The authors then used the fact that neither the p46 nor p52 isoforms of Shc influence ROS regulation or apoptosis to implicate the N-terminal region (which contains the collagen-homologous-2 (CH2) and phosphotyrosine binding (PTB) domains) of $p66^{Shc}$ in cytochrome *c* binding. Further probing uncovered a 44-residue region just N-terminal to the PTB domain which mediated cytochrome *c* binding, with the identification of E132, E133 and W134 as essential residues for redox activity and cytochrome *c* binding. Mutations that impair this redox activity not only impaired the ability of $p66^{Shc}$ to mediate ROS production, but also abrogated its ability to induce mitochondrial permeability transition and subsequent apoptosis. So $p66^{Shc}$ seems to be "an atypical signal transducer that converts proapoptotic into redox signals".

Katrin Bussell

References and links

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lines. Despite EGF stimulation, C-Raf could not be detected in caveolae in prohibitin-depleted cells. Prohibitin and C-Raf interacted *in vitro* and endogenously in cells, and this direct interaction was necessary for EGF to activate C-Raf. Prohibitin was also needed for Ras to interact with, and to activate, C-Raf.

C-Raf activation by Ras requires, among many events, a 14-3-3 protein to be displaced from Ser259-phosphorylated C-Raf. After this Ras-mediated event, Ser259 is dephosphorylated and C-Raf can associate with the membrane, where it is phosphorylated on Ser338 and Tyr341 for full activation. Adding EGF resulted in the dephosphorylation of Ser259 on C-Raf and its subsequent membrane localization only when prohibitin was present. Even Ras activation by EGF couldn't promote 14-3-3 displacement from C-Raf if prohibitin was missing. Prohibitin is therefore needed for Ras to displace 14-3-3. As a Ser259Ala mutant of C-Raf, which cannot bind 14-3-3 and binds to Ras with a high affinity, could rescue C-Raf activation in prohibitin-depleted cells, Rajalingam and colleagues ended their report by suggesting that Ser259 is the site from which 14-3-3 is displaced by Ras and prohibitin.

Katrin Bussell

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CHROMATIN

Forming a silent structure

Heterochromatin is essential for correct centromere and telomere function, and has an important role in silencing genes at specific loci. In the current model for heterochromatin formation in fission yeast, the protein Rik1 recruits the histone methyltransferase Clr4 to a target locus. Clr4 then methylates histone 3 (H3) on K9, and this modification results in the recruitment of other proteins that nucleate the structural spreading of heterochromatin.

In *Genes & Development*, Peterson and colleagues now give new insights into heterochromatin formation through the identification of novel Rik1-associated proteins. Using tandem affinity purification and mass spectrometry, they showed that the following proteins associate with Rik1: Clr4, histone H2B, two novel proteins, which they named Rik1-associated factor-1 (Raf1) and Raf2, and Pcu4 and Pip1, which are components of cullin-dependent ubiquitin ligases.

The finding that Pcu4 and Pip1 copurified with Rik1 indicated that Rik1 might be associated with ubiquitylation processes, and the authors showed that, in the presence of recombinant ubiquitin-activating and ubiquitin-conjugating enzymes, the Rik1 complex polyubiquitylated the candidate substrate H2B *in vitro*. They also showed that Raf2 and Clr4 are components of this ubiquitin-ligase complex.

Next, Peterson and co-workers studied the role of the Rik1-associated proteins in heterochromatin-related functions. First, they studied the effect of the novel proteins Raf1 and Raf2 on the transcriptional silencing of transgenes that had been inserted into centromeric heterochromatic regions. They showed that deleting *rik1*, *clr4*, *raf1* or *raf2* resulted in the derepression of these transgenes, but that such deletions did not affect the expression of a transgene in a euchromatic region. These proteins therefore specifically function on heterochromatic loci.

Rik1 and Clr4 are required for the centromeric pattern of H3 methylation — an enrichment of H3-K9 methylation and a reduction in H3-K4 methylation. So, do Raf1 and Raf2 affect this methylation pattern? Deleting *raf1* or *raf2* had the same affect as deleting *rik1* or *clr4* — that is, it caused a decrease in H3-K9 methylation and an increase in H3-K4 methylation. Together with the transgene silencing assays, these data indicate that Raf1 and Raf2 are novel components of the silencing

machinery that affect the regulation of H3 methylation in heterochromatin.

Peterson and colleagues then studied the role of the ubiquitin-ligase activity of the Rik1 complex in heterochromatin formation. Using a dominant-negative allele of Pcu4, they showed that Pcu4 contributes to heterochromatic silencing. More specifically, a Pcu4-dependent ubiquitin ligase has a role in establishing heterochromatin domains by preventing inappropriate H3-K4 methylation.

So, these authors have identified two novel proteins — Raf1 and Raf2 — that are essential for transcriptional silencing in centromeric heterochromatin, as well as a "...novel Rik1-associated E3 ubiquitin ligase that is required for heterochromatin formation". Future studies will address the precise molecular roles of these proteins.

Rachel Smallridge

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WEB SITE

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