Do Bucentaur family proteins represent true monsters in the fight against stress?

The BCNT (Bucentaur) protein family, which is conserved from fungi to humans, is classified by the presence of an ~80 amino acid sequence at the C-terminus called BCNT-C. Family members in fungi, flies, fish, and chicken play essential roles in gene expression and/or development. The accidental discovery of a novel protein with a molecular size of 97 kDa in bovine brain (p97Bcnt) led to the identification of the entire BCNT protein family. During its evolution, p97Bcnt recruited a long region from a mobile gene known as RTE (retrotransposable element). Later, the human homolog was discovered to lack the RTE region, but include the BCNT-C (Fig. 1A). We named this molecule Bucentaur after a half-bull, half-man creature in Greek mythology to reflect its process of identification and the fact that it looks unusual, like a monster. It has been established that *p97Bcnt* and its once duplicated form, *p97Bcnt-2*, which are present only in ruminants, were created by a partial duplication of the ancestral *Bcnt* gene combined with the insertion of part of RTE, the apurinic/apyrimidinic (AP)-endonuclease domain. The human-type *Bcnt* gene is their ortholog and is evolutionally conserved from fungi to humans (Fig. 1 B).

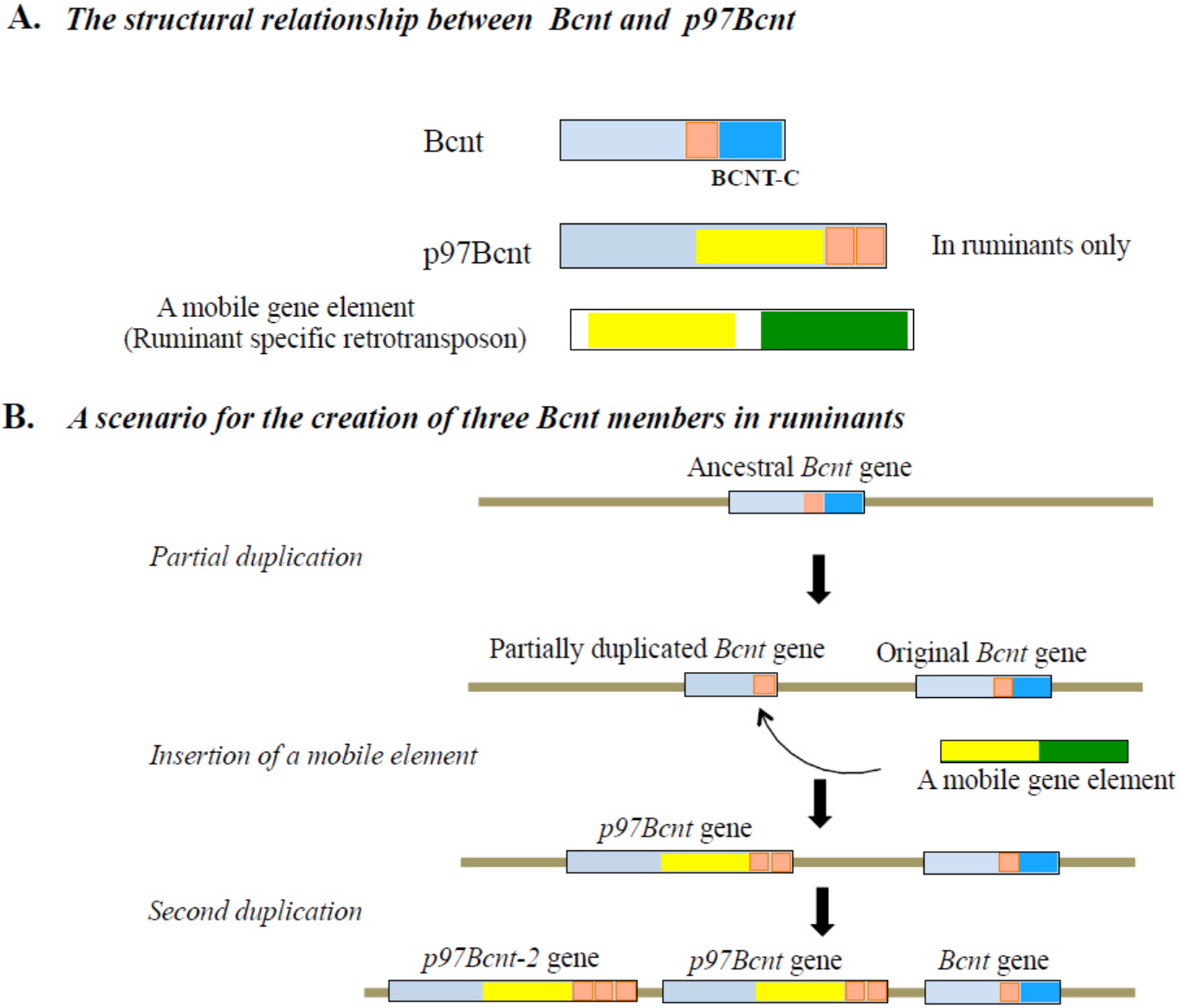

Fig. 1. A. The structural relationship between Bcnt and p97Bcnt. Human Bcnt/Cfdp1 and bovine p97Bcnt share a common acidic N-terminal region (grey box) and a 40 amino acid-unit (orange box). Although p97Bcnt contains a portion derived from a mobile gene element (yellow box), this sequence is absent in human Bcnt/Cfdp1, which instead includes the conserved C-terminus (Bcnt-C).
