Published in final edited form as:

Biochem Biophys Res Commun. 2007 November 9; 363(1): 153–158. doi:10.1016/j.bbrc.2007.08.130.

Dynamic SPR monitoring of yeast nuclear protein binding to a cis-regulatory element

Grace Mao and James P. Brody

Department of Biomedical Engineering, University of California–Irvine, Irvine, CA 92697-2715

Abstract

Gene expression is controlled by protein complexes binding to short specific sequences of DNA, called *cis*-regulatory elements. Expression of most eukaryotic genes is controlled by dozens of these elements. Comprehensive identification and monitoring of these elements is a major goal of genomics. In pursuit of this goal, we are developing a surface plasmon resonance (SPR) based assay to identify and monitor *cis*-regulatory elements. To test whether we could reliably monitor protein binding to a regulatory element, we immobilized a 16 bp region of *S. Cerevisiae* chromosome 5 onto a gold surface. This 16 bp region of DNA is known to bind several proteins and thought to control expression of the gene *RNR1*, which varies through the cell cycle. We synchronized yeast cell cultures, and then sampled these cultures at a regular interval. These samples were processed to purify nuclear lysate, which was then exposed to the sensor. We found that nuclear protein binds this particular element of DNA at a significantly higher rate (as compared to unsynchronized cells) during G1 phase. Other time points show levels of DNA-nuclear protein binding similar to the unsynchronized control. We also measured the apparent association complex of the binding to be 0.014 s^{-1} . We conclude that (1) SPR-based assays can monitor DNA-nuclear protein binding and that (2) for this particular *cis*-regulatory element, maximum DNA-nuclear protein binding occurs during G1 phase.

Introduction

Genomic DNA plays two essential roles in eukaryotic cells. It encodes both the structure (amino acid sequence) for proteins and the instructions for when and where to transcribe mRNA, the initial process in protein production. The instructions are encoded in short (5–20 base pair) sequences, called *cis*-regulatory elements. Complexes of nuclear proteins, called transcription factor complexes, bind to these *cis*-regulatory elements and enhance or repress transcription. Transcription only proceeds when the occupancy of dozens of these *cis*-regulatory elements is in an appropriate state [1,2]. Comprehensive identification and monitoring of these *cis*-regulatory elements could lead to a better understanding of fundamental biological processes like development [3] and evolution [4].

Several different approaches to the identification problem are being pursued. One can use computational analysis of genome sequence [5,6,7,8,9], or compare promoter regions of genes that show highly correlated expression patterns [10,11,12,13]. A different, but related, problem is the identification of which DNA sequence binds to known purified transcription factors [14,15,16].

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Monitoring is a different problem that cannot be solved by computational analysis. Gene expression is often transient in cells. Monitoring provides information on the occupancy of *cis*-regulatory elements as a function of the cell's state or environment.

Our approach is to both identify and monitor whether any nuclear protein binds specific regions of DNA, putative *cis*-regulatory elements. The identity of the nuclear proteins are not known *a-priori*, but the sequence of the DNAs are known. Hence, we immobilize the known DNA to a surface and pass nuclear lysate across this surface. Binding between the immobilized DNA and protein in the nuclear lysate is monitored using surface plasmon resonance. The ultimate goal of this line of research is to develop a high throughput system that would enable one to comprehensively monitor all regulatory elements. This, in turn, would lead to a better understanding of how gene expression is regulated.

Previously, we demonstrated that one could identify *cis*-regulatory elements using surface plasmon resonance[17]. We did this by showing a significant change in SPR signal correlated with both nuclear protein binding to DNA sequence representing a particular *cis*-regulatory element *and* an increased level of promoter activity. In this work, we demonstrate that we can also *monitor* dynamic changes in the occupancy of *cis*-regulatory elements. We do this by monitoring yeast nuclear protein binding to a portion of the RNR1 promoter, as the cell cycle progresses.

Surface plasmon resonance has been previously used to study DNA protein interactions [18, 19,20].

We chose to test SPR monitoring of a *cis*-regulatory region using a yeast gene, ribonucleotide reductase 1 (RNR1). Yeast ribonucleotide reductase, a multi-protein complex, catalyzes one step in the dNTP production pathway. It is activated both as a normal part of the cell cycle and also in response to DNA damage [21,22,23,24,25]. RNR1 mRNA levels vary considerably with the cell cycle; the peak level occurs in the G1 phase[26]. By collecting nuclear protein from a synchronized culture of yeast and exposing them to an SPR surface coated with different DNA sequences, we ultimately aim to identify all the specific *cis*-regulatory elements responsible for the periodic expression of RNR1. In this paper, we monitored just one well-known *cis*-regulatory element, as shown in Figure 1.

