SIGNALING

EGF, now on FM and AM

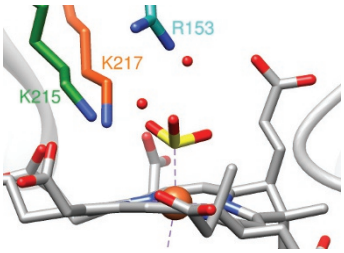
Mol. Cell, published online 5 December 2012; doi:10.1016/j.molcel.2012.11.002

The EGFR-ERK-MAPK pathway drives cell proliferation and is a major target for anticancer therapy. Although this pathway has been intensively investigated, most experiments have focused on acute responses to EGF treatment. Albeck *et al.* now evaluate the pathway (ERK activity, effector activation and cell proliferation) in more physiological steady-state conditions, with high-content immunofluorescence in single cells. ERK activity varied in frequency and duration in an EGF concentration–dependent manner, and the duration of time cells were in the ERK_{ON} state rather than the number of active pulses correlated with S phase entry. Inhibition of EGFR with the small molecule gefitinib completely inhibited ERK at high concentrations but yielded pulses of ERK activity at lower concentrations (frequency modulation). In contrast, inhibition of MEK with the small molecule PD0325901 revealed a concentration-dependent decrease in ERK output (amplitude modulation). Additional analysis revealed that pathway effectors responded to changes in the amplitude and frequency of ERK signaling and that steady-state effector activation was an excellent predictor of cell proliferation. The authors also noted that the relationship between ERK activity and proliferation was nonlinear: inhibition of up to 85% of ERK activity had less than a 2-fold effect on cell proliferation, but 95% inhibition reduced proliferation by a factor of ten. Thus, the amplitude and frequency of ERK signals affect cell proliferation, and quantitative approaches to assess pathway output in response to inhibitors will be necessary to model inhibitor efficacy for the clinic. *AD*

MECHANISMS

Reduction deduction

Biochemistry **51**, 9857–9868 (2012)



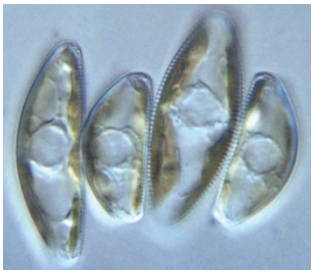
KYLE SMITH

Sulfite reductase (SiR) has a central role in sulfur geochemistry, reducing SO_3^{2-} to S^{2-}. The enzyme uses an Fe_4S_4 cluster and a siroheme cofactor to pass the necessary six electrons to the substrate, whereas some of the necessary six protons are thought to come from positively charged amino acids forming a 'cationic cage' around the substrate. To clarify how these components work together, Smith and Stroupe now report the enzymatic and structural characterization of five SiR mutants. As expected, alterations to residues in a loop that neighbors the active site and is known to undergo conformational changes upon substrate binding resulted in structural perturbations. Similarly, alterations to some of the residues postulated to be involved in the cationic cage impaired enzyme activity even though the protein structure was intact. Quantification of electrons inserted per sulfur product and analysis of hydroxylamine reduction, which requires only two electrons and two protons, then allowed the authors to identify the incoming substrate and two ordered water molecules as likely sources for the first three protons and to assign the specific order of the final three proton transfers, which are mediated by defined cationic cage residues. Analysis of the reaction products also confirmed that electrons can be transferred individually, in contrast to one proposed mechanism of transfer in pairs. These results provide a compelling deconstruction of SiR's complex reaction. *CG*

CHEMICAL ECOLOGY

Proline draws a diatom

Angew. Chem. Int. Ed. Engl., published online 12 December 2012; doi:10.1002/anie.201208175



GEORG POHNERT

Seminavis robusta is a bilaterally symmetric unicellular diatom whose life cycle is characterized by asexual reproduction punctuated by short bursts of sexual reproduction that is used to restore cell size. Cells below a sexual size threshold (SST) of ~52 μm form MT^+ and MT^- cells, where MT^+ cells are migratory and MT^- cells are attractors; both cell types produce mating signals as the process is reciprocal. Gillard *et al.* took advantage of the migratory behavior of MT^+ cells to identify the pheromone produced by MT^- cells. The authors visualized by light microscopy the migration of MT^+ cells toward beads that were loaded with extracts of MT^- cells below the SST and used this system to identify the upregulated pheromone in MT^- cell cultures by metabolomics. This approach led to the identification of di-L-prolyl diketopiperazine (diproline) as the MT^- pheromone. Diproline production was light dependent, consistent with reports that light triggers mating in pinnate diatoms. Also, diproline was only produced during the mating process, and the attraction assay was only positive when diproline was detectable. Diproline therefore represents one of the pheromones involved in MT^-/MT^+ mating and most likely binds an as-yet-unidentified receptor on MT^+ cells. *MB*

RECEPTORS

Insulin makes a move

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Insulin signaling occurs via the insulin receptor (IR), which is a receptor tyrosine kinase and a key component of cellular metabolism, growth, division, differentiation and survival. Misregulation of signaling via IR is implicated in disorders such as type 2 diabetes and Alzheimer's disease. The costructure of insulin in complex with IR has been elusive owing to difficulties in producing the receptor protein, which contains numerous glycosylated residues and disulfide bonds. Menting *et al.* now solve four crystal structures of insulin bound to a truncated IR. A previous structure of the unliganded IR ectodomain, which showed a disulfide-linked dimer with a folded-over conformation, predicted two insulin-binding surfaces per receptor monomer, and the new structures show insulin bound to the primary site. The structures also confirm an induced-fit mechanism of molecular recognition and identify a remarkable feature, namely relocation of a C-terminal segment of the α-protomer (αCT) upon hormone binding. Cross-linking data derived from the intact IR, as well as mutational analyses combined with isothermal titration calorimetry, further confirm the observed insulin-IR interactions. The mode of receptor engagement the authors visualized is unique among receptor tyrosine kinases, and the structures suggest a mechanism for initiating downstream signals. *MB*