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A role for the Myoblast city homologues Dock1 and Dock5 and the adaptor proteins Crk and Crk-like in zebrafish myoblast fusion

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Myoblast fusion follows a defined sequence of events that is strikingly similar in vertebrates and invertebrates. Genetic analysis in *Drosophila* has identified many of the molecules that mediate the different steps in the fusion process; by contrast, the molecular basis of myoblast fusion during vertebrate embryogenesis remains poorly characterised. A key component of the intracellular fusion pathway in *Drosophila* is the protein encoded by the *myoblast city* (*mbc*) gene, a close homologue of the vertebrate protein dedicator of cytokinesis 1 (DOCK1, formerly DOCK180). Using morpholino antisense-oligonucleotide-mediated knockdown of gene activity in the zebrafish embryo, we show that the fusion of embryonic fast-twitch myoblasts requires the activities of Dock1 and the closely related Dock5 protein. In addition, we show that the adaptor proteins Crk and Crk-like (Crkl), with which Dock proteins are known to interact physically, are also required for myoblast fusion.

KEY WORDS: Crk, Crkl, DOCK1, DOCK5, Myoblast city, Myoblast fusion, Zebrafish

INTRODUCTION

Skeletal muscle is composed of bundles of syncytial myotubes that arise during embryonic development by the fusion of precursor cells known as myoblasts. As well as underpinning muscle development, myoblast fusion also plays an essential role in post-embryonic life in the repair of damaged muscle fibres. Despite its fundamental importance, the molecular basis of myoblast fusion in vertebrates is still poorly understood. In *Drosophila*, by contrast, genetic analysis has provided a detailed inventory of the molecular components that underpin the process in embryonic development. Heterophilic interactions between members of the immunoglobulin superfamily – Dumbfounded (Duf; also known as Kirre), Roughest (Rst; also known as IrreC) and Sticks and stones (Sns) (Bour et al., 2000; Ruiz-Gomez et al., 2000; Strunkelnberg et al., 2001) – mediate the initial contact between fusing cells (Ruiz-Gomez et al., 2000; Galletta et al., 2004), triggering intracellular pathways that orchestrate the cytoskeletal rearrangements and cell shape changes that accompany fusion (reviewed in Taylor, 2003; Chen and Olson, 2004). A key component of the intracellular network is the protein encoded by the *myoblast city* (*mbc*) gene (Rushton et al., 1995; Erickson et al., 1997), a close homologue of the vertebrate protein dedicator of cytokinesis 1 (DOCK1, formerly DOCK180) and the *Caenorhabditis elegans* protein CED-5, which, together with Mbc, define the CDM family of proteins (Wu and Horvitz, 1998).

Myoblast fusion is much less readily amenable to experimental analysis in higher vertebrates, such as the mouse; for this reason, most of what is known about the process has been gleaned from in vitro studies using various murine and human muscle cell lines. These studies have identified a large set of proteins that appear to be required for fusion (Rosen et al., 1992; Zeschnigk et al., 1995; Schwander et al., 2003; Horsley and Pavlath, 2004; Doherty et al., 2005; Lafuste et al., 2005; Charrasse et al., 2006; Charrasse et al., 2007; Comunale et al., 2007; Georgiadis et al., 2007). Given the striking similarities between the cellular events that underpin myoblast fusion in vertebrates and invertebrates (Knudsen and Horwitz, 1977; Knudsen and Horwitz, 1978; Wakelam, 1985; Doberstein et al., 1997), there is surprisingly little, if any, overlap between these proteins and those identified by genetic analysis in *Drosophila*.

In contrast to the mouse, the zebrafish embryo has a number of qualities that makes it particularly well-suited to the in vivo analysis of myogenesis (Devoto et al., 1996; Blagden et al., 1997; Currie and Ingham, 1998; Roy et al., 2001; Henry and Amacher, 2004; Hughes, 2004). The embryonic myotome develops during the first 24 hours postfertilisation (hpf) and is comprised of two kinds of fibre: mononucleate slow-twitch fibres and multinucleate fast-twitch fibres, the latter of which arise via the fusion of precursor myoblasts (Roy et al., 2001). Muscle fibres and their progenitors can be readily imaged using confocal microscopy, and gene function can be manipulated using morpholino antisense oligonucleotides (morpholinos) (Ekker, 2000). We have taken advantage of these properties to investigate whether orthologues of the components that control myoblast fusion in *Drosophila* have a conserved function in vertebrate myogenesis. Here, we describe the establishment of a method for the quantitative analysis of fast-twitch myoblast fusion during zebrafish embryogenesis and its application to the analysis of the function of CDM family members closely related to Mbc. Because CDM proteins form part of multiple signalling pathways, the best-characterised being the Crk-CDM-Rac pathway, we have also used our assay to investigate a postulated role for the adaptor proteins Crk and Crk-like (Crkl) in myoblast fusion.

MATERIALS AND METHODS

PCR amplification and cloning

Zebrafish embryos were collected at 24 hpf, washed in water and RNA was extracted with Trizol (Invitrogen). Reverse transcription (RT) was performed using the Superscript RT kit (Invitrogen), and PCR was carried out using the following primers (all sequences are shown 5′-3′):

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Dock1: AGAGAAGCGAGAAAAAGTGTGTG and GTCATAGTTGTAGACGGCCACTC, GAGTGGCCGTCTACAACTATGAC and ACACTGGATTGAAATGATGGAAC, ACACTGGATGCTCTCTTCAACAT and TTGTCCATCAGGTTTCCATAGTC; Dock5: ATTCCACTTCACTCGAGTTTTCC and TTTCAGGGTCTAAAGTGTTTCCA, GTGCCTACAGAATTGCCTTTAGTT and TGTTGATGTATTGTGCGTAATGTG, CGGAGGGTCAATCGTACAGT and CATTGGCCAGCTCCATAGTT, TTCCAGGAATCCTCAAGTGG and GTTTTCCTCCAGCCTCACAG.

Full-length sequences of *dock1* and *dock5* were cloned by PCR using the following primers:

Dock1: AGAGAAGCGAGAAAAAGTGTGTG and GAGGTGTCAGCTGCTTTTCC; Dock5: ATTCCACTTCACTCGAGTTTTCC and CACAGCATGCAGTTGTTTGA.

A full-length construct for *crk* was cloned using the following primers: AGGATCCACCATGGCCGGAAATTTTGA and TTCTAGAAGGGCTGCTAGGTAAGACAG.

All PCR products were ligated into the TOPO-TA pCRII vector with dual promoters (Invitrogen) and sequenced.

In situ hybridisation

Anti-sense probes for *dock1* and *dock5* were transcribed from the RT-PCR products that were cloned into the TOPO-TA pCRII vector. RNAs from several sections of the genes were pooled. In situ hybridisation was performed using standard procedures (Westerfield, 2000).

Morpholino knockdown

Morpholino sequences were: *crk* ATG, GCCTTCCCTTCGCCTCTCCTTATCC; *crk* splice, GGAGCAAGCCCTGCGGGATGACATT; *crkl* ATG, AGGAGTCGAACCGTGCAGACGACAT; *crkl* splice, TCATTTAGCCACTTACCTCCAGTGC; *dock1* ATG, TTTTTGTAGGCACCCAGCGCGACAT; *dock1* splice, CATCACCTGCAAACACACAACACAC; *dock5* splice 1, TGTTGATGTCTTACTATGTAGGGAG; *dock5* splice 2, AAAACAGCGCTCACCTTCTGGAATG.

Morpholinos (Gene Tools) were injected at the one- to two-cell stage as previously described (Wolff et al., 2003) at 0.1-2.0 mM, in solution containing 40 ng/μl *mylz2:GFP* plasmid. This plasmid contains *EGFP* (Clontech) preceded by 2239 bp of the *mylz2* upstream region (Ju et al., 2003) amplified from genomic DNA using the following primers: TCAATCAAATATCACCCCATATGTC and TGTGAAGTCTAAGAAGATCAAGAAGAGA. Embryos expressing the *mylz2:GFP* plasmid were washed in ice-cold PBS then fixed in 4% paraformaldehyde/PBS at 4°C overnight. Subsequently, embryos were washed in PBS + 0.1% Tween20 and mounted laterally in 75% glycerol with yolk removed. An Olympus BX61 microscope with Fluoview FV1000 confocal system and 60× water lens was used. Counting nuclei was easiest when embryos were viewed without any additional staining procedure through the microscope eyepieces at high magnification (600× total).

For morpholinos targeting splice junctions, groups of 20 injected embryos were collected at 26-28 hpf, RNA extraction and RT were performed as above, then PCR was carried out using the following primers: *crk*, GTGCTGTCTGTGTCGGAGAA and TGGTCACCTTTACCATGTCG; *crkl*, TTGGATACGACCACCCTGAT and CCTTCCCACTGACCACTGAT; *dock1*, GCTGAAGCTTCTTCCAGGTG and CTTGAAGGTGAAACGCAGGT; *dock5* splice 1, CGGAGGGTCAATCGTACAGT and TTCTCAGGCGAGAAGGACTC; *dock5* splice 2, CGGCTTCAAGCTGAGAGATA and CGGATATAAATGTCCTCCCTCTT.

PCR products were ligated into the TOPO-TA pCRII vector with dual promoters (Invitrogen), and sequenced.

mRNA injection

For in vitro transcription, a full-length *crk* cDNA amplified by PCR (see above) was subcloned into pCS2+ with *Bam*HI and *Xba*I. The *crkl* full-length construct was made by subcloning *crkl* from the plasmid IRAKp961A10118Q (RZPD clone) with *Spe*I and *Xba*I into the *Xba*I site of pCS2+. Full-length constructs were linearised with *Not*I and transcribed in vitro using SP6 and the mMessage Machine kit (Ambion), and the mRNA was injected into newly fertilised eggs as previously described (Krauss et al., 1993).

Antibodies

Antibodies used were F59 [developed by Frank E. Stockdale (Miller et al., 1985) and obtained from the Developmental Studies Hybridoma Bank] (1:50) and rabbit anti-β-catenin (Sigma; 1:50). Secondary antibodies were Alexa-Fluor-488 goat anti-rabbit IgG (Molecular Probes; 1:500), goat anti-mouse Cy3 (Jackson ImmunoResearch; 1:500). DAPI (Sigma; 1:1000) was also used. Staining was performed using standard procedures.

RESULTS

Myoblast fusion in the zebrafish embryo

The process of fast-twitch myoblast fusion was analysed both by the global staining of fixed zebrafish embryos and by scatter labelling of fast fibres and their precursors using a *mylz2:GFP* transgene (Xu et al., 2000; Ju et al., 2003). Prior to the onset of fusion, the fast-fibre precursors were seen as small rounded cells that extended thin protrusions (Fig. 1A,D). These protrusions could best be seen when isolated cells were labelled by the *mylz2:GFP* transgene (Fig. 1D). At the 18-somite stage (18 hpf), mononucleated slow-twitch fibres were already elongated and spanned the entire length of the somite (Fig. 1B). Fast-twitch fibres elongated later; elongated fibres could be seen as fusion began, at approximately 19 hpf (20 somites) (Fig. 1E). By 26 hpf, fusion had largely finished and most fast fibres were multinucleated (Fig. 1C,F).

