The iron response regulator (Irr) protein from Bradyrhizobium japonicum is a conditionally stable protein that degrades in response to cellular iron availability. This turnover is heme-dependent, and rapid degradation involves heme binding to a heme regulatory motif (HRM) of Irr. Here, we show that Irr confers iron-dependent instability on glutathione S-transferase (GST) when fused to it. Analysis of Irr-GST derivatives with C-terminal truncations of Irr implicated a second region necessary for degradation, other than the HRM, and showed that the HRM was not sufficient to confer instability on GST. The HRM-defective mutant IrrC29A degraded in the presence of iron but much more slowly than the wild-type protein. This slow turnover was heme-dependent, as discerned by the stability of Irr in a heme-defective mutant strain. Whereas the HRM of purified recombinant Irr binds ferric (oxidized) heme, a second site that binds ferrous (reduced) heme was identified based on spectral analysis of truncation and substitution mutants. A mutant in which histidines 117–119 were changed to alanines severely diminished ferrous, but not ferric, heme binding. Introduction of these substitutions in an Irr-GST fusion stabilized the protein in vivo in the presence of iron. We conclude that normal iron-dependent Irr degradation involves two heme binding sites and that both redox states of heme are required for rapid turnover.

Heme is the prosthetic group or active moiety of proteins involved in a wide range of cellular functions that include catalysis, electron transport, and oxygen transport. In addition, heme proteins can be sensors that detect and respond to O2 (1, 2), CO (3, 4), NO (5), and the cellular redox state (6). Moreover, the heme moiety itself can have regulatory functions that control gene expression at the levels of transcription (7, 8), translation (9), protein localization (10), protein stability (11–13), and cell differentiation (14). Heme (protopheme) biosynthesis involves a multistep pathway culminating with the insertion of ferrous iron into protoporphyrin IX by the enzyme ferrochelatase (15). Iron can be a limiting nutrient for both prokaryotes and eukaryotes. The iron response regulator (Irr)1 protein from the bacterium Bradyrhizobium japonicum coordinates the heme biosynthetic pathway to prevent the accumulation of toxic porphyrin precursors under iron limitation (16). Loss of function of the irr gene is sufficient to uncouple the pathway from iron-dependent control as discerned by the accumulation of protoporphyrin under iron limitation. Irr belongs to the Fur family of transcriptional regulators involved in metal-dependent control of gene expression. The Irr protein accumulates in cells under iron limitation, where it negatively regulates the pathway at hemB, the gene encoding the heme biosynthetic enzyme δ-aminolevulinic acid dehydratase (16).

Irr is a conditionally stable protein that degrades rapidly when cells are exposed to iron, allowing derepression of heme synthesis (13). This iron-dependent degradation is mediated by heme (13). Accordingly, Irr persists in heme synthesis mutant strains in the presence of iron, and a mutation in an Irr heme binding site stabilizes the protein in the presence of the metal (13). Thus, heme is an effector molecule in Irr degradation that reflects the availability of iron for heme synthesis. Irr interacts directly with the heme biosynthesis enzyme ferrochelatase (17). Moreover, a regulatory activity for ferrochelatase was identified, distinct from its catalytic function, which allows Irr function to be controlled by the substrates of ferrochelatase. In the presence of iron, ferrochelatase inactivates Irr followed by heme-dependent degradation. Under iron limitation, protoporphyrin relieves the inhibition of Irr by ferrochelatase, probably by promoting protein dissociation, allowing genetic repression. Thus, Irr responds to iron via the status of protoporphyrin and heme locally at the site of heme synthesis. This allows heme to regulate a cellular process in the absence of a free heme pool (17).

Heme binds directly to Irr near the N terminus at a region termed the heme regulatory motif (HRM), which is necessary for rapid degradation (13). HRMs are found in functionally diverse proteins, and Cys-Pro is the only absolutely conserved sequence within the short motif. Numerous heme-mediated functions of proteins require an HRM, including mitochondrial targeting of mammalian δ-aminolevulinic acid synthase precursor (10), transcriptional activation of yeast HAP1 (18), repressor activity and nuclear export of human Bach1 (8, 19, 20), and the activities of cytochrome c heme lyase (21) and heme oxygenase-2 (22). It is not known how heme exerts its effect through the HRM.

