Detection of a tandem BRCT in Nbs1 and Xrs2 with functional implications in the DNA damage response

Emmanuella Becker†, Vincent Meyer†, Hocine Madaoui and Raphaël Guerois

1Service de Biophysique des Fonctions Membranaires, URA CNRS 2096, Département de Biologie Joliot-Curie and 2Département d’Etude et d’Ingénierie des Protéines, CEA Saclay, 91191 Gif-Sur-Yvette, Cedex, France

Received on January 30, 2006; revised and accepted on February 27, 2006

ABSTRACT

Motivation: Human Nbs1 and its homolog Xrs2 in Saccharomyces cerevisiae are part of the conserved MRN complex (MRX in yeast) which plays a crucial role in maintaining genomic stability. NBS1 corresponds to the gene mutated in the Nijmegen breakage syndrome (NBS) known as a radiation hyper-sensitive disease. Despite the conservation and the importance of the MRN complex, the high sequence divergence between Nbs1 and Xrs2 precluded the identification of common domains downstream of the N-terminal Fork-Head Associated (FHA) domain.

Results: Using HMM–HMM profile comparisons and structure modeling, we assessed the existence of a tandem BRCT in both Nbs1 and Xrs2 after the FHA. The structure-based conservation analysis of the tandem BRCT in Nbs1 supports its function as a phosphoserine binding domain. Remarkably, the 5 bp deletion observed in 95% of NBS patients cleaves the tandem at the linker region while preserving the structural integrity of each BRCT domain in the resulting truncated gene products.

Contact: guerois@cea.fr

Supplementary information: http://www-spider.cea.fr/Groups/si6661/view.html

1 INTRODUCTION

Nbs1 (or Xrs2 in yeast) is an essential component of the so-called MRN complex associating Mre11, Rad50 and Nbs1 (Petrini and Stracker, 2003; van den Bosch et al., 2003) and plays a crucial role in DNA repair pathways (Kobayashi et al., 2004). The human Nbs1 protein is a 754 amino acid long protein composed of several functional domains identified from sequence analysis and biochemical experiments (Fig. 2A). At the N-terminus, a Fork-Head Associated (FHA) domain (Durocher and Jackson, 2003) followed by a single BRCA1 C-terminal (BRCT) domain (Bork et al., 1997; Callebaut and Mornon, 1997) can be detected from sequence to profile searches. The C-terminus of Nbs1 contains a Mre11 binding region (Desai-Mehta et al., 2001) and an ATM recruitment motif (Falck et al., 2005). In Xrs2, the Saccharomyces cerevisiae functional homolog of Nbs1, the FHA domain together with the Tel1 (ATM homologue) and Mre11 binding regions are conserved but the existence of a BRCT domain was never detected from sequence analysis. As a matter of fact, the sequences of Xrs2 and Nbs1 are highly divergent in the 250 amino acids following the FHA domain (10% sequence identity). Using a specific strategy, new sequences of Xrs2 homologs not present in databases such as GenBank or EMBL could be retrieved and aligned to human sequences. From the resulting multiple sequence alignment, we show that in fact two BRCT domains are present in both human Nbs1 and yeast Xrs2 right behind the FHA domain.

Tandem BRCT have been recently recognized as major mediators of phosphorylation-dependent protein–protein interactions in processes related to cell-cycle checkpoint and DNA repair functions (Glover et al., 2004). The ability of the tandem BRCT of Nbs1 to bind phospho-peptides was never probed before since the existence of the second BRCT was not suspected. The model-based analysis of the tandem BRCT of Nbs1 strongly suggests that it is a phosphoserine binding module. The 5 bp deletion observed in 95% of NBS patients splits up the tandem at position 218. Remarkably, this mutation preserves the structural integrity of the second BRCT at plus or minus one residue. Altogether, our findings suggest that the NBS disease could be partly linked to a disruption of the interaction properties of the tandem BRCT: cleavage of the tandem BRCT may alter the selectivity of target recognition by Nbs1 and hence affect the signaling network required for efficient DNA damage responses.

2 METHODS

An initial profile containing close homologs of Nbs1 was built from searches of the non-redundant database using PSI-BLAST (Altschul et al., 1997) on the MPI server (Soding et al., 2005). For Xrs2, the initial profile gathered three sequences retrieved from tblastn searches on the Saccharomyces comparative genomic database (Kellis et al., 2003). The profiles were enriched by aligning profiles of more divergent sequences using the profile–profile alignment method HHAlign (Soding, 2005).

