

Rapid report

The prion-like protein Doppel fails to interact with itself, the prion protein and the 37 kDa/67 kDa laminin receptor in the yeast two-hybrid system

Christoph Hundt, Stefan Weiss*

*Laboratorium für Molekulare Biologie-Genzentrum-Institut für Biochemie der Ludwig-Maximilians-Universität München,
Feodor-Lynen-Str. 25, D-81377 Munich, Germany*

Received 2 December 2003; received in revised form 13 February 2004; accepted 17 February 2004

Available online 12 March 2004

Abstract

The prion-like protein termed Doppel (Dpl) shows approx. 25% sequence identity with all known prion proteins (PrP). We recently showed that the cellular PrP is dimeric under native conditions, a finding which was confirmed by the investigation of its crystal structure. Human PrP further interacts with its cellular receptor, the 37 kDa/67 kDa laminin receptor (LRP/LR). Here we report that human Doppel fails to interact with (i) itself, (ii) the human 37 kDa/67 kDa LRP/LR, and (iii) the human cellular prion protein (huPrP) in the yeast two-hybrid system. Our findings suggest that Dpl and PrP are not related or are only marginally related with respect to their ligand binding behaviour.

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Keywords: Doppel; Dpl; Prion; PrP; 37 kDa/67 kDa laminin receptor; LRP/LR; Yeast two-hybrid system; Scrapie; Ligand

Prion diseases are fatal neurodegenerative disorders which occur in man or animals (for review see Refs. [1–3]). A major feature of these diseases is the conversion of the non-pathogenic, cellular prion protein PrP^c into the pathogenic isoform PrP^{Sc} (for review see Ref. [4]). This isoform has a strong tendency to polymerize forming amyloid aggregates. Recent publications demonstrated that PrP^c shows dimerization properties: (i) PrP dimers have been observed as intermediate states during PrP-multimerization [5,6], (ii) the crystal structure of human PrP reveals a dimeric nature of the prion protein [7], and (iii) we recently showed that PrP is dimeric under native conditions, interacts with itself in the yeast two-hybrid system and in recombinant Semliki Forest virus (SFV)-transfected BHK cells [8]. Furthermore, antibodies directed against dimeric PrP interfere with PrP^{Sc} propagation in cultured cells [9] and a soluble dimeric PrP generated by fusion to immunoglobulin Fc γ binds PrP^{Sc} in vivo and antagonizes prion disease [10]. Covalently linked

dimers might act as powerful tools to interfere with the prion life cycle [11].

Besides the self-interaction of the prion protein, a series of ligands for the prion protein have been identified. Among them are protein X [12], the 37 kDa/67 kDa laminin receptor [13] acting as the cellular receptor for PrP^c [14] (for reviews: see Refs. [15,16]) and proven to be required for PrP^{Sc} propagation in cultured cells [17], as well as HSPGs acting as co-factors/co-receptors for PrP [18]. Transgenic ectopic expression of specific antisense LRP RNA in the mouse brain resulted in a knock-down of the 37 kDa/67 kDa laminin receptor in the hippocampus and the cerebellum [19].

Recently, a prion-like protein was identified termed Doppel (Dpl) [20]. Dpl is approx. 25% homologous to PrP and has a series of common features with normal PrP (for summary see Table 1). Although both proteins differ in size (for review, see Ref. [21]), both are processed during maturation, a process in which signal peptides and signal sequences become removed. Both proteins are glycosylated at two C-terminal residues. Whereas PrP reveals three α -helices and two β -sheets, Doppel encompasses four α -helices and two β -sheets (see Table 1). Within the *Prn*-p-gene defined mutations/polymorphisms exist causing trans-

* Corresponding author. Tel.: +49-89-2180-76951; fax: +49-89-2180-76999.

E-mail address: Weiss@lmb.uni-muenchen.de (S. Weiss).