Experimental Section

Yeast cell cultures

We used the PY1 strain of *Saccharomyces cerevisiae* (yeast)[28]. It has the BF264-Dau genetic background. BF264-Dau is a derivative of BF264-15D[29] (ARG4, bar1 Δ , ura3 Δ ns, ade1, his2, leu2-3,112, and trp1-1a). Bar1 Δ is required in the yeast strain to inactivate Bar1p, a protease secreted in **a** cells to cleave and inactivate **a** factor. **a** cells that lack Bar1p are ultra-sensitive to G1 arrest induced by α factor.

Yeast cultures were grown in YPD medium (2% yeast extract, 4% peptone, and 4% dextrose) at 30°C with a temperature controlled heater and shaker. One liter of YPD growth medium was in each two liter flask, to allow sufficient aeration. Cultures were grown for approximately 20 hours until cells reached late-log phase.

Synchronization of yeast cultures

Following [26], yeast cells were synchronized by adding 12 ng/ml of α -factor to culture for 3 hours. α -factor, a 13 amino acid peptide (TRP-HIS-TRP-LEU-GLN-LEU-LYS-PRO-GLY-GLN-PRO-MET-TYR), arrests **a** cells in G1 of the cell cycle. This α -factor is removed by centrifugation of the cells, replacing the supernatant with fresh medium, and re-suspending the

cells. This process is repeated twice to ensure that all α -factor has been removed. Samples were taken at regular intervals after establishing synchronous cultures and then processed through the nuclear protein extraction protocol.

The yeast suspension was pelleted by centrifugation and washed in 1 ml water. The suspension was centrifuged, washed by 500 μ l RNase solution (40 mg RNase A and 20 ml 50 mM Tris pH 8.0/15 mM NaCl) and incubated at 37°C overnight. On the next day, the solution was centrifuged, resuspended in 300–500 μ l protease solution (50 mg pepsin, 45 μ l concentrated HCl, 10 ml water), and incubated at 37°C for 15–30 min. Again, this solution was spun out and resuspended in 500 μ l water [34].

Flow Cytometry

Flow cytometry monitored the degree of synchronization. 50 μ l solution was transferred to a FACS tube that contained 1 ml of 1 $\times$ Sytox (1 mM Sytox Green in DMSO from Molecular Probes) in 50 mM Tris at pH 7.5. It was then incubated at room temperature for at least one hour. Before using flow cytometry, the sample solution was sonicated for 5 seconds on low power. The samples were illuminated at 488 nm by the flow cytometer and fluorescence near 530 nm was recorded. We used WinMDI software to convert fluorescent intensity to DNA content frequency histograms. The histograms were further analyzed by utilizing Cylchred [30,31] to quantify the percentage of cells in different phases of the cell cycle (G1, S, and G2/M).

Nuclear protein extraction

Nuclear protein was extracted from each sample of the synchronous culture. Detailed protocols exist [32,33] for the isolation and purification of yeast nuclei. Following these, yeast cells were first converted to spheroplasts by adding Zymolyase, which digests the cell walls by disrupting β -glucan linkages. Spheroplasts were then lysed and recovered from the glucanase treatment. Centrifugation at 13,000 rpm for 30 minutes separated the cellular lysate from the nuclear contents. The nuclear extract was further purified by gradient centrifugation and finally by dialysis. A protease inhibitor cocktail (Sigma P8215, which contains 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, and 1,10-phenanthroline) was added to the extraction buffer to inhibit any protease activities.

Concentration of Nuclear Protein

To determine the concentrations of nuclear proteins, we used the BioRad protein assay, which is based upon the method of Bradford [35]. This is a dye-binding assay in which various concentrations of protein are indicated by a differential color change of the dye. First, three to five dilutions of a protein standard were prepared. Then, 10 μ l of each standard and sample solution was pipetted into a clean, dry well of a microplate. Afterward, 200 μ l of dye reagent concentrate was added, mixed, and incubated at room temperature for 5 minutes. Finally, the absorption is measured at 595 nm.

