The resurgence of stem rust on wheat and barley, caused by Puccinia graminis f. sp. tritici (Pgt), via the highly virulent race TTKSK (aka Ug99) and its variants has brought renewed interest in stem rust resistance genes and their function. The barley Rpg1 gene has conferred durable resistance against many Pgt races for over 60 years (1, 2). The Rpg1 gene was cloned by a map-based approach (3) and validated by haplotype sequencing and stable transformation of a susceptible cultivar, “Golden Promise”, which was rendered completely resistant (4). Rpg1 is a novel disease resistance gene with a pseudokinase domain (pK1) and an active kinase domain (pK2), both of which are required for disease resistance (5). The RPG1 protein autophosphorylates in vitro, but its in vivo significance was not known. However, failure to autophosphorylate under in vitro conditions correlates with lack of RPG1 protein degradation in vivo (6) and results in disease susceptibility (5, 6). Therefore, to understand how RPG1 perceives the signal from the fungus, we investigated the in vivo phosphorylation status of RPG1 upon inoculation with the rust pathogen. Here we show that RPG1 is rapidly phosphorylated only in response to the avirulent, but not virulent, rust fungus spores and that this phosphorylation is required for disease resistance. The rapidity of the phosphorylation and apparent autophosphorylation suggest that this is the signal to initiate a cascade of biochemical events leading to activation of the defense response.

**MATERIALS AND METHODS**

Phospho-immunoprecipitation and western blot analysis: Barley seedlings were collected at different time points post-inoculation with the stem rust fungus as indicated and the total phosphorylated proteins precipitated using phospho-specific antibodies and subjected to SDS-PAGE. The phosphorylated RPG1 was detected by subsequent western blot analysis using RPG1 specific polyclonal antibodies. For the protein kinase inhibitor experiments, plants of the resistant cv. Morex were treated separately with 5 μM of seven different specific protein kinase inhibitors for 30 minutes post-inoculation with the avirulent stem rust race, MCCF. Samples were collected 15 min. post-inoculation and subjected to RPG1 phosphorylation assay or were scored for disease reaction on the 14th day after inoculation. RPG1 phosphorylation is triggered exclusively by the avirulent rust pathotypes.

**REFERENCES**

4) Horvath et al., 2003. PNAS., USA, 100: 364-369.
5) Nirmala et al., 2006. PNAS., USA, 103: 7518-7523.
6) Nirmala et al., 2007. PNAS., USA, 104: 10276-10281.

**CONCLUSIONS**

**RPG1** is phosphorylated within 5 minutes post-spore landing on the plant surface.

**RPG1 phosphorylation** is a highly race specific and takes place only in response to viable spores.

**RPG1 phosphorylation is required for resistance and probably acts as a very early signal in pathogen perception.**