Generation of a cDNA Library for a High-Copy Suppressor Screen to Identify Genes that Code for Proteins that Interact with Histone H2A during DNA Repair

The purpose of this project was to identify genes that rescue the growth sensitivity of a serine-to-alanine mutant (S122A) of histone H2A in the presence of a double-strand break (DSB) inducing agent. Histones are proteins that DNA wraps around to form nucleosomes, the basic unit of chromatin. Chromatin is important because it is the compacted form of DNA that allows it to fit in the nucleus of a cell. Histones are globular proteins with flexible tail regions, which contain residues that can be covalently modified. Covalent modifications act as signals for enzymes involved in different cellular processes, such as DNA repair.[1] Previous research in this lab has shown that S122 of H2A is important for the repair of DNA DSBs, possibly via phosphorylation of this residue. DSBs are dangerous because of potential loss of genetic information. To identify genes that interact with H2A S122 during DNA repair, we initiated a high-copy suppressor screen using the S122A mutant. We made a cDNA library from mRNAs isolated from both untreated cells and from cells exposed to MMS (methyl methanesulfonate), a DNA damaging agent, to create a library enriched for DNA repair factors. The cDNA library was constructed and will be screened with a plating assay. Due to time constraints, we were not able to get to this part of the project. S122A cells will be transformed with the libraries, and genes of interest will be identified by colonies that grow more robustly than the S122A control when plated on MMS-containing media.

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Introduction

Chromatin is the condensed form of DNA, allowing 2 meters of DNA to fit in a nucleus 10 μm in diameter. Chromatin is the combination of DNA and proteins, which form a nucleosome, the basic unit of chromatin. The proteins that make up the core of the nucleosome are the core histones and are named H2A, H2B, H3, and H4. The core histones have flexible tail regions that contain modifiable residues, and these residues can be covalently modified in a number of ways. Two common types of covalent modifications are phosphorylation and acetylation. H2A, unlike the other histones, has two tails. The Krebs lab has a suite of H2A mutants in which the modifiable residues on each tail have been mutated to alanine so they can no longer be covalently modified.[2] We were interested in the H2A serine 122 to alanine mutant, because previous research in our lab has shown us that H2A S122 is important for the repair of chemically induced DNA damage. Our model organism was *Saccharomyces cerevisiae*. This yeast is easy to work with in the sense that it is easy to grow and easy to extract nucleic acids from. Yeast cells also have chromatin structures and DNA repair mechanisms very similar to those of human cells.

Methods

Acid Phenol Extraction

When we collected our RNA, we started with an acid phenol extraction. We grew up a 20 mL culture, diluted it down to Optical Density$_{600}$ (OD_{600}): 0.2, put in a shaking incubator for three hours at 30°C to get OD_{600}: 0.5, added 20 μL of MMS to one culture to a concentration of 0.1% MMS of total volume and incubated the +MMS samples for two hours at 30°C with shaking. After incubating, we transferred both cultures evenly into two culture tubes. We pelleted the cells and resuspended them with 400 μL of RNA extraction buffer (0.5 M NaCl; 0.2 M Tris, pH 7.5; 0.01 M EDTA; 1% SDS). We added acid phenol, pH 4.5 (Ambion) with a 1:1 ratio of acid phenol to the cell suspension, and boiled the cells, following a standard procedure.

Oligo (dT) Cellulose Purification

In the next step, we performed an oligo (dT) cellulose (MP Biomedical) purification. We prepared our oligo (dT) cellulose by washing it four times with elution buffer (50 mL: 500 μL 1 M Tris; 100 μL 0.5 M EDTA; 250 μL 10% SDS), and did another wash with 1× binding buffer (50 mL: 6.25 mL 4 M NaCl; 500 μL 1 M Tris; 100 μL 0.5 M EDTA; 250 10% SDS). We mixed total RNA 1:1 with 2× binding buffer and then we applied the RNA to the oligo (dT) cellulose. There were two washes with 1× binding buffer and 2 washes with wash buffer (50 mL: 2.5 mL 4 M NaCl; 500 μL 1 M Tris, 100 μL 0.5 EDTA; 250 μL of 10% SDS). After washing the oligo (dT) cellulose we eluted RNA with an elution buffer to collect our RNA and we repeated the protocol one time. We used the purified mRNA to synthesize a cDNA library, using Superscript II Plasmid System with Gateway Technology for cDNA Synthesis and Cloning (Invitrogen), following the suggested protocol.

