AtrR, an essential regulator of azole resistance in *Aspergillus fumigatus*, acts via the TR34 element in the *cyp51A* promoter

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Introduction: Previous work identified AtrR as an important regulator of azole resistance in *A. fumigatus* via its transcriptional control of an ABC transporter gene and the *cyp51A* locus encoding the azole target enzyme. Here we identify the binding site for AtrR called the AtrR Response Element (ATRE) with a focus on the TR34 element found in the *cyp51A* promoter.

Figure 2. **Mutant forms of AtrR are hyperactive.** A. Western blot of AbcG1 ABC transporter expression in the indicated strains is shown along with the MIC for voriconazole (Numbers above each lane). The two hyperactive alleles of *atrR* correspond to either an epitope-tagged allele (*atrR-3X HA*) or an overproduced but untagged form (*hspA-atrR*). B. Levels of the indicated mRNAs were determined by RT-qPCR.

Figure 1. **Dnase I protection analysis using recombinant AtrR and SrbA on the TR34 element from *cyp51A*.** The location of the SrbA response element (SRE) and ATRE are indicated at the left.

Figure 3. **Epistatic relationship between *atrR* and *srbA*.** The indicated strains were plated on minimal media and a disk containing 1 mg/ml voriconazole placed in the center.

Figure 4. **Model for transcriptional regulation of *cyp51A*.** A. The location of the ATRE and SRE are indicate as colored boxes. The repression via the CBC/HapX complex (Gsaller, et al PLOS Pathogens 14:12, 2016) is indicated by the solid T bar. The presence of the TR34 duplication is postulated to weaken this repression leading to elevated *cyp51A* transcription. SrbA response element (SRE) and ATRE are indicated at the right. B. DNA sequence of the elements in A.

Figure 5. **Genetic relationships between AtrR, SrbA and selected target genes.** Both AtrR and SrbA control expression of ergosterol pathway genes (*erg*) but only AtrR regulates transcription of the ABC transporter-encoding gene *abcG1*.

Acknowledgements: This work was carried out by Dr. Sanjoy Paul and supported by NIH GM49825 and AI113748. I thank Drs. Daisuke Hagiwara, Mike Bromley and Paul Bowyer for sharing unpublished data and important discussions.