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Review article

A symbiogenetic basis for the centrosome?

Michael Chapman[1] and Mark C. Alliegro[2,3*]

[1]Department of Biology, Holy Cross College, Worcester, MA 01610, USA;
[2]Department of Cell Biology, LSU Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112, USA,
Tel. +1-504-568-7618, Fax. +1-504-568-2759, Email. mallie@lsuhsc.edu;
[3]Marine Biological Laboratory, Woods Hole, MA 02543, USA

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Abstract

The modern eukaryotic cell is derived from its ancestors both by direct filiation (accumulation of random mutations) and by symbiogenesis. In symbiogenesis, one cell, internalized by another, persists in the cytoplasm as an obligate relationship develops. Through mutation, lateral gene transfer, and selective processes the fused individuals become interdependent. The partners tend to relinquish portions of their genomes, thus minimizing redundancy, such that once free-living microbes cement their status as organelles. The first step in this process is internalization of the smaller symbiotic partner by the larger. Since internal cell motility systems are required for endocytosis, internal cell motility is likely one of the earliest features of the eukaryotic branch. But by what pathway did this motility system arise? Some postulate that the cytoskeleton evolved by direct filiation, as a specialization from within. Others hold that the cell motility system, or at least parts of it, were derived exogenously, by symbiogenesis. Especially because of its mode of duplication and cytoplasmic inheritance, the centriole and related structures have long been at the center of evolutionary debate. If any exogenous viral or bacterial model for the origin of the centriole is true, then one would predict at least remnants of a genome to be associated with the organelle. Current hypotheses for the evolutionary origin of the centriole, the centrosome, and associated nucleic acids, are discussed.

Keywords: Centriole, kinetosome, undulopodium, symbiogenesis, centrosomal RNA

1. The Centrosome, the Centriole and the Kinetosome

The centrosome is the principal microtubule organizing center (MTOC) of animal cells and cells from other phyla, including some plants and many protists (Chapman et al., 2000). A number of detailed reviews are available on aspects of centrosome structure and activities (Fulton, 1971; Hartman, 1975; Palazzo et al., 2000; Hinchcliffe and Sluder, 2001; Doxsey, 2001; Doxsey et al., 2005; Tsou and Stearns, 2006). Only a brief summary is provided here.

The mammalian centrosome is typically, but not always, composed of a pair of [9(3)+0] centrioles surrounded by a pericentriolar matrix (PCM). The centrioles themselves consist of nine microtubule triplets, arranged to form a short cylinder (Fig. 1). Centrioles range in size from approximately 300–700 nm in length and 250 nm in diameter. When viewed in cross section, the centriole's microtubule triplets resemble the angled blades of a pinwheel. The nine-fold symmetry is conserved across taxa. The mother centriole serves as a progenitor for ciliary and flagellar[1] basal bodies (kinetosomes[2]). Although a number of centriolar proteins have been identified, centriole composition and the PCM remain poorly defined. The

*The author to whom correspondence should be sent.

[1]Although the term "flagellum" is often used for both the bacterial and eukaryotic organelle, it is important to note the lack of similarity between the two. The bacterial flagellum is composed of few, if not a single protein, whereas the eukaryotic flagellum is composed of hundreds. The structure and mechanisms of action are, therefore, vastly different.

[2]"Basal body" and "kinetosome" are two names for the same structure. The latter is most often used in reference to ciliates.

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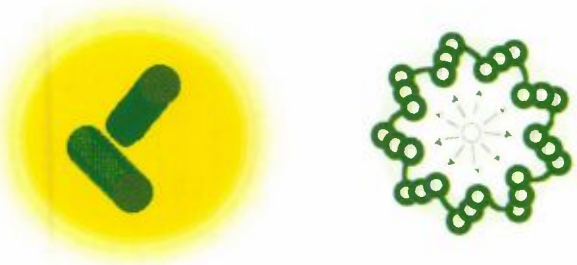

Figure 1. The animal centrosome, shown on the left, is composed of paired centrioles surrounded by a pericentriolar matrix (PCM). The centrioles are barrel-shaped structures lying at right angles to each other, and are connected by linking proteins during most of the cell cycle. The two centrioles separate slightly and replicate during S-phase, daughter centrioles growing perpendicularly from the vicinity of each existing (mother) centriole's wall. During M-phase the centrosome divides into two distinct centrosomes that migrate to opposite poles of the cell in preparation for spindle assembly. Each migrating centrosome carries with it one mother and one daughter centriole, plus a portion of the PCM. A cross section through a centriole is shown on the right. The wall is composed of nine microtubule triplets. The innermost, designated the "A" tubule, is a complete ring. The more lateral "B" and "C" microtubules have an incomplete wall. Nine radial spokes emanate from the center of the structure. Protein-containing structures (most of which are not depicted in this diagram) connect the spokes and the microtubule triplets and link the triplets themselves.

advent of methods to isolate centrosomes and related structures from several species has led to a recent growth in information (Mitchison and Kirschner, 1984; Bornens et al., 1987; Moritz et al., 1995; Rout and Kilmartin, 1990; Vogel et al., 1997).

