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RFC3 regulates cell proliferation and pathogen resistance in Arabidopsis

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Replication factor C subunit 3 (RFC3) is one of the small subunits of the RFC complex originally purified from the HeLa cells that is essential for the in vitro replication of Simian virus 40 (SV40). Although RFC has been reported to be involved in DNA replication, DNA repair and check-point control of cell cycle progression in yeast, little is known about the precise function of each subunit of the RFC in plants. We recently reported the identification of *rfc3-1*, which carries a point mutation leading to plants with enhanced expression of *Pathogenesis-Related* (*PR*) genes and resistance against the virulent oomycete *Hyaloperonospora arabidopsidis* (*H.a.*) Noco2. The mutant is hypersensitive to SA and has enhanced pathogen resistance independent of Nonexpressor of *PR* genes 1 (NPR1). The *rfc3-1* mutation caused a substitution from a nonpolar aliphatic amino acid (Gly-84) to a negatively charged amino acid (Asp) in functional domain III, which is one of eight conserved domains in the RFC. This may interfere with the interaction between RFC3 and other subunits, compromising the function of the protein complex, and leading to cell proliferation defects in the leaves and roots of Arabidopsis. Furthermore, enhanced expression of *PR* genes and induction of systemic acquired resistance in *rfc3-1* may be caused by a partial loss of RFC function through its involvement in replication-coupled chromatin assembling.

DNA replication is essential for all organisms with DNA genomes. Replication factor C (RFC) is a protein complex originally purified from HeLa cells as a host factor essential for the in vitro replication of Simian virus 40 (SV40) DNA.[1-3] RFC can bind to a DNA template-primer junction and load the proliferating-cell nuclear antigen (PCNA) clamp onto DNA with the assistance of ATP. PCNA loading recruits DNA polymerase to the site of DNA synthesis.[4] The five subunits of RFC were identified as one large subunit (RFC140/RFC1) and four small subunits (RFC37/RFC2, RFC36/RFC3, RFC40/RFC4 and RFC38/RFC5), and have been found in all eukaryotes.[5-10] RFC plays an essential role in DNA replication, DNA damage repair and check-point control during cell cycle progression.[11-15] In recent years, three RFC-like complexes (RLCs), Rad24-RLC, Ctf18-RLC and Elg1-RLC, have been identified in yeast. Each RLC is made up of the four small subunits of the archetypal RFC, but the large subunit, RFC1, is replaced with an RFC-related protein (Rad24 or Ctf18 or Elg1). Rad24-RLC or Ctf18-RLC have distinct functions in checkpoint signaling and the establishment of chromosome cohesion, whereas Elg1-RLC plays a role in sister chromatid cohesion and maintenance of genome stability. The unique C-terminus and N-terminus of Elg1 were found to be important for its function.[16-19] However, the precise function of each subunit is largely unclear in plants.

Systemic acquired resistance (SAR) is a plant immune response that is activated in many plant species by necrotizing pathogens. In 1961, Ross described SAR after finding tobacco plants challenged with tobacco mosaic virus (TMV) subsequently developed increased resistance to secondary infection in distal tissues.[20] SAR is long-lasting, sometimes for the

lifetime of the plant, and effective against a broad-spectrum of pathogens including viruses, bacteria, fungi and oomycetes. During the onset of SAR, salicylic acid (SA) levels increase in both local and systemic tissues, causing upregulation of a set of *Pathogenesis-Related* (*PR*) genes.[21] Recently, in a novel genetic screen to search for mutants that are hypersensitive to SA induction, we identified *rfc3-1*, a dwarf mutant that exhibits enhanced induction of *Pathogenesis-Related* (*PR*) genes and resistance against the virulent oomycete *Hyaloperonospora arabidopsidis* (*H*.a.) Noco2. Enhanced pathogen resistance in *rfc3-1* is independent of Nonexpressor of *PR* genes1 (NPR1). The phenotypic analysis of *rfc3-1* revealed that RFC3 negatively regulates the expression of *PR* genes and SAR.[22]

