Roles of SWI1, SWI2, and SWI3 Proteins for Transcriptional Enhancement by Steroid Receptors

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The SWI1, SWI2, and SWI3 proteins, which are required for regulated transcription of numerous yeast genes, were found also to be essential for rat glucocorticoid receptor function in yeast; the receptor failed to activate transcription in strains with mutations in the *SWI1*, *SWI2*, or *SWI3* genes. Certain mutations in genes encoding components of chromatin, identified as suppressors of *swi* mutations, partially relieved the SWI$^-$ requirement for receptor function. Immunoprecipitation of glucocorticoid receptor derivatives from wild-type (*SWI*$^+$) yeast extracts coprecipitated the SWI3 protein; such receptor-SWI3 complexes were not detected in *swi1*$^-$ or *swi2*$^-$ mutant strains, implying that a complex of multiple SWI proteins may associate with the receptor. Prior incubation of a *Drosophila* embryo transcription extract with the yeast SWI3-specific antibody inhibited receptor function in vitro whereas the antibody had no effect if added after initiation complex formation. Thus, positive regulation by the glucocorticoid receptor in vivo and in vitro appears to require its interaction, at an early step, with one or more SWI proteins.

The rate of transcription initiation by RNA polymerase II at specific eukaryotic genes is governed by multiprotein complexes that form at promoters and linked regulatory sites on the DNA template (*1*). Although not yet completely identified, the components of these complexes appear to be dynamic structures composed of sequence-specific DNA-binding proteins together with non-DNA–binding factors that associate via protein-protein contacts. The glucocorticoid receptor is an example of a sequence-specific regulatory protein. Upon association with its steroid hormonal ligands, the receptor migrates to the cell nucleus, binds to specific DNA sequences termed glucocorticoid response elements (GRE's), and regulates transcription from nearby promoters (*2*). In the course of its signaling and regulatory functions, the receptor interacts with other proteins, such as the heat shock protein Hsp90 (*3*) and transcription factors such as AP1 (*4*), CREB (*5*), and probably others (*6*). In vitro studies indicate that one action of the receptor may be to facilitate assembly of functional initiation complexes at hormone-regulated promoters (*7, 8*). In addition, it has been shown that receptor action is accompanied by alterations in the structure of chromatin overlying the functional GRE's and regulated promoters (*9*). Thus, while it is not known how the structural effects of the receptor relate to its effects on initiation complex formation (or to receptor function in general), it is conceivable that the receptor may operate through both chromatin-independent and chromatin-dependent mechanisms.

Despite the apparent complexity of transcription initiation and its regulation, these processes appear to have been conserved during evolution. The rat glucocorticoid receptor expressed in the yeast *Saccharomyces cerevisiae* confers hormone-dependent regulation on yeast promoters linked to GRE's (*10, 11*). The receptor also functions when expressed in distantly related fungi (*12*) and in insect (*13*) and plant cells (*14*). These observations imply that many organisms carry functional homologs of the factors with which the receptor interacts in mammalian cells. The demonstration of receptor function in *S. cerevisiae* suggests that it might be possible to exploit the genetic manipulability of yeast to identify other gene products essential for receptor function, including those with which it might interact directly. For example, Picard *et al.* (*15*) used this approach to establish that Hsp90 is essential for receptor function in vivo.

Genetic studies in yeast have led to the identification of three genes whose products appear to facilitate the enhancement of transcription by sequence-specific DNA binding proteins. These genes—*SWI1*, *SWI2*, and *SWI3*—were identified originally as mutants that failed to induce transcription of the *HO* gene, which is required for cell type switching (*16*). The proteins SWI1, SWI2, and SWI3 also potentiate the transcription of many other yeast genes that are regulated positively by sequence-specific DNA-binding proteins, although these SWI proteins themselves have not been shown to bind DNA (*17*).

The *SWI1*, *SWI2*, and *SWI3* genes are functionally similar to the *SNF2*, *SNF5*, and *SNF6* genes, which were identified by their requirement for transcription of the *SUC2* gene (*18*). In fact, the *SNF* genes are necessary for *HO* transcription, and the *SWI* genes are required for *SUC2* transcription (*17*); indeed, SWI2 and SNF2 are identical (*19, 20*). It has been suggested that these five gene products, SWI1, SWI2, SWI3, SNF5, and SNF6, may form a protein complex that modulates various promoters (*17*).

The transcriptional defects of *swi1*$^-$, *swi2*$^-$, or *swi3*$^-$ mutants are suppressed by particular mutant alleles of two genes, *SIN1* and *SIN2*. Thus, partial inducibility of *SWI*-dependent genes is observed in appropriate *swi*$^-$ *sin*$^-$ double mutant strains (*21*). Sequence analysis reveals that *SIN1* encodes a protein that contains regions of amino acid similarity with HMG-1 (*22*), and that *SIN2* encodes histone H3 (*23*). Furthermore, Winston and colleagues have identified other suppressor mutants in this phenotypic class; one such mutant carries a deletion of a gene cluster encoding histones H2A and H2B (*24*). It may be therefore that the products of *SWI1*, *SWI2*, and *SWI3* collaborate with sequence-specific regulators and antagonize the repressive properties of chromatin components.

In that the glucocorticoid receptor may affect chromatin structure, we have tested whether mutations in the *SWI* and *SIN* genes affect transcriptional regulation by the glucocorticoid receptor in yeast. In addition, we have extended these in vivo studies to biochemical analyses by assessing putative SWI function during receptor-mediated transcriptional enhancement in vitro, and by testing for physical interactions between the receptor and the SWI proteins.

SWI-dependent receptor action. We first determined whether transcriptional enhancement by the rat glucocorticoid receptor (GR) in yeast requires *SWI* gene products. Expression and reporter constructs (shown in Fig. 1) were used to analyze receptor function in wild-type yeast and in isogenic yeast strains containing mutations in *SWI1*, *SWI2*, or *SWI3*. Expression plasmid pG-N795, in which the wild-type receptor cDNA is transcribed from the yeast glyceraldehyde-3-phosphate dehydrogenase gene *GPD* promoter (*25*), was transformed into wild-type (*SWI*$^+$) and mutant (*swi*$^-$) strains, together with a high copy β-galactosidase reporter plasmid, pΔS26X, which

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contains three rat tyrosine aminotransferase (*TAT*) GRE's upstream of the minimal (UAS$^-$) yeast *CYC1* promoter. Cultures of the transformed strains were incubated with a synthetic glucocorticoid, deacylcortivazol (DAC), for 10 to 12 hours; measurements of β-galactosidase activity revealed that in the *swi*$^-$ mutant strains, the receptor was only 2 to 7 percent as active as it is in the *SWI*$^+$ strain (Fig. 2A).