In the present study, we further addressed the mechanism of iron-dependent degradation of Irr. We provide evidence for a second heme binding site that is necessary for degradation. The HRM and the new heme site bind the ferric and ferrous forms of heme, respectively, showing that normal Irr degradation involves both redox states of heme.
IrrC29A was grown with 50 μg/ml of GST, pSK/Irr1–135-GST, pSK/Irr1–150-GST, and the control pSK/Pirr from pSK/GST by QuikChange using two complementary primers that contained EcoRV and PstI sites at 5' ends, respectively. The product was ligated into HindIII/EcoRV sites of pSK to construct pSK/Irr. Mutant irr genes with His-α-Ala mutations were amplified from pSK/Irr using QuikChange (Stratagene) using two complementary primers that contained the nucleotide changes. Nucleotide sequences of mutant irr genes were confirmed by sequencing. The mutations changed the histidine codon (CAA) to an alanine codon (GCC) used highly in B. japonicum. Thus, single mutants (H117A, H118A, and H119A), double mutants (H117A/H118A, H117A/H119A, and H118A/H119A), and the triple mutant (H117A/H118A/H119A) were constructed in pSK with HindIII and EcoRV sites at 5' and 3' ends, respectively.

Construction of GST Fusion Proteins—The irr-GST gene fusion contains a GST coding region at the C terminus and an irr gene, encoding a 211-bp promoter region and a 492-bp coding region at the N terminus. To do this, the coding region of GST was amplified by PCR from pGEX-6P-2 (Amersham Biosciences) with EcoRV and PstI sites at 5' and 3' ends, respectively. The product was ligated into EcoRV/PstI sites of pSK/Irr-GST. The nucleotide sequence of GST was confirmed by sequencing. To construct GST gene fusions, the EcoRV/PstI fragment from pSK/Irr-GST was ligated into the EcoRV/PstI sites of pSK constructs of irr genes, yielding pSK/Irr-GST, pSK/C29A-GST, and pSK/H117A-H118A-H119A-GST.

GST fusions of C-terminal truncations of the irr gene were amplified from pSK/Irr-GST by QuikChange using two complementary primers that encompassed the deleted region, yielding pSK/Irr1–135-GST, pSK/Irr1–150-GST, and the control pSK/Irr-GST. The primers were complementary to both sides of the deleted region. Nucleotide sequences of C-terminal truncations and the GST control were confirmed by sequencing. Truncations encode proteins that retain the N-terminal 36, 116, 135, and 150 residues of Irr, respectively. Nucleotide sequences of C-terminal truncations were confirmed by sequencing. To construct GST fusion proteins, the EcoRV/PstI fragment from pSK/Irr-GST was ligated into the EcoRV/PstI sites of pSK constructs of irr genes, yielding pSK/Irr-GST, pSK/C29A-GST, and pSK/H117A-H118A-H119A-GST.

Analysis of Irr-GST Fusion Proteins—GST fusion protein levels were measured in cells grown under varying conditions of iron or heme. Aliquots of cells grown to mid-log phase were analyzed for Irr-GST protein on 12% SDS-PAGE gels run under non-reducing conditions and visualized by autoradiography using x-ray film or a Bio-Rad phosphorimaging device.
labeled with [35S]methionine and [35S]cysteine in low iron media. At anti-GroEL antibodies.

was used as a control for an unregulated protein and was detected with detected by immunoblot analysis using anti-GST antibodies. GroEL

either low iron (Fe) or heme (H), high iron (Fe) and high heme (H) concentrations. Irr levels in cells were analyzed by immunoblot analysis using anti-Irr antibodies. Thirty micrograms of protein were loaded/lane. C, the protocol is the same as for A, except Irr was followed over 4 h.

requirement can be overridden by supplementation of the growth medium with heme. Irr-GST and the truncations that were iron-responsive also degraded in response to heme (Fig. 2A). However, Irr-(1–116) was stable in the presence of heme as well as iron. These observations indicate that the stability of Irr-(1–116) was not because of an inability to interact with ferrochelatase, but rather the truncation is missing a heme-dependent instability element. We attempted to make N-terminal truncation fusions, but none of these constructs were expressed

An HRM Mutant of Irr Degrades Slowly in a Heme-dependent Manner—Iron-dependent degradation of Irr is mediated by heme, which binds directly to the HRM (13). Irr accumulates under low iron conditions but disappears within 60–90 min upon exposure of cells to iron (Fig. 2A) (13). By contrast, IrrC29A, which contains a Cys→Ala substitution within the HRM, does not degrade within this time frame in response to iron (Fig. 2A) (13).

