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Putative roles of glutaredoxin-BolA holo-heterodimers in plants

Tiphaine Dhalleine[1,2], Nicolas Rouhier[1,2], and Jérémy Couturier[1,2]*

[1]Université de Lorraine; Interactions Arbres-Micro-organismes; UMR1136; Vandoeuvre-lès-Nancy, France;
[2]INRA; Interactions Arbres-Microorganismes; UMR1136; Champenoux, France

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Several genomic analyses, high-throughput or targeted interaction studies including the purification of protein complexes indicated a physical and functional link between BolAs and monothiol glutaredoxins (Grxs) that is conserved both in prokaryotes and eukaryotes. In a recent work, we confirmed that several *Arabidopsis* protein couples, used as plant representatives, also physically interact. More interestingly, we determined that two BolA proteins, BolA2 and SufE1, contain a conserved cysteine that is sensitive to oxidizing treatments, unraveling a possible redox-control of BolA2 and SufE1 by monothiol glutaredoxins. By coexpressing physiological partners in *E. coli*, Grx-BolA heterodimers binding a labile, oxygen sensitive iron-sulfur cluster were isolated. Altogether, these results illustrate the existence of different modes of interaction between monothiol glutaredoxins and BolA proteins in plants and probably in other organisms. Incidentally, the function of each partner could be differentially modulated depending on the type of interaction.

Besides their classical oxidoreductase activity, it is now well recognized that monothiol glutaredoxins (Grxs) with CGFS active sites participate to the maturation of iron-sulfur (Fe-S) proteins, at least in yeast and human, through their ability to bind Fe-S clusters, likely receiving it from scaffold proteins before transferring it to apoproteins.[1-6] In addition, by interacting with transcription factors such as Fep1, Php4, HapX, Aft1 or Aft2, CGFS Grxs also are involved in iron sensing and homeostasis in yeast and fungal species.[7-11] Concerning BolAs, several pieces of evidence indicate that they can act themselves as transcriptional regulators, possessing a helix-turn-helix (HTH) motif necessary for nucleic acid-binding.[12] Supporting this observation, some bacterial BolAs can bind to promoters of genes playing in particular a role in the maintenance of cell morphology.[13-16] Moreover, a *Chlamydomonas reinhardtii* ortholog likely exhibits an endonuclease activity.[17]

In *S. cerevisiae*, monothiol Grx3 and Grx4 form a complex with a BolA protein, FRA2, but also with an aminopeptidase-like protein named FRA1,[18] confirming the initially suggested role of FRA2 in the regulation of iron homeostasis.[19] By interacting with and regulating the nuclear translocation of Aft1, this complex links the status of the mitochondrial Fe-S cluster biogenesis to the regulation of the iron regulon *i.e.*, genes responsible of iron uptake, transport and storage.[18] From mutagenesis studies of Fe-S cluster ligands (the active site Cys of Grxs and an invariant His of BolA), it was proposed that the binding of the Fe-S cluster in the Grx-FRA2 complex is essential for Aft1 regulation.[7,20,21] Using Aft2 as a model, it was recently demonstrated that it can accept an Fe-S cluster from a Grx3-FRA2 holo-heterocomplex leading to its dimerization and activity inhibition.[11] It is not yet clear whether the regulation of other Grx-regulated transcription factors rely on BolAs, but studies performed with *E. coli* and human proteins confirmed that the ability of monothiol Grx-BolA to form holo-heterocomplexes is widespread.[22-24]

In our recent study, the physical interaction between monothiol Grxs and BolAs from plants, initially suggested for some protein couples by a global yeast-two hybrid screening,[25] was confirmed by binary yeast-two hybrid and bimolecular fluorescence complementation approaches.[26] Moreover, we have demonstrated that recombinant monothiol Grxs can reduce oxidized forms of AtBolA2 and to a lesser extent of AtSufE1 thereby suggesting that the in vivo activity of BolA proteins could be modulated in a redox manner. As all eukaryote BolA2 orthologs possess this conserved cysteine, this redox-regulation could well represent a general regulatory mechanism. However, we did not determine whether plant proteins also have the capacity to form Fe-S-bridged heterocomplexes. For this purpose, several *A. thaliana* Grx and BolA protein couples were expressed in *E. coli* using a co-expression vector, one partner bearing an N-terminal poly-His tag enabling complex purification by immobilized metal ion affinity chromatography if proteins indeed stably interact. Complexes formed by physiological couples (His_6AtGrxS14-AtBolA1, AtGrxS16-His_6AtBolA1, or His_6AtGrxS17-AtBolA2) were indeed purified from red colored bacterial pellets (**Fig. 1**). For all constructs, although

