Human and rat testis express two mRNA species encoding variants of NRD convertase, a metalloendopeptidase of the insulinase family

Véronique HOSPITAL*, Annik PRAT*, Catherine JOULIE*, Dorra CHÉRIF†, Robert DAY‡ and Paul COHEN*[1]

*Laboratoire de Biochimie des Signaux Régulateurs Cellulaires et Moléculaires, Unité de Recherche Associée au Centre National de la Recherche Scientifique 1682, Université Pierre et Marie Curie, 96 Bd Raspail, 75006 Paris, France, †Centre d'Etude du Polymorphisme Humain, 27 rue Juliette Dodu, 75018 Paris, France, and ‡JA DeSève Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, 110 Pine Ave West, Montreal, Que, Canada H2W 1R7

Rat testis NRD convertase (EC 3.4.24.61) is a Zn^{2+}-dependent endopeptidase that cleaves, *in vitro*, peptide substrates at the N-terminus of Arg residues in dibasic sites. This putative processing enzyme of the insulinase family of metallopeptidases exhibits a significant degree of similarity to insulinase and two yeast processing enzymes, Axl1 and Ste23. We report the cloning of two human testis cDNA species encoding isoforms of NRD convertase, hNRD1 and hNRD2. Whereas the hNRD1 transcript (3.7 kb) is equivalent to the previously characterized rat cDNA (rNRD1), hNRD2 and rNRD2 are 3.9 kb novel forms containing a nucleotide insertion encoding a 68-residue segment. This motif, which is inserted N-terminal of the Zn^{2+}-binding site, HXXEH, is contained within the most conserved region among the insulinase family members. Analysis of the deduced primary sequences revealed 92% identity between rat and human orthologues. The human gene encoding NRD convertase was localized to chromosome 1p32.1–p32.2. Whereas NRD convertase is mostly expressed in testis and in 24 cell lines, low mRNA levels were detected in most of the 27 other tissues tested.

INTRODUCTION

The activation of numerous proproteins during their transit through the secretory pathway requires limited proteolysis at specific sites that most commonly comprise basic residues organized as singlets or doublets (reviewed in [1–3]). Seven mammalian proprotein convertases involved in this type of post-translational processing, and related to bacterial subtilisin and yeast kexin, have been characterized [4–7]. These enzymes cleave at the C-terminus of basic amino acids and the exposed basic residues are subsequently removed by carboxypeptidase E or carboxypeptidase D [8,9].

The search for the enzyme(s) responsible for the processing of somatostatin 14 led us to purify and characterize a novel Zn^{2+}-metalloendopeptidase [10–13] and clone its cDNA [14]. *In vitro* this enzyme cleaves various peptide substrates such as somatostatin 28, dynorphin A and atrial natriuretic factor at the N-terminus of Arg (R) residues in dibasic sites and was thus named NRD convertase (Nardilysin, EC 3.4.24.61; reviewed in [15]). Initially isolated from rat cortex, the enzyme was purified and cloned from testis, where it is expressed abundantly [13,14]. The predicted 1161-residue rat NRD convertase sequence is characterized by the presence of a putative signal peptide, a highly acidic stretch of 71 residues (79% Glu and Asp) and a zinc-binding motif, HXXEH [14]. The presence of this motif, together with an overall 35% similarity to *Escherichia coli* protease III (Pitrilysin, EC 3.4.24.55) [16] and 48% similarity to rat or human insulinase (Insulysin, EC 3.4.24.56) [17,18], clearly identified NRD convertase as a member of the insulinase family of metalloendoproteases [14,19]. Several members of this family are implicated in the limited proteolysis of proteins or peptides, such as the mitochondrial matrix-processing protease, which clips nuclear encoded protein precursors [20–22], and yeast Axl1 and Ste23, which produce mature a-factor [23,24]. Although the substrates *in vivo* of NRD convertase have not been yet identified, its cleavage specificity and its membership of the insulinase family suggest its involvement in proprotein processing events. Interestingly, the acidic stretch, unique to NRD convertase, is inserted 35 residues upstream of the Zn^{2+}-binding motif, within the most conserved region of the insulinase family members.

