Many proteins are post-translationally modified by lipid moieties such as palmitoyl or prenyl (e.g., farnesyl) groups, creating functional proteolipids. Lipid modifications share the property of increasing a protein’s hydrophobicity and thus the propensity of that protein to associate with a membrane. These modifications are used to control the localization and activity of membrane-associated proteins. A well-recognized paradigm is farnesylation of the Ras GTPase that helps target this critical signaling protein to the plasma membrane.1

Protein palmitoylation, the addition of a 16-carbon fatty acid side chain to cysteines via a reversible thioester linkage, has received attention because of the relatively recent discovery of a conserved family of palmitoyltransferases (PATs for protein acyl transferase) that catalyze this modification.2,3 PATs are integral membrane proteins that contain a catalytic domain with the signature asp-his-his-cys (DHHC) sequence that faces the cytosol. Mammalian cells are estimated to encode over 20 PATs, the sheer number suggesting expansive regulatory roles for palmitoylation in biology. The budding yeast Saccharomyces cerevisiae, where PATs were originally discovered, encodes seven such enzymes. Each shows a distinct cellular localization pattern, with individual PATs enriched in either the endoplasmic reticulum (ER), Golgi, vacuole or plasma membranes.4 In general, each PAT modifies a distinct set of proteins in vivo.5

Palmitoylation in the nucleus
A little fat around the edges

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Palmitoylation helps anchor substrate proteins or protein domains to membranes and in this capacity often influences vesicular trafficking between membranes. For instance, while farnesylation of some forms of Ras is sufficient to anchor Ras to the ER membrane, subsequent palmitoylation is required to target these Ras proteins to the plasma membrane.3 Similarly, trafficking of the integral membrane protein yeast chitin synthase from the ER to the plasma membrane requires palmitoylation.4 As these two examples show, palmitoylation regulates the specificity of protein-membrane interactions in the cytosol.

Proteolipids also act in the nucleus but the list of examples is considerably less extensive. Foremost are the metazoan lamins, a set of intermediate filaments that form a fibrous network at the inner nuclear membrane (INM) called the nuclear lamina.7 The mature B-type lamins are farnesylated constitutively whereas lamin A is farnesylated only during maturation. Lamins stabilize the shape of the nucleus while binding specific chromatin domains at the nuclear edge, and thus influence a variety of chromosomal processes. Mutations of the lamin-encoding genes, such as those responsible for Hutchinson-Gilford progeria syndrome, yield misshapen nuclei, changes in chromatin distribution and modification, as well as loss of perinuclear heterochromatin.7

While the model eukaryote yeast Saccharomyces cerevisiae lacks lamins, significant aspects of chromosome organization at the INM are preserved. In particular, heterochromatin regions are
anchored to the INM where assembly of these complex networks transitional silent chromatin structures is favored. Telomeres, which contain adjacent domains of heterochromatin, are also anchored to the INM, thereby enhancing the genomic stability of chromosome ends. By virtue of the precedent set by proteolipids in the cytosol and lamina, one might expect that proteolipids also influence the localization, assembly or regulation of these chromosomal landmarks. However, while extensive studies in budding yeast have identified a set of proteins that anchor telomeres and other heterochromatic domains, to date none of these proteins have been shown to function as proteolipids.

Here we summarize our recent study, published in the August 2011 issue of *PNAS*, in which we show that the conserved nuclear protein Rif1 is in the budding yeast *Saccharomyces cerevisiae* is palmitoylated, and that this modification governs the localization of Rif1 to the INM. Rif1 is a telomere binding protein that regulates both telomere length and heterochromatin-mediated gene silencing near telomeres and other internal chromosomal sites. We found that some but not all of these roles for Rif1 are influenced by palmitoylation. We recovered *PFA4* mutants, from these loci suggested that palmitoylation frequently regulates protein localization, we turned to biochemical analysis, these results support the hypothesis that *PFA4* influences heterochromatin-mediated gene silencing through palmitoylation of Rif1.

