Heat shock transcription factor 1 (HSF1) mediates the induction of heat shock protein gene expression in cells exposed to elevated temperature and other stress conditions. In response to stress HSF1 acquires DNA binding ability and localizes to nuclear stress granules, but the molecular mechanisms that mediate these events are not understood. We report that HSF1 undergoes stress-induced modification at lysine 298 by the small ubiquitin-related protein called SUMO-1. Antibodies against SUMO-1 supershift the HSF1 DNA-binding complex, and modification of HSF1 in a reconstituted SUMO-1 reaction system causes conversion of HSF1 to the DNA-binding form. HSF1 colocalizes with SUMO-1 in nuclear stress granules, which is prevented by mutation of lysine 298. Mutation of lysine 298 also results in a significant decrease in stress-induced transcriptional activity of HSF1 in vivo. This work implicates SUMO-1 modification as an important modulator of HSF1 function in response to stress.

Exposure of cells to stress conditions results in conversion of HSF1 from an inactive monomeric form to a trimeric DNA-binding form, which then interacts with promoters of heat shock protein (hsp) genes to up-regulate transcription (1, 2). Stress also causes localization of HSF1 to punctate nuclear bodies (3–5). However, the underlying mechanism(s) by which stress causes these changes in HSF1 structure, activity, and subcellular localization are not understood.

SUMO-1 is an 11-kDa protein with homology to ubiquitin whose conjugation to proteins appears to be involved in regulating the functional properties of these proteins, including subcellular localization and protection against degradation (6–10). We recently discovered that another member of the HSF family, HSF2, is constitutively modified by SUMO-1 in vivo (11). Thus, we hypothesized that HSF1 may be subject to stress-regulated SUMO-1 modification and that this may be responsible for converting the factor to the DNA-binding form and/or its localization to nuclear bodies in response to stress.

Here we report that in contrast to HSF2, the HSF1 protein is not constitutively modified by SUMO-1 and instead is only modified after cells are exposed to stress conditions. HSF1 colocalizes with SUMO-1 in nuclear bodies after stress treatment with kinetics that closely parallels that of heat shock treatment and recovery. We have identified lysine 298 as the site of stress-induced SUMO-1 modification in HSF1. Mutation of this residue significantly decreases stress-inducible HSF1 activation of hsp gene transcription, suggesting that SUMO-1 modification at this site is important for proper stress-induced HSF1 function. Finally, our results suggest that SUMO-1 modification modulates HSF1 function by regulating its DNA binding activity.

**Experimental Procedures**

Immunofluorescence Analysis—Control and heat-treated (42 °C for 30 min) HeLa cells or HSF1−/− MEF cells (12, 13) grown on coverslips were fixed using 4% paraformaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4) + 2% BSA at room temperature (5). Coverslips were then incubated for 60 min with one of the following primary antibodies in PBS containing 2% BSA: HSF1 rat monoclonal antibody (1:1000 dilution) (14), or PML mouse monoclonal antibody from Santa Cruz Biotechnology Inc. (1:100 dilution). After washing with PBS + 2% BSA, the coverslips were incubated for 30 min with a 1:200 dilution of the appropriate secondary antibody linked to either the Texas Red fluorochrome or fluorescein isothiocyanate (Vector Laboratories, Inc.). Immunostaining was visualized using a Nikon fluorescence microscope with a ×60 objective and a Nikon Spotcam digital imaging camera. As a control, we verified that addition of purified recombinant HSF1 to the coverslips eliminated HSF1 staining in nuclear granules.