The G-to-A partial loss-of-function mutation in *rfc3-1* occurred within the second exon of *At1g77470*. This point mutation caused a nonpolar alphatic amino acid (Gly-85) substitution to a negatively charged amino acid (Asp) in one of the eight conserved RFC motifs (box III) (**Fig. 1**, see the arrow). The most conserved motif within RFC box III forms a phosphate-binding loop (P loop, also known as Walker A) with the consensus sequence GxxxxGK(S/T). This loop usually contains additional glycines and prolines and has the consensus sequence phUUuyGPPGtGKT(S/T)t (where U stands for a bulky aliphatic residue such as I, L, V or M).[8] Substituting the third Gly for Asp in *rfc3-1* mutant may affect the interaction between RFC3 and other subunits. Alternatively, changing the P-loop may affect the spatial stucture of the whole RFC and its affinity for the binding of the target. As a consequence of these scenarios, partial function of the protein complex could be lost, potentially leading to *rfc3-1* phenotype.

Supporting this idea, the Arabidopsis RFC3 was found to localize to the nucleus and is essential for plant survival, as a null mutant of *RFC3* is lethal.[22] Since *RFC3* encodes a putative replication factor, we tested whether the partial loss-of-function of RFC leads to replication related phenotypes. As expected, *rfc3-1* plants are dwarfed and have smaller and narrower leaves compared with wild-type plants. Inside the mutant root, the length of cortex cells in *rfc3-1* is twice the size of that of wild type and thus the root cell production rate of *rfc3-1* is only half of the wild type plants. In the leaf epidermis, the epidermal cell area of *rfc3-1* both on the abaxial and adaxial surface of the third true leaf is significantly bigger than that of the corresponding cell area of the wild type plants ($p < 0.01$). Consequently, the epidermal cell number of *rfc3-1* both on the abaxial and adaxial surface of the third true leaf is significantly smaller than that of the corresponding cell number of the wild type plants ($p < 0.01$). Similar signifant differences of the interior cells of the leaf (palisade parenchyma or spongy mesophyll) were found between *rfc3-1* and the wild type plants (Xia and Zhang, unpublished data). Taken together, partial loss-of-function *rfc3-1* plants are smaller in size due to the reduced number of cells, suggesting defects in replication. We therefore concluded that RFC3 plays an essential role in the process of cell proliferation.

It is not clear how RFC3 regulates cell proliferation and pathogen resistance. During cell division, epigenetically defined chromatin structure is often propagated with high fidelity through replication-coupled chromatin assembly. Failure to transmit epigenetic modifications such as histone modifications and DNA methylations would lead to changes of gene expression patterns in the daughter cells. On one hand, RFC3 may function with RFC in DNA replication, DNA damage repair and check-point signaling. When the function of these complexes were compromised by RFC3 mutation, DNA replication and check-point control signaling were affected, cell proliferation was slowed down, and thus fewer cells were produced in *rfc3-1* plants relative to wild type. On the other hand, since the phenotypes of *rfc3-1* are highly similar to those of *sni1* (suppressor of *npr1-1*, inducible 1), and we did not detect interactions between SNI1 and RFC3 in the yeast two-hybrid assays, RFC3 may negatively regulate *PR* genes and SAR by indirect protein-protein interaction with SNI1.[22,23] It has been suggested that SNI1 represses transcription through affecting chromatin modifications.[24] Loss of SNI1 function leads to an increased abundance of activating histone modifications such as AcH3 and MeH3K4 at the *PR-1* promoter, which may induce chromatin at promoter to adopt a more accessible conformation and lead to elevated gene expression.[24,25] The *rfc3-1* mutation probably causes defects in chromatin assembly and remodeling, leading to alterations of chromatin structure in the promoters of *PR* genes. In *rfc3-1* mutant plants, promoters of *PR* genes may adopt more accessible conformations, which result in elevated gene expression. Further characterization and identification of the other related mutants and careful investigations into chromatin modifications in *rfc3-1* might provide more detailed mechanistic insights in the future.

