

Drosophila Ana2 is a conserved centriole duplication factor

Naomi R. Stevens,^{1,2} Jeroen Dobbelaere,¹ Kathrin Brunk,¹ Anna Franz,^{1,2} and Jordan W. Raff^{1,2}

¹The Gurdon Institute, Cambridge, England CB2 1QN, UK

²Sir William Dunn School of Pathology, University of Oxford, Oxford, England OX1 3RE, UK

In *Caenorhabditis elegans*, five proteins are required for centriole duplication: SPD-2, ZYG-1, SAS-5, SAS-6, and SAS-4. Functional orthologues of all but SAS-5 have been found in other species. In *Drosophila melanogaster* and humans, Sak/Plk4, DSas-6/hSas-6, and DSas-4/CPAP—orthologues of ZYG-1, SAS-6, and SAS-4, respectively—are required for centriole duplication. Strikingly, all three fly proteins can induce the de novo formation of centriole-like structures when overexpressed in unfertilized eggs. Here, we find that of eight candidate

duplication factors identified in cultured fly cells, only two, Ana2 and Asterless (Asl), share this ability. Asl is now known to be essential for centriole duplication in flies, but no equivalent protein has been found in worms. We show that Ana2 is the likely functional orthologue of SAS-5 and that it is also related to the vertebrate STIL/SIL protein family that has been linked to microcephaly in humans. We propose that members of the SAS-5/Ana2/STIL family of proteins are key conserved components of the centriole duplication machinery.

Introduction

The centriole is composed of a radial array of nine microtubule (MT) triplets, doublets, or singlets depending on species and cell type. Centrioles are required to make two important cellular structures: centrosomes and cilia. The centrosome consists of a pair of centrioles surrounded by pericentriolar material (PCM) and is the major MT organizing center in many animal cells (Rieder et al., 2001; Doxsey et al., 2005). Cilia are formed when the centriole pair migrates to the cell cortex, and the older, mother, centriole forms a basal body that nucleates the ciliary axoneme. Many different cell types possess cilia, and they have multiple roles in development (Gerdes et al., 2009).

To ensure their inheritance by each daughter cell, centrioles duplicate precisely once per cell cycle. This process must be tightly regulated. Failure in centriole duplication leads to catastrophic errors during embryogenesis in both worms and flies (O'Connell et al., 2001; Stevens et al., 2007), and an increasing number of human diseases have been linked to defects in centrosome and/or cilia function (Badano et al., 2005; Sharma et al., 2008). Centriole overduplication can be equally damaging,

as excess centrioles are frequently observed in human tumors (Nigg, 2002), and there appears to be a direct causative relationship between centriole overduplication and tumorigenesis in flies (Basto et al., 2008).

In canonical centriole duplication, a new daughter centriole grows at a right angle to the mother centriole. A series of genome-wide RNAi and genetic screens in worms have found just five proteins essential for centriole duplication: SPD-2, ZYG-1, SAS-5, SAS-6, and SAS-4 (O'Connell et al., 2001; Kirkham et al., 2003; Leidel and Gönczy, 2003; Dammermann et al., 2004; Delattre et al., 2004; Kemp et al., 2004; Pelletier et al., 2004; Leidel et al., 2005). SPD-2 is required to recruit the kinase ZYG-1 to the centriole, and both proteins then recruit a complex of SAS-5 and SAS-6 (Delattre et al., 2006; Pelletier et al., 2006). SAS-5 and SAS-6 are mutually dependent for their centriolar localization and are in turn needed to recruit SAS-4 (Leidel et al., 2005).

Although DSpd-2 is not essential for centriole duplication in flies (Dix and Raff, 2007; Giansanti et al., 2008), and no SAS-5 homologues have been identified outside worms, proteins related to ZYG-1, SAS-6, and SAS-4 have a conserved

Correspondence to Jordan W. Raff: jordan.raff@path.ox.ac.uk

Abbreviations used in this paper: 3AT, 3-aminotriazole; Asl, Asterless; BLAST, basic local alignment search tool; Cnn, centrosomin; D-PLP, *Drosophila* pericentrin-like protein; MBP, maltose-binding protein; MT, microtubule; PACT, pericentrin/AKAP450 centrosomal-targeting domain; PCM, pericentriolar material; STIL/SIL, SCL/TAL1 interrupting locus; UAS, upstream activation sequence; Ubq, ubiquitin; WT, wild type; Y2H, yeast two-hybrid.

© 2010 Stevens et al. This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date [see <http://www.jcb.org/misc/terms.shtml>]. After six months it is available under a Creative Commons License [Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>].

role in centriole duplication in other systems. In *Drosophila*, for example, the kinase Sak, which is related to ZYG-1, and the homologues of SAS-6 (DSas-6) and SAS-4 (DSas-4) are required for centriole duplication (Bettencourt-Dias et al., 2005; Basto et al., 2006; Peel et al., 2007; Rodrigues-Martins et al., 2007a). Recently, however, several additional proteins have been identified in cultured fly cells that are potentially involved in centriole duplication (Goshima et al., 2007; Dobbelaere et al., 2008). Here, we set out to identify which of these potential duplication factors are likely to function as upstream regulators of centriole formation.

Results and discussion

Ana2 and Asterless (Asl) can drive the de novo formation of centriole-like structures

Genome-wide RNAi screens in cultured fly cells identified just 18 proteins that, when depleted, gave a reduced number of centrioles (Goshima et al., 2007; Dobbelaere et al., 2008). This list includes Sak, DSas-6, and DSas-4, as well as eight other proteins that specifically localize to centrosomes (Ana1, Ana2, Ana3, Asl, DCP110, DCep135/Bld10, DCep97, and Rcd4): these eight are therefore good candidates to play a direct role in centriole duplication.

GFP-Sak, GFP-DSas-6, and DSas-4-GFP share the unique ability to drive de novo formation of centriole-like structures in unfertilized eggs when highly overexpressed from the upstream activation sequence (UAS) promoter (Peel et al., 2007; Rodrigues-Martins et al., 2007b). UAS-GFP-Sak and UAS-GFP-DSas-6 induce these structures in ~95% of unfertilized eggs, whereas UAS-DSas-4-GFP does so in ~60% of unfertilized eggs (Peel et al., 2007). We wondered if we could use this assay to identify other components likely to function upstream in the centriole duplication pathway. We therefore generated transgenic lines carrying GFP fusions to all eight potential duplication factors under the control of the UAS promoter, which allowed us to overexpress them in unfertilized eggs (Fig. S1). Strikingly, only Ana2 (in 97% of eggs) and Asl (in 33% of eggs) were able to drive de novo formation of centriole-like structures (Fig. 1).

Asl has recently been shown to be essential for centriole duplication in flies (Blachon et al., 2008), whereas, of the six proteins unable to induce de novo centriole formation, two, DCep135/Bld10 and Ana3, are now known not to be essential for centriole duplication in flies (Mottier-Pavie and Megraw, 2009; Stevens et al., 2009). These findings indicate that our overexpression assay can identify those proteins likely to be most intimately involved in centriole duplication. As Asl has already been shown to be required for centriole duplication (Blachon et al., 2008), we focused on investigating the function of Ana2.

Ana2 is an important regulator of canonical centriole duplication

Ana2 can drive de novo formation of centriole-like structures as efficiently as DSas-6 and Sak (Peel et al., 2007; Rodrigues-Martins et al., 2007b). We wanted to verify, however, that it also has a role in canonical centriole duplication. Overexpressing

GFP-Sak or GFP-DSas-6 from the ubiquitin (Ubq) promoter induces centriole overduplication in brains and embryos, respectively (Peel et al., 2007). Surprisingly, however, overexpression of Sak, DSas-6, or DSas-4 cannot drive centriole overduplication in primary spermatocytes (Peel et al., 2007), which suggests that another duplication protein is limiting. To test if Ana2 might be this limiting factor, we generated Ubq-GFP-Ana2 transgenic lines. Strikingly, we found that in spermatocytes expressing Ubq-GFP-Ana2, in addition to the normal centriole pairs (doublets), we observed centriole triplets, quadruplets, and even quintets (Fig. 2, A–G). The extra centrioles in these clusters appeared to be fully functional; they separated from one another by the end of meiosis I (as centriole doublets normally do), and the extra centrioles inherited by secondary spermatocytes recruited PCM and nucleated MT asters, and so formed multipolar spindles during meiosis II (Fig. 2, H and I).

