Identification of the Human YVH1 Protein-tyrosine Phosphatase Orthologue Reveals a Novel Zinc Binding Domain Essential for in Vivo Function

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A human orthologue of the Saccharomyces cerevisiae YVH1 protein-tyrosine phosphatase is able to rescue the slow growth defect caused by the disruption of the S. cerevisiae YVH1 gene. The human YVH1 gene is located on chromosome 1q21-q22, which falls in a region amplified in human liposarcomas. The evolutionary conserved COOH-terminal noncatalytic domain of human YVH1 is essential for in vivo function. The cysteine-rich COOH-terminal domain is capable of coordinating 2 mol of zinc/mol of protein, defining it as a novel zinc finger domain. Human YVH1 is the first protein-tyrosine phosphatase that contains and is regulated by a zinc finger domain.

Levels of cellular phosphorylation are controlled by the coordinated actions of protein kinases and protein phosphatases. The protein phosphatases can be divided into two large families: the Ser/Thr phosphatases, which are metalloproteins, and the protein-tyrosine phosphatase family (here referred as CysX₅Arg), which proceeds via a thiol-phosphate enzyme intermediate (1). The CysX₅Arg family includes: 1) tyrosine specific phosphatases (PTP); 2) VH1-like dual specificity phosphatases; 3) CDC25 phosphatases, which regulate the cell cycle; and 4) the low molecular weight phosphatases (1, 2).

The prototypic VH1-like phosphatase was identified from Vaccinia virus (3) and upon expression in Escherichia coli, VH1 was shown to dephosphorylate phosphotyrosine as well as phosphoserine and phosphothreonine containing substrates. Several members of this growing family of dual-specificity tyrosine phosphatases have been identified in mammals and yeast (1, 2). Among the yeast VH1-like phosphatases, MSG5 (4) and Pmp1 (5) have been shown to regulate the phosphorylation state of specific mitogen-activated protein kinases, FUS3 and Pmk1, respectively. The Saccharomyces cerevisiae dual specificity phosphatase YVH1 is of particular interest because inactivation of the YVH1 gene results in a striking increase in the yeast doubling time (6). Moreover, YVH1 has also been suggested to play a role in controlling meiosis and sporulation (7).

Analysis of the S. cerevisiae genome suggests that there are 17 open reading frames corresponding to PTPs and dual specific phosphatases. The number of mammalian phosphatases currently identified in the human genome appears to be greater than 200 and is likely to increase as more sequence information is deposited in the data bases. Yeast Cys(X)₅Arg phosphatases that have clearly defined mammalian orthologues are particularly interesting, because they are likely to function as regulators of important cellular functions that have been conserved over an extensive evolutionary period. This report identifies the human orthologue of yeast YVH1, named hYVH1, and demonstrates that the human protein can complement the yeast slow growth phenotype. The noncatalytic COOH terminus of hYVH1 is essential for the complementation of the yeast yvh1Δ phenotype. The COOH terminus of hYVH1 encodes a novel domain, which binds 2 mol of zinc/mol of protein. This is the first example of a phosphatase that harbors a novel zinc finger regulating motif. Although the exact function of hYVH1 is unknown, it maps to chromosome 1q21-q22, a region that is amplified in human liposarcomas (8, 9).

EXPERIMENTAL PROCEDURES

Strains and Yeast Method—S. cerevisiae haploid strains used in this study, MYMY22 (MATa, his3, leu2, trpl, ura3, yvh1Δ::G418) and MYMY21 (MATa, his3, leu2, trpl, ura3, YVH1) were derived from GYC86 (MATa, his3, leu2, trpl, ura3, YVH1/ YVH1) (6). The haploid MYMY22 strain carrying the null mutation yvh1Δ::G418, was obtained following PCR-based gene disruption (10). This null mutation removes the entire coding sequence of YVH1 within the S. cerevisiae genome. G418-resistant colonies, obtained from GYC 86 using the above procedure, were screened by PCR to identify yvh1Δ::G418/YVH1 transformants. Segregants were selected from tetrads with a 2 G418-resistant: 2 G418-sensitive segregation pattern. Strain MYMY21 and MYMY22 were derived from the same tetrad.

