Aspergillus fumigatus Septation Initiation Network (SIN) kinases contribute to fungal pathogenesis, cell wall construction, and rRNA metabolism

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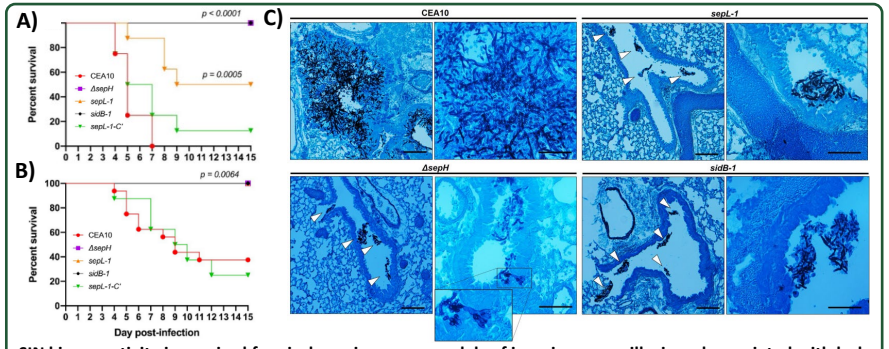
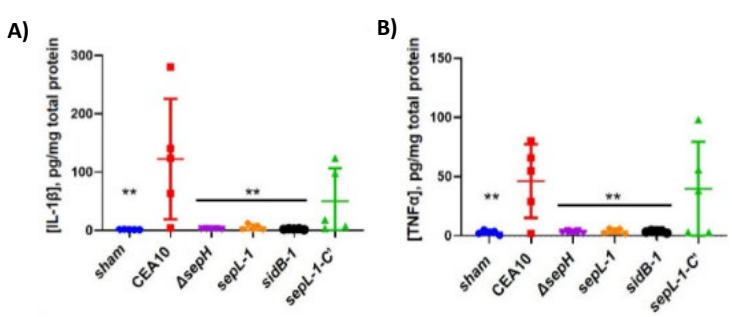
Introduction

Aspergillus fumigatus is the main causative agent of invasive aspergillosis. The therapeutic armamentarium to fight against this disease is limited. Therefore, we sought to identify genetic pathways that might serve as novel therapeutic targets through generating and studying an *A. fumigatus* protein kinase disruption mutant library. The aim of the current work is to understand the importance of the Septation Initiation Network (SIN) kinase genes, *sepH*, *sepL* and *sidB*, in *A. fumigatus* pathobiology.

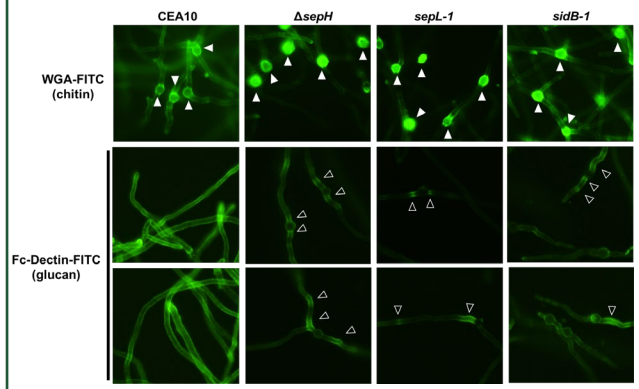
Results



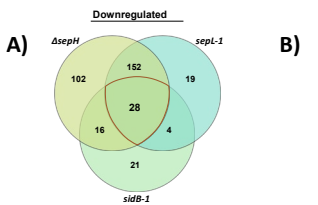
SIN kinase activity is required for virulence in mouse models of invasive aspergillosis and associated with lack of tissue invasion. Mice (n = 8 / group for *ΔsepH*, *sepL-1*, *sidB-1* and *sepL-1-C'*; n=16 for CEA10) were chemotherapeutically immune suppressed with both cyclophosphamide and triamcinolone acetonide (**A**) or triamcinolone acetonide alone (**B**) and inoculated with 1 x 10^5 conidia of the indicated strain. Survival was followed for 15 days post-inoculation. Statistical analyses (Mantel-Cox Log-rank test) identified significantly reduced virulence for all SIN kinase mutant strains *vs.* the Cea10 control. (**C**) photomicrographs of Gomori methenamine silver (GMS)-stained lung tissue sections from the Cea10, *ΔsepH*, *sepL-1* and *sidB-1* at day +4 post-inoculation.



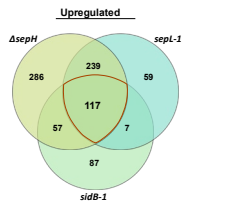
Loss of virulence and the lack of tissue invasion of the SIN kinase mutants is characterized by decreased hot response to infection.
Quantitation of IL-1β (**A**) and TNFα (**B**) revealed decreased host response in SIN kinase mutant infected mice. Mice (n=5/group) were immune suppressed as indicated for fungal burden analysis and lung tissue was removed at day +4 post inoculation, homogenized and analyzed by ELISA. **p = 0.0024 for (**A**); **p = 0.0031 for (**B**).