We performed a quantitative analysis of the process, counting nuclei in individual fast-twitch fibres highlighted by the transient mosaic expression of the *mylz2:GFP* transgene. Because varying numbers of fibres were labelled in each embryo, the mean number of nuclei/fibre for each embryo was treated as one sample value, weighting each embryo equally. Overall means of these samples are shown in Table 1, together with the total numbers of fibres scored, total number of nuclei and number of embryos analysed. From the distributions of the individual embryo means shown in Fig. 1G, it can be seen that the majority of fusion events occurred between 20 somites and 24 hpf. Mann-Whitney rank tests confirmed significant increases in the mean number of nuclei/fibre between embryos at 15 and 20 somites ($P<0.02$), 20 and 22 somites ($P<0.01$), and 22 somites and 24 hpf ($P<0.02$). Further fusion events occurred over the next 24 hours; there was a significant increase in nuclei/fibre between 30 hpf and 48 hpf ($P<0.04$). The raw data upon which this analysis is based can be found in Tables 1-6 in the supplementary material.

Identification of the zebrafish *dock1* and *dock5* genes

Given the key role of *mbc* in myoblast fusion in *Drosophila*, we searched for orthologues of the human DOCK180 sub-family (Cote and Vuori, 2002; Meller et al., 2005) in zebrafish. One

Table 1. Progression of fusion in wild-type embryos, from 15 somites to 48 hpf

Stage	Total fibres	Total nuclei	Number of embryos	Mean of embryo means*
15 somites	113	113	6	1
20 somites	269	301	14	1.19
22 somites	225	400	7	1.73
24 hpf	384	929	12	2.48
26-28 hpf	2531	6730	77	2.63
30 hpf	709	1954	22	2.81
34 hpf	443	1156	12	2.71
48 hpf	130	419	8	3.23

*Mean nuclei/fibre for each embryo used as sample values, to weight each embryo equally, *n*=number of embryos. hpf, hours postfertilisation.

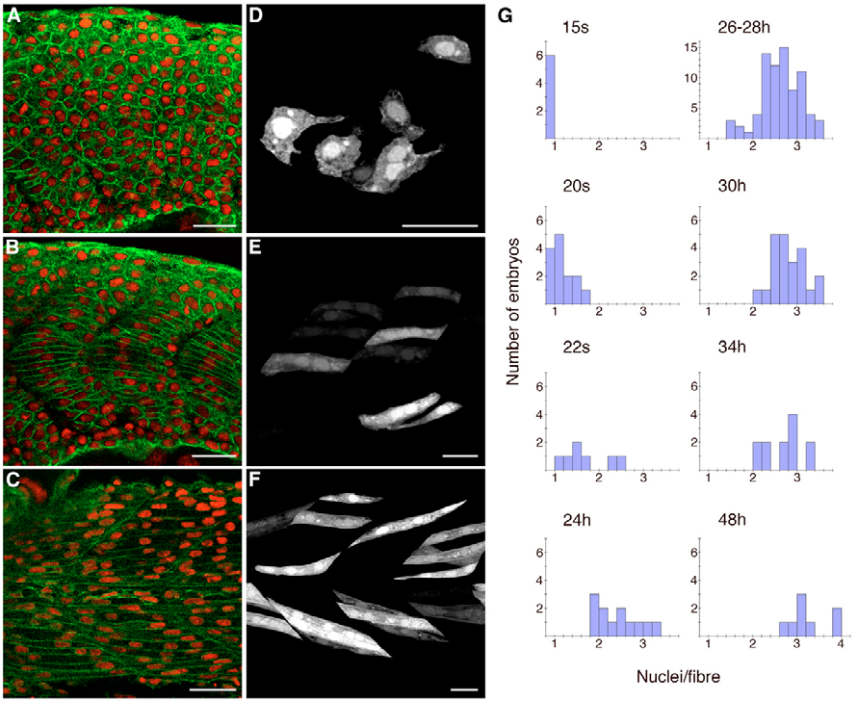

Fig. 1. Wild-type fusion of fast-twitch myoblasts. (**A-C**) Mid-trunk; β-catenin (green) and DAPI (red). (A) At 18 somites; optical section mid-way between the midline and the lateral surface, through fast precursors, showing unfused small rounded cells. (B) At 18 somites; optical section through medial adaxial cells, the slow-fibre precursors, which have elongated. (C) At 26 hpf; optical section through fast muscle fibres, showing multinucleate fused cells. (**D-F**) *mylz2:GFP* transgene expression in isolated cells. GFP labelling is found almost exclusively in fast-twitch muscle fibres, the few slow fibres that express the transgene being distinguishable by their shape and more-advanced differentiative state. (D) At 17 somites, showing small rounded mostly mononucleate cells with projections. (E) At 20 somites, showing that fusion has started to occur. Some cells are multinucleate. (F) At 26 hpf, showing elongated multinucleate fibres. (**G**) Quantitative data showing distributions of mean nuclei/fibre for individual wild-type embryos fixed at progressive embryonic stages, from 15 somites (15s) to 48 hpf (48h). Scale bars: 25 μm.

member of this family, *dock2*, has previously been annotated in zebrafish and shown to be expressed specifically in macrophages (Thisse et al., 2001), a pattern reminiscent of the haematopoietic restriction of human DOCK2 (Nishihara et al., 1999). We therefore focussed our attention on the identification of the other two sub-family members, *dock1* and *dock5*. Using BLAST searches of the Sanger Ensembl zebrafish database, we identified nucleotide sequences whose conceptual translation products showed significant levels of similarity to the mammalian DOCK1 and DOCK5 proteins. By assembling overlapping database sequences, an RT-PCR strategy was devised to amplify sections of the putative zebrafish *dock1* and *dock5* genes, allowing the sequence of the full-length coding region of each gene to be determined.

Both genes encode predicted proteins with CZH1 and CZH2 domains. The CZH1 domain of CDM proteins has been shown to interact with PtdIns(3,4,5)P_3 (Cote et al., 2005), whereas the CZH2 domain is a novel guanine-nucleotide exchange factor (GEF) domain that is implicated in the regulation of the small GTPase Rac1 (Reddien and Horvitz, 2000; Brugnera et al., 2002; Cote and Vuori, 2002; Meller et al., 2005). Each predicted protein also contains one or two C-terminal proline-rich sequences conforming to the SH3 consensus binding motif (P)PXLPXK (Schumacher et al., 1995; Matsuda et al., 1996), which has been shown to mediate interaction with Crk; PPPLPVK and PPPLPSK in the case of Dock1 and MPPLPAK in the case of Dock5. These are signature features of CDM proteins, although in humans, only DOCK1 contains exact (P)PXLPXK motifs. On the basis of this domain organisation and of the high level of protein similarity between the human DOCK1 (Ensembl accession ENSP00000280333) and DOCK5 (ENSP00000276440) and the corresponding zebrafish proteins (84.1% and 70.8% identity respectively), we conclude that these transcripts encode the zebrafish Dock1 and Dock5 orthologues (see Fig. S1 in the supplementary material). Exon boundaries were inferred using homology to the human proteins, and confirmed with the trace search facility on the Sanger website, which searches the genomic shotgun sequence database. RT-PCR analysis showed expression of both genes throughout development, from the two-cell stage to 27 hpf (Fig. 2A). In situ hybridisation analysis revealed that both genes are ubiquitously expressed during the early somitogenesis stages (Fig. 2B,D), with some modulation of expression becoming apparent by 22 somites (20 hpf) (Fig. 2C,E).

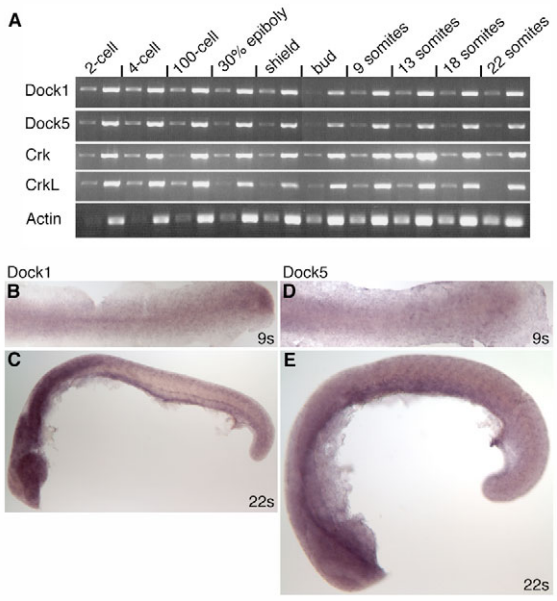

Fig. 2. Expression of Dock1, Dock5, Crk and Crkl during embryogenesis. (**A**) Reverse transcriptase (RT)-PCR analysis of *dock1*, *dock5*, *crk* and *crkl* expression, from the two-cell to 22 somite stage. Two lanes per stage; left-hand lane amplified with five PCR cycles fewer than the right-hand lane. (**B-E**) In situ hybridisation analysis of *dock1* and *dock5*. (B) *dock1*, 9 somites (9s). (C) *dock1*, 22 somites. (D) *dock5*, 9 somites. (E) *dock5*, 22 somites.

Dock1 and Dock5 are required for myoblast fusion in zebrafish embryos

To investigate a possible requirement for either of the Dock proteins in myoblast fusion, we used the injection of morpholino oligonucleotides into newly fertilised wild-type embryos to block the function of these proteins. Simultaneously, we injected plasmid DNA carrying the *mylz2:GFP* reporter gene in order to scatter-label fast-twitch myoblasts. The injected embryos were fixed at 26-28 hpf and analysed by confocal microscopy.

To assess the function of *dock5*, we injected splice morpholinos targeting the junctions between exons 27 and 28 (splice 1) and between exons 33 and 34 (splice 2) of this gene. The majority of labelled myocytes in the morphants were elongated and spanned the somite width (Fig. 3A,B,E), although some failed to elongate and appeared more rounded (Fig. 3A). In both cases, a substantial proportion were mononucleate. The *dock5* splice 1 morpholino caused a decrease in overall mean nuclei/fibre from 2.63 (wild-type control) to 1.36 at 0.5 mM, and the *dock5* splice 2 morpholino overall mean nuclei/fibre was 1.88 at 0.5 mM (see Table 2 for mean nuclei/fibre and Fig. 4 for distributions of individual embryo means). Using the Mann-Whitney test, the distributions of embryo means were shown to be significantly different from wild type for both groups ($P<0.0001$). Higher doses of the second splice morpholino gave a greater effect on fusion but led to a higher frequency of early arrest.

