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Thermal Stabilization of Putative Karyoskeletal Protein-Enriched Fractions from *Saccharomyces cerevisiae*

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Elevated growth temperature (heat shock) promoted the structural stability of karyoskeletal protein-enriched fractions isolated from *Saccharomyces cerevisiae*. Similar stabilization could be induced by brief incubation of nuclei at 37°C in vitro. These results are similar to those reported for higher eucaryotes and have practical implications for investigation of the karyoskeleton in *S. cerevisiae*.

Karyoskeletal protein-enriched fractions can be prepared from a variety of higher eucaryotic nuclei by a combination of nuclease digestion and extraction with nonionic detergents and solutions of high salt concentration (for a recent review, see reference 5). These fractions contain morphologically recognizable remnants of nuclear pore complexes and peripheral lamina, as well as relatively amorphous insoluble material, apparently derived from the nuclear interior. The biological significance of this internal material, often referred to as the internal nuclear matrix or chromosome scaffold, remains to be determined.

Recently, a number of reports (1, 4, 8) have suggested that the composition and structural stability of karyoskeletal protein-enriched fractions isolated from higher eucaryotic cells can be profoundly affected either by brief incubation of nuclei at moderately elevated temperatures in vitro or in response to heat shock conditions in vivo. These observations have both practical and biological implications. From a practical perspective, it seems possible that previously documented associations between putative karyoskeletal elements and a number of nuclear proteins and/or specific DNA sequences may in fact represent thermal denaturation artifacts. On the other hand, we have suggested (8) that regulation of karyoskeletal form and function may be a significant component of the cellular response to thermal stress (i.e., heat shock).

When *Saccharomyces cerevisiae* nuclei are subjected to nuclease digestion and extraction with nonionic detergents and solutions of high salt concentration, an insoluble proteinaceous fraction is obtained which is at least morphologically similar to karyoskeletal protein-enriched fractions prepared from higher cells (9, 11). Pore complex remnants and a putative internal matrix were tentatively identified in these fractions; it is not certain whether *S. cerevisiae* possesses a nuclear lamina. However, the REP1 protein, a 2μm circle plasmid-encoded polypeptide of 45 kilodaltons (kDa) with significant homology of both primary and secondary structure to mammalian lamins and intermediate filament proteins was identified as a component of this fraction.

To extend the operational definition of putative karyoskeletal protein-enriched fractions from *S. cerevisiae*, we performed experiments analogous to those previously reported for higher eucaryotes (4, 8). For all of the experiments reported, yeast strain DC04 (*MATα adel leu2-04* Gal$^+$) carrying plasmid pSS4 (3) was grown in rich medium containing 2% yeast extract, 2% Bacto-Peptone, and 2% glucose (YEPD). Cells were grown at 23°C to a density of 2×10^7 cells per ml, harvested by centrifugation, and washed in water. The washed cells were suspended in 100 mM Tris hydrochloride (pH 8.0)–100 mM EDTA–0.5% 2-mercaptoethanol, incubated for 20 min at 23°C, pelleted, and suspended in 1.1 M sorbitol–20 mM $KHPO_4$ (pH 6.8)–0.5 mM $CaCl_2$. Glusulase and Zymolyase (Miles Laboratories) were added to final concentrations of 1% and 1 mg/ml, respectively, and spheroplasts were generated by incubation of the cells at 23°C for 30 to 40 min. Conversion of cells to spheroplasts was monitored by phase-contrast microscopy, and when conversion was >90% complete, the spheroplasts were collected by gentle centrifugation and suspended in 10 volumes of 40 mM PIPES [piperazine-N-*N*′-bis(2-ethanesulfonic acid)]–10 mM $MgCl_2$–0.2% Triton X-100–1 mM–phenylmethylsulfonyl fluoride–1 μg of pepstatin A per ml (buffer A).

