Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation

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Sequence analyses of fungal genomes have revealed that the potential of fungi to produce secondary metabolites is greatly underestimated. In fact, most gene clusters coding for the biosynthesis of antibiotics, toxins, or pigments are silent under standard laboratory conditions. Hence, it is one of the major challenges in microbiology to uncover the mechanisms required for pathway activation. Recently, we discovered that intimate physical interaction of the important model fungus *Aspergillus nidulans* with the soil-dwelling bacterium *Streptomyces rapamycinicus* specifically activated silent fungal secondary metabolism genes, resulting in the production of the archetypal polyketide orsellinic acid and its derivatives. Here, we report that the streptomycete triggers modification of fungal histones. Deletion analysis of 36 of 40 acetyltransferases, including histone acetyltransferases (HATs) of *A. nidulans*, demonstrated that the Saga/Ada complex containing the HAT GcnE and the AdaB protein is required for induction of the orsellinic acid gene cluster by the bacterium. We also showed that Saga/Ada plays a major role for specific induction of other biosynthesis gene clusters, such as sterigmatocystin, terrequinone, and penicillin. Chromatin immunoprecipitation showed that the Saga/Ada-dependent increase of histone 3 acetylation at lysine 9 and 14 occurs during interaction of fungus and bacterium. Furthermore, the production of secondary metabolites in *A. nidulans* is accompanied by a global increase in H3K14 acetylation. Increased H3K9 acetylation, however, was only found within gene clusters. This report provides previously undescribed evidence of Saga/Ada dependent histone acetylation triggered by prokaryotes.

histone modification | microbial communication | secondary metabolism gene clusters

Microorganisms have an enormous potential to produce all kinds of different low molecular-weight molecules. Many of these molecules have found their way as important compounds to medicine or agriculture, such as the penicillins and avermectins, respectively (1, 2). However, a major question is how many potentially useful compounds are actually overlooked, not only because the majority of microorganisms has not yet been cultivated, but also because the increasing number of genome sequences of fungi and bacteria indicates that the genetic capability of these organisms to produce different compounds has been largely underestimated. The latter conclusion was based on the presence of genes coding for thiotemplate assembly lines, such as polyketide synthases (PKSs) (3) and nonribosomal peptide synthetases (NRPSs) (4) in various microorganisms. For example, the important model fungus *Aspergillus nidulans* harbors 28 putative PKS and 24 NRPS gene clusters (4), giving this fungus the potential to produce at least 52 different secondary metabolites. Recent data further complicate a serious estimation because communication occurs between gene clusters, which could potentially lead to even more compounds (5). A major hurdle to identifying this untapped reservoir, however, is the observation that most of these gene clusters are silent under standard laboratory conditions (6–8). To address this problem, methods were established to activate silent gene clusters by genetic engineering (6, 7, 9). The most challenging question, however, is the identification of the true function of these compounds in the habitat. Because these functions are largely unknown, it is not possible to develop strategies on a rationale basis for the activation of their biosyntheses. It is highly likely that many of these compounds are produced as chemical signal molecules or for defending the habitat (10, 11). In this context, we recently discovered that the intimate physical interaction of *A. nidulans* with a distinct soil-dwelling bacterium identified from a collection of 58 species of actinomycetes, i.e., *Streptomyces hygroscopicus* (renamed *S. rapamycinicus*) (12) led to activation of a silent polyketide synthase (PKS) gene cluster. This finding indicated that communication between microorganisms can indeed play a key role in activating silent gene clusters (13). The identified gene cluster encodes the production of the archetypal polyketide orsellinic acid, its derivative lecanoric acid, and the cathepsin K inhibitors F-9775A and F-9775B.

Because *A. nidulans* is a model organism well-suited to study various fundamental biological questions, this opened up the opportunity to shed light on the molecular mechanisms that form the basis of communication between the fungus and the bacterium. Previously, it was reported that chromatin modifications also contribute to the regulation of gene clusters (9, 14, 15). However, none of these studies addressed the question of whether this regulation is important in a natural setting of interacting microorganisms.

Here, our integrative study led to the discovery that the bacterium induces the histone modification via the main histone acetyltransferase (HAT) complex Saga/Ada in *A. nidulans*. Furthermore, because Saga/Ada is also required for the biosynthesis of penicillin, sterigmatocystin, and terrequinone, we expand the function of this complex to a set of genes not previously known.

Our work provides insight into bacteria-triggered histone modification in a fungus as a potential basis for a crosstalk between different species of microorganisms.