Identification, Sequencing, and Mammalian Plasmid Construction—Human sequences similar to the S. cerevisiae YVH1 protein were identified by BLAST searches of the human expressed sequence tag data base. A cDNA clone corresponding to ESTB188030 was obtained from American Type Culture Collection (ATCC, Manassas, VA) and completely sequenced on both strands. For mammalian expression, the hYVH1 open reading frame was amplified by PCR using primers containing HindIII and BamHI restriction sites and cloned into the corresponding sites in pEGFP (CLONTECH, Palo Alto, CA). Yeast and Bacteria Plasmid Construction—For bacterial expression, the hYVH1 open reading frame was subcloned into pGEX4T1 (Amer sham Pharmacia Biotech) to generate pGEXhYVH1, pGEXhYVH1CS, pGEX/hYVH1AC, pGEX/hYVH1CSAC as follows. PCR primers containing EcoRI and PstI restriction sites were used to subclone the ~1-kilobase PCR product into the corresponding sites of pBSK (Stratagene, La Jolla, CA). The plasmid obtained was cut with EcoRI and NotI, and the fragment containing hYVH1 was subcloned into the corresponding vector.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF119226.

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1 M. Muda, M. Wishart, B. Ernsting & J. E. Dixon, unpublished observation.

2 The abbreviations used are: PCR, polymerase chain reaction; EST, expressed sequence tag; ICP, inducibly coupled plasma emission spectroscopy; GST, glutathione S-transferase; GFP, green fluorescent protein.
sites of pGEX4T1. hYVH1ΔC was obtained by PCR using a specific primer that introduces a stop codon at amino acid 191. To generate an enzymatically inactive hYVH1 and hYVH1CSAΔ the catalytic essential Cys\(^{115}\) was mutated to a serine residue. Site-directed mutagenesis was performed using the Quick-Change Kit (Stratagene) according to manufacturer’s instructions. The hYVH1, hYVH1CS, hYVH1ΔC, hYVH1CSAΔ inserts were isolated from pGEX4T1 using BamHI and XhoI and subcloned into p416ADH (11). The XbaI fragment encoding YVH1, from pGE-KG/YVH1 was used to generate p416ADH/YVH1. All PCR-generated constructs were verified by sequence analysis.

**GST Fusion Proteins Expression and Purification in E. coli—**PGEX/hYVH1 wild type and mutants fusion proteins were transformed into E. coli BLR (Novagen, Madison, WI). Cells were grown in 2× YT medium containing 100 \(\mu\)g/ml ampicillin. Following induction with isopropyl-\(\beta\)-D-thio-\(\beta\)-galactopyranoside cells were harvested, resuspended in phosphate-buffered saline containing 1% (w/v) Triton X-100, 5 mM dithiothreitol, supplemented with complete EDTA-free protease inhibitor tablets (Roche Molecular Biochemicals), and proteins were purified as described previously (12). Following elution from glutathione-agarose beads, protein samples were concentrated using Centriprep 30 Concentrators (Amicon, Inc., Beverly, MA) buffer exchanged to storage buffer, 5 mM dithiothreitol, 100 mM NaCl in 50 mM Tris, pH 7.5, and stored at 4 °C. The purity of all proteins in this study was ~ 80–90%, as judged by SDS-polyacrylamide gel electrophoresis analysis. Protein concentrations were determined by amino acid analysis at the University of Michigan Protein Core Facility.

**Metal Quantitation—**Inductively coupled plasma atomic emission spectroscopy (ICP) was used to determine the metal species bound to the purified protein. ICP of GST/hYVH1, GST/hYVH1CS, GST/hYVH1ΔC, GST/hYVH1CSAΔ was performed by Ted J. Huston, Department of Geological Sciences, University of Michigan, Ann Arbor.

