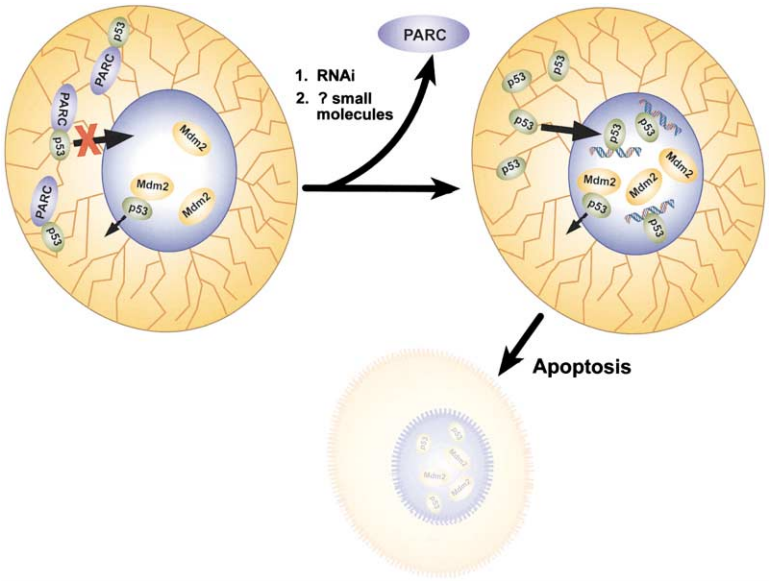

Cytoplasmic Sequestration of p53 by Parc

Neuroblastomas can overexpress Parc, which sequesters p53 within the cytoplasm, possibly by tethering p53 to the cytoskeleton. In addition to facilitating p53 degradation, Mdm2 also fosters p53 cytoplasmic localization by enhancing its export from the nucleus. Interference with Parc expression through RNAi results in nuclear translocation of p53 and apoptosis.

damaging agents still caused a rapid stabilization and translocation of p53 into the nucleus, despite sequestration of p53 by Parc in the cytoplasm of cells before exposure. Parc's lack of effect on the translocation of p53 in cells after DNA damage will need to be reconciled with its apparently profound effect on apoptosis after DNA damage. Future studies addressing this and other questions will likely provide interesting mechanistic insights about the interactions between stress responses and p53-Parc interactions.

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Toxin-Antitoxin Pairs in Bacteria: Killers or Stress Regulators?

Plasmid toxin-antitoxin systems, which kill daughter cells that fail to inherit the plasmid genome, have chromosomal homologs in eubacteria and archaea. In this issue of *Cell*, Pederson et al. show that the *E. coli* RelE toxin cleaves mRNA in the ribosomal A site, potentially allowing it to function as a stress regulator during amino acid starvation.

Bacterial toxin-antitoxin (TA) systems generally consist of a toxic protein and a cognate antitoxin or antidote protein that is proteolytically unstable (Gerdes, 2000; Zielenkiewicz and Ceglowski, 2001). Because the antitoxin is degraded continuously, new protein synthesis is required to maintain a high steady-state level of antitoxin, which forms an inhibitory complex with the toxin. Some TA systems affect the inheritance of the plasmids that encode them by acting as "addiction modules." In daughter cells that fail to inherit the plasmid, degradation of the antitoxin in the absence of new protein synthesis leads to cell death. Intriguingly, TA systems are also encoded in the genomes of a variety of bacteria and archaeabacteria. *Escherichia coli* has at least three TA systems, *mazEF* (*chpAI-chpAK*), *relBE*, and *dinJ-yafQ* (Gotfredsen and Gerdes, 1998). Transcription of the *mazEF* operon is inhibited during amino acid starvation, leading to diminished levels of the MazE antitoxin and apparent cell killing by MazF (Aizenman et al., 1996). Based on this work, it was postulated that starved *E. coli* undergo altruistic cell death, helping to ensure survival of the population. However, recent studies suggest that chromosomal TA toxins may be bacteriostatic rather than bacteriocidal. Specifically, Pedersen et al. (2002) demonstrated that expression of the MazF or

RelE toxins halts cell growth, which can resume if the cognate antitoxin is expressed at a later time. Hence, under inclement nutritional or environmental conditions, chromosomal TA systems could temporarily inhibit specific cellular processes, arresting growth until more favorable conditions return.

In *E. coli*, the *relBE* TA system is part of the complex cellular response to amino acid deprivation that includes increased synthesis of stress proteins and amino acid biosynthetic enzymes, increased proteolysis, and the inhibition of tRNA and rRNA transcription (Cashel et al., 1996; Christensen et al., 2001). After amino acid starvation, the levels of the RelB antitoxin fall as a result of decreased translation and Lon-dependent proteolysis (Christensen et al., 2001). This reduction in RelB concentration frees the RelE toxin (RelB inhibits RelE by complex formation), which then acts to decrease translation in some fashion.

