RESEARCH ARTICLE

Interaction of Nup53 with Ndc1 and Nup155 is required for nuclear pore complex assembly

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ABSTRACT

Nuclear pore complexes (NPCs) are the gateways for nucleocytoplasmic exchange. The ordered assembly of these huge complexes from several hundred individual components into an intricate protein interaction network which deforms the two membranes of the nuclear envelope into a pore is only rudimentarily understood. Here, we show that the interaction between Nup53 and the integral pore membrane protein Ndc1 is essential for vertebrate NPC assembly. The Ndc1 binding site on Nup53 overlaps with a region that induces membrane bending and is specifically required to modulate this activity, suggesting that the membrane-deforming capability of Nup53 is adjusted during the NPC assembly process. We further demonstrate that the interaction of Nup53 and Nup155 has a crucial role in NPC formation as the main determinant of recruitment of Nup155 to the assembling pore. Overall, our results pinpoint the diversity of interaction modes accomplished by Nup53, highlighting this protein as an essential link between the pore membrane and the NPC, and as a crucial factor in the formation of the pore membrane.

KEY WORDS: Ndc1, Nuclear pore complex, Nup35, Nup53, Nup155

INTRODUCTION

The compartmentalization of eukaryotic cells entails the spatial organization of specialized functions in distinct membrane-bound organelles. The characteristic organelle of eukaryotes is the nucleus, which is enclosed by the double membrane of the nuclear envelope (NE). The NE forms a barrier that separates nuclear gene transcription from cytoplasmic protein synthesis, thereby ensuring accurate processing of the genetic information. Nuclear pore complexes (NPCs) intercept the NE at fusion sites of the two membranes and mediate the selective transport of macromolecules. In most eukaryotic cells, NPCs represent the largest protein complexes but contain only about 30 individual proteins, which are called nucleoporins (Nups), and are found in up to 32 copies per NPC (Cronshaw et al., 2002; Ori et al., 2013; Rout et al., 2000). They can be categorized into two groups: (1) scaffold nucleoporins, which form the NPC structural backbone and (2) barrier nucleoporins, which execute transport functions and exclude inert molecules from passage.

The core NPC scaffold resides close to the pore membrane and is formed by two conserved subcomplexes, the Nup107–Nup160 complex and the Nup93 complex (Nup84 and Nic96 complexes in S. cerevisiae). Nucleoporins of these subcomplexes show structural similarities to vesicle coats (Brohawn et al., 2008; Devos et al., 2004; Mans et al., 2004) and might stabilize the highly curved pore membrane. The core scaffold is linked to the pore membrane through integral membrane proteins. So far, four membrane nucleoporins have been identified in different organisms: Pom34, Pom152, Pom33 and Ndc1 in yeast (Chadrin et al., 2010; Lau et al., 2004; Miao et al., 2006; Wozniak et al., 1994) and Gp210, Pom121 and Ndc1, which is the only one conserved as an integral pore membrane protein, in vertebrates (Gerace et al., 1982; Hallberg et al., 1993; Mans et al., 2004; Mansfeld et al., 2006; Stavrul et al., 2006).

Ndc1 is crucial for NPC assembly in vertebrates and yeast (Madrid et al., 2006; Mansfeld et al., 2006; Stavrul et al., 2006). It interacts with Nup53 (also referred to as Nup35), a member of the Nup93 complex, in metazoa and its two S. cerevisiae orthologs, Nup53 and Nup59 (Hawryluk-Gara et al., 2008; Mansfeld et al., 2006; Onischenko et al., 2009; Uetz et al., 2000; Vollmer et al., 2012). This interaction might link NPCs to the pore membrane. However, in some organisms Ndc1 is dispensable for NPC assembly or absent from the genome (DeGrasse et al., 2009; Liu et al., 2009; Mans et al., 2004; Neumann et al., 2010; Stavrul et al., 2006). In vertebrates, the interaction between Ndc1 and Nup53 seems dispensable for NPC formation because Nup53 truncations lacking the Ndc1 binding region are functional in NPC assembly (Hawryluk-Gara et al., 2008; Vollmer et al., 2012). Hence, alternative interactions might connect the NPC with the pore membrane. Indeed, Nup53 and both of its yeast homologs can bind membranes directly (Patel and Rexach, 2008; Vollmer et al., 2012). In vertebrates, two independent membrane binding sites mediate the Nup53 interaction to the pore membrane and this feature is crucial for NPC assembly (Vollmer et al., 2012). Although either one of these membrane binding sites is sufficient for NPC assembly at the end of mitosis, they are not completely interchangeable because the C-terminal site deforms membranes and is exclusively required for NPC assembly during interphase.

In addition to its membrane-binding ability and its interaction with Ndc1, Nup53 is part of multiple protein–protein interactions within the Nup93–Nic96 complex. The metazoan protein, as well as its yeast counterparts Nup53 and Nup59, binds Nup155 (or its corresponding yeast proteins Nup157 and Nup170) and Nup93/Nic96 (Fahrenkrog et al., 2000; Hawryluk-Gara et al., 2005; Onischenko et al., 2009; Sachdev et al., 2012; Uetz et al., 2000; Vollmer et al., 2012). In a thermophilic fungus, additional direct interactions between Nup53 and Nup192, corresponding to Nup205 in metazoa, as well as Nup188 have been described (Amlacher et al., 2011). Nup53, Nup93 and Nup155 are each...
essential for assembly of vertebrate NPCs and form a trimeric complex in which Nup93 stabilizes the interaction between Nup53 and Nup155 (Franz et al., 2005; Grandi et al., 1997; Hawryluk-Gara et al., 2008; Hawryluk-Gara et al., 2005; Krull et al., 2004; Mitchell et al., 2010; Sachdev et al., 2012; Vollmer et al., 2012). Additionally, Nup155 can interact with the transmembrane nucleoporins Ndc1 and Pom121 (Mitchell et al., 2010; Yavuz et al., 2010). Altogether, there seems to be a broad and at least partially redundant interaction network between the pore membrane including transmembrane nucleoporins and the Nup93 complex.

