whether E2F3 is a repressor of *Arf* also in other cell types and other species. Notwithstanding these open questions, the identification of E2F3 as a pivotal repressor of *Arf* is an important addition to our understanding of how E2Fs control cell proliferation and apoptosis in normal and transformed cells.

The data presented by Aslanian et al. also imply that, similarly to the *INK4a/Arf* locus, the E2F3 locus affects the activity of the pRB/E2F and ARF/p53 pathways. However, while the products of the *INK4a/Arf* locus upregulate the activities of pRB and p53, the products of the E2F3 locus, E2F3a and E2F3b, antagonize pRB and ARF/p53 activities, respectively. This raises the interesting possibility that under some settings amplification of the E2F3 locus may contribute to tumor development, similarly to inactivation of the *INK4a/Arf* locus that is observed in many human cancers (Ruas and Peters, 1998). Indeed, amplification of the E2F3 locus was documented in some human bladder tumors. It remains to be determined whether such amplification exists also in other tumors.

E2F1 can induce both proliferation and apoptosis, and understanding how its proapoptotic activity is inhibited during normal proliferation is a challenging problem. Of note, the E2F3-mediated repression of *Arf* most probably does not fully account for this inhibition: First, the role of ARF in E2F1-induced p53-dependent apoptosis is debatable. Second, E2F1 induces apoptosis also in a p53-independent manner and this is attributed, at least in part, to direct transcriptional activation of proapoptotic genes including p73, Apaf-1, caspases, and BH3-only proteins (Ginsberg, 2002). Unlike *Arf*, expression of these proapoptotic genes is growth regulated peaking at or near the G1/S transition. This implies that mechanisms acting downstream to their transcriptional activation may play a critical role in inhibition of E2F-induced apoptosis during normal growth. Unraveling these molecular mechanism(s) remains a challenging problem.

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GPI-Anchor Synthesis: Ras Takes Charge

A new study shows that Ras2 regulates GPI-anchor synthesis in the ER. Reciprocally, the targeted enzyme GPI-GlcNAc transferase regulates Ras2 signal output. This novel intersection of Ras2 signaling and an ER-localized protein complex has interesting implications for Ras function.

Eri1 (for endoplasmic reticulum-associated Ras inhibitor 1) was recently isolated in a genetic screen for yeast with growth defects that were additive with defective protein kinase C: a regulator of cell wall biogenesis (Sobering et al., 2003). Interestingly, *eri1*Δ yeast are heat shock sensitive and grow invasively on agar, phenotypes that indicate hyperactive Ras signaling. The heat shock sensitivity of *eri1*Δ strains is milder than that induced by constitutively active $Ras2^{Val19}$ but is suppressed by deleting Ras2 or overexpressing Ira2 (a GTPase activating protein). Eri1 and Ras2 form a complex when coexpressed in yeast, although immunofluorescence microscopy shows Eri1 is localized to the endoplasmic reticulum (ER).

*eri1*Δ cells have an additional phenotype that cannot be explained by increased Ras signaling: they grow normally at 23°C but not at 37°C. It is the investigation of this *ts* (temperature-sensitive) phenotype that forms the intriguing second part of the story published in the May 28 issue of *Cell* (Sobering et al., 2004). Levin and colleagues show that *eri1*Δ cells are extremely sensitive to lysis at the nonpermissive temperature, a phenotype that is rescued by increasing the osmotic pressure of the growth media and therefore indicative of a cell wall defect. A genetic screen for multicopy suppressors of *ts* growth then turned up GFA1, an enzyme that catalyzes production of glucosamine-6-phosphate—the rate-limiting step for UDP-N-acetylglucosamine (UDP-GlcNAc) production. The conclusion that GFA1 drives up cellular levels of glucosamine-6-phosphate to compensate for loss of Eri1 was confirmed by showing a similar rescue of growth at 37°C if *eri1*Δ cells were supplied with an exogenous source of glucosamine.

UDP-GlcNAc is used for protein N-glycosylation, and the synthesis of chitin and GPI-anchors. Chitin and GPI-anchored proteins are, respectively, minor and major structural components of the yeast cell wall. Defects in N-glycosylation and chitin synthesis were excluded by showing normal maturation of N-glycosylated CPY and by the finding that chitin synthesis is actually increased, partly because of an upregulation of endogenous GFA1

in *eri1*Δ cells. There was also no increase in the severity of the *ts* phenotype when chitin synthase was deleted. In contrast, maturation of the GPI-anchored Gas1 protein was severely retarded, with a concomitant failure to transport Gas1 out of the ER. Biochemical analysis identified the cause as failure of GPI-anchor synthesis.