**Phosphatase Activity—**GST/hYV1 wild type and mutants fusion proteins were assayed for intrinsic phosphatase activity. Briefly, GST fusion proteins were incubated at 37 °C for 10 min in a reaction volume of 500 \(\mu\)l containing 0.1 mM 3-O-methylfluorescein phosphate, or 20 mM \(p\)-nitrophenyl phosphate, 50 mM Tris-HCl, pH 7.5, and 5 mM dithiothreitol. Reactions were monitored by measuring hydrolysis at 477 or 410 nm for 30 min using a Milton Roy Spectroline multiwell plate reader. A total of 500 \(\mu\)l containing 0.1 mM 3-O-methylfluorescein phosphate, or 20 mM \(p\)-nitrophenyl phosphate, respectively, as described previously (13).

**Preparation of hYVH1 Antisera and Antibodies Purification—**Purified GST/hYVH1 (above) was used to immunize two rabbits (Cocalico Biologicals, Inc., Reamstown, PA). Polyclonal antibodies were prepared and purified by pre-absorption on GST-coupled Affi-Gel-15 (Bio-Rad) followed by absorption on GST/hYVH1-coupled Affi-Gel-15. Elution and storage of antibodies was as described (14).

**Mapping the hYVH1 Gene—**A sequence tagged site with forward primer AGCTTGGGAAGAAACTTGC and a reverse primer GAT-

**Characterization of hYVH1—**To identify the human protein sequences that were similar to the S. cerevisiae YVH1, a

**RESULTS AND DISCUSSION**

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**FIG. 1. Subcellular localization of GFP and endogenous hYVH1.** A, COS-7 cells were transfected with pEGFP-hYVH1, fixed, and observed 24 h after transfection using fluorescence microscopy. B, HeLa cells were fixed and permeabilized, and endogenous hYVH1 was detected using affinity-purified hYVH1 polyclonal antibody followed by fluorescein isothiocyanate-conjugated goat anti-rabbit antibody. The specificity of staining was determined in the absence of primary antibody (C).
BLAST computer search of the expressed sequence tag data base was performed. Several human sequences were found that had fragment similarity to YVH1. One clone corresponding to EST188030 contained an insert of 1,271-base pairs; nucleotide sequence analysis revealed a putative start codon followed by an open reading frame of 1,021 and 228-base pairs of 3'-untranslated sequence with an uncommon poly(A) signal, AT-TAAA, preceding the 3'-poly(A) tail. Using a probe encompassing the first 998 nucleotides of the cDNA, a major RNA of 1.4 kilobases was detected in most of the tissues analyzed, the strongest signals were present in spleen, testis, ovary, and peripheral blood leukocytes (data not shown). In lung and liver, the 1.4-kilobase band was only detectable following long exposure times. The size of this band is in agreement with the observed size of the cDNA clone. The open reading frame encodes a protein of 340 amino acids, and comparison of the human and yeast sequences revealed an overall identity of 31%. Western blot analysis using hYVH1 antisera demonstrated that a protein of approximately 38,000 Da, consistent with the predicted mass of the protein (37,687 Da), was expressed in a variety of cell lines (data not shown). The subcellular localization of hYVH1 was determined both by overexpressing hYVH1 fused to the green fluorescent protein (GFP) in COS-7 cells and indirect immunofluorescence detection of the endogenous protein in a HeLa cell line. The endogenous as well as the recombinant hYVH1 proteins localized predominantly to

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**Fig. 2.** Amino acid similarity between YVH1 phosphatases and identification of the conserved cysteine-rich domain. A, the deduced amino acid sequences of hYVH1, S. cerevisiae and S. pombe YVH1 were aligned using the ClustalW tool of MacVector (Oxford Molecular Group). Boxes indicate regions of amino acid similarity. Sequences comprising the phosphatase domain are boxed in red, and in green is the COOH-terminal conserved cysteine-rich domain. The catalytic essential cysteine is highlighted in red. The seven conserved cysteines and histidine residue in the COOH-terminal domain are highlighted in yellow. B, sequence alignment of the cysteine-rich domain found in hYVH1, S. cerevisiae, S. pombe, C. elegans, P. falciparum YVH1 phosphatases and a protein of unknown function from A. thaliana.