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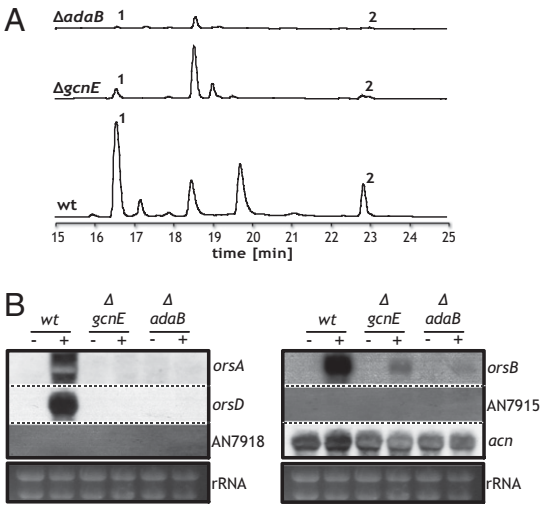

Fig. 1. Orsellinic acid and lecanoric acid biosynthesis in *gcnE* and *adaB* deletion strains of *A. nidulans* during cocultivation with *S. rapamycinicus*. (*A*) HPLC profiles of ethyl acetate extracts of Δ*gcnE* and Δ*adaB* and the wild type cocultivated with *S. rapamycinicus*. 1, orsellinic acid; 2, lecanoric acid. (*B*) Northern blot analysis of *ors* cluster genes in wild-type and deletion strains incubated without (–) or with (+) *S. rapamycinicus*. The genes AN7915 and AN7918 lie next to the *ors* gene cluster. The β-actin–encoding gene *acn*, which is not induced by *S. rapamycinicus* and not affected by Saga/Ada, served as an internal control.

Results and Discussion

A Functional Saga/Ada Complex Is Required for *ors* Gene Cluster Activation. Posttranslational modifications of histones are involved in the regulation of secondary metabolism in filamentous fungi (9). Therefore, we analyzed a number of chemical epigenetic modifiers for their impact on orsellinic and lecanoric acid production induced by the interaction of *A. nidulans* with *S. rapamycinicus*. The addition of the HAT inhibitor anacardic acid blocked the transcription of the *orsA* gene (Fig. S1), but not the actin gene analyzed as a control. By contrast, the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) activated the *orsA* gene without the need for coincubation of *A. nidulans* with *S. rapamycinicus* (Fig. S1*C*). These effects were confirmed by HPLC analysis of the fungal culture extracts (Fig. S1 *A* and *B*).

Based on these inhibitor studies, it was likely that acetylation of histones or other proteins, such as transcription factors, is required for the induction of the *ors* gene cluster by the streptomycete. By genome mining, we identified 40 genes encoding putative acetyltransferases. Deletion of 36 of these genes was successful; deletion of the remaining four appeared to be lethal (Table S1). Importantly, one of the deletion mutants showed a dramatically decreased ability to induce the *ors* genes after coincubation with the streptomycete. This mutant lacks the *gcnE* gene (16) that encodes a HAT orthologous to the *Saccharomyces cerevisiae* GCN5 (17, 18), also named KAT2 (19) (Table S1). GCN5 and its orthologs are members of several multisubunit coactivator complexes, such as SAGA, ADA, and NuA4, involved in histone acetylation and chromatin restructuring [reviewed in Baker and Grant (20)]. Gcn5 acetylates various lysine residues of histones H3 and H2B. Thereby, Gcn5 is involved in global acetylation, but also facilitates gene transcription via specific targeted acetylation (21). In *S. cerevisiae*, SAGA is recruited to promoter regions by interaction of its Tra1 subunit with transcription factors (22) such as Gal4 and Gcn4. Genome-wide expression profiling suggested a role for SAGA in