The first step in the biosynthesis of GPI-anchors is transfer of GlcNAc from UDP-GlcNAc to an acceptor phosphatidylinositol molecule, catalyzed by a multisubunit ER-localized enzyme complex: GPI-GlcNAc transferase. Direct assay of this enzyme in *eri1*Δ cells revealed 2% residual activity, identifying the phenotype as a problem of GPI-anchor synthesis. Four proteins make up the yeast GPI-GlcNAc transferase, Gpi1, Gpi2, Gpi3, and Gpi15. The new data suggest that Eri1 may be a fifth: for example, Gpi2 is present in Eri1 immunoprecipitates when the proteins are coexpressed, and, analogous to the original results with Eri1, $Ras2^{Val19}$ can form a complex with Gpi2 if the Ras effector domain is intact. Furthermore, deletion of Gpi1 faithfully replicates the *eri1*Δ phenotype, including hyperactive Ras signaling. Ras2 also regulates UDP-GlcNAc activity: ectopic expression of $Ras2^{Val19}$ inhibits UDP-GlcNAc activity to <5% wild-type, whereas in *ras2*Δ cells its activity is increased 8- to 11-fold.

The results show that GPI-GlcNAc is a novel Ras effector, but one whose activity is negatively regulated by activated Ras. Simultaneously, GPI-GlcNAc negatively regulates Ras. The authors propose that Ras, in parallel, inhibits GPI-GlcNAc and stimulates adenyl cyclase, the established Ras effector. They also propose that the hyperactive Ras phenotype in *eri1*Δ and *gpi1*Δ cells is a consequence of losing an inhibitor of Ras signaling—GPI-GlcNAc transferase—thus in this model the hyperactive Ras phenotype is mediated by adenyl cyclase. Ras2 therefore regulates cell wall architecture by inhibiting GPI-anchor protein synthesis and perhaps by increasing chitin production. The resulting changes in cell wall structure could be important in the change to filamentous/invasive growth.

This crosstalk between Ras signaling, GPI-anchor synthesis, and cell wall structure adds an exciting new perspective to Ras signal transduction. There are, however, some as yet unanswered questions. For example, exactly what is the mechanism whereby Ras2 and GPI-GlcNAc are reciprocally regulated? The authors advocate that this occurs through a direct protein/protein interaction; the data show that complex formation between Eri1, Gpi2, and Ras2 is possible, although precisely which component(s) of GPI-GlcNAc directly contacts Ras2 remains to be identified. Nevertheless, an important issue here is that Ras and GPI-GlcNAc are localized to nonoverlapping membranes (plasma membrane and ER, respectively), and even in cells ectopically expressing Eri1 and Ras2 less than 20% of the proteins colocalize on the ER (Sobering et al., 2003). It therefore remains formally possible that the actual regulation of GPI-GlcNAc activity by Ras2 is downstream of adenyl cyclase; if so, then GPI-GlcNAc activity in $Ras2^{Val19}$ cells should be restored by high-copy expression of PDE2; a cAMP phosphodiesterase.

What are the implications of this novel intersection of Ras2 signaling and GPI-anchor production in the ER for Ras function in mammalian cells? Although Ras2 and mammalian Ras utilize different effector proteins, the Levin study will surely prompt investigation of whether mammalian GPI-GlcNAc transferase is also regulated by Ras. Mammalian Ras proteins transiently associate with the ER for posttranslational processing of the C-terminal CAAX motif. Although it is possible that during biosynthetic transit GlcNAc transferase and Ras could reciprocally inhibit each other's activity, this presupposes that there is sufficient Ras present in the ER at steady state to regulate GlcNAc transferase. To date, only ectopically expressed mutant forms of Ras appear to stably associate with the ER (Chiu et al., 2002).

If the regulation of GPI-GlcNAc by Ras2 is indirect, the hyperactive Ras2 phenotype in *eri1*Δ and *gpi1*Δ cells may reflect a change in the composition of the yeast cell membrane that allows increased Ras2 signaling from the plasma membrane rather than loss of a direct inhibitory interaction with GPI-GlcNAc. In mammalian cells, the lateral segregation of Ras proteins to different plasma membrane microdomains has a profound effect on signal output. The nature of these domains is debated, but cholesterol, actin, and scaffold proteins are all important in their organization (Hancock, 2003). Mutations in GPI-GlcNAc that block GPI-anchor synthesis cause substantial increases in plasma membrane cholesterol (Abrami et al., 2001) that could influence Ras microlocalization and signal output. Given that yeast also have ergosterol-dependent (rather than cholesterol-dependent) lipid raft domains (Bagnat and Simons, 2002), these concepts from mammalian biology may be relevant.