The centrosome is positioned adjacent to the nuclear envelope in interphase. It divides once per cell cycle in animal cells, at the onset of mitosis. The daughter centrosomes then migrate to opposite ends of the cell to organize the spindle poles. Centrosome duplication is tightly regulated and under the control of both cytoplasmic and intrinsic factors (Sluder and Hinchcliffe, 1999; Wong and Stearns, 2003). This is necessary to ensure that a normal bipolar spindle is formed during mitosis and the genome is divided equally between offspring cells. Boveri proposed 90 years ago that misregulation of centrosome numbers leads to genomic instability and cancerous tumors (Boveri, 1914; cited in Brinkley and Goepfert, 1998). This hypothesis has gained considerable attention recently (Doxsey et al., 2005; Emdad et al., 2005).

The first observable event in centrosome duplication is centriole replication, which typically occurs during S-phase of the mammalian cell cycle. Like mitochondria and chloroplasts, centriole replication is independent of the nucleus, but unlike these membrane-bounded organelles, centrioles replicate in a semiconservative, generative (not fissive) manner. Its mode of replication has led some investigators to propose that the centrosome (or perhaps more specifically, the centriole) is of endosymbiotic origin (reviewed in Chapman et al., 2000). In this article, we review the general theory of symbiogenesis, then apply its principles to the centrosome and microtubule-based cytoskeleton. A corrollary to the hypothesis that the centrosome arose as an endosymbiont is that, like the mitochondrion and chloroplast, the organelle should possess at least the remnant of a genome. The status of centriole/kinetosome-associated nucleic acids was last comprehensively reviewed seven years ago (Marshall and Rosenbaum, 2000). Recent results now mandate a new review.

2. Evolution by Symbiogenesis: General Concepts

Symbiosis is defined as the living together of unrelated organisms which share a cooperative niche. As such it differs from competitive, classical Darwinian natural selection, which acts on variation within a single species such that genetically superior types proliferate over other types within a niche. Although the term (*symbiotismus*) was coined by Albert Bernhard Frank in 1877, mention of the phenomenon itself is far more ancient: Herodotus and others saw mutualism as evidence of the balance of nature. Among other examples, he described the relationship between Nile crocodiles and Egyptian plovers; the crocodile does not harm the plover, which lives virtually inside the crocodile's mouth, eating the leeches which attach themselves there (Egerton, 1977). Characteristics used for the study of symbioses include relative size and position of the partners, necessity of the association to the partners, duration, stability and mode of perpetuation of the symbiosis, specificity, recognition and mode of interaction between the partners, and degree of integration; i.e. the number of characteristics displayed by the partners in association, but not living separately (Smith and Douglas, 1987). Nutrient-poor environmental conditions appear to favor evolution of symbioses such that primary producers in nitrogen-deficient soils, oceanic littoral zones, and arctic/xeric ecosystems are overwhelmingly symbiotic in origin (e.g. mycorrhizae, scleractinian corals/*Symbiodinium microadriaticum*, and lichens).

Symbiogenesis is defined as new form or function as a direct consequence of symbiosis. Symbiogenetic associations also tend to be obligate rather than facultative. While the plover and crocodile (a case of symbiosis) each benefit from their association, neither partner is totally dependent on the other: the crocodile can live in plover-free habitats and vice versa. In lichens, by contrast, neither the fungal nor the algal partner could survive in extreme habitats typical of lichens (e.g. alpine, arctic or xeric climates; bare rock surfaces) without the other. The lichen

is a classic example of symbiogenesis, and forms the basis of an ecosystem in the arctic tundra. Similarly, corals have formed symbiogenetic associations with photosynthetic algae, and form the basis of ecosystems in reefs and atolls described by Darwin.