Table 1
Comparison of the prion protein with its homolog Doppel

Feature	Prion protein ^a	Doppel protein
Universal properties		
protein size (human)	253 aa	179 aa [20]
mature form (human)	aa 23–230	aa 27–154 [29]
signal peptide/signal sequence	yes	yes [20]
disulfide bridge	one	two [20]
octarepeat region	yes	no [20]
copper binding	yes	yes [22]
glycosylation sites	two	two [20]
mutations/polymorphisms	at least 22 (human)	four (human) [37]
structure	three α -helices/ two β -sheets	four α -helices/ two β -sheets (murine) [38] (human) [39]
pK treatment	sensitive (PrP ^c)	sensitive [40]
Expression		
during RNA embryogenesis	yes	yes [20]
in the CNS	high level	low level [20]
PrP ^{0/0} mice	no	upregulation [20]
main local expression	brain	testis [29]
PrP ^{Sc} propagation	PrP ^c -dependent	Dpl dispensable [25]
Oxidative stress	SOD activity	induction of oxidative stress markers [41]
Ligands		
binding to PrP	yes [8]	no ^b
binding to Dpl	no ^b	no ^b
binding to 37 kDa/67 kDa LRP/LR	yes [13,18]	no ^b

^a For PrP citations see Ref. [42].

^b Data described in this manuscript.

missible spongiform encephalopathies (TSEs) in humans. Defined mutations within the *Prn-d*-gene have also been observed (Table 1). However, their relevance for the development of TSEs is speculative. In contrast to PrP, Doppel lacks the octarepeat region which is responsible for copper binding. Surprisingly, it was recently shown that Dpl binds copper via the α B/B' -loop- α C subregion [22]. The proposed transmembrane region of PrP (aa 106–126) which seems to have neurotoxic effects is also missing in Dpl [20]. In contrast to PrP which exhibits one disulfide bridge, Dpl contains four cystein residues which are able to form two disulfide bridges [20]. The role of Dpl in prion diseases remains speculative since expression of Dpl in the CNS of mice did not influence the development of TSEs [23], its expression is not modified in scrapie-infected cells and in brains of CJD patients [24] and Dpl is not required for PrP^{Sc} generation and prion disease progression [25]. Although Doppel and PrP are raft-associated proteins, they do not seem to share the same membrane microenvironment [26]. The onset of ataxia and Purkinje cell loss—originally described as a phenotype for the loss of function of PrP^c in PrP knock-out mice [27]—has been

shown to be a consequence of Dpl expression in the brain [28]. Doppel is mainly expressed in testis [29] and Dpl knock-out mice reveal male sterility [30].

Very little is known about the potential binding partners of Doppel. PrP is able to interact with itself [8] and with the 37 kDa/67 kDa laminin receptor [13], acting as the cellular receptor for PrP^c [14] required for PrP^{Sc} propagation in neuronal cells [17]. A potential direct interaction between Dpl and PrP has not been investigated so far. Therefore, we wanted to investigate whether Doppel is able to interact with itself (i) the prion protein (ii) and the 37 kDa/67 kDa LRP/LR (iii) employing the yeast two-hybrid system as a powerful method for the investigation of protein–protein interactions (for review, see Ref. [31]).

The Dpl27–154 protein failed to interact with GST::huPrP23–230 (Fig. 1, row 2). As a control, GST failed also to interact with Dpl27–154 (Fig. 1, row 1). Dpl27–154 did also not interact with LRP44–295 the cellular receptor for PrP [14] (Fig. 1, row 3), whereas GST::huPrP23–230 interacts with its receptor LRP44–295 (Fig. 1, row 5). Dpl27–154 shows also no interaction with itself in the yeast two-hybrid system (Fig. 1, row 4), whereas huPrP23–230 interacts with itself in the same system [8].

In order to exclude the possibility that the failure of Doppel to interact with PrP, LRP and itself might be due to the lack of expression in the yeast two-hybrid system, we investigated whether Dpl is expressed in bait and prey position. After co-transformation of yeast cells with the bait plasmid pSH2–1-Dpl27–154 and the prey plasmid pJG4–5-Dpl27–154, we analyzed the crude lysate of the yeast cells by Western blotting employing a polyclonal anti-Dpl antibody. We observed molecular weights of approx. 21 kDa for the bait and approx. 36 kDa for the prey protein (Fig. 2), which is in good harmony with the expected molecular weights of 21 kDa (lexA-DNA-binding domain: 7 kDa plus Dpl27–154: 14 kDa) and 36 kDa (acidic activation domain B42: 22 kDa plus Dpl27–154: 14 kDa). The band at approx. 70 kDa might be due to a cross-reactivity of the anti-Dpl-antibody. These data demonstrate that the failure of Doppel to interact with itself, the prion protein and the LRP/LR is not due to the lack of Doppel expression.