Finally, there is an interesting, albeit rare hematological disorder, PNH, characterized by somatic mutations in the catalytic subunit of mammalian GlcNAc transferase. GPI-deficient hematopoietic stem cells have a growth advantage over normal GPI-anchor-expressing stem cells that allows for their clonal expansion (Inoue et al., 2003). The reason why is poorly understood and the subject of much speculation; in light of the current paper, we can add the suggestion of increased basal Ras signaling—this would at least explain the recently reported ubiquitous upregulation in PNH cells of EGR-1, a MAPK-regulated transcription factor (Inoue et al., 2003). Whatever the molecular mechanisms in play, crosstalk between Ras signaling and GPI-anchor synthesis opens a Pandora's box of intriguing possibilities for yeast and mammalian cell biologists.

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Putting the Squeeze on Mechanotransduction

Both mechanical and chemical stimuli guide tissue function. In a recent paper, Tschumperlin et al. proposed that pressure acting on airway epithelium elicits mechanotransduction not by directly altering biochemical signaling but by regulating extracellular fluid volume to modulate ligand-receptor interactions.

Asthma is generally considered an inflammatory disease, often triggered by allergy or infection. It involves progressive airway remodeling to thicken the bronchial wall and hyperresponsiveness of airways to contractile stimuli. Remodeling contributes to decreased airflow during constriction and possibly to smooth muscle hyperresponsiveness. Asthma research and treatment have focused largely on mechanisms of inflammation. However, based on the notion that airway constriction is an intrinsically mechanical event, some investigators have begun to explore the idea that remodeling could be a consequence of airway constriction. Their work has used a variety of model systems to show that mechanical stimulation of airway epithelium can trigger many of the events involved in airway thickening, including cell proliferation and deposition of a collagenous extracellular matrix. This group has now published evidence for a novel mechanism for mechanotransduction that radically departs from current thinking in this area.

The ability of cells to convert mechanical forces into biochemical regulatory information is crucial for normal physiology and pathological processes in many tissues. Examples include bone adaptation to exercise or microgravity and the strong link between locations of atherogenesis and regional hemodynamics. Force or strain can activate mechanosensitive ion channels at the cell surface, alter the conformation of extracellular matrix proteins, or alter the function of tension-sensitive components of the cytoskeleton (Davies, 1995). Forces may also alter physical properties of membrane microdomains such as lipid rafts or caveolae to control assembly of signaling complexes (Ferraro et al., 2004). Thus, it is generally thought that cells contain force-sensing elements and are capable of integrating a complex array of environmental cues.

A recent paper (Tschumperlin et al., 2004) provides evidence for a distinct model for mechanotransduction. Tschumperlin and coauthors study cultured airway epithelium using application of hydrostatic pressure as a model for mechanotransduction. Previous studies from these authors have shown that application of hydrostatic pressure from the apical side of cells cultured on filters stimulates a variety of events including metalloproteinase-dependent growth factor secretion and collagen secretion by nearby mesenchymal cells, events that may model airway thickening in asthma (Swartz et al., 2001). They now report that the space between the cells narrows in response to pressure and suggest that if the growth factor HB-EGF is secreted at a constant rate into this compartment, narrowing will result in increased HB-EGF concentration. They show that EGF receptors are activated, that blocking these receptors inhibits some of the responses to pressure, and that these events can be accurately predicted by a simple first-order diffusion model for this process.

What is the evidence that such a mechanism exists in vivo? Acetylcholine stimulation of canine bronchiolar smooth muscle results in intraluminal pressures of $\sim$30 cm H_2O (Gunst and Stropp, 1988), corresponding to average circumferential stresses of $\sim$45 cm H_2O (Ressler et al., 2000). Tschumperlin et al. confirmed that maximal bronchoconstriction with methacholine in the mouse trachea significantly increased EGFR phosphorylation, implicating the bronchial epithelium as a mechanochemical transducer of intraluminal pressure. The validity of a pressure-induced decrease in lateral intercellular space (LIS) volume is less clear, since the expected mechanical environment differs among adjacent epithelial cells in the constricted airway wall. Smooth muscle contraction causes the epithelium to buckle and fold so that regions of epithelial cells might be compressed against each other along their apical surfaces (Figure 1). Cells at the tops of the folds (facing the airway lumen) are not likely to experience similar pressure increases, and the potential effects of this heterogeneity in force distribution at the cell length scale is not addressed. On the other hand, these cells may experience increased circumferential tension on tight junctions and compression of their basolateral surfaces due to the high inverted radius of curvature underlying the fold. Further work will be required to determine whether these circumferential stresses narrow the LIS in these regions and whether these regions correlate with remodeling. Interestingly, in mammary epithelium where milk accumulation at the end of nursing induces local epithelial cell apoptosis, hydrostatic pressure has been proposed to mediate this effect (Marti et al., 1997). The mechanism proposed by Tschumperlin et al. could be involved.

If autocrine signaling within the LIS is functionally relevant, then paracellular movement of fluid is likely to influence the process. Tissues such as intestine and glandular epithelium have regulated fluid flow through the LIS that should affect ligand concentrations within