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Nuclear Import Substrates Compete for a Limited Number of Binding Sites

EVIDENCE FOR DIFFERENT CLASSES OF YEAST NUCLEAR IMPORT RECEPTORS*

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A nuclear receptor likely involved in nuclear protein import is described. Purified ATP-depleted yeast nuclei show saturable high-affinity binding of the yeast nuclear protein Mcm1. The dissociation constant for the binding is 0.5 μM, and the number of binding sites is approximately 3,500 per nucleus, equivalent to 10–30 binding sites per nuclear pore. Mcm1 competes with other yeast nuclear proteins Ste12 and Swi5, but not with Rap1 or Nop1, indicating that there may be different types of import receptors. Bound Mcm1 is resistant to extraction by nucleases, salt, and non-ionic detergent, but can be released by 5 M urea, suggesting that Mcm1 binds to a yeast equivalent of the nuclear pore complex-lamina fraction of higher eukaryotes.

The selectivity of protein transport is conferred by receptors that recognize a signal in proteins destined to be transported to a given cellular compartment or to be exported. However, only in a few cases have the binding parameters of presumed transport receptors been studied. In bacteria, SecA binds to a precursor-SecB complex with a dissociation constant (K_d) of 60 nM (1). Mitochondrial protein import receptors have been shown to have dissociation constants of 3 and 2 nM for a chemically treated form of the mitochondrial porin and the ATP/ADP translocator, respectively (2–4). A yeast microsomal receptor binds the bacterial outer membrane protein proOmpA with a K_d of 8 nM (5). In higher eukaryotes, the signal recognition particle (SRP) binds nascent prepro-lactin with a K_d of 8 nM (6), and the KDEL receptor of the so-called salvage compartment binds protein disulfide isomerase with a K_d of 23 μM (7).

Nuclear protein import is a two-step process, an energy-independent accumulation of an import substrate at the nuclear periphery followed by an energy-dependent translocation across the nuclear envelope (8–10). The poorly characterized energy-independent step requires a functional targeting signal and is assumed to be a binding to specific receptors on the surface of the nucleus. This step is now amenable to study with recently established cell-free systems (9, 11–15). In these systems, nuclear protein import is specific and ATP-dependent, indicating that the machinery which recognizes and translocates import substrates is functional. In the absence of ATP, only a small amount of import substrate associates with the nuclear surface, presumably with a receptor component of the import machinery (8, 9, 13).

Here we investigate the energy-independent binding of a purified nuclear protein to isolated yeast nuclei. We find a limited number of high-affinity binding sites which are also receptors for other, but not all, nuclear proteins. These binding sites are a strong candidate for a nuclear protein import receptor.

EXPERIMENTAL PROCEDURES

Protein Substrates and Nuclei—The purified nuclear protein used as a ligand to determine binding affinities and number of binding sites is Mcm1, a yeast transcriptional regulator (16–20). Mcm1, obtained from S. Tan (ETH Zürich) was greater than 90% pure and active, as determined by gel electrophoresis and band shift DNA-binding assays (18). Dimeric Mcm1 (molecular mass 66 kDa), which is the active species of Mcm1 and which is stably formed in solution,[1] was considered the ligand species. Mcm1 was labeled by iodination with $Na^{125}I$ (100 mCi/ml, Du Pont de Nemours International, Switzerland) using iodobeads (Pierce Europe, The Netherlands) according to the procedure recommended by the manufacturer. Typical specific activities were approximately 600 cpm/fmol.

For competition assays between unlabeled Mcm1 and other nuclear proteins, the latter were synthesized by *in vitro* transcription and translation. Plasmids were transcribed using T7 RNA polymerase (Biofinex, Switzerland), and the mRNA was translated in a rabbit reticulocyte lysate as described (15). *In vitro* translated proteins were biosynthetically labeled with [^{35}S]methionine (12 mCi/ml, Du Pont de Nemours International, Switzerland).

Nuclei were purified from the haploid, protease-deficient *Saccharomyces cerevisiae* strain BJ1994 (*α leu2 trp1 ura3-52 prb1-1122 pep4-3*) (provided by E. Jones, Carnegie-Mellon University, Pittsburgh) using previously described procedures (15). Nuclei could be frozen and stored in liquid nitrogen in 25% glycerol without detectable changes in binding activity.