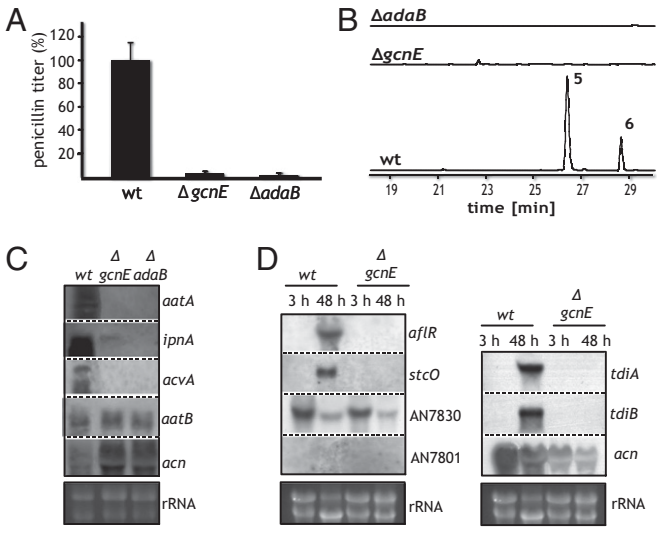

Fig. 2. Saga/Ada activity is required for the production of penicillin, sterigmatocystin, and terrequinone A in *A. nidulans*. Calculated penicillin titers (*A*) and HPLC profiles of ethyl acetate extracts (*B*) of wild type, Δ*gcnE*, and Δ*adaB*. The wild-type penicillin titer was set to 100%. 5, sterigmatocystin; 6, terrequinone A. Northern blot of penicillin biosynthesis genes *acvA*, *ipnA*, *aatA*, and *aatB* (*C*) and of selected sterigmatocystin and terrequinone cluster genes (*D*) in wild-type and deletion strains. Strains were cultivated in fermentation medium for 48 h (*C*) and under nonproducing (3 h of cultivation in AMM) and producing (48 h of cultivation in AMM) conditions (*D*). *aflR* and *stcO* are members of the sterigmatocystin cluster; the genes AN7830 and AN7801 are located directly adjacent to the cluster. *tdiA* and *tdiB* represent central genes of the terrequinone gene cluster. The actin gene *acn* served as a control for a gene not affected by Saga/Ada.

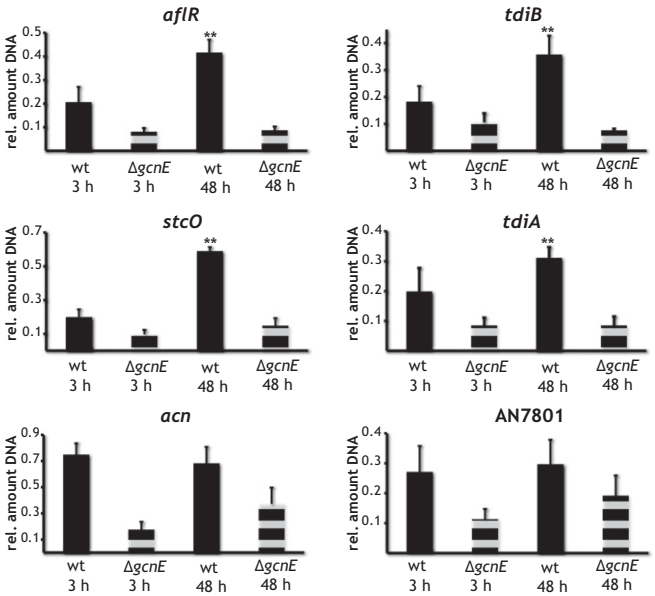

Fig. 3. Saga/Ada-mediated increase of H3 acetylation of sterigmatocystin and terrequinone cluster genes. Wild-type and Δ*gcnE* strains were incubated under nonproducing (3-h incubation) and producing (48-h incubation) conditions. ChIP was carried out by probing acetylated K9 and K14 at H3 followed by qRT-PCR analysis of the promoter regions of the genes *aflR*, *stcO*, *tdiA*, *tdiB*, AN7801, and *acn*. Data are given as the amount of DNA obtained from acetylated H3 relative to the total amount of H3 protein. SDs of the mean of three biological replicates are indicated. The missing increase of the DNA amounts of both the sterigmatocystin cluster genes *aflR* and *stcO* and the terrequinone cluster genes *tdiA* and *tdiB* under production conditions in Δ*gcnE* indicates the need for Saga/Ada for the expression of these two gene clusters. Statistical significance of data are given by the *P* value (**P* < 0.05; ***P* < 0.01).

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the regulation of stress-related genes responding to environmental stress, such as metabolic starvation, DNA damage, and heat (23). Depending on the organism, Saga/Ada consists of about 20 subunits. In *A. nidulans*, it was shown that GcnE and AdaB are essential core subunits (16). To provide evidence that GcnE is part of the Saga/Ada complex, we also deleted the *adaB* gene (Fig. S2). The *gcnE* and *adaB* deletion strains generated here were viable, indicating that the complex is not required for essential cellular functions. Furthermore, both mutants exhibited the previously reported phenotype (16), i.e., strongly reduced formation of spores and a slightly reduced growth rate. As demonstrated by HPLC analysis of the culture supernatants, both mutants showed significantly reduced production of orsellinic and lecanoric acid when cocultured with *S. rapamycinicus* (Fig. 1*A*). Consistently, in the *gcnE* and *adaB* mutant strains, there were hardly any transcripts of the *ors* genes analyzed, i.e., *orsA*, *orsB*, and *orsD* (Fig. 1*B*). The reintegration of the *gcnE* gene into the Δ*gcnE* mutant strain fully restored the wild-type phenotype, including expression of *ors* cluster genes and orsellinic acid production during cocultivation of *A. nidulans* with *S. rapamycinicus* (Fig. S3*A*). These data proved that GcnE is responsible for the described phenotypes and confirmed the function of the Saga/Ada complex for induction of the *ors* gene cluster. By contrast, the actin gene used for normalization of expression did not show a major differential regulation in both the *gcnE* and *adaB* mutant strains, suggesting that the Saga/Ada complex specifically regulates expression of the *ors* genes.