The spheroplasts were lysed in buffer A by Dounce homogenization (10 strokes, tight pestle), and nuclei were prepared as described by Wu et al. (11). DNase I and RNase A were then added (6), and in the first experiment, the nuclei were digested for 15 min either at 23 or 37°C. The digested nuclei were extracted with 2% Triton X-100 and 1 M NaCl as previously described (6, 11), and samples of the various fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis, using standard procedures (2, 6, 7, 10). A Coomassie blue-stained gel (Fig. 1A) and an immunoblot of a gel run in parallel and probed with anti-yeast topoisomerase II immunoglobulin G (IgG) (Fig. 1B) are shown. Topoisomerase II was chosen for this analysis to allow direct comparison with data obtained for the homologous protein in *Drosophila melanogaster*. Anti-yeast topoisomerase II was the generous gift of James Wang, Harvard University, Cambridge, Mass., and was prepared by immunizing a rabbit with the purified yeast enzyme. The specificity of this antibody on immunoblots has previously been demonstrated (11). As reported for *D. melanogaster* (8), when nuclei (lane N) were digested at 23°C as indicated, virtually all of the nuclear protein was solubilized by sequential extractions with 2% Triton X-100 and 1 M NaCl. In contrast, and also consistent with results obtained with *D. melanogaster*, when nuclei were digested with nucleases at 37°C, a majority of the

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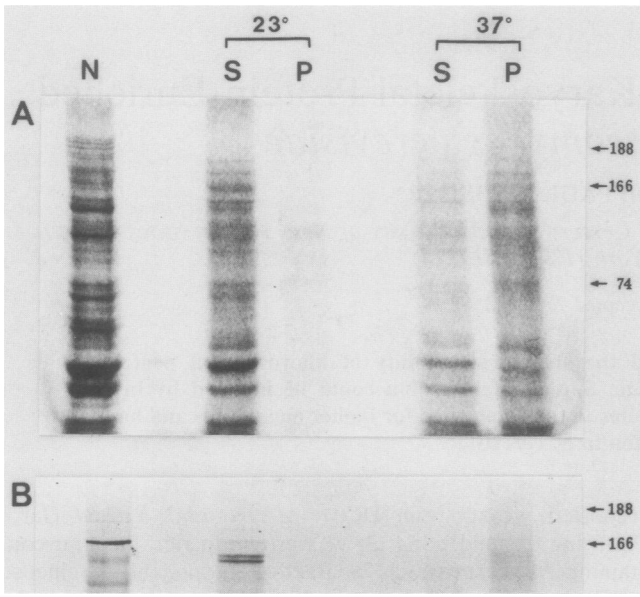

FIG. 1. SDS-PAGE and immunoblot of nuclear protein fractionation after nuclease digestion at 23 and 37°C. Nuclei were incubated with 10 μg of DNase I per ml and 8 μg of RNase A per ml for 15 min at either 23 or 37°C. Extractions with nonionic detergents and 1 M NaCl were performed after nuclease treatment, and the various fractions generated were subjected to SDS-PAGE and immunoblot analysis on 7% polyacrylamide minigels (Hoefer Scientific). Fractions loaded in each lane were as follows: lane N, purified nuclei prior to nuclease treatment; lanes S, pooled supernatant fractions from nuclease digestion and detergent and salt extraction; lanes P, putative karyoskeletal protein-enriched pellet fraction remaining after detergent and salt extraction. Equivalent amounts (20 U; 1 U is defined as the amount derived from 1 μl of starting material) of each fraction were loaded on the gel. (A) Coomassie blue-stained gel; (B) immunoblot prepared from an identical gel loaded and run in parallel and probed with anti-yeast topoisomerase II IgG at a dilution of 1:1,000 relative to the antiserum from which it was derived. Molecular mass markers to the right of the gel are *D. melanogaster* nuclear proteins gp-188 (188 kDa), DNA topoisomerase II (166 kDa), and lamins (74 kDa).