Immunoprecipitation Analysis—Control and heat-treated (42 °C, 1 h) HeLa cells or cells transfected with pcDNA3 constructs encoding wild-type and lysine mutant HSF1 proteins were lysed in a solution containing 0.5 mM Tris-HCl (pH 6.7), 5% SDS, and 0.25 M glycerol, 0.5 M MgCl2, 0.5 M EDTA, 0.5 M phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol (12, 13). The extracts of control and heat-treated (42 °C, 1 h) HeLa cells made in Buffer C (20 mM HEPES, pH 7.9, 25% (v/v) glycerol, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol) were sub-
jected to gel mobility shift assay in the absence or presence of anti-SUMO-1 monoclonal antibodies (21C7) (14). The binding reaction contained 20 μl of binding buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 5% glycerol), 0.1 ng of 32P-end-labeled DNA probe, 0.5 μg of poly(dI·dC)/poly(dI·dC), and 10 μg of BSA and was incubated at 20 °C for 10 min. Binding reactions were subjected to electrophoresis on native 4% polyacrylamide gels in 0.5× Tris-borate/EDTA, and HSF1 DNA-binding complexes were visualized by autoradiography. The oligonucleotide probe contained four inverted repeats of the heat shock element consensus sequence 5′-nGAAn-3′.

In Vitro SUMO-1 Modification—Full-length HSF1 protein was in vitro translated in a rabbit reticulocyte lysate system and then subjected to in vitro SUMO-1 modification assay as previously described (15).

Transient Transfection Analysis—HeLa cells (4 × 10^6) were transfected with 1 μg of pcDNA3-wild-type HSF1 expression plasmid, pcDNA3-LysXE mutant HSF1 plasmid, or empty pcDNA3 plasmid (vector control), 1 μg of hsp promoter-luciferase plasmid, and 50 ng of β-galactosidase internal control plasmid, using Polyfect (Qiagen) as the transfection reagent. After 18 h the cells were heat-treated at 42 °C for 1 h and recovered at 37 °C for 6 h to allow expression of the reporter protein, and then the amounts of luciferase and β-galactosidase activities in the transfected cells were determined by assay of cell extracts.

RESULTS

A number of SUMO-1-modified proteins localize in discrete structures in the nucleus including PML, HIPK2, and Sp100 (15–19). Therefore, to determine whether there is a relationship between HSF1 and SUMO-1 in stress-induced nuclear granules, we performed double labeled immunofluorescence analysis on normal and heat-treated HeLa cells using antibodies that recognize each of these proteins. The results demonstrate that the HSF1-containing nuclear granules observed in response to heat treatment colocalized with SUMO-1 protein (Fig. 1A). Analysis of cells at various times of heat treatment and following recovery indicated a tight correlation between HSF1 and SUMO-1 localization in nuclear granules (Fig. 1B). HSF1 also colocalized with SUMO-1 in response to treatment with the heavy metal cadmium (Fig. 1C), indicating that this result is not limited to heat stress (4, 5).

Among the SUMO-1-modified proteins that localized to nuclear bodies, most appeared to localize in PML bodies (15–18), prompting us to examine whether HSF1-containing nuclear stress granules co-localize with PML. The data reveal the interesting result that some of the HSF1-containing stress granules colocalized with PML although a subset did not, suggesting the existence of at least two distinct classes of HSF1-containing nuclear stress granules (Fig. 1D).

To directly test whether HSF1 is modified by SUMO-1 in vivo, we immunoprecipitated HSF1 protein from extracts of non-stressed and heat-treated cells and then subjected it to Western blot using anti-SUMO-1 antibodies. The results indicate that the heat-treated sample contained a band of ~80 kDa, a size consistent with SUMO-1-modified HSF1 (which is not present in the non-stressed sample), indicating that HSF1 undergoes stress-induced SUMO-1 modification (Fig. 2A). Western blot using anti-HSF1 polyclonal antibodies revealed the decrease in the SDS-polyacrylamide gel electrophoresis mobility characteristic of the HSF1 protein in heat-treated cells (lower panel). In addition, gel shift assay of extracts of heat-treated cells revealed that the mobility of the HSF1-DNA complex is retarded by the addition of antibodies against SUMO-1, indicating that the active DNA-binding form of HSF1 present in heat-treated cells is modified by SUMO-1 (Fig. 2B). Antibodies that recognize the related HSF family member HSF2 (but not HSF1) did not show this effect (3).

