Reverse recruitment: The Nup84 nuclear pore subcomplex mediates Rap1/Gcr1/Gcr2 transcriptional activation

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The recruitment model for gene activation presumes that DNA is a platform on which the requisite components of the transcriptional machinery are assembled. In contrast to this idea, we show here that Rap1/Gcr1/Gcr2 transcriptional activation in yeast cells occurs through a large anchored protein platform, the Nup84 nuclear pore subcomplex. Surprisingly, Nup84 and associated subcomplex components activate transcription themselves *in vivo* when fused to a heterologous DNA-binding domain. The Rap1 coactivators Gcr1 and Gcr2 form an important bridge between the yeast nuclear pore complex and the transcriptional machinery. Nucleoporin activation may be a widespread eukaryotic phenomenon, because it was first detected as a consequence of oncogenic rearrangements in acute myeloid leukemia and related syndromes in humans. These chromosomal translocations fuse a homeobox DNA-binding domain to the human homolog (hNup98) of a transcriptionally active component of the yeast Nup84 subcomplex. We conclude that Rap1 target genes are activated by moving to contact compartmentalized nuclear assemblages, rather than through recruitment of the requisite factors to chromatin by means of diffusion. We term this previously undescribed mechanism ''reverse recruitment'' and discuss the possibility that it is a central feature of eukaryotic gene regulation. Reverse recruitment stipulates that activators work by bringing the DNA to an nuclear pore complex-tethered platform of assembled transcriptional machine components.

chromatin boundaries | leukemia | silencing | synthetic genetic array | gene regulation

An underlying assumption of both the stepwise and preassembly alternatives (1) of the recruitment model of *in vivo* gene activation (2–6) is that activators work by bringing the transcriptional machinery to the DNA, i.e., that the machinery itself diffuses relatively freely within the nuclear compartment. We have been studying the repressor/activator protein Rap1 of *Saccharomyces cerevisiae*, which recognizes identical motifs in mediating either transcriptional activation (of glycolytic genes and ribosomal protein genes; refs. 7–9) or repression (of silent mating type loci and telomeres; refs. 10–15) and with its coactivators Gcr1 and Gcr2 participates in coordination of growth with cell-cycle progression (16, 17). Numerous aspects of Rap1 activation have conformed poorly with the ''free diffusion'' aspect of the recruitment model for transcriptional activation. One such aspect is the presence of an unusually large activation domain that is easily inactivated by means of mutations throughout the N-terminal 280 residues of Gcr1, spanning four distinct hypomutable regions (8, 17, 18); two of these hypomutable regions overlap with putative transmembrane domains.

We report here independent approaches demonstrating that the Rap1/Gcr1/Gcr2 activation assemblage (7–9, 19), like its silencing counterpart, is anchored at the nuclear periphery. For example, synthetic genetic array (SGA) analysis identified a robust genetic network that connects the Rap1 coactivators Gcr1 and Gcr2 with the Nup84 subcomplex (20, 21) and other nuclear envelope components; some of these same perinuclear factors coimmunoprecipitated with Gcr1 and Gcr2.

We also report here that the nucleoporin Nup84 and all but one of its subcomplex components activate transcription when fused to a DNA-binding domain, a result that recapitulates oncogenic nucleoporin–HoxA9 gene fusions in acute leukemias in humans (22, 23). Transcriptional activation by most nucleoporins is severely impaired by removing either the Rap1 coactivator Gcr1 or the nuclear pore complex (NPC) component Nup84.

Recent evidence for the *GAL* (24) and *INO1* (25) genes has now confirmed in *S. cerevisiae* what was first observed by Hutchison and Weintraub (26) in mouse L cells: active genes are localized to the nuclear periphery. In conjunction with these studies, our work clearly demonstrates that the nuclear rim in general and NPCs in particular play a critical role in activation of gene transcription. We also report here that Rap1, Gcr1, and perhaps many more transcription factors occupy relatively fixed positions in the nucleus as components in one or more highly networked gene expression machines (27). We therefore propose the term ''reverse recruitment'' to reflect the most important departure from previous models of transcriptional activation, that chromatin must locate the peripherally anchored expression machinery rather than the other way around.