Our finding that Dpl failed to interact with itself in the yeast two-hybrid system was predicted by modelling the potential Dpl and PrP interaction interfaces suggesting that Dpl might fail to dimerize [32]. The model states that PrP dimerization might take place via a β -hairpin structure located between aa 119 and 128 of PrP, a non-polar region which is lacking in the Dpl protein suggesting that dimerization here might take place via another domain. We have recently shown that one major interaction domain for the PrP/PrP interaction represents the octarepeat region [8], which is highly conserved in different mammalian species [33]. One feature of the octarepeat region is the binding of copper [34], which might be triggering a copper-mediated protein–protein interaction. Copper-in-

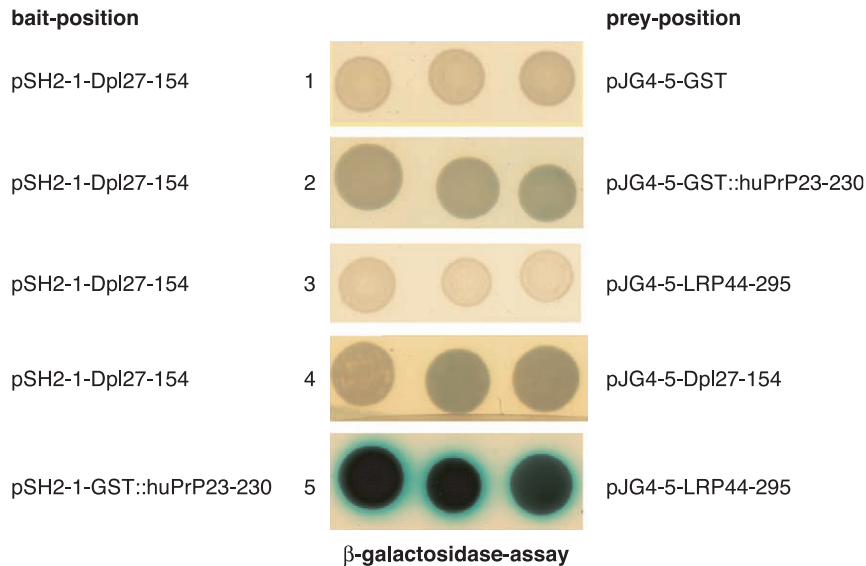


Fig. 1. Interaction study of the prion-like protein Dpl. HuDpl27–154 in bait-position (rows 1–4) was co-expressed with GST (row 1), GST::huPrP23–230 (row 2), LRP44–295 (row 3) and Dpl27–154 (row 4) in prey-position of the yeast two-hybrid system. For positive control GST::huPrP23–230 in bait position and LRP44–295 in prey position was co-expressed (row 5) [13,18]. Interactions were detected by the β -galactosidase reporter system. We amplified the Dpl27–154 (the mature form of Doppel [29]) encoding cDNA by RT-PCR using oligodeoxyribonucleotides flanking the Dpl-sequence and introducing *EcoRI* (5') and *SalI* (3') restriction sites from mRNA isolated from cultivated HeLa cells. The PCR-product was cloned into the vector pSH2-1 via *EcoRI* and *SalI* restriction sites resulting in pSH2-1-Dpl27–154. The Dpl27–154 encoding cDNA was excised from pSH2-1 via *EcoRI* and *SalI* and subcloned into pJG4-5 restricted with *EcoRI* and *XhoI* resulting in pJG4-5-Dpl27–154. All plasmids were confirmed by dideoxy sequencing. The construction of pSH2-1-GST, pSH2-1-GST::huPrP23–230 and pJG4-5-LRP44–295 was described previously [13]. The different bait and the prey plasmids as well as the reporter plasmid pSH18–34 (*lacZ*) were co-transformed into EGY48 yeast cells and transformants were tested in a β -galactosidase assay as described [8,18].

duced aggregation was also observed in Alzheimer's disease [35]. This octarepeat region is missing within the Dpl protein and therefore metal-induced dimerization is unlikely. A hypothetical Dpl/Dpl interaction might take place via the core region of the protein since human PrP/PrP interactions also take place via the PrP90–230 region [8]. However, results from the prion protein cannot be transferred to Doppel due to a more rigid structure of the latter which might result from two disulfide bridges. In addition, structural changes, e.g. domain swapping observed in the dimeric crystal structure of PrP [7] should be more hampered by the two disulfide bridges in the Dpl protein. Dimeric forms of Dpl did not appear under denaturing conditions, e.g. during analysis of the expression whereas dimeric forms of PrP were observed when PrP was expressed in neuroblastoma cells and in scrapie-infected hamster brains [36]. In contrast to PrP [8], Dpl indeed failed to interact with itself in the yeast two-hybrid system suggesting that both proteins have a different self-interacting behaviour probably due to significant structural differences.