Here, we analyze the function of the different Nup53 interactions and their contribution to NPC assembly. By dissecting the direct membrane interaction of Nup53 and its ability to bind Ndc1, we find that the Ndc1–Nup53 interaction is indispensable for NPC formation. This interaction is not required for Nup53 recruitment to the NPC. Instead, Ndc1 is needed to modulate the membrane-deformation capability of Nup53 to promote NPC assembly. Similarly, we demonstrate that the direct binding of Nup53 to Nup155 is crucial for NPC assembly. This interaction localizes Nup155 to the nascent pore, a crucial step for downstream events in NPC biogenesis.

RESULTS
Ndc1 interacts directly with Nup53 through its N-terminal transmembrane regions

Xenopus Nup53 interacts directly with nuclear membranes and this membrane binding is crucial for NPC assembly (Vollmer et al., 2012). Nup53 is embedded in the complex protein–protein interaction network of the NPC, where different interactions might influence or regulate each other during NPC assembly or within the intact pore. In this respect, interaction of Nup53 with the integral pore membrane protein Ndc1 might be relevant for its membrane binding, but its functional importance is unknown. In Xenopus laevis the last eight amino acids of Nup53 are required for its interaction with Ndc1. This region also contains one of the two direct membrane binding sites. It is therefore important to determine whether these two features of Nup53 influence each other or act independently.

We reconstituted the interaction between Xenopus Nup53 and Ndc1 using recombinant proteins overexpressed in E. coli. Because Ndc1 is an integral membrane protein, it is important to maintain its membrane environment to preserve its functionality. To this end, a MISTIC tag (Roosild et al., 2005) was used to direct Ndc1 to the inner E. coli membrane. After harvesting the bacteria, membrane vesicles were generated and used as preys in GST pull-downs. The bait constructs comprised the C-terminal half of Nup53, including an RNA recognition motif (RRM) because this fragment interacts with Ndc1 (Vollmer et al., 2012 and supplementary material Fig. S1). In Nup53, the RRM domain acts as a dimerization domain and strengthens the interaction with other Nup93 complex members, including Nup93 and Nup155, and most relevant here – is required for direct membrane binding (Handa et al., 2006; Vollmer et al., 2012). Hence, we used a dimerization mutant (F172E W203E, Nup53 162–320 RRM mutant, Fig. 1A) to discriminate protein–protein-mediated interactions from direct membrane binding of Nup53. Accordingly, E. coli–derived membrane vesicles containing a fragment of the pore membrane protein Gp210 did not bind Nup53 above background levels (Fig. 1B). Inhibition of Nup53 dimerization does not affect Ndc1 interaction (Vollmer et al., 2012). Indeed, full-length Ndc1 eluted from the Nup53 bait, indicating a direct interaction.

Ndc1 consists of two parts: the N-terminal half comprises six predicted transmembrane helices and the C-terminal part is a conserved globular domain facing the nucleoplasmic side of the pore (Lau et al., 2006; Mansfeld et al., 2006; Stavru et al., 2006). To map the region required for the direct Nup53 interaction, we used membrane vesicles containing the N-terminal half of Ndc1 including all transmembrane helices (aa 1–301, Ndc1-N) or the C-terminal part starting from the last transmembrane helix including the globular C-terminal domain (aa 234–660, Ndc1-C, Fig. 1C). The Ndc1-N fragment bound Nup53 more strongly than the Ndc1-C fragment did (Fig. 1D). Further truncations of the transmembrane domain in which single transmembrane helices were sequentially deleted showed residual Nup53 binding (data not shown). This suggests that the Nup53 interaction surface is probably formed by multiple loops within the N-terminal region of Ndc1. Nup53 also interacted with the N-terminal half of Ndc1 in lysates of transfected HeLa cells (Fig. 1E). Here, full-length Ndc1 as well as Ndc1-N co-immunoprecipitated with Nup53. By contrast, Ndc1-C did not co-immunoprecipitate with Nup53. Accordingly, Nup53 co-immunoprecipitated with Ndc1 and Ndc1-N, but not Ndc1-C. Taken together, these data show that Xenopus Ndc1 and Nup53 interact directly and the N-terminal transmembrane domain of Ndc1 is necessary and sufficient for Nup53 binding.

The N- and the C-terminal halves of Ndc1 are required for NPC assembly

Having observed that the N-terminal half of Ndc1 mediates the interaction with Nup53, we tested whether this part is required for NPC assembly. For this, we used a cell-free system in which NPC assembly can be faithfully reconstituted. Incubation of cytosol and purified membranes from Xenopus laevis egg extracts with sperm heads gives rise to nuclei with closed NEs and functional NPCs (Gant and Wilson, 1997; Lohka and Masui, 1983; Wilson and Newport, 1988). Endogenous Ndc1 was specifically immunodepleted from the membrane fraction (Fig. 2A). This treatment did not affect other transmembrane proteins, including the pore membrane proteins Gp210 and Pom121. If mock-treated membranes and cytosol were used, a closed NE formed around the chromatin template, as indicated by membrane staining that had a smooth appearance (Fig. 2B,C). These nuclei contained NPCs as detected by the antibody mAB414, which acts as a read-out for NPC formation because it recognizes several nucleoporins that represent a major subfraction of the NPC. In the absence of Ndc1, a closed NE was not formed and mAB414 staining was markedly reduced, indicating a block in NE and NPC formation, consistent with a previous study (Mansfeld et al., 2006). Similar phenotypes have been observed upon depletion of other nucleoporins crucial for NPC assembly, including Pom121 (Antonin et al., 2005), Nup53 (Hawryluk-Gara et al., 2008; Vollmer et al., 2012), Nup93 (Grandi et al., 1997; Sachdev et al., 2012) and Nup155 (Franz et al., 2005). Upon depletion of Ndc1, Pom121 was still detected on the chromatin, showing that nuclear membrane recruitment to chromatin was not blocked in general (Fig. 2B,C). Re-addition of recombinant EGFP-tagged Ndc1 purified from E. coli and reconstituted into the Ndc1-depleted membranes rescued the phenotype at a concentration that was approximately endogenous. This demonstrates the specificity of the depletion and the functionality of the recombinant protein.