We did not observe centriole overduplication in embryos or brain cells expressing Ubq-GFP-Ana2 (unpublished data), which is consistent with DSas-6 and Sak levels, respectively, limiting centriole formation in these tissues. Nevertheless, that Ana2 overexpression can drive centriole overduplication in spermatocytes demonstrates that it is an important regulator of canonical centriole duplication.

Ana2 shows a unique asymmetric localization to the daughter centriole

We next wanted to compare the localization of Ana2 with that of the other *Drosophila* centriole duplication factors. DSas-4-GFP, GFP-DSas-6, and GFP-Sak are all enriched at the proximal and distal ends of the large spermatocyte centrioles (Peel et al., 2007). We found that, likewise, Ana2-GFP localized preferentially to the proximal and distal centriole tips. Strikingly, however, Ana2-GFP (and GFP-Ana2) also exhibited a unique asymmetric distribution, consistently localizing preferentially along one centriole barrel (Fig. 3, A and B).

In primary spermatocytes, it is possible to distinguish mother and daughter centrioles, as the daughter can often be observed associating end-on with the side of the mother (Blachon et al., 2008). In 25 centriole pairs where we could unambiguously distinguish mother and daughter centrioles, Ana2-GFP was always enriched on the daughter (Fig. 3 A). Mother and daughter centrioles can show important differences in their behavior in vertebrate cells (Piel et al., 2000) and during asymmetric stem cell divisions in *Drosophila* (Rebollo et al., 2007; Rusan and Peifer, 2007; Yamashita et al., 2007). Although mother and daughter centrioles are morphologically and molecularly distinguishable in vertebrates (see, for example, Vorobjev and Chentsov, 1982; Chang et al., 2003; Gromley et al., 2003; Graser et al., 2007), this is not the case in *Drosophila* (Callaini and Riparbelli, 1990; Callaini et al., 1997; Vidwans et al., 2003). To our knowledge, Ana2-GFP is the first fly protein shown to localize asymmetrically to mother and daughter centrioles in this manner.

Interestingly, as spermatocytes progressed through meiosis I, this centriolar asymmetry became less pronounced, and this appeared to reflect the selective loss of GFP-Ana2 from the daughter centriole, bringing its levels down to that of the mother (compare Fig. 3 B, showing a G2 centriole pair, to Fig. 3 C,

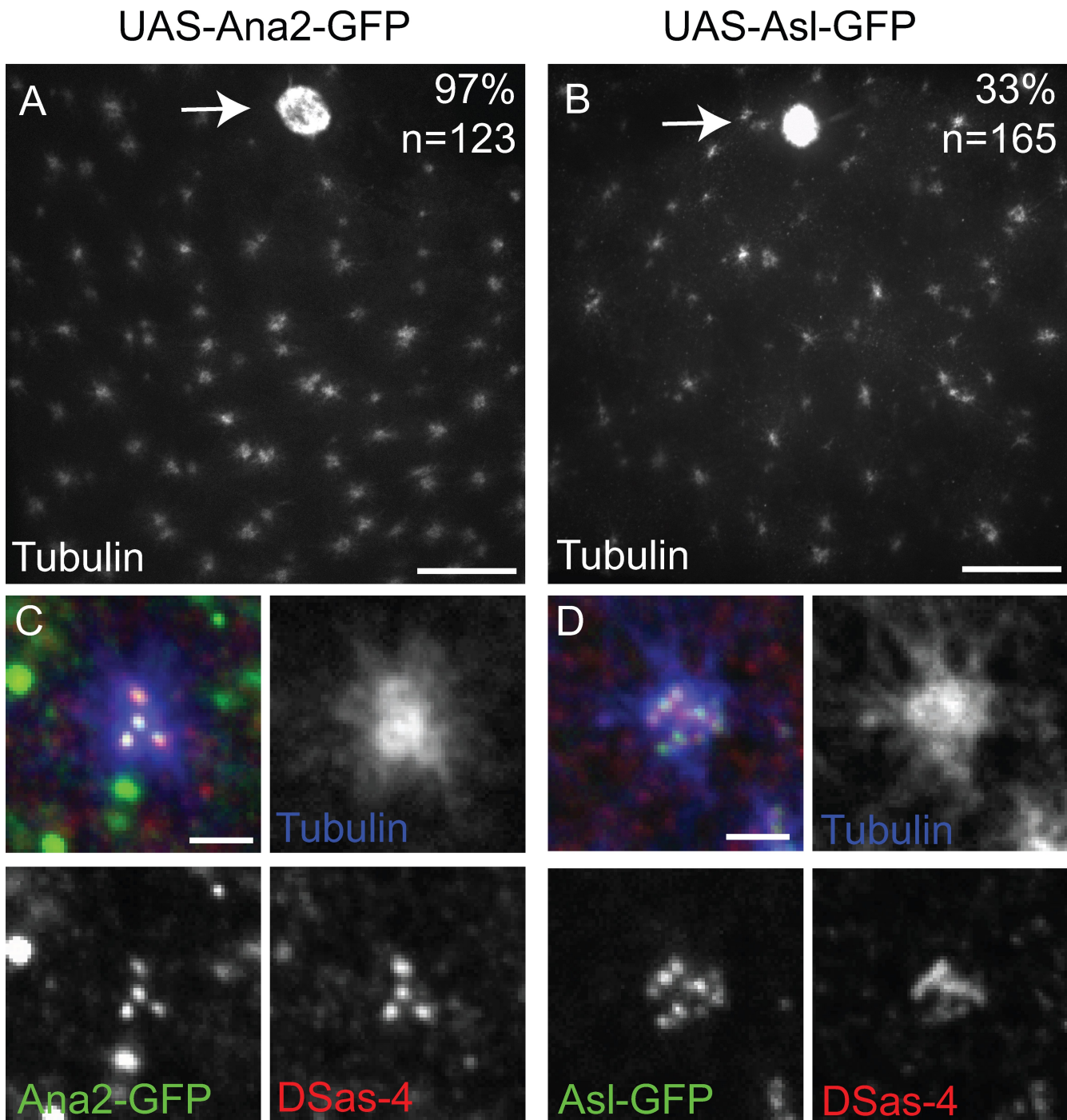


Figure 1. **Overexpression of Ana2 or Asl drives de novo formation of centriole-like structures.** (A and B) Unfertilized eggs laid by UAS-Ana2-GFP (A) or UAS-Asl-GFP (B) mothers containing numerous MT asters (stained for tubulin). Arrows indicate the polar bodies. (C and D) Single asters from UAS-Ana2-GFP (C) or UAS-Asl-GFP (D) eggs stained for tubulin (blue) and DSas-4 (red). GFP is in green. Each aster contains several structures that stain for centriole markers. Bars: (A and B) 20 μm ; (C and D) 2 μm .

showing a centriole pair separating at the end of meiosis I). As overexpression of Ana2 can lead to centriole overduplication, Ana2 levels presumably must normally be tightly regulated to prevent the formation of extra centrioles.

After exit from meiosis II, each spermatid inherits a single centriole, which acts as a basal body to nucleate the flagellar axoneme. Structural components of the centriole, like Ana3 (Stevens et al., 2009) and *Drosophila* pericentrin-like protein

(D-PLP; Martinez-Campos et al., 2004), continue to localize along the basal body. In contrast, Ana2, like the conserved duplication proteins (Blachon et al., 2009), was undetectable along the basal body (Fig. 3 D). Ana2 did, however, colocalize with GFP-DSas-6 at the proximal centriole-like structure (Fig. 3 D), a small nodule adjacent to the basal body that has been proposed to be an early intermediate in centriole formation (Blachon et al., 2009).