Materials and Methods

Strains, Plasmids, and Assays. Strains and plasmids are listed in Table 2, which is published as supporting information on the PNAS web site. The tagged polypeptides used in this study are all fully functional *in vivo* when substituted for their native counterparts (data not shown). β-galactosidase assays were performed as described in refs. 8 and 9. Protein concentrations were determined by using the Bradford assay (Bio-Rad). Reporter assay values in WT cells for lexA fusions (units/mg of β-galactosidase activity) were as follows: empty vector, <0.1; Nup42, <0.1; Seh1, <0.1; Nup84, 5,573; Nup145N, 848; Nup145C, 2,077; hNup98, 2,685; Nup85, 961; Nup120, 939; Nup133, 273; Sec13, 105; Nup53, 24; Gcr1, 14,157; Gcr2, 10,619; Gcn4, 4,827; Swi4, 9,626; Ssn8, 5,478; and Sin4, 3,578.

SGA Analysis. Screening of Consortium strains with the Δ*gcr1* query strain SD8 (generated by backcrossing KW1433 to Y2454) was performed as described in ref. 28. After the final selection, double-mutant colonies that had a synthetic growth defect were

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Abbreviations: NPC, nuclear pore complex; SGA, synthetic genetic array.

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scored. Genes that were identified in at least two of three independent screens were scored as hits. Each SGA double mutant was generated independently to confirm the synthetic growth defects. Double mutants were scored as exhibiting a synthetic defect if a >20% reduction in colony size was observed (relative to those formed by the Δ*gcr1* mutant, which was invariably the slower growing of the two parents).

Immunoprecipitation, Western Blotting, and Nuclear Envelope Isolation. Immunoprecipitation was done as described in ref. 7 by using whole cell extracts of KW1433 expressing Gcr1-myc or, as a control, untagged Gcr1 (data not shown). All samples were resolved on 7% polyacrylamide/0.1% SDS gels. Western blotting was performed as described in ref. 7; coimmunoprecipitated polypeptides were detected by using native or α-lexA antibodies. Bands were visualized with the Pierce SuperSignal West Pico chemiluminescent substrate detection kit. Nuclear envelope fractions were isolated as described by Kipper *et al.* (29). The fractionation pattern of Rap1, Gcr1, Gcr2, Pom152, and Nop1 was determined with Western blots. The amount of total protein loaded in lanes marked cytosol, nucleus, and nuclear envelope was 75, 10, and 150 μg, respectively. Gcr1- and Gcr2-myc were detected with α-myc antibody (9E10; Santa Cruz Biotechnology); native antibodies were used to detect Rap1, Pom152, and Nop1.

Results

A Synthetic Genetic Interaction Network Suggests a Functional Link Between Rap1 Activation and the Nuclear Rim. SGA analysis (28) of a Δ*gcr1* or Δ*gcr2* query strain revealed a surprising genetic connection between transcriptional activation and nuclear pore function. Deletion of any 1 of 12 nonessential genes encoding nucleoporins or NPC-associated factors caused a synthetic growth defect when combined with *GCR1* deletion (Fig. 1). The gene encoding the eponymous subunit of the Nup84 NPC subcomplex (*NUP84*) was independently identified by SGA analysis with a Δ*gcr2* query strain. Deletion of *NUP120* or *NUP133*, which encode two other components of the Nup84 subcomplex (20, 21), also caused a synthetic defect in combination with the Δ*gcr1* mutation. Disruption of *NUP84*, *NUP120*, or *NUP133* is known to cause defects in nuclear membrane and NPC organization (21) and, like the Δ*gcr1* and Δ*gcr2* mutations (16), results in defective growth on glucose. Finally, the *NUP100* product also was identified by SGA analysis of the Δ*gcr1* query strain; it shares homology with the N terminus of Nup145, a third component of the Nup84 subcomplex that is essential for viability (30). Thus, the Rap1 transcriptional activation complex is functionally linked to nuclear pores and exhibits a particularly robust genetic connection to the Nup84 subcomplex.

