SPRY domains in ryanodine receptors (Ca^{2+}-release channels)

Intracellular Ca^{2+} signalling is critical to the control of diverse cellular processes[1] and depends, in many instances, on the release of Ca^{2+} ions from intracellular stores via either inositol 1,4,5-trisphosphate receptor (IP_3R) or ryanodine receptor (RyR) subtypes[2]. In excitable striated muscle cells, membrane depolarisation induces a signal via the dihydropyridine receptor (DHPR) that triggers RyR-mediated Ca^{2+}-release from the sarcoplasmic reticulum. The DHPR-mediated signal is coupled to RyR-1 and/or RyR-2 homotetramers by one or both of two mechanisms, dependent on the RyR subtype: Ca^{2+}-induced Ca^{2+} release is accomplished by either RyR-1 or RyR-2, whereas a mechanical coupling of DHPR with RyR is mediated solely by RyR-1 (Ref. 3). IP_3R and RyR proteins are homologues, with sequence-similar amino-terminal regions, encompassing the IP_3-binding site in IP_3Rs, and carboxy-terminal regions, containing between 4–12 membrane-spanning sequences[1,2,4]. The intervening central regions of RyRs and IP_3Rs, however, are sequence-dissimilar and are likely to contain domains with modulatory and transducing functions.

Here, we report the identification of a domain that is present in three copies in each of the three mammalian RyR subtypes. The triplicated domain is located in the central IP_3R-dissimilar region of RyRs, and is also present three times in a *Dictyostelium discoideum* dual-specificity kinase termed splA. Owing to the repeats in splA and RyR, we refer to these sequences as SPRY domains. Our interest in these domains arose from a recent collation of proteins containing a domain termed a 'sterile alpha motif' (SAM)[5]. SplA (also called DPYK1) contains a single SAM and is a dual-specificity kinase that regulates spore cell differentiation[6]. A Blastp[7] search with the amino-terminal region that precedes the SAM domain