In rat testis, hybridization and immunocytochemical studies *in situ* showed that NRD convertase expression is restricted to germ cells and is maximal in elongated spermatids. In this cell type the enzyme is present in the cytoplasm, where it is strongly associated with two microtubular structures: the manchette and the flagellum [25]. In addition, NRD convertase is still present in the flagella of mature spermatozoa. These results suggest its possible participation in the morphological differentiation of the spermatid and/or in spermatozoon motility.

In view of the possible implication of NRD convertase in male spermatogenesis, in sperm motility and in other aspects of reproductive biology that could influence male sterility [26,27], we have undertaken the characterization of the human cDNA. Here we report the cloning and sequencing of a second form of human and rat cDNA encoding novel NRD convertase species. These isoforms differ from the previously characterized rat enzyme [14] by a 68-residue segment inserted within the most conserved region of the insulinase family of metallopeptidases, containing the HXXEH motif. The possible role of such an insertion on NRD convertase biochemical properties will be discussed.

EXPERIMENTAL

Isolation and characterization of cDNA clones encoding human NRD convertase

A human testis λgt10 cDNA library (Clontech, Palo Alto, CA, U.S.A.) was screened by using standard procedures with a

Abbreviations used: NRD convertase, endopeptidase cleaving peptide substrates at the N-terminus of Arg residues in dibasic sites; SLIC, single-strand ligation of cDNA.

[1] To whom correspondence should be addressed.

The nucleotide sequence results reported here will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers X93207 (hNRD2), X93208 (rNRD2) and X93209 (hNRD1).

615 bp *Pst*I/*Bam*HI fragment of rNRD convertase cDNA (nt 734–1348) [14] labelled by random priming in accordance with the manufacturer's recommendations (Rediprime DNA labelling system; Amersham, Little Chalfont, Bucks., U.K.). Approx. 10^6 recombinant phages were screened and the insert size of the purified positive clones was determined by the use of PCR with a pair of amplimers (Clontech). After restriction mapping analysis, inserts were cloned in the pCRII vector (TA Cloning System; Invitrogen, Carlsbad, CA, U.S.A.). A second screening of the library was performed with a 5′-specific probe corresponding to the 438 bp *Eco*RI/*Bgl*II fragment of the rNRD convertase cDNA (nt 1–438) [14].

Sequencing of the human NRD convertase cDNA and computer analysis

Restriction fragments subcloned into pBluescript vectors (Stratagene, La Jolla, CA, U.S.A.) were sequenced by the dideoxynucleotide chain-termination method [28] with the Sequenase (Version 2.0) DNA-sequencing kit (United States Biochemical/Amersham, Little Chalfont, U.K.). Computer sequence analysis was performed with BISANCE [29] and DNA Strider [30]. Similarity searches were performed with the FASTA algorithm [31] and the GenPro databank (version 95).

Cloning by anchored PCR of mRNA 5′ ends

This strategy (referred to as SLIC for single-strand ligation of cDNA) was performed as described in [32]. All the NRD convertase-specific oligonucleotides used (O1 to O9) are shown in Figure 1 and the SLIC primers (A5′NV, A5′1 and A5′2) were as described in [32]. Briefly, the first cDNA strand was synthesized by avian myeloblastosis virus reverse transcriptase (New England Biolabs, Hitchin, U.K.) using O1 and 1 μg of human testis poly(A)$^+$ RNA (Clontech). The 50-mer A5′NV oligonucleotide was ligated to the cDNA 3′ end with T4-RNA ligase (New England Biolabs). The ligation product was amplified by PCR with the oligonucleotide pair A5′1/O2. A second nested PCR amplification was then performed on an aliquot of the first amplification with the oligonucleotide pair A5′2/O3. Both amplifications were performed for 30 cycles and thermal cycling was as follows: denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s and elongation at 72 °C for 90 s. PCR products were analysed on 2 % (w/v) agarose gels, subcloned into pCRII vector (TA Cloning System; Invitrogen) and sequenced on both strands. A second round of SLIC/PCR was conducted in the same conditions with O7 to initiate the reverse transcription and O8 and O9 to perform nested PCR amplifications.

