A genetic screen for *blm* suppressors in *Ustilago maydis* identifies novel proteins effecting DNA repair and recombination

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Introduction

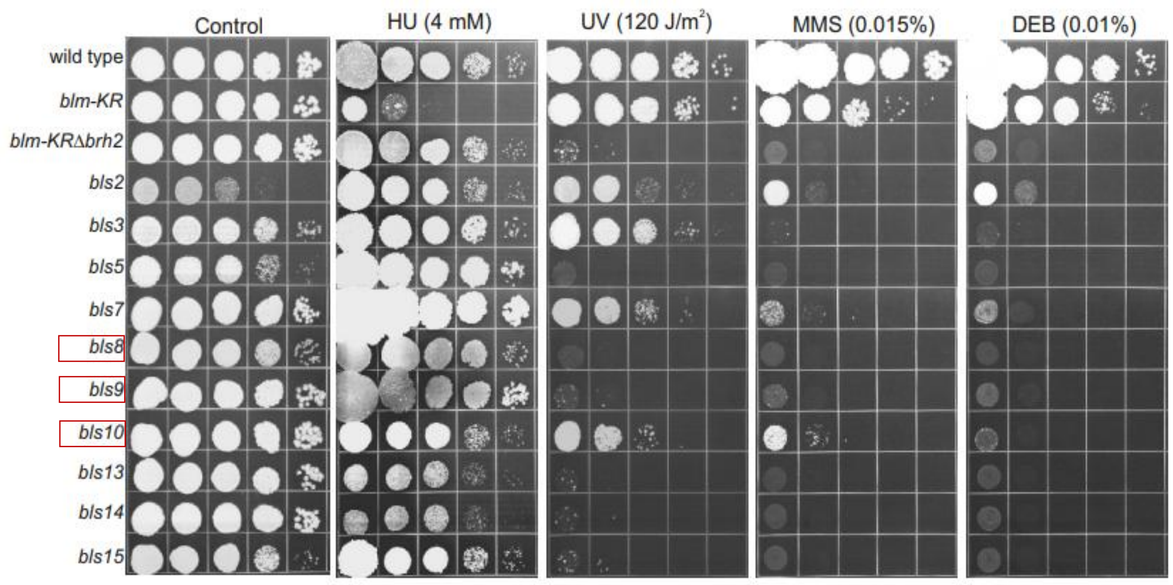
The maintenance of genome integrity is a fundamental cellular process and it is highly conserved among all domains of life. Since the DNA molecule is under constant threat from endogenous and exogenous factors that cause its damage, organisms have evolved several DNA repair mechanisms. Homologous recombination (HR) is essential for the error-free repair of DNA double strand breaks, which are the most deleterious lesions.

Despite extensive research of HR in different organisms, not only that all functions of known HR factors and interactions among them are still unknown, but also the question arises as whether there is a possibility of the existence of unknown factors. The focus of our research is to uncover novel cellular factors that regulate HR, by isolating suppressors of ***blm*** in ***Ustilago maydis***, a unicellular phytopathogen, which is extremely resistant to radiation and has DNA repair system similar to that in human, with highly conserved BRCA2 (named Brh2). .

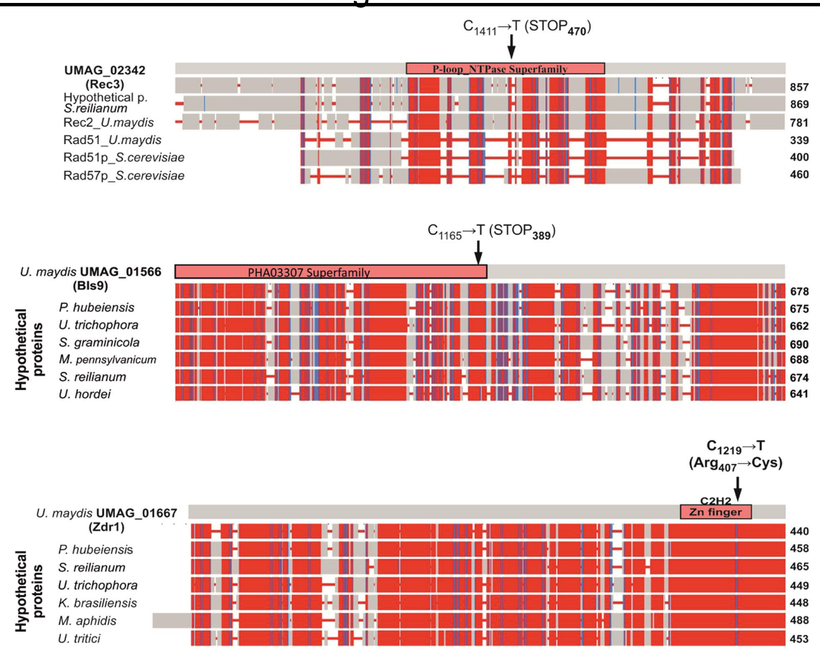
Given that inactivation of HR genes fully suppresses the lethality of *blm* on hydroxyurea (HU), these mutants could be used to identify novel HR-related genes.

