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Review

The NHR domains of Neuralized and related proteins: Beyond Notch signalling

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ABSTRACT

Neuralized Homology Repeats (NHRs) were first identified in Neuralized, an E3-ubiquitin ligase that plays a key role in the Notch signalling pathway. Since their original discovery, NHR domains have been shown to regulate protein-protein interactions in a broad range of developmental processes and in a wide variety of species from flies to humans. The NHR family of proteins can be categorized into three groups: (1) those that contain a RING finger, (2) those that contain a SOCS box and, (3) those that only have NHR domains. Here we review the structure and function of NHR domains in various cellular and developmental processes.

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1. Introduction

Neuralized Homology Repeat (NHR, also referred as NEUZ) domains are highly conserved and found in a variety of proteins from flies to humans. The NHR domain was first identified using the *prospero* algorithm looking for internal duplications in *Drosophila* proteins [1]. The NHR domains were identified in three *Drosophila* proteins; Neuralized (dNeur), Neuralized homologue 4 (Neurl4, originally mistakenly referred to as bluestreak) and another uncharacterized protein (CG3894). There are five NHR domain containing proteins in mammals: Neuralized-1, Neuralized-2 (two dNeur homologues), OzzE3 (CG3894 homologue), a homologue of Neurl4 (also referred to as KIAA1787) and the Lung Inducible Neuralized related C3CH4 RING domain protein (LINCR), which has no homologue in flies (Fig. 1). Structural and functional studies indicate that the NHR domains function as protein interaction domains that mediate a diverse range of processes. All identified NHR domain-containing proteins contain between one and six NHR domains. Neuralized homologues in flies and mammals have two NHR domains (NHR1 and NHR2). LINCR, OzzE3 and the *Drosophila* homologue of OzzE3 (CG3894), each contain a single NHR domain while Neurl4 homologues in both flies and mammals have a tandem

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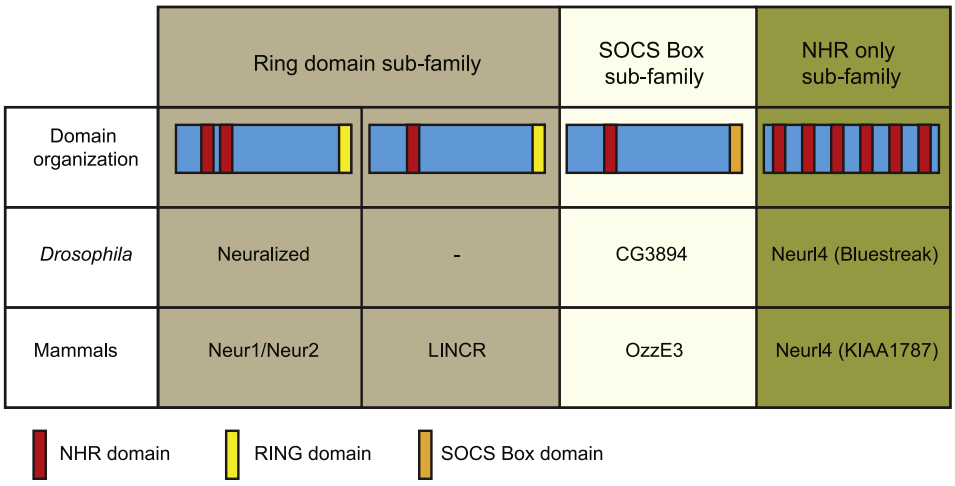

Fig. 1. Schematic of the three NHR domain containing protein sub-families. Members of the RING domain sub-family have a C-terminal RING domain and one or two N-terminal NHR domains. This sub-family consists of a single Neuralized homologue in flies and two Neuralized homologues and a related paralogue, LINCR (lung-inducible Neuralized-related C3HC4 RING domain protein) in higher vertebrates. Members of the SOCS-box sub-family have a C-terminal SOCS box domain and a single N-terminal NHR domain. This sub-family consists of the mammalian homologue OzzE3 and the uncharacterized *Drosophila* homologue CG3894. Proteins in the NHR only sub-family have six NHR domains. This sub-family consists of fly and mammalian homologues of Neurl4 (the fly homologue was formerly referred to as Bluestreak). Abbreviations: NHR, neuralized-homology repeat; RING, really interesting new gene; SOCS, suppressor of cytokine signalling.

of six NHR domains (Table 1). The NHR domain containing proteins can be sub-divided into three sub-families based on the domain organization of the proteins. The RING-domain sub-family consists of a group of proteins that contain one or two N-terminal NHR domains and a C-terminal C3HC4 RING finger domain. The SOCS box sub-family of NHR domain containing proteins is comprised of proteins with a single N-terminal NHR domain and a C-terminal Suppressor of Cytokine Signalling (SOCS) box domain. The final sub-family is the NHR only sub-family, consisting of proteins with six NHR domains.