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**Table 1.**

| Species       | Amino Acid Sequence | Conserved Cysteines |
|---------------|---------------------|---------------------|
| Human         |                     |                     |
| S. cerevisiae |                     |                     |
| S. pombe      |                     |                     |
| C. elegans    |                     |                     |
| P. falciparum |                     |                     |
| A. thaliana   |                     |                     |

Note: The table provides a comparison of the amino acid sequences of human YVH1 and other species, highlighting the conserved cysteine-rich domain.
TABLE I

| Metal ion | hYVH1 | hYVH1CS | hYVH1AC | hYVH1CSAC |
|-----------|-------|---------|---------|-----------|
| Zinc      | 2.1 ± 0.2 | 2.4 ± 0.2 | 0.0161 | 0.0122 |
| Magnesium | 0.041 | 0.017 | 0.026 | 0.018 |
| Manganese | <0.01 | <0.01 | <0.01 | <0.01 |
| Iron      | 0.10 | 0.097 | 0.014 | 0.01 |
| Cobalt    | <0.01 | <0.01 | <0.01 | <0.01 |
| Nickel    | <0.01 | <0.01 | <0.01 | <0.01 |
| Copper    | <0.01 | 0.014 | <0.01 | <0.01 |

The nuclei (Fig. 1, A and B) but were also detected in the cytosol in a mesh-like pattern. The 3’-untranslated sequence of hYVH1 was used to design a sequence tagged site to probe the Stanford G3 Radiation Hybrid Panel. The hYVH1 gene localized to chromosome 1q21–22, which interestingly maps to a region of the genome that is amplified in liposarcomas (8, 9). It is tempting to speculate that amplification of hYVH1 in liposarcomas could lead to a positive effect on cell growth, and this is consistent with the growth defect observed in the yeast null mutant.

Using the deduced hYVH1 protein sequence the GenBank™/EMBL Data Bank was analyzed and a Schizosaccharomyces pombe YVH1 protein (accession no. 2257526) was also identified. Fig. 2A shows that the three proteins share an N-terminal phosphatase domain within the first 200 amino acids, followed by a COOH-terminal domain of approximately 100 amino acids, which shows a striking degree of amino acid sequence identity.

The COOH-terminal Domain: Identification of a Novel Cysteine-rich Motif and Its Role in Phosphatase Activity—Fig. 2A shows that the human, S. cerevisiae, and S. pombe COOH-terminal domains contain seven invariant cysteines and one histidine reminiscent of sequences observed in the RING (16), LAP/PHD (17, 18), and LIM motifs (19). Although the RING, LAP/PHD, and LIM motifs each contain seven cysteine residues and a single histidine, the spacing of the conserved residues in the COOH terminus of YVH1 is unique. An analysis of this unique pattern and spacing of the seven cysteine and one histidine residues revealed that uncharacterized YVH1 phosphatases were also present in the Caenorhabditis elegans genome (accession no. 3642004) as well as in Plasmodium falciparum genome (accession no. 3649770) (Fig. 2B). Moreover, one uncharacterized protein from Arabidopsis thaliana (accession no. 2832664) was found to contain this novel cysteine-rich motif. Interestingly, its primary sequence suggests it is not a phosphatase.

Metal Analysis of Recombinant hYVH1—The unique spacing of seven cysteine residues and a single histidine in the COOH-terminal domain is reminiscent of finger domains, which are known to bind zinc. For this reason, hYVH1 was examined for its ability to bind metal ions. Analysis of the metal content of the recombinant full-length GST/hYVH1 and a truncated GST/hYVH1AC (amino acids 1–191) was carried out using ICP (Table I). ICP analysis revealed that hYVH1 contains stoichiometric amounts of zinc, 2 equivalents of zinc/mol of protein. Moreover, zinc binding was highly specific as no other metal was detected in significant amounts. The COOH-terminal deletion mutant, hYVH1AC contained no zinc or other metals, demonstrating that the carboxyl-terminal region of the protein was required for zinc binding. Substitution of the active-site cysteine to serine, hYVH1CS, had no effect on zinc binding (Table I). To further analyze the contribution of the invariant cysteine residues present within the COOH-terminal domain, we generated three site-directed mutants, substituting pairs of the conserved cysteine residues with serine residues (hYVH1C221, 224/S, hYVH1C29, 293/S and hYVH1C308, 310/S). Unfortunately all these cysteine mutants were refractory to glutathione affinity purification preventing further characterization. This observation suggests that the conserved cysteines in the COOH-terminal zinc binding domain are necessary to generate a properly folded protein.