B. A scenario for the creation of three Bcnt members in ruminants. A scenario for the creation of the three Bcnt members includes several steps: (1) partial gene duplication of the ancestral Bcnt/Cfdp1, (2) insertion of a portion of a mobile gene element, and (3) an additional gene duplication of the ancestor p97Bcnt. Nucleotide regions corresponding to the acidic N-terminal regions, the 40-amino acid unit, and BCNT-C are indicated by the grey, orange, and blue boxes, respectively.

Whereas the function of mammalian Bcnt/Cfdp1 remains unknown, its properties have been revealed. It includes intrinsically highly disordered acidic and basic stretches in the N-terminal region, and lacks a fixed three-dimensional structure; instead, it may undergo protein modifications that trigger a transient structure that allows it to form a complex with other proteins and become functional. Eukaryotic DNA exists in a highly compacted complex form called chromatin, in which the DNA wraps around basic core proteins known as histones. Chromatin relaxation is regulated not only by modification of the histones inside the core, but also by energy-dependent and –independent changes (chromatin remodeling) carried out by many proteins outside the core. These proteins are involved in fine-tuning the relaxation without changing the underlying DNA, and are called epigenetic factors. Their protein modifications include phosphorylation (producing a negative charge), acetylation (switching-off a positive charge), methylation (excluding H_2O to allow hydrophobic interactions) and ubiquitination (a turn-over switch). Human BCNT is acetylated *in vitro* by a well-known co-transcriptional regulator (CREB-binding protein), and is phosphorylated *in vivo* at eight sites and acetylated at four sites, including in the BCNT-C. Especially, the phosphorylation of serine 250 in the BCNT-C affects the structural dynamics of the molecule, and is probably related to a critical function (Fig. 2). These properties of Bcnt identify it as an epigenetic factor.