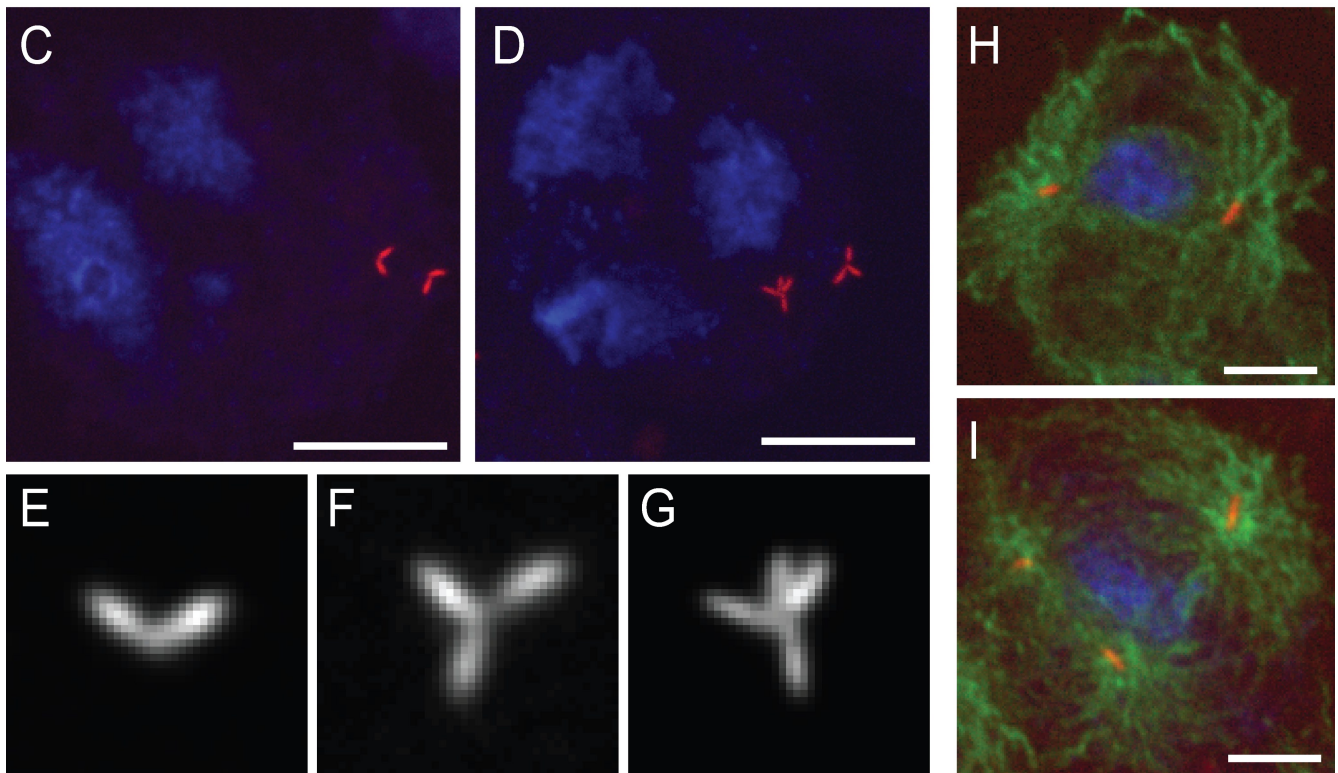
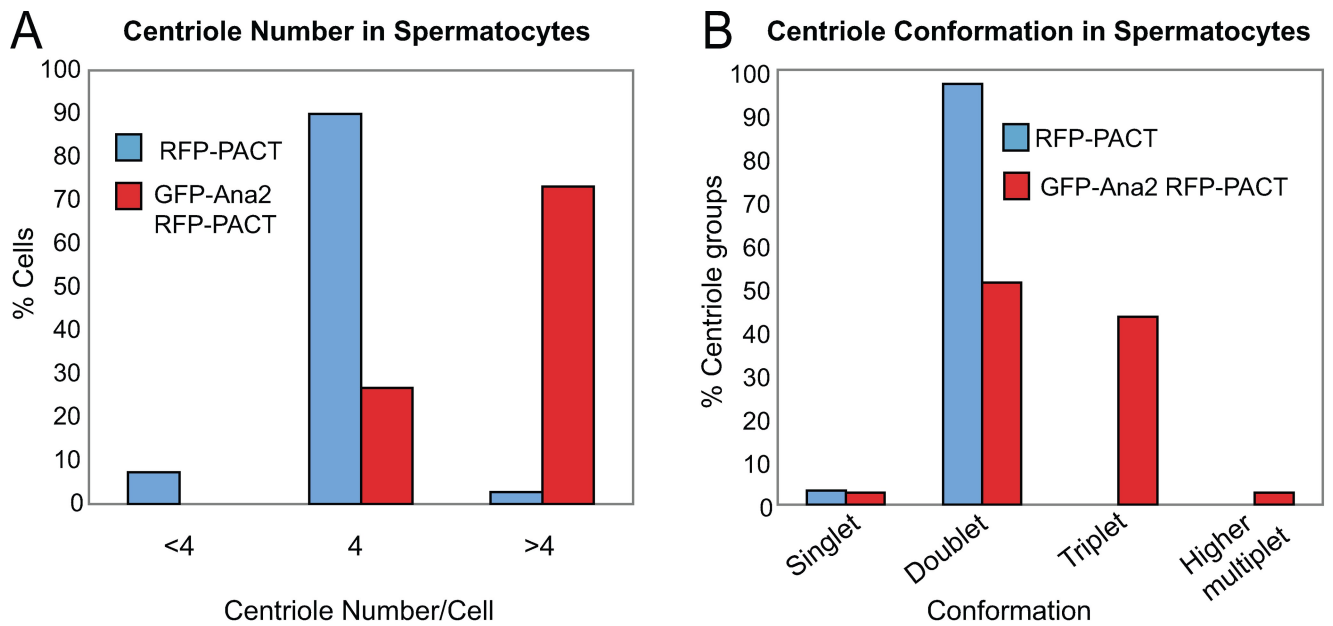


Figure 2. Overexpression of Ana2 drives centriole overduplication in spermatocytes. (A and B) Centriole number (A) and conformation (B) in G2 primary spermatocytes expressing either the centriole marker RFP-PACT alone or both RFP-PACT and GFP-Ana2. Centrioles were counted in a total of 109 RFP-PACT cells and 138 GFP-Ana2 RFP-PACT cells from seven testes per condition. (C and D) G2 primary spermatocytes expressing either RFP-PACT (red) alone (C) or both RFP-PACT and GFP-Ana2 (D). DNA is in blue. The cell in C has the normal two centriole pairs. Overexpression of GFP-Ana2 induces centriole triplets and quadruplets (D). (E–G) Magnified images of RFP-PACT-labeled doublet (E), triplet (F), and quadruplet (G) centriole groups. (H and I) Secondary spermatocytes in meiosis II expressing either RFP-PACT (red) alone (H) or both RFP-PACT and GFP-Ana2 (I). Tubulin is in green and DNA in blue. The cell in H has the normal two centrioles whereas the one in I has three centrioles forming a tripolar spindle. Bars: (C and D) 10 μ m; (H and I) 5 μ m.

Ana2 is the likely functional orthologue of *Caenorhabditis elegans* SAS-5

Intriguingly, *Drosophila* homologues have been identified for all the *C. elegans* centriole duplication factors except SAS-5, which has no clear homologues outside worms. Ana2 and SAS-5 are

similar in size and have a single central coiled-coil domain, leading Goshima et al. (2007) to suggest that Ana2 could be the *Drosophila* equivalent of SAS-5 (Goshima et al., 2007). As SAS-5 interacts with SAS-6 in worms (Leidel et al., 2005), we looked for a genetic interaction between Ana2 and DSas-6 in flies.