The HSF1 protein contains three matches to the previously identified SUMO-1 modification consensus sequence (IL/V)KXE centered around lysines 91, 126, and 298 (6–10, 20–23). To determine whether any of these lysines are sites of SUMO-1 modification, we transfected cells with HSF1 expression constructs in which each of these lysines was changed to arginine. We then compared the ability of each to undergo SUMO-1 modification in heat-treated cells by immunoprecipitation followed by SUMO-1 Western blot. The results indicate that mutation of lysine 298 significantly reduced the level of SUMO-1-modified HSF1, suggesting that this lysine is a site of SUMO-1 modification in vivo (Fig. 3A). Western blot with anti-Myc antibodies confirmed the expression of transfected HSF1 K298R protein (lower panel). Identical results were obtained from an experiment in which wild-type or lysine-mutated HSF1 proteins were subjected to SUMO-1 modification in vitro using a reconstituted reaction system (Fig. 3B). A comparison of the sequence surrounding lysine 298 of HSF1 with characterized SUMO-1 modification sites in other proteins is shown in Fig. 3C. This consensus SUMO-1 modification sequence is con-
we determined whether SUMO-1 modification in vitro had any effects on HSF1 DNA binding by performing gel shift assay on samples of wild-type HSF1 and HSF1 K298R. These were prepared identically as those shown in Fig. 3B, except we used unlabeled methionine instead of [35S]methionine. In agreement with our previous results (22), in vitro translated wild-type HSF1 exhibited little or no DNA binding activity without further treatment (Fig. 4). However, subjecting HSF1 to SUMO-1 modification in vitro resulted in a significant increase in its DNA binding activity. The increase was blocked by the K298R mutation, suggesting that it was mediated by SUMO-1 modification (Fig. 4).

We also tested the effect of the Lys298 mutation on stress-induced localization of HSF to nuclear bodies by transfecting cells with wild-type or K298R mutant HSF1, subjecting them to heat treatment, and then examining HSF1 localization by immunofluorescence. Because HSF1 trimerizes upon stress treatment, we performed this experiment using an HSF1 wild-type cell line derived from HSF1−/− knockout mice to eliminate the possibility that mutant HSF1 could be carried to nuclear bodies by heterotrimerization with endogenous wild-type HSF1 (12, 13). The results shown in Fig. 5 indicate that transfecting wild-type HSF1 is found in nuclear bodies in stressed cells but that the HSF1 K298R mutant is not, and it exhibits a diffuse nuclear staining similar to that observed for HSF1 in the absence of stress treatment (as seen in Fig. 1A). Many fields of transfected cells were examined, and we did not find any cells in which HSF1 K298R was found in nuclear bodies after heat treatment. These results suggest that stress-induced SUMO-1 modifica-

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

Fig. 2. HSF1 undergoes stress-induced SUMO-1 modification. A, extracts of HeLa cells kept at 37 °C or subjected to heat treatment at 42 °C for 1 h were immunoprecipitated using anti-HSF1 polyclonal antibodies followed by Western blot analysis using the anti-SUMO-1 monoclonal antibody 21C7. The lower panel shows a Western blot of the extracts probed with the anti-HSF1 antibodies. B, the heat-activated DNA-binding form of HSF1 is SUMO-1-modified. Extracts of heat-treated HeLa cells (42 °C, 1 h) were incubated in the absence or presence of anti-SUMO-1 antibody (Ab) 21C7 or anti-HSF2 antibody (as a negative control) and then subjected to gel mobility shift assay using a 32P-labeled heat shock element-containing oligonucleotide probe. NS and P indicate nonspecific DNA binding activity and free probe, respectively. HS, heat shock.
tion at lysine 298 is important for localization of the HSF1 protein to nuclear bodies.

To test the importance of SUMO-1 modification for regulating stress-induced HSF1 activity in vivo, cells were transfected with wild-type or K298R mutant HSF1 expression constructs or empty pcDNA3 vector (Vector Laboratories, Inc.) along with a heat shock element-driven luciferase reporter plasmid and β-gal internal control plasmid. Transfected cells were heat-treated at 42 °C for 1 h and recovered at 37 °C for 6 h to allow expression of the reporter protein, and then the amounts of luciferase and β-galactosidase activities in the transfected cells were determined by the assay of cell extracts. A, Western blot of the extracts was performed using anti-Myc antibodies to verify expression of transfected wild-type HSF1 and HSF1 K298R protein.