Copurification of Rap1 and Its Coactivators with the Nuclear Membrane, NPCs, and NPC-Associated Factors. The genetic data in Fig. 1 identified a network that includes the Gcr1/Gcr2 activation complex and nucleoporins. We therefore used biochemical analysis in an attempt to confirm the implied association between Rap1 activation and the nuclear periphery. We first assessed the capacity of Rap1 and its activation complex to cofractionate with the nuclear envelope (29). The nuclear envelope protein Pom152 and the nucleolar factor fibrillarin (Nop1 in *Saccharomyces*) served as positive and negative controls, respectively. Rap1, Gcr1, and Gcr2 all copurified with Pom152 (Fig. 2 *A–D*), which agrees with colocalization studies of fluorescently tagged components of the Rap1 activation assemblage in live cells (S.P., B.B.M., and G.M.S., unpublished data). As expected, Nop1 also was found predominantly in the nucleus but exhibited little or no association with the nuclear envelope (Fig. 2*E*).

Densitometric analysis of Fig. 2 (normalized to the amount of protein loaded) indicated that in nuclear envelope preparations Rap1 is at least as enriched, and Gcr1 is 71% as enriched, as the integral nuclear membrane protein Pom152. Although Gcr2 appeared to be less tightly associated with the nuclear envelope (19% of Pom152 enrichment), it is at least 20-fold more enriched than the Nop1 negative control (<1% enrichment). Given that Rap1 and Gcr1 possess DNA-binding activity, it is important to note that the nuclear envelope isolation protocol includes a DNase step (29), thus eliminating DNA tethering as an explanation for copurification with perinuclear factors. We repeated this analysis and added a final heparin extraction, which removes peripheral nuclear envelope proteins and yields a fraction highly enriched for integral membrane components (31). As expected, the heparin step eliminated Rap1 and Gcr1 from the nuclear envelope preparation (data not shown), identifying each as a peripheral factor associated with the nuclear membrane.

We also tested for interaction between the Rap1 activation assemblage and the products of several NPC genes that are genetically linked with *GCR1* (Fig. 1). We chose factors that represent distinct strata at the nuclear rim to investigate whether Rap1 coactivators interact specifically with a subset of perinuclear polypeptides or instead exhibit a more generic pattern of peripheral association. Pom34 and Pom152 are pore-associated integral nuclear membrane proteins; Nup84 is the eponymous subunit of an essential nuclear pore subcomplex; and Kap123 is loosely associated with NPCs and participates in cargo trafficking. Mass spectrometric analyses had already indicated that Kap123 copurifies with Gcr1 through several steps of chromato-

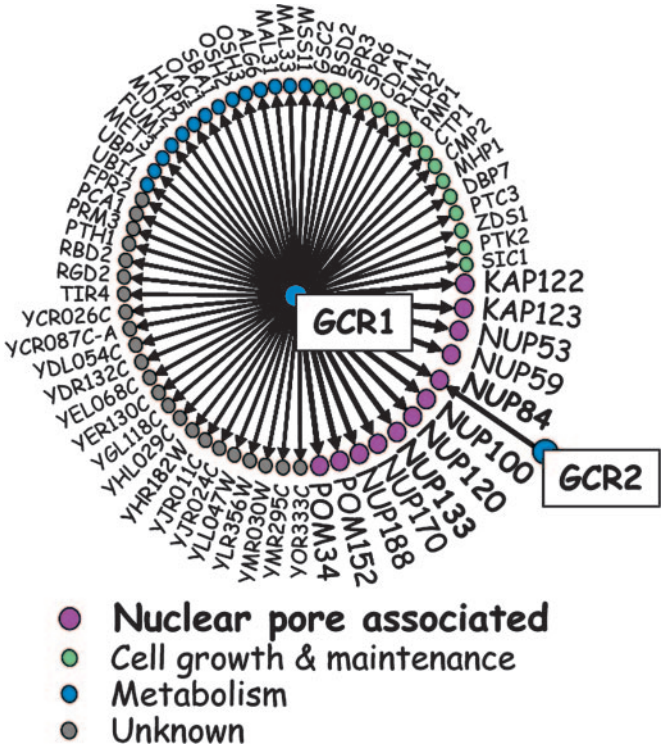

Fig. 1. Genome-wide genetic analysis of *GCR1*. A Δ*gcr1* query strain (SD8) was crossed to an array of ≈4,700 deletion mutants to screen for synthetic growth defects in the resulting double mutants. Synthetic defects that result from combination with *GCR1* deletion (arrows) fall into four categories based on Gene Ontology annotation (29) as follows: nuclear pore-associated genes (purple; large type), genes involved in cell growth and maintenance (light green), metabolic genes (blue), and uncharacterized ORFs (gray). Components of the Nup84 nuclear pore subcomplex and the related factor Nup100 are shown in bold. Deletion of *NUP84* is also synthetically defective in combination with a *GCR2* deletion, as indicated.