Next, we replaced endogenous Ndc1 with EGFP-tagged Ndc1 fragments to investigate which region is required for NPC assembly. The Ndc1-N or the Ndc1-C fragments did not support NE and NPC formation, despite the fact that membrane vesicles containing the protein fragments were recruited to chromatin.
Fig. 1. The N-terminal portion of Ndc1 interacts directly with Nup53. (A) Schematic representation of *Xenopus* Nup53 and the Nup53 mutant fragment impaired for RRM-mediated dimerization and direct membrane binding. (B) GST fusions of the nucleoplasmic region of *Xenopus* Gp210 (control) or a Nup53 fragment comprising a mutated RRM domain decreasing direct membrane binding and the subsequent C-terminus (Nup53 162–320 RRM mutant) were incubated with *E. coli* lysates overexpressing the transmembrane region and the nucleoplasmic extension of *Xenopus* Gp210 or full-length Ndc1. Eluates and 3% of the input were analyzed by western blotting using a His\(_6\) antibody. (C) Schematic representation of *Xenopus* Ndc1 (Ndc1), the N-terminal (Ndc1-N) and the C-terminal fragment (Ndc1-C) used. (D) Nup53 binding to the Ndc1-N and Ndc1-C fragments was analyzed as in B. The quantification shows the ratio of bait-bound protein to input of six independent experiments normalized to the binding of the lysate control to the Nup53 RRM mutant. Error bars show s.d. (E) HeLa cells were cotransfected with HA-tagged *Xenopus* Nup53 and full-length *Xenopus* Ndc1, the Ndc1-N or Ndc1-C fragment each fused with an N-terminal Myc and a C-terminal EGFP tag. Proteins were immunoprecipitated from cellular lysate with Myc or HA antibodies and analyzed by western blotting with indicated antibodies.
This indicates that both parts of Ndc1 are required for NE and NPC assembly.

The N-terminal half of Ndc1 is dispensable for NPC assembly if Nup53 lacks its C-terminal membrane-binding side

One obvious explanation for the necessity of both parts of Ndc1 for NPC assembly could be that the N-terminus is required for Nup53 binding, whereas the C-terminal region executes some independent mandatory function. However, the Ndc1–Nup53 interaction is described as dispensable for in vitro NPC assembly because Nup53 truncations defective in this interaction could substitute for the endogenous protein (Hawryluk-Gara et al., 2008; Vollmer et al., 2012). To resolve this discrepancy, we performed double depletion and add-back experiments.
manipulating Nup53 and Ndc1 from the egg extract cytosol and membranes, respectively. Nup53 was depleted without affecting the levels of other nucleoporins (Fig. 3A). In the absence of Nup53, formation of a closed NE was blocked (Fig. 3B,C, panels 1) as reported (Hawryluk-Gara et al., 2008; Vollmer et al., 2012). Re-addition of recombinant full-length Nup53 (Nup53 1–320) at endogenous levels (Fig. 3A) rescued this block when combined with mock-treated membranes containing endogenous Ndc1 (Fig. 3B,C panels 2). Also, a C-terminal truncation of Nup53 lacking the Ndc1 binding site (Nup53 1–312) restored NE formation together with mock-treated membranes (Fig. 3B,C, panels 3). These observations are in agreement with previous reports using untreated membranes (Hawryluk-Gara et al., 2008; Vollmer et al., 2012). If endogenous Ndc1 was depleted from the membranes, NPC formation was blocked in the presence of either Nup53 construct (Fig. 3B,C, panels 4,5). Re-addition of full-length Ndc1 in reconstituted membranes rescued NPC formation in combination with both Nup53 versions (Fig. 3B,C, panels 6,7). The Ndc1-N fragment could not replace endogenous Ndc1 in the presence of these Nup53 constructs (Fig. 3B,C, panels 8,9). This again suggests that the N-terminal half of Ndc1 is not sufficient for NE and NPC formation and that the C-terminal part is additionally required (see also Fig. 2B,C). The Ndc1-C fragment did not support formation of a closed NE in the presence of full-length Nup53 (Fig. 3B,C, panels 10). Surprisingly, in the presence of the Nup53 truncation impaired in the Ndc1 interaction (Nup53 1–312), the Ndc1-C fragment allowed NPC assembly (Fig. 3B,C, panels 11). This shows that the N-terminal half of Ndc1, which mediates Nup53 binding, is dispensable for NPC formation if Nup53 lacks the last eight amino acids.

Next, we analyzed the nuclei formed in the presence of the Ndc1-C fragment. In context of the Nup53 truncation defective in Ndc1 binding (Nup53 1–312), NPC formation is normal. All tested nucleoporins, including Nup53, localized properly to the NE (Fig. 3C, panel 11 and Fig. 3D) supporting the view that the N-terminal half of Ndc1 and thus the direct Ndc1–Nup53 interaction is not required to recruit Nup53 to reassembling NPCs. However, in the presence of full-length Nup53 (Nup53 1–320) Ndc1-C could not rescue NPC formation. Under these conditions Nup107, a nucleoporin that is recruited early to chromatin during NPC assembly, Pom121 and Gp210 were present on chromatin. Also, Nup53 and Nup155 were detected on chromatin but other Nup93 complex members, namely Nup93, Nup205 and Nup188, remained absent. This lack of recruitment of Nup93, Nup188 and Nup205 is similar to the Nup93-depletion phenotype (Sachdev et al., 2012), and suggests that Nup53 recruitment on its own cannot promote NPC assembly. Instead, the Ndc1–Nup53 interaction ensures the functionality of Nup53 in NE and NPC assembly.

The Ndc1 interaction and the C-terminal membrane-binding site of Nup53 can be separated

The previous experiments suggest that the Nup53–Ndc1 interaction is only dispensable when Nup53 lacks the last eight amino acids. This truncation not only abolishes the Ndc1 interaction, but also impairs membrane binding and deformation through the Nup53 C-terminus (Vollmer et al., 2012). Thus, the Ndc1–Nup53 interaction seems to be essential for NPC assembly if Nup53 can deform membranes. To test this, we generated a Nup53 construct that does not bind Ndc1 but retains all other features of the C-terminus. Because the Ndc1 interaction site and the C-terminal membrane binding site partially overlap (Vollmer et al., 2012), a stretch of eight amino acids upstream of the C-terminal membrane binding site (aa 275–282) was deleted. This deletion rendered Nup53 incompetent for Ndc1 interaction (Nup53 162–320 RRM mutant Δ275–282, Fig. 4A). To test for membrane binding, we inserted this deletion in a Nup53 fragment comprising the RRM domain and the C-terminal extension (aa 130–320). Because the N-terminal part of Nup53 contains another membrane-binding motif, this region was excluded. Similar to the control (Nup53 130–320), the deletion mutant (Nup53 130–320 Δ275–282) binds membranes directly when incubated with liposomes and floated through a sucrose cushion (Fig. 4B). By contrast, deletion of the last amino acid (Nup53 130–319) strongly reduced membrane binding (Vollmer et al., 2012). Similar to the wild-type fragment, the Nup53 deletion construct induced tubulation of liposomes (Fig. 4C). Notably, Nup155 binding, which also requires the C-terminal part of Nup53, remained unaffected by the deletion (supplementary material Fig. S2). Altogether, this shows that the Nup53 deletion construct is specifically impaired in its interaction with Ndc1, but its C-terminal membrane binding and deformation site remain intact.