Cloning of a partial rat NRD2 convertase cDNA

Rat testis poly(A)$^+$ RNA was purified with the mRNA isolation kit (Bioprobe Systems, Tustin, CA, U.S.A.) in accordance with the manufacturer's recommendations. Single-stranded cDNA was synthesized by using O1, 1 μg of rat testis poly(A)$^+$ RNA and avian myeloblastosis virus reverse transcriptase (New England Biolabs). A first PCR amplification was performed with the oligonucleotide pair C22a/C22b and the product was amplified in a second nested PCR with the oligonucleotide pair 5′Dac/O3. Amplification and analysis of PCR products were performed as above. The sequences corresponding to O1 and O3 are almost identical in rat and human and are shown in Figure 1. The sense C22a, anti-sense C22b and sense 5′Dac oligonucleotides correspond respectively to nt 264–280, 761–745 and 433–468 in the rNRD convertase sequence [14].

cRNA and oligonucleotide probes

Anti-sense [^{32}P]UTP-labelled cRNA probes were obtained by transcription *in vitro* (Amersham and Stratagene) of linearized plasmids bearing either the 960 bp *Pst*I fragment of rNRD convertase cDNA (probe A) [14] or the 204 bp insertion (probe B). Oligonucleotides were labelled with T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (Amersham), then purified as described [33].

Northern blot analysis

Total RNA was isolated from rat tissues or cultured cells by guanidine isothiocyanate extraction followed by precipitation with LiCl. Rat testis poly(A)$^+$ RNA was purified as described above, and human testis poly(A)$^+$ RNA was purchased from Clontech. For the analysis of tissue and cell distribution, 5 μg of total RNA was loaded in each lane of a 1.5 % (w/v) agarose gel. For the analysis of NRD1 and NRD2 expression in testis, 2 μg of poly(A)$^+$ RNA was used. Gel electrophoresis, blotting and hybridization with [^{32}P]UTP-labelled cRNA probes were performed as described previously [34]. Hybridization with 5′-^{32}P-labelled oligonucleotides was performed as follows. The filters were first prehybridized in 6 × SSC/2 × Denhardt's solution/0.25 % SDS/100 μg/ml denatured salmon sperm at 20 °C below the oligonucleotide probe's estimated dissociation temperature [T_d (°C) = 4(G + C) + 2(A + T)] for 2 h, and then hybridized with 10^7 c.p.m. labelled oligonucleotides under the same conditions for 16 h. The filters were washed in 2 × SSC/0.05 % SDS for 1 h at room temperature, then 15 min at 20 °C below the calculated T_d and finally 10 min at the calculated T_d. The RNA ladder markers (0.24–9.5 kb) were purchased from Gibco/BRL (Gailthersburg, MD, U.S.A.).

Cell lines

We examined NRD convertase expression in 24 cell lines. The constitutively secreting cells included HepG2 (hepatocellular carcinoma, human), Cos-1 (kidney, African green monkey), BSC40 (kidney epithelial cells, African green monkey), BRL-3A (liver cells, Buffalo rat), Y1 (adrenal cortex, mouse), LoVo (colon adenocarcinoma, human), DAMP (fibroblasts, mouse), NIH/3T3 (embryo, mouse), Ltk$^-$ (connective tissue, mouse), TM3 (testicular Leydig cells, mouse), TM4 (testicular Sertoli cells, mouse), Caco-2 (colon adenocarcinoma, human), LLC-PK_1 (proximal kidney tubule epithelial cells, pig) and MDCK (kidney epithelial cells, dog). The selected cell lines with regulated secretory pathways included Neuro2A (neuroblastoma, mouse), NG108 (neuroblastoma–glioma hybrids, rat–mouse), SK-N-MIXC (neuroepithelioma, human), GH4C1 (somatomammotroph, rat), GH3 (somatomammotroph, rat), AtT-20 (corticotroph, mouse), αT3-1 (gonadotrophs, mouse), Rin m5F (insulinoma, rat), PC12 (pheochromocytoma, rat) and βTC-3 (insulinoma, mouse).

Chromosomal mapping

The procedure used for fluorescence hybridization *in situ* on human metaphase chromosomes is a modification of the method described [35]. A 3′ 960 bp *Eco*RI–*Eco*RI fragment of the human cDNA was labelled by nick translation with bio-16-dUTP (Boehringer Mannheim, Germany). A 50 ng sample of the purified (Sephadex G50 column) probe was ethanol-precipitated in the presence of 20 μg of sonicated DNA from salmon sperm

and dissolved in 40 μl of a hybridization mixture containing 50 % (v/v) formamide, 2 × SSC and 10 % (v/v) dextran sulphate at pH 7. After hybridization and post-hybridization washes, the biotinylated probe was detected by avidin–FITC and amplified twice with additional layers of biotinylated goat anti-avidin and avidin–FITC. For chromosomal localization, fluorescent R-bands were obtained as described previously [35].