We also tested the estrogen receptor, a rather distantly related member of the steroid receptor family, for its dependence on the *SWI3* gene. Like the glucocorticoid receptor, the estrogen receptor enhances transcription in yeast (*26*). The expression and reporter vectors were as above except that they contained estrogen receptor cDNA and a single estrogen response element (ERE), respectively. The estrogen receptor function in *swi3*$^-$ mutant strains was only 5 percent of that in the *SWI3* wild-type parental strain (Fig. 2B). Thus, we conclude that the activity of the estrogen receptor, like that of the glucocorticoid receptor, is dependent on the *SWI3* gene product.

To determine whether the loss of receptor function in the *swi*$^-$ mutant strains might simply reflect reduced receptor production, we examined the activity of the *GPD* promoter, which drives receptor expression, and also directly measured the steady state receptor level in the *SWI*$^+$ and *swi*$^-$ strains. A *GPD LacZ* reporter was expressed at somewhat variable levels among the various *swi*$^-$ mutant strains, but the differences were neither large nor systematic relative to the changes in receptor activity observed in these strains (Fig. 3A). That is, expression was slightly higher than wild type in the *swi1*$^-$ and *swi2*$^-$ strains, whereas expression appeared to be some three times lower in the *swi3*$^-$ strain. This confirmed that not all promoters are defective when the *SWI* genes are deleted (*17*), and implied specifically that the promoter used for receptor expression was not *SWI* dependent.

To assess directly the levels of receptor protein in the *SWI*$^+$ and *swi3*$^-$ strains, we metabolically labeled the yeast proteins with [^{35}S]methionine and the labeled receptor was immunoprecipitated. Similar amounts of receptor in the *swi3*$^-$ strain (in which GR was expressed from a high copy vector) and the *SWI*$^+$ strain (in which GR was produced from a low copy vector) (Fig. 3B). Because this amount of receptor was sufficient to activate transcription from a GRE-linked reporter in the *SWI*$^+$ strain (Fig. 2A), we conclude that it is the activity, and not the expression, of the receptor that is deficient in the *swi*$^-$ mutant strains.

To define a region of the glucocorticoid receptor whose function is *SWI*-dependent, we compared the activities of various recep-

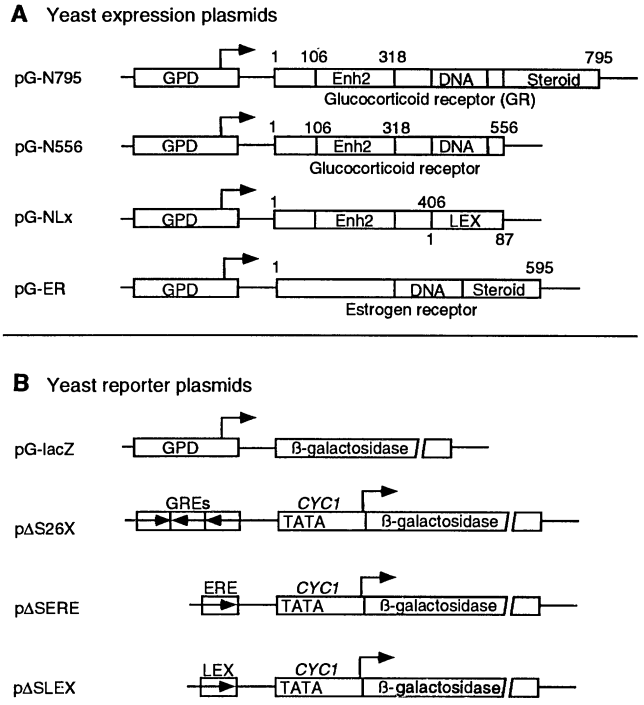


Fig. 1. Yeast expression and reporter plasmids. (**A**) Expression constructs. The full-length rat glucocorticoid receptor (GR) cDNA was expressed from the yeast glyceraldehyde-3-phosphate dehydrogenase gene (*GPD*) promoter (*10, 25*) on either low copy (centromeric, pRS314/N795) (*49*) or high copy (2μ, pG-N795) plasmids. The GR derivatives were expressed from the yeast *GPD* promoter on 2μ plasmids: pG-N556 contains GR amino acids 1 to 556 (*27*); pG-NLx contains GR sequences encoding amino acids 1 to 452 fused to sequences encoding the bacterial LexA repressor DNA binding domain (amino acids 1 to 87) (*29*). The human estrogen receptor (*15, 50*) was expressed from the *GPD* promoter on a 2μ plasmid (pHG-ER). The numbers above or below each diagram represent amino acid residues within the wild-type protein. (**B**) Reporter constructs. Reporter plasmids contained a yeast *CYC1* promoter fragment (*10, 51*) lacking the upstream activating sequence (−178 to +77) fused to the β-galactosidase (*LacZ*) coding sequence. In plasmid pΔS26X (*10*), three glucocorticoid response elements (GRE) from the rat tyrosine aminotransferase gene (*TAT*) were inserted immediately upstream of the *CYC1/LacZ* gene. Similarly, pΔSERE and pΔSLEX contains a single estrogen response element (*15*) from the *Xenopus laevis* vitellogenin A2 gene and a 26-bp DNA fragment containing the *LexA* operator (*52*), respectively, upstream of the *CYC1* promoter. Yeast cultures were grown at 30°C in complete medium (YEP, 2 percent yeast extract, and 1 percent Bactopeptone) or synthetic medium (S medium) containing either 2 percent glucose or 2 percent galactose. The S medium contains yeast nitrogen base 6.7 g/liter (Difco, amino acids) and supplemented amino acids (*16*). All strains of *S. cerevisiae* were derivatives of S288C. Strains CY26, CY57, CY58, CY73, and CY118 are isogenic; CY26 is *ura3-52 lys2 ade2-101 his3Δ200 leu2*$^-$. The *swi1Δ::LEU2, swi2Δ::HIS3*, and *swi3Δ::TRP1* alleles were constructed by gene disruptions (*17*). Strains MCY2099 and MCY2101 are isogenic and are congenic relative to CY26. The *snf5Δ* and *snf6Δ* alleles were constructed as described (*53, 54*). Yeast transformations were as described (*55*).