*Correspondence to: Jérémy Couturier; Email: jeremy.couturier@univ-lorraine.fr
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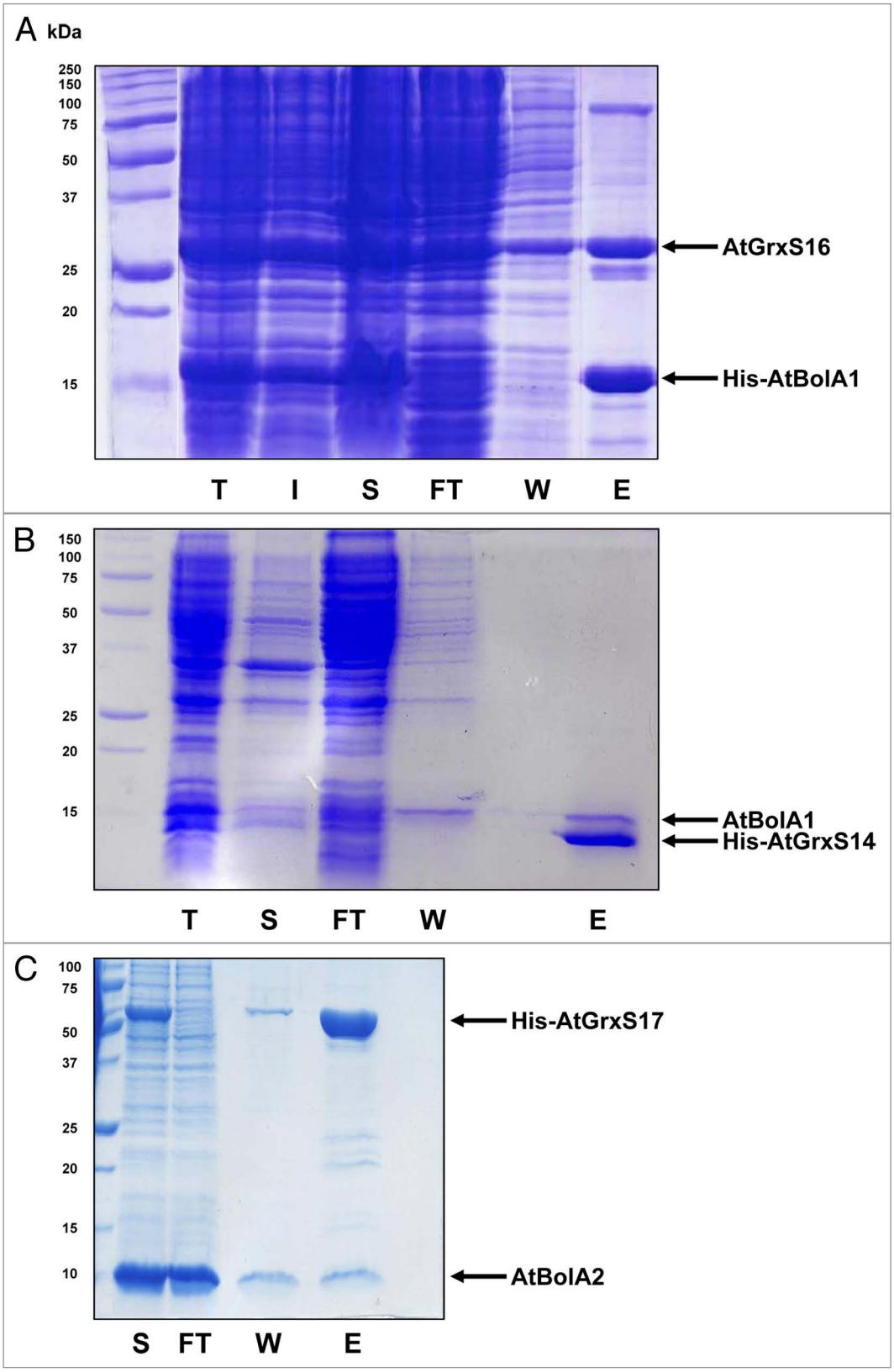

Figure 1. See next page for legend.