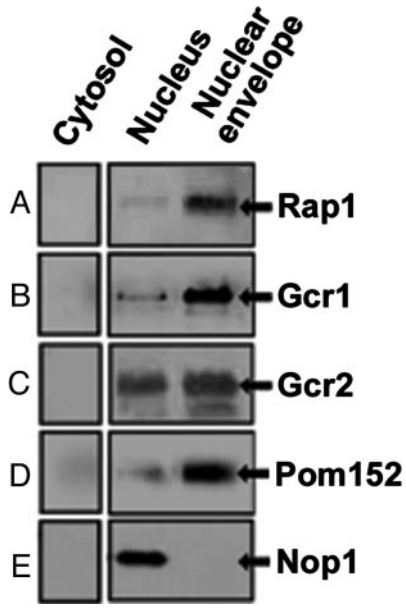

Fig. 2. Physical association between the Rap1/Gcr1/Gcr2 activation complex and the nuclear periphery. Rap1 (*A*), Gcr1-myc (*B*), and Gcr2-myc (*C*) copurify with Pom152 (*D*) in nuclear envelope fractions; the nucleolar protein Nop1 (*E*) served as a negative control.

graphic separation, including an ion-exchange step that excludes DNA (our unpublished data). Because the pattern of Gcr1-YFP nuclear localization is unaffected in a Δ*kap123* strain (data not shown), Gcr1 association with Kap123 does not reflect a reliance on the β-importin function of the latter.

Nup84, Pom34, Pom152, and Kap123 were all efficiently coimmunoprecipitated by Gcr1 (Fig. 3) and Gcr2 (data not shown), indicating that the association between Rap1 coactivators and nuclear pores can be detected at each level of perinuclear organization. An identical result was observed when the immunoprecipitation experiment was repeated with RNase- or DNase-treated cell extracts, suggesting that the association with the nuclear pores represents bona fide protein–protein interactions and not tethering by means of DNA or RNA (data not shown). Thus, our genetic and biochemical experiments both indicate a robust link between the Rap1 activation complex and the periphery of the yeast nucleus.

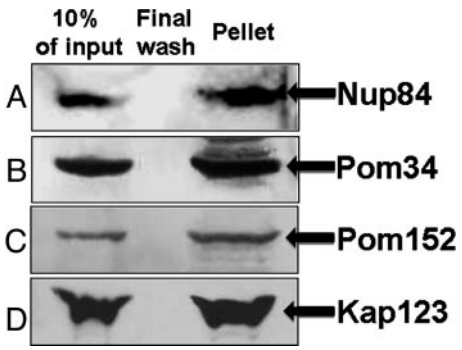

Fig. 3. Epitope-tagged Gcr1 coimmunoprecipitates with three nuclear pore factors [Nup84 (*A*), Pom34 (*B*), and Pom152 (*C*)] and the NPC-associated β-importin Kap123 (*D*). Gcr1-myc was immunoprecipitated from whole cell extracts (input; lane 1); the last of four washes before elution (final wash; lane 2) was analyzed as a control. The corresponding protein eluates (pellet) were loaded in lane 3.