Surface Plasmon Resonance Measurements

Surface plasmon resonance measurements were made with the Spreeta SPR sensor. The experimental set up includes a data acquisition and control computer, a syringe pump, and a Spreeta evaluation kit. The three channel Spreeta evaluation kit consists of several Spreeta sensor modules, a three channel flow cell, an electronic controller with comprehensive software, and an integrated flow block. The sensor modules are made by Sensata (formerly a division of Texas Instruments); other components are made by Nomadics.

The flow block was used to connect the Spreeta sensor module with the control box and to secure the flow cell to the surface of the sensor. The flow cell provides three independent flow channels. Each channel is approximately 4.5 mm long and 0.1 mm wide. The flow cell confines solution to the narrow channels, which correspond to the sensor surface.

The sensor data was analyzed to determine relative protein binding by measuring the difference in steady-state refractive index level before and after the addition of nuclear protein. Each experiment was repeated three times to provide error estimates.

Attaching DNA to the Gold Surface

Double stranded DNA representing a region of yeast chromosome 5, (see Table 1), was attached to the sensor surface using bovine serum albumin (BSA) as an intermediate. First, two complementary single-stranded DNA fragments, derivatized with biotin at the 5' end, (see Table 1) each at 450 μ M concentration, were added together into a microtube. The microtube was placed into boiling water and allowed to slowly cool to room temperature. This annealing process produces double stranded DNA.

The immobilization scheme was implemented by flowing different solutions across the sensor surface. The sensor was monitored to confirm the appropriate surface modifications took place. The solutions contained (in order) biotin-BSA (0.67 mg/ml), streptavidin (0.33 mg/ml) and biotin-DNA (either sample or control, 450 μ M) in PBS (1.37 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄ at pH 7.3), which is also the running buffer. These were stored at -20°C, and thawed before use. Changes in refractive index adjacent to the sensing surface were monitored. Solutions remained in contact with the sensing surface until a stable refractive index value was reached, indicating the binding is at equilibrium. The running buffer was injected between each solution to remove non specifically bound molecules. We previously measured the DNA surface density to be $1.0 \pm 0.1 \times 10^{11}/\text{cm}^2$ [36].

Measuring nuclear extract binding to DNA

The nuclear extract (0.33 mg/ml of protein) in protein binding buffer (20 mM HEPES (pH 7.6), 10 mM MgSO₄, 1 mM EGTA, 20% glycerol, 75 mM ammonium sulfate) flowed across the sensor. The nuclear extract was stored at -80°C to prevent any degradation. Binding buffer was then injected to remove any non-specifically bound protein.

Cleaning

To restore the surface of the sensor to its original state, it was gently wiped with a Kimwipe wet by 6 N HCL and then flushed with water. This procedure was repeated three times. Then, 70% ethanol was used to wipe the surface followed by flushing with water; this was repeated three times. This cleaning procedure effectively removed all the immobilized layers. This was confirmed by measuring the refractive index of pure water as 1.3330. After each experiment was done, all syringes and tubes were rinsed thoroughly by water three times.

Results and Discussion

Synchronous yeast cultures

Measuring dynamic changes due to the cell cycle in binding between DNA and nuclear protein requires synchronous yeast cultures. We synchronized yeast cultures by the addition (and subsequent removal) of alpha factor, as described above. We monitored the degree of synchronization by sampling the culture at regular intervals and measuring the DNA content in each cell with a flow cytometer. DNA content measurement is a reliable indicator of stage in the cell cycle. Figure 2 displays the results of the flow cytometry measurements.

Nuclear protein binding to part of the RNR1 promoter

Once we established a synchronous yeast cell culture, we extracted nuclear protein at different time points and measured the extent that the nuclear protein would bind to the selected 16 bp region of the RNR1 promoter. As a control, we compared these results to nuclear protein extracted from unsynchronized yeast cells. The results, presented in Figure 3, show that synchronous yeast cells produce a significant change in nuclear protein that is capable of binding to RNR1a:RNR1b midway through G1 phase of the cell cycle. These nuclear proteins are not present in the yeast nucleus a short time later.

Previously, others have measured levels of RNR1 mRNA. Spellman *et. al* used DNA microarrays to comprehensively estimate relative mRNA levels of all yeast genes at 18 time points across the cell cycle [26]. They found that RNR1 mRNA levels reached two relative maximums, a first at about 21 minutes after synchronization, and a second at about 77 minutes [26]. This finding is consistent with our observation that nuclear protein at this particular site reaches a maximum at about 15 minutes. We do not see a significant enhancement in nuclear protein binding at 77 minutes. Probably other *cis*-regulatory elements in the RNR1 promoter regulate the mRNA expression levels at 77 minutes.