Ndc1–Nup53 interaction is required for NPC assembly but can be bypassed if Nup53 lacks its C-terminus

After having identified a Nup53 version that was defective for the Ndc1 interaction but possessed a functional C-terminal membrane binding and bending site, we investigated the specific function of the Ndc1–Nup53 interaction in nuclear assembly. Endogenous Nup53 was depleted from the cytosol and replaced with different Nup53 versions (Fig. 5A) in the presence of untreated membranes containing endogenous Ndc1 (Fig. 5B). As before, Nup53 depletion blocked NE and NPC formation and this block was rescued by the wild-type protein (Nup53 1–320, Fig. 5C,D). However, the deletion construct (Nup53 1–320 Δ275–282) did not restore NE and NPC formation, demonstrating that the Ndc1–Nup53 interaction is necessary for NPC assembly. Interestingly, when the last amino acid of the deletion construct was removed (Nup53 1–319 Δ275–282), which impaired it for membrane interaction and deformation through the C-terminal membrane-binding site (Vollmer et al., 2012) (see also Fig. 4B,C), NE and NPC formation was rescued. By contrast, abolishing the membrane interaction through the N-terminal membrane-binding site of Nup53, which does not deform membranes, by exchange of a basic motif (Nup53 1–320 R105E K106E Δ275–282) (see Vollmer et al., 2012) did not rescue NE and NPC formation. This shows that the C-terminal membrane-binding site of Nup53 has to be specifically impaired to allow for NPC assembly if Nup53 is unable to interact with Ndc1 and that Ndc1 binding is required for NE and NPC formation if Nup53 can deform membranes.

All Nup53 constructs impaired in Ndc1 binding containing the N- or the C-terminal membrane-binding site localized to chromatin (Fig. 5C) showing that recruitment of Nup53 is independent of Ndc1. Accordingly, a Nup53 construct impaired in both membrane-binding sites and the Ndc1 interaction (Nup53 1–319 R105E K106E Δ275–282) was not detected on the chromatin and was defective in NPC and NE formation. This is consistent with previous findings whereby Nup53 membrane binding is the key determinant for its recruitment to the assembling NPC (Vollmer et al., 2012).

Altogether, these data indicate that Nup53 binding to Ndc1 is crucial for post-mitotic NPC assembly and only dispensable if
Fig. 3. Full-length Nup53 requires Ndc1 for NPC assembly. (A) Cytosol from Xenopus egg extracts was mock depleted, Nup53 depleted (ΔNup53) or Nup53 depleted and supplemented with full-length recombinant Nup53 (1–320) or a truncated version (1–312) lacking the final eight amino acids and analyzed with indicated antibodies. Note that endogenous Nup53 migrates slightly slower because of post-translational modifications absent in E. coli. Both, endogenous and recombinant Nup53 proteins are prone to C-terminal degradation (x). The Nup93 antibody recognizes a slightly slower-migrating crossreactivity (*). (B) Nuclei were assembled in Nup53-depleted cytosol (1) to which either recombinant full-length Nup53 (1–320, top panel) or a Nup53 fragment lacking the last eight amino acids (1–312, bottom panel) were added. The membranes were mock treated (2,3), depleted of endogenous Ndc1 (4,5) or Ndc1 depleted and reconstituted with either EGFP-tagged Ndc1 (6,7), Ndc1-N (8,9) or Ndc1-C (10,11). Membranes were stained with DiIC$_{18}$ (red), DNA with DAPI (blue). Nuclei were analyzed with antibodies against EGFP to detect recombinant Ndc1 proteins (green) and Nup53 antibodies. (C) Quantification of nuclei with a closed NE as in Fig. 2C. Bold numbers correspond to the experimental conditions in B. (D) Nuclei assembled as in B (10,11) were analyzed by immunofluorescence with indicated antibodies. Scale bars: 10 μm.
Nup53 cannot deform membranes. Consistent with this, the N-terminal half of Ndc1, which provides the Nup53 interaction surface, is dispensable for NPC assembly if Nup53 lacks the membrane-deformation motif (Fig. 3B, panel 11, Fig. 3C,D). Hence, we conclude that Ndc1 modulates Nup53 membrane-deformation activity to allow for NPC assembly at the end of mitosis. Accordingly, we assumed that a Nup53 construct harboring a functional membrane deformation motif, which cannot be bound and regulated by Ndc1, might act as a dominant-negative version in NPC assembly. Indeed, if we added the Nup53 deletion construct defective for Ndc1 interaction (Nup53 1–320 Δ275–282) in excess to untreated nuclear assembly reactions (i.e. in the presence of endogenous Nup53 and Ndc1) NE and NPC formation was blocked (Fig. 5E). By contrast, a Nup53 construct that is unable to interact with Ndc1 but additionally is defective for membrane deformation did not show this effect (Nup53 1–319 Δ275–282). This experiment supports our hypothesis that the Ndc1–Nup53 interaction restrains the membrane-deformation capability of Nup53.