RESULTS

Existence of two NRD convertase mRNA species in human and rat testis

A human testis cDNA library screened with a 615 bp *Pst*I–*Bam*HI fragment of the previously characterized rat cDNA (nt 734–1348) [14] led to the isolation of several partial cDNA species lacking the 5′ end of the coding sequence. A subsequent screening with a 5′ rat probe (nt 1–438) [14] did not result in the isolation of cDNA species encompassing the 5′ end. The determination of the complete cDNA sequence of human NRD convertase was finally obtained by a SLIC/PCR strategy. Starting from a single-strand cDNA initiated with oligonucleotide O1, two PCR products were obtained when the antisense oligonucleotides O2 or O3 were used (Figure 1). Sequence analysis of the longest products revealed that they contained an additional 204 nt, encoding an in-frame insertion of 68 residues (in bold in Figure 1) located between the acidic stretch (boxed in Figure 1) and the HXXEH motif. The existence of two distinct hNRD mRNA species was confirmed by Northern blot (Figure 2): the insertion-specific oligonucleotide O5 detected hNRD2 transcripts (3.9 kb; determined cDNA size 3851 bp), whereas oligonucleotide O4, hybridizing with sequences flanking the insertion site, revealed hNRD1 transcripts (3.7 kb; determined cDNA size 3647 bp) (Figure 1).

The sequence of the 135 nt 5′ untranslated region was determined by SLIC/PCR starting from a single-strand cDNA initiated with oligonucleotide O7 (Figure 1). In both human (Figure 1) and rat [14], this region presents a short open reading frame, ORF_0, adjacent to that of NRD convertase. The human 3′ untranslated region is 24 nt long, compared with 30 nt in rat [14], and both contain two overlapping polyadenylation sites, the first comprising the TAA stop codon. Finally, sequence analysis of all the clones isolated from the human testis library revealed that hNRD1 and hNRD2 cDNA species differ only by the 204 nt insertion, suggesting that the two mRNA species are generated by an alternative splicing event.

Analysis of rat testis mRNA by SLIC/PCR also revealed the presence of a similar 204 bp insertion. By using the oligonucleotide probes O4 and O5, specific to both human and rat sequences, the existence of rNRD1 and rNRD2 transcripts was confirmed (Figure 2). To estimate the relative amounts of NRD1 and NRD2 transcripts in both human and rat testicular mRNA, the common oligonucleotide O6 was used (Figures 1 and 2).