Binding Experiments—The standard incubation mixture contained, in a final volume of 50 μl, incubation buffer (50 mM Hepes[2]-KOH, pH 7.0, 25 mM potassium acetate, 5 mM $MgSO_4$ and 3 mM $CaCl_2$), 5% (v/v) glycerol, 15 mg/ml protease-free bovine serum albumin as carrier (Sigma Chemie, Buchs, Switzerland), 30 units/ml apyrase (type VII, Sigma Chemie, Buchs, Switzerland), approximately 200,000 cpm of ^{125}I-Mcm1 (approximately 7 nM), 10^8 nuclei (equivalent to 20 μg of nuclear protein), and a varying amount of unlabeled Mcm1 (see "Results"). Nuclei and apyrase were pre-mixed and incubated on ice for 10 min before adding to the other components of the incubation mixture. This ATP depletion step was included to eliminate any errors resulting from a contribution of translocation to binding measurements. Nuclei were counted in duplicate for each experiment in a Neubauer chamber. The concentration of protein in nuclei preparations was determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Glattbrugg, Switzerland), as recommended by the manufacturer.

Mcm1 binding reactions were for 30 min at 30 °C. At 30 °C and in

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[1] S. Tan and T. Richmond, personal communication.

[2] The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

the incubation mixture described above, binding of Mcm1 to isolated nuclei reaches equilibrium within 20 min. Free and bound Mcm1 were separated by centrifugation through a 100 μl 40% (w/v) sucrose cushion at 9,000 × *g* for 5 min, as described previously (15). Pelleted nuclei were resuspended in SDS-sample buffer and analyzed by electrophoresis in 10% SDS-polyacrylamide gels and autoradiography using prefogged x-ray films. Autoradiographs were scanned in a Molecular Dynamics 300A Computing Densitometer (Molecular Dynamics, Sunnyvale, CA). Measured amounts of the labeled ligand were also loaded on the gels to identify exposures in the linear range of the film and to make calibration curves relating the optical densities of film signals with ligand concentration.

Each binding experiment consisted of a series of assay tubes with a fixed amount of labeled Mcm1 and varying amounts of unlabeled Mcm1. Each series was done in duplicate and the values obtained from each pair of matched tubes was averaged in each experiment. The contribution of nonspecific binding, as measured in incubations containing an excess of unlabeled Mcm1 (130 μM), was subtracted from all binding values.

The competitive displacement assay described directly above allows determination of binding parameters even when there is uncertainty in the concentration of the labeled ligand or errors can occur when measuring binding at very low or very high ligand concentrations (21, 22). Conditions were chosen so that there was always an excess of free ligand at equilibrium, with maximum binding never exceeding 10% of the total ligand concentration.

Competition between Mcm1 and Other Nuclear Proteins—Binding assays were performed as detailed above except that all labeled ligands used to monitor binding, including Mcm1, were synthesized *in vitro* and added as 5 μl of a reticulocyte lysate translation mixture. Proteins used as labeled ligands were the yeast transcription factors Mcm1 (33 kDa), Rap1 (93 kDa), Ste12 (78 kDa), and Swi5 (78 kDa), and the yeast nucleolar protein Nop1 (34 kDa). Nop1 was incubated with 5 μg of nuclear protein, to keep total binding below 10% of the added ligand. In each experiment, two assays were set up for each ligand; one assay received unlabeled Mcm1 to a final concentration of 8 μM. In all experiments, assay tubes were duplicated and the values from the duplicate tubes were averaged.

