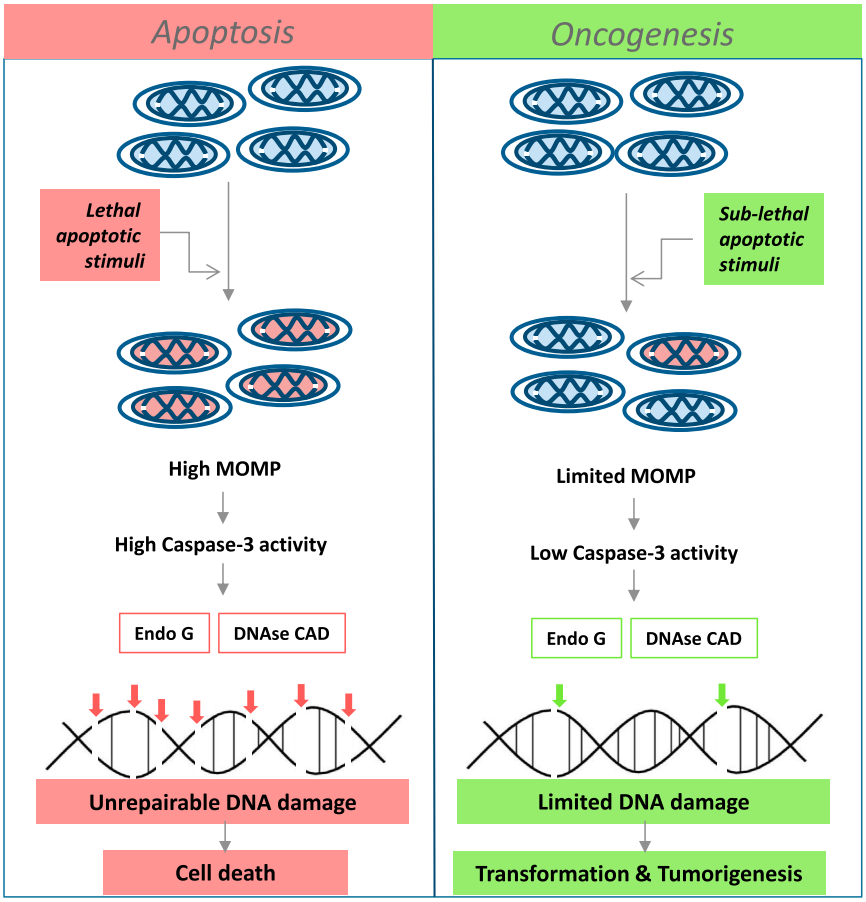

Figure 1. Events after MOMP that Induce Either Cell Death or Cellular Transformation
Sub-lethal doses of apoptotic stimuli induce limited MOMP and low levels of caspases, which paradoxically result in the increased ability of cells to mutagenize and become transformed.

A key event during apoptosis is mitochondrial outer membrane permeabilization (MOMP), which is regulated by the Bcl-2 family proteins. MOMP results in the release of a number of proteins from the mitochondrial intermembrane space. These include cytochrome *c*, which triggers activation of the caspase proteases; EndoG and AIF, which can cause DNA cleavage; and the Smac and Omi proteins, which inactivate the caspase inhibitory IAPs. Generally speaking, the event of MOMP has been considered the "point of no return" because cells become irreversibly committed to death. This is because the proteins released after MOMP are potent inducers of cell death and also because mitochondrial damage following MOMP can result in catastrophic energetic failure for most cells. Notable exceptions to this are sympathetic neurons and certain cancer cells that can survive and recover if the MOMP-inducing stimuli are removed (Colell et al., 2007; Deshmukh et al., 2000; Gama et al., 2014; Martinou et al., 1999). In HeLa cells, recovery after MOMP-inducing apoptotic stimuli was possible because a subset of mitochondria failed to undergo MOMP due to high Bcl-2/Bcl-XL levels and were maintained intact under these conditions—a phenomenon termed incomplete MOMP (iMOMP) (Tait et al., 2010). These results show that MOMP need not be an all-or-nothing event in cells exposed to apoptotic stimuli. However, what are the long-term consequences to a cell that recovers from MOMP?