Surprisingly, we observed that when strain IrrC29A cells were grown from an inoculum in medium containing FeCl3, the mutant Irr protein was expressed at very low levels, as discerned in immunoblots, similar to that observed in the wild type (Fig. 2B). Thus, it appeared that iron negatively affected Irr levels even in the HRM mutant. Because the cells were exposed to iron for a longer period of time in the growth experiments compared with the initial time course experiments, we repeated the latter over a longer time period (Fig. 2C). One hour after exposure of the cells to iron, wild-type Irr was almost undetectable in immunoblots, whereas IrrC29A was easily discerned. However, IrrC29A levels were diminished substan-

FIG. 1. Regulated degradation of Irr-GST fusion proteins. A, effects of iron and heme on Irr-GST fusion proteins. Fusion proteins were expressed under the control of native irr promoter on plasmid pLAFR3 in parent strain I110. Cells were grown in medium containing either low iron (–), high iron (Fe) or heme (H), and Irr-GST levels were detected by immunoblot analysis using anti-GST antibodies. GroEL was used as a control for an unregulated protein and was detected with anti-GroEL antibodies. B, pulse-chase pull-down of Irr-GST. Cells were labeled with [35S]methionine and [35S]cysteine in low iron media. At t = 0 during the chase, 12 μM ferric chloride was added. Samples were taken at 0 and 4 h, and GST fusion proteins were pulled down by glutathione-Sepharose beads. Aliquots were analyzed by SDS-PAGE followed by autoradiography.

12 μM FeCl3 (Fig. 1B). Although GST was detected on autoradiograms after 4 h of exposure to iron, the Irr-GST fusion turned over in an iron-responsive manner. These results are qualitatively similar to what is observed for authentic Irr (13). These findings show that the conditional instability of Irr can be conferred on a stable protein when fused to it. Furthermore, these fusions can be used to identify regions of Irr necessary for degradation.

Irr-(1–36)-GST contains the N-terminal amino acids of Irr, including the HRM, fused to GST. Unlike the full-length fusion, Irr-(1–36)-GST was stable in the presence of iron (Fig. 1), suggesting that the HRM is not sufficient to confer iron-dependent instability on GST. Additional fusions containing truncations from the C-terminal end of Irr were constructed (Fig. 1). Irr-(1–150)-GST and Irr-(1–135)-GST accumulated to very low levels when grown in iron-containing media and turned over in response to iron in pulse-chase experiments (Fig. 1). However, Irr-(1–116)-GST was unresponsive to iron in the growth and turnover experiments, indicating that a region of Irr between residues 116 and 135 is critical for iron responsiveness. Endogenous Irr disappeared in response to iron and heme in all cases (data not shown).

Irr interacts with ferrochelatase, the enzyme that catalyzes the final step of heme biosynthesis, and ferrochelatase is required for normal iron-dependent turnover of Irr (17). This
tially at 3 h and almost undetectable by 4 h. Thus, Irr responds to iron independently of the HRM, although more slowly. This observation agrees with the conclusion that Irr degradation involves elements in addition to the HRM.

To determine whether the slow disappearance is heme-dependent, we monitored the responsiveness of Irr to iron in a heme-deficient background. The hemA mutant strain MLG1 is deficient in delta-aminolevulinic acid synthase, the enzyme that catalyzes the first step in heme biosynthesis. Irr was completely stable in the hemA strain throughout the 4-h time course (Fig. 2C) and in the cultures of the mutant grown in the presence of iron (Fig. 2B), suggesting that heme is needed for iron-responsiveness of both the wild-type and mutant Irr proteins. In addition, Irr was nearly undetectable in cultures of the wild-type, IrrC29A, or the hemA strain when media were supplemented with heme (Fig. 2B). The data indicate that normal, rapid iron-dependent degradation of Irr requires the HRM but that degradation can occur slowly without the HRM in a heme-dependent manner.

Irr Binds Ferrous Heme Independent of the HRM—Heme-dependent degradation of Irr in the absence of a functional HRM raises the possibility that Irr has another heme binding site involved in turnover. Heme can exist in the ferrous (heme iron is Fe^{2+}) or ferric (Fe^{3+}) forms. We showed previously that purified recombinant Irr has two ferric heme binding sites with different affinities (13). One of them is the HRM, which is responsible for a 377-nm peak in the absorption spectrum of heme in the presence of Irr that is absent in the IrrC29A mutant protein (13) (Fig. 3A). A second ferric heme binding site was discerned from binding studies (17) and from an absorption shoulder at 419 nm in wild-type Irr and a peak at 413 nm in IrrC29A (Fig. 3A) (13). This second ferric heme site remains uncharacterized and, as described below, is distinct from the newly described heme binding site.