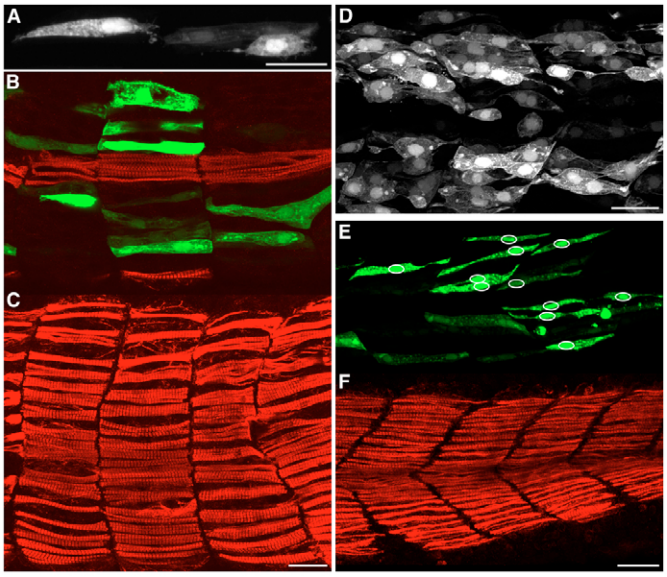

Fig. 3. Morphant embryos show defective fast-myoblast fusion. (**A**) Embryo injected with *dock5* splice 1 morpholino, showing examples in neighbouring somites of GFP-positive mononucleate cells that (left) span the somite and (right) have failed to elongate. (**B**,**C**) Embryo injected with *dock5* splice 2 morpholino, showing an example of defects in somite shape. B is more medial and shows that the GFP-positive cells are medial to the superficial slow fibres shown in C, the slow fibres in B being the medially located muscle pioneers. (**D**) Embryo injected with *crkl* ATG morpholino, showing an example with many mononucleate GFP-labelled cells that have failed to elongate and still extend thin processes. (**E**,**F**) Embryo injected with *dock5* splice 1 and *crkl* ATG morpholinos combined. Slow fibres are unaffected (F), although there are many mononucleate GFP-positive fast fibres (E). Circles in E indicate the positions of nuclei. All morpholinos were injected in solution containing *mylz2:GFP* plasmid and were fixed at 26-28 hpf. Green, GFP of *mylz2:GFP* plasmid; red, F59-antibody slow-fibre stain. Scale bars: 25 μm.

To confirm that *mylz2:GFP*-labelled mononucleate fibres are not of slow-twitch type, we stained the injected embryos with the F59 antibody, which specifically labels slow fibres at this stage (Devoto et al., 1996). None of the GFP-positive cells were labelled with the antibody. In addition, this staining revealed that the differentiation of superficial slow fibres occurs normally and on schedule in morphant embryos (Fig. 3F), although, in the more severely affected morphants, the somite shape was often disrupted such that the characteristic chevron-shape was lost (Fig. 3C). In either case, however, fusion was affected to an equal extent.

To abrogate Dock1 activity, we injected morpholinos directed against either the start ATG or the splice junction between exons 13 and 14 of the transcript. The resultant embryos were morphologically similar to the *dock5* morphants described above. Injection of the splice morpholino at two different concentrations resulted, in both cases, in a reduction in the mean number of nuclei/fibre to 1.72 (0.3 mM) or 1.31 (0.5 mM); because the higher dose resulted in a high frequency of early arrest, only the 0.3 mM data were used for statistical analysis (see Table 2 and Fig. 4). The highest number of nuclei observed in a single fibre in morphant

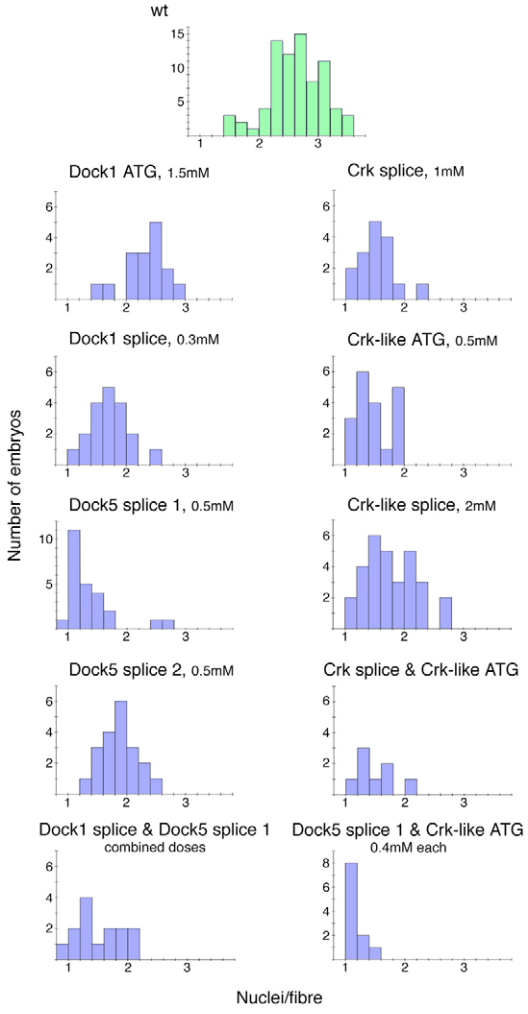

Fig. 4. Quantitative data showing the distributions of mean nuclei/fibre for individual embryos treated with *dock1*, *dock5*, *crk* and *crkl* morpholinos. These data are based upon injection of morpholinos at concentrations optimised to produce negligible early embryonic arrest.

Table 2. *dock1*, *dock5*, *crk* and *crkl* morphants all show significant increases in the number of mononucleate fast fibres, at 26-28 hpf

Morpholino	Dose (mM)	Total fibres	Total nuclei	Number of embryos	Mean of embryo means*
dock1 ATG	0.5	293	692	5	2.46
	1	368	694	12	1.89
	1.5	579	1347	16	2.35
dock1 splice	0.3	642	1168	19	1.72
	0.5	284	370	6	1.31
dock5 splice 1	0.3	307	496	11	1.66
	0.4	237	302	11	1.24
	0.5	667	862	25	1.36
dock5 splice 2	0.5	359	667	20	1.88
	1	393	722	13	1.80
	2	77	136	6	1.57
crk ATG	0.1	342	509	8	1.56
crk splice	0.5	266	451	5	1.68
	1	628	993	16	1.57
crkl ATG	0.3	404	665	13	1.66
	0.4	542	944	19	1.74
	0.5	688	1025	19	1.49
crkl splice	0.5	369	906	15	2.55
	2	773	1354	30	1.77
*dock1*spl & *dock5*spl1	0.225 & 0.3	383	649	8	1.68
	0.3 & 0.4	217	274	6	1.32
*dock5*spl1 & *crkl*ATG	0.3 & 0.3	366	477	13	1.38
	0.4 & 0.4	291	337	11	1.17
crk spl & *crkl*ATG	0.5 & 0.4	370	535	8	1.49

*Mean nuclei/fibre for each embryo used as sample values, to weight each embryo equally, *n*=number of embryos. hpf, hours postfertilisation; spl, splice.

embryos was, respectively, five and three for the two different doses, compared with a maximum of seven in wild-type controls. The *dock1* ATG morpholino had a less pronounced effect, with the number of nuclei/fibre ranging from one to six, with a mean of 2.35 for the highest dose of morpholino used (1.5 mM). Further tables of raw data are presented in Tables 1-6 in the supplementary material. Using the Mann-Whitney test, the distributions of embryo means were shown to be significantly different from wild type for all these groups ($P<0.0001$ for *dock1* splice at 0.3 mM, $P<0.003$ for *dock1* ATG at 1.5 mM).

The efficacy of each of the splice morpholinos was assayed by RT-PCR analysis of RNA extracted from the injected embryos (Fig. 5). Sequence analysis of the amplification products showed that the *dock1* splice morpholino caused the deletion of 40 bp of exon 14, leading to the introduction of a premature stop codon. The *dock5* splice 1 morpholino led to the inclusion of 273 bp of intron 27, introducing a stop codon, and *dock5* splice 2 morpholino caused deletion of 75 bp of exon 33, which also introduced a stop.

The adaptor proteins Crk and Crk-like (Crkl) are also required for myoblast fusion

CDM proteins interact physically with Crk and have been implicated in the same signalling pathways both in mammalian cell lines and in *C. elegans* embryos (Hasegawa et al., 1996; Reddien and Horvitz, 2000). A role for Crk in myoblast fusion in *Drosophila* has been hypothesised based on its biochemical interaction with Mbc, which mirrors that shown to be important in regulating the mammalian DOCK proteins (Galletta et al., 1999), but has not been tested directly by genetic inactivation. The presence of putative Crk-binding motifs in zebrafish Dock1 and Dock5 is consistent with both proteins interacting with Crk and/or the related protein Crkl. We therefore used our in vivo fusion assay to investigate the involvement of Crk and Crkl in vertebrate myoblast fusion.

The zebrafish *crk* and *crkl* orthologues have previously been identified in a cDNA expression screen and have been shown to be expressed ubiquitously (Thisse and Thisse, 2004). By RT-PCR, we confirmed that both genes are expressed throughout embryogenesis (Fig. 2A). To inactivate their function, we designed morpholino oligonucleotides that target the translation initiation codons and splice sites. The efficacy of the splice morpholinos was confirmed by RT-PCR and sequencing (Fig. 5). The *crk* morpholino caused some deletion of exon 2 and thus a frame shift in exon 3, whereas the *crkl* morpholino caused either inclusion of 57 bp of intron 2 and thus the introduction of a stop codon, or, more rarely, caused the deletion of exon 2. As with the Dock morphants, a large proportion of the fast myoblasts in injected embryos remained unfused at 26-28 hpf. The overall mean number of nuclei per *mylz2:GFP*-labelled cell following injection of the highest dose of the *crk* ATG or splice morpholino was 1.56 and 1.57, respectively (see Table 2 and Fig. 4). In the case of the *crkl* ATG and splice morpholinos, the mean nuclei/fibre was 1.49 and 1.77, respectively, at the highest doses. Each of these values is significantly different from those of the wild-type controls, as tested by the Mann-Whitney test ($P<0.0001$). The range of phenotypes caused by the *crk* and *crkl* morpholinos was very similar to that of the Dock morphant embryos described above; in most cases, mononucleate cells extended across the length of the somite, but, in some instances, many of the cells remained rounded but extended processes (Fig. 3D).

To confirm the specificity of the *crk* and *crkl* morpholinos, we assessed whether simultaneous injection of synthetic mRNA encoding either gene product could rescue the effects of the respective morpholinos. Embryos injected with *crk* mRNA at 0.3 μg/μl plus 1 mM *crk* splice morpholino had a mean number of nuclei/fibre of 2.10 ($P>0.06$ compared to wild type, i.e. not significantly different, but $P<0.02$ compared to splice morphants); embryos injected with *crkl* mRNA at 0.3 μg/μl plus 1.5 mM *crkl* splice morpholino gave a mean number of nuclei/fibre of 2.93

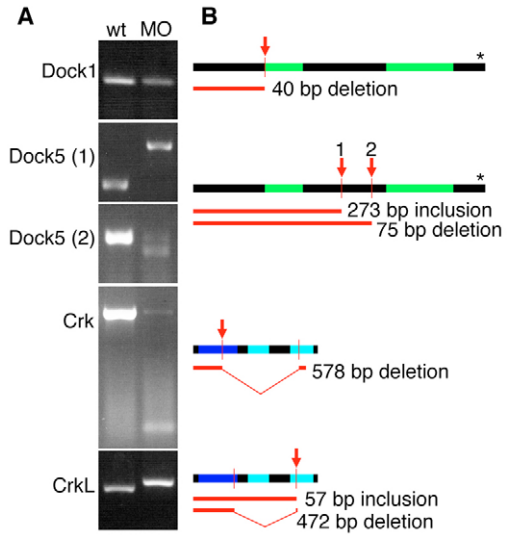

Fig. 5. RT-PCR analysis of splice morpholino function at 26-28 hpf. (**A**) Changes in size and intensity of the amplification product between wild-type (wt) and MO-injected (MO) embryos indicates effectiveness of splice morpholinos. The *dock1* wild-type band decreased in intensity when the embryos were injected with *dock1* splice MO, although a 40 bp deletion was detectable by sequencing. *dock5* splice 1 morpholino caused inclusion of 273 bp of intron. *dock5* splice 2 morpholino caused a 75 bp deletion. *crk* morpholino caused excision of 578 bp. *crkl* morpholino mainly caused inclusion of 57 bp of intron, although a deletion of 472 bp was also detected by sequencing. All changes created premature stop codons. (**B**) Diagrammatic representation of the Dock and Crk protein structures and the truncations produced by the splice morpholinos. Sites recognised by morpholinos are marked with arrows. Affected exon boundaries are shown with red vertical lines. Dock proteins of the DOCK180 subfamily possess two CZH domains (green) and one or two C-terminal proline-rich PXLPXK motifs (*), which bind Crk. The adaptor proteins Crk and Crkl contain an SH2 domain (dark blue) and two SH3 domains (pale blue). The N-terminal SH3 domain binds to DOCK180 proteins.

