Src homology adaptor proteins: more than the sum of the parts?

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The recent determination of the crystal structure of the 25 kDa growth factor receptor-bound protein 2 (Grb2) [1] brings us a step further on the road of discovery outlined by François Jacob. He described biological macromolecules as having evolved by "combining several systems to produce a more complex one", a process which "resembles not engineering but tinkering", and in which "Nature operates to create diversity by endlessly combining bits and pieces" [2]. Grb2 is an example of an apparently 'tinkered' molecule. A member of the class of Src homology (SH) domain-containing proteins known as 'adaptors', Grb2 has three SH domains in the order SH3–SH2–SH3.

Adaptor proteins have no apparent catalytic transcriptional activity, in contrast to other classes of proteins containing SH2 and SH3 domains. The first member of the adaptors identified was the oncogene product, Crk [3], and Grb2 is one of the best biologically understood adaptors, playing a key role in the pathway linking activation of cell-surface receptors to Ras activation, and subsequent modification of mitogen-activated protein (MAP) kinases and transcriptional regulation (reviewed in [4,5]). Grb2 binds to a range of tyrosine-phosphorylated targets, such as the epidermal growth factor receptor, via its SH2 domain, and to its downstream target, the guanine nucleotide exchange factor, Son of sevenless (Sos), via its SH3 domains. The structures of isolated SH3 domains from human Grb2 and homologs from other species, including their ligated forms [6–11], have been reported. Apparently, no allosteric communication occurs between SH2 and SH3 domains on binding to their respective ligands [12] in the complete Grb2 molecule. These, and other, data [13] suggest that the principal action of Grb2 is the recruitment of the Grb2–Sos complex to the plasma membrane in the vicinity of the activated receptor, followed by formation of a multicomponent complex with membrane-bound Ras.

The crystal structure of Grb2 [1] generally supports these ideas, and also raises a few intriguing issues. The protein is shown to comprise three distinct domains, with considerable separation of the SH3 domains from the SH2 domain by an interlaced junction (Fig. 1). There appears to be a better-defined interface between the two SH3 domains (Fig. 2), although owing to the relatively small area of buried surface (1000 Å^2), the authors suggest that this may permit dissociation of the two SH3 domains and adoption of different conformations, with positioning of the SH3 segments restricted solely by the length and flexibility of the linker

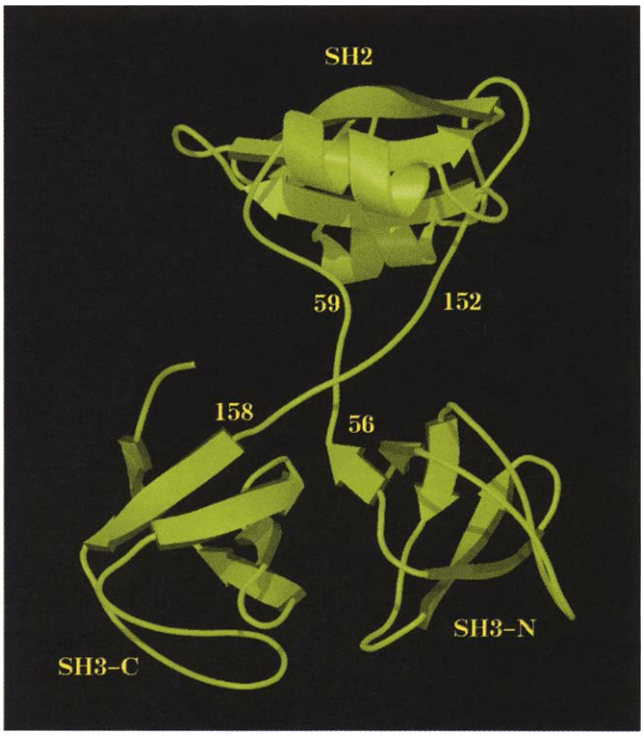

Fig. 1. Ribbon diagram of Grb2. The three SH domains are labeled, with SH3-C and SH3-N denoting the C- and N-terminal SH3 domains, respectively. (Reproduced from [1], with permission.)

segments (residues 56–59 and 152–158) as illustrated schematically in Figure 3.

The relative orientations of the SH2 and SH3 domains appear quite different from that of the regulatory apparatus [SH(3+2)] of Lck, reported previously [14]. There has been considerable speculation that the positioning of SH2 and SH3 domains may have special structural significance, mainly on the basis of the frequent juxtapositioning of the domain types in different proteins, and the relatively high sequential conservation of the linker region among families of related SH3- and SH2-containing proteins. Whereas the Grb2 results do not exclude the possibility that the linkers have special functions in some families, it seems unlikely that there is any general purpose to these linker regions, other than to bring together the various ligand-containing partners in the intracellular signaling complex.