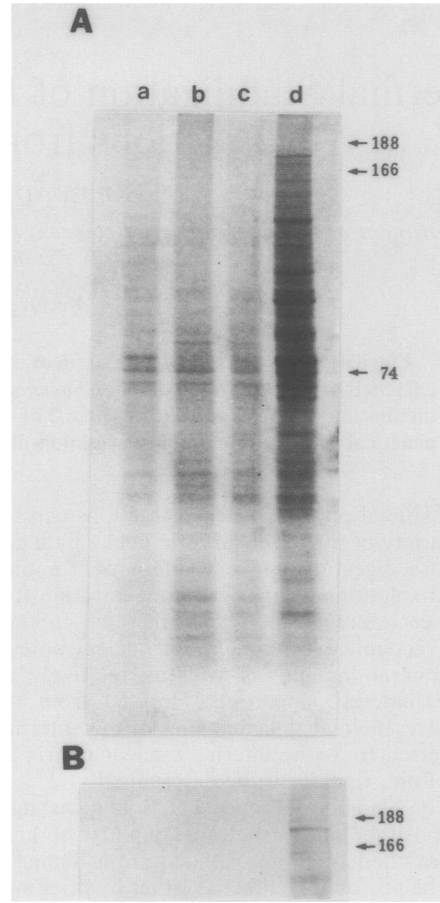

FIG. 2. Effect of growth temperature on the composition of putative karyoskeletal protein-enriched fractions. Karyoskeletal protein-enriched fractions were prepared from yeast cells grown overnight at 23°C and then maintained for 1 h at 23°C (lane a), 30°C (lane b), 37°C (lane c), or 42°C (lane d). For all preparations, the nuclease digestion step was performed at 23°C. Equivalent amounts (30 U) of the final pellet fractions were subjected to SDS-PAGE on 7 to 15% polyacrylamide gradient gels and immunoblot analysis. The gels were standard size (~20 cm long). (A) Coomassie blue-stained gel; (B) immunoblot prepared from an identical gel run in parallel and probed with anti-yeast topoisomerase II IgG as in Fig. 1. DNA topoisomerase II was less sensitive to in vitro proteolysis following in vivo heat shock (see also Fig. 3). Molecular mass standards are the same as those in Fig. 1.

total nuclear protein was found in the insoluble pellet material remaining after detergent and salt extraction.

The results of the immunoblot analysis with anti-topoisomerase II IgG (Fig. 1B) are also consistent with those reported previously for *D. melanogaster*, although they were complicated somewhat by proteolysis which took place during the nuclease incubations. After nuclease digestion at 23°C, DNA topoisomerase II was recovered entirely in the soluble fraction following detergent and salt extraction (Fig. 1B, 23°C, lane S). It was apparently degraded to a doublet of about 150 kDa from a mass of about 180 kDa as determined in unfractionated nuclei prior to incubation with nucleases (Fig. 1B, lane N). When nuclease incubations were carried out at 37°C, degradation was more pronounced; it was difficult to conclude with certainty that the faint bands detectable at the 150-kDa position in the pellet fraction (Fig. 1B, 37°C, lane P) did in fact represent DNA topoisomerase II. Nevertheless, there was virtually no detectable immunoreactive material in the supernatant fractions (Fig. 1B, 37°C, lane S). Parallel blots probed with preimmune IgG failed to show any bands in the high-molecular-mass region of the gel for any of the fractions for which analyses are shown in Fig. 1B (results not shown).

In vitro incubation analyses were complemented by experiments performed in vivo. The first of two experiments (Fig. 2) was done to evaluate the effects of temperature shift from 23°C to each of three different temperatures, 30, 37, and 42°C. Cells were grown overnight at 23°C and then shifted to the indicated temperature for 1 h; an aliquot was allowed to continue growing at 23°C. Cells from all four culture aliquots were collected by centrifugation, washed, converted to spheroplasts, and fractionated exactly as described for the experiment for which results are shown in Fig. 1; the nuclease digestion step was done at 23°C. Initial cell pellets were adjusted so that equal amounts of starting material were used for each time point. The results of SDS-PAGE and Coomassie blue staining are shown in Fig. 2A; an immunoblot prepared in parallel and stained with anti-topoisomerase II IgG is shown in Fig. 2B. Coomassie blue staining showed slight increases in protein detectable in the final pellet fraction at both 30°C (lane b) and 37°C (lane c); dramatic increases were seen after incubation at 42°C (lane

