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Studing of factors affecting the stoichiometry of the ATP synthase c ring.

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ATP synthase is an essential protein complex integral to the bioenergetics of all living organisms. Located within the membrane, it facilitates the conversion of ADP into ATP using an inorganic phosphate, leveraging the proton concentration gradient across the membrane [1]. The proton flow induces rotation in the membrane domain of ATP synthase (specifically, the rotor c-ring). This rotation triggers conformational shifts in the F_1 segment of ATP synthase, catalyzing ATP synthesis.

A critical bioenergetic metric is the ATP/H^+ ratio, which represents the number of ATP molecules synthesized for every proton passing through the c-ring. This ratio is influenced by the stoichiometry of the c-ring, specifically the number of c_1 monomers constituting the c_n-ring, and is generally represented as 3/n. While evidence suggests that the stoichiometry of cn is influenced by the amino acid sequence of c_1 [2], several questions remain about the primary determinants of c-ring stoichiometry. The impact of the surrounding protein environment on stoichiometry, for instance, remains elusive. Additionally, for many organisms, the stoichiometry of c-ring is yet to be determined, and current algorithms, such as Alphafold, have been inadequate in predicting it.

In this study, we examined a recombinant c-ring derived from spinach chloroplasts, produced in *Escherichia coli* cells. The gene was cloned into *E.coli* TG-1 cells, followed by the isolation and purification of c_1 monomers. Utilizing gel electrophoresis and Western blotting, we analyzed the proteins. Our findings confirm the successful expression of the c_1 protein from spinach chloroplasts in *E.coli* cells. We further discuss methodologies for assembling the c ring within *E.coli* cells.

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[1] Vlasov, A. V., et al. "Unusual features of the c-ring of F_1F_o ATP synthases." Scientific reports 9.1 (2019): 1-11.
[2] Pogoryelov, Denys, et al. "Engineering rotor ring stoichiometries in the ATP synthase." Proceedings of the National Academy of Sciences 109.25 (2012): E1599-E1608.

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