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Mini-Review

Staying in the fold

The SGT1/chaperone machinery in maintenance and evolution of leucine-rich repeat proteins

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The conserved eukaryotic protein SGT1 (suppressor of G_2 allele of *skp1*) participates in diverse physiological processes such as cell cycle progression in yeast, plant immunity against pathogens and plant hormone signalling. Recent genetic and biochemical studies suggest that SGT1 functions as a novel co-chaperone for cytosolic/nuclear HSP90 and HSP70 molecular chaperones in the folding and maturation of substrate proteins. Since proteins containing the leucine-rich repeat (LRR) protein-protein interaction motif are overrepresented in SGT1-dependent phenomena, we consider whether LRR-containing proteins are preferential substrates of an SGT1/HSP70/HSP90 complex. Such a chaperone organisation is reminiscent of the HOP/HSP70/HSP90 machinery which controls maturation and activation of glucocorticoid receptors in animals. Drawing on this parallel, we discuss the possible contribution of an SGT1-chaperone complex in the folding and maturation of LRR-containing proteins and its evolutionary consequences for the emergence of novel LRR interaction surfaces.

The proper folding and maturation of proteins is essential for cell viability during de novo protein synthesis, translocation, complex assembly or under denaturing stress conditions. A complex machinery composed of molecular chaperones (heat-shock proteins, HSPs) and their modulators known as co-chaperones, catalyzes these protein folding events.[1,2] In animals, defects in the chaperone machinery is implicated in an increasing number of diseases such as cancers, susceptibility to viruses, neurodegenerative disease and cystic fibrosis, and thus it has become a major pharmacological target.[3,4] In plants, molecular genetic studies have identified chaperones and co-chaperones as components of various physiological responses and are now starting to yield important information on how chaperones work. Notably, processes in plant innate immunity rely on the HSP70 and HSP90[5-7] chaperones as well as two recently characterised co-chaperones, RAR1 (required for *Mla12* resistance) and SGT1 (suppressor of G_2 allele of *skp1*).[8-11]

SGT1 is a highly conserved and essential co-chaperone in eukaryotes and is organized into three structural domains: a tetratricopeptide repeat (TPR), a CHORD/SGT1 (CS) and an SGT1-specific (SGS) domain (Fig. 1A). SGT1 is involved in a number of apparently unrelated physiological responses ranging from cell cycle progression and adenylyl cyclase activity in yeast to plant immunity against pathogens, heat shock tolerance and plant hormone (auxin and jasmonic acid) signalling.[7-9,12,13] Because the SGT1 TPR domain is able to interact with Skp1, SGT1 was initially believed to be a component of SCF (Skp1/Cullin/F-box) E3 ubiquitin ligases that are important for auxin/JA signalling in plants and cell cycle progression in yeast.[13,14] However, mutagenesis of *SGT1* revealed that the TPR domain is dispensable for plant immunity and auxin signalling.[15] Also, SGT1-Skp1 interaction was not observed in Arabidopsis.[13] More relevant to SGT1 functions appear to be the CS and SGS domains.[16] The former is necessary and sufficient for RAR1 and HSP90 binding. The latter is the most conserved of all SGT1 domains and the site of numerous disabling mutations.[14,16,17]

We recently demonstrated that Arabidopsis SGT1 interacts stably through its SGS domain with cytosolic/nuclear HSP70 chaperones.[7] The SGS domain was both necessary and sufficient for HSP70 binding and mutations affecting SGT1-HSP70 interaction compromised JA/auxin signalling and immune responses. An independent in vitro study also found interaction between human SGT1 and HSP70.[18] The finding that SGT1 protein interacts directly with two chaperones (HSP90/70) and one co-chaperone (RAR1) reinforces the notion that SGT1 behaves as a co-chaperone, nucleating a larger chaperone complex that is essential for eukaryotic physiology. A future challenge will be to dissect the chaperone network at the molecular and subcellular levels. In plant cells, SGT1 localization appears to be highly dynamic with conditional nuclear localization[7] and its association with HSP90 was recently shown to be modulated in vitro by RAR1.[16]