Dissociation of Bound Mcm1—Mcm1 was bound to purified nuclei as described above, with the exception that the amount of nuclei was increased (final amount equivalent to 40 μg of nuclear protein) to increase the signal. Nuclei and bound Mcm1 were centrifuged at 4 °C through a 40% (w/v) sucrose cushion onto a 15 μl cushion of 68% (w/v) sucrose. The incubation mixture and the 40% cushion were removed and the volume of the lower cushion plus the nuclei-containing interphase was adjusted to 50 μl with cold incubation buffer. To dissociate bound Mcm1, 50 μl of a solution containing either 1 M NaCl, 1% (v/v) Nonidet P-40 (Sigma Chemie, Switzerland), 1 M NaCl plus 1% Nonidet P-40, 10 M urea, or a nuclease mixture was added. The nuclease mixture contained 200 μg/ml DNase I (Sigma Chemie, Switzerland), 200 μg/ml RNase A (Boehringer Mannheim, Germany), 0.5 M NaCl, and 0.4% (v/v) Nonidet P-40. All treatment solutions were made up in incubation buffer containing 4 mM spermidine (Sigma Chemie, Switzerland) to improve the stability of the nuclei. Control incubations received incubation buffer with only spermidine. Tubes were incubated in ice water for 20 min and then centrifuged at 122,000 × *g* (average) in an Airfuge (Beckman, Palo Alto, CA) for 15 min at 4 °C. Mcm1 does not sediment when incubated in the above solutions in the absence of nuclei. Pellets and supernatants were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described above. In all experiments results from duplicate assays were averaged.

Pretreatment of Nuclei—The extractability of the nuclear Mcm1 receptor with 5 M urea was tested by incubating nuclei (40 μg of nuclear protein) with 5 M urea, 25% glycerol, and 2 mM spermidine in incubation buffer for 20 min in ice water. Control nuclei were treated identically but without urea. The mixture was underlaid with 15 μl of cold 68% sucrose and the nuclei were concentrated on the sucrose cushion by centrifugation at 9,000 × *g* for 15 min at 4 °C. The upper phase was aspirated and the interphase with the nuclei was washed with 200 μl of 25% glycerol, 1 mM spermidine in incubation buffer. Nuclei were again concentrated by centrifugation and then resuspended directly in the binding assay incubation mixture. Subsequent steps were as described above.

RESULTS

If protein translocation into the nucleus is indeed receptor-mediated, an import substrate should exhibit saturable high-affinity binding to nuclei and should, assuming a general import receptor, compete the binding of other nuclear proteins.

Saturable High-affinity Binding of Mcm1 to Nuclei—We analyzed the binding of Mcm1 to a yeast nuclear preparation previously shown to import this protein in a temperature- and energy-dependent manner. Nuclear binding was assayed by incubating isolated ATP-depleted nuclei with a fixed amount of labeled Mcm1 and varying amounts of unlabeled Mcm1. Binding of purified Mcm1 was found to be saturable (Fig. 1). The competitive displacement curve has a sigmoidal shape with only one inflection point, indicating the existence of a single class of binding sites. The concentration of cold Mcm1 required for half-maximal competition ($C_{0.5}$)(x intercept) was obtained by logarithmic transformation of the binding curve. This value, minus the concentration of labeled protein used, represents the dissociation constant (K_d) for the binding of Mcm1 to yeast nuclei (22). The average value obtained from five independent experiments like the one illustrated in Fig.1 is $K_d = 0.5$ μM (S.D. = 0.1). Thus, Mcm1 binds to nuclei with high affinity.

The total concentration of binding sites (B_{max}) was calculated from the estimated concentration of labeled protein in the binding assay (a), the total binding in the absence of cold Mcm1 (B_a), and the $C_{0.5}$, using the formula $B_{max} = B_a(C_{0.5}/a)$ (22). The number of binding sites per nucleus, B_{max} divided by the concentration of nuclei present in the assay, is 3,500 sites. This value is an average obtained from five independent experiments (S.D. = 500). Considering the published figures for the number of nuclear pores in *S. cerevisiae*, 119, 200, and 350 pores/nucleus (23–25), the above result represents an average of 10–30 binding sites/pore.

Competition between Nuclear Proteins for Receptor Binding—Because different nuclear localization signal peptides have been shown to bind a common protein (26), and furthermore, because it is unlikely that there is a different import receptor for each nuclear protein, it is assumed that different proteins share import receptors. To investigate if different proteins compete for binding to nuclei, the binding of several labeled yeast nuclear proteins was tested in the presence and absence of 8 mM unlabeled Mcm1. As shown in Fig. 2, Mcm1 competes weakly, if at all, the binding of Rap1 and Nop1. In contrast, Mcm1 competes its own binding and that of Swi5 and Ste12 , showing that these three proteins share a common receptor.