Kinetic constants

The dynamic data collected by the surface plasmon resonance sensor allows one to estimate kinetic binding parameters. While, these measurements are limited by mass transport effects, we can still place limits on kinetic binding parameters. We used a model for the association phase [17]

$$R = R_s - (R_s - R_o) \exp^{-k_A(t-t_o)}, \quad (1)$$

where R is the response of the sensor, R_s the response at steady state, R_o the initial response, and k_A , is the apparent association constant, which is related to the true association constant by the relation $k_A = k_a C - k_d$, where C is the concentration of protein. Since we are working with nuclear lysate, rather than purified protein, C cannot be uniquely identified and the true association constant, k_a , cannot be measured. However we estimate that $k_A = 0.014 \text{ s}^{-1}$.

Conclusion

In conclusion, this demonstrates that surface plasmon resonance sensors are capable of dynamic monitoring of *cis*-regulatory elements and that apparent association rates can be estimated from this data.

Acknowledgments

This work was supported by grant HG-00047 from the National Institutes of Health. We thank Peter Kaiser for providing the yeast strain and advice.

References

1. Davidson, Eric H. Genomic Regulatory Systems. Academic Press; 2001.
2. Davidson EH, Rast JP, Oliveri P, Ransick A, Caestani C, Yuh CH, Minokawa T, Amore G, Hinman V, Arenas-Mena C, Otim O, Brown CT, Livi CB, Lee PY, Revilla R, Rust AG, Pan Z, Schilstra MJ, Clarke PJ, Arnone MI, Rowen L, Cameron RA, McClay DR, Hood L, Bolouri Hq. A genomic regulatory network for development. Science 2002;295:1669–78. [PubMed: 11872831]