A co-chaperone function suits SGT1 diverse physiological roles better than a specific contribution to SCF ubiquitin E3 ligases. Because SGT1 does not affect HSP90 ATPase activity, SGT1 was proposed rather as a scaffold protein.[16,19] In the light of our findings

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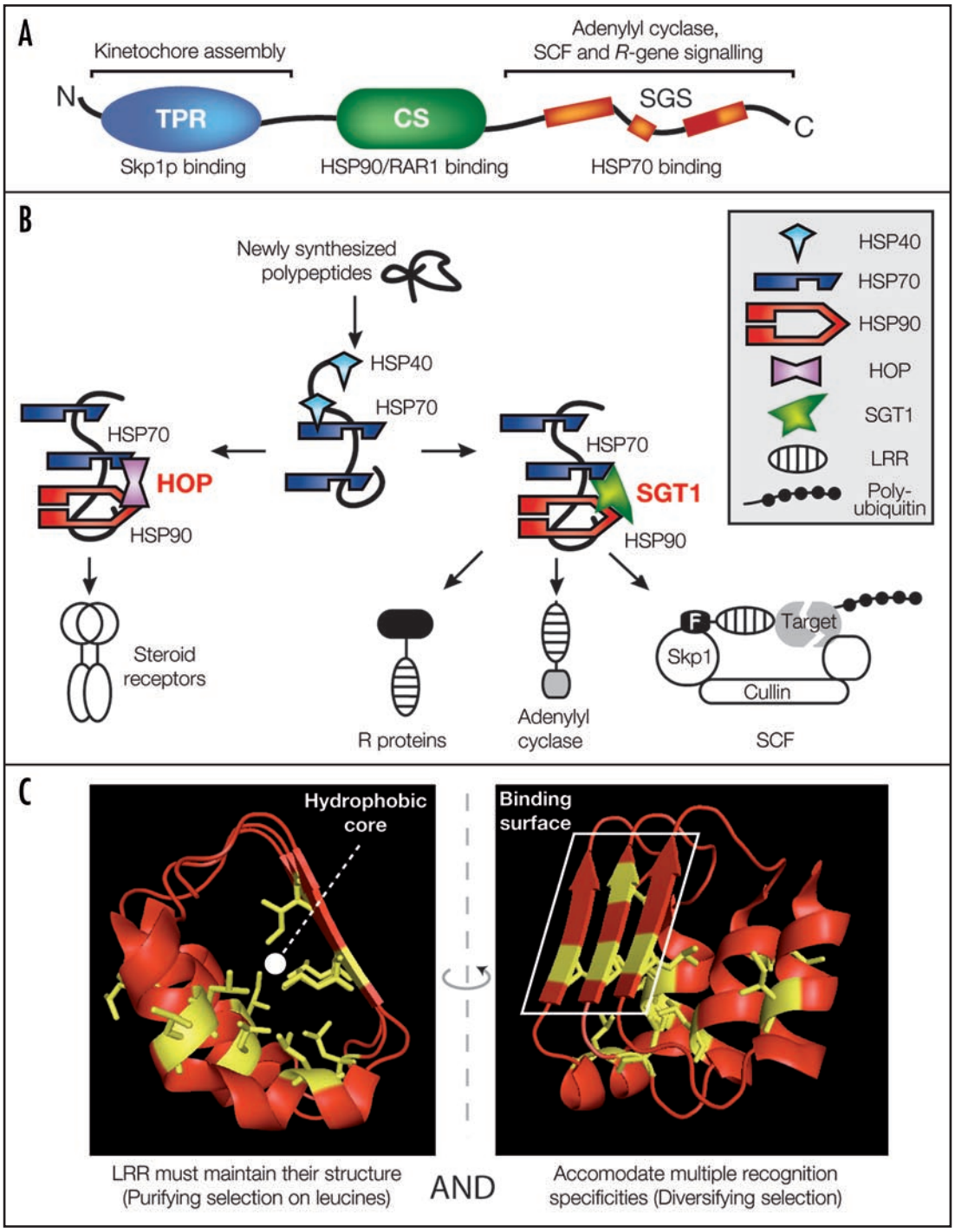