Mcm1 Binds Tightly to a Nuclease-resistant Fraction—The association of nuclear proteins with a nuclear import receptor could involve ionic interactions between the known positively charged import signals and possibly negatively charged receptors (27, 28). To investigate if ionic interactions or other types of binding forces contribute to Mcm1 binding, nuclei with bound Mcm1 were treated with different solutions and the amount of bound and released Mcm1 was determined. Most Mcm1 remained bound after treating the nuclei with 0.5 M NaCl, ruling out ionic interactions as the sole force responsible for the observed binding (Fig. 3). Extraction of the nuclear membranes with 0.5% non-ionic detergent or disruption of nuclear structure by nuclease digestion in the presence of 0.25 M NaCl and 0.2% detergent solubilized only approximately 30% of the bound protein. As expected, nuclease treatment eliminated all microscopically recognizable, 4′-6-diamidine-2-phenylindole dihydrochloride-stainable nuclei (data not shown). A combination of 0.5 M salt and 0.5% detergent

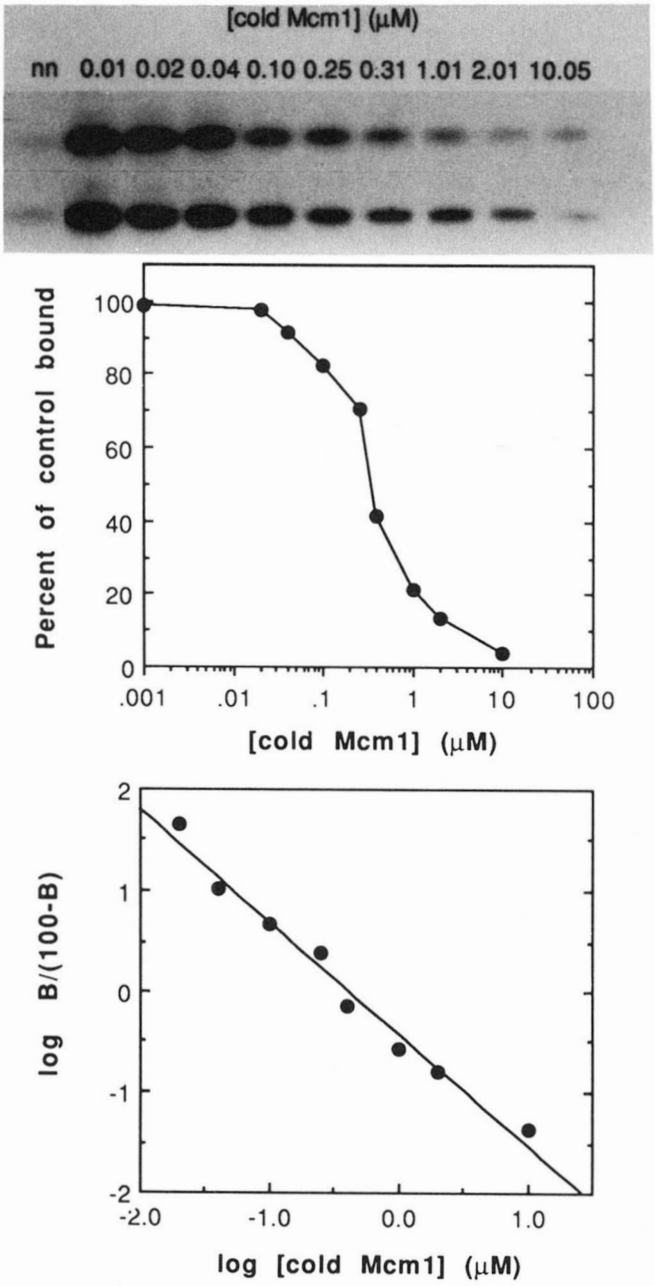

FIG. 1. **Competition displacement curves for the binding of Mcm1 to nuclei.** ^{125}I-iodinated Mcm1 was allowed to bind to isolated nuclei in the presence of variable amounts of unlabeled Mcm1 as described under "Experimental Procedures." Representative results from one out of five experiments performed are illustrated (see text for statistical variation). The photograph on top shows autoradiographs of a duplicate series of assays. The concentration of unlabeled Mcm1 is indicated. *nn*, no nuclei blank. The *upper graph* indicates the amount of labeled Mcm1 bound relative to the control incubation without unlabeled protein. The lower graph, a linearization (logit/log plot) of the upper graph, was used to calculate the K_d for binding (see text). The interpolated line has a regression coefficient of 0.98. *B* is percent of control bound.