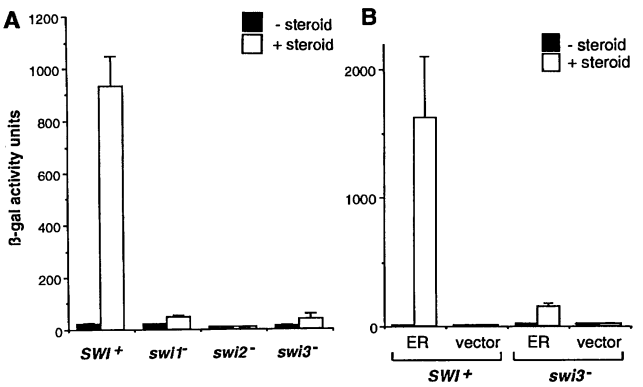


Fig. 2. Effect of *swi*$^-$ mutations on steroid receptor function in yeast. (**A**) Transcriptional activation by the glucocorticoid receptor. Isogenic *SWI*$^+$ (CY26), *swi1*$^-$ (CY58), *swi2*$^-$ (CY57), and *swi3*$^-$ (CY73) strains were transformed with a centromeric GR expression vector (pRS314/N795) and a GRE β-gal reporter vector (pΔS26X). (**B**) Transcriptional activation by the estrogen receptor. A yeast estrogen receptor expression vector (pG-ER) and an ERE-linked β-galactosidase reporter plasmid (pΔSERE) were transformed into *SWI* (CY26) and *swi3* (CY73) yeast strains. As a control, the expression vector (vector) lacking the estrogen receptor cDNA was cotransformed with pΔSERE. Transformants were grown overnight in minimal medium and then diluted (1:5) in minimal medium containing (for A) 200 nM DAC added as a concentrated 10^3× stock in 100 percent ethanol; (for B) 100 nM β-estradiol (Sigma; added as a 10^3× stock in ethanol); control cultures received vehicle only. Cultures were generally harvested after 10 to 12 hours of growth to an optical density ($OD_{600\ nm}$) of 0.6 to 0.8 (late log phase); the β-galactosidase activity from three independent transformants was then assayed (*56*). Units are defined as 10^3 times the change in $OD_{420\ nm}$ due to the hydrolysis of *o*-nitrophenyl-β-D-galactosidase, divided by the product of the assay time (minutes) multiplied by the culture volume (ml) times the $OD_{600\ nm}$ of the culture.

tor derivatives in *SWI*$^+$ and *swi*$^-$ yeast strains. We first examined a GR derivative, N556, which lacks the COOH-terminal hormone-binding domain; N556 activates transcription constitutively from GRE-linked reporter constructs in mammalian (*27*), *Drosophila* (*28*), and yeast (*10*) cells. The N556 protein was inactive in a *swi*$^-$ mutant strain (Fig. 4A); parallel experiments confirmed that *SWI2* and *SWI3* were also required under these conditions. Thus, the SWI proteins were essential for DNA binding or transcriptional activation by the receptor, and not solely for the transduction of the steroid signal.

We next tested the zinc finger DNA-binding domain of the receptor for the effects of the *SWI* products on receptor action. We used a chimeric N556 derivative, NLx, in which the zinc fingers of the receptor were substituted by the helix-turn-helix DNA-binding domain of the bacterial LexA repressor (Fig. 1, pG-NLx) (*29*). Although NLx enhanced transcription from a *LexA* operator-linked promoter in the *SWI*$^+$ strain, it displayed only 0.02 percent as much activity in a *swi3*$^-$ mutant background (Fig. 4B). While these findings cannot exclude action of the SWI proteins on these distinct DNA-binding motifs (as discussed below), the simplest interpretation is that the SWI factors facilitate function of the NH_2-terminal region of GR; this domain includes a strong transcriptional activation signal (*29*).

Yeast strains carrying *snf5*$^-$ or *snf6*$^-$ lesions are phenotypically similar to those with *swi1*$^-$, *swi2*$^-$, or *swi3*$^-$ mutations (*17*). We examined receptor function in *snf5*$^-$ and *snf6*$^-$ mutant strains and found in each case that the receptor displayed ~20 percent of the activity observed in the wild-type *SNF*$^+$ strain (Fig. 5) whereas receptor activity was only 2 to 7 percent of that in wild type in *swi1*$^-$, *swi2*$^-$, or *swi3*$^-$ strains. Induced expression from the yeast *HO* and *SUC2* genes is at least 30 times lower in *snf5*$^-$ or *snf6*$^-$ strains; in contrast, GAL4 activity is decreased only slightly in a *snf5*$^-$ strain, while it is profoundly affected in *swi1*$^-$, *swi2*$^-$, *swi3*$^-$, and *snf6*$^-$ strains (*17*). Thus, different sequence-specific regulators display distinct requirements for the various SWI and SNF proteins.

The *sin1*$^-$ and *sin2*$^-$ mutations were identified initially because they alleviate the transcriptional defects due to *swi1*$^-$, *swi2*$^-$, and *swi3*$^-$ mutations (*21*); sequencing indicated that *SIN1* and *SIN2* encode components of chromatin (*22, 23*). To determine whether these *sin*$^-$ mutations could relieve the loss of receptor function in a *swi3*$^-$ strain, we transformed that strain with plasmids that express either wild-type histone H3 or the partially dominant mutant gene *sin2-1*. The *sin2-1* transformants recovered 25 percent of receptor activity (Fig. 6), a degree of suppression similar to that observed for *HO* expression in *swi1*$^-$ *sin2*$^-$ strains (*23, 30*). Mutations in *SIN1* also partially suppressed the *swi3*$^-$ phenotype, allowing recovery of ~30 percent of the wild-type activity (*31*). These results are consistent with a model in which SWI proteins facilitate receptor action by antagonizing transcriptional repression by chromatin components.