Figure 1. Model for SGT1/chaperone complex functions in the folding of LRR-containing proteins. (A) The structural domains of SGT1, their sites of action (above) and respective binding partners (below) are shown. N- and C-termini are indicated. TPR, tetratricopeptide repeat; CS, CHORD/SGT1; SGS, SGT1-specific. (B) Conceptual analogy between steroid receptor folding by the HOP/chaperone machinery and LRR protein folding by the SGT1/chaperone machinery. LRR motifs are overrepresented in processes requiring SGT1 such as plant immune receptor signalling, yeast adenylyl cyclase activity and plant or yeast SCF (Skp1/Cullin/F-box) E3 ubiquitin ligase activities. (C) Opposite forces drive LRR evolution. Structure of LRRs 16 to 18 of the F-box auxin receptor TIR1 is displayed as an illustration of the LRR folds.[30] Leucine/isoleucine residues (side chain displayed in yellow) are under strong purifying selection and build the hydrophobic LRR backbone (Left). By contrast, solvent-exposed residues of the ß-strands define a polymorphic and hydrophilic binding surface conferring substrate specificity to the LRR (Right) and are often under diversifying selection.

and earlier studies,[20] SGT1 is reminiscent of HOP (Hsp70/Hsp90 organizing protein) which links HSP90 and HSP70 activities and mediates optimal substrate channelling between the two chaperones (Fig. 1B).[21] While the contribution of the HSP70/HOP/HSP90 to the maturation of glucocorticoid receptors is well established,[21] direct substrates of an HSP70/SGT1/HSP90 complex remain elusive.

It is interesting that SGT1 appears to share a functional link with leucine-rich repeat- (LRR) containing proteins although LRR

domains are not so widespread in eukaryotes. For example, plant SGT1 affects the activities of the SCF^{TIR1} and SCF^{COI1} E3 ligase complexes whose F-box proteins contain LRRs.[13] Moreover, plant intracellular immune receptors comprise a large group of LRR proteins that recruit SGT1.[8,9] LRRs are also found in yeast adenylyl cyclase Cyr1p and the F-box protein Grr1p which is required for SGT1-dependent cyclin destruction during G_1/S transition.[12,14] Yeast 2-hybrid interaction assays also revealed that yeast and plant SGT1 tend to associate directly or indirectly with LRR proteins.[12,22,23] We speculate that SGT1 bridges the HSP90-HSC70 chaperone machinery with LRR proteins during complex maturation and/or activation. The only other structural motif linked to SGT1 are WD40 domains found in yeast Cdc4p F-box protein and SGT1 interactors identified in yeast two-hybrid screens.[12]

What mechanisms underlie a preferential SGT1-LRR interaction? HSP70/SGT1/HSP90 may have co-evolved to assist specifically in folding and maturation of LRR proteins. Alternatively, LRR structures may have an intrinsically greater need for chaperoning activity to fold compared to other motifs. These two scenarios are not mutually exclusive. The LRR domain contains multiple 20 to 29 amino acid repeats, forming an α/β horseshoe fold.[24] Each repeat is rich in hydrophobic leucine/isoleucine residues which are buried inside the structure and form the structural backbone of the motif (Fig. 1C, left). Such residues are under strong purifying selection to preserve structure. These hydrophobic residues would render the LRR a possible HSP70 substrate.[25] By contrast, hydrophilic solvent-exposed residues of the β strands build a surface which confers ligand recognition specificity of the LRRs (Fig. 1C). In many plant immune receptors for instance, these residues are under diversifying selection that is likely to favour the emergence of novel pathogen recognition specificities in response to pathogen evolution.[26] The LRR domain of such a protein has to survive such antagonist selection forces and yet remain functional. Under strong selection pressure, LRR proteins might need to accommodate less stable LRRs because their recognition specificities are advantageous. This could be the point at which LRRs benefit most from a chaperoning machinery such as the HSP90/SGT1/HSP70 complex. This picture is reminiscent of the genetic buffering that HSP90 exerts on many traits to mask mutations that would normally be deleterious to protein folding and/or function, as revealed in Drosophila and Arabidopsis.[27] It will be interesting to test whether the HSP90/SGT1/HSP70 complex acts as a buffer for genetic variation, favouring the emergence of novel LRR recognition surfaces in, for example, highly co-evolved plant-pathogen interactions.[28,29]

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