Results and Conclusions

Isolation of *blm-KR* suppressors (HU-resistant mutants generated by UV mutagenesis)



Identification of the three genes defective in MMS sensitive *blm-KR* suppressors

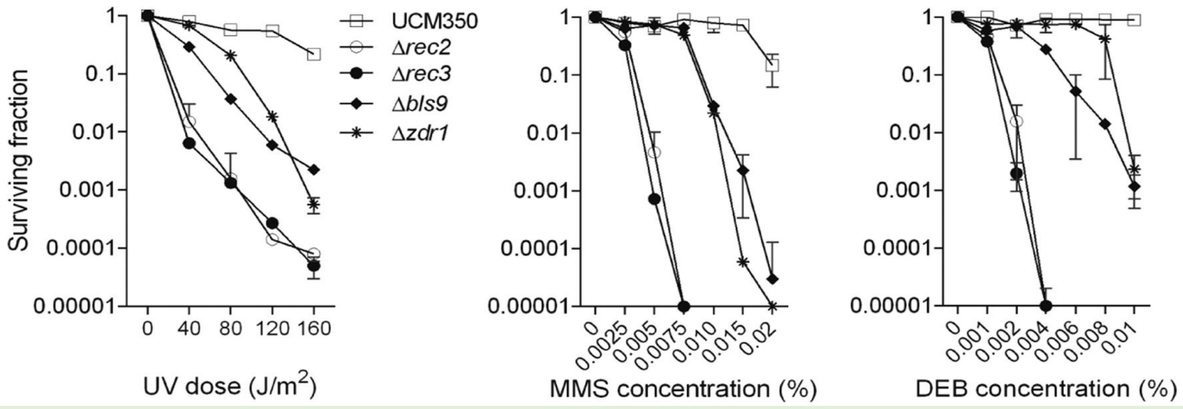


Bls8 – Rec3
- Homology within ATPase domain: new Rad51 paralogue
- Resembles rec2

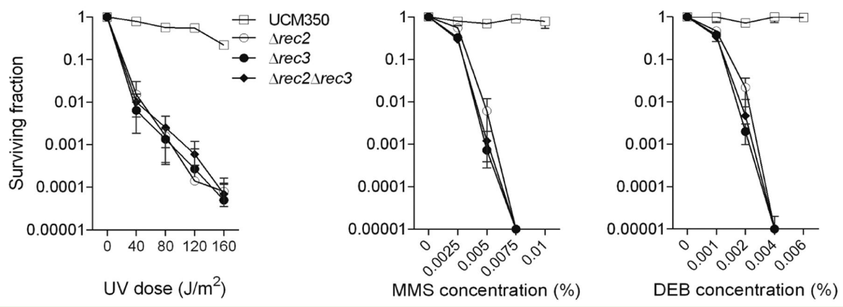
Bls9
- Homology with uncharacterized proteins of phylogenetically related fungi

Bls10 – Zdr1
- Homology with uncharacterized proteins of phylogenetically related fungi
- Conserved Cys2-His2 (C2H2) zinc finger domain