(P>0.2 compared to wild type, i.e. not significantly different, but P<0.0002 compared to splice morphants). Distributions of the embryo means for these groups injected with mRNA are shown in Fig. 6E, overall mean numbers of nuclei/fibre are in Table 3 and raw data can be found in Tables 1-6 in the supplementary material.

Significantly, we found that injection of *crk* mRNA alone (at 0.3 μg/μl) increased the overall mean number of nuclei/fibre to 3.00 (P<0.004 when compared with wild-type controls), and that *crkl* mRNA at 0.1 μg/μl also caused an increase to 3.42 nuclei/fibre (P<0.0002). Some fibres in the injected embryos contained eight or more nuclei, exceeding the maximum seven observed in wild-type fibres at the same stage. An example of fibres with an excessive number of nuclei is shown in Fig. 6C. This promotion of fusion was confined to fast-twitch progenitors; staining of slow-muscle fibres showed them to be mononucleated, as in wild type.

Taken together, these data reveal a requirement for Dock1, Dock5, Crk and Crkl during myoblast fusion in the zebrafish embryo. This could imply a partial redundancy of function between the pairs of related proteins; simultaneous knockdown of either Dock1 and Dock5 or of Crk and Crkl, however, resulted in only a slight enhancement of the phenotype relative to that of each single morphant (Table 2). Notably, the highest level of fusion suppression that we observed occurred in embryos injected with both *dock5* and *crkl* morpholinos (see Figs 3 and 4). The failure to suppress fusion completely most probably reflects the fact that, in no cases, was the activity of the various genes completely eliminated, as indicated by our RT-PCR analysis of splicing. Injection of higher combined doses of morpholinos also tended to increase mortality, precluding analysis of the effects of total inhibition of gene function.

DISCUSSION

Two types of skeletal muscle fibres are formed during zebrafish embryogenesis: mononucleated slow-twitch fibres, the majority of which form a superficial layer on the surface of each somite, and multinucleated fast-twitch fibres, which constitute the bulk of the embryonic myotome. Previous studies have shown that these latter fibres arise by the fusion of myoblasts (Roy et al., 2001), a process that is specifically suppressed in the slow-twitch myoblasts by the Prdm1 transcription factor encoded by the *ubo* gene (Roy et al., 2001; Baxendale et al., 2004). Here, we have analysed the temporal progression of the fusion process and have begun to dissect its genetic control. At 18 hpf, fast-twitch myoblasts remain unfused but can be seen to be probing their environment by sending out processes; over the next 2 hours, fusion begins to occur and, by 26 hpf, the majority of myoblasts have fused to form multinucleated fibres, containing between two and seven nuclei.

In the *Drosophila* embryo, multinucleated muscle fibres similarly form by the fusion of myoblasts. In this organism, two distinct types of myoblasts have been identified: fusion-competent cells, which represent the majority of the muscle precursors, and founder cells, which play a key role in directing the type of muscle arising from the differentiation of the fusing myoblasts. When fusion is blocked by mutations that inactivate components of the fusion pathway, the founder cells proceed to differentiate into mononucleated muscle fibres whereas the unfused fusion-competent cells eventually undergo apoptosis and are engulfed by macrophages (Rushton et al., 1995; Doberstein et al., 1997). The first mutation affecting myoblast fusion to be recovered in *Drosophila* was *myoblast city* (*mbc*), which encodes the DOCK180 protein (Rushton et al., 1995; Erickson et al., 1997). Subsequent molecular and genetic analysis has revealed that DOCK180 occupies a pivotal role in a molecular pathway that

Table 3. *crk* and *crkl* mRNA injection causes excess fusion of fast myoblasts by 26-28 hpf, and rescues splice morphants

	Total fibres	Total nuclei	Number of embryos	Mean of embryo means*
Control	1377	3570	29	2.56
crk mRNA 0.3 μg/μl	910	2788	16	3.00
crk splice MO 1 mM	633	1129	18	1.75
crk mRNA 0.3 μg/μl & Crk splice MO 1 mM	532	1205	17	2.10
crkl mRNA 0.1 μg/μl	539	1875	15	3.42
crkl splice MO 1.5 mM	583	999	17	1.77
crkl mRNA 0.3 μg/μl & Crkl splice MO 1.5 mM	459	1408	14	2.93

*Mean nuclei/fibre for each embryo used as sample values, to weight each embryo equally, *n*=number of embryos.

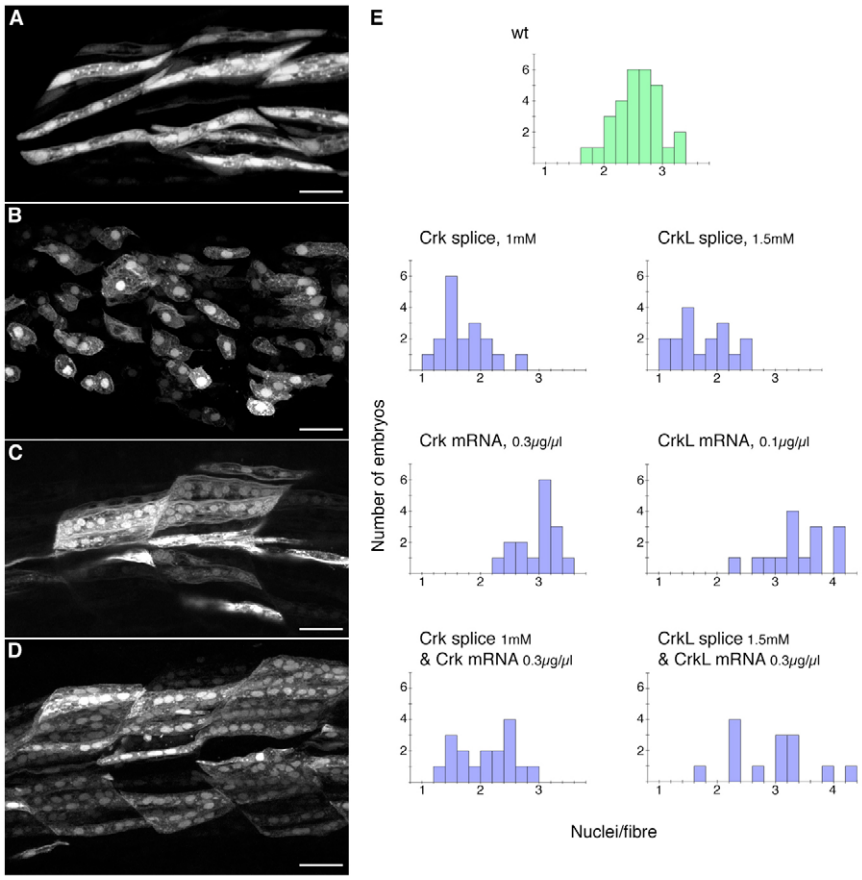

Fig. 6. Overexpression of Crk and Crkl causes excess fusion of fast myoblasts by 26-28 hpf, and rescues splice morphants. (**A-D**) Examples of GFP-positive cells in embryos injected with *mylz2:GFP* plasmid alone (A) or together with 1.5 mM of *crkl* splice morpholino (B), 0.1 μg/μl *crkl* mRNA (C), or 0.3 μg/μl *crkl* mRNA and 1.5 mM *crkl* splice morpholino combined (D). (**E**) Quantitative data showing distributions of mean nuclei/fibre for individual embryos injected with *crk* and *crkl* splice morpholinos and/or *crk* and *crkl* mRNA. Scale bars: 25 μm.

induces the rearrangement of the actin cytoskeleton in response to interactions between muscle founder and fusion-competent cells mediated by the transmembrane proteins Duf, Rst and Sns.

To investigate whether the molecular control of myoblast fusion is conserved between *Drosophila* and vertebrates, we identified homologues of DOCK180 in the zebrafish and used morpholinos to block their function. We found that knocking down two members of the DOCK180 sub-group of CDM proteins, Dock1 and Dock5, significantly compromised myoblast fusion in the zebrafish embryo. Interestingly, many of the unfused myoblasts elongated to form mononucleate muscle fibres, reminiscent of the mini-muscles formed by founder cells in *Drosophila* fusion mutants. However, we have no evidence to suggest that a similar subdivision of myoblasts into founder and fusion-competent cells occurs in the zebrafish embryo. The fact that the suppression of fusion is not complete might well reflect the failure of the morpholinos to achieve total inhibition of gene function: although those directed against splice junctions appeared more-effective than that targeting the translation start site, it seems unlikely that any of these achieved complete abrogation of function, because both *dock1* and *dock5* transcripts appeared to be maternally supplied. Indeed, given the uniform expression and probable multiple roles of both proteins in a variety of cellular processes, it would not be surprising for their complete inactivation to result in much-earlier developmental arrest. This could also explain why the simultaneous injection of *dock1* and *dock5* morpholinos resulted in only a marginal increase in the severity of the fusion phenotype.

DOCK180 was originally identified on the basis of its physical interaction with CRK, an adaptor protein that has been implicated in coupling DOCK proteins to upstream regulatory molecules (Hasegawa et al., 1996; Galletta et al., 1999; Reddien and Horvitz, 2000). On this basis, Crk has been postulated to play a role in myoblast fusion in *Drosophila* (Chen and Olson, 2004), but, for technical reasons, such a role has not been investigated experimentally. Using the same morpholino-based approach, we asked whether Crk and the related Crkl protein are involved in myoblast fusion in the zebrafish. In both cases, we found that inhibition of expression of the gene products had a significant effect on myoblast fusion. Moreover, we found not only that loss of Crk or Crkl expression inhibited myoblast fusion, but also that overexpression of the mRNAs encoding either protein resulted in an enhancement of fusion. Taken together, these data represent definitive evidence of a role for Crk and Crkl in myoblast fusion.