Perinuclear Activation of Transcription in Yeast Cells. Despite the well established connection between repression and distal location in the nucleus of *S. cerevisiae* and other eukaryotes, an increasing body of evidence has indicated that transcriptional activation is far from an unusual occurrence at the nuclear rim (22–26, 32–36). Most interestingly, peripheral activation may be oncogenic in acute myeloid leukemia and related syndromes in humans (23, 34), in which chromosomal rearrangements fuse *Hox* DNA-binding domains to the nucleoporin hNup98 (mammalian ortholog of yeast Nup145; the proteolytically cleaved Nup145 C terminus is a component of the Nup84 subcomplex). Because the above data indicated that an analogous phenomenon might exist in *S. cerevisiae*, we fused a heterologous DNA-binding domain (lexA of *Escherichia coli*) to each component of the yeast Nup84 subcomplex and measured the capacity of the resulting chimeras to activate a lexA-driven lacZ reporter gene. Nup42, one of the few strictly cytoplasmic yeast nucleoporins, was included as a control and, as expected, did not stimulate reporter transcription (Fig. 4). With the exception of the Sec13 homolog Seh1, each Nup84 subcomplex component activated transcription of the reporter gene, and Nup84 itself was comparable in strength to the conventional activator Gcn4. We also used two of the nucleoporin fusion proteins (Nup84 and Nup85) to demonstrate nucleoporin transcriptional activity during normal localization at the nuclear periphery (see Fig. 6, which is published as supporting information on the PNAS web site). These data strongly suggest that nucleoporin activation takes place in the normal context of nuclear pores and is not due to mislocalization of the fusion protein to the interior of the nucleus.

Interestingly, nucleoporin activation was not limited to the Nup84 subcomplex. Both Nup145N and Nup53 also stimulated transcription. Deletion of *NUP53* was synthetically defective with the Δ*gcr1* mutation (*NUP145* is an essential gene and, therefore, could not be identified by the SGA analysis shown in Fig. 1).

Furthermore, unlike the conventional transcriptional activators Gcn4 and Swi4, activation by Nup145N, Nup145C, Nup85, Nup120, Nup133, Sec13, and Nup53 was strongly dependent on Gcr1; all but Nup85 also were severely impaired in the absence of Nup84 (Table 1). We tested "artificial" activation by Ssn8 and Sin4 as well; both are Mediator components shown to associate with the RNA polymerase II C-terminal repeat domain (37). Interestingly, Sin4 activation depended on Gcr1 but not Nup84, whereas transcriptional stimulation by the C-type cyclin Ssn8 depended on both perinuclear factors.

Activation by Gcr1 and Gcr2 also was reduced in a Δ*nup84* strain. Loss of Rap1/Gcr1/Gcr2 activation in the Δ*nup84* background was independently confirmed by measuring expression of two glycolytic target genes, *ADH1* and *CDC19* (*PYK1*); in the absence of Nup84, the level of each of these gene products was reduced to ≈50% of WT levels. Further, activation of a heterologous reporter gene by either Gcr1 or Gcr2 is reduced by >5-fold in the absence of Nup84 (Table 1). Microarray analyses demonstrated that the impaired levels of transcriptional activation shown in Table 1 were not due to reduced expression in Δ*nup84* or Δ*gcr1* cells (K.E.B. and G.M.S., unpublished data). Gcr1-dependent activation by Nup84 and associated subcomplex components therefore likely reflects a normal aspect of perinuclear function that had previously gone undetected.

Discussion

Rap1 has long been known to participate in two opposing roles in global gene regulation, activation, and silencing. It has been well established that silencing assemblages normally localize and function at the nuclear periphery (10–15). The perinuclear localization of Rap1 and its coactivators, presented herein, suggest that Rap1 transcriptional activation also occurs at the

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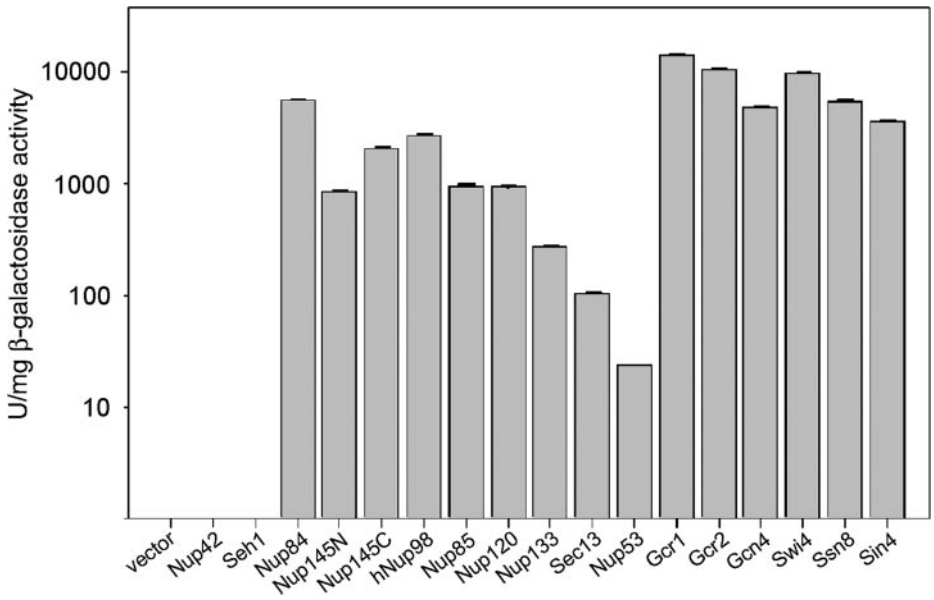