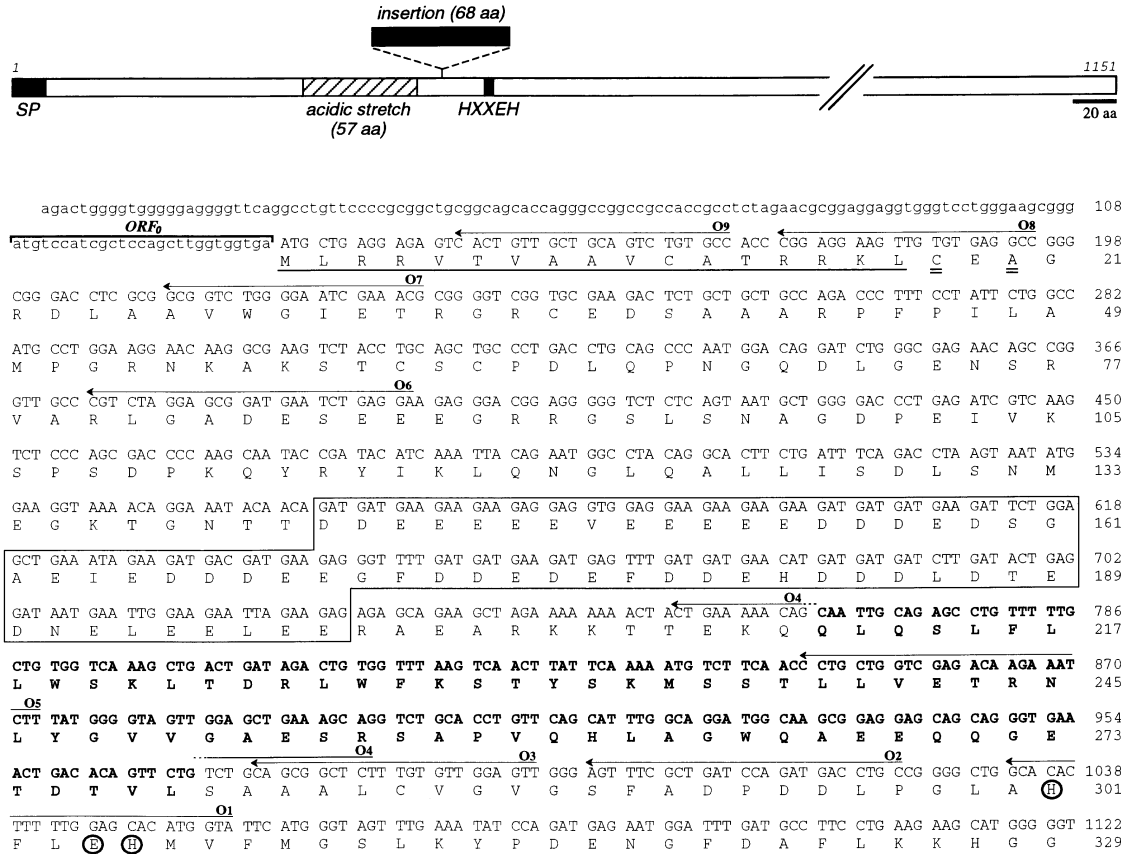

Figure 1 Schematic representation of human NRD1 or NRD2 convertases and of the 5′ region of their cDNA and the corresponding amino acid sequence

The putative signal peptide (SP) is underlined in the sequence and the potential excision sites are doubly underlined. Circled are the HXXEH residues implicated in Zn^{2+} binding. The various oligonucleotides (O1 to O9) are shown by arrows. The small upstream open reading frame (ORF_0) is indicated. The sequence encoding the 68-residue insertion specific to NRD2 convertase is in bold type; the acidic stretch is boxed. Abbreviation: aa, amino acid residues.

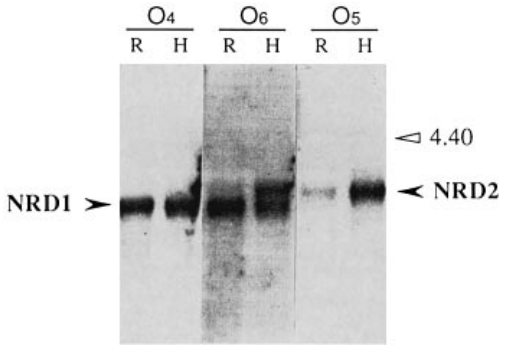

Figure 2 Evidence for NRD1 and NRD2 transcripts in rat and human poly(A)$^+$ RNA

A Northern blot analysis of 5 μg of rat (R) or human (H) poly(A)$^+$ RNA was performed with either O4 and O5 oligonucleotide probes, which are specific to NRD1 and NRD2 isoforms respectively, or O6 oligonucleotide probe recognizing both isoforms. The position of the size marker is shown at the right (in kb). The X-ray exposure time for each blot was one week.

Scanning densitometry suggested that, whereas NRD1 and NRD2 transcripts are expressed at the same level in human testis, NRD2 transcript is a minor species (less than 10%) in rat testis.

Expression of NRD convertase in rat tissues and cell lines

Expression of NRD convertase was analysed by Northern blot (Figure 3) with a cRNA probe (probe A) common to NRD1 and NRD2 transcripts. Except in testis, where they are remarkably abundant, NRD transcripts were detected at low levels in most of the 27 other tissues tested. Hybridization *in situ* confirmed their presence in a number of tissues such as brain and adrenal (P. Cohen, unpublished work).

