Supplemental Information

I. SCAR system

1. Assessment of phenotypic consequences resulting from zygotic disruption of SCAR-complex function. Zygotic disruption of the D-WIP locus provides a good means to assess Wsp pathway function during myoblast fusion, given the exclusively zygotic mode of D-WIP gene expression, and the close similarities between D-WIP and Wsp myogenic phenotypes (Massarwa et al, 2007). Use of the Wsp$^{3D3}$ allele (see below), provides an alternative method to achieve this end. However, choosing an appropriate genetic background for parallel study of SCAR NPF function is less straightforward, given apparent variabilities in maternal contributions and lack of complete information regarding SCAR regulatory factors. We therefore surveyed phenotypes resulting from complete zygotic disruption of all Drosophila homologs of SCAR regulatory complex elements, as well as SCAR itself. Nuclei of the dorsal embryonic muscle DAI express the transcription factor Even-skipped (Eve), and determination of the number of such Eve-expressing nuclei serves as an established and sensitive assay for the severity of fusion defects (Paululat et al., 1999). As shown in Supp. Table 1, zygotic disruption of kette, the Drosophila NAP-1 homolog, provides the strongest phenotype (see also (Schroter et al., 2004)), and this background was therefore chosen for the analyses presented in the main text.

Differences in the severity of fusion phenotypes obtained via assays of this type, have been put forward as evidence that distinct cellular mechanisms underlie the initial and subsequent rounds of embryonic muscle fusion (Rau et al., 2001; Schroter et al., 2004). A recent study has analyzed Arp2/3 system function in the context of this two-step
model (Berger et al., 2008). It is important to note, however, that the issue is controversial, and that alternative models for the temporal progression of fusion events have been proposed (Beckett and Baylies, 2007). While our data does not impinge directly on these matters, we wish to emphasize that our analyses throughout this study were performed on properly aged embryos, in which an initial fusion round should be complete.

2. Generation of a SCAR dominant-negative allele. The pleiotropic nature of SCAR-complex phenotypes motivated us to obtain specific functional impairment of SCAR during myogenesis, based on a dominant negative approach and the GAL4-UAS system for regulated expression (Brand and Perrimon, 1993). An initial attempt involved expression of a SCAR protein construct lacking the C-terminal “VCA” Arp2/3 and actin binding domains. This approach, which proved successful with Wsp (Massarwa et al., 2007), failed in this case (data not shown), possibly due to instability of SCAR when subjected to structural alterations. However, a second construct, in which the full SCAR coding region is fused to GFP at its N terminus, was found to cause a severe fusion arrest in the majority of embryos (Supp Fig 1), when expressed in developing muscles using the muscle specific driver Mef2-GAL4 (Ranganayakulu et al., 1996). The fusion arrest phenotype can be overcome by co-expression of the intact SCAR protein (Supp Fig 1M). We therefore suggest that the GFP-SCAR construct generates a stable, inactive form of the SCAR NPF, which competes with the endogenous SCAR protein in binding to the other SCAR-complex members.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nuclei in DA1 muscle</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>9.8</td>
<td>1.7</td>
</tr>
<tr>
<td>kette^{J4-48} **</td>
<td>3.3</td>
<td>1.75</td>
</tr>
<tr>
<td>SCAR^{A37} *</td>
<td>7.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Df(3R)red3l (abi) *</td>
<td>6.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Df(2R)Chi^{p230} (HSPC300) *</td>
<td>6.75</td>
<td>1.9</td>
</tr>
<tr>
<td>Sra-1^{85.1}</td>
<td>10.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Supp Table 1. Quantification of myoblast fusion arrest in SCAR-complex mutants. The DA1/Eve assay was applied to wild-type embryos and embryos homozygous for the following genotypes: kette^{J4-48}, an amorphic kette allele (Hummel et al., 2000; Schroter et al., 2004; kette is the Drosophila homolog of vertebrate NAP-1); SCAR^{A37}, a full deletion of the SCAR locus (Zallen et al., 2002); Df(3R)red3l, a chromosomal deficiency spanning the abi locus at cytogenetic band 88A9; Df(2R)Chi^{p230}, a chromosomal deficiency spanning the SIP1/HSPC300 locus at cytogenetic band 60B4; and Sra-1^{85.1}, a null allele of the Sra-1/CYFIP gene (Schenck et al., 2003). The kette mutant displays a severe fusion phenotype (see also Supp. Fig 1 below), while the phenotypes of the other mutants are substantially weaker. Asterisks mark values that differ in a statistically significant manner from wild-type. SD= standard deviation.
Supp Fig 1. Dominant-negative effects of GFP-SCAR. (A-L) Wild-type (A-C), kette^{14-48} (D-F), SCAR^{37} (G-I) and Mef2-GAL4::UAS-GFP-SCAR (J-M) stage 16 embryos. Panels A,D,G,J (anti-MHC, red) document the dispersed, unfused myoblasts in four-segment-wide embryonic sections of these genotypes. Panels B,E,H,K (anti-Eve, red) are representative images of the Eve/DA1 assay, quantified in Supp. Table 1. Images from an equivalent assay, quantifying the extent of fusion in muscle DO1 and using the Runt nuclear marker (blue), is shown in panels C,C’,F,F’,I,I’,L,L’. A bar diagram summarizing the DA1 and DO1 assays is shown in panel N. (M) Co-expression of UAS-SCAR overcomes the effects of UAS-GFP-SCAR (compare with panel J).
II. Wsp system