Nup53 interacts directly with Nup155
Nup53 combines different modes of interactions that are crucial for NPC assembly such as direct membrane binding and deformation, as well as interactions with other proteins within the NPC. We have demonstrated that the Ndc1 interaction is required to modulate the membrane-deformation ability and thus membrane binding through the C-terminus and Ndc1 interaction influence each other. Nup53 also interacts with Nup155 through its C-terminus (Amlacher et al., 2011; Hawryluk-Gara et al., 2008; Hawryluk-Gara et al., 2005; Marelli et al., 1998; Onischenko et al., 2009; Sachdev et al., 2012; Uetz et al., 2000; Vollmer et al., 2012). Similar to Ndc1 and Nup53, Nup155 is crucial for NPC assembly (Franz et al., 2005). Nup53 binding to Nup155 is thought to be essential for NPC assembly because...
Fig. 5. Ndc1 functionally interacts with Nup53 membrane-bending activity. (A) Schematic representation of full-length Xenopus Nup53 (1–320) and the different mutants and fragments used. (B) Xenopus egg extracts were mock-treated, Nup53-depleted or Nup53-depleted and supplemented with recombinant wild-type Nup53 (1–320) or different constructs impaired for Ndc1 interaction (1–320 D275–282). Variants of the deletion construct deficient for membrane deformation (1–319 D275–282), membrane interaction through the N-terminal membrane binding site (1–320 R105E K106E D275–282) or both membrane interaction and deformation (1–319 R105E K106E D275–282) were included. Nup53 degradations are indicated (x). (C) Nuclei were assembled in mock, Nup53-depleted extracts or Nup53-depleted extracts supplemented with Nup53 constructs as in B. Membranes were analyzed by immunofluorescence. Nuclei were assembled in mock, Nup53-depleted extracts or Nup53-depleted extracts supplemented with Nup53 constructs as in B. Membranes were analyzed by immunofluorescence. DNA was stained with DAPI (blue), membranes with DiIC18 (red). Scale bar: 10 μm. (D) Quantification of nuclear assembly reactions from C performed as in Fig. 2C. (E) Recombinant full-length Nup53 (1–320), the Nup53 deletion construct impaired for Ndc1 interaction (1–320 D275–282) and a Nup53 construct impaired for Ndc1 interaction and defective in membrane deformation (1–319 R105E K106E D275–282) were added to untreated nuclear assembly reactions in 10-fold excess over endogenous Nup53 levels. More than 300 nuclei from three independent experiments were quantified. Error bars represent s.d.
Nup53 truncations lacking the Nup155 interaction site failed to replace the endogenous protein in nuclear assembly reactions (Hawryluk-Gara et al., 2008). However, these truncated versions also lacked the RRM domain that is required for Nup53 dimerization. This dimerization is necessary for binding of Nup53 to the membrane and, in turn, for NPC formation. Hence, it is unresolved whether the Nup53–Nup155 interaction is indeed essential for NPC formation and independent of other interactions of Nup53. To answer this, we separated the membrane binding and Nup155 interaction functions of Nup53 after mapping the Nup155 interaction site on Nup53 more precisely. Nup53 binds Nup53 upstream of Ndc1 (Vollmer et al., 2012). Therefore, we tested C-terminal truncations of Nup53 (Fig. 6A) in GST pull-downs. These constructs covered the RRM domain which might contribute to the interaction (Vollmer et al., 2012) and sections closer to the C-terminus. Nup53 truncations lacking the last eight (Nup53 162–312) or 25 amino acids (Nup53 162–295) bound Nup155 (Fig. 6B). Further C-terminal truncation up to amino acid 267 (Nup53 162–267) resulted in a loss of the interaction. If the RRM domain (aa 163–243 in the Xenopus protein) was absent, Nup53 failed to bind Nup155 (Fig. 6C) showing that the RRM domain of Nup53 is required but not sufficient for the Nup155 interaction. Thus, the RRM domain and an additional region (between aa 267–295) within the C-terminus of Nup53 form the Nup155 interaction surface.

Next, we generated a Nup53 point mutant to impair its interaction with Nup155. By exchanging the conserved lysine residue of position 262 to alanine, Nup53 binding to Nup155 was compromised both in vitro and in vivo without affecting the interaction with Ndc1 (Fig. 6D,F; supplementary material Fig. S3). Thus, the K262A mutation on Nup53 specifically affects Nup155 binding and provides a useful tool to study the role of the Nup53–Nup155 interaction in NPC assembly.

**Nup53–Nup155 interaction is essential for NPC assembly**

The Nup53 K262A point mutant was used in nuclear assemblies with Nup53-depleted egg extracts (1–320 K262A, Fig. 7A). As before, depletion of Nup53 blocked NE and NPC formation and wild-type Nup53 (Nup53 1–320) rescued this phenotype (Fig. 7B,C). By contrast, the Nup53 mutant deficient for the Nup155 interaction (1–320 K262A) did not support formation of a closed NE and NPCs. Interestingly, the Nup53 point mutant was recruited to the chromatin template, albeit at reduced levels. This indicates that localization of Nup53 to the reassembling NPC is independent of Nup155. By contrast, recruitment of Nup155 mainly depends on its interaction with Nup53 because Nup155 was hardly detectable on the chromatin in the presence of the mutant Nup53 construct (1–320 K262A). Other Nup93 complex members, including Nup93, Nup205 and Nup188 were absent from the chromatin if the Nup53 mutant was used (Fig. 7D). It is unlikely that this lack of recruitment was caused by disrupting the direct protein interaction between Nup53 and Nup93 because Nup93 binds to the N-terminal part of Nup53 (Amlacher et al., 2011; Fahrenkrog et al., 2000; Hawryluk-Gara et al., 2008; Vollmer et al., 2012), which was unaffected by the mutation, and rather indicates that the recruitment of Nup93 as well as of Nup188 and Nup205 requires a functional Nup53–Nup155 interaction. Similarly, Nup58, a member of the Nup62 complex, was not recruited because this requires the presence of Nup93 (Sachdev et al., 2012). Nup107, Ndc1, Pom121 and Gp210 were present on the chromatin but did not show smooth rim staining because no closed NE was formed. Therefore, the interactions of Nup155 with Ndc1 and Pom121 or the Nup107–Nup160 complex (Mitchell et al., 2010; Yavuz et al., 2010) are insufficient to locate Nup155 at the nuclear rim. These data show that binding of Nup53 to Nup155 is required for efficient recruitment of Nup155 to the assembling pore and is crucial for NPC assembly at the end of mitosis.

**DISCUSSION**

We have functionally analyzed the interactions of three essential nucleoporins in NPC assembly: Ndc1, Nup53 and Nup155. *Xenopus* Nup53 and Ndc1 interact directly. This interaction is mediated by the transmembrane regions of Ndc1 and the C-terminal part of Nup53. Our data indicate that this interaction is crucial for NPC reassembly at the end of mitosis and regulates the membrane-deformation capability of Nup53. Furthermore, our study reveals a crucial role for the direct interaction of Nup53 and Nup155 for NPC formation because abolishing this interaction by specific point mutation of Nup53 blocks NPC assembly.