Whether the requirement for Crk and Crkl in myoblast fusion that we have uncovered involves interaction with Dock1 and/or Dock5 remains to be determined. Based on its role in coupling DOCK180 proteins to upstream regulators in other signalling contexts, one possibility is that Crk and/or Crkl act to couple the transmembrane proteins required for myoblast fusion to the Mbc/DOCK1/5 proteins via other molecules, such as Rolling pebbles (also known as Antisocial; human orthologue known as TANC2) (Chen and Olson, 2001). Against this, however, a recent study has demonstrated that, in *Drosophila*, mutated forms of Mbc that lack Crk-binding motifs can rescue the *mbc* mutant phenotype, implying that, at least in flies, direct interaction between Crk and Mbc is not necessary for myoblast fusion (Balagopalan et al., 2006). Similarly, the deficiency in engulfment of apoptotic cells seen in *C. elegans* lacking the Dock1 orthologue CED-5 can be rescued by mutated forms of the protein that are unable to bind the Crk orthologue CED-2 (Tosello-Trampont et al., 2007). Nevertheless, functional analysis has

demonstrated that both Crk and Dock1 are required in *C. elegans* for this phagocytic activity, even if direct physical interaction between the two proteins is dispensable (Wu and Horvitz, 1998; Reddien and Horvitz, 2000). Thus, by analogy, the requirement for Crk and Crkl in myoblast fusion might be independent of a direct interaction with Mbc/DOCK1/5 proteins. In this context, it is notable that human CRKL and *Drosophila* Crk have been shown to interact directly with WIP (WASP-interacting protein) (Sasahara et al., 2002; Kim et al., 2007). WIP has recently been shown to have a role in myoblast fusion in *Drosophila* (Kim et al., 2007; Massarwa et al., 2007), leading to the proposal that Crk acts as a link between the transmembrane protein Sticks and stones and the WIP-WASP-Arp2/3 actin nucleation complex (Kim et al., 2007) in a pathway parallel to that mediated by Mbc. In this regard, it is notable that we found that simultaneous knockdown of Crkl and Dock5 blocked fusion almost completely in the zebrafish embryo. Further analysis of the function of these and other orthologues of the *Drosophila* myoblast fusion genes using the zebrafish model that we have established will help to elucidate further the functional relationships between these proteins.

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Note added in proof

While this paper was under review, a related study describing the role of a homologue of Duf/Kirre in zebrafish myoblast fusion was published (Srinivas et al., 2007).

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/17/3145/DC1

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Zf_Dock1                    MSRWVPTKKE-KYGVAVYNYDARSNEELSLQIGDTVHILETYEGWYRGYR
ENSP00000280333_Hs_Dock1    MTRWVPTKREEKYGVAFYNYDARGADELSLQIGDTVHILETYEGWYRGYT
Zf_Dock5                    MTRWIPTKRE-KYGVAIYNYDAKGEPELSLQVGDTVHILETFEGWYRGYT
ENSP00000276440_Hs_Dock5    MARWIPTKRQ-KYGVAIYNYNASQDVELSLQIGDTVHILEMYEGWYRGYT
                            *:**:***:: *****.***:*    *****:******** :*******

Zf_Dock1                    LRRKSKKGVFPACYIHLKEATVEGSGQKETVIPTELPLVHEVTTTLREWA
ENSP00000280333_Hs_Dock1    LRKKSKKGIFPASYIHLKEAIVEGKGQHETVIPGDLPLIQEVTTTLREWS
Zf_Dock5                    LRQKSQKGIFPASYIHLKEAKVEGTGKQETVVPTELPLVQELGTTLREWT
ENSP00000276440_Hs_Dock5    LQNKSKKGIFPETYIHLKEATVEDLGQHETVIPGELPLVQELTSTLREWA
                            *:.**:**:**  ******* **. *::***:* :***::*: :*****:

Zf_Dock1                    AIWRDLYVGDKREMFNTVRDMIYDLIEWRSQILSGTLPQDELTELKQRVT
ENSP00000280333_Hs_Dock1    TIWRQLYVQDNREMFRSVRHMIYDLIEWRSQILSGTLPQDELKELKKKVT
Zf_Dock5                    QIWHKLYVNNKSTLFRNVQQMAYSLIEYRSQIVSGTLPNDDLVELKKKVT
ENSP00000276440_Hs_Dock5    VIWRKLYVNNKLTLFRQLQQMTYSLIEWRSQILSGTLPKDELAELKKKVT
                             **:.*** ::  :*. ::.* *.***:****:*****:*:* ***::**

Zf_Dock1                    SKMDYGNKYLDLDLVVRDKDGNILDPDLTSTVNLFRAHETASKQIEARIQ
ENSP00000280333_Hs_Dock1    AKIDYGNRILDLDLVVRDEDGNILDPELTSTISLFRAHEIASKQVEERLQ
Zf_Dock5                    AKIDYGNRILGLDLVVRDEAGNTLDPESSSTVSLFRAHESASHSIDERIQ
ENSP00000276440_Hs_Dock5    AKIDHGNRMLGLDLVVRDDNGNILDPDETSTIALFKAHEVASKRIEEKIQ
                            :*:*:**: *.*******. ** ***: :**: **:** **: :: ::*

Zf_Dock1                    EEKSQKQNLDLNRLAKFASTPSFALFVTLKNVVCKIGEDAEVLMSLYDPI
ENSP00000280333_Hs_Dock1    EEKSQKQNIDINRQAKFAATPSLALFVNLKNVVCKIGEDAEVLMSLYDPV
Zf_Dock5                    EEKMRLQNLEKRRQSLFSGVHTYSLLMNLRNFVCNIGEDAELFMSLYDPD
ENSP00000276440_Hs_Dock5    EEKSILQNLDLRGQSIFSTIHTYGLYVNFKNFVCNIGEDAELFMALYDPD
                            ***   **:: .  : *:   : .* :.::*.**:******::*:****

Zf_Dock1                    ESKFISENYLVRWSSSGLVKDIDQLHNLRAVFTDLGSEDLKREKISFVCQ
ENSP00000280333_Hs_Dock1    ESKFISENYLVRWSSSGLPKDIDRLHNLRAVFTDLGSKDLKREKISFVCQ
Zf_Dock5                    KSEFISENFLVRWDSTGMPKEIEKLNNLPALFTDLNSSDLLRQRLFLVCQ
ENSP00000276440_Hs_Dock5    QSTFISENYLIRWGSNGMPKEIEKLNNLQAVFTDLSSMDLIRPRVSLVCQ
                            :* *****:*:**.*.*: *:*::*:** *:****.* ** * :: :***

Zf_Dock1                    IVRVGRMELRDNNTKKLTSGLRRPFGVAVMDVTDIITGKMDDEDKQHFIP
ENSP00000280333_Hs_Dock1    IVRVGRMELRDNNTRKLTSGLRRPFGVAVMDVTDIINGKVDDEDKQHFIP
Zf_Dock5                    IIRVGCMELKEG--KKHTGGLRRPFGVAVMDITDIAHGKTDDDDKQYFIP
ENSP00000276440_Hs_Dock5    IVRVGHMELKEG--KKHTCGLRRPFGVAVMDITDIIHGKVDDEEKQHFIP
                            *:*** ***::.  :* * ************:***  ** **::**:***

Zf_Dock1                    FQPVA---------------------GESDFLQSVINKVIAAKEVNHKGQ
ENSP00000280333_Hs_Dock1    FQPVA---------------------GENDFLQTVINKVIAAKEVNHKGQ
Zf_Dock5                    FQQIAMETYIRQRQLIMSPLIPSRVIGENEPLVAVFNKVIATREVNHKGQ
ENSP00000276440_Hs_Dock5    FQQIAMETYIRQRQLIMSPLITSHVIGENEPLTSVLNKVIAAKEVNHKGQ
                            ** :*                     **.: * :*:*****::*******

Zf_Dock1                    GLWVTLKLLPGDIHQIRKDFPHLVDRSTAVARKMGFPEIIMPGDVRNDIY
ENSP00000280333_Hs_Dock1    GLWVTLKLLPGDIHQIRKEFPHLVDRTTAVARKTGFPEIIMPGDVRNDIY
Zf_Dock5                    GLFVTLKLLPGDVFQVQKDYPHFVDRTTAIVRKMGFPEIILPGYVRNDIY
ENSP00000276440_Hs_Dock5    GLWVSLKLLPGDLTQVQKNFSHLVDRSTAIARKMGFPEIILPGDVRNDIY
                            **:*:*******: *::*::.*:***:**:.** ******:** ******

Zf_Dock1                    VTLVQGDFDKGSKTTPKNVEVTMSVHDEDGKKLESVIFPGAGDEGILEYK
ENSP00000280333_Hs_Dock1    VTLVQGDFDKGSKTTAKNVEVTVSVYDEDGKRLEHVIFPGAGDEAISEYK
Zf_Dock5                    VTLLQGEFDRGKKKTPKNVEVIVSVLDEEGNPMEKAIFPGAGYEGITEYK
ENSP00000276440_Hs_Dock5    VTLIHGEFDKGKKKTPKNVEVTMSVHDEEGKLLEKAIHPGAGYEGISEYK
                            ***::*:**:*.*.*.***** :** **:*: :* .*.**** *.* ***