**FIG. 4.** SUMO-1 modification regulates HSF1 DNA binding. Wild-type (WT) HSF1 and K298R HSF1 were subjected to SUMO-1 modification in vitro, and then the DNA binding activities of unmodified and SUMO-1-modified wild-type and mutant HSF1 were measured by gel mobility shift assay using a specific HSF-binding oligonucleotide probe.

**FIG. 5.** Mutation of lysine 298 prevents localization of HSF1 to nuclear bodies after heat treatment. HSF1<sup>−/−</sup> cells were transfected with pcDNA expression constructs encoding wild-type or K298R mutant HSF1. Transfected cells were heat-treated at 42 °C for 1 h and then subjected to immunofluorescence analysis using HSF1 antibodies.

**FIG. 6.** Mutation of lysine 298 decreases heat-induced HSF1 activity in vivo. A, HeLa cells were transfected in duplicate with pcDNA3 (Vector Laboratories, Inc.) or pcDNA expression constructs encoding wild-type or K298R mutant HSF1 along with a heat shock element-driven luciferase reporter plasmid and β-gal internal control plasmid. Transfected cells were heat-treated at 42 °C for 1 h and recovered at 37 °C for 6 h to allow expression of the reporter protein, and then the amounts of luciferase and β-galactosidase activities in the transfected cells were determined by the assay of cell extracts. B, Western blot of the extracts was performed using anti-Myc antibodies to verify expression of transfected wild-type HSF1 and HSF1 K298R protein.

**DISCUSSION**

The results in this study indicate that HSF1 undergoes stress-induced SUMO-1 modification and that this modification regulates the DNA binding of this transcription factor. Thus, we propose SUMO-1 modification as a candidate for the mechanism that regulates HSF1 DNA binding in response to stress, leading to induction of the cellular stress response. Regarding potential mechanisms by which SUMO-1 modification could regulate HSF1 DNA binding, we note that the lysine 298 SUMO-1 modification site lies between the trimerization domain of HSF1 and a C-terminal leucine zipper motif previously implicated in maintaining HSF1 in the monomeric non-DNA-binding form by interacting with and thus masking the trimerization domain (27). It is possible that SUMO-1 modification causes a conformational change that disrupts the ability of these two leucine zipper motifs to interact, leading to trimerization and DNA binding.

Interestingly, the region of HSF1 containing lysine 298 has been implicated in negatively regulating HSF1 transactivation activity in the absence of stress, suggesting that this region may be involved in more than one mechanism of HSF1 regulation (28–30). These studies showed that mutation of serines 303 and 307 results in derepression of the repressed phenotype, resulting in elevated basal transactivation function at non-stress temperatures, which suggests a role for basal phosphorylation of these serines in maintaining repressed transactivation. In one of these studies it was also found that mutation of lysine 298 or glutamic acid 300 to alanine also gave a dere-
pressed phenotype and elevated transactivation (31). The authors proposed that these charged residues may be involved in interaction with a protein that somehow blocks function of the C-terminal activation domain. If true, this suggests that stress-induced SUMO-1 modification at this site, in addition to its role in regulating HSF1 DNA binding, might have a second function by acting to disrupt this interaction and thus allow the maximal transactivation ability that would be expected by HSF1 during the stress response.

Alternatively, because lysine 298 and glutamic acid 300 represent required residues in the SUMO-1 modification consensus sequence (6–10, 20, 21), another possibility is that SUMO-1 modification plays some role in the phenotype displayed by the Lys 

298 and Glu 

300 mutants in these experiments. As the authors note, a known consequence of HSF1 transfection into cells is that a portion of the transfected HSF1 protein is converted to the trimeric DNA-binding form even at normal temperatures, but this HSF1 lacks the ability to activate hsp promoter transcription until the cells are heat shocked, indicating that some stress-induced change is required for full HSF1 transactivation ability in vivo (3, 27–31). Similarly, we hypothesize that in this previous experiment the transfected HSF1 proteins may have undergone inappropriately regulated SUMO modification and that without the heat treatment necessary to confer full transactivation competence the presence of these SUMO groups interfered with the overall transactivation ability of HSF1. The Lys 

298 and Glu 

300 mutants unable to undergo SUMO modification would be expected to exhibit the observed higher relative transactivation ability. However, we think it unlikely that this relates to physiological HSF1-SUMO modification, as our results did not detect modification in the absence of stress, although we cannot rule out the possibility that either a small fraction of HSF1 is modified in each cell or is modified in a cell cycle-dependent manner, for example.