3. Ben-Tabou de Leon, Smadar; Davidson, Eric H. Gene regulation: Gene control network in development. *Annu Rev Biophys Biomol Struct.* Jan. 2007 URL <http://dx.doi.org/10.1146/annurev.biophys.35.040405.102002>
4. Prud'homme, Benjamin. Nicolas Gompel, and Sean B Carroll. Emerging principles of regulatory evolution. *Proc Natl Acad Sci U S A.* May. 2007 URL <http://dx.doi.org/10.1073/pnas.0700488104>
5. Niwa, Nao; Hiromi, Yasushi; Okabe, Masataka. A conserved developmental program for sensory organ formation in *Drosophila melanogaster*. *Nat Genet Mar;2004* 36(3):293–297. [PubMed: 14981517]
6. Ruvinsky, Ilya; Ruvkun, Gary. Functional tests of enhancer conservation between distantly related species. *Development Nov;2003* 130(21):5133–5142. [PubMed: 12944426]
7. Sharan, Roded; Ovcharenko, Ivan; Ben-Hur, Asa; Karp, Richard M. CREME: a framework for identifying cis-regulatory modules in human-mouse conserved segments. *Bioinformatics 2003;19* (Suppl 1):283–291. [PubMed: 12538251]
8. Philippakis, Anthony A.; Busser, Brian W.; Gisselbrecht, Stephen S.; Sherry He, Fangxue; Estrada, Beatriz; Michelson, Alan M.; Bulyk, Martha L. Expression-guided in silico evaluation of candidate cis regulatory codes for *drosophila* muscle founder cells; *PLoS Comput Biol.* May. 2006 p. e53 URL <http://dx.doi.org/10.1371/journal.pcbi.0020053>
9. Hardison RC. Conserved noncoding sequences are reliable guides to regulatory elements. *Trends Genet Sep;2000* 16(9):369–372. [PubMed: 10973062]
10. Dickmeis, Thomas; Plessy, Charles; Rastegar, Sepand; Aanstad, Pia; Herwig, Ralf; Chalmel, Frederic; Fischer, Nadine; Strehle, Uwe. Expression profiling and comparative genomics identify a conserved regulatory region controlling midline expression in the zebrafish embryo; *Genome Res.* Feb. 2004 p. 228-238. URL <http://dx.doi.org/10.1101/gr.1819204>
11. Hutton, John J.; Jegga, Anil G.; Kong, Sue; Gupta, Ashima; Ebert, Catherine; Williams, Sarah; Katz, Jonathan D.; Aronow, Bruce J. Microarray and comparative genomics-based identification of genes and gene regulatory regions of the mouse immune system; *BMC Genomics.* Oct. 2004 p. 82 URL <http://dx.doi.org/10.1186/1471-2164-5-82>
12. Jegga, Anil G.; Sherwood, Shawn P.; Carman, James W.; Pinski, Andrew T.; Phillips, Jerry L.; Pestian, John P.; Aronow, Bruce J. Detection and visualization of compositionally similar cis-regulatory element clusters in orthologous and coordinately controlled genes; *Genome Res.* Sep. 2002 p. 1408-1417. URL <http://dx.doi.org/10.1101/gr.255002>
13. Jegga, Anil G.; Gupta, Ashima; Gowrisankar, Sivakumar; Deshmukh, Mrunal A.; Connolly, Steven; Finley, Kevin; Aronow, Bruce J. Cismols analyzer: identification of compositionally similar cis-element clusters in ortholog conserved regions of coordinately expressed genes; *Nucleic Acids Res.* Jul. 2005 p. W408-W411. URL <http://dx.doi.org/10.1093/nar/gki486>
14. Mukherjee, Sonali; Berger, Michael F.; Jona, Ghil; Wang, Xun S.; Muzzey, Dale; Snyder, Michael; Young, Richard A.; Bulyk, Martha L. Rapid analysis of the DNA-binding specificities of transcription factors with DNA microarrays; *Nat Genet.* Dec. 2004 p. 1331-1339. URL <http://dx.doi.org/10.1038/ng1473>
15. Bulyk, ML.; Gentalen, E.; Lockhart, DJ.; Church, GM. Quantifying DNA-protein interactions by double-stranded DNA arrays; *Nat Biotechnol.* Jun. 1999 p. 573-577. URL <http://dx.doi.org/10.1038/9878>
16. Bulyk, Martha L. DNA microarray technologies for measuring protein-DNA interactions; *Curr Opin Biotechnol.* Aug. 2006 p. 422-430. URL <http://dx.doi.org/10.1016/j.copbio.2006.06.015>
17. Lin, Limin. PhD thesis. University of California; Irvine: 2005. An SPR-Based Sensor to Measure DNA-Protein Interactions.
18. Bondeson K, Frostell-Karlsson A, Fugerstam L, Magnusson G. Lactose repressor-operator dna interactions: kinetic analysis by a surface plasmon resonance biosensor. *Anal Biochem* Oct;1993 214 (1):245–251. [PubMed: 8250230]
19. Majka, Jerzy; Speck, Christian. Analysis of protein-DNA interactions using surface plasmon resonance. *Adv Biochem Eng Biotechnol* 2007;104:13–36. [PubMed: 17290817]
20. Teh, Huey Fang; Peh, Wendy YX.; Su, Xiaodi; Thomsen, Jane S. Characterization of protein–dna interactions using surface plasmon resonance spectroscopy with various assay schemes; *Biochemistry.* Feb. 2007 p. 2127-2135. URL <http://dx.doi.org/10.1021/bi061903t>