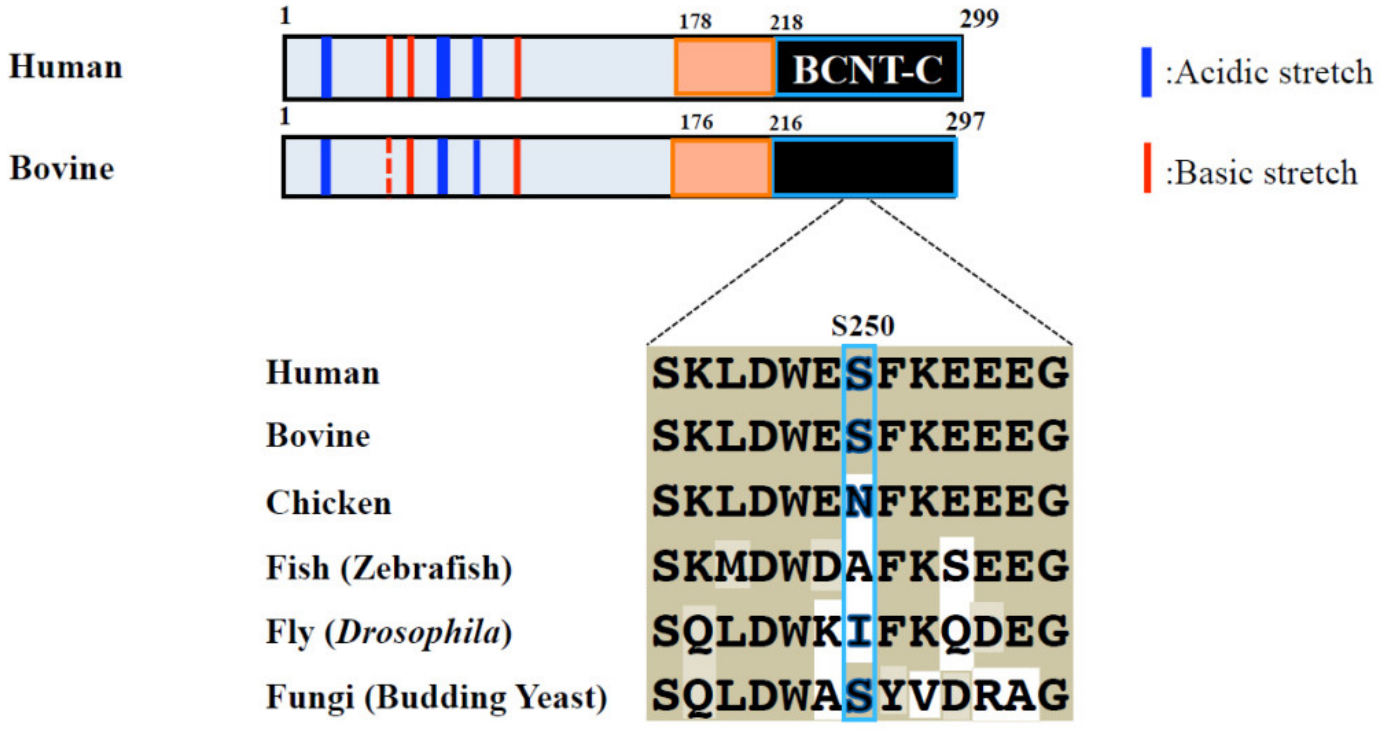

Fig. 2. Structures of human and bovine BCNT and alignment of amino acid residues around the Ser250 phosphorylation site

The molecular architectures of human and bovine Bcnt/cfdp1 are very similar to one another: an N-

terminal region, a 40-amino acid stretch, and the exact same C-terminal region (BCNT-C). Alignment of the amino acid sequence around serine 250 in human BCNT/CFDP1, the phosphorylation of which affects the molecular dynamics and may play a critical role in protein function, is shown.

Living things are always threatened by oxidative stress (OS) and have evolved anti-oxidant machinery to cope. BCNT family members have been suggested to function preferentially under conditions of OS. Fungal Bcnt, which functions in energy-dependent chromatin remodeling, shuttles between the nucleus and cytoplasm in response to oxygen, and regulates proper gene expression under OS. Cerebellar granule neurons, which are known to be vulnerable to OS, are the target tissue of a *Bcnt/Cfdp1* defect in zebrafish. Additionally, the two ruminant-specific pseudo BCNT members, p97Bcnt and p97Bcnt2, include an AP-endonuclease-like domain in the middle of their molecules (Fig. 1A&B). The canonical functions of this domain lie in DNA repair and as a redox factor acting to counteract OS. It is also a biomarker of cancer. Therefore, the existence of three family members in ruminants raises the intriguing question of whether the BCNT family represents a unique anti-oxidative network in the animal kingdom.

Comment:

Although the *Bcnt* gene is officially called *Cfdp1*, so far solid evidence that the gene is involved in craniofacial development has not been provided. Thus the name *Bcnt* or *Bcnt/Cfdp1* is used in this article.

Publication

Mammalian Bcnt/Cfdp1, a potential epigenetic factor characterized by an acidic stretch in the disordered N-terminal and Ser250 phosphorylation in the conserved C-terminal regions.
Iwashita S, Suzuki T, Yasuda T, Nakashima K, Sakamoto T, Kohno T, Takahashi I, Kobayashi T, Ohno-Iwashita Y, Imajoh-Ohmi S, Song SY, Dohmae N
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