To further analyze the global meaning of GcnE on gene expression, we performed full genome microarray analysis. We showed that GcnE plays a major role in the fast fungal response to the streptomycete (*SI Results and Discussion* and Fig. S4).

Saga/Ada Complex Is a Specific Regulator of Secondary Metabolism of *A. nidulans*. To address the question of whether the Saga/Ada complex is also required for the regulation of other secondary metabolite gene clusters in *A. nidulans*, we incubated the wild-type and deletion strains in *Aspergillus* minimal medium (AMM) for 72 h. Within this time period, the fungus started to produce compounds like sterigmatocystin and terrequinone A (24). HPLC profiles of whole-culture extracts indicated that both the *gcnE* and *adaB* mutant strains were impaired in the production of sterigmatocystin and terrequinone A (Fig. 2*B*). Northern blot analysis confirmed this observation, because in the Δ*gcnE* mutant, mRNA of sterigmatocystin and terrequinone biosynthesis genes was not detected (Fig. 2*D*). The specific role played by the Saga/Ada complex for the biosynthesis of these compounds was further supported by the observation that the mRNA levels of genes not belonging to these clusters, such as the genes AN7830 and AN7801, were not affected by a nonfunctional Saga/Ada complex (Fig. 2*D*).

Another well-studied secondary metabolite produced by *A. nidulans* is penicillin. Four genes, namely *acvA*, *ipnA*, *aatA*, and *aatB*, encode the enzymes required for penicillin's biosynthesis (25, 26). Whereas *acvA*, *ipnA*, and *aatA* are located in a cluster on chromosome VI, *aatB* is separately located on chromosome I. As measured by a bioassay, the Saga/Ada mutant strains Δ*gcnE* and Δ*adaB* produced only ∼3% of this antibiotic compared with the wild type (Fig. 2*A*). This finding was reflected by a strongly re-

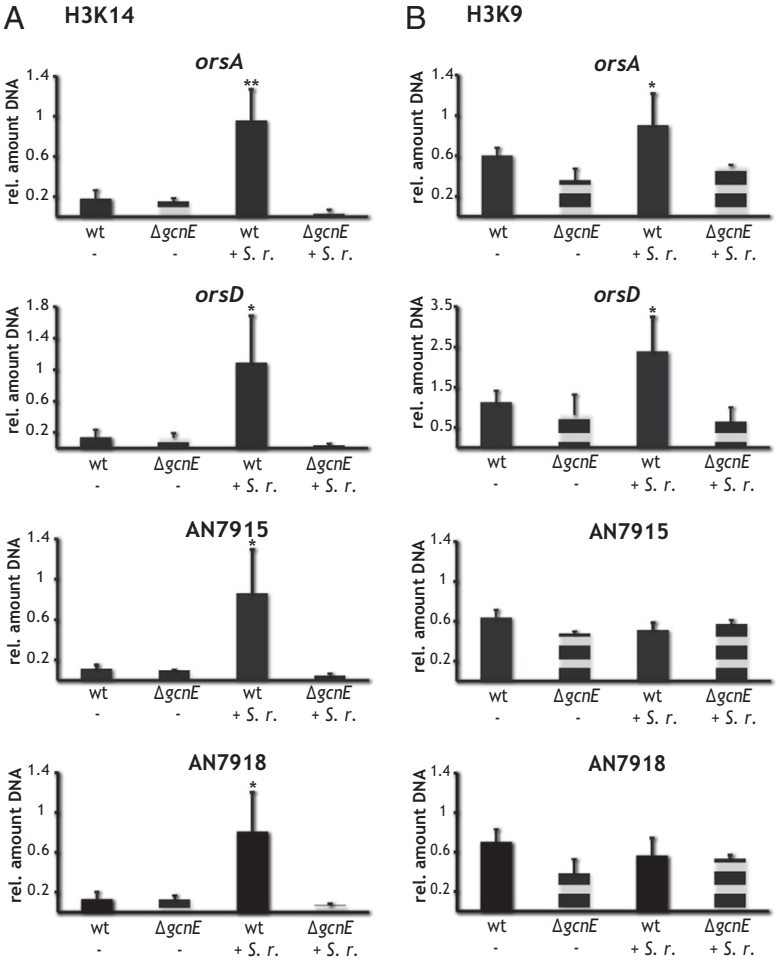

Fig. 4. H3K9 and H3K14 acetylation during interaction of *S. rapamycinicus* and *A. nidulans*. ChIP experiment of wild type and Δ*gcnE* incubated without (–) or with (+) *S. rapamycinicus* (*S. r.*). Promoter regions of *orsA*, *orsD*, AN7915, and AN7918 were analyzed by qRT-PCR. SDs of two (H3K14) and three (H3K9) biological replicates are shown. Data are given as the amount of DNA obtained from acetylated H3 protein relative to the total amount of H3 protein. Equal amounts of DNA in the wild-type and Δ*gcnE* samples under inducing conditions show the Saga/Ada-mediated H3K14 (*A*) and H3K9 (*B*) acetylation of the *ors* genes during cocultivation of *A. nidulans* and *S. rapamycinicus*. Statistical significance of data are given by the *P* value (*$P < 0.05$; **$P < 0.01$).