21. Elledge SJ, Davis RW. Two genes differentially regulated in the cell cycle and by DNA-damaging agents encode alternative regulatory subunits of ribonucleotide reductase. *Genes Dev* May;1990 4 (5):740–751. [PubMed: 2199320]
22. Elledge SJ, Zhou Z, Allen JB. Ribonucleotide reductase: regulation, regulation, regulation. *Trends Biochem Sci* Mar;1992 17(3):119–123. [PubMed: 1412696]
23. Elledge, SJ.; Zhou, Z.; Allen, JB.; Navas, TA. DNA damage and cell cycle regulation of ribonucleotide reductase; *Bioessays*. May. 1993 p. 333-339. URL <http://dx.doi.org/10.1002/bies.950150507>
24. Yao, Ruojin; Zhang, Zhen; An, Xiuxiang; Bucci, Brigid; Perlstein, Deborah L.; Stubbe, JoAnne; Huang, Mingxia. Subcellular localization of yeast ribonucleotide reductase regulated by the dna replication and damage checkpoint pathways; *Proc Natl Acad Sci U S A*. May. 2003 p. 6628-6633. URL <http://dx.doi.org/10.1073/pnas.1131932100>
25. Zhou Z, Elledge SJ. Dun1 encodes a protein kinase that controls the dna damage response in yeast. *Cell* Dec;1993 75(6):1119–1127. [PubMed: 8261511]
26. Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B. Comprehensive identification of cell cycle-regulated genes of the yeast *saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* Dec;1998 9(12):3273–3297. [PubMed: 9843569]
27. Harbison, Christopher T.; Gordon, D Benjamin; Lee, Tong Ihn; Rinaldi, Nicola J.; Macisaac, Kenzie D.; Danford, Timothy W.; Hannett, Nancy M.; Tagne, Jean-Bosco; Reynolds, David B.; Yoo, Jane; Jennings, Ezra G.; Zeitlinger, Julia; Pokholok, Dmitry K.; Kellis, Manolis; Rolfe, P Alex; Takusagawa, Ken T.; Lander, Eric S.; Gifford, David K.; Fraenkel, Ernest; Young, Richard A. Transcriptional regulatory code of a eukaryotic genome; *Nature*. Sep. 2004 p. 99-104. URL <http://dx.doi.org/10.1038/nature02800>
28. Kaiser P, Sia RA, Bardes EG, Lew DJ, Reed SI. Cdc34 and the f-box protein met30 are required for degradation of the cdk-inhibitory kinase swe1. *Genes Dev* Aug;1998 12(16):2587–2597. [PubMed: 9716410]
29. Reed SI, Hadwiger JA, Lerincz AT. Protein kinase activity associated with the product of the yeast cell division cycle gene cdc28. *Proc Natl Acad Sci U S A* Jun;1985 82(12):4055–4059. [PubMed: 3889921]
30. Watson, JV.; Chambers, SH.; Smith, PJ. A pragmatic approach to the analysis of dna histograms with a definable g1 peak; *Cytometry*. Jan. 1987 p. 1-8. URL <http://dx.doi.org/10.1002/cyto.990080101>
31. Ormerod, MG.; Payne, AW.; Watson, JV. Improved program for the analysis of dna histograms; *Cytometry*. Nov. 1987 p. 637-641. URL <http://dx.doi.org/10.1002/cyto.990080617>
32. Dove JE, Brockenbrough JS, Aris JP. Isolation of nuclei and nucleoli from the yeast *Saccharomyces cerevisiae*. *Methods Cell Biol* 1998;53:33–46. [PubMed: 9348503]
33. Rieder, SE.; Emr, SD. Current Protocols in Molecular Biology, chapter Subcellular fractionation and isolation of organelles. John Wiley & Sons; 2004.
34. Sazer S, Sherwood SW. Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fission yeast. *J Cell Sci* Nov;1990 97(Pt 3):509–516. [PubMed: 2074269]
35. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* May;1976 72:248–254. [PubMed: 942051]
36. Lin, Limin; Harris, Joseph W.; Garrett, H.; Thompson, R.; Brody, James P. Surface plasmon resonance-based sensors to identify cis-regulatory elements; *Anal Chem*. Nov. 2004 p. 6555-6559. URL <http://dx.doi.org/10.1021/ac049371e>

Figure 1.

We monitored the binding of nuclear protein to a 16 bp region of the RNR1 promoter. The origin of this 16 bp sequence is shown in this figure. It lies on yeast chromosome 5 approximately half way between the coding regions for the genes ARG5,6 and RNR1. Others [27] have previously identified the MBP1, SWI4, SWI6, and FKH1 binding sites within this 16 bp sequence, as indicated.

Figure 2.

After the synchronization protocol, flow cytometry measurements quantified the degree of synchronization obtained in the yeast cell culture. This figure depicts the flow cytometry data, which is a histogram of DNA content per cell. As cells progress through the cell cycle, they synthesize DNA and DNA content per cell increases.

Figure 3.

Surface plasmon measurements indicate that a significant increase in nuclear protein binding to a 16 bp sequence found in the RNR1 promoter occurs midway through G1 of the cell cycle. Each measurement was repeated three times and the data points represent the mean value, while the error bars indicate the standard deviation in the mean. The time ranges for different phases of the cell cycle, G1, S, and G2/M were determined from the flow cytometry data shown in Figure 2. A t-test indicates that measured binding at times $t = 30$ and $t = 45$ minutes differed significantly from the control.

Figure 4.

The top compares the response due to mass transport with that measured due to nuclear protein binding as measured at the 45 minute time point. The bottom compares the best fit of Equation 1, with an apparent association constant of 0.014 s^{-1} to the data. Deviations from the equation are probably due to mass transport effects.

Table 1

Oligonucleotides used in the RNR1 promoter experiments. RNR1 contains a biotin on the 5' end of the strand, which is used to immobilize the strand to the surface.

RNR1a:	5'-Biotin- AAA AAA CGC GTA AAC A-3'
RNR1b:	3'- TTT TTT GCG CAT TTG T-5'