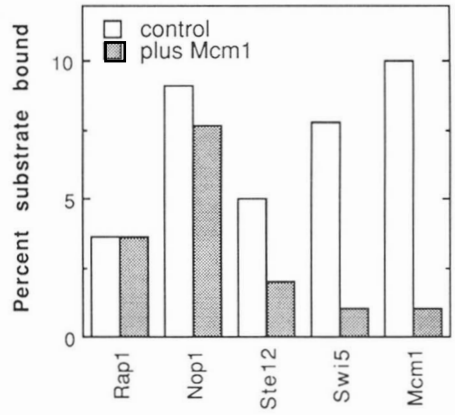

FIG. 2. **Competition between Mcm1 and other nuclear proteins for binding to nuclei.** *In vitro* translated ^{35}S-labeled proteins were incubated with nuclei in the presence or absence (control) of 8 μM unlabeled Mcm1. *Bars* represent the percentage of total labeled substrate bound. Values are the average of four assays from two independent experiments.

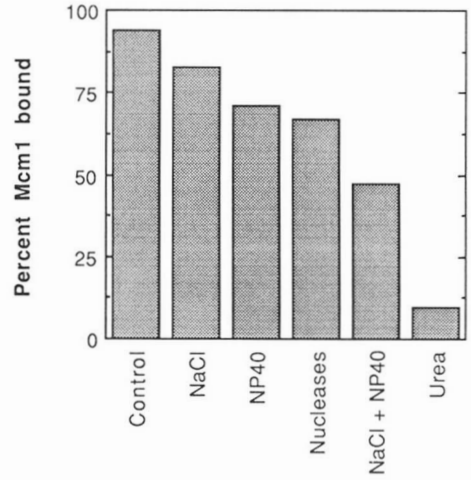

FIG. 3. **Extractability of Mcm1 pre-bound to nuclei.** ^{125}I-Mcm1 was pre-bound to nuclei as described under "Experimental Procedures." Re-isolated nuclei were then treated (see "Experimental Procedures") at 4 °C with 0.5 M NaCl (NaCl); 0.5% Nonidet P-40 (*NP40*); 0.25 M NaCl, 0.2% Nonidet P-40, DNase and RNase (*Nucleases*); 0.5 M NaCl and 0.5% Nonidet P-40 (*NaCl + NP40*); or 5 M urea (*Urea*). *Bars* represent the percentage of Mcm1 still bound after each treatment. Results are the average of two assays from one out of three similar experiments, all of which gave essentially the same results.

extracted half the bound protein. Only a protein denaturant, 5 M urea, released most (90%) of the bound Mcm1 (Fig. 3). Urea most likely breaks the ligand-receptor interaction and does not release the receptor itself from the nucleus, since prewashing nuclei with 5 M urea does not reduce binding (data not shown). The findings that bound Mcm1 is not released by nucleases in the presence of salt and detergent and that it is released by a protein denaturant are indicative of an association with a proteinaceous karyoskeletal structure (29, 30), possibly a yeast equivalent of the nuclear pore complex-lamina fraction of higher eukaryotes (31, 32).

DISCUSSION

Isolated yeast nuclei specifically accumulate nuclear proteins in the presence of hydrolyzable nucleotide triphosphates (13, 15). Here we present evidence that in the absence of ATP, the purified yeast nuclear protein Mcm1 binds to a limited number of specific nuclear receptors. Furthermore, we find that these receptors are common to some, but not all, nuclear proteins tested.