The importance of the COOH-terminal domain in regulating enzymatic activity was analyzed in vitro by comparing the phosphatase activity of full-length and truncated recombinant GST fusion proteins. The initial rate of hydrolysis of the truncated and full-length forms of hYVH1 were determined with two artificial substrates, p-nitrophenyl phosphate and 3-O-methylfluorescein phosphate. At several different substrate concentrations, the initial rates for the full-length and catalytic...
Human YVH1 Protein-tyrosine Phosphatase

23995

domain of hYVH1 differed by less than a factor of two, suggesting that the COOH-terminal domain does not play a major role in the enzymatic activity of the YVH1-like phosphatases (data not shown). In contrast the COOH-terminal domain is essential for YVH1 function in vivo (see below).

Functional Analysis of hYVH1 Using yvh1Δ S. cerevisiae Strains—We disrupted the YVH1 gene in the diploid S. cerevisiae strain, GYC86, replacing the YVH1 coding region with a kanamycin cassette. Diploid strains in which one YVH1 copy was inactivated did not display any growth defect. However, consistent with our previous report, subsequent to sporulation, all of the haploid cells carrying the yvh1Δ allele displayed a strikingly slow growth phenotype (6). We then tested the ability of an episomal copy of hYVH1 to complement this growth defect. Strains carrying the yvh1Δ allele were transformed with a centromeric yeast expression vector expressing the hYVH1 protein under the control of the ADH promoter. Following transformation, colonies were grown to saturation and serial dilutions were replica plated on SC-uracil or YPD plates. Colony size, indicative of growth rate, was scored by visual inspection following 2 days of incubation at 30 °C. Using this semi-quantitative analysis, we observed that the hYVH1 protein was able to restore the normal growth phenotype in the yvh1Δ strain, MYV22 (Fig. 3A).

Complementation by hYVH1 was indistinguishable from yeast YVH1 protein expressed from the same vector (Fig. 3A). Surprisingly, the catalytically inactive versions of hYVH1 and YVH1 proteins were also able to restore normal growth phenotype to strain carrying the yvh1Δ mutation (Fig. 3A). One explanation of this phenomena is that both the human and yeast Cys-Ser mutant phosphatases act such that the mutated phosphatase complexes with the endogenous downstream target/effector blocking its activity (20). Hence, this would be functionally equivalent to dephosphorylation/inactivation of the target/effector by the wild type enzyme.

In contrast, expression of the truncated phosphatase (hYVH1ΔC) in S. cerevisiae disrupted strains did not restore the normal growth phenotype. Likewise, a catalytic inactive truncated protein, hYVH1CSΔC, was also unable to rescue the growth defect (Fig. 3A), suggesting that the COOH-terminal domain is required for YVH1 function in vivo. Importantly the lack of complementation was not due to differences in the protein expression levels, because Western blot analysis demonstrated that all proteins were expressed in comparable amounts (Fig. 3B). Expression of the “double” point mutated proteins, hYVH1C221,224/S, hYVH1C290,293/S and hYVH1C308,310/S were unable to restore a normal growth rate. However, all these mutated forms, in contrast to the full-length and truncated versions, were extremely unstable when expressed in yeast and barely detectable by Western blot.

Taken together, these results suggest that although the COOH-terminal domain is largely dispensable for the intrinsic enzymatic activity, its putative interaction with the endogenous target/effectorors is essential for the in vivo function. Roles for the COOH-terminal domain include correct localization of the catalytic core within cellular compartments or, more directly, docking of the phosphatase domain to a target molecule.