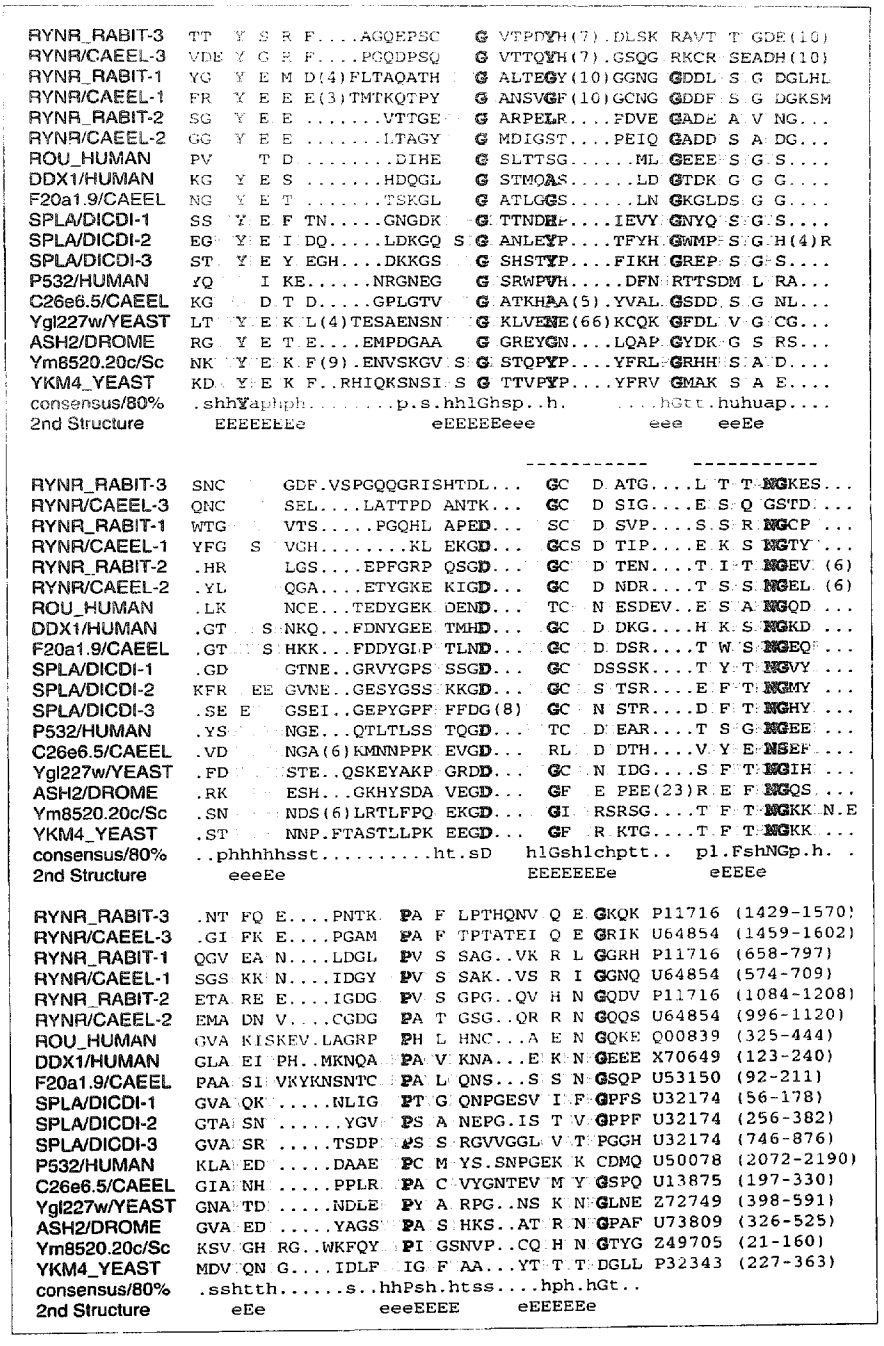

Figure 1.

Multiple alignment of representative SPRY-domain sequences (apparent orthologues have been removed from the alignment). β-strands β3–β4–β5 are expected to be aligned with less accuracy than the remainder of the β-strands. Secondary structure prediction, using the PHD server[16] [H/h denotes an α-helix and E/e a β-strand with an expected accuracy higher than 82% (upper case)/72% (lower case)] is shown beneath the alignment, as is the consensus sequence (threshold = 80%; a = aromatic, c = charged, h = hydrophobic, p = polar, s = small, u = tiny). Dots represent insertions/deletions. Residue limits and SWISS-PROT, EMBL and PIR accession codes are shown following the alignment. RYNR_RABIT represents rabbit RyR subtype 1. Numbers in parentheses represent residues excised from the alignment. Species: CAEEL, *Caenorhabditis elegans*; DICDI, *Dictyostelium discoideum*; DROME, *Drosophila melanogaster*; RABIT, *Oryctolagus cuniculus*; YEAST and Sc, *Saccharomyces cerevisiae*. Repeats in splA were also detected using REPRO[17] (three pairwise alignments scored > 300). A motif search using MoST[18] and an alignment block (overlined) of the three splA repeats, identified similar sequences in five RyRs, a *D. melanogaster* DEAD-box protein, *C. elegans* F20a1.9, and yeast YGL227w and YM8520.20c (E < 0.01) in an initial iteration (parameters E = 0.02, I = 80%); subsequent iterations yielded all sequences shown in Fig. 1, with the exception of RyR SPRY3, hnRNP U, p532 and C26e6.5. A human SPRY domain appears to be partially encoded by expressed sequence tags H26869 and R73437. The SPRY domain carboxy-terminal limit is defined by the SPRY domain in C26e6.5 (residues 197–330 of a total of 332); the amino-terminal limit is more poorly defined and is likely to extend further than shown here.

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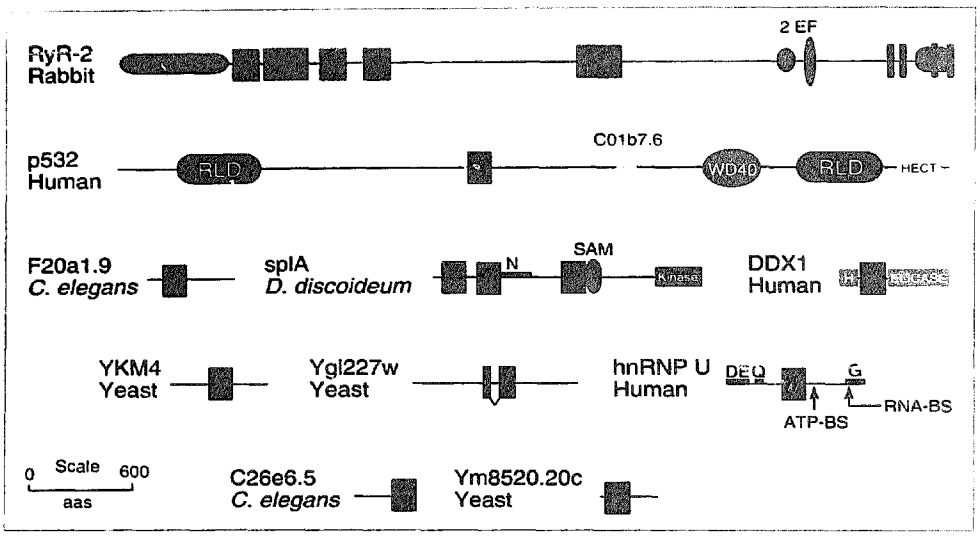