duced expression of the biosynthesis genes *aatA*, *ipnA*, and *acvA* (Fig. 2*C*). Interestingly, no difference for *aatB* expression was observed between the wild-type and the Saga/Ada-deficient strains. Consistent with the data on *aatB*, the actin gene is also not part of the cluster and thus not regulated by Saga/Ada (Fig. 2*C*), further confirming the specificity of the complex for distinct gene clusters. Penicillin production was restored to that of the wild type in the complemented *gcnE*^{c} strain (Fig. S3*C*).

We also analyzed whether the *laeA* gene, encoding a potential methyltransferase involved in the expression of several secondary metabolism gene clusters (27), influences activation of the *ors* gene cluster. As shown in Fig. S5, the *laeA* gene was neither expressed during bacterial–fungal cocultivation nor influenced by Saga/Ada during sterigmatocystin production. Importantly, deletion of the *laeA* gene did not affect either the *ors* gene cluster expression or orsellinic acid and lecanoric acid production, thus making an involvement of LaeA in the bacteria-induced activation of the *ors* gene cluster unlikely.

Saga/Ada Complex Catalyzes Gene Cluster-Specific Acetylation. Prominent targets of histone acetylation by the Saga/Ada complex are lysine K9 and K14 of histone 3 (H3). Recently, Reyes-Dominguez et al. (28) showed that acetylation of these H3 residues in the promoter region of the sterigmatocystin biosynthesis regulatory gene *aflR* was increased when the fungus was shifted to sterigmatocystin production conditions. However, a high level of acetylation did not necessarily lead to a high expression of cluster genes. It thus remained elusive whether these histone marks play an important role in secondary metabolite regulation and whether this acetylation is catalyzed by the Saga/Ada complex. Therefore, we determined the histone acetylation of both sterigmatocystin and terrequinone cluster genes by ChIP and used antibodies targeted against both K9 and K14 of H3. In the wild-type strain, for all promoter regions of genes residing inside the aflatoxin or terrequinone gene cluster (i.e., *aflR*, *stcO*, *tdiA*, and *tdiB*), we detected increased acetylation at both lysines of H3 after 48 h compared with the 3-h incubation (Fig. 3). This increased acetylation coincided with the expression of the genes after 48 h (Fig. 2*D*). Promoters of genes not confined to the clusters, such as *acn* and AN7801, did not show any change in acetylation of H3 (Fig. 3). These results indicate a cluster-specific acetylation of H3 in response to secondary metabolite production conditions. Furthermore, in the Saga/Ada deletion strain, at both time points, i.e., 3 h and 48 h, we observed a significant reduction in K9/K14 acetylation of H3 compared with the acetylation level in the wild-type strain (Fig. 3). Hence, Saga/Ada is responsible for these histone marks, which are likely required for secondary metabolite gene cluster activation. In addition, Saga/Ada also affected promoter acetylation of genes whose regulation did not change and that are not organized in clusters, as seen for the genes *acn* and AN7801. Similar results were obtained for proline utilization genes. Deletion of *gcnE* drastically reduced the degree of acetylation, but not the level of gene activation (16). Despite reduced H3K9K14 acetylation levels in the proline utilization genes, the loss of nucleosome positioning associated with the gene activation event was identical between the wild-type and the *gcnE* mutant. It was proposed that the strength of interaction between the activator and its target in the transcription complex elicits the extent to which activation will depend on the global acetylation function (29). We suggest the same is true for targeted acetylation by the Saga/Ada complex reported here.