Fig. 4. Transcriptional activation at the nuclear rim. The exclusively cytoplasmic nucleoporin Nup42 and the Seh1 component of the Nup84 subcomplex failed to activate transcription above background levels (vector) in WT cells. All other nucleoporins tested, including the human nucleoporin hNup98 (protooncogenic homolog of Nup145C), stimulated transcription of the reporter gene. The conventional activators Gcr1, Gcr2, Gcn4, and Swi4, as well as the mediator components Ssn8 and Sin4, are shown for comparison. Error bars represent standard error of the mean.

periphery. It remains to be determined whether a given Rap1 molecule can switch from one transcriptional state to the other. The alternative is that distinct Rap1 silencing and activation assemblages operate at the nuclear rim, perhaps in segregated subdomains (Fig. 5).

Interestingly, a "molecular latch" proposed to control gating of nuclear pore translocation channels (35, 38) may influence Rap1 perinuclear function. Each component of the proposed "gate" (Nup120–Nup85), "hinge" (Nup170), and control mechanism (Nup145C–Nup84–Nup133) is a constituent of the *GCR1* genetic network (Fig. 1) and (with the exception of Nup170, which has not yet been tested) activates transcription in a Gcr1-dependent fashion, suggesting a functional link between such a latch and Rap1 activation (Fig. 5). We currently envision a latching apparatus regulating Rap1 function in one of two ways, either by mediating a switch in the transcriptional state of a single functionally bipartite complex or by acting as a barrier between segregated perinuclear silencing and activation assemblages.

Table 1. Activation of a reporter gene by nucleoporins in Δ*nup84* or Δ*gcr1* cells

lexA fusion	Δ*nup84*	Δ*gcr1*
Nup84	—	74.2 ± 1.4
Nup145N	9.5 ± 0.1	19.4 ± 0.4
Nup145C	18.4 ± 0.1	22.2 ± 0.2
hNup98	19.6 ± 0.3	52.2 ± 0.9
Nup85	58.1 ± 1.4	8.7 ± 0.1
Nup120	7.2 ± 0.1	25.6 ± 0.7
Nup133	<0.1*	6.4 ± 0.1
Sec13	<0.1*	30.2 ± 0.3
Nup53	<0.1*	28.9 ± 0.1
Gcr1	19.8 ± 0.1	—
Gcr2	18.1 ± 0.5	50.2 ± 1.5
Gcn4	102.7 ± 1.4	180.3 ± 4.0
Swi4	57.3 ± 1.7	130.4 ± 7.9
Ssn8	32.4 ± 0.5	13.3 ± 0.8
Sin4	93.2 ± 0.2	11.9 ± 0.1

Activity of lexA fusions was determined in either a Δ*nup84* or a Δ*gcr1* strain; the percent of β-gal activity in these strains relative to that in WT cells (see Fig. 4) is shown ± SD. —, Not determined.
*No detectable activity.

In addition to demonstrating that Rap1 and its coactivators are subcompartmentalized at the nuclear periphery, we also have shown that many yeast nucleoporins function as transcriptional activators and that Nup84 and Nup85 do so at their normal perinuclear location. The surprising finding that nucleoporin activation is entirely dependent on the Rap1 coactivators Gcr1 (Table 1) and Gcr2 (data not shown) supported the independently derived functional (Fig. 1) and physical (Figs. 2 and 3) connection between the Rap1/Gcr1/Gcr2 assemblage and the membrane-embedded nuclear pores. Interestingly, Gcr1 contains two predicted transmembrane domains that overlap with hypomutable regions; point mutations or small in-frame deletions in either of these regions eliminate *GCR1* function (ref. 18 and data not shown). Further investigation is needed to determine whether Gcr1 requires intermediates to interact with the nuclear membrane or instead does so directly, by means of one or both of its essential transmembrane domains. The fact that transcriptional activation by nucleoporins is disrupted by *GCR1* deletion (Table 1) suggests that alteration of the Rap1 activation assemblage interferes with the normal organization of basal transcription factors at the yeast nuclear periphery (see below).