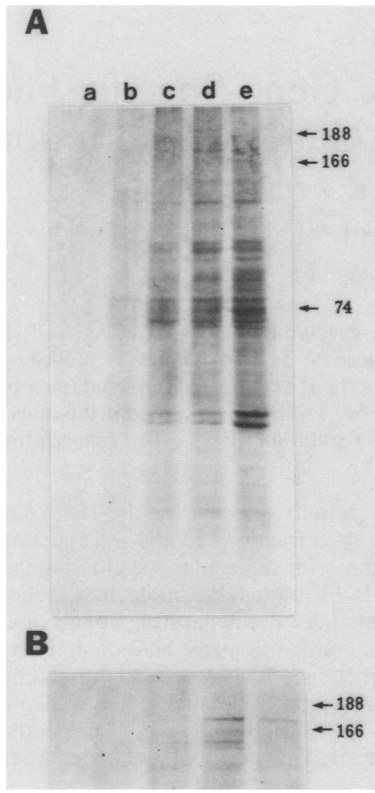

FIG. 3. Effect of duration of in vivo heat shock on the composition of putative karyoskeletal protein-enriched fractions. Karyoskeletal protein-enriched fractions were prepared from yeast cells grown overnight at 23°C and subjected to heat shock at 42°C in vivo for 0 (lane a), 30 (lane b), 60 (lane c), 120 (lane d), or 240 (lane e) min. SDS-PAGE and immunoblot analysis with anti-yeast topoisomerase II IgG were done exactly as described in the legend to Fig. 2.

d). Topoisomerase II was detectable in the final pellet fraction only after incubation at 42°C.

In a second in vivo experiment, we examined the effects of growth at an elevated temperature as a function of time. Cells were grown at 23°C overnight and shifted to 42°C, and samples were taken over a 4-h time course as indicated in the legend to Fig. 3. The cells were fractionated exactly as described for the experiment for which results are shown in Fig. 1; nuclease digestion was done at 23°C. Samples were subjected to SDS-PAGE followed either by Coomassie blue staining (Fig. 3A) or immunoblot analysis with anti-topoisomerase II IgG (Fig. 3B). Coomassie blue staining showed a cumulative effect over time on the appearance of protein in the detergent-insoluble, salt-insoluble pellet derived from nuclease-digested nuclei. This is similar to observations made with *D. melanogaster* (8). The results of immunoblot analysis with anti-topoisomerase II IgG are roughly comparable except that at the last time point (Fig. 3B, lane e), there was apparently a decrease in the amount of topoisomerase II. This observation is of uncertain significance but may reflect either increased proteolysis upon prolonged incubation or sample-to-sample variability.

The practical implications of the observation that the composition and structural stability of karyoskeletal protein-enriched fractions from higher eucaryotic cells can be markedly affected by either in vivo heat shock or incubation at moderately elevated temperatures in vitro have been discussed in detail (4, 8). A number of concerns have been raised. Our current results suggest that similar concerns pertain to lower eucaryotes, in particular the yeast *S. cerevisiae*. These concerns need not be explicitly reiterated here.

From a biological perspective, it now appears that the tendency of a certain class or classes of nuclear proteins to form insoluble aggregates in response to thermal stress may be a universal feature of living cells. It remains to be determined exactly what this tendency signifies, whether it be random denaturation, regulated changes in the in vivo structural stability of karyoskeletal elements, or some combination of the two. The exact relationship between the effects on karyoskeletal stability reported here and previously (4, 8) and the biological heat shock response also remains to be elucidated. The identification of this phenomenon in *S. cerevisiae* allows us to consider systematic genetic approaches as a means of further study.

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