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## ***Chlamydomonas reinhardtii* tubulin-gene disruptants for efficient isolation of strains bearing tubulin mutations**

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### Abstract:

The single-cell green alga *Chlamydomonas reinhardtii* possesses two  $\alpha$ -tubulin genes (*tua1* and *tua2*) and two  $\beta$ -tubulin genes (*tub1* and *tub2*), with the two genes in each pair encoding identical amino acid sequences. Here, we screened an insertional library to establish eight disruptants with defective *tua2*, *tub1*, or *tub2* expression. Most of the disruptants did not exhibit major defects in cell growth, flagellar length, or flagellar regeneration after amputation. Because few tubulin mutants of *C. reinhardtii* have been reported to date, we then used our disruptants, together with a *tua1* disruptant obtained from the *Chlamydomonas* Library Project (CLiP), to isolate tubulin-mutants resistant to the anti-tubulin agents propyzamide (pronamide) or oryzalin. As a result of several trials, we obtained 8 strains bearing 7 different  $\alpha$ -tubulin mutations and 12 strains bearing 7 different  $\beta$ -tubulin mutations. One of the mutations is at a residue similar to that of a mutation site known to confer drug resistance in human cancer cells. Some strains had the same amino acid substitutions as those reported previously in *C. reinhardtii*, however, the mutants with single tubulin genes showed slightly stronger drug-resistance than the previous mutants that express the mutated tubulin in addition to the wild-type tubulin. Such increased drug-resistance may have facilitated sensitive detection of tubulin mutation. Single-tubulin-gene disruptants are thus an efficient background of generating tubulin mutants for the study of the structure-function relationship of tubulin.

### ■ 理工学研究所との関連

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