Nuclear binding of Mcm1 is saturable (Fig. 1). The presence of only one inflexion point in the competitive displacement curve indicates the presence of a single class of binding sites with a dissociation constant of 0.5 μM. This affinity is in agreement with the previously measured K_m of 1.8 μM for the nuclear import of signal peptide-bovine serum albumin con-

jugates injected into *Xenopus* oocytes (33). The K_d for the binding of Mcm1 to isolated nuclei is approximately 10 times higher than that for the binding of SV40 nuclear localization signal peptides to certain rat hepatocyte proteins (34). Assuming that these proteins are indeed involved in nuclear import, the difference could be due to different signal sequences, to differences between rat and yeast receptors, or to both. Mechanisms of nuclear protein import in yeast are not strictly equivalent to those in higher eukaryotes. Proteins that bind nuclear localization signals have been found in the nucleus and cytosol of higher eukaryotic cells (reviewed in Ref. 35) but only in the nucleus of yeast cells (29, 36). Accordingly, yeast cytosol appears not to be required for nuclear protein import in yeast *in vitro* systems (13, 15) and cannot fulfill the cytosol requirement of a HeLa cell *in vitro* import system (37).

Nuclear protein import seems to be able to function with receptors that have lower affinity for their substrates (Ref. 33 and this work) than the receptors described in mitochondria (2, 3) and the endoplasmic reticulum (6) (see Introduction), perhaps indicating that import substrates are concentrated near the nuclear pore before an interaction with a nuclear receptor takes place. This view is compatible with electron micrographs showing nuclear import substrates bound to skeletal elements in the vicinity of pores (8, 38).

The number of receptors detected per nucleus, 3,500, is relatively low; even if the precise figure is affected by uncertainties in the concentration of labeled ligand, the variation cannot realistically be larger than 20%. This represents approximately 300 binding sites/μm^2 of nuclear surface or, depending on the figure used for the number of pores per yeast nucleus (23–25), 10–30 sites/nuclear pore. This is in remarkable agreement with the number of receptor sites, 8–16, extrapolated from electron microscopic visualization of nuclear protein-coated gold particles bound to nuclear pore complexes (39).

The competition between Mcm1, Swi5, and Ste12 indicates that these proteins share a common receptor. The observed binding is most likely to a nuclear import receptor because the only specific property that the three competing ligands have in common is that they are nuclear proteins and because the receptor is part of a yeast equivalent of the nuclear pore complex-lamina fraction. Accordingly, the binding of the proteins which are not competed by Mcm1 could define additional types of nuclear import receptors.

It is conceivable that there are structurally and functionally distinct receptors and that each type of receptor recognizes a different group of ligands, analogous to amino acid transporters. The notion of different receptors agrees with previous suggestions of multiple nuclear import pathways (40–44) and is consistent with the lack of a single consensus nuclear localization signal (35).