Here, the effects of Irr on the absorption spectrum of reduced (ferrous) heme were examined. A peak at 423 nm was observed in the presence of Irr; whereas free heme had only a very slight, broad feature in that region (Fig. 3B). Unlike ferric heme, the spectrum of ferrous heme bound to IrrC29A was identical to the wild-type protein, showing that Cys^{29} within the HRM was not a ligand for reduced heme. Thus, Irr binds ferrous heme independently of the HRM.

Effects of Irr Truncations on Ferrous and Ferric Heme Binding—It seemed plausible that a heme binding site on Irr other
than the HRM participates in turnover and that one or more residues necessary for heme binding was absent in the truncations. To address this, we analyzed the effects of truncations of purified recombinant Irr derivatives on the spectral properties of heme (Fig. 4). The 419-nm shoulder of ferric heme bound to Irr was lost in Irr-(1–80), and the 377-nm peak was shifted slightly to 380 nm, indicating that this truncation retained the HRM but lost the other ferric heme binding site (Fig. 4A). The ferric heme spectra of Irr-(1–116) was similar to the wild type, with spectral features at 377 and 419 nm. However, the 423-nm peak of the ferrous heme spectrum of wild-type Irr was lost in Irr-(1–116), as well as in Irr-(1–80) (Fig. 4B), showing that this truncated protein retained the ability to bind ferric heme but not ferrous heme. These data show that one or more ligands binding ferrous heme must be different from those binding either of the ferric heme binding sites. In addition, the loss of ferrous heme binding in Irr-(1–116) in vitro correlates with the loss of iron- and heme-dependent degradation of the Irr-(1–116) fusion protein in vivo.

Ferrozine Heme Binds an Irr Histidine and Is Required for Degradation—Histidine residues are often involved in heme binding (25). As an initial search for candidate histidine residues in Irr, we aligned numerous Irr homologs from different organisms using ClustalW (26). Histidines are completely conserved at positions 63, 117, and 119 (B. japonicum numbering) and His118 is conserved in many, but not all, of the Irr homologs. We mutagenized each of the three conserved histidines, as well as His118, to alanine and analyzed the spectra of ferrous heme bound to each of the four mutants. None of the single mutations abrogated the 423-nm peak (data not shown). It is possible that mutation of a heme binding ligand can be compensated by another residue (e.g. Ref. 27), which seems particularly plausible with the three adjacent histidines. Therefore, we studied that region further by constructing and analyzing the three double mutations and the triple mutation H117A/H118A/H119A. All of the double mutations retained the ferrous heme peak, but it was slightly diminished (data not shown). However the 423-nm peak was severely diminished in the triple mutant (Fig. 5A), indicating that this region is involved in ferrous heme binding. Unlike ferrous heme, ferric heme binding was retained in the triple mutant as discerned by the spectral features that were very similar to the wild type (Fig. 5B). These data reinforce the conclusion that ferrous heme binds to different residues on Irr than does ferric heme.

We addressed the effects of the H117A/H118A/H119A mutant on iron responsiveness in vivo using fusion proteins (Fig. 6). Unlike the wild-type or IrrC29A fusions, the triple mutant was stable in cells grown in high iron medium (Fig. 6A). Furthermore, the triple mutant did not turnover in response to iron in pulse-chase experiments (Fig. 6B). Two lines of evidence suggest that the stability of the H117A/H118A/H119A fusion is not caused by the lost ability to interact with ferrochelatase. First, supplementation of the media with heme overrides the need for ferrochelatase for turnover, and the triple mutant was stable in heme-containing cultures (Fig. 6A). In addition, we addressed the interaction between Irr and ferrochelatase by examining the ability of a GST-ferrochelatase fusion to pull down purified recombinant Irr proteins (Fig. 7). Wild-type Irr

![Image](http://www.jbc.org/)

**FIG. 5.** Effects of His→Ala substitutions at various positions on the absorption spectra of ferrous (A) or ferric (B) heme. A, absorption spectra of ferrous heme were taken between 380 and 480 nm, with 30 mM dithionite as the reductant in all reactions. B, absorption spectra of ferric heme were taken between 320 and 450 nm. The spectra are as follows: 8 μM protein alone, 4 μM heme alone, H117A/H118A/H119A plus heme, C29A plus heme, and Irr plus heme.