Next, we addressed the question of which lysine of H3, K9 or K14, is more important for the cluster-specific gene activation. Previously, it was reported that K14 of H3 is an activation mark for light-induced gene expression in the filamentous fungus *Neurospora crassa* (30). Therefore, by ChIP analysis we studied this modification for the promoter of the sterigmatocystin regulatory gene *aflR* (Fig. S6*A*). We found a highly increased acetylation level under sterigmatocystin production conditions that was dependent on the Saga/Ada component GcnE. Surprisingly, we observed a significant increase of K14 acetylation for genes transcriptionally not regulated by Saga/Ada, i.e., *acn* and AN7801. By contrast,

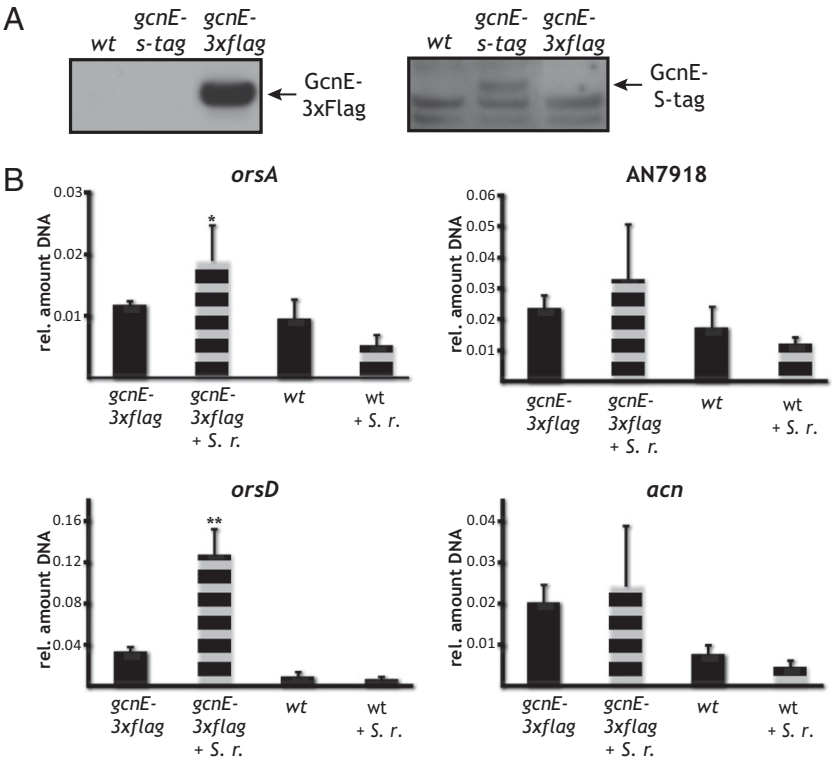

Fig. 5. GcnE is localized to the *ors* gene cluster promoters during cocultivation of *A. nidulans* with *S. rapamycinicus*. (*A*) Western blot detection of GcnE-tagged proteins. Protein lysates of wild type, *gcnE-S-tag*, and *gcnE-3xflag* coincubated with *S. rapamycinicus* were analyzed with either monoclonal anti-FLAG antibody or polyclonal anti–S-tag antibody. The anti-FLAG antibody showed high specificity for GcnE-3xFLAG, whereas the anti–S-tag antibody showed lower specificity, resulting in further bands in strains not producing S-tagged GcnE. (*B*) ChIP analysis of wild type and *gcnE-3xflag* incubated with or without *S. rapamycinicus* using an anti–GcnE-3xFLAG monoclonal antibody followed by qRT-PCR analysis of the *orsA*, *orsD*, AN7818, and *acn* promoter regions. Data are given as the amount of DNA obtained from FLAG-tagged GcnE protein relative to the amount of total DNA obtained from input protein. SDs of the mean of three biological replicates are indicated. Statistical significance of data obtained for *gcnE-3xflag* and *gcnE-3xflag* + *S. rapamycinicus* for *orsA* and *orsD* promoter was given by calculating the *P* value (*$P < 0.05$; **$P < 0.01$).

ChIP experiments with K9-specific antibodies only revealed an augmentation in precipitated DNA for promoter regions residing inside the secondary metabolite gene cluster. As seen in Fig. S6*B*, and consistent with the results obtained with the combined H3K9K14-specific antibody (Fig. 3), the cluster genes *aflR* and *stcO* showed a strong increase of K9 acetylation after 48 h that was absent in the *gcnE* deletion strain. This increase was not observed in promoters of genes that are not part of the clusters, such as *acn* and AN7801. Taken together, our data indicate that the production of secondary metabolites in *A. nidulans* is associated with a global increase in H3K14 acetylation. Specificity for cluster genes, however, depends on H3K9 acetylation.

S. rapamycinicus-Induced Activation of the _ors_ Cluster in _A. nidulans_ Is Mediated by Targeted Histone Acetylation Dependent on Saga/Ada. Our data pointed to the question of whether the acetylation level of H3 is decisive for induction of the *A. nidulans ors* gene cluster by *S. rapamycinicus*. We approached this question by performing ChIP experiments with antibodies targeted against either K9 or K14. Two promoter regions of genes located next to the *ors* cluster and of genes within the *ors* cluster were analyzed (Fig. 4). The acetylation pattern reflected the data seen for the sterigmatocystin gene cluster. Both acetylation marks (K9 and K14 of H3) increased during the bacterial–fungal interaction, but were reduced in the *gcnE* deletion strain, indicating the requirement of a functional Saga/Ada complex for (*i*) the acetylation triggered by the streptomycete and (*ii*) for acetylation of K9 and K14 (Fig. 4).