Connecting Rif1 to Pfa4: Forward Genetics Gets a Boost from Proteomics

We recovered *PFA4* in a transposon mutagenesis screen to identify genes that enhance heterochromatin-mediated gene silencing. When *PFA4* was disrupted, transcriptional repression of genes at two extensively examined yeast heterochromatic (silent) loci, *HMR* and *HML*, dropped several fold. A corresponding loss of Sir3, a heterochromatin structural protein, from these loci suggested that the transcriptional phenotype resulted from a chromatin defect. *PFA4* encodes a DHHC-palmitoyltransferase and a catalytically defective allele showed that Pfa4’s palmitoyltransferase activity was required for silencing. Thus we concluded that palmitoylation of some protein(s) was required for at least one nuclear event in yeast: efficient heterochromatin formation at the *HM* loci, *HMR* and *HML*.

Pfa4 resides in the ER. Conceptually, the enzyme could modify a protein in the cytoplasm that would then migrate to the nucleus to affect silencing. However, the protein of budding yeast that the INM is nevertheless contiguous with it. Thus an alternative hypothesis is that Pfa4 may or may not contribute to heterochromatin-mediated gene silencing also indicated that other aspects of telomere behavior might also be influenced by *PFA4*. We first examined telomere clustering, measured by tagging teleric heterochromatin with Sir3-GFP. The average number of clusters (between 7–9) was reduced in yeast strains lacking *PFA4* or *RIF1* but the difference was not dramatic. A change in another aspect of telomere clustering, on the other hand, was strikingly obvious. Specifically, a substantial number of nuclei contained single spots of unusually bright Sir3-GFP intensity. Such foci, termed SBFs for single bright foci, are rarely seen in wild-type cells but have been observed in some mutants known to perturb telomere anchoring and silencing. At present we do not know if the SBFs in *PFA4* and *RIF1* mutants represent an unusual clustering of telomeric heterochromatin, abnormal enrichment of Sir3 on a single telomere, or whether the Sir3 aggregate is even chromosome-associated. However, earlier studies by the Shore lab demonstrated that Rif1 does indeed regulate the distribution of heterochromatin proteins among various chromosomal target sites, as discussed below.

That a strain lacking both *PFA4* and *RIF1* produced an equivalent number of SBFs as strains lacking either gene alone indicated that *PFA4* and *RIF1* normally act in a common pathway to suppress large aggregates of Sir3. An epistasis analysis of *HML* and *HMR* silencing also indicated
that PFA4 and RIF1 act in a common genetic pathway to mediate heterochromatic repression. Taken together, these results show that PFA4 controls the distribution of both RIF1 and heterochromatin proteins in the nucleus, and that this distribution has consequences on gene expression.

**Palmitylation Controls Some but Not All of RIF1's Nuclear Roles: Unexpected Outcomes Raise New Mechanistic Issues**

Rif1 was first discovered as a Rap1-interacting factor. Rap1 initiates heterochromatin assembly by recruiting the heterochromatin structural Sir proteins to specific chromosomal domains. Rap1 binding sites are densely situated within telomeres whereas individual sites are found within regulatory sequences of the HM loci, and at numerous other sites unrelated to heterochromatin repression. At telomeres, Rif1 binding is thought to attenuate silencing by impeding Sir protein recruitment by Rap1. Since Sir proteins are limiting in the nucleus, gain or loss of silencing at some locations must be accompanied by a balanced change in silencing elsewhere in the genome. Thus, a simple explanation for the HM silencing defect in PFA4 mutants is that palmityl-deficient Rif1 allows for a redistribution of Sir proteins away from the HM loci and toward telomeres.

The Sir protein redistribution model predicts that RIF1 and PFA4 mutants should behave similarly with respect to telomeric silencing, just as they do with HM silencing. We, however, did not observe this result. Whereas deletion of RIF1 increased silencing of several telomeric reporter genes, deletion of PFA4 bore little impact. This disconnect extends to other RIF1 functions. Specifically, Rif1 also inhibits telomerase, the enzyme that catalyzes lengthening of telomeric DNA ends. While loss of Rif1 promotes telomere length extension, loss of PFA4 did not. How could a gene that has an obvious effect on Rif1 and Sir3 localization, as well as Rif1-mediated regulation of HM silencing, have such a small effect on Rif1's most recognized roles in telomere biology? One explanation is that PFA4 modulates Rif1 function but that significant Rif1 activity remains in the absence of palmitylation. In other words, delet- ing PFA4 is equivalent to creating a RIF1 hypomorphic allele. Indeed, chromatin immunoprecipitation analysis revealed that significant levels of Rif1 remain bound at telomeres in a PFA4 mutant even when the bulk Rif1 population redistribu-
sites into the nucleoplasm. According to this interpretation, PFA4 loss reduces the effective concentration and/or activity of Rif1 at telomeres but not to a threshold low enough to cause measurable defects in Rif1's roles at telomeres.