Of the 30 known yeast genes encoding nucleoporins, 15 are nonessential (39), and the corresponding knockouts therefore can be screened with the SGA method; deletion of any 1 of 10 (67%) of these nonessential genes caused a synthetic growth defect when combined with *GCR1* deletion. Significantly, SGA analysis identified three of the four nonessential components of the Nup84 nuclear pore subcomplex (*NUP84*, *NUP120*, or *NUP133*); the other three components are essential (there are a total of seven known Nup84 subunits). The only Nup84 subcomplex component that was not identified as a member of the *GCR1* genetic network (*Seh1*) was also the only one that failed to activate transcription (Fig. 5).

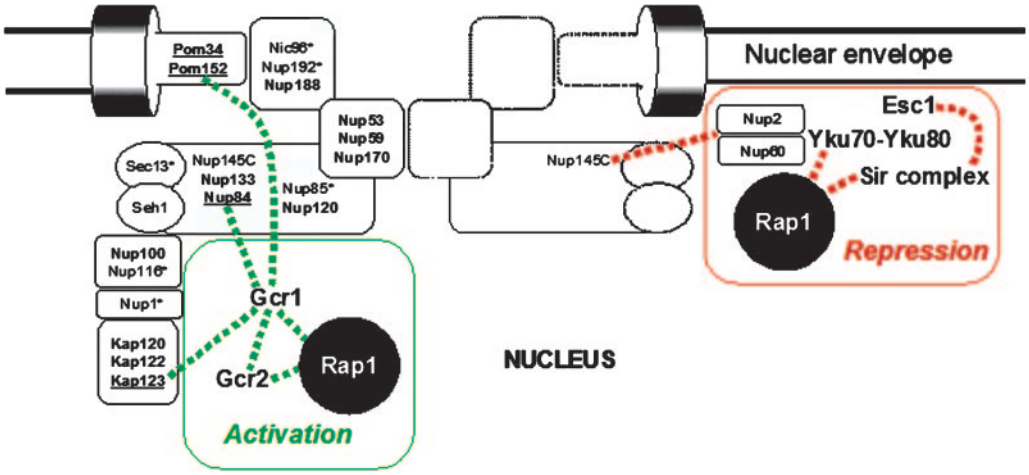

Fig. 5. Activation and repression by the multifunctional regulator Rap1 at the nuclear periphery of *S. cerevisiae*. Known components of the Rap1 activation (Gcr1/Gcr2; refs. 7–9, 16, 18, and 19) or silencing (Sir complex; refs. 10–12, 14, 15, and 19) assemblages are shown. Essential nucleoporins are indicated with an asterisk; components of the *GCR1* genetic network identified by SGA analysis (Fig. 1) are shown in bold. Note that combining deletion of *GCR1* with deletion of each of several genes encoding components of the Rap1 silencing assemblage did not result in a synthetic growth defect (*NUP2*, *NUP60*, *YKU70*, *YKU80*, and *SIR1* were tested by tetrad dissection). Dashed lines highlight presumptive perinuclear tethering interactions; NPC-associated factors shown to interact with Gcr1 (Fig. 3) are underlined. The representation shown here is not intended to rule out the existence of a unified complex that can switch between activation and repression of transcription (see text for further discussion).

Our discovery of Gcr1-dependent transcriptional stimulation by nucleoporins also helps to explain the synthetic growth defects resulting from removal of Gcr1 and each NPC-associated factor (Fig. 1). Such pairwise synthetic defects between deletion mutants typically occur because the absent gene products act either in parallel or redundantly. Impairment of parallel mechanisms of gene expression would explain our SGA data without departing from the conventional view that the function of Rap1 coactivators and nuclear pore-associated factors (mRNA production and transport, respectively) exhibit little or no overlap. However, we favor the redundancy explanation for synthetic defects, which implies that either Rap1 coactivators or nuclear pore-associated factors possess a heretofore hidden role in the yeast nucleus; either the Gcr1/Gcr2 complex participates in nuclear export, or there is a nucleoporin role in transcriptional activation. The capacity of many nucleoporins to activate a reporter gene clearly suggests the existence of the latter.

