Mass Spectrometry & Purification Techniques

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Opinion Article

A General Technique for Rapidly Purifying Native Chromatin Fragments

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ABOUT THE STUDY

Saccharomyces cerevisiae (budding yeast) is a single-celled eukaryote that has proven valuable for epigenetic research due to its powerful genetic tractability and thoroughly annotated genome. Because yeast can be produced in vast quantities in liquid culture and under a range of experimentally controlled circumstances, it is also ideal for biochemical investigations of chromatin, which would substantially supplement its genetic and genomic advantages. Unfortunately, for a variety of reasons, the isolation of chromatin from yeast has proven exceedingly difficult. There are various aspects of yeast cell physiology that make quantitative isolation of pure nucleosomes extremely difficult. For example, yeast has a cell wall that must be removed to access subcellular components, yet many typical cell wall removal treatments, including proteolytic clipping on the N-terminal histone tails, accelerate chromatin breakdown. Therefore, maintaining intact chromatin from spontaneous breakdown during subsequent purification procedures can be difficult.

The majority of published techniques for direct purification of native yeast chromatin are based from protocols previously utilized for mammalian chromatin separation. When applied to yeast, these approaches provide low yields, incomplete and/or nonreproducible purity levels, and/or substantial histone degradation. To address the difficulties of direct yeast chromatin purification, metal affinity and micro-ChIP purification approaches were previously developed. These approaches, however, necessitate the overexpression of tagged histones, which might place a metabolic strain on the cell, and still result in low yield and material with abnormally tagged histones.

Yeast have a thick cell wall made up of glucans, mannoproteins, and chitin. The cell wall must be removed to gain access to nuclei, which are subsequently separated from other subcellular components. In the presence of osmotic stabilizers, such as sorbitol, digested yeast cells (spheroplasts) are protected against mechanical stress. To improve lytic enzyme effectiveness, yeast cells are pretreated with a thiol-containing buffer that removes the outer shell of mannoproteins. To breakdown the major cell wall components, commercially available lytic enzyme combinations such as lyticase or zymolyase are utilized. We discovered that even when a protease-deficient BJ2168 strain was employed in combination with a variety of inhibitor additions, a routinely used zymolyase-based approach for yeast cell wall removal resulted in significant proteolytic clipping of the N-terminal tail of histone H3. Several studies have shown that histone H3 is clipped during zymolyase therapy. Histone tail clipping destabilises native nucleosomes and causes the loss of essential post-translational modifications. The time course of H3 breakdown was determined using small aliquots of zymolyase-containing yeast solution. The time course of cell wall digestion revealed that histone H3 degradation was visible after only 5 minutes of zymolyase treatment and that more than half of the histone H3 tail had been cut after 30 minutes. The entire zymolyase treatment time required for complete spheroplasting exceeds 30 minutes, and other nucleosomal and chromatin-associated proteins may be affected. Because zymolyase's proteolytic action was partially responsible for histone H3 degradation, and both zymolyase and lyticase commercial blends contain protease components, we used purified recombinant 1,3-glucanase to remove yeast cell walls. The active form of glucanase, which cleaves the major 1,3-glucan component of the yeast cell wall, may be overexpressed and isolated from *Escherichia coli*.

Recombinant glucanase is a low-cost alternative to commercial enzymes (lyticase and zymolyase) that is compatible with a wide range of inhibitors used to protect chromatin and histone modifications. Glucanase takes longer incubation durations than zymolyase to complete cell wall digestion, however it did not cause detectable H3 degradation even after 4 hours.

Many present chromatin purification techniques are time-consuming, expensive, inefficient, and, while applicable for higher eukaryotic cells, insufficient for isolating yeast chromatin at sufficient yield and purity for quantitative biochemical or proteomic research. Several variables connected to yeast biology contribute to these constraints. Essentially, since yeast cells have a short, compact genome, they have significantly less chromatin per cell than higher eukaryotes. Furthermore, because yeast nuclei lack lamins, they are weak and vulnerable to detergents and mechanical destruction. Isolation of intact yeast nuclei is a difficult and low-yielding method. During the isolation procedure,

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the yeast nuclear membrane provides relatively inadequate protection for chromatin, which greatly leads to contamination with cationic cytoplasmic contaminants. Finally, when compared to higher eukaryotes, yeast core nucleosomes are innately unstable. These characteristics, when combined, represent major hurdles, imposing additional constraints on the time and circumstances utilized for chromatin purification.