**FIG. 6.** Effects of mutations in the HRM or the ferrous heme binding site on iron-dependent degradation of Irr-GST fusion proteins. A, effects of iron and heme on Irr-GST fusion proteins. Fusion proteins were expressed under the control of native irr promoter on plasmid pLAFR3 in parent strain I110. Cells were grown in medium containing either low iron (−), high iron (+Fe) or heme (+H), and Irr-GST levels were detected by immunoblot analysis using anti-GST antibodies. B, pulse-chase pull-down of Irr-GST fusions. The protocol was the same as described in the legend to Fig. 1.
Although Irr has been studied extensively only in *B. japonicum*, an *irr* mutant in *Rhzobium leguminosarum* has the same fluorescent colony phenotype as the *B. japonicum* mutant (34), and the genomes among the *irr* homologs show a high degree of synteny. Thus, Irr likely has a similar function in other organisms. However, examination of the Irr homologs reveals that only some of them have the Cys-Pro sequence and an HRM-like domain, whereas His^{117} and His^{119} of *B. japonicum* Irr are completely conserved in all of the homologs. The current work suggests that the proteins lacking an HRM may degrade nevertheless. It is possible that the second, uncharacterized ferric heme binding site partially compensates for the defective HRM in IrrC29A, allowing slow degradation. By analogy, Irr homologs lacking an HRM may have a compensatory mechanism that allows turnover. Characterization of these other homologs should shed additional light on bacterial Irr function.

