Unravelling the mechanistic basis of methionine synthase essentiality

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The high mortality rates associated with fungal infections and the continuous emergence of resistant strains highlight the urgent need for novel antifungals. We have previously shown that the methionine synthase encoding gene (*metH*) is essential for *Aspergillus fumigatus* viability and virulence (1), which led us to propose this enzyme as a novel drug target. This protein is also essential for *Cryptococcus neoformans* virulence (2) and *Candida albicans* viability and virulence (3), suggesting that drugs targeting this enzyme may have a broad spectrum of action. However, the underlying reason for methionine synthase essentiality is not obvious and much needed to fully explore the potential of this enzyme as target for the development of novel chemotherapeutics. In this work we advance our insight of the mechanistic basis of its essentiality.

1. *metH* is essential in different *A. fumigatus* isolates

4. Supplementation of potentially depleted compounds

A) Addition of Dox to an overnight grown mycelium strongly repressed *metH* expression from 30 minutes. **B)** As little as 0.1 $\mu\text{g/ml}$ prevented colony formation on methionine containing (5 mM) plates in both the ATCC46645 and the A1160 genetic backgrounds

Of all potentially depleted compounds, only adenine was able to partially restore growth in restricted conditions. Further addition of TMP slightly improved growth ability. None of the conditions restored growth to wt level.

2. Enzymatic activity is required for methionine synthase essentiality

5. Overexpression of *mecA* to reduce homocysteine accumulation restores growth

Ectopic expression of SNP *metH* mutants in the *metH-tetOFF* background (4). Mutated protein D616A did not restore growth in restrictive conditions, suggesting that enzymatic activity is required for viability. Mutated protein Y662A triggered significant growth in methionine depleted medium, indicating that enzymatic activity is not fully disrupted; and completely restored growth in methionine containing medium, suggesting that partial activity is sufficient for viability.

A) Plasmid (pJA49) allows easy integration of any ORF in its unique StuI restriction site to episomally overexpress genes in *A. fumigatus*. **B)** Introduction of pJA61 in the *H_{OFF}* strain resulted in an overexpression of *mecA* **C)** Overexpression of cystathionine- β -synthase (*mecA*) significantly restored growth in repressed conditions. Overexpression of the S-adenosylhomocysteinase (*sahL*) did not recover growth, whilst overexpression of the *A. nidulans* bleomycin (homocysteine thiolactone) hydrolase (*blhA*) completely restored growth. Further addition of serine and adenine had no effect.

CONCLUSIONS

1. Methionine synthase is essential in *Aspergillus fumigatus*.
2. Enzymatic activity is required for essentiality
3. Supplementation of molecules which could be depleted in the absence of methionine synthase does not fully restore growth.
4. Overexpression of *mecA* restores growth, indicating that a toxic accumulation of homocysteine takes place.
5. Overexpression of *blhA* restores growth, indicating that conversion to homocysteine thiolactone is likely the reason for toxicity.

REFERENCES

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