A receptor-SWI3 interaction. We used co-immunoprecipitation assays to examine the interaction between the *SWI* proteins and the glucocorticoid receptor. Purified receptor derivatives produced in *Escherichia coli* were mixed with extracts from *SWI*$^+$ and *swi3*$^-$ yeast strains, and a receptor-specific monoclonal antibody was used to precipitate the receptor and associated proteins. Precipitated proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose, and probed with antibody to SWI3 protein. The SWI3 protein coprecipitated from *SWI*$^+$

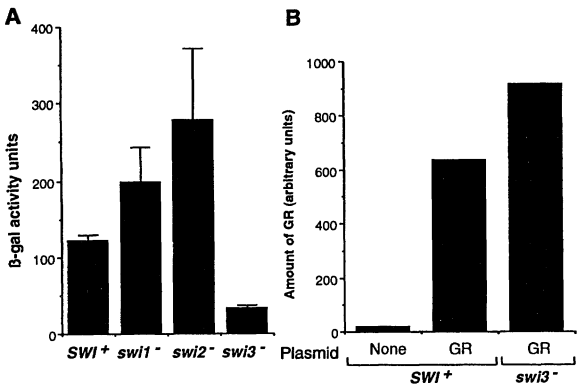


Fig. 3. Effect of *SWI* deletions on *GPD* promoter activity and GR expression in yeast. (**A**) *GPD* promoter activity in *swi1*$^-$, *swi2*$^-$, and *swi3*$^-$ strains. Plasmid pG-lacZ, containing the *GPD* promoter fused to the *lacZ* gene was transformed into *SWI*$^+$ (CY26), *swi1*$^-$ (CY58), *swi2*$^-$ (CY57), and *swi3*$^-$ (CY73) strains. Three independent transformants of each strain were grown overnight in minimal medium, diluted (1:10), grown for an additional 10 to 12 hours, harvested, and assayed for β-galactosidase activity as in Fig. 2. (**B**) Glucocorticoid receptor expression in *SWI*$^+$ and *swi3*$^-$ strains. Proteins in the *SWI*$^+$ (CY26) and *swi3*$^-$ (CY73) strains were labeled with [^{35}S]methionine (Amersham; 100 μCi/ml, 5 minutes) (*17*). The *SWI*$^+$ strain was either not transformed (None) or transformed with a centromeric glucocorticoid receptor expression plasmid (GR; pRS314/N795); the *swi3*$^-$ strain was transformed with a 2μ glucocorticoid receptor expression plasmid (GR; pG-N795). Labeled glucocorticoid receptor was immunoprecipitated (as in Fig. 7) with the receptor-specific monoclonal antibody BUGR2 (*57*). Precipitated receptor was fractionated by SDS-PAGE and quantitated (ImageQuant software; PhosphorImager).

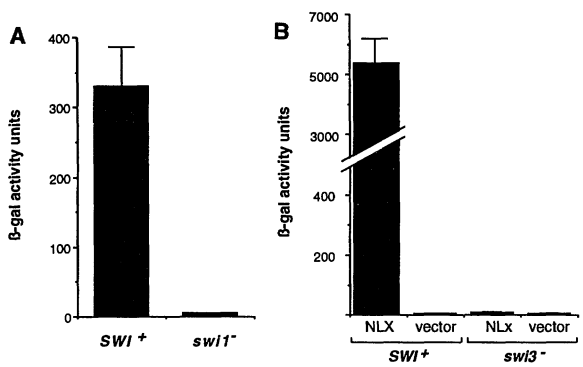


Fig. 4. Function of glucocorticoid receptor derivatives in *SWI*$^+$ and *swi*$^-$ strains. (**A**) pG-N556, which expresses a GR that lacks the hormone binding domain, and a GRE-linked reporter plasmid (pΔS26X) were transformed into *SWI*$^+$ (CY26) and *swi1*$^-$ (CY58) strains. (**B**) pG-NLx, which expresses a fusion protein containing GR(1–452) and the LexA DNA-binding domain (see Fig. 1), and a *LexA* operator-linked reporter plasmid (pΔSLex) were transformed into *SWI*$^+$ (CY26) and *swi3*$^-$ (CY73) strains. Transformants were cultured and β-galactosidase activities from three independent isolates were assayed as in Fig. 2.

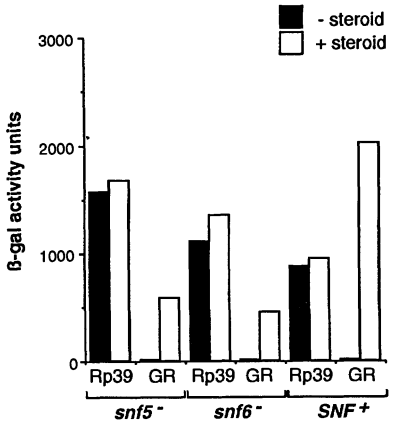


Fig. 5. Effect of *snf5*$^-$ and *snf6*$^-$ mutations on GR function. *SNF*$^+$ (CY26), *snf5*$^-$ (MCY2099), and *snf6*$^-$ (MCY2101) strains were transformed with GR expression (pG-N795) and reporter (pΔS26X) plasmids. Transformants were selected, cultured, treated with DAC, and assayed for β-galactosidase activity as in Fig. 2. As a control, a plasmid carrying the promoter for a ribosomal protein gene, *RP39* (*58*), fused to *LacZ* (RP39), was transformed into the strains.

extracts together with receptor derivatives EX556 or X556 (*7*) (Fig. 7A, lanes 2 and 3). X556 is a 150–amino acid (aa) fragment centered on the 60-aa zinc finger region of the receptor. In addition, EX556 contains a 213-aa segment from the receptor NH_2-terminal region that includes a transcriptional activation domain (*29*). Approximately 5 percent of the SWI3 protein in the wild-type yeast extract was precipitated with receptor (*32*). In control experiments, no SWI3 coprecipitate was observed in extracts from a *swi3*$^-$ strain (Fig. 7A, lane 1), or when receptor was omitted (lane 4). Thus, both receptor derivatives appear to interact with the SWI3 protein under these in vitro conditions (*33*).