**REFERENCES**

1. Gong, W., Hao, B., Manye, S. S., Gonzalez, G., Gilles-Gonzalez, M. A., and Chan, M. K. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 15177–15182
2. Rifai, S. L., Larson, C. W., Boulca, D., Black, S., Karathan, E., Zimmer, M., Or dol, G. W., and Alam, M. (2000) *Science* 403, 549–544
3. Lanzilotta, W. N., Schuller, D. J., Thorsteinsson, M. V., Kerby, R. L., Roberts, G. P., and Pouls, T. L. (2000) *Nat. Struct. Biol.* 7, 870–880
4. Beu, M. R., Ruthe, J., Tacklun, J. R., Gonzalez, G., Gilles-Gonzalez, M. A., and McKnight, S. L. (2002) *Science* 298, 2385–2387
5. Bretti, D. S., and Snyder, S. H. (1994) *Annu. Rev. Biochem.* 63, 175–195
6. Delgado-Nixon, V. M., Gonzalez, G., and Gilles-Gonzalez, M. A. (2000) *Biochemistry* 39, 2685–2691
7. Hach, A., Hon, T., and Zhang, L. (2000) *J. Biol. Chem.* 275, 248–254
8. Ogawa, K., Sun, J., Taketa, S., Nakajima, O., Nishitani, C., Sassa, S., Hayashi, N., Yamamoto, M., Shibahara, S., Fujita, H., and Igarashi, K. (2001) *EMBO J.* 20, 2835–2843
9. Raffi-Kolpin, M., Chelal, P. J., Hussen, Z., Hahn, J., Uma, S., Matts, R. L., and Chen, J.-J. (2000) *J. Biol. Chem.* 275, 5171–5178
10. Latham, J. T., and Timko, M. P. (1993) *Science* 259, 522–525
11. Wang, L., Elliott, M., and Elliott, T. (1999) *J. Bacteriol.* 181, 1211–1219
12. Wang, L., Wilson, S., and Elliott, T. (1999) *J. Bacteriol.* 181, 6033–6041
13. Qi, Z., Hamza, I., and O’Brien, M. R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 13056–13061
14. Nakajima, O., Takahashi, S., Harigai, H., Furumaya, K., Hayashi, N., Sassa, S., and M., Y. (1999) *EMBO J.* 18, 6282–6289
15. Panek, H., and O’Brien, M. R. (2002) *Microbiology* (U. K.) 148, 2273–2282
16. Hamza, I., Chauhan, S., Hasset, R., and O’Brien, M. R. (1998) *J. Biol. Chem.* 273, 21669–21674
17. Qi, Z., and O’Brien, M. R. (2002) *Mol. Cell* 9, 155–162
18. Lee, H. C., Hon, T., Lan, C., and Zhang, L. (2003) *Mol. Cell. Biol.* 23, 5657–5666
19. Suzuki, H., Tashiro, S., Hira, S., Sun, J., Yamazaki, C., Zenke, Y., Beda-Saito, V., Yoshida, M., and Igarashi, K. (2004) *EMBO J.* 23, 2544–2553
20. Sun, J., Brand, M., Zenke, Y., Tashiro, S., Grouinie, M., and Igarashi, K. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 1461–1466
21. Steinier, H., Kispal, G., Zoller, A., Hafler, A., Neupert, W., and Lill, R. (1996) *J. Biol. Chem.* 271, 32005–32011
22. McCoubrey, W. K., Jr., Huang, T. J., and Maines, M. D. (1997) *J. Biol. Chem.* 272, 12568–12574
23. Guerrinot, M. L., and Chelm, B. K. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 1837–1841
24. Chauhan, S., and O’Brien, M. R. (1995) *J. Biol. Chem.* 270, 19823–19827
25. Dawson, J. H., Andersson, L. A., and Somo, M. (1982) *J. Biol. Chem.* 257, 3906–3917
26. Pearson, W. R. (1990) *Methods Enzymol.* 155, 63–98
27. Letoffe, S., Deniau, C., Wolff, N., Dassa, E., Delepelaire, P., Lecroisey, A., and Ordal, G. W., and Alam, M. (2000) *Science* 293, 522–525
28. Iwai, K., Drake, S. K., Wehr, N. B., Weissman, A. M., La Vaute, T., Minato, N., Ishimori, K., and Iwai, K. (2003) *Proc. Natl. Acad. Sci. U. S. A.* 100, 21669–21674
29. Qi, Z., Hamza, I., and O’Brien, M. R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 13056–13061
30. Kurokawa, H., Lee, D. S., Watanabe, M., Sagami, I., Mikami, B., Raman, C. S., Shi, J., and Shimizu, T. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 1357–1365
31. Sato, J., Shima, T., and Chen, J.-J. (2000) *Mol. Cell* 5, 336–340
32. Aft, R. L., and Mueller, G. C. (1844) *J. Biol. Chem.* 259, 301–305
33. Iwai, K., Drake, S. K., Wehr, N. B., Weissman, A. M., La Vaute, T., Minato, N., Klauser, R. D., Levine, R. L., and Rouault, T. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 4923–4928
34. Geissling, L. S., Mascott, D. P., and Thac, R. E. (1998) *J. Biol. Chem.* 273, 12555–12557
35. Bourdon, E., Kang, D. K., Ghosh, M. C., Drake, S. K., Wey, J., Levine, R. L., and Rouault, T. A. (2003) *Blood Cells Mol. Dis.* 31, 247–255
36. Yamanaka, K., Ishikawa, H., Megumi, Y., Tokunaga, F., Kanie, M., Rouault, T. A., Morishima, I., Minato, N., Ishimori, K., and Iwai, K. (2003) *Nat. Cell Biol.* 5, 336–340
37. Kuramata, H., Lee, D. S., Watanabe, M., Sagami, I., Mikami, B., Raman, C. S., and Shimizu, T. (2004) *J. Biol. Chem.* 279, 20186–20193
38. Wexler, M., Todd, J. D., Kolade, O., Bellini, D., Hemmings, A. M., Sawers, G., and Johnston, A. W. (2000) *Microbiology* (U. K.) 146, 1357–1365
39. Frustaci, J. M., Sangwan, L., and O’Brien, M. R. (1993) *J. Bacteriol.* 173, 1145–1150

---

**Fig. 7. Interaction of Irr and mutant derivatives with GST-Ferrochelatase.** The GST-FC fusion protein was used to precipitate purified recombinant Irr and mutant derivatives in solution. GST was used as a negative control. 2.5 μg of GST, 5 μg of GST-FC, and 10 μg of Irr, C29A, and H117A/H118A/H119A were used for the corresponding individual experiment. Irr proteins were detected by immunoblots using antibodies against Irr.
Two Heme Binding Sites Are Involved in the Regulated Degradation of the Bacterial Iron Response Regulator (Irr) Protein

Jianhua Yang, Koichiro Ishimori and Mark R. O'Brian

J. Biol. Chem. 2005, 280:7671-7676.  
doi: 10.1074/jbc.M411664200 originally published online December 21, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M411664200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 24 of which can be accessed free at http://www.jbc.org/content/280/9/7671.full.html#ref-list-1