An alternative and not mutually exclu-
sive explanation for the disparity in the two RIF1 and PFA4 mutant phenotypes is that palmitylation influences the specificity of Rif1 site selection. According to this view, palmitylated Rif1 is largely restricted to telomeres where it hinders Sir recruitment. When loss of palmitylation causes Rif1 to redistribute, the protein gains access to ectopic sites, like the HM loci, where it inappropriately inhibits Sir accumulation. Indeed, Rif1 has been reported to bind the HM loci and we observed that PFA4 loss enhances Rif1 binding at HMR in conjunction with a reduction in silencing and Sir3 binding. Thus, the scope of Rif1 inhibition sites broadens in a PFA4 mutant without losing substantial inhibi-
tory activity at the original telomeric sites (Fig. 1). Gauging the relevance of these two models will require mapping the palmitylation sites on Rif1 and creating Rif1 alleles specifically defective for pal-
mitoylation. In addition, a high-resolution genome-wide analysis of the effects of a PFA4 mutant on Rif1 binding should help reveal whether there is significant redistri-
bution of Rif1 to more internal target sites in the genome.

**Palmitylation Contributes to Telomere Anchoring**

If palmitylation secures Rif1 to the INM then it follows that this telomere-binding factor might define a new pathway for INM anchorage of telomeres. Two redun-
dant telomere pathways are already recog-
nized and described in great detail. Briefly, the first involves Ku, a DNA end-binding factor that interacts with telomerase that
Figure 1. Pfa4 in the anchoring of telomeres and the dynamics of heterochromatin in budding yeast. (A) A simplified view of pathways that anchor telomeres at the nuclear periphery. A single chromosome is shown for simplicity, and the dynamics of heterochromatin proteins (Sir proteins) between telomeric and internal (e.g., HMR) heterochromatic loci are depicted. Additional details, including cell cycle specificity of the relevant pathways (depicted by dotted lines) are comprehensively discussed in reference 8. At the ends of chromosomes, Ku interacts with telomerase that in turn associates with the integral membrane protein Mps3 (Mps3-Ku via telomerase). Ku interacts with Sir4 and it also participates in another anchoring pathway involving as yet unidentified factors at the INM (Ku-other). Sir4-based anchoring pathways are used at internal heterochromatic loci such as HMR but are not shown. Based on our study, we propose that palmitoylated Rif1 binds to telomeric-bound Rap1 proteins and thereby contributes to telomeric anchoring, presumably because the palmitoyl group is imbedded in the INM. Rif1 also attenuates the recruitment of Sir proteins by DNA-bound Rap1. Pfa4, the palmitoyltransferase required to modify Rif1, is an integral membrane protein shown in the ER. (B) Nuclear consequences of loss of Pfa4 are depicted. Rif1 is no longer anchored in the membrane, allowing it to dissemble from telomeric clusters and redistribute into the nucleoplasm. Rif1 is now able to sample other sites in the genome with the consequence of influencing silencing or other functions at other loci. While enrichment of Rif1 at telomere clusters is diminished, interactions between Rif1 and Rap1 maintain sufficient levels of Rif1 at telomeres to leave telomeric silencing and length regulation relatively unaffected. However, the increased dynamics of Rif1 that result from loss of Pfa4 leads to a perturbation in Sir protein dynamics (bolder arrows) that in turn leads to changes in transcriptional silencing and the appearance of unusual Sir3 aggregates that are molecularly uncharacterized (not depicted, please see text).
of Sir protein recruitment at the loci to or the
unregulated sequestration of the het-
erochromatin proteins elsewhere (Fig. 1).
Either way, our recent work shows that palmitoylation of nuclear proteins like Rif1 can influence the transcriptional
deregulation of the genome.

Rif1 can influence the transcriptional
localization and partitioning of human
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