In summary, the human orthologue of the S. cerevisiae YVH1 protein-tyrosine phosphatase was identified and its function is beginning to be dissected. Remarkably, the COOH-terminal domain is dispensable for in vitro phosphatase activity but is essential for function in vivo. ICP analysis of purified hYVH1 proteins revealed that the COOH-terminal domain binds 2 mol of zinc/mol of protein, identifying it as a novel zinc finger domain. Because zinc finger domains (such as the RING finger and LIM domains) are broadly known for their role in mediating multiprotein complex assembly, it is tempting to speculate that the COOH-terminal domain of hYVH1 is involved in protein-protein interactions. Specific subcellular targeting by the noncatalytic portion of intracellular protein tyrosine phosphatases is a proposed mechanism for assuring enzymatic specificity (21). Recently, direct substrate binding of protein phosphatases via noncatalytic extensions, has been described as another means of restricting enzyme action within the cell (22). Results presented in this report suggest that the COOH-terminal domain may tether YVH1 protein phosphatases to their corresponding intracellular target/substrate, and this interaction is necessary for physiological function within the cell. We are currently investigating these possibilities and experiments are under way to identify the targets of the human and S. cerevisiae YVH1 proteins.

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REFERENCES
1. Barford, D., Das, A. K. & Egly, M. P. (1998) Annu. Rev. Biophys. Biomol. Struct. 27, 133–164
2. Fauman, E. B. & Saper, M. A. (1996) Trends Biochem. Sci. 21, 413–417
3. Guan, K.-L., Broyles, S. S. & Dixon, J. E. (1991) Nature 350, 359–362
4. Doi, K., Gartner, A., Ammerer, G., Errede, B., Shinkawa, H., Sugimoto, K. & Matsumoto, K. (1994) EMBO J. 13, 61–70
5. Sugura, R., Toda, T., Sluntsh, H., Yanagida, M. & Kuno, T. (1998) EMBO J. 17, 140–148
6. Guan, K.-L., Hakes, D. J., Wang, Y., Park, H.-D., Cooper, T. G. & Dixon, J. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12175–12179
7. Park, H.-D., Besser, A. E., Clancy, M. J. & Cooper, T. G. (1996) Yeast 12, 1135–1151
8. Forus, A., Wegluhuis, D. O., Smeets, D., Fostad, Ø., Myklebost, O. & van Kessel, A. G. (1996) Genes Chromosomes Cancer 4, 8–14
9. Forus, A., Berner, J.-M., Meza-Zepeda, L. A., Saeter, G., Mischke, D., Fostad, Ø. & Myklebost, O. (1998) Br. J. Cancer 78, 495–503
10. Goldsman, U., Becker, S., Fiedler, T., Beinhauer, J. & Hegemann, J. H. (1996) Nucleic Acids Res. 24, 2519–2524
11. Mukherjee, S., Muller, R. & Funk, M. (1995) Genet. 156, 119–122
12. Mudae, M., Boschert, U., Smith, A., Antonsson, B., Gillieron, C., Chabert, C., Camps, M., Martinou, I., Ashworth, A. & Arkinstall, S. (1997) J. Biol. Chem. 272, 5141–5151
13. Gottlin, E. R., Xu, X., Epstein, D. M., Burke, S. P., Eckenstein, J. W., Ballou, D. & Dixon, J. E. (1996) J. Biol. Chem. 271, 27445–27449
14. Harlow, E. & Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
15. Oei, C. E., Rabinovich, E., Dancis, A., Bonifacino, J. S. & Klausner, R. D. (1996) EMBO J. 15, 3515–3523
16. Bordner, K. L. B. & Freemont, P. S. (1996) Curr. Opin. Struct. Biol. 6, 395–401
17. Saha, V., Chaplin, T., Gregorini, A., Ayton, P., & Young, B. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9737–9741
18. Mullersman, J. E. & Pfeffer, L. M. (1995) Trends Biochem. Sci. 20, 56–59
19. David, I. B., Breen, J. J. & Toyama, R. (1998) Trends Genet. 14, 156–162
20. Tomkus, N. K. & Neel, B. (1996) Cell 87, 365–368
21. Mauro, L. J. & Dixon, J. E. (1994) Trends Biochem. Sci. 19, 151–155
22. Camps, M., Nichols, A., Gillieron, C., Antonsson, B., Mudae, M., Chabert, C., Boschert, U. & Arkinstall, S. (1998) Science 280, 1262–1265