A collaborative effort by the groups of Kenn Gerdes and Måns Ehrenberg, reported in the current issue of *Cell*, has begun to uncover the molecular details of how RelE inhibits translation and affects reversible cell stasis (Pedersen et al., 2003). RelE has or induces a novel ribonucleolytic activity that cleaves mRNA codons between the second and third nucleotides in the A site of the ribosome in vitro. This cleavage occurs with a marked specificity for certain codons, with the UAG (amber) stop codon and the CAG (glutamine) codon being cleaved at the highest rates. RNA messages cleaved in this manner are no longer competent to direct further protein synthesis and are not recognized by the protein release factors that catalyze normal translation termination. Hence, after RelE cleavage, ribosomes become trapped in an inactive state at the 3′ ends of truncated mRNAs. Without intervention (see below), these ribosomes cannot participate in further rounds of translation. One interesting question concerns the specificity of RelE-mediated mRNA cleavage in cells. If some mRNAs were more sensitive to RelE-mediated cleavage than others, then this could provide a way of saving scarce amino acids for translation of messages encoding proteins needed to recover from amino acid starvation.

Is RelE a nuclease or does it induce a nucleolytic activity in the ribosome? The latter possibility needs to be considered because purified RelE does not cleave mRNA in the absence of ribosomes (Pedersen et al., 2003), and ribosome binding normally protects mRNA in and around the A site from RNase cleavage (Huttenhofer and Noller, 1994). Of course, RelE could have a shape that allows access to codons in the A site, and binding to the ribosome could activate RelE nuclease activity. Moreover, RelE-mediated cleavage of mRNA codons can also be observed in the ribosomal E site and in initiation complexes with the 30S subunit (Pedersen et al., 2003). Hence, if a part of the ribosome itself catalyzes mRNA cleavage in a RelE-dependent manner, then this element would need to be flexible enough to act in each distinct ribosomal environment where cleavage is observed.

How do cells rescue ribosomes trapped on RelE-cleaved mRNAs and recover from translation arrest? Ribosomes stalled at the 3′ ends of mRNA are typically substrates for SsrA (tmRNA), which contains a tRNA-like domain that can be charged with alanine and a reading frame for a peptide tag (Karzai et al., 2000). SsrA engages stalled ribosomes like a tRNA, adds its charged alanine to the nascent chain, directs translational addition of the SsrA tag to the C terminus of the polypeptide on the stalled ribosome, and then recruits release factors that terminate translation and permit ribosome recycling. Pedersen et al. (2003) cite unpublished data that SsrA can act on RelE-trapped ribosomes in vitro and is required for efficient recovery from RelE-mediated bacteriostasis in vivo. Because SsrA-tagged proteins are substrates for several intracellular proteases (Karzai et al., 2000), the polypeptides synthesized by RelE-arrested ribosomes would be rapidly degraded after SsrA rescue. This should permit recovery of free amino acids that could then be used for translation of new proteins required for the cellular response to amino acid starvation. It is interesting that none of the codons of the SsrA tag are good targets for RelE-mediated cleavage in vitro and, as a result, this system could potentially function during the arrest period while RelE was still active. SsrA rescue of stalled ribosomes may also result in degradation of the truncated mRNA, preventing further trapping of ribosomes on damaged messages.

The regulatory circuits that control *relBE* expression are interwoven and have the potential to be quite intricate. First, RelB, which binds to and inhibits RelE, is a substrate for the Lon protease, and translational inhibition is sufficient to inactivate RelB in a Lon-dependent manner (Christensen et al., 2001). It is not known whether Lon-mediated degradation of RelB is also enhanced by amino acid starvation, whether the RelB•RelE complex is a better or worse Lon substrate than free RelB, or if feedback exists between global degradation and RelB proteolysis. Second, like many other antitoxins, RelB represses transcription of its own gene as well as the adjacent toxin gene (Gerdes, 2000; Zielenkiewicz and Ceglowski, 2001). RelB is a member of the ribbon-helix-helix family of transcription factors, but little else is known about its structure, regulatory interactions, or inhibition of RelE. Third, RelE, like many other toxins, functions as a corepressor with RelB. What is the mechanism of this corepression? Finally, RelE-mediated mRNA cleavage of the *relBE* transcript could affect translation of RelB and/or itself. Understanding this genetic and biochemical circuitry at a quantitative and predictive level presents an interesting future challenge.