The evolutionary progression from symbiosis to symbiogenesis is characterized by increasing levels of integration of the partners and increasing obligacy of the association. To use the case of the plover and crocodile once again as a simple case of symbiosis, the partners need not even be physically associated beyond the mere fact of their living in proximity to one another. When one partner makes its home on the surface of the other, however, the first step towards symbiogenesis has occurred. Parasites living on or inside their hosts' bodies are rudimentary examples of symbiogenesis, since their evolutionary niche requires them to live at the expense of a partner. But when neither partner could occupy the niche without the other (as in the example of the lichen, or the nitrogen-fixing symbioses mentioned below), the association becomes obligate, and evolution through symbiogenesis has occurred.

The floating water fern, *Azolla*, forms cavities within its leaves which are colonized by hormogonia of the cyanobacterium, *Anabaena*. Although the cyanobacteria do not physically enter the plant cells, they change in morphology and function upon entering the leaf cavity: the cylindrical, motile hormogonia cells become ovoid and sessile, twining themselves like strings of pearls around specialized transfer cells of the fern. Approximately every tenth cell differentiates as an elongated, nitrogen-fixing heterocyst. Specialized membrane and wall morphology in the transfer cells allows exchange of photosynthate and fixed nitrogen. The association is of economic importance worldwide, used by farmers for the nitrification of rice paddies.

A step up in integration from the *Azolla/Anabaena* consortium is exemplified by the well-studied legume/ *Rhizobium* association. Free-living *Rhizobium* cells undertake biochemical crosstalk with legume roots; the root hairs curl into shepherd's crooks, and bacteria enter the plant, penetrating plant cell walls but shedding their own cell walls, and differentiating as wall-less, irregular, nitrogen-fixing bacteroids. Nitrogenase produced by the bacteroid is sensitive to oxygen levels, hence the partners cooperate to produce the oxygen-scavenging molecule, leghemoglobin: the heme moiety is produced by the *Rhizobium* bacteroid, while the globin protein is produced by the plant. Inasmuch as the legume-*Rhizobium* partnership involves an exchange of gene products, and occurs within the plant cell walls (albeit not yet intracellularly), the association is one step closer in evolutionary terms to the fully obligate status of mitochondria and chloroplasts in eukaryotic cells.

3. Symbiogenetic Models for the Centrosome

There are a number of models for the origin of the centrosome and related structures, essentially variations on two or three basic themes. Each has features consistent with the available data, but each faces the nearly insoluble problem of all evolutionary models – gaps in empirical knowledge that require filling with conjecture. Autogenous theories for the origin of the microtubule-based cytoskeleton hold that microtubules evolved first, in the cytoplasm, later followed by elaborations of the axoneme and spindle (Cavalier-Smith, 1975). Such theories propose no mechanism for proto-eukaryotic cell division in the absence of a mitotic spindle, and are further hampered by the absence of any reported instances of intermediate states in evolution of eukaryotic flagella; i.e. flagella with significantly fewer than the >360 proteins listed by Pazour et al. (2005) for the *Chlamydomonas* flagellar proteome. Microtubules in such intermediate flagella might undergo dynamic instability and need periodic reestablishment, might have weaker motility due to absence of radial spokes or motor proteins, or might exhibit simpler ultrastructure than the 9(2) +2 microtubule array. Well-studied mutants with these phenotypes are not found outside the laboratory. The absence of such intermediate states, taken together with the remarkably standardized ultrastructure of the eukaryotic flagellum across taxa, suggests that it evolved once and only once, concurrent with or soon after the evolution of the eukaryotic state itself (Dolan, 2005). Moreover, the microtubule-based cytoskeleton is highly complex, including hundreds of individual gene products and multiple functions and ultrastructural configurations. The most parsimonious explanation for acquisition of this system by a single event is genetic merger by symbiogenesis, not hundreds of independent mutations.

Evidence that the basal body, or kinetosome, is a heritable system like plastids and mitochondria, can be derived from analysis of the karyomastigont. The karyomastigont (Janicki, 1915) is a unitary system comprising the nucleus, one or more MTOCs, a nuclear connector (rhizoplast) and a parabasal body (Golgi apparatus). Inasmuch as these components are interdependent, i.e. the nucleus could not undergo mitosis without a spindle, and the microtubular cytoskeleton requires nuclear-encoded genes for its production and maintenance, one may infer that the karyomastigont constitutes an evolutionary seme. By definition, semes evolve from combinations of many genes; while a seme may be inactivated by a single mutation, it virtually never arises from activity of a single gene. Since the >360 various proteins which comprise the eukaryotic flagellum do not derive from any one gene family, a symbiogenetic rather than autogenous origin for the karyomastigont is a viable alternative (Margulis et al., 2006).