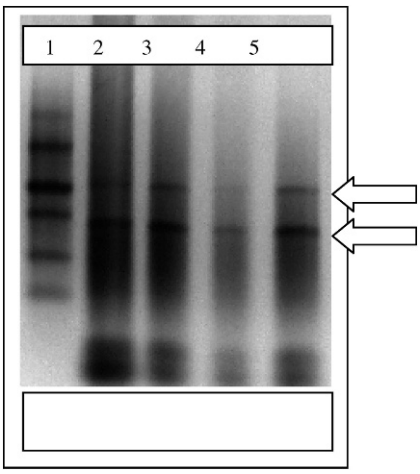

Fig 1. Total RNA extracts. Multiple extractions were performed to obtain high concentrations of non-degraded RNA, as assessed by ribosomal RNA bands. Lane 1, RNA size ladder. Lanes 2 and 3, minus MMS cultures, lanes 4 and 5, +MMS cultures. Arrows indicate ribosomal RNA. The small bands on the bottom are small RNAs such as tRNAs

RESULTS

We successfully obtained good yields of total RNA from both MMS treated and untreated samples (Figure 1). The quality of the RNA obtained was assessed by the quality of the ribosomal RNA (rRNA) bands. In degraded samples, these bands (indicated by arrows in Figure 1) are smeared or absent. Total RNA obtained from MMS treated or untreated samples were then pooled and purified using oligo (dT) cellulose as described above. In this step, polyadenylated mRNA is isolated, and other RNAs such as rRNA or tRNAs are eliminated. The purified mRNA samples are shown in Figure 2. For both the MMS-treated and untreated samples, good quality mRNA was obtained in good yields, with clear elimination of rRNA and tRNA bands. This mRNA is ready to be used for cDNA library synthesis.

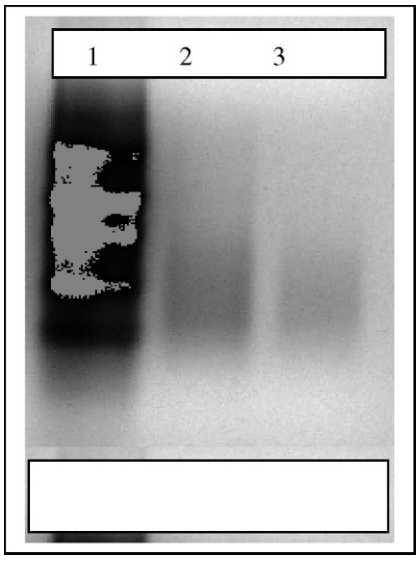

Fig 2. Purified mRNA. Samples shown in Figure 1 were purified using oligo (dT) cellulose (see text description). Lane 1, RNA size ladder, Lane 2, combined samples from lanes 2 and 3 from Figure 1. Lane 3, combined samples from lanes 4 and 5 from Figure 1

SUMMARY/FUTURE DIRECTIONS

We repeated the acid phenol extraction a few times to obtain high-quality RNA. When we isolated RNA with sharp, undegraded ribosomal bands, we carried these RNAs through to the oligo (dT) cellulose purification. We obtained good yield from our mRNA purification using the oligo (dT) cellulose, so we began cDNA library synthesis. We obtained low yield from our first strand synthesis reaction and average yield with our second strand synthesis reaction. Prepping our vector for the cloning process is still underway. Due to time constraints, we were not able to clone our library into the vector. Once this cloning is completed, the libraries will be ready for use in the high copy suppressor screen.

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