In the study, we made use of the $Wsp^{3D3}$ allele (Schafer et al., 2007), to obtain strong $Wsp$ loss-of-function circumstances without having to resort to germline clone analysis for elimination of the $Wsp$ maternal contribution. To verify that the $Wsp^{3D3}$ allele can be used in this context, we ascertained that the dominant-negative activity of $Wsp^{3D3}$ activity is directed specifically towards Wsp, by demonstrating rescue of the $Wsp^{3D3}$ myogenic phenotype by $Wsp$ over-expression (Supp Fig 2). We then carried out Eve/DA1 and Runt/DO2 fusion-arrest assays on $Wsp^{3D3}$ homozygotes, as well as on $kette^{44-48} Wsp^{3D3}$ double mutant embryos (Supp Fig 3).

Supp Fig 2. Fusion-arrest phenotypes of $Wsp^{3D3}$. (A) MHC staining reveals strong fusion-arrest phenotype of a stage 15 $Wsp^{3D3}$ hemizygous embryo, which displays multiple unfused myoblasts clustered around short, thin myofibers. (B) Rescue of the $Wsp^{3D3}$ myogenic phenotype by expression of a full-length Wsp construct (Ben-Yaacov et al., 2001) in muscle tissue via twist-GAL4.

Supp Fig 3. Quantification of the Wsp$^{3D3}$ fusion-arrest phenotype. (A,B) DA1 muscle nuclei expressing Eve (red) in Wsp$^{3D3}$ (A) and kette$^{J4-48}$ Wsp$^{3D3}$ (B) mutants. (C,D) DO2 muscle nuclei expressing Runt (red) in Wsp$^{3D3}$ (C, C’) and kette$^{J4-48}$ Wsp$^{3D3}$ (D, D’) mutants. DO2 Muscle contours can be seen via MHC staining (green) in panels C,D. A bar diagram summarizing these assays is shown in panel E.

III. Methods- Immunofluorescence

Primary antibodies and dilutions used in this study include: anti–β-galactosidase (rabbit, 1:1,000); anti-Duf (rat, 1:100); anti-GFP (mouse, 1:200; Roche); anti-MHC (rabbit,
1:500; gift from Paul Fisher, SUNY Stony Brook); anti-SCAR (guinea-pig, 1:100; Zallen et al., 2002). Secondary Cy2, Cy3 and Cy5-conjugated antibodies against the relevant species were from Jackson ImmunoResearch (West Grove, PA). Processing for experiments that included phalloidin staining involved manual devittelinization, following fixation for 15 minutes on a heptane/formaldehyde:PBS (1:1) interface. Microfilaments were visualized by a 30 min incubation in 1 unit/ml Bodipy-conjugated phalloidin (Molecular Probes). Visualization of anti-SCAR required signal amplification as in Melen et al, 2005.

References