Karyomastigonts are found in most amitochondriate protists, e.g. *Giardia*, *Trichomonas*, parabasalids, hypermastigotes, as well as in green algae (*Chlamydomonas*), chytrids, foraminiferans, dinomastigotes, invertebrate and mammalian sperm cells (Melnitsky et al., 2005). In the primitive state, all components of the karyomastigont occur together, i.e. the Golgi apparatus or parabasal body is in physical proximity to the kinetosomes and nuclear connector. In amoebomastigotes such as *Naegleria*, the kinetosomes can migrate from their perimembranous location in the flagellated phase to apical positions in the amoeboid phase, and serve as MTOCs during production of the mitotic spindle. In derived lineages (e.g. *Chlamydomonas*), the Golgi has migrated elsewhere in the cell while other components of the karyomastigont still remain attached to one another. Amitochondriate protists exhibit a continuum of states, described by the concept of mastigont multiplicity: trichomonads, for example, have a single karyomastigont; *Coronympha octonaria* has an apical ring of eight karyomastigonts; *Calonympha* has both karyomastigonts and akaryomastigonts; and finally the giant hypermastigote *Snyderella* (cell size 200–300 μm) has only akaryomastigonts, with hundreds of free nuclei. Detachment of the nuclei from the karyomastigonts in such multinucleate cells was likely required for organized mitosis, cytokinesis, and binary cell division.

4. Hypothesis for a Bacterial Origin

Archaebacteria such as *Thermoplasma* utilize elemental sulfur as the terminal electron acceptor in their metabolism, producing hydrogen sulfide. As a result, they constitute living refugia from cyclical oxygen flux. In the Proterozoic eon, habitats rich in sulfur abounded and newly evolved photosynthesizers would have constituted a strong selective pressure on anaerobic and microaerophilic eubacteria like spirochetes to form symbiotic associations with sulfide producers such as *Thermoplasma*. The hydrogen sulfide is used by many spirochetes to detoxify their environment by forming sulfur granules inside their cells. Perfiliev (1969), and later Dubinina and colleagues (1993) have described just such sulfur-redox syntrophies in white flocculence found in hot, acidic habitats exemplified by Staraya Russa hot springs north of Moscow. The microaerophilic spirochetes (eubacteria) are drawn to oxygen-, heat- and acid-mediating *Thermoplasma* archaebacteria. In one model for acquisition of the centrosome and microtubule-based cytoskeleton, the heterotrophic *Thermoplasma* capitalizes on this relationship by acquiring motility from the spirochete, gaining access to new environments and new food sources. Membrane fusion between the partners, followed by assimilation and deployment of precursors to motility proteins, may have been the first evolutionary steps leading to the eukaryotic cytoskeleton. Motility symbioses involving spirochetes (*Thiodendron, Mixotricha* and other termite hindgut symbionts) are certainly provocative; however, to date no structures resembling centrioles have been reported in spirochetes, and reports of bacterial homologues to eukaryotic motility proteins have been few (Dolan, 2005).

5. Hypothesis for a Viral Origin

A second model for the exogenous origin of the centrosome is by viral invasion (P. Satir, personal communication, and manuscript in press). In this case, the viral capsid does not degenerate on entry to the cell, but is instead assimilated as the proto-basal body. The viral envelope persists to become a sensory bud, presaging the sensory functions of the modern flagellar membrane. Following assimilation of viral genes, the host cell machinery would elaborate a 9(2) + 0 axoneme from the capsid (as exists in nonmotile mammalian primary cilia), and finally the central pair would be added as an extravasation of the spindle. Alliegro et al. (2006) have reported centrosome-specific RNAs derived from surf clam (*Spisula*) oocytes. Several of these RNAs appear to encode reverse transcriptases, suggestive of retroviral origin. Moreover, centrioles arise from a fibrogranular center which resembles a viral replication factory in sperm cells of ferns and cycads. The analogy can be also extended to assembly and modification of some viral capsid proteins, which is reminiscent of microtubule polymerization.

Unresolved issues with the viral origin hypothesis include the lack of primary cilia in any known protists. An alternative scenario is that the central pair disappeared in mammalian primary cilia through secondary loss, as these organelles became specialized for sensory functions. Likewise, the example of centriole replication in fern and cycad sperm cells, while reminiscent of viral replication, is taken from a crown taxon (plants). If either of the foregoing phenomena represented intermediate states in evolution of the centriole, one might expect to find them in protists. Lastly, since most viral genomes form extensive direct-repeat concatamers prior to entry into latent phase, if eukaryotic signature genes such as the tubulins and motor proteins were of viral origin, one might observe at least some of them to be in direct-repeat arrays. At least for the recently-published *Chlamydomonas* flagellar proteome, this has not been the case (G. Witman, personal communication).