Comparison of the results of this study with those we obtained previously on SUMO-1 modification of the HSF2 protein reveals that despite the conservation of sequence and functional domains between HSF1 and HSF2, there are important differences with respect to the SUMO-1 modification of these two proteins (11). First, HSF2 is constitutively modified, whereas HSF1 is only modified after stress treatment. Second, the lysines modified by SUMO-1 in HSF2 versus HSF1 are located in completely different regions of the proteins, with the modified lysine 82 in HSF2 found in a wing within the DNA-binding domain near the N terminus and the stress-modified lysine 298 of HSF1 located a substantial distance away in the C-terminal region of this protein (11, 32). However, despite the different locations the SUMO modifications of these two HSFs both act to alter DNA binding activity. This suggests that some SUMO-1 modification events in HSF1 and HSF2 evolved by divergent pathways and are regulated differently but serve a similar function.

The activities of a number of important transcription factors, including p53, c-Jun, and androgen receptor, are regulated by SUMO-1 modification, but the exact function(s) of these proteins that are being regulated by this modification is not clear (33–36). Thus, one question for future study is whether SUMO-1 modification also regulates the DNA binding activities of some or all of these other factors or instead regulates other functions such as the ability to interact with the transcriptional machinery. SUMO modification clearly plays a role in regulating the function of HSF1 during the cellular stress response, and it will be exciting in future studies to further characterize the details of this mechanism.