The data presented here are entirely consistent with the nuclear pore-tethering model for chromatin boundaries proposed by Ishii *et al.* (33), who found that nonsilenced domains could be established by tethering Nup2 or Nup2-associated factors to DNA sites within the yeast silent mating type locus HML. Importantly, they used fluorescence microscopy to demonstrate that the switch to transcriptional activation requires the presence of targeted chromatin at the nuclear periphery. This pioneering work, particularly when viewed in the light of recent studies of Rap1 silencing complexes (10–15) and the analysis of Rap1 activation presented here, demonstrate that the nuclear periphery is not generally repressive but is functionally segregated into at least two nuclear pore-associated domains, a silencing-telomere domain and an activating domain.

Several additional observations support a connection between the transcriptional machinery and the nuclear periphery. We have shown that artificial stimulation of transcription by the Mediator component Ssn8 is Nup84-dependent, although, interestingly, activation by a different mediator component (Sin4) was Nup84-independent; transcriptional stimulation by both factors was severely impaired in the absence of Gcr1 (Table 1). We also found significant association of both the Mediator component Med1 and the RNA polymerase II large subunit (Rpb1) with the yeast nuclear periphery (data not shown). Finally, a list of known interactions between nucleoporins and positively acting transcription factors in *S. cerevisiae* is shown in Table 3, which is published as supporting information on the PNAS web site, suggesting a variety of contacts that could account for the perinuclear accumulation of RNA polymerase II (first observed in mammalian cells; ref. 32). We noted with interest that several of these factors are Rap1-like in that they can play both positive and negative roles in transcription.

The perinuclear Rap1 activation mechanism is consistent with the proposal of Hutchison and Weintraub (26) that active eukaryotic genes are localized along interchromatin channels that communicate with the nuclear periphery. With respect to a related supposition, that Rap1 activation requires association with relatively immobile nuclear structures, fluorescence recovery after photobleaching experiments indicated that focally distributed Rap1, Gcr1, and Gcr2 molecules are slowly diffusing (see Fig. 7, which is published as supporting information on the PNAS web site). Our finding that Rap1 and its coactivators occupy relatively fixed positions in the yeast nucleus implies that structural organization plays an important role in at least some eukaryotic activation.

Further, Rap1 activation takes place at many sites in each yeast chromosome, and characterization of the transcriptome by serial analysis of gene expression has demonstrated that Rap1-up-regulated genes include 26 of the 30 most highly expressed yeast transcription units (40). Our work therefore underscores the idea that eukaryotic gene regulation often may involve movement of genes toward compartmentalized nuclear factors rather than recruitment of factors to chromatin by means of diffusion, a hypothesis that we call reverse recruitment. A recent analysis of perinuclear genes in the yeast genome provides direct substantiation for this idea (24). Chromatin immunoprecipitation of NPC-associated genes (24) resulted in a significant enrichment of those under Rap1/Gcr1 regulatory control (7–9, 16, 18); the perinuclear localization of those genes was presumed to reflect efficient mRNA export, transcriptional activation, or both. Our work clearly eliminates posttranscriptional events as the sole explanation for perinuclear Rap1-bound genes. Importantly, Casolari *et al.* (24) showed that transcriptional activation of the *GAL* genes results in their relocation to the nuclear periphery; similar results also

have been reported for *INO1* (25). It will be interesting to see whether this phenomenon is generally true of regulatory pathways in *S. cerevisiae* and other eukaryotes.

Further analysis of the reverse recruitment hypothesis, and of gene regulation at the nuclear periphery in particular, is therefore likely to yield important insights concerning the impact of transcriptional regulatory mechanisms on cell division, differentiation, and carcinogenesis. Given the oncogenicity of Nup98-*Hox* activators in acute myeloid leukemia and the identification of a cognate transcriptional activation mechanism at the nuclear periphery of yeast cells, such studies should be particularly salient with respect to the genesis of myeloid leukemias in humans.

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