Using the same cRNA probe, we observed that, after a long exposure, NRD transcripts were detected in all of the 24 endocrine and non-endocrine cell lines tested (Figure 4). Furthermore the presence of NRD2 transcripts was investigated with a cRNA probe corresponding to the 204 nt insertion (probe B; Figure 4). It should be noted that the rat cRNA probes used

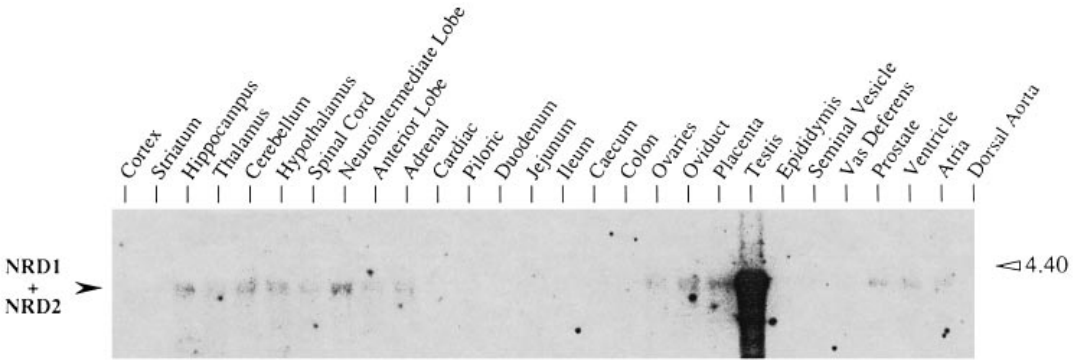

Figure 3 NRD convertase expression in rat tissues and its prevalence in the testis

Total RNA (5 μg) extracted from various rat tissues was analysed by Northern blotting with probe A common to NRD1 and NRD2 isoforms. The exposure time was 2 h. The position of the size marker is shown at the right (in kb).

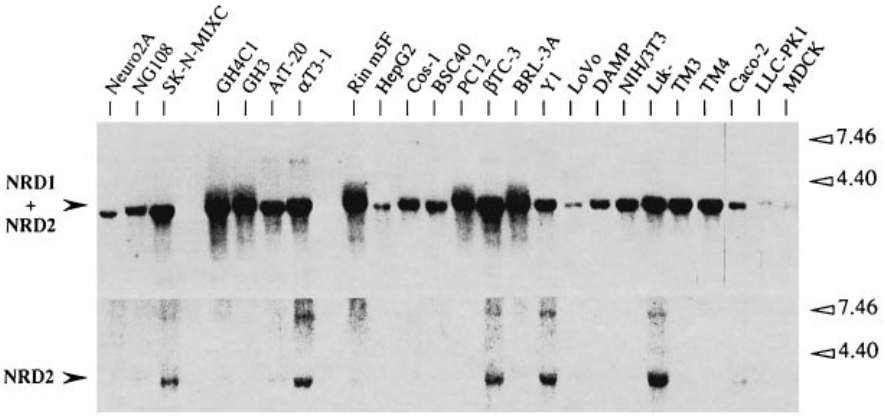

Figure 4 Analysis of the total NRD convertase transcripts and NRD2 transcripts in 24 cell lines

Total RNA (5 μg) extracted from various cell lines was analysed by Northern blotting using either probe A, allowing the detection of both NRD1 and NRD2 isoforms (top panel), or probe B, specific to NRD2 isoform (bottom panel). Exposure times were 12 h (probe A) and 48 h (probe B). The positions of the size markers are shown at the right (in kb).

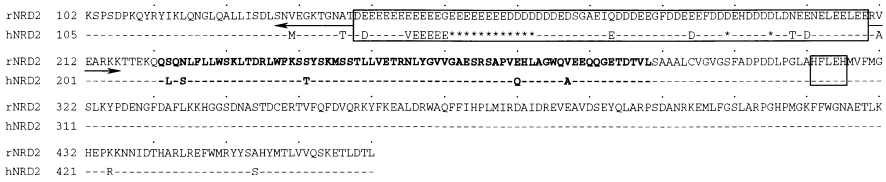

Figure 5 Alignment of rat and human NRD2 convertases in a 370-residue region containing the acidic domain, the insertion and the Zn^{2+}-binding motif

The conserved positions are represented by dashes and the gap by asterisks. The acidic domains are composed of an acidic stretch (boxed) and two short anchor regions (arrows). The insertions are in bold type and the residues (HFLEH) involved in binding Zn^{2+} are indicated.

allowed the detection of NRD transcripts in cell lines derived from human, mouse and monkey, suggesting a high degree of sequence conservation between these species. Although not quantitative, the results suggested that the ratio of NRD2 to NRD1 transcripts varies from one cell line to another: either not detectable or expressed at low levels in most of the cell lines tested, NRD2 transcripts were nevertheless well detected in five of them, including both non-endocrine (Y1 and Ltk^-) and endocrine (SK-N-MIXC, αT3-1 and βTC-3) cell lines. Finally, we noted the presence of hybridizing mRNA forms of sizes close to 7.5 kb, which were not further characterized.