Hyphal staining revealed altered distribution of chitin cell wall deposition and reduced β-glucan levels in the Sin kinase mutants.
Conidia of each strain were grown in static conditions at 37˚C for 16 hours in GMM broth. After incubation time, mycelia were stained using WGA-FITC (chitin staining) or FC-Dectin-FITC (glucan staining) respectively. White arrowheads indicate patches of chitin around initial germinated conidia. On the other hand, black arrowheads indicate intermittent bright spots along hyphae of the SIN kinase mutants.

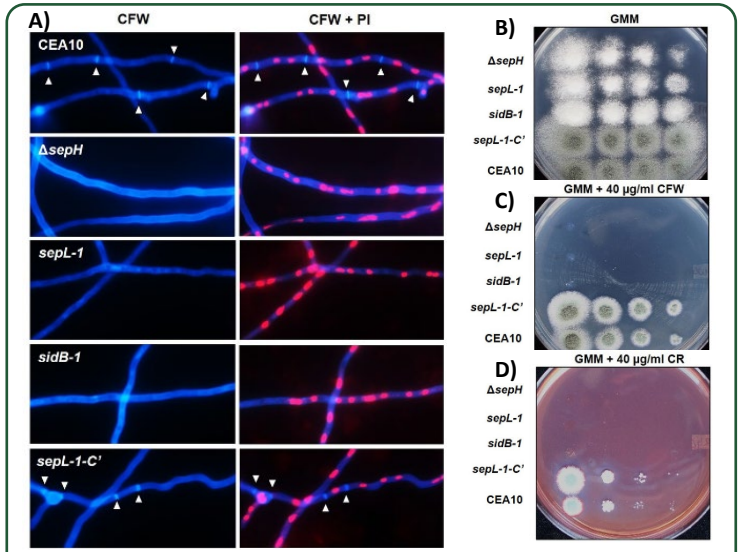


GO term	Frequency (%)
Transport	44%
RNA metabolic process	33%
Ribosome biogenesis	28%
Regulation of biological process	17%
Organelle organization	11%
Transcription, DNA-templated	11%
Response to chemical	6%
Sexual sporulation	6%
Filamentous growth	6%
Developmental process	6%
Cell cycle	6%
Asexual sporulation	6%
Cellular protein modification process	6%
Cellular homeostasis	6%
Other	17%



GO term	Frequency %)
Secondary metabolism	7
Resistance proteins	5
Metabolism of peptide derived compounds	2
Nitrogen, sulfur and selenium metabolism	6
Pyruvate dehydrogenase complex	2
Energy conversion and regeneration	3
Metabolism of beta-lactams	2
Detoxification by export	4
Catabolism of nitrogenous compounds	2
Non-vesicular cellular import	5
Regulation of glycolysis and gluconeogenesis	2
Metabolism of alanine	1
Xanthine catabolism	1

RNAseq analyses identified 997, 625 and 337 genes from the *ΔsepH*, *sepL-1*, and *sidB-1* differentially expressed when compared to the wild type.
(**A**) Genes differentially downregulated in the SIN kinases mutants when compared to the WT. We found 28 commonly downregulated genes among the SIN kinases mutant strains being transport and rRNA metabolic functions the ones most downregulated in comparison with the WT. (**B**) Genes differentially upregulated in the SIN kinases mutants when compared to the WT. We found 117 commonly upregulated genes among the SIN kinases mutant strains. In his case, Secondary metabolism alongside general metabolism genes were the categories that grouped a great number of genes upregulated.



The Septation Initiation Network (SIN) kinases are each required for hyphal septation and protection against cell wall damage in *A. fumigatus*.
(**A**) Loss of any single SIN kinase results in the absence of septa in mature hyphae. Conidia from each strain were cultured to mature hyphae and subsequently stained with calcofluor white (CFW) and propidium iodide (PI) to visualize septa and nuclei, respectively. White arrowheads indicate septa in the Cea10 and sepL-1-C' strains. Loss of any single SIN kinase results in absence of growth in the presence of the cell wall destabilizing compounds CFW (**C**) or CR (**D**). Conidia from each strain were spot inoculated in descending concentrations onto GMM alone or GMM containing either 40 μg/ml CFW or CR.

Conclusion

Our results suggest that SIN kinase activity is essential for virulence, normal germination, mitosis and for proper cell wall formation. In consequence, maybe due to cell wall defects and the lack of tissue invasion ability of the SIN kinase mutants, they showed a decreased cell host response.
Although no direct transcriptional link to cell wall biosynthesis was uncovered, RNAseq analyses suggest hyphal septation is linked to cellular transport, RNA metabolism and ribosome biogenesis.