Zf_Dock1                    SVIYYQVKQPRWYETIKVAIPIEDVNRSHLRFTFKHRSSQDCKDKSEKNF
ENSP00000280333_Hs_Dock1    SVIYYQVKQPRWFETVKVAIPIEDVNRSHLRFTFRHRSSQDSKDKSEKIF
Zf_Dock5                    SVIYYQVKQPCWNETVKVAIPIEDVCRCHLRLTFRHRSSQDSRDKSEKPF
```

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ENSP00000276440_Hs_Dock5      SVVYYQVKQPCWYETVKVSIAIEEVTRCHIRFTFRHRSSQETRDKSERAF
                              **:******* * **:**:*.**:* *.*:*:**:*****: :****: *

Zf_Dock1                      ALSFVKLMRYDGTTLRDGEHDLIVYKAEAKKLEDSSMYLSLASTKPEMEE
ENSP00000280333_Hs_Dock1      ALAFVKLMRYDGTTLRDGEHDLIVYKAEAKKLEDAATYLSLPSTKAELEE
Zf_Dock5                      GMAFVRLMKSDGTTLPDGKHELIVYKVDGKKADDAKTYLSLPGTYTEVEE
ENSP00000276440_Hs_Dock5      GVAFVKLMNPDGTTLQDGRHDLVVYKGDNKKMEDAKFYLTLPGTKMEMEE
                              .::**:**. ***** **.*:*:*** : ** :*:  **:*..*  *:**

Zf_Dock1                      R-NPSSGKNAQNLG-SFTISKDSFQISTLVCSTKLTQNVDLLGLLKWRSN
ENSP00000280333_Hs_Dock1      KGHSATGKSMQSLG-SCTISKDSFQISTLVCSTKLTQNVDLLGLLKWRSN
Zf_Dock5                      K-ERQTGKSFHHGG-AVPVTKDSFQIATLTCSTKLTQNVDLLGLLNWRSN
ENSP00000276440_Hs_Dock5      K-ELQASKNLVTFTPSKDSTKDSFQIATLICSTKLTQNVDLLGLLNWRSN
                              : .  :.*.      :   :******:** ***************:****

Zf_Dock1                      TSLLQQNLRHLMKVEGGEVVKFLQDTLDALFNIMMENSDSETFDTLVFDA
ENSP00000280333_Hs_Dock1      TSLLQQNLRQLMKVDGGEVVKFLQDTLDALFNIMMENSESETFDTLVFDA
Zf_Dock5                      TERLEEILQKLMEVEGGEIIKFLQDTLDALFNIMMETSEEETYDTLVFNA
ENSP00000276440_Hs_Dock5      SQNIKHNLKKLMEVDGGEIVKFLQDTLDALFNIMMEMSDSETYDFLVFDA
                              :. ::. *::**:*:***::*************** *:.**:* ***:*

Zf_Dock1                      LVFIIGLIADRKFHHFNPVLETYIRKHFSATLAYTKLTKVLKNYVDNAEK
ENSP00000280333_Hs_Dock1      LVFIIGLIADRKFQHFNPVLETYIKKHFSATLAYTKLTKVLKNYVDGAEK
Zf_Dock5                      LVFIISLIGDIKFQHFNPVLETYINKHFSATLAYQKLTKVLNYFVGHAED
ENSP00000276440_Hs_Dock5      LVFIISLIGDIKFQHFNPVLETYIYKHFSATLAYVKLSKVLNFYVANADD
                              *****.**.* **:********** ********* **:***: :*  *:.

Zf_Dock1                      --LSEQLLKALKAIEYIFKFIVRSRVLFNQLYENKGESD-FMESLRNLFA
ENSP00000280333_Hs_Dock1      PGVNEQLYKAMKALESIFKFIVRSRILFNQLYENKGEAD-FVESLLQLFR
Zf_Dock5                      STHTERLYAALKAFKYLFRFIVQSRVLYLRFYGKSEDGGQFQDSIRTLFL
ENSP00000276440_Hs_Dock5      SSKTELLFAALKALKYLFRFIIQSRVLYLRFYGQSKDGDEFNNSIRQLFL
                                 .* *  *:**:: :*:**::**:*: ::* :. :.. * :*:  **

Zf_Dock1                      SFNAMMNSGAEGTGGVKGAALKYVPTIVNDLKLVFDPKELSKLFSEFILK
ENSP00000280333_Hs_Dock1      SINDMMSSMSDQTVRVKGAALKYLPTIVNDVKLVFDPKELSKMFTEFILN
Zf_Dock5                      SISILMDRPLDEGVKIKGAILKYLPTIINDLQKVFDAVELSVQLSKFIES
ENSP00000276440_Hs_Dock5      AFNMLMDRPLEEAVKIKGAALKYLPSIINDVKLVFDPVELSVLFCKFIQS
                              ::. :*.   :    :*** ***:*:*:**:: ***. ***  : :** .

Zf_Dock1                      VPLGRLVKQKLHCMIDIVHSDLFTQHDCREILLPLMTDQLKLHLE---NH
ENSP00000280333_Hs_Dock1      VPMGLLTIQKLYCLIEIVHSDLFTQHDCREILLPMMTDQLKYHLE---RQ
Zf_Dock5                      IPDFQMVRQKLGCLVKIVESNLFKQPDCRDVLLPLITDQLSGQLDDNSNK
ENSP00000276440_Hs_Dock5      IPDNQLVRQKLNCMTKIVESTLFRQSECREVLLPLLTDQLSGQLDDNSNK
                              :*   :. *** *: .**.* ** * :**::***::****. :*:   .:

Zf_Dock1                      EELESCCQLLSDILEVLYRKDVGPTQWHVQIIMEKLLRTINRTVISMGRD
ENSP00000280333_Hs_Dock1      EDLEACCQLLSHILEVLYRKDVGPTQRHVQIIMEKLLRTVNRTVISMGRD
Zf_Dock5                      PDLEACVQLLSTVLDMLDCKNVGPTSGHVKLIVDRLLRRVNRTVINLSLD
ENSP00000276440_Hs_Dock5      PDHEASSQLLSNILEVLDRKDVGATAVHIQLIMERLLRRINRTVIGMNRQ
                               : *:. **** :*::*  *:**.*  *:::*:::*** :*****.:. :

Zf_Dock1                      SSLIGSFVACMTGTLRQMDDYHYTHLISTFGKMRTDVVDFLMETFIMFKD
ENSP00000280333_Hs_Dock1      SELIGNFVACMTAILRQMEDYHYAHLIKTFGKMRTDVVDFLMETFIMFKN
Zf_Dock5                      SPYIGHYLACMTAILKQMEDAHYAQYINTF-KTRQDVIDFLMETFIMFKN
ENSP00000276440_Hs_Dock5      SPHIGSFVACMIALLQQMDDSHYSHYISTF-KTRQDIIDFLMETFIMFKD
                              *  ** ::*** . *:**:* **:: *.** * * *::***********:

Zf_Dock1                      LIGKNVYPGDWVIMNMMQNKVFLRAINQYAAVLSKKFLDQTNFELQLWNN
ENSP00000280333_Hs_Dock1      LIGKNVYPFDWVIMNMVQNKVFLRAINQYADMLNKKFLDQANFELQLWNN
Zf_Dock5                      LMG-NVFPPDWMIMNLLQMKVFLRAIDQYSEVLNKGFLDYASFHLQLWNN
ENSP00000276440_Hs_Dock5      LIGKNVYAKDWMVMNMTQNRVFLRAINQFAEVLTRFFMDQASFELQLWNN
                              *:* **:. **::**: * :******:*:: :*.: *:* :.*.******
```

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Zf_Dock1                    YFHLAVAFLTQESLQLENFSSDKRAKIFHKYQDMRRQIGFEIRDMWYNLG
ENSP00000280333_Hs_Dock1    YFHLAVAFLTQESLQLENFSSAKRAKILNKYGDMRRQIGFEIRDMWYNLG
Zf_Dock5                    YFHLIVAFLTHKSLQLESFSPEKRDKILNKYGDMRKSIGFKLRDMWYNLG
ENSP00000276440_Hs_Dock5    YFHLAVAFLTHESLQLETFSQAKRNKIVKKYGDMRKEIGFRIRDMWYNLG
                            **** *****::*****.**  ** **.:** ***:.***.:********

Zf_Dock1                    PHKIKFIPEMVGPILEMTLVPEIELRKATIPIFFDMMQCEFHFRR--CFQ
ENSP00000280333_Hs_Dock1    QHKIKFIPEMVGPILEMTLIPETELRKATIPIFFDMMQCEFHSTR--SFQ
Zf_Dock5                    PQKMKFIPAMVGPILEATLVPEPELRKATIPIFFDMMQCEHHFTTNHTFQ
ENSP00000276440_Hs_Dock5    PHKIKFIPSMVGPILEVTLTPEVELRKATIPIFFDMMQCEFNFSGNGNFH
                             :*:**** ******* ** ** *****************.:      *:

Zf_Dock1                    TFENEIITKLDHEVEGGRGDEQYKILFQKILLEHCRKHKYLAKTGETFVT
ENSP00000280333_Hs_Dock1    MFENEIITKLDHEVEGGRGDEQYKVLFDKILLEHCRKHKYLAKTGETFVK
Zf_Dock5                    KFENELITKLDQEVEGGRGDEQYKILLEKTLLEHCRRHRYLSQPGEALAL
ENSP00000276440_Hs_Dock5    MFENELITKLDQEVEGGRGDEQYKVLLEKLLLEHCRKHKYLSSSGEVFAL
                             ****:*****:***********:*::* ******:*:**:..**.:.

Zf_Dock1                    LVVRLLERLLDYRTIMH-DENKENRMSCTVNVLNFYKEIEREEMYIRYLY
ENSP00000280333_Hs_Dock1    LVVRLMERLLDYRTIMH-DENKENRMSCTVNVLNFYKEIEREEMYIRYLY
Zf_Dock5                    LLSSLLENLLAYRTFTH-DESPELRMSCTVNLLNFYKEKKREDIYIRYLY
ENSP00000276440_Hs_Dock5    LVSSLLENLLDYRTIIMQDESKENRMSCTVNVLNFYKEKKREDIYIRYLY
                            *:  *:*.** ***:   **. * *******:****** :**::******

Zf_Dock1                    KLCDLHKECDNYTEAGYTLLLHAKLLKWSEEACAAHLTQRDGYQASTQGQ
ENSP00000280333_Hs_Dock1    KLCDLHKECDNYTEAAYTLLLHAKLLKWSEDVCVAHLTQRDGYQATTQGQ
Zf_Dock5                    KLRDLHLVCENYTEAAYTLLLHAELLEWSEKPCSPHLIPGDAAQVWTQQE
ENSP00000276440_Hs_Dock5    KLRDLHRDCENYTEAAYTLLLHAELLQWSDKPCVPHLLQKDSYYVYTQQE
                            ** ***  *:*****.*******:**:**:. * .**   *.  . ** :

Zf_Dock1                    LKDQLYQEIINYFDKGKMWEEAIVLGKELAEQYENEMFDFEQLSTLLRKQ
ENSP00000280333_Hs_Dock1    LKEQLYQEIIHYFDKGKMWEEAIALGKELAEQYENEMFDYEQLSELLKKQ
Zf_Dock5                    LKERLFQEIMCNLDKGKMWEKAIEMAKQLVKLHENQMFDFIELGQLLKQQ
ENSP00000276440_Hs_Dock5    LKEKLYQEIISYFDKGKMWEKAIKLSKELAETYESKVFDYEGLGNLLKKR
                            **::*:***:  :********:** :.*:*.: :*.::**:  *. **:::

Zf_Dock1                    AQFYESIVKVIRPKPDYFAVGYNGLGFPSFLRNKMFIYRGKEYERWEDFE
ENSP00000280333_Hs_Dock1    AQFYENIVKVIRPKPDYFAVGYYGQGFPTFLRGKVFIYRGKEYERREDFE
Zf_Dock5                    AQYYENIMHAMRPQPEYFAVGYYGQGFPSFLRNKMFIYRGKEYEWLEDFS
ENSP00000276440_Hs_Dock5    ASFYENIIKAMRPQPEYFAVGYYGQGFPSFLRNKIFIYRGKEYERREDFS
                            *.:**.*::.:**:*:****** * ***:***.*:*********  ***.