Sequence comparison of human and rat proteins

Alignment of the protein sequences of human and rat NRD1/2 convertases, composed of 1151/1219 and 1161/1229 residues respectively, revealed overall 92 % identity and 94 % similarity. A third of the observed residue changes reside in the first and last 50 residues. Furthermore the major difference observed between the human and rat sequences lies in the length of the acidic stretch (Figure 5). In human, this segment is indeed 14 residues shorter and exhibits only 43 acidic residues (out of 57) instead of 56 (out of 71) in the rat one. The 68 residues encoded by the 204 nt insertion is as well conserved as the rest of the sequence (five non-conservative substitutions; Figure 5) and shows no similarity with any known protein sequence reported in data banks.

Alignment of the protein sequences of all the members of the insulinase family revealed a conserved region surrounding the Zn^{2+}-binding site composed of approx. 200 residues. This alignment further emphasized the uniqueness of NRD convertase. Indeed, this metalloendopeptidase is distinguished by the presence of either one or two insertions: the acidic domain (composed of the acidic stretch plus eleven and seven residues at its N-terminus and C-terminus; Figure 5) and the 68-residue segment. As shown in Figure 6, both inserts interrupt this highly conserved region.

Chromosomal mapping of the human NRD convertase gene

Of the 25 metaphases examined, 16 (64 %) had one specific signal on chromosome 1 at band 1p32.1–p32.2, and nine metaphases (36 %) exhibited double spots on the two chromosomes 1 (results not shown). Noticeably, the NRD convertase gene locus resides in a chromosome region reported to be particularly fragile [36].

DISCUSSION

We report here the cloning of two human NRD convertase cDNA species. Whereas the first, hNRD1, corresponds to the previously characterized rat cDNA, the second, hNRD2, differs only by a 204 nt insertion. This in-frame insertion encodes a 68-residue additional segment in the vicinity of the Zn^{2+}-binding motif. An NRD2 transcript was also characterized in rat testis. Similarly to the rest of the sequence, the 68-residue insertion is highly conserved, exhibiting a 92 % identity between human and rat. By using specific oligonucleotide probes, the presence of both NRD1 and NRD2 transcripts in either rat or human testis poly(A)$^+$ RNA was confirmed. The fact that NRD2 cDNA has not been previously characterized is probably due to its low representation in rat testis cDNA libraries [14]. Indeed, whereas

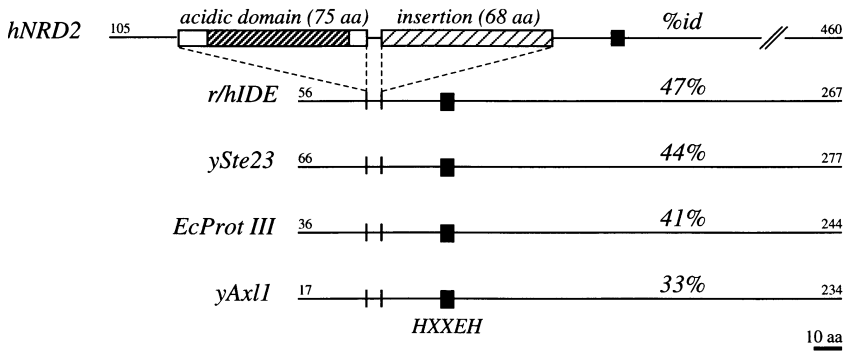

Figure 6 Alignment of hNRD2 convertase with some of its closest members of the insulinase family of metalloendopeptidases

Symbolized by a thin line is the approx. 200 residues (aa) long highly conserved region among all the members of the insulinase family, which comprises the HXXEH Zn^{2+}-binding motif symbolized by a black square. The numbers refers to the amino acid sequence of each endoprotease. hNRD2 is distinguished by the presence of an acidic domain composed of a 43-residue acidic stretch framed by eleven and seven residues on its N-terminus and C-terminus respectively, and by a 68-residue segment positioned between the acidic stretch and the HXXEH motif.