In accordance with the data obtained for the sterigmatocystin gene cluster, the increase in K14 acetylation during fungus/streptomycete coincubation was detected not only in the *ors* genes, but also in the genes AN7915 and 7918, which are located close to the *ors* cluster. By contrast, and as also observed for the sterigmatocystin genes (Fig. S6), the K9 acetylation was restricted to genes belonging to the *ors* cluster, whereas the promoters of AN7915 and AN7918 did not show a difference in this acetylation level (Fig. 4).

To unequivocally prove that Saga/Ada directly catalyzes the cluster-specific acetylation, we generated a tagged version of GcnE and performed ChIP analysis against this GcnE-3xFLAG protein. As shown in Fig. 5, GcnE-3xFLAG was specifically recruited to the *ors* cluster during cocultivation of *A. nidulans* with *S. rapamycinicus*, fully confirming the previous data obtained with the anti-H3K9/K14 antibodies. The observation that all activation events of the four different secondary metabolite gene clusters investigated here depend on H3 acetylation by Saga/Ada components GcnE/AdaB, but only the *ors* cluster is activated by bacterial–fungal interaction, deserves some additional comments. These results strengthen the hypothesis that chromatin-level regulation of secondary metabolite gene clusters represents an upper-hierarchical level of regulation, whereas each cluster requires an additional, more-specific signal to be fully activated (31). For some clusters, such as the penicillin or the sterigmatocystin biosynthesis gene cluster, several of these additional signals and regulators have been identified (25, 32). However, it is also conceivable that for some gene clusters, such as the *ors* locus, full induction only requires chromatin reprogramming induced by the bacterium, which leads to subsequent activation by the general transcription machinery (Fig. 6).

As shown here, upon direct physical contact, the bacterium induces targeted histone acetylation that requires the Saga/Ada complex. This posttranslational regulation results in the specific response of the fungus, including the production of orsellinic acid and its derivatives (Fig. 6). Based on the importance of GcnE for the production of these substances and the impact of the Saga/Ada complex on bacteria-induced secondary metabolite production, we thus anticipate a key role of GcnE in the fungal reaction to the microbial surrounding. It is intriguing that bacteria-mediated posttranslational modifications are increasingly recognized as key strategies used by pathogens to modulate factors of mammalian

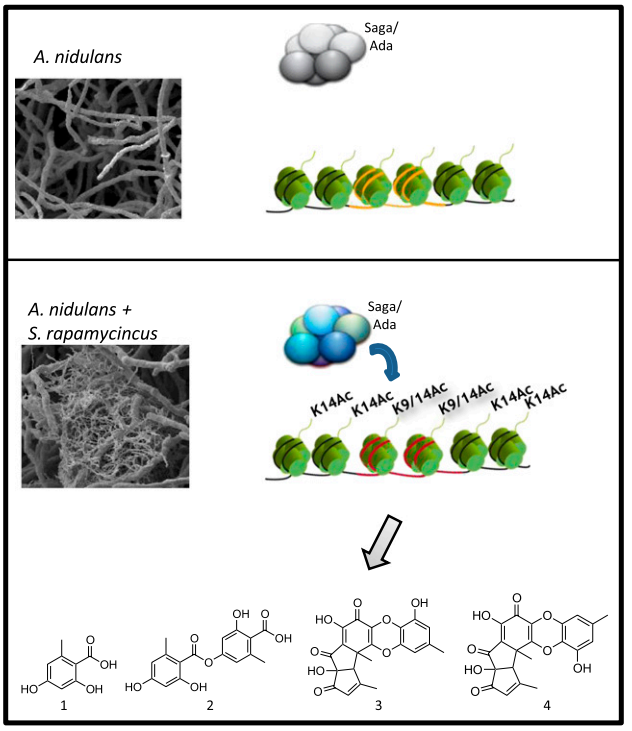

Fig. 6. Model for histone acetylation-mediated secondary metabolite gene cluster activation in *A. nidulans* by *S. rapamycinicus*. Secondary metabolite genes (orange) under noninducing conditions are characterized by deacetylated histone H3. The intimate contact between *A. nidulans* and *S. rapamycinicus* leads to an increased acetylation of histone H3 catalyzed by the Saga/Ada complex. The modification of H3K9 is specific for the secondary metabolite gene clusters (red), whereas H3K14 acetylation is not specifically targeted. Hence, Saga/Ada triggers the expression of the *ors* genes and the formation of orsellinic acid (1), lecanoric acid (2), F-9775A (3), and F-9775B (4).

hosts critical for infection (33). As shown here, such mechanisms might have been evolved very early during evolution of communication systems between microorganisms of different kingdoms.

