cryptic transcriptional initiation within ORFs (Cheung et al., 2008).

Additionally, in combination with mutations that eliminate the SHREC complex, *asf1*^{ts} mutations cause synergistic alterations in nucleosomal positioning and occupancy within heterochromatin. Therefore, HIRA/Asf1 appear to enforce silencing both by contributing to heterochromatic nucleosomal occupancy and by fostering histone deacetylation. As the repertoire of histone deposition complexes continues to grow (Campos and Reinberg, 2010), one expects that additional elegant networks of protein interactions that increase the functional specialization of chromatin remain to be discovered.

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San1-Mediated Quality Control: Substrate Recognition "Sans" Chaperones

Randolph Y. Hampton[1,*]
[1]Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
*Correspondence: rhampton@ucsd.edu
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In this issue of *Molecular Cell*, Rosenbaum et al. describe a mechanism that allows San1 to selectively detect misfolded proteins for nuclear protein quality control.

In all organisms, mechanisms exist to remove misfolded and damaged proteins harmful to cellular function. These alleviatory mechanisms are collectively known as protein quality control (PQC) pathways, with the two main strategies being to either refold the proteins or destroy the recalcitrant clients. Degradative PQC is both highly selective for misfolded proteins and broadly inclusive of the many substrates that may arise. Thus, detection of quality control substrates requires recognition of common structural hallmarks of misfolding found in a wide variety of unrelated proteins but absent in their normal counterparts. Understanding the molecular mechanisms of PQC substrate selection is of both basic and biomedical interest, since some of the most pressing maladies involve mismanagement of misfolded proteins, including inefficient destruction of misfolded protein in neurodegenerative diseases and aging (Balch et al., 2008) and overaggressive destruction of functional mutants in diseases such as cystic fibrosis (Vembar and Brodsky, 2008). The work featured in Rosenbaum et al. (2010) presents an exciting mechanism for the selective detection of misfolded proteins in the San1 nuclear QC pathway.

Most eukaryotic protein degradation occurs by the ubiquitin-proteasome pathway, by which the small protein ubiquitin is attached to the substrate as a polyubiquitin chain to target its destruction by the 26S proteasome. Ubiquitination is accomplished by an enzymatic cascade that includes a specificity-directing E3 ubiquitin ligase that brokers the transfer of ubiquitin to the substrate and growing polyubiquitin chain. Accordingly, the selectivity of a QC pathway can be understood by studying the responsible E3 ligases.

There are a small but growing number of known E3 ligases dedicated to degradative QC. These include the ER-localized Hrd1 and Doa10 (Vembar and Brodsky, 2008; Hampton and Garza, 2009), cytoplasmic Ubr1 (Heck et al., 2010), and nuclear E3 San1 (Gardner et al., 2005) ligases in yeast and the mammalian E3 CHIP (McDonough and Patterson, 2003). Some of these E3s employ chaperones in substrate recognition, including Ubr1 and CHIP and the two ER ligases for

some classes of substrates. Hrd1 also employs other proteins to discern misfolded ER lumenal substrates based on both glycosylation state and folding state, such as Yos9. These observations imply that QC ligases require the help of ''professional'' folding factors like chaperones to discern soluble misfolded proteins. In striking contrast, the featured work clearly shows that the nuclear San1 E3 ligase recognizes its diverse substrates with a different, autonomous mechanism. The model is elegant and logical and will almost certainly be recapitulated in yet-undiscovered pathways of protein folding and QC.

To understand how San1 E3 ligase selects its substrates, a two-hybrid search for San1 interactors was done. The proteins that emerged turned out to be substrates of San1, a motley crew of unrelated candidates including truncated proteins and even ''gibberish'' antisense peptides with appropriate properties for San1 interaction. Importantly, there was a complete lack of any interactors with functions related to protein folding, and they were similarly absent by mass spectrum analysis. These results say that San1 has many substrates and it functions alone to recognize them, an idea that was confirmed by direct in vitro analysis with purified San1 and a clever approach that reconstitutes the San1 pathway entirely in *E. coli*.

So how does San1 detect so many proteins without a molecular posse? Sequence analysis of San1 revealed no tell-tale motifs indicating a recognition mechanism. However, the San1s of different fungi have numerous small regions of highly conserved sequence identity, in similar positional register. The analysis of San1 sequence then veered in a less typical but highly fruitful direction. Three computational approaches all revealed that San1 has a highly disordered structure, in ~70% of its 1° sequence. Biochemical tests including circular dichroism, limited proteolysis, and gel filtration all confirm that San1 is a wild affair of great flexibility, a Perutzian nightmare occupying about 500 kDa worth of size with its 66 kDa worth of sequence.

The small conserved regions are distributed on either side of the catalytically important RING domain and often have hydrophobicity useful for interacting with misfolded regions of QC substrates. To understand how these motifs function in San1, a ''recognition matrix'' was determined using quantitative two-hybrid analysis. In this analysis, interactions between San1 substrates and San1 mutants (each with deletion of a specific motif) were assessed. A similar analysis of the San1 deletions was performed by flow cytometry of several GFP-tagged substrates. Clustering analyses were then used to provide a graphical picture of the relative role of the different sections of San1 in the recognition of different proteins. It appears that recognition of a substrate is distributed to a subset of motifs; these subsets vary for different substrates. However, there is clear grouping of different substrates to distinct subsets of motifs, indicating structural similarities between these substrates as seen by San1. In other words, this approach provides a lens to view the structural code that allows San1 to discern an impressive variety of misfolded protein without any help from its friends. The authors propose a model in which a substrate is recognized by a subset of motifs being able to simultaneously engage separate regions of misfolding through the flexibility of San1's disordered structure. Thus, the combination of motif number and structural disorder provides a ''dynamic code'' for recognition of diverse substrates, sans chaperones.

The idea that binding motifs with high positional plasticity determines the breadth of San1's recognition powers suggests further experiments. The disordered regions should be amenable to replacement with diverse sequences of similar disorder, while alteration of the recognition motifs should cause large changes in substrate recognition. The need for a large amount of disorder in San1 action may explain why a simple BLAST search does not reveal conserved San1s of high primary sequence homology in diverse organisms: maintained disorder can be attained with a wide variety of sequences, so there could be great diversity in the family of San1-related E3s. It is likely that the crystallography of this ''octopus with Velcro gloves'' will be nontrivial. Perhaps the answer lies in copurification of the San1 protein with high-affinity substrates or sets of substrates that lock the protein into particular shapes.

Some interesting questions are suggested by this exciting work. Is this approach of binding motifs in a sea of disorder used in other instances of misfolded protein recognition? Can San1 also function in protein folding? Is there an allosteric component to San1 action, or is binding sufficient for ubiquitination? Can San1 serve as a design platform to engineer nanodevices that detect and destroy misfolded proteins? Whatever the answers to these questions, San1 provides the most detailed collection of glyphs in the Rosetta stone of QC substrate recognition. Further study of this and other E3s will lead to a new understanding of how nature detects these dangerous proteins and how we might harness these ideas for both technical and clinical purposes.

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