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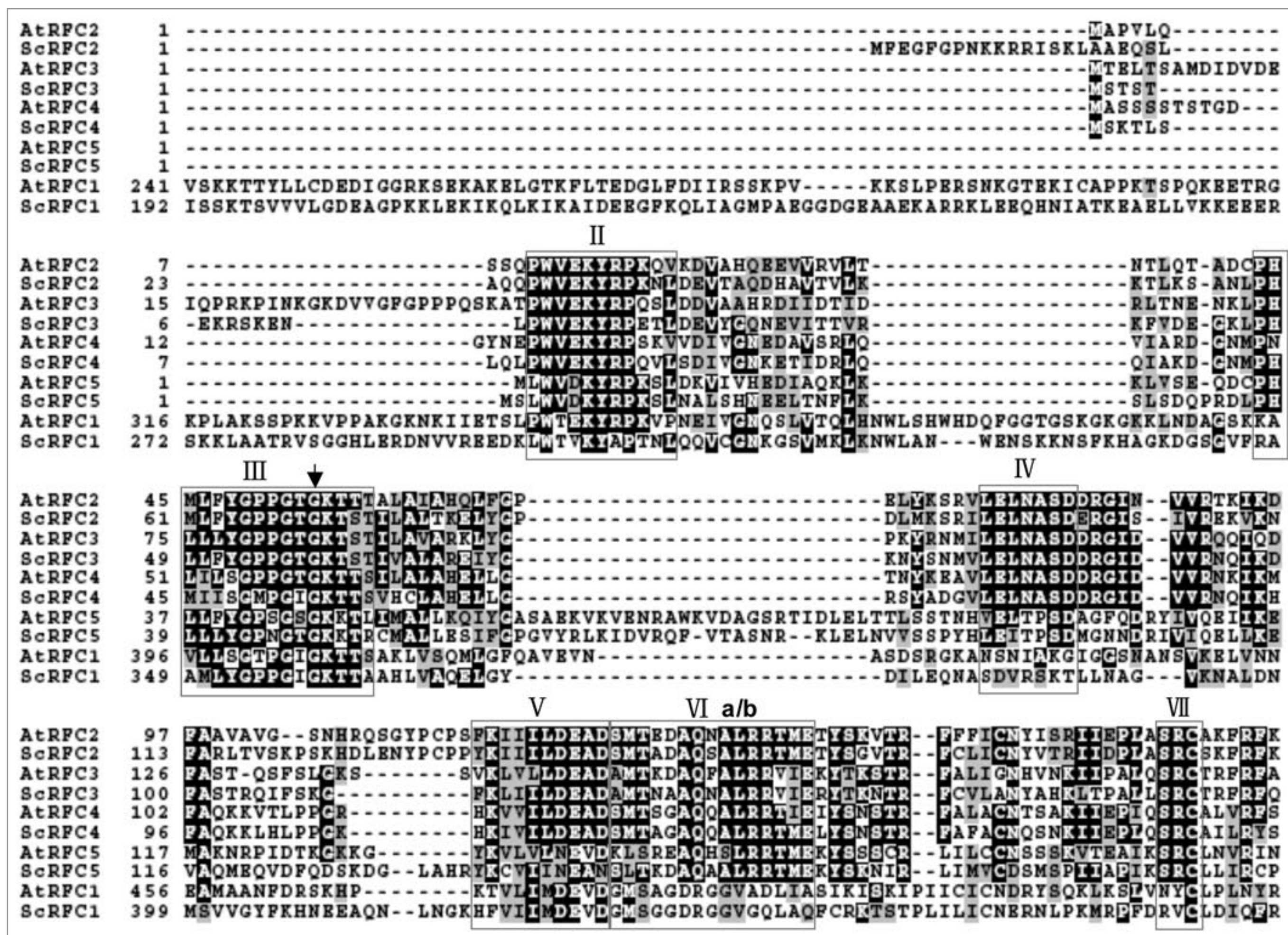

Figure 1. RFC boxes II to VII of RFC proteins from *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. Alignment was carried out using ebi ClustalW (www.ebi.ac.uk/clustalw/). The amino acids enclosed in the red frame indicate RFC boxes II to VII, which are amino acid sequence motifs conserved in all RFC subunits. Box VIa is conserved in the large RFC subunits, and box VIb is conserved in the other proteins. The arrow points to the mutation site of *At*RFC3 in *rfc3-1* mutant.

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