Nup53 is a component of the Nup93 complex forming the inner ring of NPCs adjacent to the pore membrane (Alber et al., 2007; Brohawn et al., 2009). As a result of its many interactions, Nup53 acts as a connection point between the pore membrane and other nucleoporins. *Xenopus* Nup53 and the yeast counterparts bind membranes directly and this interaction in vertebrates is essential for NPC assembly (Patel and Rexach, 2008; Vollmer et al., 2012). Moreover, Nup53 interacts with the transmembrane nucleoporin Ndc1 (Hawryluk-Gara et al., 2008; Mansfeld et al., 2006; Onischenko et al., 2009; Uetz et al., 2000; Vollmer et al., 2012), as well as with Nup155 and Nup93, two other members of the Nup93 complex (Amlacher et al., 2011; Fahrenkrog et al., 2000; Hawryluk-Gara et al., 2005; Onischenko et al., 2009). In metazoa, Nup53 and Nup155 are both essential components of the Nup93 complex (Franz et al., 2005; Galy et al., 2003; Hawryluk-Gara et al., 2008; Hawryluk-Gara et al., 2005; Kiger et al., 1999; Mitchell et al., 2010; Ródenas et al., 2009; Vollmer et al., 2012). *S. cerevisiae* possesses two Nup53 orthologs, Nup53 and Nup59, and two Nup155 orthologs, Nup157 and Nup170. Double deletions of Nup53 and Nup59 in *S. cerevisiae* are viable (Marelli et al., 1998) but co-deletion of either one with Nup157 or Nup170 causes severe growth defects or lethality, respectively (Marelli et al., 1998). These observations suggest an important role of the Nup53–Nup155 interaction and, indeed, Nup155 binding to Nup53 is thought to be crucial for vertebrate NPC assembly (Hawryluk-Gara et al., 2008). However, the experiments defining the requirement of the vertebrate Nup53–Nup155 interaction used Nup53 constructs that not only lack the Nup155-interaction site but also the RRM domain, which is important for membrane binding. Also, a deletion in the gene encoding Nup53 in *C. elegans* that causes NPC defects (Ródenas et al., 2009) probably affects interaction with both the membrane and Nup155, because the major part of the RRM domain was absent from the protein product. By generating a Nup53 point mutant specifically impaired in Nup155 binding without modifying the RRM domain, we separated these two functions of Nup53 and show that the Nup53–Nup155 interaction is specifically required for NE and NPC assembly (Fig. 6,7). Nup53 and Nup155 form a conserved trimeric complex with Nup93 in which Nup93 stabilizes the Nup53–Nup155 interaction (Amlacher et al., 2011; Sachdev et al., 2012). We found that Nup93 is absent from the chromatin in the context of the Nup53 point mutant deficient for Nup155 binding (Fig. 7D). This suggests that Nup53–Nup155 binding recruits Nup93 to the
Fig. 6. Nup53 interacts with Nup155 through its C-terminal portion. (A) Schematic representation of Xenopus Nup53 constructs used. (B) GST pull-downs with the nucleoplasmic domain of Xenopus Gp210 (control) or fragments of Xenopus Nup53 comprising the RRM domain and parts of the C-terminus (Nup53 162–312, 162–295 and 162–267) and E. coli lysates overexpressing NusA-tagged Xenopus Nup155. Eluates and 3% of the input were analyzed using a His$_6$ antibody. Three independent experiments were quantified (bottom) for the bait-bound protein to input ratio normalized to Nup53 162–312. Error bars represent s.d. (C) GST pull-downs as in B, using Nup53 constructs comprising the RRM domain and the C-terminus (162–320) or lacking the RRM domain (242–320, 252–320). Three independent experiments were quantified and normalized to Nup53 162–320 as in B. (D) GST pull-downs as in B, using a Nup53 fragment comprising the RRM domain and the C-terminus (162–320) and a mutant impaired in the Nup155 interaction (162–320 K262A). (E) GST pull-downs with baits as in D and E. coli lysates as in Fig. 1B. Quantification was carried out as in Fig. 1D.
assembling NPC, which in turn recruits the Nup62 complex (Amlacher et al., 2011; Grandi et al., 1995; Sachdev et al., 2012). Interestingly, Nup53, Nup155 and Nup93 are identified in all eukaryotic super-groups (Neumann et al., 2010) and could have been present in the last common eukaryotic ancestor. Altogether, this implicates an essential, evolutionary conserved role of the three proteins and their interaction in NPC assembly and function.

Ndc1 is a crucial factor for NPC assembly both in metazoa (Mansfeld et al., 2006; Stavru et al., 2006) and yeast (Madrid et al., 2006). In S. cerevisiae, it binds directly both Nup53 orthologs, Nup53 and Nup59 (Onischenko et al., 2009). Our data demonstrate that this direct interaction is evolutionarily conserved (Fig. 1) and important for NPC assembly (Fig. 5). Upon separation of the Ndc1–Nup53 interaction from the Nup53 C-terminal membrane binding and deformation activity, we determined the specific requirement of the Nup53–Ndc1 interplay during NPC formation. This revises previous observations, suggesting a redundant role of this interaction in NPC assembly (Hawryluk-Gara et al., 2008; Vollmer et al., 2012). We show that the Ndc1 interaction is specifically required if Nup53 can deform membranes (Fig. 5). The Ndc1 transmembrane domain, which contains the Nup53 binding site, becomes dispensable if endogenous Nup53 is replaced by a Nup53 truncation that is defective in membrane deformation (Fig. 3). Our finding that the N-terminal part of Ndc1 is the Nup53 interaction surface (Fig. 1) is surprising at first glance because the C-terminal part of Ndc1 is much better conserved and exposed to the NPC (Lau et al., 2006; Mansfeld et al., 2006; Stavru et al., 2006). This region serves as an interaction platform for two other nucleoporins, Aladin and Nup155 (Kind et al., 2009; Mitchell et al., 2010; Yamazumi et al., 2009). The N-terminal half of Ndc1 comprises the six predicted transmembrane helices embedded in the pore membrane and interacts with the C-terminus of Nup53, which in turn is required for membrane deformation. Similarly, yeast Nup53 and Nup59
both interact with Ndc1 through their C-terminal ends (Onischenko et al., 2009). These regions are predicted to fold into amphipathic α-helices and might also deform membranes (Marelli et al., 2001; Patel and Rexach, 2008; Vollmer et al., 2012). Thus, the Ndc1–Nup53 interaction could take place within the lipid bilayer and membrane integration of the Nup53 binding site might hence be required. Our data highlight the fact that the Ndc1–Nup53 interaction is only required for post-mitotic NPC assembly if Nup53 can deform membranes (Figs 3, 5). This membrane deformation activity of the Nup53 C-terminus is dispensable for NPC formation at the end of mitosis but essential if NPCs integrate into the closed NE during interphase (Vollmer et al., 2012). We propose that Nup53 membrane deformation activity needs to be counteracted or fine-tuned and this requires Ndc1 binding. During NPC formation, both at the end of mitosis and in interphase, nuclear membranes might initially show high positive curvature at local sites of pore formation. However, during the process of NPC formation these highly curved membranes have to convert into the specific structure of the nuclear pore (Fig. 8). This final membrane shape is characterized by the coexistence of a positive and a negative curvature. Because the negative curvature could energetically balance the positive curvature (Terasaki et al., 2013), the final pore membrane structure might be passively adopted and might not require active membrane deformation as induced by Nup53. In this regard, Ndc1 might be the crucial factor regulating the membrane-deformation activity of Nup53 in the NPC.