Viral and spirochete symbiogenetic origins for the cytoskeleton are not, in any case, mutually exclusive. A consortium similar to the present-day case of *Thiodendron* could well have acquired competitive advantage through assimilation of viral genes for rapid assembly/disassembly of protein subunits for the nascent cytoskeleton, for

example. Regardless of whether the microtubule-based cytoskeleton or selected parts are of viral or bacterial endosymbiotic origin, it is expected that the centrosome, centriole, or other eukaryotic relatives of the karyomastigont would contain remnants of a once-independent genome. The following discussion summarizes the evidence for and against the presence of nucleic acids in the centrosome and related organelles.

6. The Case for Centrosomal Nucleic Acids

Early efforts to define the molecular composition of the eukaryotic mitotic apparatus by Shimamura (1956), Mazia (1955), Stich (1954), Rustad (1959), Zimmerman (1960) and others revealed evidence for "pentosenucleic acid" in the polar regions. These studies had several limitations that have been largely overcome in later years due to the development of high resolution imaging and more specific histochemical probes. The early studies were also subject to the ubiquitous problem faced even today: when the cell and its compartments are disrupted, molecules that do not normally associate with certain organelles and complexes may adsorb to them artifactually. Nonetheless, they paved the way for more refined dissection of the spindle and associated structures. In their wake have been a number of reports describing the presence of both DNA and RNA in the centrosome. We cannot address all of these studies. Thus, the following discussion is confined to a few of the most straightford descriptive reports, and then on a small group of functional studies that overlap technically or conceptually. Additional reports on RNA in centrosomes not described below are listed in the bibliography and marked with an asterisk.

Evidence for the presence of DNA in centrosomes was met almost from the outset with evidence to the contrary (see, for example, Younger et al., 1972). Thus, the consensus in the field today is that centrosomes do not contain DNA; the arguments are discussed in some detail in a comprehensive review article by Marshall and Rosenbaum (2000). The debate can be summarized as follows: Histochemical, biochemical, and functional assays have been used to demonstrate the presence of DNA in centrioles, centrosomes, and kinetosomes from a variety of organisms. The results are difficult to interpret because: 1) The probes used in these studies typically lack specificity (e.g. they bind both DNA *and* RNA); 2) It is possible (indeed likely) that the DNA demonstrated biochemically was derived from nuclear or mitochondrial contamination of preparations; 3) Results shown in one set of studies in support of centrosome-associated DNA were contradicted in similar studies. It is important to note, however, that an inability to demonstrate centrosomal DNA does not stand alone as evidence that it does not exist. Also, it is not possible to know if functional studies failing to show an effect of DNase or DNA-binding reagents were focused on the correct biological activity. An experimental result of "no effect" is therefore an uninterpretable result in most cases. It is fair to say that evidence for centrosomal DNA is lacking, but it may be premature to arrive at a firm conclusion.

Much of the historical evidence concerning the existence of centrosomal RNA is indirect and, similar to the case for DNA, is sometimes contradictory. However, there remain several basic observations that withstand reasonable scrutiny. Perhaps the two most straightforward reports are those by Dippel (1976) and Rieder (1979). In the former case, the author performed a detailed ultrastructural analysis of basal bodies, and then repeated her observations after treating sections with RNase, DNase, and protease for various time periods. Among the structural features described by Dippel was the luminal complex, a "twisted or looped 90Å diameter fiber, or more problably pair of fibers in association with dense granules." Prolonged DNase treatment had no detectable effect on basal body structure, including the luminal complex. Pronase treatment, as would be expected for a largely proteinaceous structure, had a series of time-dependent effects. RNase was found to dissolve the luminal complex. Dippel's interpretation was that the luminal complex contains RNA. An alternative explanation is that the commercial RNase preparation used in these experiments was contaminated with proteolytic enzymes, particularly since the luminal complex was affected by pronase treatment as well (although differentially). However it is also possible that the luminal complex is a ribonucleoprotein (RNP) complex, and its structural integrity is therefore susceptible to both enzymes. The contamination hypothesis to explain Dippel's result requires the assumption that the RNase preparation was selectively compromised with protease and that the DNase was not contaminated. Of course, one might also hypothesize that the protease preparation was contaminated with RNase, but no evidence has been presented for either case. The simplest explanation is that RNA may be a structural component of the basal body (centriole) lumen. Occam would recommend the simplest explanation, at least as a starting point.

Rieder (1979) employed Berhard's method of uranyl staining followed by EDTA bleaching (Bernhard, 1962) to visualize RNP complexes at the ultrastructural level. RNP staining was followed by RNase treatment. Organelles and molecular assemblies in the same section known *a priori* to either lack or contain RNA served as internal controls for both histochemical staining and its subsequent abolition. RNP-stained structures included ribosomes, kinetochores, and centrioles. In centrioles, RNP staining was seen on the inner surface of the centriole triplet blades and on the foot of the A tubule. This pattern was abolished by RNase treatment. It could again be postulated that the RNase preparation contained protease contamination, or, in this

case, that Berhanrd's RNP method is not adequately specific. There is merit in each of these arguments. However, in the absence of contradictory evidence, we would again propose the results may just as easily be taken at face value, if only as impetus for additional study.

7. Functional Studies

RNA has been proposed to play a direct role in centriole architecture, and function in centriole replication, microtubule nucleation, and spindle assembly. These studies have, for the most part, similar approaches to those discussed above – demonstrating RNase sensitivity of certain centriole- or centrosome-based activities. Added complexity (and therefore added points of contention) arise in interpreting these studies since assays for biological activity were conducted *in vitro* and/or were artificially induced.

Functional evidence for the presence of RNA in basal bodies and a possible role for RNA in aster formation was described by Heidemann et al. (1977). Isolated basal bodies induce aster formation when injected into *Xenopus* oocytes. Heidemann et al. found that pretreatment of the basal bodies with RNase, unlike that with DNase, eliminated their aster-forming activity. Thus, aster formation appeared to be dependent upon the presence of intact RNA. One concern with this interpretation is that isolated basal bodies can nucleate microtubule outgrowth from the triplet microtubule ends, even in the apparent absence of PCM. The mode of outgrowth from isolated basal bodies may therefore have been non-physiological. Moreover, the injected basal bodies are clustered, so what appears to be aster formation may truly represent non-physiological microtubule outgrowth from randomly oriented basal bodies in a dense cluster (Marshall and Rosenbaum, 2000). This would discount the results as evidence of a role for RNA in at least some aspects of aster formation. However, similar evidence using lysed cells has been reported by other investigators which could partially alleviate this criticism. Pepper and Brinkley (1980) showed that RNase T_1 and RNase A both degrade pericentriolar material in lysed PtK2 cell preparations with a concomittant loss in microtubule nucleation potential. Neither enzyme had any apparent effect upon the structure or microtubule nucleating (or capture) activity of kinetochores. Conversely, DNaseI affected both the structure and nucleating activity of kinetochores, but had no effect on centrosomes. Snyder (1980) also showed that RNase A or T_2, but not DNase 1, inhibited microtubule nucleation in PtK1 cells. One point of uncertainty in both of these studies is whether the effect of RNase was on microtubule assembly rather than nucleation at the centrosome.

There are a number of other reports implicating RNA in centrosome function. Zackroff et al. (1976) demonstrated the effects of RNase on aster symmetry and fiber length and was able to reverse the observed effects with the RNase inhibitor polyguanylic acid. Peterson and Berns (1978) inhibited spindle formation using the light-activated nucleic acid binding dyes, psoralens. In these studies, PtK2 cells were treated with psoralens of varying affinity, followed by laser microbeam targeting of the centriolar region. Psoralens selective for DNA had no effect, while those of broader specificity – for both RNA and DNA – inhibited spindle formation.

Two reports are often overlooked in the debate over centrosome-associated nucleic acids. Neither directly addresses whether there are nucleic acids in centrosomes, but both are compatible with that hypothesis. The presence of purine nucleoside phosphorylase (PNP) was demonstrated in centrioles and basal bodies (Oliver et al., 1981). PNP is an important component in the purine salvage pathway, converting purine + ribose 1-phosphate to purine nucleoside + inorganic phosphate. Ultrastructural enzyme histochemistry and immunocytochemistry were used to visualize PNP in the centrioles and basal bodies of mammalian, avian, and protozoan cells. These findings beg the question of why an enzyme specifically involved in nucleic acid metabolism would be localized in centrioles and kinetosomes if there were no nucleic acids present in these organelles. Perhaps PNP is collected at these sites prior to distribution elsewhere in the cell, but it is also possible that the enzyme is present to carry out its native function in nucleic acid metabolism, on site. In another study, a fluorescent analogue of α-amanatin was used to localize RNA polymerase II in PtK1 cells (Wulf et al., 1980). The enzyme is distibuted primarily in the nucleus during interphase, but during mitosis it appears to be concentrated on the spindle and centrosomes. An antibody to RNA polymerase II used in conjunction with the fluorescent α-amanatin probe shows an overlapping distribution, but without concentration at these two sites. The identity of the α-amanatin binding protein as RNA polymerase II is, therefore, not confirmed, although the amanatin probe was carefully characterized as part of the study.

8. Recent Evidence

Several more recent reports bear on the subject of centrosome-associated RNA. Lambert and Nagy (2003) showed that the centrosome is a trafficking hub for the asymmetric distribution of certain mRNAs between sister blastomeres in embryos of the snail, *Ilyanassa obsoleta.* Transcripts of the developmental patterning genes, *even-skipped* (*Eve), decapentaplegic* (*Dpp),* and *tolloid* (*Tld)* are distributed diffusely in the cytoplasm of all four blastomeres after the second cleavage. They become localized to the centrosome during interphase in a

microtubule-dependent manner, and are then transported to a region of the blastomere cortex inherited by only one of the two sister cells during prophase of the third cleavage division. The transcripts in question are all well characterized molecules, defined and known for years as cytoplasmic transcripts, so it does not seem likely that they represent a set of RNAs intrinsic to the centrosome. Nevertheless, the study demonstrates clearly that RNA can be localized at the centrosome, even if the association is transient and exemplifies, rather than regulates, centrosome function.

Evidence of RNA playing a role in spindle assembly comes from a study of chromatin-dependent microtubule polymerization by Blower et al. (2005). In the absence of centrosomes, spindle assembly can be initiated in the vicinity of chromosomes. This microtubule organizing activity is regulated by Ran GTPase and by the binding of several downstream effectors to the nuclear transport receptor, importin β. Binding of these effector molecules to importin β is indirect, requiring the activity of importin α. Blower et al. discovered additional elements of the chromatin-induced spindle assembly pathway that are independent of importin α, including the protein Rae1. Rae1 was found to be part of a ribonucleoprotein (RNP) complex, and Rae1 RNP spindle promoting activity is RNA-dependent. In this sense the study by Blower et al. is similar to the earlier reports of Heidemann et al. (1979), Snyder (1980), Pepper and Brinkley (1980), and others, utilizing RNase to block spindle-assembly. One addition that strengthens this study and bolsters the earlier reports is the use of S protein or S peptide (proteolytic products of RNase A with reduced catalytic activity). Alone, each is without effect on spindle assembly. However, S protein and peptide added together, which reconstitutes RNase A activity, had the same effect as the native enzyme. It is worth reiterating that the subject of this study is chromatin-dependent, not centrosome-dependent spindle assembly. The result is nevertheless consistent with the earlier studies, and it can be reasonably expected that some of the same mechanisms underlie both spindle assembly pathways.

The presence of specific RNA in centrosomes was reported by Alliegro et al. (2006). These studies were facilitated by the relatively large size of surf clam (*Spisula solidissima*) zygote centrosomes, their precise developmental regulation (absent in unfertilized oocytes, assembled at four minutes post-activation), and a reliable method for their isolation in preparative quantities (Palazzo and Vogel, 1999). RNA was extracted directly from isolated centrosomes, amplified by random RT-PCR, and cloned to create a library of centrosome associated RNAs (cnRNAs). Five of these transcripts were analyzed, comprising a unique family, perhaps intrinsic to the centrosome. These RNAs are present in small amounts. Unlike *Ilyanassa Eve, Dpp,* and *Tld,* they were indetectable by northern analysis, and virtually indetectable in whole cell RNA isolates by RT-PCR. This suggests their presence in the general cytoplasm is vanishingly small. The five original clones had no significant matches in DNA or protein databases. The first transcript to be fully sequenced and localized in cells was subsequently found to contain a conserved reverse transcriptase domain and colocalizes with the centrosome marker protein, gamma tubulin (Fig. 2).

Characterization of the full library is ongoing. Extensive BLAST analysis reveals few database matches; only one-fourth of cnRNAs show significant similarity to current database entries (unpublished observations). By far, the major two groups of molecules represented in this one-

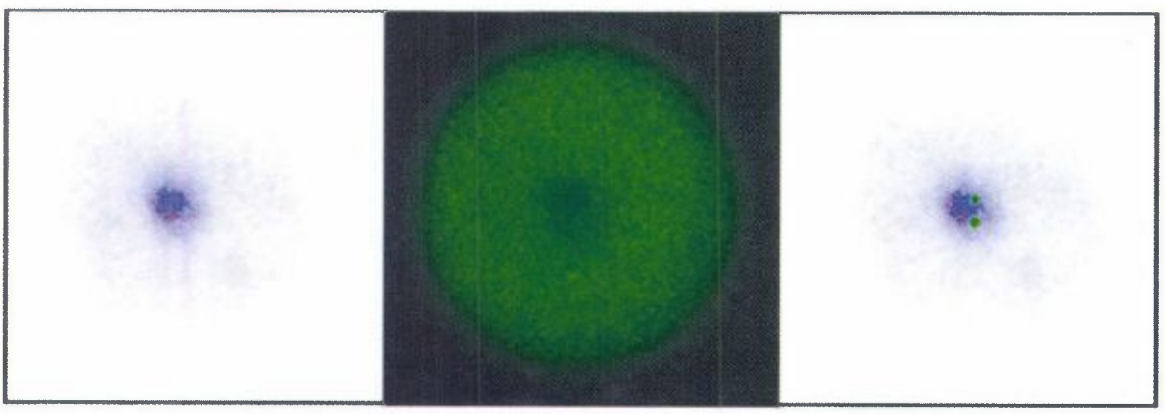

Figure 2. *In situ* localization of cnRNA11. The left-hand panel shows a *Spisula* zygote labeled with a cnRNA11 hybridization probe. The middle panel is an immunofluorescent image of the same cell co-labeled with antibodies to the centrosome marker protein, gamma tubulin. The right-hand panel is an overlay showing the two gamma-tubulin labeled centrosomes embedded within the cnRNA11 hybridization patch (reprinted from Alliegro et al., 2006). *See cover illustration.*

fourth were hypothetical proteins or untranslated DNA sequences derived from various genome projects, and proteins involved in nucleic acid metabolism. Preliminary data indicate that DNA corresponding to the cnRNAs is present in *Spisula* oocytes. The location, cytoplasmic or nuclear, is not yet known. The emerging picture, however, is that the structure of these DNA elements is distinct from other known *Spisula* sequences (as well as the *Ilyanassa* sequences localized to the centrosome by Lambert and Nagy). There are few, if any, *bona fide* introns, and conservation around the one or two possible splice sites found thus far is at best modest compared to the consensus sequence at the intron/exon border of known *Spisula* nuclear genes.

Finally, a report by Groismann et al. (2000) echos the earlier studies of Mazia and others, indicating the presence of RNA on the mitotic spindle. The newer study, however, had at its disposal the molecular techniques to permit identification of the specific transcripts involved. Translational control of certain proteins in the embryo, including a panel of cell cycle regulators, is mediated by cytoplasmic polyadenlylation. *Cis*-acting sequences that mediate cytoplasmic polyadenylation are found in the *Xenopus* homologues of Bub3 and cyclin B1 mRNAs. The proteins that regulate cytplasmic polyadenylation at these sites (CPEB and maskin, among them) localize to the spindle, leading the investigators to seek, and confirm, a similar distribution for the target mRNAs. This is clearly a case of specific mRNAs localizing to a discrete site within the cell for functional purposes, a circumstance with ample precedent. But from a different perspective, neither is it inconsistent with the notion that these molecules are homing to a site and function imprinted in their evolutionary history.

9. Conclusion and Outlook

Centrosomes and mitotic spindles contain nucleic acids. This has now been demonstrated in three independent studies using *in situ* probes selective not just for RNA, but for specific RNAs (Groismann et al., 2000; Lambert and Nagy, 2003; Alliegro et al., 2006). Some of these transcripts may be targeted to centrosomes transiently for localized distribution and function within and/or between cells, others appear to be associated uniquely with the centrosome. A fourth recent and carefully controlled study (Blower et al., 2005) supports the long-held notion that RNA plays a role in spindle assembly, albeit chromatin-mediated spindle assembly. The presence of RNA in centrosomes is an important corrollary to the hypothesis that the organelle arose by symbiogenesis. While none of these studies constitute proof of the central hypothesis, they do represent an important step. Specific sequences have been identified that can now be manipulated selectively to examine the consequences of interference on both the localization of the RNA and cell behavior. Sequence information will permit us to determine if and where corresponding DNA genes exist in the cell, and if they more closely resemble the (presumptive host) species from which they were derived, or a more ancient relative of the presumptive endosymbiont. The tools to answer an old and perplexing question are now at hand.

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