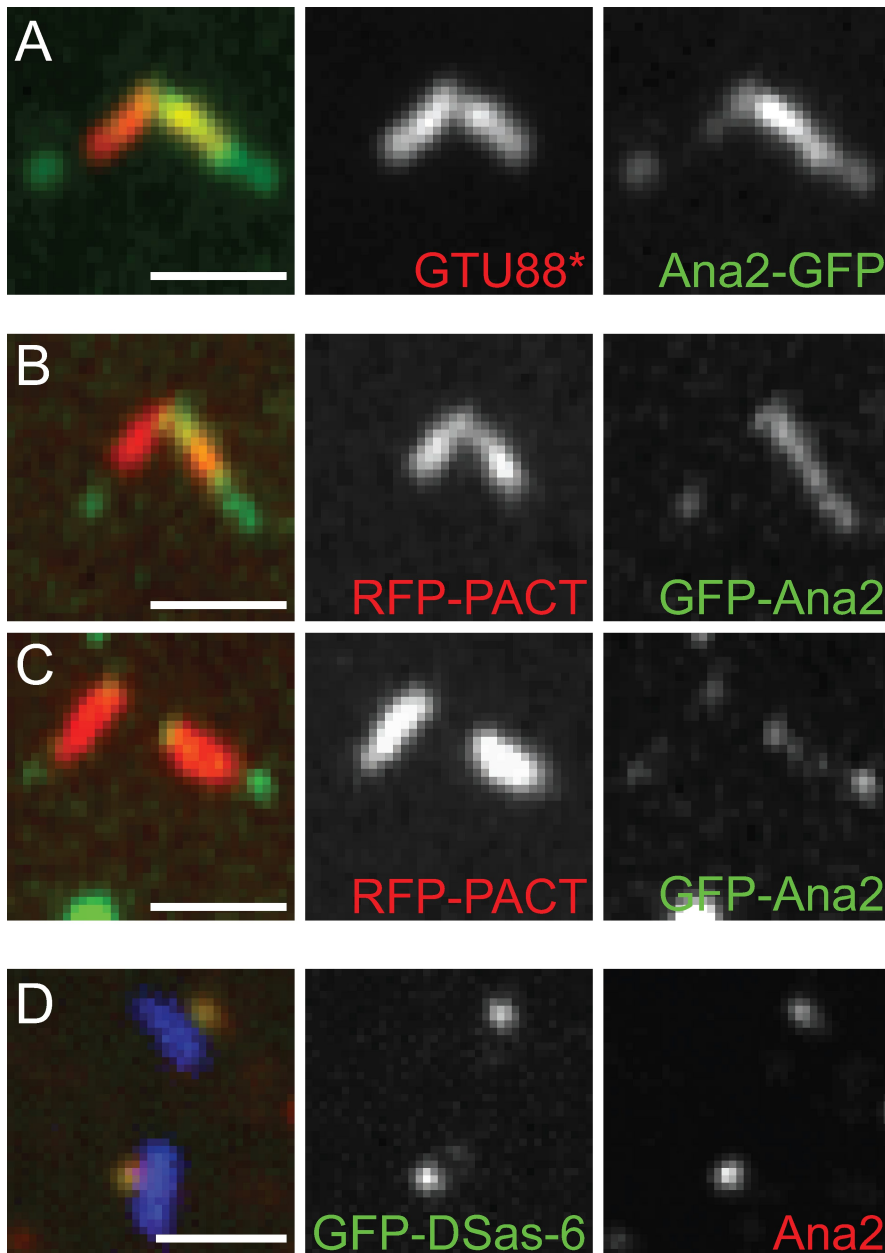


Figure 3. Ana2 is a centriole component with a unique asymmetric localization. (A) Centriole pair from a G2 primary spermatocyte expressing Ana2-GFP (green) stained for the centriole marker GTU88* (red). Ana2-GFP localizes to the proximal and distal centriole ends and also exhibits a unique asymmetric distribution, localizing preferentially along one centriole barrel, which can be identified as the daughter from the GTU88* staining (see main text). (B) Centriole pair from a G2 primary spermatocyte expressing GFP-Ana2 (green) and RFP-PACT (red). GFP-Ana2 localization is indistinguishable from Ana2-GFP. (C) Centriole pair from a primary spermatocyte at anaphase of meiosis I: the centrioles are beginning to separate. The cell is expressing RFP-PACT (red) and GFP-Ana2 (green), which is no longer obviously asymmetric. (D) Two basal bodies from spermatids expressing RFP-PACT (blue) and GFP-DSas-6 (green), and stained for Ana2 (red). GFP-DSas-6 and Ana2 colocalize at the proximal centriole-like structure, a nodule adjacent to the basal body marked by RFP-PACT. Bars, 2 μ m.

A small percentage of eggs laid by mothers carrying two copies of a Ubq-GFP-DSas-6 transgene (as opposed to the much stronger UASp-GFP-DSas-6 discussed above) assemble centriole-like structures (Peel et al., 2007). To see if we could enhance this effect, we generated flies carrying one copy of Ubq-GFP-DSas-6 and one copy of Ubq-Ana2-GFP, neither of which alone (as a single copy) induces the assembly of centriole-like structures (Fig. 4 A). Strikingly, almost all the unfertilized eggs laid by these females contained hundreds of large structures that stained for centriole markers, recruited PCM, and nucleated asters (Fig. 4, A–D; and Fig. S2 A). Importantly this interaction was specific to Ana2 and DSas-6. In eggs from mothers carrying one copy of either Ubq-Ana2-GFP or Ubq-GFP-DSas-6 together with one copy of either Ubq-GFP-Sak, Ubq-Asl-GFP, or Ubq-DSas-4-GFP, we observed at most a very small number of asters in very few eggs (Fig. 4 A).

Interestingly, the centriole-like structures produced by overexpressing UASp-GFP-DSas-6 differ significantly from those resulting from the overexpression of GFP-Sak, DSas-4-GFP, Asl-GFP, or Ana2-GFP in that they are much larger and often appear ring-shaped, and that only one structure is contained within each aster (Fig. 4 E; Peel et al., 2007; Rodrigues-Martins et al., 2007a). The structures in the eggs from females expressing both Ubq-GFP-DSas-6 and Ubq-Ana2-GFP were similar to this DSas-6 type (Fig. 4, D and E). This suggests that Ana2-GFP acts to promote the assembly of GFP-DSas-6 into these structures.

Having shown that Ana2 functionally interacts with DSas-6, we looked for a physical interaction. Using a yeast two-hybrid (Y2H) assay, we found that Ana2 and DSas-6 interact and that the N-terminal region of DSas-6 and the C-terminal region of Ana2 are necessary and sufficient for this

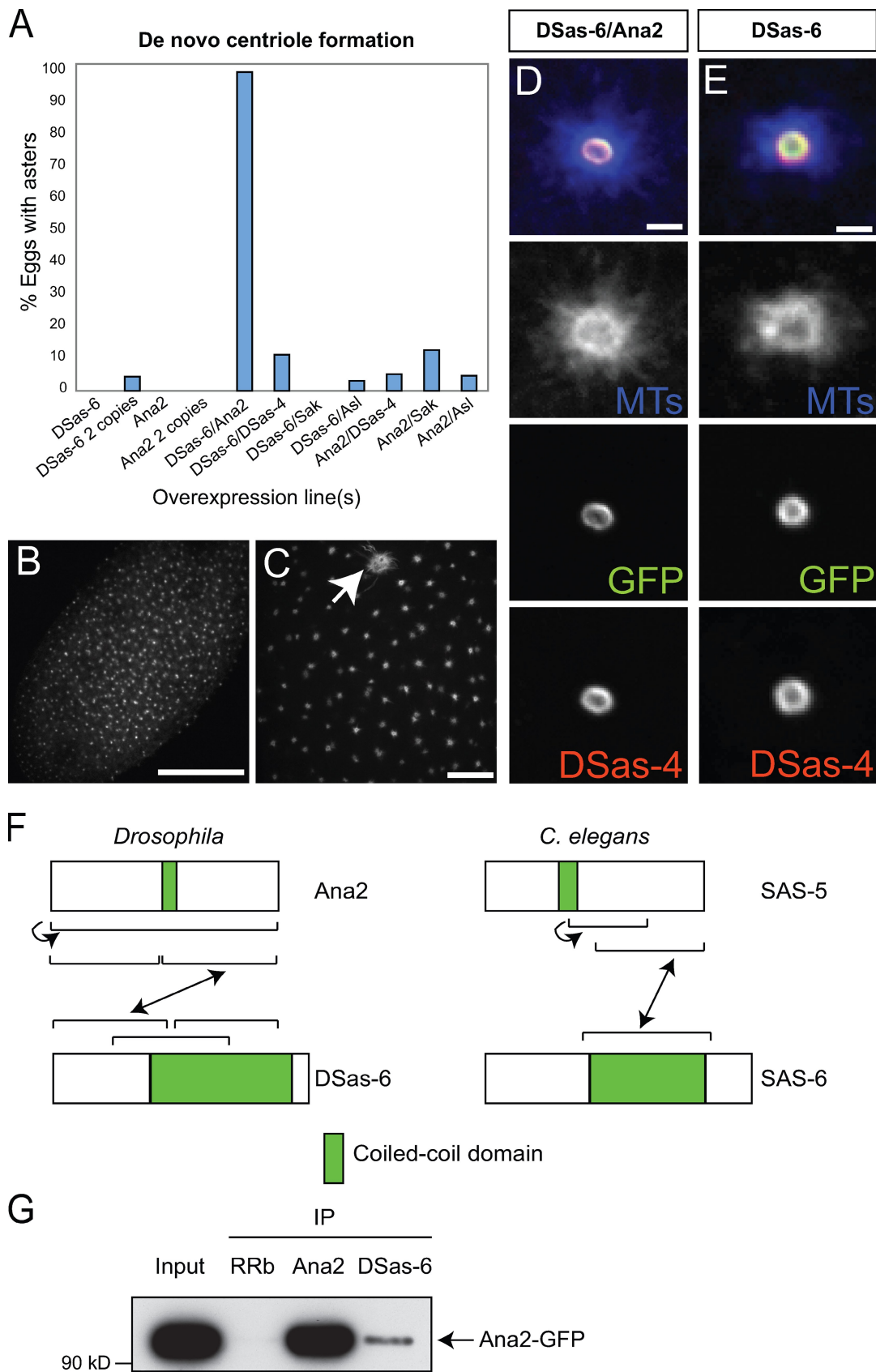


Figure 4. **Ana2 and DSas-6 functionally and physically interact.** (A) Percentage of unfertilized eggs laid by mothers of the given genotypes that contained MT asters. All transgenes were GFP fusions with a Ubq promoter. Eggs from mothers expressing one or two copies of Ubq-GFP-DSas-6 and Ubq-Ana2-GFP were analyzed; all combinations expressed one copy of each transgene. $n > 80$ eggs per genotype (for values, see Materials and methods). (B and C) Almost all unfertilized eggs from mothers expressing one copy of Ubq-GFP-DSas-6 and one copy of Ubq-Ana2-GFP assemble large numbers of