The crystal structure of Grb2 contains two molecules in the asymmetric unit with a local two-fold axis. A substantial dimer interface (4100 Å^2) and the lack of overlap

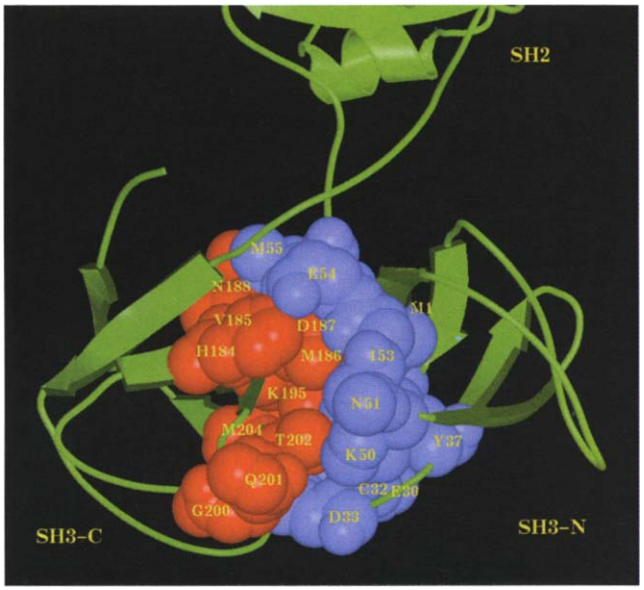

Fig. 2. Interface between the two SH3 domains of Grb2. Atoms in contact are represented by space-filling model. (Figure courtesy of Sébastien Maignan.)

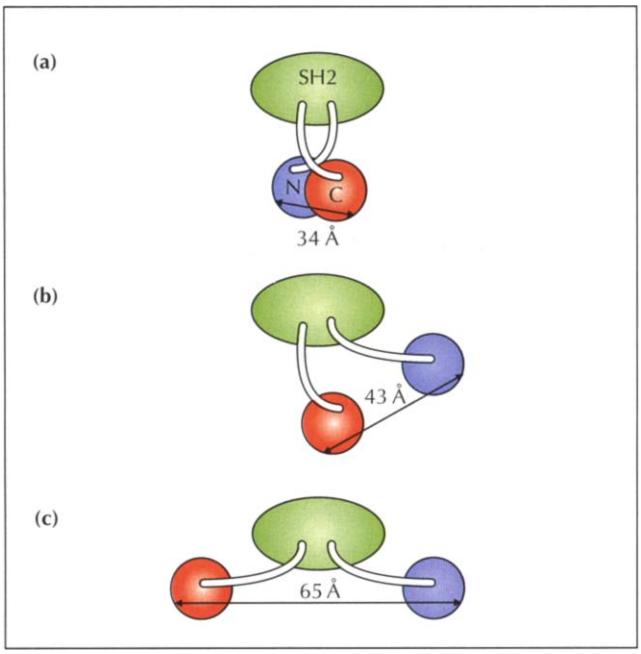

Fig. 3. Schematic representation of the possible relative orientations of the N-terminal (N) and C-terminal (C) SH3 domains of Grb2. Arrows indicate the distance between SH3 peptide binding sites. **(a)** Closed: as observed in Grb2 X-ray structure; **(b)** half open; **(c)** open. (Adapted from [1].)

of this interface with putative binding sites for ligands to the SH2 and SH3 domains raises the question that this dimerization has some functional role. This is reminiscent of the suggested role for dimerization in the Lck kinase regulation [14]. Both Grb2 and Lck SH(3+2) are observed as dimers in the crystal form, but not, apparently, in solution. As both proteins are membrane associated, could this relatively weak dimerization have a significant role in biological activity, by, for example, limiting the dissociation of complexes incompletely occupied by their full set of ligands?

While such speculations are intriguing, we should probably recall Jacob's concept of molecular evolution [2], and put our bets on a methodical test of these and other notions. The paper by Maignan *et al.*, by providing the first description of a multiple SH domain-containing protein in its entirety, not only represents a significant advance in our knowledge of Grb2, but may provide insights into complex adaptor proteins in general and related multiple SH and other domain-containing proteins.

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