2. The structure of NHR domains

Structural characterization of NHR1 domains from dNeur (residues 106-266) and hKIAA1787 (Neurl4) (residues 43-205) showed that both NHR domains adopt a characteristic β-sandwich fold (Fig. 2). The dNeur NHR1 has 12 β-strands that are organized into five-stranded and seven-stranded antiparallel β-sheets. The hKIAA1788 NHR1 domain adopts a similar two-layered β-sheet structure with 14 β-strands that form into two seven-stranded antiparallel β-sheets [2]. The β-strands are connected by loops of various length ($L1-11$), which extend out from the β-sandwich. There are also four short alpha helices contained within the loop regions. In both NHR1 domains, the upper β-sheet (5 stranded in dNeur and 7-stranded in hKIAA1787) forms a concave hydrophobic patch that is covered by amino acids from the long L6 loop region. He et al. [2] showed that the hydrophobic amino acids in the L6 loop interact strongly with the upper β-sheet and concluded that the L6 loop structure is a common feature of NHR domains.

Another point of interest is the Gly167 residue, which is the only amino acid that is absolutely conserved amongst all NHR domains. Previous work from this laboratory has shown that mutation of Gly167 within the NHR1 domain, or Gly430 residue within the NHR2 domain, abolishes dNeur activity [3–5]. This conserved glycine residue was shown to interact strongly with the hydrophobic amino acids in the L6 loop. Based on the strength of the interactions between this conserved glycine residue and the hydrophobic residues of the L6 loop, it was suggested that mutation of this amino acid may affect the arrangement of the hydrophobic core and de-stabilize the β-sandwich fold [2].

Comparison of both the sequence and solution structure of the NHR1 domains from dNeur and hKIAA1787 to other known protein domains indicates a strong similarity to B30.2/SPRY domains [2,6,7]. Like the B30.2/SPRY domains which are known to mediate protein-protein interactions [8], the NHR domains have also been shown to mediate numerous protein-protein interactions (see below). Comparisons of the target binding regions of the NHR1 domain of dNeur and the B30.2/SPRY domain of GUSTAVUS suggest that these domains bind to their relative targets (TOM and VASA respectively) in similar locations [2,8]. The target-binding site in the NHR1 domain of dNeur is located on the L3, L5, L11 loops and the tip of L6 loop [2,8]. In the B30.2/SPRY domain of GUSTAVUS, the target-binding site is located in loops L3, L5, L13 and the top of the L6 loop [2]. Despite the similarity in the location of the target-binding pocket of these domains, NMR titration experiments confirmed significant substrate specificity for each domain. A 20 amino acid peptide derived from the Bearded family member Tom interacted with the NHR1 domain of dNeur but showed no interaction with the NHR1 domain of hKIAA1787 [2]. Similarly, the DDINNNN peptide derived from VASA interacted with the GUSTAVUS B30.2/SPRY domain, but did not interact with either of the NHR1 domains from dNeur or hKIAA1787 [2,8]. Despite the overall similarities in the structures of these domains and the location of the target binding pockets, differences in key amino acids appears to be important for substrate specificity. For example, the Tyr183 residue on Loop 6 in the dNeur NHR domain was found to be a key residue mediating the interaction with

Table 1
Function of neuralized and other NHR-containing proteins from different species.

Species	Protein	Function
Drosophila	Neuralized	Notch signalling, learning and memory, epithelial cell polarity, GSC maintenance
	CG3894	Unknown
	Neurl4 (formerly Bluestreak)	germ cell migration
Mouse	Neurl1	Notch signalling, ethanol hypersensitivity, olfactory discrimination, mammary gland maturation, apoptosis, synaptic plasticity and memory storage
	Neurl2	Notch signalling (cooperates with Mib-1)
	OzzE3	β-Catenin regulation during myogenesis
	Neurl4	Centrosome maintenance
	LINCR	Glucocorticoid-attenuated response gene
Human	hNeur1	Tumor suppressor
	hNeur2	Notch (interact with Dl-like ligand)
	LINCR	Glucocorticoid-attenuated response gene
	Neurl4 (hKIAA1787)	MTOC formation, centrosome architecture