DNA-repair phenotype of the Δrec3, Δbls9 and Δzdr1 mutants

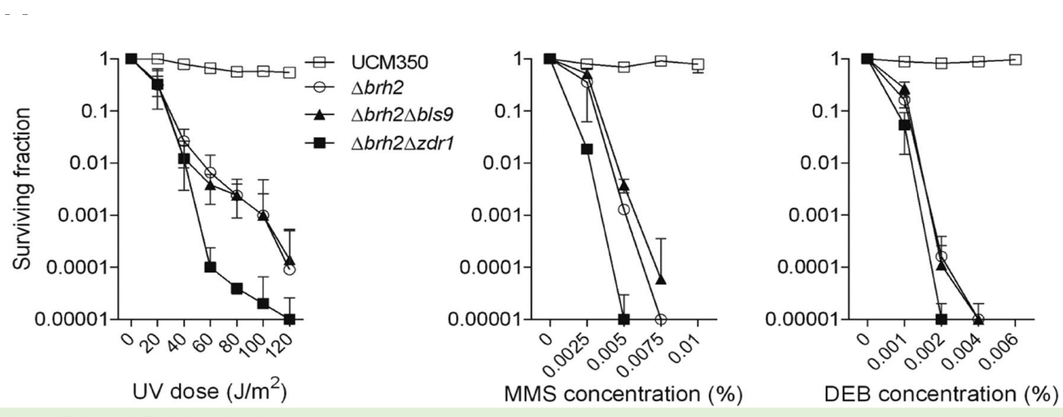


- A common feature among these novel factors (Rec3, Bls9, and Zdr1) is their prominent role in UV, MMS and DEB resistance.
- *Δrec3* has the most severe phenotype when challenged by all three DNA-damaging agents; about the same (extreme) sensitivity to the DNA-damaging agents as does the *Δrec2* - Rec3 is crucial for DNA repair proficiency in *U. maydis* cells.

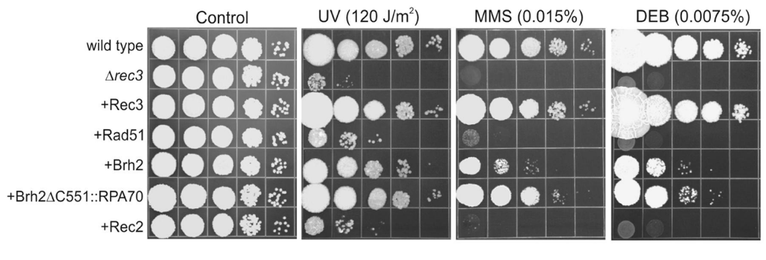


- Double Δ*rec2*Δ*rec3 mutant is no more sensitive than the single mutants* ⟶ *These two genes share the same function in DNA repair.*

The Brh2 connection

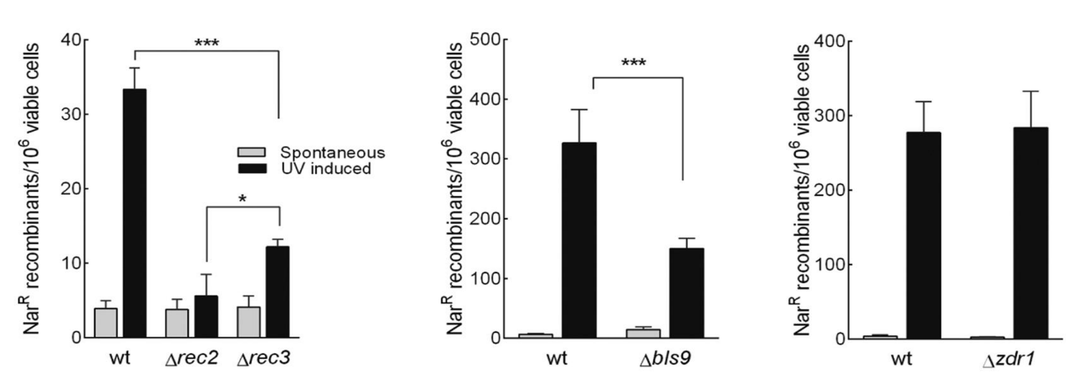


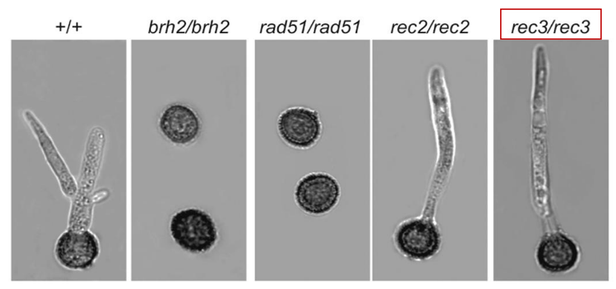
- Function of Brh2 and Bls9 is epistatic to one another.
- Brh2 and Zdr1 operate in different DNA-repair pathways.