Iteratively, the profile–profile alignment procedure led to a global multiple sequence alignment gathering 25 sequences from human Nbs1 to S. cerevisiae Xrs2 (see Supplementary information). The profile consisting of 25 sequences was compared against a database of profiles built from the PDB using the HHpred server (Soding et al., 2005). Three structures of
tandem BRCT were detected with significant scores and confidence levels >96% (PDB codes 1l0b, 2ado, 1kzy). Over the major length of the profile, the built alignment was consistent with the structural alignment of the templates. Yet, significant divergence could be observed at the N-terminus (first strand) and C-terminus (last strand) motif. At the N-terminus, only the alignments with 1l0b and 2ado were compatible with the presence of an upstream FHA domain. At the C-terminus, only the alignment with the 1kzy template suggested the existence of a long insertion in the $b_3/a_2$ loop of the second BRCT, consistent with the conservation profile in the whole Nbs1 family. A global sequence to structure alignment between the 25 sequences and the structural alignment of the three templates (1l0b, 2ado and 1kzy) was created based on these features. Models were generated for both human Nbs1 and S.cerevisiae Xrs2 with Modeller 8v2 (Sali and Blundell, 1993) using the three structures 1kzy, 2ado and 1l0b as templates (max. Seq. ID: 13.2%). The quality of the models was assessed using Verify3D (Luthy et al., 1992), Prosa2003 (Sippl, 1993), ProQ and MaxSub (Wallner and Elofsson, 2003). The profile–profile alignment between the tandem BRCT of the Nbs1/Xrs2 family and that of the structural alignment of the three templates was iteratively refined in order to reduce the alignment errors pinpointed by the four evaluation scores. To further assess the physical relevance of the model built for the tandem BRCT of Nbs1, a 5 ns molecular dynamic simulation was performed at 300 K in explicit solvent using GROMACS 3.2 (Van Der Spoel et al., 2005) (see Supplementary information for details). Conservation analyses were carried out using the Rate4site algorithm (Pupko et al., 2002). Possible arrangements of the FHA domain with respect to the tandem BRCT were explored using the HADDOCK program (Dominguez et al., 2003) by docking models of the FHA domain onto models of the tandem BRCT while constraining the distance between their C- and N-termini in respect of the Nbs1 sequence. 3 RESULTS Models of the Nbs1 and Xrs2 tandem BRCT were built from the multiple sequence alignment in Figure 1 and assessed using standard evaluation tools. The scores of Nbs1 model are (Prosa2003: 1.92), (Verify3D: 0.395), (ProQ : 3.51) and (MaxSub : 0.348) and those of Xrs2 (Prosa2003: 1.16), (Verify3D: 0.332), (ProQ : 3.75) and (MaxSub : 0.338). The absence of residues with Verify3D scores below 0.1 together with ProQ and MaxSub scores significantly above 1.5 and 0.1, respectively, ensures the absence of major issues in the models of both tandem BRCT (Wallner and Elofsson, 2003). The physical quality of the Nbs1 model was further assessed by running a 5 ns simulation of molecular dynamics in an explicit solvent. The Cα rmsd stabilizes around 4 Å from the initial model structure (3.2 Å excluding the long loops 201–216 and 273–291) and secondary structures are overall preserved after a 5 ns of simulation as illustrated in Figure 2B (see also Supplementary information). 3.1 Functional insights from the tandem BRCT model 3.1.1 Clues for phosphoserine binding in Nbs1 So far, the tandem BRCT repeats of MDC1, PTIP, BARD1, 53BP1, RAD4, Ect2, TOPBP1, DNA ligase IV, S.pombe Crb2 and S.cerevisiae Rad9 have been shown to have phospho-serine (pSer) binding properties in vitro (Manke et al., 2003; Yu et al., 2003). The consensus signature for the pSer binding property was described as [S/T-G] in $b_1/a_1$ loop.
A tandem BRCT in Nbs1 and Xrs2

3.1.2 Location of the phosphorylated sites in Nbs1

In response to ionizing radiation, Nbs1 is phosphorylated at Ser278 and Ser343 by the ATM kinase, and this event is required for activation of the intra S phase checkpoint (Kobayashi et al., 2004). From the structural model, Ser278 is located in the long $\beta$3/$\alpha$2 loop of the second BRCT (Fig. 2B) and Ser343 is found 13 residues after the last residue of the tandem BRCT. Interestingly, the flexible linkers surrounding Ser278 and Ser343 are not long enough to allow for an intramolecular recognition of the pSer by the tandem BRCT.