(Izraeli et al., 1999). Most importantly, it has recently been shown that mutations in human *STIL* cause primary microcephaly (MCPH), a congenital disorder characterized by reduced brain size (Kumar et al., 2009). Mutations in four other genes, *MCPH1*, *CDK5RAP2*, *ASPM*, and *CPAP/CENPJ*, are known to cause MCPH, and all are centrosomal proteins (Bond et al., 2002, 2005; Jackson et al., 2002; Zhong et al., 2005, 2006), which strongly suggests that *STIL* is required for efficient centrosome function in humans.

Conclusions

Here, we show that of eight centrosomal proteins identified as potential duplication factors in *Drosophila* tissue culture cells, only two, *Asl* and *Ana2*, appear to be able to induce de novo formation of centriole-like structures in unfertilized eggs. *Asl* has recently been shown to be essential for centriole duplication (Blachon et al., 2009), and we provide evidence that *Ana2* is also a key centriole duplication factor. Thus, *Ana2* and *Asl* join *Sak*, *DSas-6*, and *DSas-4* to make up a module of just five proteins known to drive centriole duplication in flies.

Our data strongly suggest that the *Ana2/STIL* family of centrosomal proteins are the long-sought functional orthologues of *SAS-5*. Thus, four of these five components (*Sak/ZYG-1*, *DSas-6/SAS-6*, *Ana2/SAS-5*, and *DSas-4/SAS-4*) are functionally conserved between flies and worms. Moreover, three of these proteins are required for centriole duplication in humans (Habedanck et al., 2005; Leidel et al., 2005; Kleylein-Sohn et al., 2007; Strnad et al., 2007; Kohlmaier et al., 2009), whereas the fourth, *SAS-5/Ana2/STIL*, also appears likely to be required for this process in vertebrates (Izraeli et al., 1999; Pfaff et al., 2007; Kumar et al., 2009).

Both flies and worms have an additional protein (*SPD-2* in worms, *Asl* in flies) that appears to be essential for centriole duplication. Intriguingly, both *SPD-2* (Kemp et al., 2004; Pelletier et al., 2004) and *Asl* (Bonaccorsi et al., 1998; Varmark et al., 2007; Blachon et al., 2008) are not only required for centriole duplication, but also for PCM recruitment. There is evidence that the PCM promotes centriole duplication (Dammermann et al., 2004; Loncarek et al., 2008), so *SPD-2* and *Asl* could play a more indirect role in centriole duplication via their ability to recruit PCM. Alternatively, both proteins may act directly in centriole duplication, with the function of *SPD-2* in worms perhaps being performed by *Asl* in flies.

In summary, we show that *Ana2* acts as a centriole duplication factor in *Drosophila* and is likely to have a conserved role in other species. Overall, centriole duplication appears to be a highly conserved process, at the heart of which is a small number of key proteins. The challenge will now be to tease apart how these components cooperate to build a centriole of the right size, in the right place, and at the right time.

Materials and methods

Generation of GFP fusions and transgenic lines

P element-mediated transformation vectors containing GFP-fusions to *Ana1*, *Ana2*, *Ana3*, *Asl*, *DCP110*, *DCep135*, *DCep97*, and *Rcd4* were generated as follows. The complete coding region of each protein was amplified from either cDNA (*Ana1*, *Ana2*, *Asl*, *DCP110*, *DCep135*, and

Rcd4) or genomic DNA (*Ana3* and *DCep97*), with *att* sites at either end for Gateway cloning (Invitrogen). These fragments were inserted into the Gateway pDONR Zeo vector. The two *Ana3* exons were amplified separately from genomic DNA before being ligated together to produce a vector with the complete coding sequence. The pDONR vectors were then recombined with UASp and, for *Ana2* and *Asl*, Ubq plasmids (Peel et al., 2007), with each coding sequence placed in frame with GFP at the N or C terminus. The following transgenic lines were generated by BestGene, Inc.: UASp-GFP-*Ana1*, UASp-*Ana2*-GFP, UASp-*Ana3*-GFP, UASp-*Asl*-GFP, UASp-*DCP110*-GFP, UASp-GFP-*DCep135*, UASp-GFP-*DCep97*, UASp-GFP-*Rcd4*, Ubq-*Ana2*-GFP, Ubq-GFP-*Ana2*, and Ubq-*Asl*-GFP. The Ubq promoter drives moderate expression in all tissues (Lee et al., 1988), whereas the UASp lines were crossed to V32a, which expresses a *Gal4/VP16* fusion protein from a maternal tubulin promoter; this drives very high-level overexpression in the female germline (Peel et al., 2007).

We also used the previously described transgenic lines Ubq-GFP-*DSas-6*, Ubq-GFP-*Sak*, Ubq-*DSas-4*-GFP, and Ubq-mRFP-pericentrin/AKAP450 centrosomal-targeting domain (PACT; Peel et al., 2007).

Generation and use of *Ana2* antibodies

A maltose-binding protein (MBP; New England Biolabs, Inc.) fusion of aa 1–201 of *Ana2* was purified according to the manufacturer's instructions, and antisera were raised in two rabbits by Eurogentec. To affinity purify antibodies, the antiserum was first depleted of anti-MBP antibodies by passing over an Aminolink MBP column (Thermo Fisher Scientific). Specific antibodies were then purified by passing the antiserum over a column of MBP-*Ana2*(1–201) fusion protein. The column was washed with PBS + 0.5 M KCl, and antibodies were eluted in 0.1 M glycine, pH 2.1. The antibodies were neutralized with 1 M Tris, pH 8.5, and glycerol was added to 50%, then materials were stored at -20°C .

The antibody was used at 1:250 for immunofluorescence experiments. It weakly stained centrosomes in embryos and the proximal centriole-like structure in spermatids, but it did not stain primary spermatocyte centrioles. To investigate why this was the case, we used the antibody to stain *Ana2*-GFP-expressing spermatocytes. Here, the antibody stained the distal tips of the centrioles, but not the proximal ends of the centrioles or the single centriole barrel, even though the *Ana2*-GFP labeling was clearly visible at these sites. This suggests that the antibody does not stain spermatocyte centrioles for a combination of reasons. First, endogenous *Ana2* must be present at centrioles at very low levels, as we cannot detect it even in the distal portion of the centrioles where we can detect *Ana2*-GFP. Second, endogenous *Ana2* is probably not easily accessible to antibodies at the proximal end of the centrioles and along the centriole barrel, as we cannot detect *Ana2*-GFP with the antibody at these sites even though *Ana2*-GFP is localized there. For other uses see the "Electrophoresis and immunoblotting" and "Immunoprecipitation" sections.