No interaction partners of Doppel have been identified so far. In contrast, a series of interaction partners for the prion protein have been described (for review, see Ref. [15]). One of which represents the 37 kDa/67 kDa laminin receptor which acts as the cell surface receptor for the cellular prion protein [14]. A hypothetical direct interaction between Doppel and the prion protein has not been described. The lack of interaction between Doppel and

PrP as well as with 37 kDa/67 kDa LRP/LR together with the findings that (i) expression of Doppel in the CNS of mice does not modulate TSEs [23], (ii) Dpl expression is not modified in scrapie-infected cells and in the brain of CJD patients [24], and (iii) Dpl is dispensable for prion disease progression and PrP^{Sc} generation [25] suggests that Doppel might not play a crucial role in the life cycle of prions.

We excluded that the failure of Doppel to interact with itself, the prion protein and LRP/LR might be due to the lack of Doppel expression in the yeast two-hybrid system, since we proved Dpl expression in both prey and bait position. We assume that structural differences between Dpl and PrP might account for the different interaction behaviours of both proteins. Although our data do not support an essential role of the Doppel protein in prion diseases, further studies are required to investigate in more detail a possible contribution of Dpl in transmissible spongiform encephalopathies.

Acknowledgements

We thank Hermann M. Schätzl (TU, Munich) for providing us with the polyclonal anti-Dpl antibody. This work was supported by grant BMBF (Bundesministerium für Bildung und Forschung) 01-KO-0106, FAIR-CT-98-7020, QLRT-2000-02085 and the NoE NeuroPrion 506579 (all European Union) and LMU4 (Bavarian Prion Research

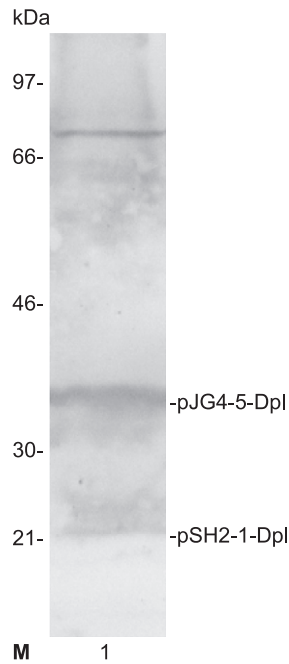


Fig. 2. Expression of Dpl in bait and prey position of the yeast two-hybrid system analyzed by Western blotting. Crude cell lysate (30 μ l) of EGY48 yeast cells were analyzed on a 12.5 % SDS PA gel, blotted and developed by a polyclonal anti-Dpl antibody (lane 1). Marker proteins are indicated (M). EGY48 yeast cells were co-transformed with pSH2–1-Dpl27–154 and pJG4–5-Dpl27–154 as described [8,18]. After 3 days of incubation in the presence of glucose at 200 rpm and 30 °C, cells were collected, washed (ddH₂O) and incubated at 30 °C at 200 rpm in the presence of galactose. After a further 3 days, cells were collected, washed (ddH₂O) and resuspended in 150 μ l SDS sample buffer. 30 μ l were loaded onto a SDS-polyacrylamide gel containing 12.5% acrylamide. Proteins were blotted on a polyvinylidene difluoride membrane, blocked and incubated overnight with a polyclonal anti-Dpl antibody (provided by Prof. Dr. H.M. Schätzl, TU Munich, Germany) (diluted 1:1000 in blocking solution). After washing with TBS/0.05% Tween 20, incubation of the blot with a peroxidase-conjugated secondary antibody (Sigma; dilution 1:2500) for 1 h was performed. Detection was done by enhanced chemiluminescence (Western Lightning, NEN).

Foundation). We thank Katharina Krüger and Annette Pahlich for excellent technical assistance.

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