3.1.3 Tandem BRCT and disease related mutations

Of the NBS patients, 95% carry a 5 bp deletion in exon 6 of the NBS1 gene, which results in the expression of two truncated proteins of 26 (p26) and 70 kDa (p70) (Fig. 2A). The mutation splits the tandem precisely in the linker between the two BRCT domains. P26 moiety includes the region 1–218 spanning the FHA and the integrality of the first BRCT domain. P70 corresponds to the C-terminal half of Nbs1 and is produced by an alternative initiation of translation upstream of the 5 bp deletion. After a 18 residue extension at the N-terminus, the sequence of p70 is identical to that of the wild-type Nbs1 from I221 to the end (Williams et al., 2002).

I221 sharply corresponds to the beginning of the second BRCT and is the first residue fully buried in its hydrophobic core. Several structures of well-folded single C-terminal BRCT domains isolated from a tandem support that each BRCT domain can adopt its structure independently (Gaiser et al., 2004; Zhang et al., 1998). Hence, despite the severe sequence variations induced by the mutation in the linker, elements crucial for the structural integrity of the second BRCT have been preserved. It suggests that the second BRCT may not only fold independently but also hold a function important for
viability in NBS patients. Regarding the first BRCT, it has been shown that the FHA/BRCT could bind in vitro the histone H2AX phosphorylated by ATM (Kobayashi et al., 2002). Phosphorylation of H2AX at Ser129 is among the first events of the repair of double strand breaks (Lowndes and Toh, 2005). Our data suggest that the p26 fragment (Fig. 2A) may still be able to bind pSer residues in NBS cells but with a loss of binding selectivity due to the truncation of the second BRCT. This novel hypothesis would be interesting to test in the light of the results obtained on animal models of the NBS pathology (Difilippantonio et al., 2005; Williams et al., 2002).

3.1.4 Nbs1 and Mdm2 interaction Mdm2 has been extensively studied as a negative regulator of p53 tumor suppressor (Vousden and Prives, 2005). Mdm2 overexpression was recently shown to inhibit the DNA repair function of the MRN complex and this effect required the binding of Mdm2 to Nbs1 (Alt et al., 2005). The region 198–314 of Mdm2 was shown to associate with the MRN complex through the central region of Nbs1 221–540. This region encompasses the newly identified second BRCT domain 221–330 but not the first one. Downstream of the second BRCT, the region 330–540 is predicted to be largely unfolded (see Supplementary information). We hypothesize that the second BRCT of Nbs1 by itself may be involved in the interaction with Mdm2.

3.2 Functional implications from the FHA-tandem BRCT structural model A striking feature of the domain organization among all Nbs1 homologs is the absence of a linker between the FHA and the tandem BRCT modules. Despite the high versatility in position and length of the insertions inside the FHA or the BRCT and between the two BRCT, not even a single amino acid was ever added at the hinge between the two modules. A structural model of the ensemble composed by the FHA and the tandem BRCT domains was built to probe the potential organization of the modules (Fig. 2B). Owing to steric hindrance, the phospho-binding sites of both domains are constrained on opposite sides of the whole assembly and could hardly be closer than 45 Å (see Supplementary information). It excludes the possibility to bind simultaneously a pThr neighboring a pSer at <15 residues. The structural constraint between the domains may originate from a specific evolutionary constraint coupling both pThr and pSer binding functions. Interestingly, the FHA and the BRCT were shown to be both required for optimal chromatin association of the MRN complex (Kobayashi et al., 2002; Zhao et al., 2002). Moreover, a mutation disrupting the FHA pThr binding site revealed that this domain is involved in a signal amplification step crucial for DNA repair after low doses of irradiation (Difilippantonio et al., 2005). The coupling between pThr and pSer binding functions suggested from the model might as well contribute to this amplification process.

ACKNOWLEDGEMENTS

The authors are grateful to F. Ochsenbein, M.-C. Marsolier-Kergoat and S. Zinn-Justin for their useful comments about the manuscript. This work is partly funded by the ACI IMPBio 2004, V.M. is supported by an AFM fellowship (Association Française contre les Myopathies). H.M. is supported by a DGA fellowship. Funding to pay the Open Access publication charges was provided by the CEA Saclay.

Conflict of Interest: none declared.

REFERENCES