A similar regulation of membrane-deforming activity was reported for reticulons, which are ER-tubulating proteins that associate with highly curved membranes (Hu et al., 2008; Voeltz et al., 2006). These proteins are displaced from the reforming NE at the end of mitosis. In human cells, overexpression of reticulons delays re-formation of the NE and a reduction in reticulons accelerates it (Anderson and Hetzer, 2008). Hence, it might be generally important to attenuate membrane deformation during re-formation of the NE and NPCs. This could be achieved by the removal of tubulating proteins or regulation by other proteins. How this happens mechanistically for the Ndc1–Nup53 interaction can only be speculated. Ndc1 might deform the pore membrane in the opposite way as a result of its shape or by oligomerization, which then obliterates the membrane curvature induced by Nup53 (Fig. 8A). Alternatively, Ndc1 binding could induce a conformational change in Nup53, resulting in a rearrangement of its C-terminus, which might then be buried inside the protein or the interaction surfaces (Fig. 8B).

Surprisingly, Ndc1 is not found in all eukaryotes (DeGrasse et al., 2009; Neumann et al., 2010) and is dispensable in the fungus Aspergillus nidulans (Liu et al., 2009). *A. nidulans* also lacks any obvious Nup53 ortholog, which is in agreement with our observation that the Ndc1–Nup53 interaction is only essential if Nup53 can deform membranes. Some organisms contain a Nup53 but no Ndc1 ortholog (Neumann et al., 2010). In these organisms Nup53 might be regulated by other proteins or their Nup53 orthologs could lack the membrane-deformation ability. Indeed, Ndc1 is absent from all Amoebozoa tested (Neumann et al., 2010) and their Nup53 proteins show only little sequence homology to the yeast or metazoan proteins in their C-termini, suggesting that they might not be able to deform membranes. Overall, it seems that not only is the direct Ndc1–Nup53 interaction conserved, but the function of this complex in controlling the membrane-deformation activity of Nup53 is also maintained.

The analyses of the different Nup53 interactions reveal its diverse functions in NPC assembly and elucidate the step-wise process of re-formation of the metazoan NPC at the end of mitosis (for a review, see Schooley et al., 2012). NPC reassembly starts on the decondensing chromatin by DNA binding of the nucleoporin Mel28/ELYS, which in turn recruits the Nup107–Nup160 complex (Franz et al., 2007; Galy et al., 2006; Gillespie et al., 2007; Rasala et al., 2006). Subsequently, NE precursor membranes bind to the NPC assembly sites, including the integral pore membrane proteins Ndc1 and Pom121 (Antonin et al., 2005; Mansfeld et al., 2006; Rasala et al., 2008). Pom121 was detected on the chromatin templates in the absence of Ndc1 (Fig. 2). This suggests that recruitment of Ndc1 and Pom121 can occur independently and supports the previously observed redundancy in the membrane recruitment step. Next, the Nup93 complex is localized to the nascent pore. This step is initiated by the recruitment of Nup53 for which its membrane interaction is crucial (Vollmer et al., 2012). Nup53 recruitment is independent of the Ndc1 interaction because Nup53 is also localized to assembling NPCs if both proteins cannot interact or if Ndc1 is depleted (Figs 3, 5). The Nup155 recruitment and subsequent establishment of the Nup53–Nup155 interaction is the next decisive step in NPC assembly. In Nup53-depleted extracts or if Nup53 is defective in binding Nup155, staining for Nup155 on the chromatin is strongly decreased and other members of the Nup93 complex as well as the Nup62 complex are not detectable (Fig. 7 and Hawryluk-Gara et al., 2008; Vollmer et al., 2012). Consistently, knockdown of Nup53 by siRNA in HeLa cells...
decreases the levels of Nup155 at the nuclear rim, as well as of Nup93 and Nup205 (Hawryluk-Gara et al., 2005), and in *C. elegans*, localization of Nup155 requires Nup53 (Rodenas et al., 2009). Together, this indicates that recruitment of Nup155 predominantly depends on the presence of Nup53, despite the fact that it also interacts with Ndc1 and Pom121. If Nup53 and Nup155 are present in an intact complex, NPC assembly proceeds further, probably by recruiting Nup93 and subsequently, the Nup62 complex (Sachdev et al., 2012). Eventually, further components of the nuclear basket and cytoplasmic filaments are recruited by yet ill-defined steps, completing the process of NPC assembly at the end of mitosis and yielding fully assembled functional NPCs.

**MATERIALS AND METHODS**

Antibodies against Ndc1 (Mansfeld et al., 2006), Pom121 and Gp210 (Antonin et al., 2005), Nup53, Nup188 and Nup205 (Theerthagiri et al., 2010), Nup93 and Nup58 (Sachdev et al., 2012), Nup107 (Franz et al., 2005) and Nup107 (Walther et al., 2003) have been described previously. MAB414 was obtained from BAbCO (Richmond, CA), antibodies against EGFP, His6, Myc and HA were from Roche (Mannheim, Germany), Alexa Fluor 488 goat anti-rabbit IgG, Cy3 goat anti-mouse IgG and 1,1’-Diocadecyl-3,3’,3’,3’-tetramethylindocarbocyanine perchlorate (DiIC18) were from Invitrogen (Eugene, OR).