In conclusion, we show that bacteria are able to trigger alterations of histone modification in fungi. This reprogramming leads to specific activation of the biosynthesis of distinct secondary metabolites derived from orsellinic acid. Our conclusion was based on HAT inhibitor studies and the deletion of most of the acetyltransferase genes encoded by *A. nidulans*, including HATs. Among these genes, we identified the *gcnE* gene required for the induction of the *ors* gene cluster by HPLC, liquid chromatography–mass spectrometry (LS-MS), and Northern blot analysis. Because deletion of the *adaB* gene gave the same phenotype as *gcnE* deletion, we concluded that the Saga/Ada complex is the regulatory target of bacteria-induced histone modification. Impressively, Saga/Ada is also required for production of other natural products, such as penicillin, sterigmatocystin, and terrequinone A. The production of these compounds and the mRNA levels of their biosynthesis genes were massively down-regulated in both the *gcnE* and *adaB* mutant strains. Thus, besides of one of the proposed functions of Saga/Ada in stress regulation, we expanded the function of the Saga/Ada complex to the induction of secondary metabolism gene clusters. It remains to be elucidated whether this induction caused by the bacterium represents a type of stress signal or a communication signal. We applied extensive ChIP experiments to successfully determine the histone modifications catalyzed by the Saga/Ada complex and associated with the bacterial control of orsellinic acid production. Our data suggests that specificity for activation of cluster genes depends on

H3K9 acetylation. Several distinct interactions between fungi and bacteria have been identified thus far, and only a few of them have been investigated in detail, such as the symbiosis of bacteria and fungi in lichens and the symbiosis of intracellular bacteria in zygomycetes (34, 35). Here, by the finding that the bacteria-induced secondary metabolite production in *A. nidulans* is mediated by histone modification via the Saga/Ada complex, we postulate a possible mechanism by which fungi integrate stimuli from interacting microbes.

Materials and Methods

Strains. *A. nidulans* and bacterial strains used in this study are listed in Table S2. Deletion strains were obtained by transformation (36) using the *argB* gene of *A. nidulans* as a selectable marker. Deletion cassettes were produced as described in Szewczyk (37). Strains with tagged gene variants were obtained using the *pabaA1* gene of *A. nidulans* as a selectable marker.

Media, Cultivation Conditions, and Penicillin Bioassay. *A. nidulans* was incubated in *Aspergillus* minimal medium (AMM) (38). When required, supplements were added as follows: arginine (final concentration 50 μM), *p*-aminobenzoic acid (3 $\mu g \cdot mL^{-1}$), pyridoxine HCl (5 $\mu g \cdot mL^{-1}$), and biotin (0.6 $\mu g \cdot mL^{-1}$). Each experiment was started with an AMM overnight culture that was inoculated with 4×10^6 spores mL^{-1} and incubated at 37 °C. The *gcnE* and *adaB* deletion strains were incubated 4 h longer than the wild type to warrant the formation of equal amounts of biomass. The biomass was separated from the medium using Miracloth and inoculated into fresh AMM. For sterigmatocystin and terrequinone production experiments, experimental cultures were grown at 37 °C for 72 h. Samples for RNA isolation were taken after 3 h and 48 h. Cross-linking for ChIP experiments was carried out at the same time points. For coincubation experiments, freshly prepared *S. rapamycinicus* was added to an *A. nidulans* culture, as described in Schroeckh et al. (13). If required, SAHA (4 mM) and anacardic acid (100 μM) were added at the same time point. For both RNA isolation and cross-linking for ChIP, mycelia were harvested after 3 h of coincubation. After 24 h of cultivation, the coculture was prepared for HPLC analysis. For penicillin production, fermentation media were applied as previously described (39). Penicillin bioassay was carried out with *Bacillus calidolactis* C953 as an indicator organism (40). Three biological replicates were analyzed to calculate SDs.

Preparation of Chromosomal DNA, Total RNA, Northern and Southern Blot Analyses, and Quantitative RT-PCR. Isolation of chromosomal DNA and of total RNA of *A. nidulans*, Northern and Southern blot analysis, and quantitative RT-PCR (qRT-PCR) were performed as described in Schroeckh et al. (13). Oligonucleotides are listed in Table S3.

Western Blot, ChIP Coupled to qRT-PCR Analysis, Generation of Transformation Cassettes, Microarray, and Analysis of Secondary Metabolites. Western blot analysis, ChIP coupled to qRT-PCR analysis, generation of deletion cassettes, microarrays, and extraction, HPLC, and LC-MS of secondary metabolites are described in *SI Materials and Methods*.

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