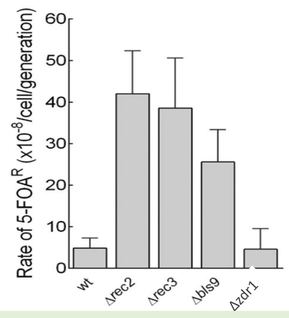


- Recombination proficient forms od Brh2 suppress *rec3* phenotype (resembles *rec2*).

The roles of *Rec3, Bls9 and Zdr1* in homologous recombination

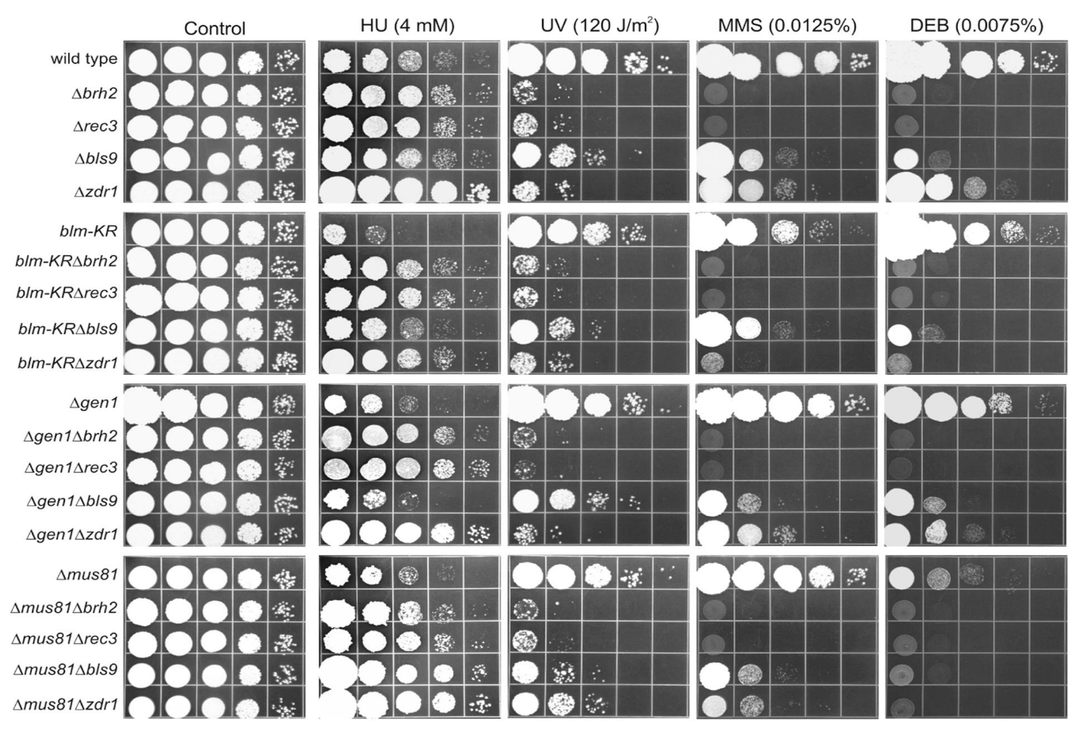






- Rec3 and Bls9 play roles in induced allelic recombination.
- Rec3, like Brh2, Rad51 and Rec2, is absolutely required for completion of meiosis, and by inference, meiotic recombination (spore germinated, formed promycelia, but no further nuclear division).
- Rec3 and Bls9 play an important role in protecting the genome from mutations .

Ability to suppress HU-sensitivity of Δ*gen1 and Δmus81 mutants*



- Deletion of *Rec3* and *Zdr1* can suppress HU-sensitivity of *blm-KR,* Δ*gen1,* and Δ*mus81* mutants.
- Loss of Bls9 does not rescue HU-sensitivity of Δ*gen1.*

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