Purification of the hexameric Smc5/6 complex from S. cerevisiae

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Background and aim:

The genomic DNA in every living cell is orders of magnitude longer than the cell itself. This fact necessitates extensive organisation of the genetic material to ensure unperturbed transcription, replication, DNA repair, and chromosome segregation. Sophisticated multi-protein complexes from the Structural Maintenance of Chromosomes (SMC) family play important roles in this organisation by folding the DNA into loops, a process known as 'loop extrusion'.

One of these complexes is the Smc5/6 complex, which contains six distinct proteins (Smc6, Smc5, Nse4, Nse3, Nse2, and Nse1). We had previously purified this complex of yeast origin (S. cerevisiae) after over-expression in bacteria (E. coli), and characterized its DNA-dependent ATPase, DNA-interaction, and loop-extrusion activities (1, 2).

However, since we worked on a eukaryotic complex expressed in a prokaryotic host, the possibility remained that these activities might be influenced by post-translational modifications (e.g. phosphorylation) which would not be present in our purified material. Furthermore, other groups obtained different results when analyzing the loop-extrusion activities of a complex purified directly from yeast. We therefore wanted to also purify the complex directly from S. cerevisiae and compare its activities (first and foremost the loop-extrusion activity) to the material produced in E. coli.

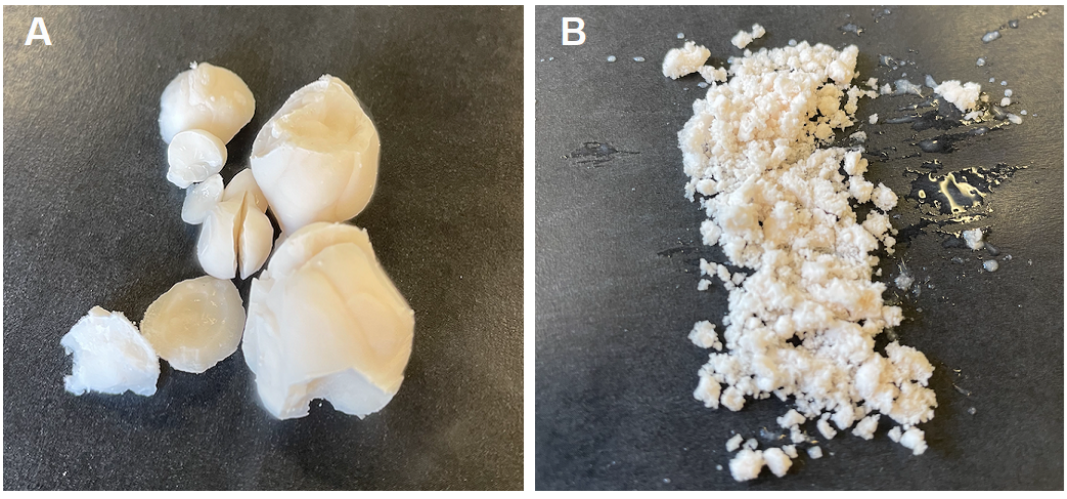

Figure 1: (A) frozen drops of yeast cells in lysis buffer (`popcorn`)
(B) material after freezer-milling

Reason for choosing the Freezer Mill and experimental overview:

What is problematic with purification from yeast is that the tough cell wall makes efficient cell lysis difficult. Enzymatic removal of the cell wall is possible, but the large amount of enzyme needed to lyse cell pellets from several liters of yeast culture makes this approach very expensive. The best alternative is mechanical lysis by freezer-milling.

We obtained a yeast strain that contains galactose-inducible genes for all 6 subunits of the yeast Smc5/6 hexamer (one of which contains an affinity tag), which had previously been successfully used to obtain the complex (3). This strain was grown in 4 liters of glucose-free medium and expression was induced by addition of 2 % galactose. The cells were then harvested, and the pellet resuspended in an equal volume of a suitable lysis buffer.

This suspension was then added dropwise into a liquid nitrogen bath to freeze it into small drops ('yeast popcorn', Fig. 1A). The frozen cells were added into a grinding vial with an impactor, loaded into the pre-cooled 6875 Freezer Mill, and ground to a fine powder using 3 grinding cycles (Fig. 1B). The powder was then transferred to 50 ml Falcon-tubes (pre-cooled on dry ice) and thawed on ice. Cell debris was removed by centrifugation at 40,000 g at 4°C, and the proteins were purified using an established protocol (3).

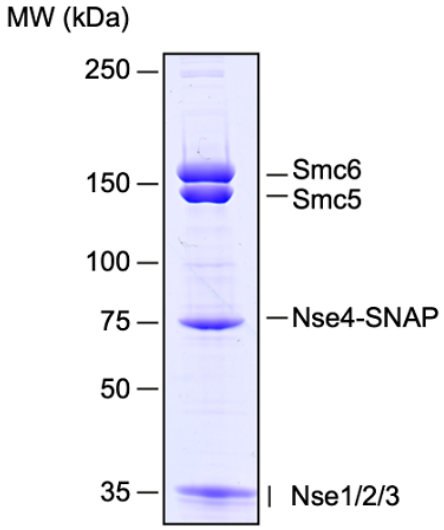

Figure 2: Representative SDS-PAGE gel showing the purity of the obtained material. All 6 subunits of the hexameric S. cerevisiae Smc5/6 complex are present.

Experimental outcome:

This purification yielded several milligrams of enzymatically active material (Fig. 2), and its analysis by single-molecule loop-extrusion assays confirmed that its activity was indistinguishable from the bacterially expressed version (4). We could thus conclude that the loop-extrusion activity is not noticeably influenced by potential modifications.

References:

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