Fixed analysis of eggs and pupal testes

0–4-h collections of unfertilized eggs were made from mothers expressing UASp-GFP-*Ana1* ($n = 77$), UASp-*Ana2*-GFP ($n = 123$), UASp-*Ana3*-GFP ($n = 206$), UASp-*Asl*-GFP ($n = 165$), UASp-*DCP110*-GFP ($n = 239$), UASp-GFP-*DCep135* ($n = 177$), UASp-GFP-*DCep97* ($n = 172$), UASp-GFP-*Rcd4* ($n = 214$), Ubq-GFP-*DSas-6* ($n = 90$), Ubq-GFP-*DSas-6* 2 copies ($n = 92$), Ubq-*Ana2*-GFP ($n = 90$), Ubq-*Ana2*-GFP 2 copies ($n = 84$), Ubq-GFP-*DSas-6/Ubq-Ana2*-GFP ($n = 84$), Ubq-GFP-*DSas-6/Ubq-DSas-4*-GFP ($n = 81$), Ubq-GFP-*DSas-6/Ubq-GFP-Sak* ($n = 81$), Ubq-GFP-*DSas-6/Ubq-Asl*-GFP ($n = 96$), Ubq-*Ana2*-GFP/Ubq-*DSas-4*-GFP ($n = 117$), Ubq-*Ana2*-GFP/Ubq-GFP-*Sak* ($n = 96$), and Ubq-*Ana2*-GFP/Ubq-*Asl*-GFP ($n = 86$). Eggs were dechorionated in 60% bleach for 2 min, washed in water + 0.05% Triton X-100, then washed into a small glass bottle with 1 ml heptane. 1 ml methanol + 5% 0.25 M EGTA was added and the bottle was shaken gently until most eggs fell into the lower methanol/EGTA layer. Eggs were stored in methanol at 4°C . For immunostaining, eggs were rehydrated by washing in PBT (PBS + 0.1% Triton X-100), blocked in PBS + 5% BSA, and incubated with primary antibodies in PBS/BSA overnight at 4°C . Eggs were then washed in PBT before incubation with secondary antibodies diluted 1:1,000 in PBT for 4 h at room temperature. After final washes in PBT, eggs were mounted in mounting medium (85% glycerol and 2.5% *n*-propylgallate).

Pupal testes were dissected in PBS, placed on a coverslip, and cut open. A slide was then placed over the coverslip and then flash frozen in liquid nitrogen. Coverslips were removed and the slides were incubated for 5 min in methanol at -20°C , and then in acetone for 1–2 min at -20°C . This was followed by incubation in PBT for 10 min, washes in PBS, and blocking in PBS/BSA (1%). Slides were incubated in primary antibody

(diluted in PBS/BSA) under a mounted coverslip in a moist chamber overnight at 4°C. After washes in PBS, slides were incubated in secondary antibodies (diluted 1:300 in PBS) for 1 h at 25°C. After final washes, slides were mounted in mounting medium.

Slides were observed at room temperature on a spinning disc confocal system (ERS; PerkinElmer), mounted on an inverted microscope (Axiovert 200M; Carl Zeiss, Inc.) with a charge-coupled device camera (Orca ER; Hamamatsu), using a 63×/1.25 NA objective (Carl Zeiss, Inc.) with Immersol oil (Carl Zeiss, Inc.). Images were acquired using Ultraview ERS software (PerkinElmer), imported into Photoshop CS2 (Adobe), and adjusted to use the full range of pixel intensities.

Identification of centriole-like structures in eggs

Unfertilized eggs were initially stained for α -tubulin and the centriole marker DSas-4. In wild-type (WT) eggs, the only MTs visible are those surrounding the polar bodies (Peel et al., 2007; Stevens et al., 2009). Under some of the GFP overexpression conditions, MT asters were formed that contained GFP dots at the center, which stained for DSas-4. For these conditions, we went on to stain unfertilized eggs with antibodies against a second centriole marker, D-PLP, and the PCM proteins centrosomin (Cnn) and γ -tubulin. Structures were considered to be centriole-like if they stained for both of the centriole and both of the PCM markers, and nucleated MT asters. We then quantified the percentage of unfertilized eggs containing these structures for each overexpression condition.

Antibodies

The following antibodies were used: 1:1,000 rabbit anti-D-PLP (Martinez-Campos et al., 2004), 1:250 rabbit anti-DSas-4 (Basto et al., 2006), 1:500 guinea pig anti-Cnn (Dix and Raff, 2007), 1:1,000 mouse monoclonal anti- α -tubulin (DM1 α ; Sigma-Aldrich), and 1:1,000 GTU88*, a batch of the mouse monoclonal anti- γ -tubulin GTU88 (Sigma-Aldrich) antibody that cross-reacts with centrioles in flies (Martinez-Campos et al., 2004). Alexa Fluor 488, Cy3, and Cy5 secondary antibodies were obtained from Invitrogen or Jackson ImmunoResearch Laboratories, Inc.

Y2H assay

pDEST22 (prey) and pDEST32 (bait) vectors containing full-length Ana2, full-length DSas-6, DSas-6 NT (aa 1–210), DSas-6 M (aa 104–317), DSas-6 CT (aa 224–414), Ana2 NT (aa 1–200), or Ana2 CT (aa 201–420) were generated as described for the GFP constructs in the “Generation of GFP fusions and transgenic lines” section. Prey vectors were transformed into Y8800 (α) and bait vectors into Y8930 (α) (Boxem et al., 2008). Yeast containing bait or prey constructs were arrayed into 96-well plates. Baits were tested on $-Leu$, $-His$ medium with different 3-aminotriazole (3AT) concentrations for autoactivation. Only full-length Ana2 showed strong autoactivation. For the final screen, the yeast strains were mated overnight on yeast extract, peptone, adenine, and dextrose (YPAD) plates, and diploids were selected twice on $-Leu$, $-Trp$ plates. Diploids containing both bait and prey vectors were then screened on $-Leu$, $-Trp$, $-His$ with 5 mM or 20 mM 3AT $-Leu$, $-Trp$, $-Ade$ plates and by performing an X-gal assay (Invitrogen) according to manufacturer’s instructions. Krev1 was used as a control bait, and interaction was tested with RalGDS-WT (positive) and RalGDS-m2 (negative) according to manufacturer’s instructions (ProQuest2; Invitrogen).

Drosophila cell culture

S2 cells were cultured in Schneider’s medium (Sigma-Aldrich) with 10% FBS (Sigma-Aldrich) and 1% penicillin/streptomycin (Invitrogen). pUbq-GFP-DSas-6 and pUbq-Ana2-GFP vectors were transfected into S2 cells using cellfectin (Invitrogen) using the pCoBlast vector for selection. After 3 wk of selection with blasticidin, stable cell lines were obtained.

Immunoprecipitation

An 80-cm³ flask of cells was grown for 3 d and harvested by centrifugation (5 min at 100 rpm). Cells were washed once in immunoprecipitation (IP) buffer (PBS, 5 mM EDTA, 1× PMSF, 1× protease inhibitor [Roche], 25 mM NaF, 1 mM Na₃VO₄, 20 mM β -glycerol phosphate, and 1× phosphatase inhibitor cocktail [Sigma-Aldrich]) and resuspended in 500 μ l of IP buffer. Keeping the cells on ice, they were broken by syringing up and down 20 times using a G24 syringe. Extracts were then centrifuged twice for 15 min at 15,000 rpm. 30 μ l of Dynabeads (Invitrogen) were prepared and coupled with 10 μ g of antibody (Ana2, DSas-6 [Peel et al., 2007], and random rabbit IgG) using the cross-linker BS3 (Thermo Fisher Scientific) according to the manufacturer’s instructions. 150 μ l of extract was added to 30- μ l beads and incubated

overnight at 4°C. The beads were washed five times with IP buffer and then resuspended in 60 μ l of loading buffer. 15 μ l was then loaded on a 4–12% gradient precast NuPAGE (Invitrogen) acrylamide gel and analyzed by Western blotting.

Electrophoresis and immunoblotting

WT, UASp-GFP-Ana1, and UASp-Ana2-GFP methanol-fixed eggs were rehydrated, and 30 eggs were selected. Eggs were then boiled in SDS sample buffer. The proteins were separated in a 4–12% gradient precast NuPAGE (Invitrogen) acrylamide gel and transferred to a Hybond-P membrane (GE Healthcare). After transfer, the membrane was blocked in ECL advance blocking solution (PBST [PBS + 0.1% Tween-20] and 2% blocking agent [GE Healthcare]) before incubation with primary antibody overnight at 4°C (diluted to 5 ng/ml for Ana1 and 1 ng/ml for Ana2 in blocking solution). The membrane was then washed in PBST and incubated with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare) diluted 1:1,500,000 in PBST for 1 h at room temperature. Finally, the membrane was washed in PBST, incubated with ECL advance chemiluminescent substrate (GE healthcare) according to manufacturer’s instructions, and exposed to x-ray film.

Identification of Ana2 homologues and sequence alignments

The position-specific iterated BLAST (PSI-BLAST) algorithm (Altschul et al., 1997) from the National Center for Biotechnology Information was used to search for homologues of Ana2. Multiple sequence alignments were performed using ClustalW2 (Larkin et al., 2007) and visualized in Jalview (Waterhouse et al., 2009) using the Blosum62 coloring scheme.

Online supplemental material

Fig. S1 is a Western blot showing that both UAS-GFP-Ana1 and UAS-Ana2-GFP are overexpressed at very high levels in unfertilized eggs. Fig. S2 shows the raw data from our Y2H analysis of Ana2 and DSas-6. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.200910016/DC1>.