Figure 1. Co-expression of *A. thaliana* monothiol Grx and BolA in *E. coli*. For each protein couple, sequences coding for each protein were cloned in pCDFDuet vector allowing co-expression of an N-terminal His-tagged version of one protein and an untagged version of the other. The purification was achieved under aerobic conditions for each of the following co-expression experiments, AtGrxS16-HisAtBolA1 (A), HisAtGrxS14-AtBolA1 (B) and HisAtGrxS17-AtBolA2 (C). After bacterial cell lysis, the soluble and insoluble fractions were then separated by centrifugation for 30 min at 27,000 g. The soluble fraction was then loaded onto a Ni^{2+} Hitrap chelatin resin (Sigma) pre-equilibrated with 50 mM Tris-HCl buffer pH 8.0 containing 300 mM NaCl, 10 mM imidazole. After extensive washing with the same buffer, the proteins were eluted with 50 mM Tris-HCl buffer pH 8.0 containing 300 mM NaCl, 250 mM imidazole. The presence of both AtGrx and AtBolA in the different fractions was analyzed after protein separation on SDS-PAGE gels. T: total extract, I: insoluble fraction, S: soluble fraction, FT: flow through fraction, W: washing fraction eluted with 10 mM imidazole, E: eluted fraction with 250 mM imidazole.

time-dependent color losses were observed because of the aerobic conditions, the presence of an Fe-S cluster was clearly evident from the red/brown color of the sample but also from the presence of absorption bands around 410 nm in the UV-visible spectra. Overall, although further spectroscopic analyses are required on anaerobically-purified complexes, these results established the capacity of plant proteins to form holo-heterodimers and suggested that, as Grx holo-homodimers,[4] Grx-BolA holo-heterodimers are also quite labile or oxygen sensitive.

We have already largely discussed in our seminal paper the functions attributed to BolAs and Grxs and how they could affect each other. Hence, we will not detail everything again but will concentrate on the new information obtained by us and others that could help clarifying the putative physiological roles associated to Grx-BolA holo-heterodimers in plants. The current view is that holo-homodimer formed by monothiol Grxs would preferentially act as scaffold and/or carrier proteins, whereas, from studies conducted in *S. cerevisiae*, Grx-BolA holo-heterodimers would represent sensor systems of cellular iron status. These different functions supposedly reflected a difference in Fe-S cluster lability, holo-heterodimers being more stable,[22] and less efficient for Fe-S cluster transfer than Grx holo-homodimers.[23] A sensing function for the cytosolic GrxS17/BolA2 couple is not evident first because there is no plant ortholog of Aft1 and second because *A. thaliana* plants with knockout or knock-down expression for GrxS17, although having pleiotropic phenotypes, do not have phenotypes clearly and easily attributed to a dysfunctioning of iron homeostasis.[27] Moreover, several recent reports suggest that holo-heterodimers could also have a role in the Fe-S cluster biogenesis systems. Indeed, human patients having a mutation in the gene coding for the mitochondrial BolA3 present defects in Fe-S proteins from respiratory complexes, in particular complex I, and very likely in lipoic acid synthase, which affects all lipoate-dependent enzymes.[28] Also, there are evidence for Fe-S cluster transfer reactions involving holo-heterodimers since a ScGrx3-FRA2 apo-heterodimer can accept an Fe-S cluster from an A-type carrier protein,[29] and can efficiently and rapidly transfer it to Aft2.[11] Interestingly, the assumed presence of A-type proteins in plant organelles but not in the cytoplasm,[30] suggests that Grx-BolA holo-heterodimers might fulfill different functions depending on the subcellular compartmentation. Overall, these biochemical evidence further increase the roles possibly associated to Grx-BolA complexes. An in-depth genetic investigation of plants mutated for monothiol Grxs and BolA, alone or in combination, is clearly required to differentiate all these possibilities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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