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Pars, PI 3-kinase, and the Establishment of Neuronal Polarity

As reported in this issue of *Cell* (Shi et al., 2003), the protein complex consisting of mPar3, mPar6, and atypical protein kinase C is selectively localized to the axonal growth cone of cultured hippocampal neurons and is required for specification of the axon.

As recent ceremonies in Stockholm attest, analysis of genetically tractable organisms has proved immeasurably valuable as a starting point for unraveling signaling processes in mammalian cells. The report by Shi et al. (2003) offers yet another example of the power of this approach. They show that genes first identified in *C. elegans* play a central role in governing the development of neuronal polarity.

Most nerve cells develop a single axon and several dendrites, which are structurally and molecularly distinct. Neurons developing in culture, where spatially organized extracellular cues are absent, follow an intriguing internal program that leads to cell polarization (Fukata et al., 2002). Cells initially establish several, short, identical neurites, which apparently compete to become the cell's axon. The neurites undergo brief spurts of growth followed by retraction until one neurite undergoes a period of protracted growth, breaking the initial morphological symmetry. This neurite becomes the axon and the remaining neurites become dendrites (Dotti et al., 1988). It is thought that small, randomly occurring growth events are amplified by positive feedback into further growth, eventually allowing one neurite to exceed a critical length and become specified as the axon; negative feedback prevents other axons from forming (Goslin and Banker, 1989; Andersen and Bi, 2000).

While the evidence supporting this view emerged more than a decade ago, the molecular mechanisms that underlie the development of polarity have remained annoyingly elusive. Enhanced actin-based motility and microtubule extension are required to support elongation of the emerging axon, which involves local activation of cdc42 and rac1, small GTPases that induce actin-driven lamellipodial activity, and local accumulation of CRMP-2, a tubulin binding protein (Bradke and Dotti, 2000; Fukata et al., 2002). But the upstream signaling pathways that restrict growth to a single neurite are not known.

Here is where the genetics-inspired strategy of Shi et al. has come into play. These authors begin by focusing on mPar3 and mPar6, the mammalian homologs of two genes required for the generation of anterior-posterior polarity in early *C. elegans* embryos ("par" from "partitioning defective"). Homologous genes were subsequently found to play essential roles in asymmetric cell division in Drosophila and in the development of polarity by mammalian epithelial cells in culture (Wodarz, 2002). mPar3 and mPar6, which interact via their PDZ domains, form a scaffolding complex for atypical protein kinase C (aPKC) and for the activated forms of rac1 and cdc42. Using neuronal cultures, Shi et al. show that before the establishment of polarity, endogenous mPar3 and mPar6 are present in all neurites. In polarized neurons, they are highly concentrated in the axonal growth cone and virtually undetectable in the future dendrites. When expressed by transfection prior to the establishment of polarity, mPar3 and mPar6 fail to become restricted to a single growth cone (presumably a dominant negative effect arising from overexpression), and the cells fail to become polarized. The activity of the aPKC component of the complex also appears to be required for polarity to develop normally. Inhibitors of aPKC block the development of polarity and prevent mPar3/mPar6 from becoming concentrated in a single growth cone.

What signaling components lie upstream of mPar3/mPar6, and how might they govern localization of the Par complex? Based on analogies with the signaling pathways that lead to the development of asymmetry and directed migration in cells undergoing chemotaxis, Shi et al. make the inspired guess that PI 3-kinase plays a role. Local activation of PI 3-kinase generates $PI(3,4,5)P_3$ in the cytoplasmic leaflet of the membrane and recruits proteins with $PI(3,4,5)P_3$ binding domains. Shi et al. provide evidence that PI 3-kinase activity is concentrated in the axonal growth cone (as well as in the cell body), and demonstrate that inhibitors of PI 3-kinase block the development of polarity and cause mislocalization of mPar3 and mPar6. Overexpression of PTEN, which dephosphorylates $PI(3,4,5)P_3$, also disrupts the development of polarity. Shi et al. suggest that mislocalization of the Par complex and disruption of polarity are direct consequences of altering $PI(3,4,5)P_3$ metabolism in the growth cone. Alternatively, they could result from changes in membrane trafficking, which is also regulated by phosphoinositides (Simonsen et al., 2001).

Shi et al. hypothesize that local PI 3-kinase activity recruits the Par complex to the growth cone, which could enhance actin activity via its affinity for activated cdc42/rac1 and influence microtubule polymerization via the activity of aPKC. Previous studies have demonstrated that activation of cdc42 and rac1 enhances PI 3-kinase activity and can stimulate formation of the Par complex. Such a signaling loop could contribute to the positive feedback pathway required to amplify small fluctuations in growth cone signaling into a symmetry-breaking event (Andersen and Bi, 2000).

As with any important study, these observations raise many questions and possible directions for future research. What signals locally activate PI 3-kinase in the