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REFERENCES

1. Hartl, F. U., Iecker, S., Shiebel, E., Hendrick, J. P., and Wickner, W. (1990) *Cell* **63,** 269–279
2. Pfaller, R., and Neupert, W. (1987) *EMBO J.* **6,** 2635–2642
3. Pfaller, R., Steger, H. F., Rassow, J., Pfanner, N., and Neupert, W. (1988) *J. Cell Biol.* **107,** 2483–2490
4. Sollner, T., Griffiths, G., Pfaller, R., Pfanner, N., and Neupert, W. (1989) *Cell* **59,** 1061–1070
5. Sanz, P., and Meyer, D. I. (1989) *J. Cell Biol.* **108,** 2101–2106
6. Walter, P., Ibrahimi, I., and Blobel, G. (1981) *J. Cell Biol.* **91,** 545–550
7. Vaux, D., Tooze, J., and Fuller, S. (1990) *Nature* **345,** 495–502
8. Richardson, W. D., Mills, A. D., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1988) *Cell* **52,** 655–664
9. Newmeyer, D. D., and Forbes, D. (1988) *Cell* **52,** 641–653
10. Hall, M. N., Craik, C., and Hiraoka, Y. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 6954–6958
11. Markland, W., Smith, A. E., and Roberts, B. L. (1987) *Mol. Cell. Biol.* **7,** 4255–4265
12. Shirakawa, F., and Mizel, S. B. (1989) *Mol. Cell. Biol.* **9,** 2424–2430
13. Kalinich, J. F., and Douglas, M. G. (1989) *J. Biol. Chem.* **264,** 17979–17989
14. Parnaik, V. K., and Kennady, P. K. (1990) *Mol. Cell. Biol.* **10,** 1287–1292
15. Garcia-Bustos, J. F., Wagner, P., and Hall, M. N. (1991) *Exp. Cell Res.* **192,** 213–219
16. Keleher, C. A., Goutte, C., and Johnson, A. D. (1988) *Cell* **53,** 927–936
17. Passmore, S., Maine, G. T., Elble, R., Christ, C., and Tye, B.-K. (1988) *J. Mol. Biol.* **204,** 593–606
18. Tan, S., Ammerer, G., and Richmond, T. J. (1988) *EMBO J.* **7,** 4255–4264
19. Jarvis, E. E., Clark, K. L., and Sprague, G. F. (1989) *Genes & Dev.* **3,** 936–945
20. Ammerer, G. (1990) *Genes & Dev.* **4,** 299–312
21. Jacobs, S., Chang, K. J., and Cuatrecasas, P. (1975) *Biochem. Biophys. Res. Commun.* **66,** 687–692
22. Akera, T., and Cheng, V. J. (1977) *Biochim. Biophys. Acta* **470,** 412–423
23. Moor, H., and Mühlethaler, K. (1963) *J. Cell Biol.* **17,** 609–628
24. Jordan, E. G., Severs, N. J., and Williamson, D. H. (1977) *Exp. Cell Res.* **104,** 446–449
25. Maul, G. G., and Deaven, L. (1977) *J. Cell Biol.* **73,** 748–760
26. Yamasaki, L., Kanda, P., and Lanford, R. E. (1989) *Mol. Cell. Biol.* **9,** 3028–3036
27. Yoneda, Y., Imamoto-Sonobe, N., Matsuoka, Y., Iwamoto, R., Kiho, Y., and Uchida, T. (1988) *Science* **242,** 275–278
28. Imamoto-Sonobe, N., Matsuoka, Y., Semba, T., Okada, Y., Uchida, T., and Yoneda, Y. (1990) *J. Biol. Chem.* **265,** 16504–16508
29. Lee, W. C., and Melese, T. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 8808–8812
30. Hurt, E. C. (1990) *J. Cell Biol.* **111,** 2829–2837
31. Allen, J. L., and Douglas, M. (1989) *J. Ultrastruct. Mol. Struct. Res.* **102,** 95–108
32. Georgatos, S. D., Maroulakou, I., and Blobel, G. (1989) *J. Cell Biol.* **108,** 2069–2082
33. Goldfarb, D. S., Gariepty, J., Shoolnik, G., and Kornberg, R. D. (1986) *Nature* **322,** 641–644
34. Adam, S. A., Lobl, T. J., Mitchell, M. A., and Gerace, L. (1989) *Nature* **337,** 276–279
35. Garcia-Bustos, J. F., Heitman, J., and Hall, M. N. (1991) *Biochim. Biophys. Acta* **1071,** 83–101
36. Silver, P., Sadler, I., and Osborne, M. A. (1989) *J. Cell Biol.* **109,** 983–989
37. Adam, S. A., Marr, R. S., and Gerace, L. (1990) *J. Cell Biol.* **111,** 807–816
38. Feldherr, C. M., Kallenbach, E., and Schultz, N. (1984) *J. Cell Biol.* **99,** 2216–2222
39. Akey, C. W., and Goldfarb, D. S. (1989) *J. Cell Biol.* **109,** 971–982
40. Knipe, D. M., and Smith, J. L. (1986) *Mol. Cell. Biol.* **6,** 2371–2381
41. Dabauvalle, M. C., Shulz, B., Scheer, U., and Peters, R. (1988) *Exp. Cell Res.* **174,** 291–296
42. Schneider, J., Schindewolf, C., van Zee, K., and Fanning, E. (1988) *Cell* **54,** 117–125
43. White, E. M., Allis, C. D., Goldfarb, D. S., Srivastva, A., Weir, J. W., and Gorovsky, M. A. (1989) *J. Cell Biol.* **109,** 1983–1992
44. Michaud, N., and Goldfarb, D. S. (1991) *J. Cell Biol.* **112,** 215–223