Zf_Dock1                    ARLLTQFPNAEKMKTTTPPSEDIRNSSGQYIQCFTVKPLLELPPKFQNKP
ENSP00000280333_Hs_Dock1    ARLLTQFPNAEKMKTTSPPGDDIKNSPGQYIQCFTVKPKLDLPPKFHR-P
Zf_Dock5                    LKLLSQFPNAVRMTSTSPPADNICSSTGQHIQCFTVKPVLNLPSHFKDKG
ENSP00000276440_Hs_Dock5    LRLLTQFPNAEKMTSTTPPGEDIKSSPKQYMQCFTVKPVMSLPPSYKDKP
                             :**:***** :*.:*:**.::* .*. *::******* :.**. ::

Zf_Dock1                    VSEQIVSFYTVNEVQKFQYSRPVRKGEKDPDNEFSNMWIERTTYVTAYKL
ENSP00000280333_Hs_Dock1    VSEQIVSFYRVNEVQRFEYSRPIRKGEKNPDNEFANMWIERTIYTTAYKL
Zf_Dock5                    LPEQILNYYRTNEVDKFQYSRPFRKGEKDPDNEFATMWIERTTFITAYRF
ENSP00000276440_Hs_Dock5    VPEQILNYYRANEVQQFRYSRPFRKGEKDPDNEFATMWIERTTYTTAYTF
                            :.***:.:* .***::*.****.*****:*****:.****** : *** :

Zf_Dock1                    PGILRWFEVLSVSAEEISPLENAMETMQLTNEKISNMVQRHLNDPNLPIN
ENSP00000280333_Hs_Dock1    PGILRWFEVKSVFMVEISPLENAIETMQLTNDKINSMVQQHLDDPSLPIN
Zf_Dock5                    PGILKWFEVKSMSVEEISPLDNAIETMELANEKLSNLVQQQACDRSCPVH
ENSP00000276440_Hs_Dock5    PGILKWFEVKQISTEEISPLENAIETMELTNERISNCVQQHAWDRSLSVH
                            ****:**** .:   *****:**:***:*:*::::.. **::  * . .::

Zf_Dock1                    PLSMLLNGIVDPAVMGGFTNYEKAFFTEKYFLEHPNDQEKIERLKDLIAW
ENSP00000280333_Hs_Dock1    PLSMLLNGIVDPAVMGGFANYEKAFFTDRYLQEHPEAHEKIEKLKDLIAW
Zf_Dock5                    PLSMMLNGIVDPAVMGGFSNYEKAFFTEAYMKKNPDHLERIEVLKHLIAL
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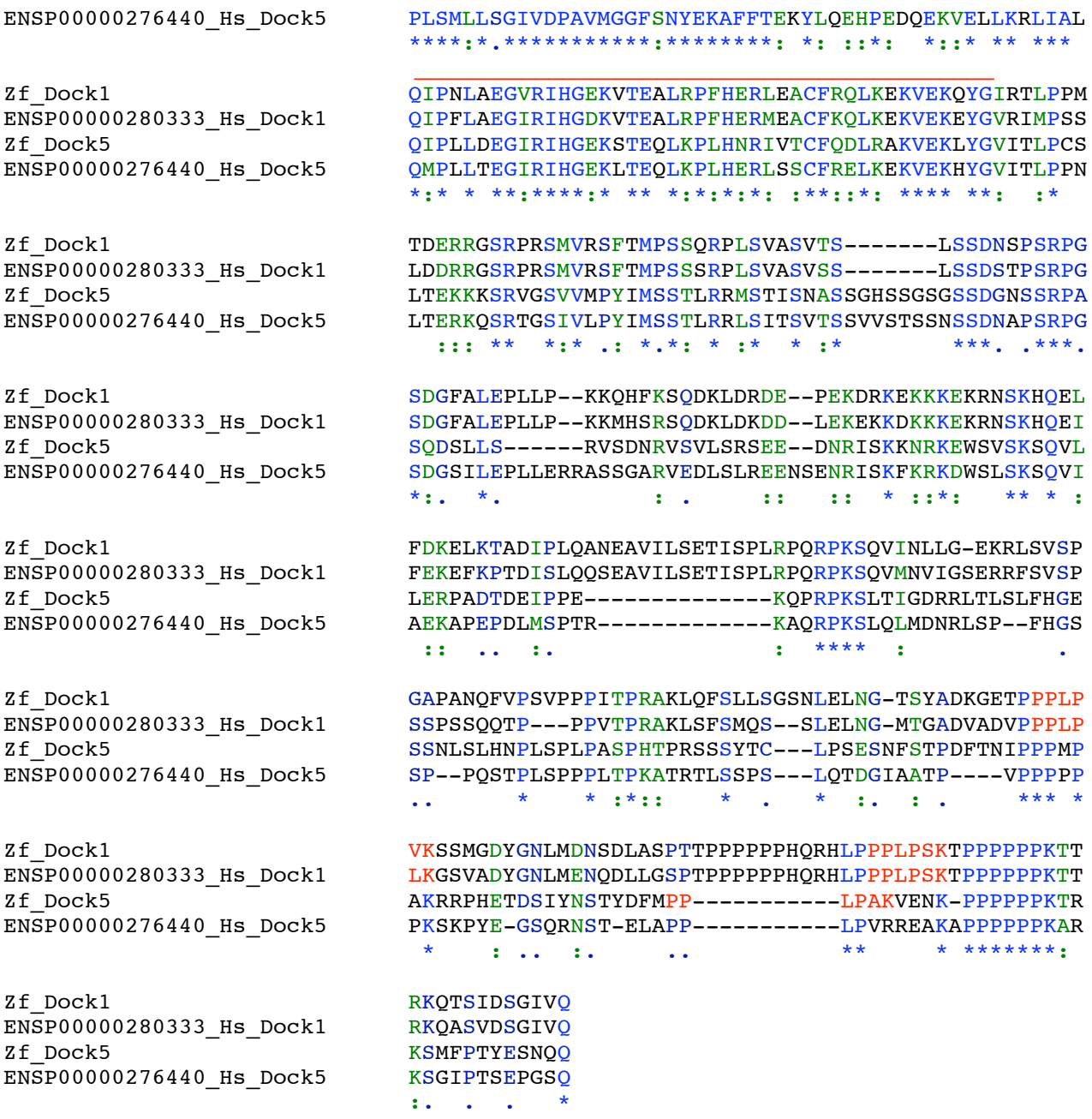
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ENSP00000280333_Hs_Dock1 QIPFLAEGIRIHGDKVTEALRPFHERMEACFKQLKEKVEKEYGVRIMPSS
Zf_Dock5 QIPLLDEGIRIHGEKSTEQLKPLHNRIVTCFQDLRAKVEKLYGVITLPCS
ENSP00000276440_Hs_Dock5 QMPLLTEGIRIHGEKLTEQLKPLHERLSSCFRELKEKVEKHYGVITLPPN
Zf_Dock1 TDERRGSRPRSMVRSFTMPSSQRPLSVASVTS-------LSSDNSPSRPG
ENSP00000280333_Hs_Dock1 LDDRRGSRPRSMVRSFTMPSSSRPLSVASVSS-------LSSDSTPSRPG
Zf_Dock5 LTEKKKSRVGSVVMPYIMSSTLRRMSTISNASSGHSSGSGSSDGNSSRPA
ENSP00000276440_Hs_Dock5 LTERKQSRTGSIVLPYIMSSTLRRLSITSVTSSVVSTSSNSSDNAPSRPG
Zf_Dock1 SDGFALEPLLP--KKQHFKSQDKLDRDE--PEKDRKEKKKEKRNSKHQEL
ENSP00000280333_Hs_Dock1 SDGFALEPLLP--KKMHSRSQDKLDKDD--LEKEKKDKKKEKRNSKHQEI
Zf_Dock5 SQDSLLS------RVSDNRVSVLSRSEE--DNRISKKNRKEWSVSKSQVL
ENSP00000276440_Hs_Dock5 SDGSILEPLLERRASSGARVEDLSLREENSENRISKFKRKDWSLSKSQVI
Zf_Dock1 FDKELKTADIPLQANEAVILSETISPLRPQRPKSQVINLLG-EKRLSVSP
ENSP00000280333_Hs_Dock1 FEKEFKPTDISLQQSEAVILSETISPLRPQRPKSQVMNVIGSERRFSVSP
Zf_Dock5 LERPADTDEIPPE--------------KQPRPKSLTIGDRRLTLSLFHGE
ENSP00000276440_Hs_Dock5 AEKAPEPDLMSPTR-------------KAQRPKSLQLMDNRLSP--FHGS
Zf_Dock1 GAPANQFVPSVPPPITPRAKLQFSLLSGSNLELNG-TSYADKGETPPPLP
ENSP00000280333_Hs_Dock1 SSPSSQQTP---PPVTPRAKLSFSMQS--SLELNG-MTGADVADVPPPLP
Zf_Dock5 SSNLSLHNPLSPLPASPHTPRSSSYTC---LPSESNFSTPDFTNIPPPMP
ENSP00000276440_Hs_Dock5 SP--PQSTPLSPPPLTPKATRTLSSPS---LQTDGIAATP----VPPPPP
Zf_Dock1 VKSSMGDYGNLMDNSDLASPTTPPPPPPHQRHLPPPLPSKTPPPPPPKTT
ENSP00000280333_Hs_Dock1 LKGSVADYGNLMENQDLLGSPTPPPPPPHQRHLPPPLPSKTPPPPPPKTT
Zf_Dock5 AKRRPHETDSIYNSTYDFMPP----------LPAKVENK-PPPPPPKTR
ENSP00000276440_Hs_Dock5 PKSKPYE-GSQRNST-ELAPP----------LPVRREAKAPPPPPPKAR
Zf_Dock1 RKQTSIDSGIVQ
ENSP00000280333_Hs_Dock1 RKQASVDSGIVQ
Zf_Dock5 KSMFPTYESNQQ
ENSP00000276440_Hs_Dock5 KSGIPTSEPGSQ

Table S1. Data for wild-type embryos at progressive stages

Number of nuclei	15 somites	20 somites (19 hpf)	22 somites (20 hpf)	24 hpf	26-28 hpf	30 hpf	34 hpf	48 hpf
1	113	240	119	71	432	108	55	3
2	0	26	53	150	733	197	170	22
3	0	3	38	111	824	234	129	61
4	0	0	14	37	390	116	72	33
5	0	0	1	13	119	40	16	9
6	0	0	0	2	26	12	1	2
7	0	0	0	0	7	2	0	0
Total number of fibres	113	269	225	384	2531	709	443	130
Total number of nuclei	113	301	400	929	6730	1954	1156	419
Mean nuclei/fibre	1	1.12	1.78	2.42	2.66	2.76	2.61	3.22

Table S2. Data for morphant embryos at 26-28 hpf

	Dock1 ATG			Dock1 splice		Dock5 splice 1			Dock5 splice 2			D1spl & D5spl1	
Number of nuclei	0.5 mM	1 mM	1.5 mM	0.3 mM	0.5 mM	0.3 mM	0.4 mM	0.5 mM	0.5 mM	1 mM	2 mM	0.225 & 0.3 mM	0.3 & 0.4 mM
1	66	134	116	279	209	176	185	520	159	192	38	198	172
2	111	150	220	226	64	91	41	111	122	109	21	116	35
3	74	76	191	113	11	28	9	28	56	61	16	59	8
4	33	8	44	22	0	6	2	5	15	26	2	8	2
5	6	0	6	2	0	6	0	2	6	5	0	2	0
6	1	0	2	0	0	0	0	1	1	0	0	0	0
7	2	0	0	0	0	0	0	0	0	0	0	0	0
Total number of fibres	293	368	579	642	284	307	237	667	359	393	77	383	217
Total number of nuclei	692	694	1347	1168	370	496	302	862	667	722	136	649	274
Mean nuclei/fibre	2.36	1.89	2.33	1.82	1.30	1.62	1.27	1.29	1.86	1.84	1.77	1.69	1.26

	Crk ATG	Crk splice		Crk-like ATG			Crk-like splice		D5spl1 & CrklATG		Crk spl & CrklATG
Number of nuclei	0.1 mM	0.5 mM	1 mM	0.3 mM	0.4 mM	0.5 mM	0.5 mM	2 mM	0.3 & 0.3 mM	0.4 & 0.4 mM	0.5 & 0.4 mM
1	214	127	336	215	262	439	97	367	296	253	245
2	92	97	227	128	187	173	98	270	36	31	91
3	33	38	58	51	71	67	107	106	27	6	30
4	3	4	6	9	16	6	47	21	7	1	2
5	0	0	1	1	5	3	17	9	0	0	2
6	0	0	0	0	1	0	2	0	0	0	0
7	0	0	0	0	0	0	1	0	0	0	0
Total number of fibres	342	266	628	404	542	688	369	773	366	291	370
Total number of nuclei	509	451	993	665	944	1025	906	1354	477	337	535
Mean nuclei/fibre	1.49	1.70	1.58	1.65	1.74	1.49	2.46	1.75	1.30	1.16	1.45

Distribution of fibres according to number of nuclei. Mean nuclei/fibre calculated from total numbers of fibres and nuclei, i.e. *n*=number of fibres.