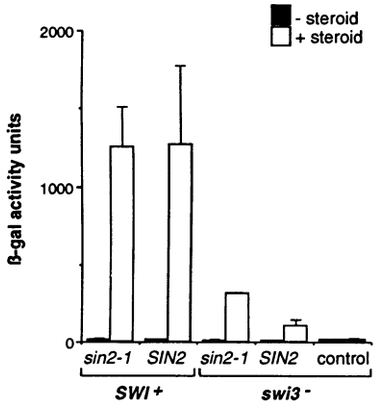


Fig. 6. Effect of *sin2*$^-$ mutant gene expression on GR function in a *swi3*$^-$ strain. The wild-type *SIN2* gene (which encodes histone H3), or *sin2-1* (a point mutation in amino acid 119 with a partial dominant phenotype) (*23*), was cotransformed into *SWI*$^+$ and *swi3*$^-$ strains with GR expression (pG-N795) and reporter (pΔS26X) plasmids. As a control, the *swi3*$^-$ strain was transformed with only pG-N795 and pΔS26X. Cultures were analyzed as in Fig. 2.

Genetic evidence implied that the SWI1, SWI2, and SWI3 proteins might function either as a complex or in the same regulatory pathway. For example, the SWI3 protein appears to be relatively unstable in strains lacking SWI1 or SWI2 (*17*), consistent with the possibility that these proteins might form a complex. We therefore tested the effects of *swi1*$^-$ or *swi2*$^-$ mutations on the efficiency of GR-SWI3 complex formation. Because these mutant strains normally show lower amounts of SWI3 protein, we constructed strains that overexpress SWI3 from a high-copy plasmid (Fig. 7B, lanes 6 and 9). Nevertheless, we found that SWI3 did not interact with the EX-556 receptor derivative in the absence of SWI1 or SWI2 (Fig. 7B, lanes 7 and 10). This dependence of the GR-SWI3 interaction on the SWI1 and SWI2 proteins provides support for their functional interdependence and suggests that a complex of SWI proteins may associate with the receptor.

Action of a *Drosophila* SWI3-like protein in vitro. The *Drosophila* gene *brahma* (*brm*) appears to share certain functional properties with *SWI2* (see below); moreover, the deduced sequence of the 185-kD *brm* protein reveals 40 percent identity with the 198-kD *SWI2* gene product (*34*). On the basis of these findings, we surmised that *Drosophila* might produce additional *SWI* homologs as well. Indeed, antibodies to the yeast *SWI1* and *SWI3* gene products revealed cross-reacting polypeptides in *Drosophila* cell extracts that were similar in size to the *bona fide* yeast SWI proteins. We therefore tested antibodies to yeast SWI3 protein for their effect on receptor action in a *Drosophila* in vitro transcription system.

Freedman *et al.* (*7*) demonstrated that GR derivatives could enhance transcription in *Drosophila* embryo extracts (*35*). Thus, purified EX556 protein stimulated transcription ~10-fold from a GRE-linked reporter construct in vitro (Fig. 8B, lanes 1 and 2; quantitation is shown in Fig. 8C). Addition of affinity-purified SWI3-specific antibody to the transcription reaction inhibited receptor activity (Fig. 8B, lanes 3 and 4). As a control, affinity-purified antibody to the SWI4 protein (whose actions are unrelated to those of SWI1, SWI2, and SWI3) (*36*) had little effect on receptor-mediated transcription (Fig. 8B, lanes 5 and 6, and Fig. 8C). We conclude that our *Drosophila* extracts contain a SWI3 homolog that stimulates receptor function in vitro.

Previous studies implied that steroid receptors may facilitate the assembly or stability of transcription preinitiation com-

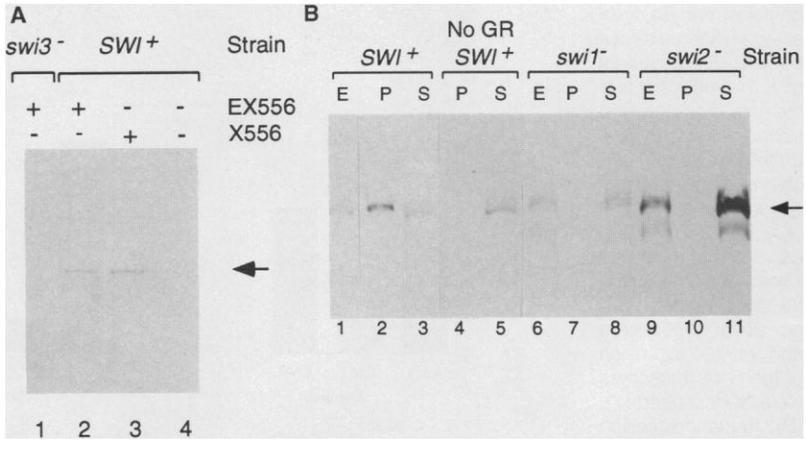
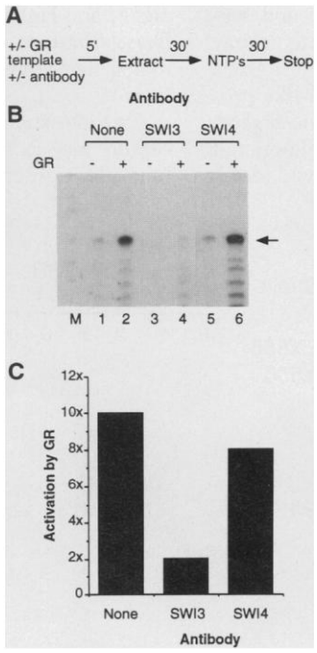


Fig. 7. Interaction of GR and SWI3 protein in vitro. (**A**) Co-immunoprecipitation and immunoblotting. GR derivatives X556, which encompasses GR(407–556) and includes the DNA-binding domain, and EX556, which contains GR(106–318) fused to GR(407–556) (*7*), were expressed in *E. coli*, purified to homogeneity, and mixed with extracts from *SWI*$^+$ (CY26) or *swi3*$^-$ (CY73). GR was precipitated with a monoclonal antibody [BUGR2; (*57*)], and the precipitated proteins were fractionated by SDS–polyacrylamide gel electrophoresis and assayed for SWI3 protein by immunoblotting; arrow indicates the SWI3 protein signal. (**B**) Effect of *swi1*$^-$ or *swi2*$^-$ mutations on GR-SWI3 interaction. Co-immunoprecipitation and immunoblotting were conducted as in (A) with EX556 protein and extracts from *SWI*$^+$, *swi1*$^-$ or *swi2*$^-$ strains. Lanes 1, 6, and 9 contained 200 μg of extract from each strain (E, extract). Lanes 2, 4, 7, and 10 contained proteins that coprecipitate (P) with EX556. Lanes 3, 5, 8, and 11 contained 200 μg of protein from the supernatant (S) after immunoprecipitation. Lanes 4 and 5 contained the precipitate and the supernatant, respectively, from an incubation without added EX556 (no GR). Arrow indicates SWI3 protein signal. Yeast extracts were prepared from 100-ml cultures grown at 30°C and harvested in late log phase. The cells were disrupted by shaking with glass beads for 30 minutes in 10 mM tris-HCl (pH 7.5), 1 mM EDTA, 50 mM NaCl, 2 mM DDT, 20 percent glycerol, 1 mM PMSF, and, aprotinin, leupeptin, and pepstatin A at ≥1 μg/ml each, (*22*). After a brief low-speed centrifugation, the extracts were clarified by high-speed centrifugation at 35,000 rpm (Beckman Ti60 rotor; 30 minutes at 4°C). The GR derivatives (2 μg) were incubated with 3 mg of yeast extract in 400 μl for 30 minutes at 4°C; these amounts of receptor (5 μg/ml and 0.07 percent of extract protein) are similar to those deduced in vivo. GR-specific monoclonal antibody (BUGR2) coupled to protein A Affi-Prep beads (Bio-Rad) was added, and the mixture was incubated at 4°C for 2 hours. Antibody beads were sedimented and washed three times with HEGNDT buffer (10 mM Hepes, pH 8.0, 1 mM EDTA, 10 percent glycerol, 50 mM NaCl, 2 mM DTT, 0.1 percent Triton X-100) and once with HEGNDT buffer without Triton X-100. Bound proteins were eluted with 2× protein sample buffer (120 mM tris-HCl, 4 percent SDS, 1.4 M β-mercaptoethanol, 20 percent glycerol, 0.2 percent bromophenol blue) and separated on an 8 percent SDS-polyacrylamide gel. SWI3 protein was detected by immunoblotting with affinity-purified rabbit polyclonal antibodies (*17*) as the primary probe, and secondary goat antibody to rabbit secondary immunoglobulin (Bio-Rad) coupled to horseradish peroxidase. Immunoblots were developed with chemiluminescent substrates (Amersham, ECL).

plexes (*7, 8*). If putative GR-SWI complexes functioned at this stage of the initiation pathway in vitro, the SWI3 antibodies should inhibit receptor-mediated enhancement when added before but not after complex formation. Therefore, we added the SWI3 antibody to the transcription reaction either before or after a 30-minute incubation of the extract with the DNA template, in either the presence or absence of receptor (Fig. 9A). After another 30 minutes, polymerization was triggered by addition of nucleotide precursors; reactions were stopped after 1 minute, probably limiting synthesis to a single round of transcription. In the absence of antibody, the receptor increased transcription by eight times (Fig. 9B, lanes 1 and 2). When the antibody was added before the addition of extract, receptor activity was diminished; in contrast, the antibody had no effect if it was added only after incubation of the extract with the template (Fig. 9B, lanes 3 to 6; Fig. 9C). These results suggest either that SWI3 in a preformed complex is inaccessible to the antibody, or, in our favored view, that SWI3 acts prior to complex formation. We conclude that GR collaborates with a presumptive *Drosophila* SWI3 homolog in vitro, and that the GR-SWI complex acts at an early step in transcription initiation.

Various determinants of transcription and its regulation (*37*) and factors that control the cell division cycle (*38*) can be functionally interchanged between yeasts, insects, and mammals. Our study, in which we have demonstrated a functional relation between the rat glucocorticoid receptor and the *S. cerevisiae* SWI1, SWI2, and SWI3 proteins, is an example of this broad conservation. In parallel with our experiments, Laurent and Carlson have described a derivative of the *Drosophila* Bicoid protein that depends on SWI2, SNF5, and SNF6 for regulatory activity in yeast (*39*). Our findings that GR function in yeast requires SWI action and that receptor activity in *swi*$^-$ mutants is partially restored by specific lesions in *SIN* genes, imply that mammalian cells produce structural and functional homologs of these yeast gene products.



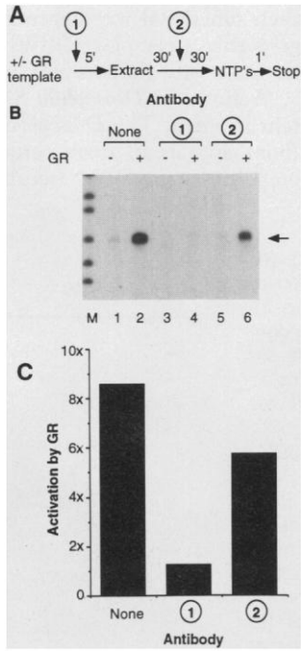


Fig. 8. Effect of SWI3-specific antibodies on GR function in vitro. (**A**) Schematic representation of the transcription reaction. *Drosophila* embryo extracts were used for in vitro transcription of plasmid GACO, which contains six rat tyrosine aminotransferase gene GRE's upstream of a minimal promoter element (from the *Drosophila* distal *Adh* gene) linked to the chloramphenicol acetyltransferase gene (*13*). The transcription reaction with crude *Drosophila* embryo extracts (20 μg of protein per 25 μl of reaction) and primer extension of the RNA transcripts were as described (*7*). (**B**) Primer extension products from transcripts produced in vitro. GACO (250 ng), was incubated with (+) or without (−) 400 ng of EX556 in the absence (none; lanes 1 and 2) or presence of 1 μl of antibody to either SWI3 (lanes 3 and 4) or SWI4 (lanes 5 and 6); the two antisera display similar activities, detecting their cognate antigens at 1:1000 in immunoblotting experiments. Arrow indicates 89 nucleotide primer extension product from correctly initiated transcripts. (**C**) Primer extension products of the assay in (B) were determined (ImageQuant software; PhosphorImager), and fold activation by GR is presented.

Fig. 9. Effects of SWI3-specific antibodies on receptor function before or after the assembly of preinitiation complexes. (**A**) Schematic representation of the transcription reaction. Antibody to SWI3 was added either before, ①, or after, ②, a 30-minute incubation of the DNA template with the extract. (**B**) Templates, proteins, antibodies, and reaction conditions were as described for Fig. 8, except that the SWI3 antibody was added either before (lanes 3 and 4) or after (lanes 5 and 6) a 30-minute incubation of the DNA template with the extract. Reactions were for 1 minute, probably limiting transcription to a single round. Lanes 1 and 2 are products from reactions without antibody. (**C**) Primer extension products from (B) were quantitated as in Fig. 8.