Figure 2

Schematic representation of the domain organisations of SPRY domain-containing proteins. SPRY domains are shown as purple boxes, and a previously-described fourfold repeat in RyRs is represented by a green box. The DDX1 SPRY domain is present as an insertion within a helicase homologous sequence[12]. The YGL227w SPRY contains a 60-residue insertion. Inositol 1,4,5-trisphosphate receptor (IP_3R)-similar regions in RyR are represented by red ovals. Black stripes represent the minimum number of transmembrane segments thought to be present in RyRs. Regions rich in a particular type of amino acid (D or E, N, Q or G) are indicated by a solid line. P532 contains two regions ('RLD') that are homologous to the RCC1 cell-cycle regulator, and a region containing seven WD40 repeats[13]. A region of p532 that is similar to a hypothetical *Caenorhabditis elegans* protein (C01b7.6) is represented by a brown box. Putative ATP- and RNA-binding sites within hnRNP U[10] are indicated. Mutations in the *RyR-1* gene that are associated with malignant hyperthermia susceptibility[19] do not map to any of its three SPRY domains. Results of profile, motif and HMM methods (not shown) indicate the presence of two EF-hands in RyRs, of which the first was reported to bear 'some resemblance' to EF-hands in a previous publication[20]. The amino-terminal EF-hand is strongly predicted by these methods [e.g. score of 35 bits for *C. elegans* Ryr-1 using an EF-hand HMM[21], whereas the carboxy-terminal EF-hand is more poorly predicted (10 bits)]. However, the latter prediction is made given that EF-hands almost invariably occur in pairs and that PROSITE[22] predicts two EF-hands in *C. elegans* Ryr-1.

(residues 1–905) revealed significant similarities with the *Caenorhabditis elegans* Ryr-1 ($P = 1.9 \times 10^{-8}$), a hypothetical *Saccharomyces cerevisiae* protein (YGX7; $P = 2.6 \times 10^{-7}$), a *Drosophila melanogaster* helicase homologue ($P = 4.5 \times 10^{-5}$) and other RyR isoforms ($10^{-4} < P < 10^{-2}$). In addition, the Blastp output revealed regions of self-similarity within the splA sequence (pairwise alignments with HSP scores 121, 114 and 81), indicating the presence of three repeats. The presence of triplicated repeats in splA was further indicated by MACAW[8]-derived three-way alignments that yielded P-values of 0.0 and 6.2×10^{-11}; similar methods were used to suggest three repeats in rabbit skeletal muscle and in other RyR isoforms ($P = 1.1 \times 10^{-9}$ and 1.0×10^{-2}). A profile, prepared from an alignment of the three splA SPRY domains, when compared in a single iteration with databases using SWise[9] indicated SPRY domains in RyRs and a further nine proteins (Figs 1, 2). These and other results (see Fig. 1 legend) lead us to propose that these proteins contain homologous domains.

Although no information concerning the functions of SPRY domains is yet available, their presence in two proteins suggests a possible RNA-binding role. Heterogeneous nuclear ribonucleoprotein U (hnRNP U) is a nucleoplasmic RNA-binding protein that is thought to participate in RNA processing[10], and DDX1 is a putative RNA helicase that has been suggested to contribute to the control of cell growth and division[11,12]. However, it is not clear what functional benefit might accrue to RyRs from their possession of three RNA-binding SPRY domains. The remaining proteins' functions are uncharacterised, except for two proteins: p532 (previously called p619), which stimulates guanine nucleotide exchange factor on ARF1 and may perform multiple roles in membrane trafficking processes[13]; and the nuclear protein ash2 from *Drosophila*, a trithorax group gene product that is required for imaginal disc pattern formation[14].

In conclusion, a novel domain has been identified in RyRs and in other proteins. These results, and evidence for the presence of two EF-hands in RyRs (see Fig. 2 legend), will facilitate both the investigation of the molecular mechanisms underlying Ca^{2+}-induced Ca^{2+}-release and the identification of RyR domains that bind regulatory components of the Ca^{2+}-release complex, such as triadin and FK506-binding protein (reviewed in Ref. 15).

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CHRIS PONTING

Fibrinolysis Research Unit, University of Oxford, The Old Observatory, South Parks Road, Oxford, UK OX1 3RH.
Email: ponting@molbiol.ox.ac.uk

JÖRG SCHULTZ AND PEER BORK

EMBL, Heidelberg and Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany.