Lessons from yeast: the spindle pole body and the centrosome

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The yeast spindle pole body (SPB) is the functional equivalent of the centrosome. Most SPB components have been identified and their functions partly established. This involved a large variety of techniques which are described here, and the potential use of some of these in the centrosome field is highlighted. In particular, very useful structural information on the SPB was obtained from a reconstituted complex, the \( \gamma \)-tubulin complex, and also from a sub-particle, SPB cores, prepared by extraction of an enriched SPB preparation. The labelling of SPB proteins with GFP at the N or C termini, using GFP tags inserted into the genome, gave informative electron microscopy localization and fluorescence resonance energy transfer data. Examples are given of more precise functional data obtained by removing domains from one SPB protein, Spc110p, without affecting its essential function. Finally, a structural model for SPB duplication is described and the differences between SPB and centrosome duplication discussed.

1. Introduction

The functional equivalent of the centrosome in budding yeast is called the spindle pole body (SPB). It lies embedded in the nuclear membrane, which remains intact during mitosis. The SPB is a cylindrical multi-layered organelle, with the two most visible layers being the outer and central plaques (figure 1a). Attached to one side of the SPB is a specialized part of the nuclear membrane called the half-bridge. Here, the lipid bilayers are darkly stained on thin sections, and a thin cytoplasmic half-bridge outer layer lies just above the outer nuclear membrane. This part of the SPB has an important role in SPB duplication.

Almost all of the components of the SPB have been identified and their locations are shown in figure 1b. This review will describe the approaches to identifying SPB components, excluding transiently associated components such as those involved in mitotic exit and checkpoints. It will describe how the functions of some of these SPB components were established, and what lessons can be learnt that be helpful in establishing the functions of centrosome components.

2. Enrichment and extraction to prepare sub-particles

The first SPB components were identified from a monoclonal antibody screen of a 600-fold enriched preparation [1]. The monoclonals were later used to screen expression banks to identify the corresponding genes [2,3]. Subsequently, further SPB genes were identified by mass spectrometry screening of a more enriched SPB preparation [4]. This approach of enrichment [5,6] followed by mass spectrometry [7,8] has also been successful with the centrosome. There are additional benefits of enrichment. First, the enriched preparation can be very useful in characterizing antibodies against low abundance components of both SPBs [1] and centrosomes [9,10], which were not detectable in western blots of whole cell lysates. Second, enriched preparations are very useful in high-resolution cryo-electron microscopy (cryoEM) structural work [11–14], where a high density of SPBs or centrosomes is required. Lastly, the enriched preparation can be extracted to prepare sub-particles: for instance, treatment of the enriched SPBs with heparin [1] gave sub-particles, SPB cores, morphologically similar to central plaques, which were later made essentially pure, and contained Spc110p, Cnm67p, Spc42p, Spc29p, calmodulin (Cmd1p) and...
homologue [29]. A further suppressor analysis using spc98-2 identified SPC97 [30]. These three proteins constitute the yeast γ-tubulin complex (see §4).

Analysis of ts phenotypes could also be helpful if the phenotype was clear; thus mps2-1 showed a very clear defect in the insertion of the SPB into the nuclear membrane [25]. In this work and subsequently it was essential to examine the cells by serial sectioning to obtain a clear view of the phenotype.

Another very useful genetic approach in yeast was the tagging of most of the genes with GFP [31]. Although this screen did not identify any new SPB components, it did give reassurance that probably all the components had been identified, except possibly for very low abundance SPB proteins encoded by the 25% of the genome where the tagging procedure was unsuccessful.

In the case of the centrosome, an RNAi screen for mitotic phenotypes in Caenorhabditis elegans was very productive in identifying several genes involved in the initial stages of centriole assembly [32]. In mammalian cells, the increasing ease with which genomes can be edited [33–36] suggests that both a deletion analysis and tagging with GFP are possible and would be useful in defining centrosomal components.

4. Isolation of complexes

A very productive way of identifying and establishing the function of SPB components was to tag them and use the tag in a pulldown to identify binding partners. This isolation can be the basis for a reconstitution giving larger quantities and allowing a structural and in vitro functional approach. All of this is illustrated most clearly in the case of the yeast γ-tubulin complex. This is composed of three proteins: Tub4p, the γ-tubulin homologue in Saccharomyces cerevisiae [29,37,38], together with Spc98p and Spc97p [28,30]. The γ-tubulin complex was purified in a pulldown after attaching protein A to either Spc98p or Spc97 [39]. This complex was later reconstituted in vitro [40], and cryoEM reconstruction showed that the complex had 13-fold symmetry and probably acted as a template for microtubule initiation [41].