Table S3. Data for embryos injected with crk and *crkl* mRNA, at 26-28 hpf

Number of nuclei	Control	*crk* mRNA 0.3 μg/μl	Crk splice MO 1 mM	*crk* mRNA 0.3 μg/μl & Crk spl MO 1 mM	*crkl* mRNA 0.1 μg/μl	Crkl splice MO 1.5 mM	*crkl* mRNA 0.3 μg/μl & Crkl spl MO 1.5 mM
1	258	158	280	172	84	307	84
2	403	169	243	157	64	173	99
3	453	278	82	126	151	76	122
4	191	175	24	51	120	20	87
5	52	90	3	19	78	5	35
6	17	28	1	7	26	1	27
7	3	12	0	0	16	1	5
8	0	7	0	0	9	0	5
Total number of fibres	1377	910	633	532	539	583	459
Total number of nuclei	3570	2788	1129	1205	1875	999	1408
Mean nuclei/fibre	2.59	3.06	1.78	2.27	3.48	1.71	3.07

Control group injected with *mylz2:GFP* alone. Distribution of fibres according to number of nuclei. Mean nuclei/fibre calculated from total numbers of fibres and nuclei, i.e. *n*=number of fibres.

Table S4. Data for wild-type embryos at progressive stages

15 somites	20 somites (19 hpf)	22 somites (20 hpf)	24 hpf	26-28 hpf	26-28 hpf	26-28 hpf	30 hpf	34 hpf	48 hpf
1.00	1.00	2.32	2.70	2.46	3.26	1.93	3.18	2.34	3.17
1.00	1.09	1.50	2.36	2.89	3.18	2.36	3.43	2.90	2.67
1.00	1.09	1.42	2.00	2.29	3.06	2.71	2.67	2.12	2.88
1.00	1.06	1.19	1.90	2.29	2.67	3.22	2.91	2.09	3.83
1.00	1.00	1.36	2.11	2.50	2.18	3.04	3.14	2.75	3.83
1.00	1.00	1.79	3.32	2.05	2.88	3.06	2.63	2.80	3.14
	1.00	2.54	3.00	2.51	2.76	2.39	2.43	2.97	3.25
	1.57		2.00	2.76	2.26	3.12	3.36	2.76	3.13
	1.25		2.53	3.22	2.25	2.70	3.47	3.21	
	1.43		2.19	2.07	2.52	2.72	2.90	3.36	
	1.12		3.20	2.71	2.38	2.78	3.13	2.33	
	1.09		2.46	2.55	2.29	2.70	2.58	2.83	
	1.67			3.25	2.26	2.38	3.14		
	1.26			3.00	2.05	2.54	2.97		
				2.22	2.25	3.19	2.45		
				2.63	1.76	2.50	2.59		
				3.16	1.44	2.56	2.64		
				2.53	1.53	2.87	2.62		
				2.54	3.18	2.24	2.78		
				2.63	2.55	2.63	2.28		
				2.92	2.93	2.96	2.47		
				2.61	3.42	2.95	2.08		
				2.70	2.36	1.50			
				3.09	3.58	2.56			
				3.09	3.15	2.62			
				3.41	1.80				
N 6	14	7	12		77		22	12	8
Mean 1	1.19	1.73	2.48		2.63		2.81	2.71	3.23

Mean nuclei/fibre values for individual embryos. Means are calculated using these values as sample points, to weight each embryo equally, i.e. *n*=number of embryos.

Table S5. Data for morphant embryos at 26-28 hpf

Morpholino:	Dock1 ATG			Dock1 splice		Dock5 splice 1			Dock5 splice 2			D1spl & D5spl1	
Dose:	0.5 mM	1 mM	1.5 mM	0.3 mM	0.5 mM	0.3 mM	0.4 mM	0.5 mM	0.5 mM	1 mM	2 mM	0.225 & 0.3 mM	0.3 & 0.4 mM
	2.76	2.03	1.79	1.40	1.10	1.19	1.42	1.16	1.58	1.14	1.14	1.38	1.02
	2.78	2.14	2.49	1.64	1.33	1.71	1.50	1.26	1.23	1.50	1.33	2.12	1.10
	2.34	1.72	2.15	1.45	1.51	2.14	1.23	1.07	2.06	2.15	2.10	1.93	1.22
	2.21	1.43	2.55	1.08	1.17	1.62	1.00	1.09	2.28	1.14	2.00	1.24	1.89
	2.20	1.69	2.51	1.59	1.13	1.36	1.15	1.60	2.00	1.78	1.33	2.18	1.68
		2.05	2.76	1.35	1.62	1.59	1.26	1.18	1.72	1.87	1.50	1.55	1.00
		2.15	2.09	2.50		1.31	1.04	1.26	1.83	1.86		1.74	
		1.78	2.23	1.63		1.88	1.22	1.04	1.71	2.32		1.31	
		2.17	3.00	1.50		1.67	1.07	1.07	2.13	1.03			
		3.00	2.32	2.00		2.33	1.00	2.64	2.00	1.26			
		1.29	1.59	1.56		1.50	1.76	2.53	1.93	3.06			
		1.26	2.47	1.67				1.43	2.33	2.42			
			2.41	1.73				1.62	1.67	1.92			
			2.65	2.04				1.00	1.52				
			2.12	1.91				1.62	1.81				
			2.39	1.93				1.26	2.10				
				2.01				1.46	1.54				
				2.00				1.11	1.85				
				1.77				1.11	2.55				
								1.19	1.69				
								1.19					
								1.17					
								1.54					
								1.25					
								1.26					
N	5	12	16	19	6	11	11	25	20	13	6	8	6
Mean	2.46	1.89	2.35	1.72	1.31	1.66	1.24	1.36	1.88	1.80	1.57	1.68	1.32

Morpholino:	Crk ATG	Crk splice		Crk-like ATG			Crk-like splice		D5spl1 & CrklATG		Crk spl & CrklATG
Dose:	0.1 mM	0.5 mM	1 mM	0.3 mM	0.4 mM	0.5 mM	0.5 mM	2 mM	0.3 & 0.3 mM	0.4 & 0.4 mM	0.5 & 0.4 mM
	1.56	1.74	1.49	2.46	1.50	1.17	2.78	1.38	1.27	1.32	1.61
	1.47	1.54	1.79	1.97	2.00	1.07	2.53	2.00	2.54	1.09	1.15
	1.47	1.88	1.31	1.58	1.87	1.26	1.96	1.13	2.08	1.15	1.24
	1.31	1.48	1.17	2.33	1.70	1.29	3.67	1.85	2.19	1.04	1.34
	2.42	1.76	1.27	1.41	1.41	1.65	3.33	1.74	1.25	1.50	1.74
	1.37		1.54	1.57	2.58	1.22	2.92	2.26	1.07	1.12	1.22
	1.71		1.19	1.40	1.69	1.96	2.26	2.05	1.41	1.04	1.44
	1.18		1.58	1.67	1.76	1.55	3.33	1.47	1.04	1.07	2.19
			1.76	1.44	1.23	1.41	1.83	2.18	1.02	1.02	
			1.59	1.57	1.80	1.86	2.00	1.42	1.00	1.35	
			1.37	1.54	1.67	1.11	2.88	1.30	1.00	1.15	
			1.79	1.56	2.04	1.58	2.00	2.10	1.00		
			2.25	1.06	1.64	1.93	1.96	1.69	1.11		
			1.65		1.17	1.83	2.21	1.45			
			1.83		2.00	1.51	2.65	1.69			
			1.52		1.54	1.83		2.13			
					1.59	1.35		1.44			
					1.67	1.34		1.21			
					2.30	1.40		1.10			
								1.29			
								2.24			
								2.67			
								2.27			
								1.65			
								2.69			
								1.46			
								1.50			
								1.73			
								1.82			
								2.05			
N	8	5	16	13	19	19	15	30	13	11	8
Mean	1.56	1.68	1.57	1.66	1.74	1.49	2.55	1.77	1.38	1.17	1.49

Mean nuclei/fibre values for individual embryos. Means are calculated using these values as sample points, to weight each embryo equally, i.e. *n*=number of embryos.

Table S6. Data for embryos injected with *crk* and *crkl* mRNA, at 26-28 hpf

Control		*crk* mRNA 0.3 μg/μl	Crk splice MO 1 mM	*crk* mRNA 0.3 μg/μl & Crk spl MO 1 mM	*crkl* mRNA 0.1 μg/μl	Crkl splice MO 1.5 mM	Crkl mRNA 0.3 μg/μl & Crkl spl MO 1.5 mM
2.72	2.71	3.09	1.87	2.54	3.64	1.13	3.96
2.95	2.55	3.15	2.76	2.32	4.13	1.85	2.74
2.20	2.34	2.46	2.18	2.41	4.04	2.52	3.15
3.01	2.86	3.60	1.59	2.91	3.54	1.42	3.23
2.81	2.91	3.29	2.30	1.60	3.39	2.22	2.28
2.05	2.86	2.92	1.50	2.06	2.66	1.66	3.14
2.62	2.22	3.17	1.78	2.61	2.95	2.00	2.37
3.28	2.37	3.36	2.04	2.58	3.72	2.50	4.21
1.73	2.48	3.10	1.40	2.58	3.29	2.04	3.23
2.50	2.42	2.58	1.37	1.60	2.39	1.16	2.40
2.19	2.64	3.04	1.72	1.62	3.27	1.58	2.27
1.86	2.80	2.80	1.59	1.53	3.71	2.18	1.61
2.22	2.52	2.30	1.82	1.78	3.10	1.36	3.22
3.24	2.53	2.74	1.43	2.10	3.26	1.33	3.20
2.71		3.05	1.52	1.21	4.20	2.17	
		3.30	1.59	2.00		1.47	
			1.16	2.31		1.45	
			1.88				
N	29	16	18	17	15	17	14
Mean	2.56	3.00	1.75	2.10	3.42	1.77	2.93

Control group injected with *mylz2:GFP* alone. Mean nuclei/fibre values for individual embryos. Means are calculated using these values as sample points, to weight each embryo equally, i.e. *n*=number of embryos.