The difficulty in directly addressing this question has stemmed, at least in part, from the lack of sensitive assays that can detect very low levels of MOMP or caspase activity at the single-cell level. Ichim et al. and Liu et al. tackle these issues by utilizing clever approaches to detect MOMP and caspase-3 activity using fluorescent protein re-localization, chemically dimerizable FKBP/FRB domains (Ichim et al., 2015), and a non-invasive caspase-3 reporter (Liu et al., 2015). The first approach uses two fluorescent fusion probes: a cytosolic probe containing GFP-FKBP and a mitochondrial inner membrane-localized probe containing a mCherry-FRB. These two fusion proteins can dimerize only when the cell undergoes MOMP and the cytosolic probe has access to the inner mitochondrial membrane. In the second approach, EGFP was made unstable by linking it to a polyubiquitin domain. As a caspase-3 cleavage site was introduced in between EGFP and the polyubiquitin domain, caspase activity would result in the stabilization of EGFP because of removal of the polyubiquitin domain. Using these imaging techniques, which provide a sensitive readout of MOMP and caspase-3 activation in live cells, the authors set out to examine what happens in cells exposed to sub-lethal apoptotic stimuli. First, they found that sub-lethal doses of apoptotic stimuli induced MOMP only in a subset of mitochondria. These surviving cells only exhibited limited caspase-3 activation. Second, both groups examined the fate of cells that survived caspase-3 activation and found low levels of caspase-3 activity to *induce* DNA damage. The consequence of this non-lethal DNA damage was the accumulation of mutations that *increased* chromosomal instability. Third, since genomic instability is known to promote carcinogenesis, the authors then tested whether the limited MOMP-induced DNA damage could lead to increased oncogenic transformation. Indeed, both studies found that limited MOMP-induced genomic instability *potentiated* cellular transformation (assays of E1A/KRAS- and $p19^{Arf}$-induced transformation and anchorage-independent growth in soft agar). Liu et al. also found chemical-induced skin carcinogenesis to be reduced in the absence of caspase-3. These results are striking and challenge the notion that caspase activation is always anti-tumorigenic. While both groups reach the same overall conclusions, some mechanistic details were different. For example, Ichim et al. report that DNA damage induced by

caspase-3 was mediated via CAD (caspase-activated DNase), which is also the effector of caspase-3-mediated DNA fragmentation during apoptosis. In contrast, Liu et al. found EndoG to be important for the DNA damage in their model (Figure 1).

What do these studies mean for cancer therapy? If the model holds true, then anticancer therapies aimed at inducing apoptosis would have the risk of actually increasing the likelihood of further malignant progression or development of secondary cancer. While most cells would undergo apoptosis and die with adequate doses of chemotherapy or radiation therapy, cells that may survive post-MOMP could play a significant role in cancer relapse and drug resistance by acquiring new mutations. While these have been previously attributed to the direct mutagenic effects of the genotoxic cancer therapy, they could also arise via limited MOMP and caspase activity in the surviving cells. Importantly, such mutations may also accumulate in non-genotoxic cancer therapy where MOMP occurs.

Beyond tumorigenesis, could this phenomenon be important in a physiological context? Could limited MOMP be engaged in cells to permit low levels of caspase activity? There have been an increasing number of non-apoptotic contexts in which low levels or locally restricted caspase activity is utilized. In the brain, for example, caspase activity has been linked with axon pruning as well as synaptic plasticity during learning and memory (Hyman and Yuan, 2012). Thus, cells may be capable of exquisitely controlling the extent and location of MOMP to allow limited caspase activation. Could there be situations where limited MOMP is also engaged to induce low levels of DNA damage? It is plausible that sub-lethal levels of caspase activation and DNA damage play a role in priming cell populations to environmental stresses by heightening the DNA repair pathways. Low levels of MOMP and non-lethal DNA damage could also be physiologically beneficial in some contexts as it would be predicted to promote genetic diversity. While these are still speculations, life after MOMP may be a reality for many cells and perhaps one with even more opportunities.

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