Some centrosome complexes have been isolated, such as the γ-tubulin ring complex [42] and the CP110 complex [43], although at present for the latter only the identity of the other components has been reported.

5. Two-hybrid analysis and fluorescence resonance energy transfer

Two-hybrid analysis was very useful in establishing the pattern of interactions between the domains of different proteins, for example the N and C terminal domains of the coiled-coil proteins within the SPB core [15,44]. Only one SPB component was found from a two-hybrid genomic screen, Spc72p [45]; this protein was also found from a mass spectrometry screen [4].

Fluorescence resonance energy transfer (FRET) has also been used to examine the interactions between the N and C termini of core SPB components [46] after labelling with CFP or YFP. This method has the additional advantage that it is possible to estimate the distance between the domains.
6. Location of components

From the earliest days in the characterization of SPB components, a considerable effort was put into their localization within the organelle by immunoEM [1,22]. Accurate localization has always been very helpful in establishing the function of the SPB components; for instance, in the case of Spc110p, suggesting particular experiments to show that it acted as a spacer protein (see §7). One problem with immunoEM is often antibody accessibility, but this can be helped by the use of the small secondary probe Fab-Nanogold followed by silver intensification [47]. However, in some instances, for example the localization of Cnm67p, no signal was obtained with an anti-Cnm67p, presumably because the antigen is buried deep within the SPB. This problem was overcome by labelling Cnm67p with GFP at the C terminus [15], and this fusion was shown to be functional, as were all the SPB proteins tested (except Cdc31p and Tub4p), since they complemented the deletions. The success with using GFP-labelled protein in immunoEM is probably for two reasons: first, the compact GFP is probably on the exterior and so more accessible in a densely packed structure; and second, the anti-GFP antibodies always have a very high titre [48] which facilitates the immunoEM. An additional advantage of GFP labelling is that the GFP can be placed on either the N or the C terminus; thus, in the case of the filamentous centrin-binding protein Sfi1p, it was possible to determine the distinct locations of both the N and C termini [49,50]. Placement of GFP at the C terminus was not always successful, thus Spc29p-GFP was functional but gave no immunoEM signal; however, when GFP was at the N terminus, staining was seen both at the SPB and the satellite [15].

Given the increasing ease with which homologous recombination can be applied to the genomes of vertebrate and mammalian cells [33–36], it should be possible to tag genes with GFP or other direct labels [51,52]. CryoEM of centrosomes isolated from cell lines with directly labelled centrosomal components should give high-resolution localization of these components. These approaches will complement the new super-resolution light microscopy techniques [53–57], as they can be carried out on unfixed material.

7. Structural role of components

SPBs and centrosomes are very large organelles whose construction relies on many coiled-coil proteins, and since these are very often filamentous, they could be the equivalent of connectors in the construction of these organelles. A useful way of clearly establishing this is to change the length of the coiled coil, which may lead to a predictable alteration in the morphology of the organelle. If this change has little effect on the function, a clearer conclusion can be drawn. This approach is in contrast to the normal one of removal or disabling of the protein, which can lead to a local or complete collapse of the organelle structure. This shows the important structural role of the protein but does not lead to a detailed definition of its role.

There was an early illustration of this approach for the SPB in the case of Spc110p [2], a protein with probable globular N- and C-terminal domains and a long central coiled coil. ImmunoEM with monoclonal antibodies, most of which recognized epitopes in the coiled coil [2], located this part of the protein to the region between the central plaque and the inner plaque [1] where the spindle microtubules are initiated, suggesting the coiled coil might bridge this gap. Partial deletion of the coiled coil decreased the gap, while an almost complete deletion removed the gap. So the spindle microtubules appear to grow directly from the central plaque (figure 2), suggesting that the coiled coil of Spc110p was responsible for the spacing of this gap.

Another illustration of this approach was in establishing the essential function of calmodulin in yeast mitosis [59]. Calmodulin has several essential functions in yeast [60] including one in mitosis which was defined by particular ts alleles including cmd1-1 [61]. Because of the role of calmodulin in calcium signalling, there was considerable interest in its role in mitosis. Mutations in the essential calcium-binding amino acids reduced the calcium binding to negligibly low amounts yet this calmodulin was still functional [62], eliminating any role for calcium. Calmodulin’s exact role in mitosis was investigated further by identifying a binding partner, Spc110p, and further identifying the binding site of Spc110p which was close to the C terminus [59,63]. Suppressors of cmd1-1 included truncated versions of Spc110p, which removed the calmodulin-binding site and were functional since they fully complemented the ts mutation [59]. This suggested that the essential function of calmodulin in mitosis is to stabilize an α-helix in the calmodulin-binding site of Spc110p, and that removal of this unstable helix produces a functional protein which no longer requires calmodulin binding.