**Protein expression and purification**

Constructs are listed as supplementary material Table S1. Nup53 constructs were generated from a synthetic DNA optimized for *E. coli* codon usage (Vollmer et al., 2012). For pull-down reactions, Nup53 fragments were cloned into a pET28a vector (EMD, Darmstadt, Germany) with an N-terminal His6 tag and a TEV-protease recognition site upstream of the respective protein and a C-terminal His6 tag with Ni-NTA agarose (Qiagen, Hilden, Germany). For nuclear assembly, liposome flotation or tubulation Nup53 fragments were cloned into a pET28a vector with a yeast SUMO tag (SMT3) and a TEV-protease recognition site upstream of the protein fragments. His6 and SUMO tags were cleaved off after purification from *E. coli*; the proteins were concentrated using Vivaspin 500 (Sartorius, Gottingen, Germany) and purified by gel filtration, either in PBS for liposome flotation and tubulation or in sucrose buffer for nuclear assembly. The Nup53 deletion constructs impaired in Ndc1 interaction were generated by replacing the eight amino acids of positions 275–282 (RAASMRPL) with the linker sequence GGSGSGGS and cloned into the respective vectors for pull-down, flotation, liposome tubulation and NPC assembly experiments.

To express integral membrane proteins, *Xenopus* Ndc1, the respective Ndc1 fragments or the Gp210 fragment comprising the transmembrane region and the nucleosparsemic part were cloned into a pET28a vector with an N-terminal Mistic (membrane-integrating sequence for translation of integral membrane protein constructs) sequence to enhance expression at the *E. coli* membrane (Roosild et al., 2005; Theerthagiri et al., 2010) followed by an EGFP tag upstream of the respective protein and a C-terminal His6 tag. Proteins were expressed in *E. coli* and used as lystate in pull-downs or further purified in the presence of 1% (w/v) cetyltrimethylammoniumbromide (Calbiochem, Darmstadt, Germany) using Ni-NTA agarose and dialyzed against sucrose buffer (Theerthagiri et al., 2010).

**Pull-down experiments**

*E. coli* lysates overexpressing the transmembrane nucleoporin constructs were adjusted with lysates from *E. coli* transformed with empty vector to equal protein concentrations and incubated with 1.5 μM of GST baits in a final volume of 800 μl. For Fig. 1B, 0.5 μM GST baits were incubated in 2 ml of the respective *E. coli* lysates. After 1 hour, 9 μl of magnetic glutathione beads (Pierce, Rockford, IL) were added for another hour, washed six times with 20 mM Tris-HCl, pH 7.5 and 150 mM NaCl and eluted in 30 μl total volume by TEV protease cleavage (0.5 mg/ml) for 1 hour at 25°C. For pull-downs with recombinant Nup155, 1.5 μM of respective GST baits were incubated with *E. coli* lysates overexpressing NusA-fused full-length Nup155 (Sachdev et al., 2012). Pull-downs with Xenopus-derived cytosol and membranes were performed as described previously (Vollmer et al., 2012).

**Immunoprecipitation**

Full-length *Xenopus* Ndc1, the respective fragments or full-length Nup155 were cloned with an N-terminal Myc tag into pEGFP-N1 (Clontech, Saint-Germain-en-Laye, France) containing a C-terminal EGFP-tag. HA-tagged *Xenopus* Nup53 has been described previously (Vollmer et al., 2012) and the K262A point mutation was introduced in this construct. Transfection of HeLa cells and immunoprecipitation were carried out as described (Vollmer et al., 2012).

**Other methods**

Nuclear assembly and immunofluorescence (Antonin et al., 2005; Theerthagiri et al., 2010), generation of affinity resins, sperm heads and floated membranes (Franz et al., 2005) as well as prelabelled membranes (Antonin et al., 2005) were all carried out as described previously. For add-back of recombinant Ndc1 constructs, purified proteins were reconstituted into solubilized membranes (Mansfeld et al., 2006). For testing dominant-negative effects in nuclear assembly, recombinant proteins were added to untreated *Xenopus* egg extracts at the beginning of the reaction. Liposome flotation and tubulation were done as described previously (Vollmer et al., 2012).

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**Author contributions**

N.E. and W.A. designed and performed experiments and wrote the manuscript. J.R. developed the methods to express and purify transmembrane nucleoporins from *E. coli*.

**Competing interests**

The authors declare no competing interest.

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**Supplementary material**

Supplementary material available online at http://jcs.biologists.orglookup/supp://doi.10.1242/jcs.141739/-DC1

**References**

Alber, F., Dokudovskaya, S., Veenhoff, L. M., Zhang, W., Kipper, J., Devos, D., Suprapto, A., Karni-Schmidt, O., Williams, R., Chait, B. T. et al. (2007). The molecular architecture of the nuclear pore complex. *Nature* 450, 695-701.

Amici, C., Sarges, P., Fleming, D., van Noort, V., Kunze, R., Devos, D. P., Arumugam, M., Bork, P. and Hurt, E. (2011). Insight into structure and assembly of the nuclear pore complex by utilizing the genome of a eukaryotic thermophile. *Cell* 146, 277-289.

Anderson, D. J. and Hetzer, M. W. (2008). Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation. *J. Cell Biol.* 182, 911-924.

Antonin, W., Franz, C., Haselmann, U., Antony, C. and Mattaj, I. W. (2005). The integral membrane nucleoporin pom121 functionally links nuclear pore complex assembly and nuclear envelope formation. *Mol. Cell* 17, 83-92.

Brogow, S. G., Leksia, N. C., Spear, E. D., Rajashankar, K. R. and Schwartz, T. U. (2006). Pharmacology of nuclear assembly and nuclear envelope formation. *J. Cell Biol.* 182, 1389-1397.

Brogow, S. G., Partridge, J. R., Whittle, J. R. and Schwartz, T. U. (2009). The nuclear pore complex has entered the atomic age. *Structure* 17, 1156-1168.

Chadri, A., Hess, B., San Roman, M., Gatti, X., Lombard, B., Loew, D., Barral, Y., Palancade, B. and Doyle, V. (2010). Pom33, a novel transmembrane nucleoporin required for proper nuclear pore complex distribution. *J. Cell Biol.* 189, 795-811.