Targeting and function of SWI complexes. Two previous results indirectly suggested that SWI1, SWI2, and SWI3 may form a multiprotein complex: First, a triple deletion *swi1*$^-$, *swi2*$^-$, and *swi3*$^-$ strain is phenotypically similar to strains carrying only single gene lesions. Second, the rate of intracellular turnover of SWI3 protein is substantially higher in *swi1*$^-$, *swi2*$^-$ mutants than in wild-type strains (*17*). Our immunoprecipitation studies provide additional support for the existence of multiprotein SWI complexes. In particular, the SWI3 protein binds the glucocorticoid receptor in vitro in a reaction that requires the SWI1 and SWI2 proteins. Thus, a complex of all three of these SWI proteins may be required for interaction with the receptor. Although suitable antibodies are not yet available to assess directly the presence of SWI2, SWI1 has been detected in the GR coprecipitates (*40*). In fact, other proteins, such as SNF5 and SNF6, might also associate with these complexes. The dependence of receptor function on *SNF5* and *SNF6*, however, appeared to be less dramatic than on the *SWI1*, *SWI2*, and *SWI3* genes. That the activities of other site-specific regulators are also affected differentially by mutations in the various *SWI* and *SNF* genes (*17*) suggests that the composition or the functional requirements for these putative multiprotein complexes may be determined by regulator or promoter context.

Clearly, the SWI proteins act selectively; indeed, certain promoters (such as the *GPD* promoter examined here) appear independent of *SWI1*, *SWI2*, and *SWI3* function. Efforts to detect DNA binding by SWI1, SWI2, or SWI3 have been unsuccessful (*17*), and there are no evident DNA binding motifs. Because GR and SWI3 interact in vitro, we suggest that SWI protein complexes are targeted to specific loci by interacting with factors, such as GR, that are themselves sequence-specific regulators. Consistent with this model is the finding (see below) that fusions of the SWI2-SNF2, SNF5, or SNF6 proteins to a bacterial repressor DNA binding domain produce chimeras that activate transcription in yeast from promoters linked to the bacterial operator (*20*).

The *swi1*$^-$, *swi2*$^-$, or *swi3*$^-$ lesions compromise receptor-mediated enhancement of transcription, but have little or no effect on basal transcription in the absence of hormone. These genes are not essential for yeast viability under growth conditions in which induction of the affected genes is not required (*16, 18, 41*). This is consistent with the idea that these factors affect the activity of regulatory components, rather than their acting directly on the general transcription machinery.

How might GR-SWI complexes regulate transcription? Five findings seem relevant. (i) Chimeras of SWI2, SNF5, or SNF6 with the LexA protein DNA-binding domain activate transcription from a *LexA* operator-linked promoter in yeast (*20*). This result implies that the SWI proteins themselves are transcriptional activators and may not merely stimulate the activities of site-specific activators with which they interact. (ii) Sequence analysis of the SWI2 protein reveals homology to a large family of putative helicases (*42*); other *SWI2*-homologues from yeast (*42*), *Drosophila* (*34*), and mammals (*43*) also share some similarities within the putative helicase domains. Helicase enzymatic activity has not been tested, and the functional significance of these similarities is unknown. (iii) Defects in SWI proteins are partially suppressed by *sin*$^-$ mutations, suggesting that SWI factors may function by antagonizing chromatin structure. This result is consistent with a previous demonstration that glucocorticoid receptor function is accompanied by chromatin alterations (*9*). (iv) The GR-SWI3 interaction in vitro requires SWI1 and SWI2, perhaps indicating that a complex of SWI proteins joins the receptor. (v) GR and SWI appear to collaborate in vitro to modulate an early step in transcription initiation.

Our results do not distinguish between chromatin-dependent and chromatin-independent mechanisms for SWI function. At present, the involvement of chromatin is inferred solely from genetic studies: the loss of various SWI activities in yeast can be compensated by particular defects in chromatin components (*21–24*); mutations in a putative SWI2 homologue in *Drosophila* (*brm*) suppress defects in *Polycomb*, a repressor of specific homeotic genes (*44*). The Polycomb protein sequence contains a short region of homology with HP1, a *Drosophila* heterochromatin protein (*45, 46*); mutations in this region block association of Polycomb protein with chromatin (*47*). These findings can be interpreted in terms of a model in which SWI and brahma factors may facilitate transcriptional induction by neutralizing repression by chromatin-associated proteins. We find this chromatin-dependent mechanism appealing, especially in view of studies correlating GR function with chromatin alterations (*9*). However, biochemical tests of this hypothesis have not yet been described.

On the contrary our biochemical studies could be taken as evidence that alteration of nucleosomal structure is an unlikely mechanism for SWI action. That is, our transcription extracts contain large amounts of histone H1 and Polycomb (*48*), but only small amounts of the nucleosomal histones, making it unlikely that template DNA is packaged into chromatin under these conditions. (Because only a very small fraction of the added templates are actually transcribed, bulk measurements of the packaging structure of the added templates are not informative.) Conceivably, SWI factors might function in vitro by clearing the template of various nonnucleosomal proteins bound to the template, and this same activity might act on chromatin in vivo. Alternatively, SWI proteins may operate without dependence on chromatin, for example by increasing GR-GRE affinity, or by stimulating preinitiation complex assembly. Although an effect of SWI on DNA binding by GR has not been ruled out, the latter view seems most compatible with our data because the SWI effect is evident in vitro even if GR is first loaded onto the template DNA.

Of course, it is possible that the SWI complexes, like GR, might function in both chromatin-dependent and chromatin-independent regulatory modes. The use of fractionated extracts or purified components and defined templates, especially under conditions in which the SWI and SIN factors can be quantitated and manipulated, may elucidate the mechanisms of the chromatin-related regulatory modes.