We thank Gill Howard and Jenny Richens for help with antibody purification and Y2H, respectively, and the Raff laboratory for comments on the manuscript.

This work was funded by Wellcome Trust PhD studentships (to N.R. Stevens and A. Franz), European Molecular Biology Organization and Human Frontier Science Program fellowships (to J. Dobbelaere), a Cancer Research UK Programme Grant (C5395/A10530 to J. Dobbelaere and J.V. Raff), and a Cancer Research UK Project Grant (C5395/A8819 to K. Brunk).

Submitted: 2 October 2009

Accepted: 3 January 2010

References

- Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402. doi:10.1093/nar/25.17.3389
- Badano, J.L., T.M. Teslovich, and N. Katsanis. 2005. The centrosome in human genetic disease. *Nat. Rev. Genet.* 6:194–205. doi:10.1038/nrg1557
- Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C.G. Woods, A. Khodjakov, and J.W. Raff. 2006. Flies without centrioles. *Cell.* 125:1375–1386. doi:10.1016/j.cell.2006.05.025
- Basto, R., K. Brunk, T. Vinadogrova, N. Peel, A. Franz, A. Khodjakov, and J.W. Raff. 2008. Centrosome amplification can initiate tumorigenesis in flies. *Cell.* 133:1032–1042. doi:10.1016/j.cell.2008.05.039
- Bettencourt-Dias, M., A. Rodrigues-Martins, L. Carpenter, M. Riparbelli, L. Lehmann, M.K. Gatt, N. Carmo, F. Balloux, G. Callaini, and D.M. Glover. 2005. SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* 15:2199–2207. doi:10.1016/j.cub.2005.11.042
- Blachon, S., J. Gopalakrishnan, Y. Omori, A. Polyanovsky, A. Church, D. Nicastro, J. Malicki, and T. Avidor-Reiss. 2008. *Drosophila* asterless and vertebrate Cep152 Are orthologs essential for centriole duplication. *Genetics.* 180:2081–2094. doi:10.1534/genetics.108.095141
- Blachon, S., X. Cai, K.A. Roberts, K. Yang, A. Polyanovsky, A. Church, and T. Avidor-Reiss. 2009. A proximal centriole-like structure is present in *Drosophila* spermatids and can serve as a model to study centriole duplication. *Genetics.* 182:133–144. doi:10.1534/genetics.109.101709
- Bonaccorsi, S., M.G. Giansanti, and M. Gatti. 1998. Spindle self-organization and cytokinesis during male meiosis in asterless mutants of *Drosophila melanogaster*. *J. Cell Biol.* 142:751–761. doi:10.1083/jcb.142.3.751
- Bond, J., E. Roberts, G.H. Mochida, D.J. Hampshire, S. Scott, J.M. Askham, K. Springell, M. Mahadevan, Y.J. Crow, A.F. Markham, et al. 2002. ASPM is