in human testis NRD1 and NRD2 transcripts seem to be expressed at the same level, the NRD2 transcript is a minor species in rat testis (Figure 2). Our Northern blot analysis of 28 different tissues defines the testis as the major site of NRD convertase expression (Figure 3). This conclusion agrees with a previous analysis of the enzyme activity in five selected tissues [13]. In addition, NRD transcripts were also detected in all endocrine and non-endocrine cell lines tested (Figure 4). Although not quantitative, the specific detection of NRD2 transcripts in the 24 cell lines tested suggests that this isoform is in most cases a minor species and that its ratio to total NRD transcripts varies independently of the constitutive or regulated character of the selected cell lines. Surprisingly, the TM3 and TM4 cell lines, which derive from Leydig and Sertoli cells respectively, display significant levels of NRD transcripts, whereas their original primary cells do not express either the mRNA or the protein [25]. This suggests an up-regulation of the enzyme expression in these established cell lines and possibly in others (Figure 4). In addition, our results suggest that no strict association of one isoform with a given cell type exists, because in almost all cases both isoforms are co-expressed.

The well-conserved 5′ unstranslated region is characterized by a small upstream open reading frame, ORF_0 (Figure 1), which might have a role in the regulation of the translation initiation of NRD convertase, as reported for *GCN4* mRNA [37] and *CPA1* mRNA [38]. The acidic domain and/or the 68-residue insertion, characteristic of NRD convertase, might yet represent other regulatory sequences, which could influence the activity and/or specificity of the enzyme. Several Ca^{2+}-binding proteins exhibit clusters of acidic amino acid residues, suggesting that they might be involved in the observed divalent cation binding [39,40]. Therefore it is possible that cationic compounds could bind to the acidic stretch, thereby modulating the activity of the enzyme and/or its interaction with other proteins. Indeed, in a number of other proteins, including nuclear histone-binding protein N1/N2 [41] and nucleolin [42], clusters of acidic residues have been implicated to have a key role in possible interactions with other proteins. In this context it is interesting to note that the rat acidic stretch is 12 residues longer than the human one, resulting in a greater number of negative charges in this region: 56 instead of 43 (Figure 5). The role of a such a domain *in vivo*, lying 35 or 103 residues upstream of the HXXEH motif in the NRD1 or NRD2 isoforms, is yet to be determined.

The 68-residue insertion, specific to the NRD2 isoform, lies between the acidic domain and the Zn^{2+}-binding motif. No suggestion about its possible function could be derived from either data bank sequence comparisons or secondary structure predictions. A careful characterization *in vitro* was previously performed on the purified rat testis enzyme [13]. If the ratio of rat NRD2 to NRD1 convertases reflects that of their transcripts, the observed properties would essentially reflect that of NRD1 convertase. Production of each isoform via the vaccinia virus expression system is now being undertaken and a characterization *in vitro* will be performed to measure the possible impact of the presence of the insertion on the catalytic properties of NRD2 convertase. Attempts to generate antibodies against this novel sequence were unsuccessful, possibly because of its hydrophobic nature. NRD convertase has previously been shown to be localized in two distinct subcellular compartments: the cytoplasm of rat spermatids [25] and the endoplasmic reticulum of endocrine cells (C. Tougard, personal communication). The targeting of NRD1 or NRD2 convertases to the cytoplasm and/or the secretory pathway, which is possibly correlated with the presence or absence of the 68-residue insertion, remains to be investigated by transfection experiments.

The acidic stretch and the 68-residue insertion are both localized in the most conserved region among the members of the insulinase family of metalloendopeptidases (Figure 6) and constitute unique features of NRD convertase. A number of metallopeptidases were reported to exhibit functions distinct from their enzymic one: for example, Axl1 [24], mitochondrial matrix-processing proteases [22] and carboxypeptidase E [43] also intervene in processes such as yeast budding, electron transfer and protein sorting respectively, implicating domains distinct from the catalytic one. In view of the existence of NRD convertase isoforms and the dual subcellular localization of this enzyme, it is possible that one or more functions unrelated to its enzymic activity could be discovered in the future. Nevertheless, the results presented here provide the first indication of the existence of isoforms of NRD convertase in both human and rat, and it can be expected that their expression in heterologous cells will permit the study of their comparative properties and of the general function(s) of this complex enzyme.

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