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30. The presumed overproduction of the wild-type histone H3 from a high copy yeast expression plasmid (*SIN2*) also partially alleviated the defect in receptor activation in the *swi3*$^-$ mutant. This result appears analogous to previously described results in which imbalances in the gene dosage of histones partially suppress transcriptional defects (*57*). The amount of suppression by the *sin2-1* mutation was three times greater than the suppression by wild-type histone H3.
31. S. K. Yoshinaga, unpublished results.
32. We might have been misled in such an assay if the receptor and SWI3 had associated nonspecifically with common DNA fragments present in the extracts. Although this explanation seemed unlikely, especially in view of the failure to detect DNA binding by the SWI proteins, we did control assays in the presence of ethidium bromide at concentrations sufficient to disrupt DNA-protein interactions (*60*). This treatment did not inhibit GR-SWI3 coimmunoprecipitation. In other experiments, we were unable to detect co-precipitation of GR with the SWI3-specific antibody. The failure of this reciprocal experiment has not been investigated further but might indicate that the SWI3 epitope is inaccessible under nondenaturing conditions in which SWI3 protein is complexed with GR or with other cell proteins such as SWI1 or SWI2.
33. The receptor-Lex chimera NLx, which retained strong SWI-dependence for transcriptional regulation (see Fig. 4B), lacks the receptor zinc fingers, but it contains a 46-amino-acid segment (aa 407 to 452) in common with X556. Thus, if the GR-SWI3 interaction detected in vitro is significant for regulation in vivo, we might infer that the interaction occurs within this short segment. However, neither the significance nor the detailed mapping have yet been determined.
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11 September 1992; accepted 30 October 1992

Phthalate Dioxygenase Reductase: A Modular Structure for Electron Transfer from Pyridine Nucleotides to [2Fe-2S]

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Phthalate dioxygenase reductase (PDR) is a prototypical iron-sulfur flavoprotein (36 kilodaltons) that utilizes flavin mononucleotide (FMN) to mediate electron transfer from the two-electron donor, reduced nicotinamide adenine nucleotide (NADH), to the one-electron acceptor, [2Fe-2S]. The crystal structure of oxidized PDR from *Pseudomonas cepacia* has been analyzed at 2.0 angstrom resolution resolution; reduced PDR and pyridine nucleotide complexes have been analyzed at 2.7 angstrom resolution. NADH, FMN, and the [2Fe-2S] cluster, bound to distinct domains, are brought together near a central cleft in the molecule, with only 4.9 angstroms separating the flavin 8-methyl and a cysteine sulfur ligated to iron. The domains that bind FMN and [2Fe-2S] are packed so that the flavin ring and the plane of the [2Fe-2S] core are approximately perpendicular. The [2Fe-2S] group is bound by four cysteines in a site resembling that in plant ferredoxins, but its redox potential (−174 millivolts at pH 7.0) is much higher than the potentials of plant ferredoxins. Structural and sequence similarities assign PDR to a distinct family of flavoprotein reductases, all related to ferredoxin NADP$^+$-reductase.

A change in the currency of reducing equivalents, in which a flavin prosthetic group accepts two electrons as hydride ion (H^-) and donates one electron at a time to iron-sulfur centers or to cytochromes, is essential in the reaction pathways of respiration, photosynthesis, and biodegradation. To simplify crystallization and x-ray analysis of a protein catalyzing this conversion, we chose a bacterial oxidoreductase that is a natural fusion of flavoprotein and [2Fe-2S] domains. Phthalate dioxygenase reductase (PDR) is a component of the electron transfer chain involved in pyridine nucleotide–dependent dihydroxylation of phthalate in *Pseudomonas cepacia* (*1*, *2*). Thus PDR should provide a model for the many [2Fe-2S] flavoproteins that catalyze the initial steps in electron transfer chains. The geometry for electron transfer between flavins and other one-electron acceptors, including [4Fe-4S], cytochrome b, and cytochrome c, has been established from previously determined structures (*3*–*5*), and the mechanism of two-electron reduction of flavins by pyridine nucleotides (*6*) has been correlated with structure analyses of glutathione reductase (*7*).

Oxygenase systems typically comprise reductase, electron carrier, and terminal oxygenase components. When grown on phthalate, *P. cepacia* expresses two proteins: phthalate dioxygenase (PDO), a tetramer of 48-kD chains, and PDR, a 36-kD monomeric species (*1*, *8*). In this and related oxygenase systems (*2*, *9*), oxygen binding and hydroxylation occur at a mononuclear Fe^{2+} site in the dioxygenase component (*2*). Electrons from nicotinamide adenine nucleotide (NADH) are conveyed to the site for phthalate hydroxylation via three centers: flavin mononucleotide (FMN) and [2Fe-2S] in PDR and a Rieske [2Fe-2S] center (*10*) in PDO (*1*, *2*). The [2Fe-2S] center of PDR is presumed to be the site for electron transfer to PDO, since NADH-dependent substrate hydroxylation requires an intact [2Fe-2S] species to transfer electrons to the oxygenase component in related systems (*2*, *9*, *11*). Phthalate hydroxylation is catalyzed at a rate of at least 90 min^{-1} at 4°C (*12*) in the presence of both PDR and PDO; electron donors other than PDR do not support efficient oxygenation (*1*, *2*).

Redox equilibria favor electron transfer in the physiological direction: NADH → FMN → [2Fe-2S] → dioxygenase. The one-electron midpoint potentials at pH 7.0, determined by titration with dithionite, are −174 mV for the $FMNH^{\cdot}$-FMN couple, −284 mV for the $FMNH^-$-$FMNH^{\cdot}$ couple, −174 mV for the [2Fe-2S] cluster of PDR, and approximately −120 mV for the Rieske center in PDO (*12*). The potential of the [2Fe-2S] cluster of PDR, which is much higher than the potentials of −325 to −460 mV for plant ferredoxins (*9*), allows substantial population of the reduced [2Fe-2S] species during turnover.

Conversion of H^- to one-electron equivalents occurs in the reductive half-reaction of NADH with PDR (Fig. 1); the reaction has been studied by stopped-flow techniques. The internal electron transfer, which distributes one electron from fully reduced FMN to the [2Fe-2S] cluster of PDR (step 3), is rapid. A lower limit of 200 s^{-1} for the electron transfer rate has been derived from simulations with the stopped-flow data (*2*). The actual rate constant is likely to be much larger, since the structure now reveals that the FMN and a cysteine sulfur ligated to an iron are separated by an edge-edge distance of only 1.5 Å. Electron transfer in the analogous complex of ferredoxin with ferredoxin-NADP$^+$ reductase (FNR) occurs at 1500 to 7000 s^{-1} (*13*). The three rate-determining steps in the

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