Both of these experiments illustrate that clear conclusions can be obtained if domains are removed from a protein without affecting its main function.

8. Conservation

Although the SPB and centrosome are functionally equivalent, most of the internal components, in particular some of those in the SPB core, are highly divergent. Thus, Spc42p and Spc29p homologues can only be found in the Saccharomyces clade [64] and have not been identified in other fungi with SPBs. SPB components which have a more direct involvement with microtubules are much more conserved.

9. Spindle pole body duplication

One of the most interesting problems in the case of both the SPB and the centriole is their duplication at the start of the cell cycle to produce a single copy. Centriole duplication...
is currently a very active area. Its control by the kinase Plk4 seems clear [65], although what is happening structurally is less clear, particularly in what exactly connects the procen- 

trioloe at a set distance to the mother. SPB duplication has 

been examined by EM and immunoEM. The process, 

shown schematically in figure 3, starts with a doubling in 

length of the half-bridge and the formation of a small struc-

ture called the satellite at the distal cytoplasmic end of the 

bridge [66]. ImmunoEM showed the satellite contains SPB 

core components [15] which then assemble into a larger struc-

ture called the duplication plaque which contains the same 

SPB core components. In some unknown way, this plaque 

is inserted into the nuclear membrane and the rest of the 

SPB then assembles. The transition from satellite to dupli-

cation plaque and insertion and spindle assembly is very 

fast, and rare examples of these stages could only be found 

from observations at the right time point in synchronized 

cells [15]. Based on these results, a structural model was 

proposed for SPB duplication [15] and later modified after the 

centrin-binding protein Sfi1p was characterized [50]. Yeast 

centrin (Cdc31p) has an essential function in SPB duplication 

[21] and localizes to the half-bridge [22]. Sfi1p was found 

from a centrin pulldown in yeast [49]; it, like centrin, localizes 

to the half-bridge and has an essential function during SPB 

duplication [49]. Sfi1p has about 20 continuous centrin-

binding repeats which form a long \( \alpha \)-helical filament [50], 

long enough to span the half-bridge where the centrin is 

localized [22]. This was confirmed by immunoEM using 

GFP placed at either the N or C terminus [49,50], which 

showed the N terminus of Sfi1p close to the SPB while the 

C terminus was at the distal end of the half-bridge. This 

suggested that the doubling in length of the half-bridge 

during SPB duplication was due to an end-to-end dimeriza-

tion of Sfi1p at its C terminus (figure 3) and led to a 

structural model for SPB duplication [50]. This model pro-

poses that since the N terminus of Sfi1p is close to the SPB, 

it can bind directly or indirectly to the core SPB components. 

The end-to-end dimerization of Sfi1p during the initial stages 

during SPB duplication, when the half-bridge doubles in length, 

would then give a fresh N terminus of Sfi1p at the distal 

d end of the bridge to initiate the assembly of the daughter 

SPB. Provided the critical concentration for the assembly of 
core SPB components is high, then they would only assemble 
at the mother SPB or at the new Sfi1p N terminus, and in this 

way only a single copy of the daughter SPB would be pro-
duced. Once the daughter SPB is assembled the bridge is 
split so each SPB now has a half-bridge and a spindle is 

now formed. It is interesting that while ts mutants in the cen-

trin-binding domains of Sfi1p described above show a block 
in SPB duplication, ts mutants in the C-terminal domain 
arrest with paired SPBs [67,68], suggesting that the fission 
of the bridge is blocked. After cell division, the single SPBs 

with half-bridges can then go through another cycle of 
elongation and daughter SPB assembly.

Figure 3. Role of Sfi1p in SPB duplication.
Sfi1p is conserved and when expressed localized to the centrosome in HeLa cells [49], but it does not seem likely that vertebrate Sfi1p is involved in centriole duplication because it is possible to delete all the centrin genes in chicken DT40 cells without affecting duplication [69]. Also there are clear structural differences in the nature of the connection between mother and daughter in SPBs and centrioles. In SPBs the connection is like-to-like, that is from central plaque to central plaque via the bridge. In centrioles the connection is different, from the proximal side of the mother to the proximal end of the daughter. However, despite these differences, there is a fundamental similarity in which a single point of assembly from a single organelle, the mother, ensures a single copy of the daughter.

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