A novel method for monitoring assembly of multisubunit complexes *in vivo*

- **Multisubunit complexes conduct most essential cellular functions.** Disruptions to the biogenesis or composition of numerous multisubunit complexes underlie human diseases.
- **Assessing the assembly state and dynamics of multisubunit complexes *in vivo* has proven exceptionally challenging.** Typical methods to investgate macromolecular structure rely on biochemical analyses of lysed cells, which introduces artifacts and compromises temporal and spatial information.
- **We describe a split protein-based reporter system to assess multisubunit complex assembly status *in vivo*.** This system relies on reconstitution of a methotrexate resistance gene (DHFR, shown) or a fluorescent protein (mCherry, not shown) to yield growth or fluorescence, respectively.

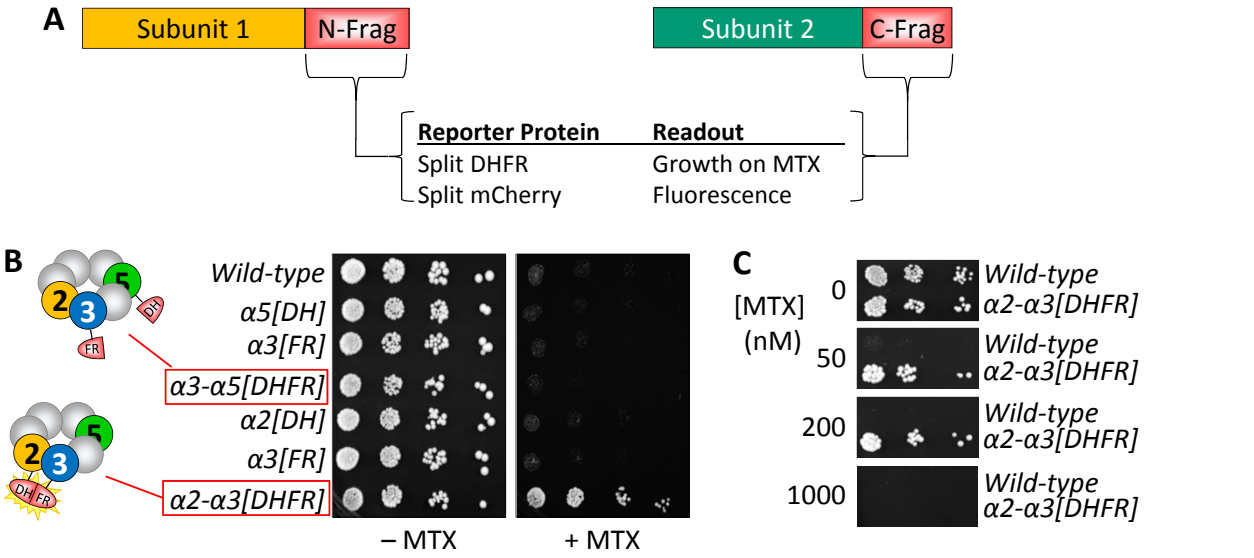

Figure 1. Assembly-dependent protein complementation. Two subunits whose assembly is to be measured are tagged with N- or C-terminal fragments of a split protein reporter. Upon assembly into a complex, the two fragments are juxtaposed, the reporter is reconstituted, and its activity is restored. **B,** In wild-type cells, proteasome subunit α2 is adjacent to α3 in the proteasome, whereas α5 is not. Assembly of proteasomes containing tagged α2 and α3 reconstitutes DHFR and allows growth of these yeast when they are spotted onto media containing 200 nM methotrexate (MTX). In contrast, α3 and α5 do not reconstitute DHFR or restore growth because they are not juxtaposed. **C,** Growth of both wild-type and reporter strains can be titrated with MTX, providing substantial control over the sensitivity of this growth-based assay.

- **This system can be exploited to identify novel genes involved in multi-subunit complex assembly or disassembly, to assess the dynamics or compartmentalization of assembly events, or to follow the fates of defective or misassembled complexes *in vivo*.**

Interested in macromolecular complexes, protein degradation, or their ties to disease? Let's discuss collaboration!

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