- a major determinant of cerebral cortical size. *Nat. Genet.* 32:316–320. doi:10.1038/ng995
- Bond, J., E. Roberts, K. Springell, S.B. Lizarraga, S. Lizarraga, S. Scott, J. Higgins, D.J. Hampshire, E.E. Morrison, G.F. Leal, et al. 2005. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nat. Genet.* 37:353–355. doi:10.1038/ng1539
- Boxem, M., Z. Maliga, N. Klitgord, N. Li, I. Lemmens, M. Mana, L. de Lichtervelde, J.D. Mul, D. van de Peut, M. Devos, et al. 2008. A protein domain-based interactome network for *C. elegans* early embryogenesis. *Cell.* 134:534–545. doi:10.1016/j.cell.2008.07.009
- Callaini, G., and M.G. Riparbelli. 1990. Centriole and centrosome cycle in the early *Drosophila* embryo. *J. Cell Sci.* 97:539–543.
- Callaini, G., W.G. Whitfield, and M.G. Riparbelli. 1997. Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*. *Exp. Cell Res.* 234:183–190. doi:10.1006/excr.1997.3618
- Chang, P., T.H. Giddings Jr., M. Winey, and T. Stearns. 2003. Epsilon-tubulin is required for centriole duplication and microtubule organization. *Nat. Cell Biol.* 5:71–76. doi:10.1038/ncb900
- Dammermann, A., T. Müller-Reichert, L. Pelletier, B. Habermann, A. Desai, and K. Oegema. 2004. Centriole assembly requires both centriolar and pericentriolar material proteins. *Dev. Cell.* 7:815–829. doi:10.1016/j.devcel.2004.10.015
- Delattre, M., S. Leidel, K. Wani, K. Baumer, J. Bamat, H. Schnabel, R. Feichtinger, R. Schnabel, and P. Gönczy. 2004. Centriolar SAS-5 is required for centrosome duplication in *C. elegans*. *Nat. Cell Biol.* 6:656–664. doi:10.1038/ncb1146
- Delattre, M., C. Canard, and P. Gönczy. 2006. Sequential protein recruitment in *C. elegans* centriole formation. *Curr. Biol.* 16:1844–1849. doi:10.1016/j.cub.2006.07.059
- Dix, C.I., and J.W. Raff. 2007. *Drosophila* Spd-2 recruits PCM to the sperm centriole, but is dispensable for centriole duplication. *Curr. Biol.* 17:1759–1764. doi:10.1016/j.cub.2007.08.065
- Dobbelaere, J., F. Josué, S. Suijkerbuijk, B. Baum, N. Tapon, and J. Raff. 2008. A genome-wide RNAi screen to dissect centriole duplication and centrosome maturation in *Drosophila*. *PLoS Biol.* 6:e224. doi:10.1371/journal.pbio.0060224
- Doxsey, S., D. McCollum, and W. Theurkauf. 2005. Centrosomes in cellular regulation. *Annu. Rev. Cell Dev. Biol.* 21:411–434. doi:10.1146/annurev.cellbio.21.122303.120418
- Gerdes, J.M., E.E. Davis, and N. Katsanis. 2009. The vertebrate primary cilium in development, homeostasis, and disease. *Cell.* 137:32–45. doi:10.1016/j.cell.2009.03.023
- Giansanti, M.G., E. Bucciarelli, S. Bonaccorsi, and M. Gatti. 2008. *Drosophila* SPD-2 is an essential centriole component required for PCM recruitment and astral-microtubule nucleation. *Curr. Biol.* 18:303–309. doi:10.1016/j.cub.2008.01.058
- Goshima, G., R. Wollman, S.S. Goodwin, N. Zhang, J.M. Scholey, R.D. Vale, and N. Stuurman. 2007. Genes required for mitotic spindle assembly in *Drosophila* S2 cells. *Science.* 316:417–421. doi:10.1126/science.1141314
- Graser, S., Y.D. Stierhof, S.B. Lavoie, O.S. Gassner, S. Lamla, M. Le Clech, and E.A. Nigg. 2007. Cep164, a novel centriole appendage protein required for primary cilium formation. *J. Cell Biol.* 179:321–330. doi:10.1083/jcb.200707181
- Gromley, A., A. Jurczyk, J. Sillibourne, E. Halilovic, M. Mogensen, I. Groisman, M. Blomberg, and S. Doxsey. 2003. A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. *J. Cell Biol.* 161:535–545. doi:10.1083/jcb.200301105
- Habedanck, R., Y.D. Stierhof, C.J. Wilkinson, and E.A. Nigg. 2005. The Polo kinase Plk4 functions in centriole duplication. *Nat. Cell Biol.* 7:1140–1146. doi:10.1038/ncb1320
- Izraeli, S., L.A. Lowe, V.L. Bertness, D.J. Good, D.W. Dorward, I.R. Kirsch, and M.R. Kuehn. 1999. The SIL gene is required for mouse embryonic axial development and left-right specification. *Nature.* 399:691–694. doi:10.1038/21429
- Jackson, A.P., H. Eastwood, S.M. Bell, J. Adu, C. Toomes, I.M. Carr, E. Roberts, D.J. Hampshire, Y.J. Crow, A.J. Mighell, et al. 2002. Identification of microcephalin, a protein implicated in determining the size of the human brain. *Am. J. Hum. Genet.* 71:136–142. doi:10.1086/341283
- Kemp, C.A., K.R. Kopish, P. Zipperlen, J. Ahringer, and K.F. O'Connell. 2004. Centrosome maturation and duplication in *C. elegans* require the coiled-coil protein SPD-2. *Dev. Cell.* 6:511–523. doi:10.1016/S1534-5807(04)00066-8
- Kirkham, M., T. Müller-Reichert, K. Oegema, S. Grill, and A.A. Hyman. 2003. SAS-4 is a *C. elegans* centriolar protein that controls centrosome size. *Cell.* 112:575–587. doi:10.1016/S0092-8674(03)00117-X
- Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y.D. Stierhof, and E.A. Nigg. 2007. Plk4-induced centriole biogenesis in human cells. *Dev. Cell.* 13:190–202. doi:10.1016/j.devcel.2007.07.002
- Kohlmaier, G., J. Loncarek, X. Meng, B.F. McEwen, M.M. Mogensen, A. Spektor, B.D. Dynlacht, A. Khodjakov, and P. Gönczy. 2009. Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. *Curr. Biol.* 19:1012–1018. doi:10.1016/j.cub.2009.05.018
- Kumar, A., S.C. Girimaji, M.R. Duvvari, and S.H. Blanton. 2009. Mutations in STIL, encoding a pericentriolar and centrosomal protein, cause primary microcephaly. *Am. J. Hum. Genet.* 84:286–290. doi:10.1016/j.ajhg.2009.01.017
- Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics.* 23:2947–2948. doi:10.1093/bioinformatics/btm404
- Lee, H.S., J.A. Simon, and J.T. Lis. 1988. Structure and expression of ubiquitin genes of *Drosophila melanogaster*. *Mol. Cell. Biol.* 8:4727–4735.
- Leidel, S., and P. Gönczy. 2003. SAS-4 is essential for centrosome duplication in *C. elegans* and is recruited to daughter centrioles once per cell cycle. *Dev. Cell.* 4:431–439. doi:10.1016/S1534-5807(03)00062-5
- Leidel, S., M. Delattre, L. Cerutti, K. Baumer, and P. Gönczy. 2005. SAS-6 defines a protein family required for centrosome duplication in *C. elegans* and in human cells. *Nat. Cell Biol.* 7:115–125. doi:10.1038/ncb1220
- Loncarek, J., P. Hergert, V. Magidson, and A. Khodjakov. 2008. Control of daughter centriole formation by the pericentriolar material. *Nat. Cell Biol.* 10:322–328. doi:10.1038/ncb1694
- Martinez-Campos, M., R. Basto, J. Baker, M. Kernan, and J.W. Raff. 2004. The *Drosophila* pericentriolar-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis. *J. Cell Biol.* 165:673–683. doi:10.1083/jcb.200402130
- Mottier-Pavie, V., and T.L. Megraw. 2009. *Drosophila* bld10 is a centriolar protein that regulates centriole, basal body, and motile cilium assembly. *Mol. Biol. Cell.* 20:2605–2614. doi:10.1091/mbc.E08-11-1115
- Nigg, E.A. 2002. Centrosome aberrations: cause or consequence of cancer progression? *Nat. Rev. Cancer.* 2:815–825. doi:10.1038/nrc924
- O'Connell, K.F., C. Caron, K.R. Kopish, D.D. Hurd, K.J. Kempfues, Y. Li, and J.G. White. 2001. The *C. elegans* zyg-1 gene encodes a regulator of centrosome duplication with distinct maternal and paternal roles in the embryo. *Cell.* 105:547–558. doi:10.1016/S0092-8674(01)00338-5
- Peel, N., N.R. Stevens, R. Basto, and J.W. Raff. 2007. Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation. *Curr. Biol.* 17:834–843. doi:10.1016/j.cub.2007.04.036
- Pelletier, L., N. Ozlü, E. Hannak, C. Cowan, B. Habermann, M. Ruer, T. Müller-Reichert, and A.A. Hyman. 2004. The *Caenorhabditis elegans* centrosomal protein SPD-2 is required for both pericentriolar material recruitment and centriole duplication. *Curr. Biol.* 14:863–873. doi:10.1016/j.cub.2004.04.012
- Pelletier, L., E. O'Toole, A. Schwager, A.A. Hyman, and T. Müller-Reichert. 2006. Centriole assembly in *Caenorhabditis elegans*. *Nature.* 444:619–623. doi:10.1038/nature05318
- Pfaff, K.L., C.T. Straub, K. Chiang, D.M. Bear, Y. Zhou, and L.I. Zon. 2007. The zebra fish cassiopeia mutant reveals that SIL is required for mitotic spindle organization. *Mol. Cell. Biol.* 27:5887–5897. doi:10.1128/MCB.00175-07
- Piel, M., P. Meyer, A. Khodjakov, C.L. Rieder, and M. Bornens. 2000. The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. *J. Cell Biol.* 149:317–330. doi:10.1083/jcb.149.2.317
- Rebollo, E., P. Sampaio, J. Januschke, S. Llamazares, H. Varmark, and C. González. 2007. Functionally unequal centrosomes drive spindle orientation in asymmetrically dividing *Drosophila* neural stem cells. *Dev. Cell.* 12:467–474. doi:10.1016/j.devcel.2007.01.021
- Rieder, C.L., S. Faruki, and A. Khodjakov. 2001. The centrosome in vertebrates: more than a microtubule-organizing center. *Trends Cell Biol.* 11:413–419. doi:10.1016/S0962-8924(01)02085-2
- Rodrigues-Martins, A., M. Bettencourt-Dias, M. Riparbelli, C. Ferreira, I. Ferreira, G. Callaini, and D.M. Glover. 2007a. DSAS-6 organizes a tube-like centriole precursor, and its absence suggests modularity in centriole assembly. *Curr. Biol.* 17:1465–1472. doi:10.1016/j.cub.2007.07.034
- Rodrigues-Martins, A., M. Riparbelli, G. Callaini, D.M. Glover, and M. Bettencourt-Dias. 2007b. Revisiting the role of the mother centriole in centriole biogenesis. *Science.* 316:1046–1050. doi:10.1126/science.1142950
- Rusan, N.M., and M. Peifer. 2007. A role for a novel centrosome cycle in asymmetric cell division. *J. Cell Biol.* 177:13–20. doi:10.1083/jcb.200612140
- Sharma, N., N.F. Berbari, and B.K. Yoder. 2008. Ciliary dysfunction in developmental abnormalities and diseases. *Curr. Top. Dev. Biol.* 85:371–427. doi:10.1016/S0070-2153(08)00813-2

- Stevens, N.R., A.A. Raposo, R. Basto, D. St Johnston, and J.W. Raff. 2007. From stem cell to embryo without centrioles. *Curr. Biol.* 17:1498–1503. doi:10.1016/j.cub.2007.07.060
- Stevens, N.R., J. Dobbelaere, A. Wainman, F. Gergely, and J.W. Raff. 2009. Ana3 is a conserved protein required for the structural integrity of centrioles and basal bodies. *J. Cell Biol.* 187:355–363. doi:10.1083/jcb.200905031
- Strnad, P., S. Leidel, T. Vinogradova, U. Euteneuer, A. Khodjakov, and P. Gönczy. 2007. Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. *Dev. Cell.* 13:203–213. doi:10.1016/j.devcel.2007.07.004
- Varmark, H., S. Llamazares, E. Rebollo, B. Lange, J. Reina, H. Schwarz, and C. Gonzalez. 2007. Asterless is a centriolar protein required for centrosome function and embryo development in *Drosophila*. *Curr. Biol.* 17:1735–1745. doi:10.1016/j.cub.2007.09.031
- Vidwans, S.J., M.L. Wong, and P.H. O'Farrell. 2003. Anomalous centriole configurations are detected in *Drosophila* wing disc cells upon Cdk1 inactivation. *J. Cell Sci.* 116:137–143. doi:10.1242/jcs.00204
- Vorobjev, I.A., and Y.S. Chentsov. 1982. Centrioles in the cell cycle. I. Epithelial cells. *J. Cell Biol.* 93:938–949. doi:10.1083/jcb.93.3.938
- Waterhouse, A.M., J.B. Procter, D.M. Martin, M. Clamp, and G.J. Barton. 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics.* 25:1189–1191. doi:10.1093/bioinformatics/btp033
- Yamashita, Y.M., A.P. Mahowald, J.R. Perlin, and M.T. Fuller. 2007. Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science.* 315:518–521. doi:10.1126/science.1134910
- Zhong, X., L. Liu, A. Zhao, G.P. Pfeifer, and X. Xu. 2005. The abnormal spindle-like, microcephaly-associated (ASPM) gene encodes a centrosomal protein. *Cell Cycle.* 4:1227–1229.
- Zhong, X., G.P. Pfeifer, and X. Xu. 2006. Microcephalin encodes a centrosomal protein. *Cell Cycle.* 5:457–458.