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REFERENCES

1. Cotto, J. J., and Morimoto, R. I. (1999) Biochem. Soc. Symp. 64, 105–118
2. Morano, K. A., and Thiele, D. J. (1999) Gene Expr. 7, 271–282
3. Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 12350–12355
4. Cotto, J. J., Fox, S., and Morimoto, R. I. (1997) J. Cell Biol. 110, 2925–2934
5. Jolly, C., Usson, Y., and Morimoto, R. I. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6769–6774
6. Krete-Benny, C., and Tanguay, R. M. (1999) Biochem. Cell Biol. 77, 299–309
7. Yeh, E. T., Gong, L., and Kamitani, T. (2000) (Gene Annot.) 248, 1–14
8. Melchior, F. (2000) Annu. Rev. Cell Dev. Biol. 16, 591–626
9. Hay, R. T. (2001) Trends Biochem. Sci. 26, 322–333
10. Muller, S., Hoege, C., Pyrowolakis, G., and Jenatsch, S. (2001) Nat. Rev. Mol. Cell. Biol. 2, 202–210
11. Goodson, M. L., Hong, Y., Rogers, R., Matunis, M. J., Park-Sarge, O. K., and Sarge, K. D. (2001) J. Biol. Chem. 276, 18533–18538
12. McMillan, D. R., Xian, X., Shao, L., Graves, K., and Benjamin, I. J. (1998) J. Biol. Chem. 273, 7523–7528
13. Xiao, X., Zuo, X., Davis, A. A., McMillan, D. R., Curry, R. B., Richardson, J. A., and Dejean, J. J. (1999) EMBO J. 18, 5943–5952
14. Matunis, M. J., Coutavas, E., and Blobel, G. (1996) J. Cell Biol. 133, 1457–1470
15. Duprez, E., Saurin, A. J., Desterro, J. M., Lalemen-Breitenbach, V., Howe, K., Boddy, M. N., Solomon, E., de The, H., Hay, R. T., and Freemont, P. S. (1999) J. Cell Sci. 112, 381–393
16. Sterndorf, T., Jensen, K., and Will, H. (1997) J. Cell Biol. 139, 1621–1634
17. Muller, S., Matunis, M. J., and Dejean. A. (1998) EMBO J. 17, 61–70
18. Kamitani, T., Nguyen, H. P., Kito, K., Fukuda-Kamitani, T., and Yeh, E. T. (1998) J. Biol. Chem. 273, 3117–3120
19. Kim, Y. H., Choi, C. Y., and Kim, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13922–13927
20. Rodriguez, M. S., Dargemont, C., and Hay, R. T. (2001) J. Biol. Chem. 276, 12654–12660
21. Sampson, D. A., Wang, M., and Matunis, M. J. (2001) J. Biol. Chem. 276, 21664–21669
22. Sarge, K. D., Zimarino, V., Holm, K., Wu, C., and Morimoto, R. I. (1991) Genes Dev. 5, 1902–1911
23. Rabindran, S. K., Giorgi, G., Clos, J., and Wu, C. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6906–6910
24. Nakai, A., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 1893–1997
25. Rabergh, C. M., Airaksinen, S., Soitamo, A., Bjorklund, H. V., Johansson, T., Nikkinen, M., and Sistonen, L. (2000) J. Exp. Biol. 203, 1817–1824
26. Stump, D. G., Landsberger, N., and Wolfe, A. P. (1995) (Gene Annot.) 160, 207–211
27. Rabindran, S. K., Haroun, R. I., Clos, J., Wisniewski, J., and Wu, C. (1995) Science 259, 230–234
28. Green, M., Schuetz, T. J., Sullivan, E. K., and Kingston, R. E. (1995) Mol. Cell. Biol. 15, 3354–3362
29. Zuo, J., Rungger, D., and Voellmy, R. (1995) Mol. Cell. Biol. 15, 4319–4330
30. Shi, Y., Kroeger, P. E., and Morimoto, R. I. (1995) Mol. Cell. Biol. 15, 4309–4318
31. Knafel, U., Newton, E. M., Kyriakis, J., and Kingston, R. E. (1996) Genes Dev. 10, 2782–2793
32. Littlefield, O., and Nelson, H. C. (1999) Nat. Struct. Biol. 6, 446–470
33. Muller, S., Berger, M., Lehembr, F., Seeler, J. S., Haupt, Y., and Dejean, A. (2000) J. Biol. Chem. 275, 13321–13329
34. Rodriguez, M. S., Desterro, J. M., Lain, S., Midgley, C. A., Lane, D. P., and Hay, R. T. (1999) EMBO J. 18, 6455–6461
35. Gostissa, M., Hengstermann, A., Vogel, V., Sand, P., Schwarz, S. E., Scheffner, M., and Del Sal, G. (1999) EMBO J. 18, 6462–6471
36. Poukka, H., Karvonen, U., Janne, O. A., and Palvimo, J. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14145–14150
Regulation of Heat Shock Transcription Factor 1 by Stress-induced SUMO-1 Modification
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Dr. Goodson’s middle initial was omitted from the author line. The corrected author line is shown above.

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Characterization of two 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/inosine monophosphate cyclohydrolase isozymes from Saccharomyces cerevisiae.

Anne S. Tibbetts and Dean R. Appling

Page 20924, Table III: The 10-CHO-THF $K_m$ value for Ade16p was miscalculated. The correct value is 38 $\mu$M. The corrected table is shown below.

| Enzyme   | AICAR transformylase activity $^a$ | $K_m$ AICAR | 10-CHO-THF | IMP cyclohydrolase activity $^b$ |
|----------|-----------------------------------|-------------|------------|----------------------------------|
| Ade16p   | 1.0 ± 0.1                         | 26          | 38         | 1.7 ± 0.4                        |
| Ade17p   | 0.9 ± 0.1                         | 22          | 63         | 2.0 ± 0.5                        |
| Human Pur H$^c$ | 1.2                              | 16.8        | 60.2       | 2.8                              |

$^a$ AICAR transformylase units expressed as $\mu$mol of THF formed/min (mean ± S.D.). 10-CHO-THF, 10-formyltetrahydrofolate.

$^b$ IMP cyclohydrolase units expressed as $\mu$mol of IMP formed/min (mean ± S.D.).

$^c$ From Rayl et al. (12).

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