*Chromosoma (*review)

Kinetochore fiber formation in animal somatic cells: dueling mechanisms come to a draw

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Abstract

The attachment to and movement of a chromosome on the mitotic spindle is mediated by the formation of a bundle of microtubules (MTs) that tethers the kinetochore on the chromosome to a spindle pole. The origin of these "kinetochore fibers" (K-fibers) has been investigated for over 125 years. As noted in 1944 by Schrader, there are only three possible ways to form a K-fiber: either it a) grows from the pole until it contacts the kinetochore; b) grows directly from the kinetochore; or c) it forms as a result of an interaction between the pole and the chromosome. Since Schrader's time it has been firmly established that K-fibers in centrosome-containing animal somatic cells form as kinetochores capture MTs growing from the spindle pole (route a). It is now similarly clear that in cells lacking centrosomes, including plants and many animal oocytes, K-fibers "self-assemble" from MTs generated by the chromosomes (route b). Can animal somatic cells form K-fibers in the absence of centrosomes by the "self-assembly" pathway? In 2000 the answer to this question was shown to be a resounding "yes". With this result, the next question became whether the presence of a centrosome normally suppresses K-fiber self-assembly, or if this route works concurrently with centrosome-mediated K-fiber formation. This question, too, has recently been answered: observations on untreated live animal cells expressing GFP-tagged tubulin clearly show that kinetochores can nucleate the formation of their associated MTs in the presence of functional centrosomes. The concurrent operation of these two "dueling" routes for forming K-fibers in animals helps explain why the attachment of kinetochores and the maturation of K-fibers occur as quickly as it does on all chromosomes within a cell.

Introduction

In 1879 Walther Flemming (Flemming, 1879) wrote in regards to mitosis that "we do not know, in the movement or changes of position of the threads of a nuclear figure (i.e., chromosomes), whether the immediate causes lie within the threads themselves, outside of them, or both". Since Flemming's time the question of how the force(s) are generated to move the chromosomes during nuclear division (karyokinesis) has been actively pursued by many investigators.

In his fixed preparations of newt cells (Fig. 1A), Flemming could not see that during anaphase each chromatid was connected to a pole of the mitotic apparatus by a prominent fiber. Instead, Van Beneden (1883), Hermann (1891) and Druner (1894) made this observation (Fig. 1B), and Hermann even predicted that these "mantle" fibers were the principle agents by which the daughter chromosomes were "dragged" apart (see Wilson, 1911; pp 78-79).

As late as 1925, Edmund B. Wilson (Wilson, 1925), in the 3rd edition of his monumental treatise on the "Cell in Development and Heredity", characterized the point that a mantle fiber inserts into the chromosome simply as "a small area" (pg 131). However, it was noted as early as 1894 by Metzner (Metzner, 1894) that this area contained a small, discrete staining structure or "kinetic region" that led the way during poleward chromosome motion (Fig.1C). Later reports noted that chromosome fragments lacking this region were expelled from the spindle and exhibited no directed motion (Carlson, 1938). Thus, by the time Franz Schrader published the first edition of his book on mitosis in 1944 (Schrader, 1944), it was widely accepted that Metzner's kinetic region was responsible for attaching a chromosome to, and somehow moving it on, the spindle. In his book Schrader listed 27 terms that had been used previously to describe this region and he finally settled on Moore's term "*kinetochore*" (see (Sharp, 1934).

Near the time that Schrader published his book, polarized light microscopy (LM) of living cells by Schmidt (Schmidt, 1939), and later Inoue (Inoue, 1952; Inoue, 1953) proved that the fibrous nature of the spindle seen in fixed preparations was not

an artifact. These studies also revealed that the spindle is highly dynamic in that it grows or shrinks (assembles/ disassembles) in response to certain drugs and environmental changes. Thus, by the mid 20th century it was evident that mantle fibers, which were now termed chromosomal or *kinetochore fibers* (K-fibers), were real, dynamic, and responsible for generating and/or transmitting the forces for chromosome motion. The prevailing idea at this time was that a K-fiber "pulled" on its associated kinetochore, and thus on the chromosome, with a force that was proportional to its length (Ostergren, 1945).

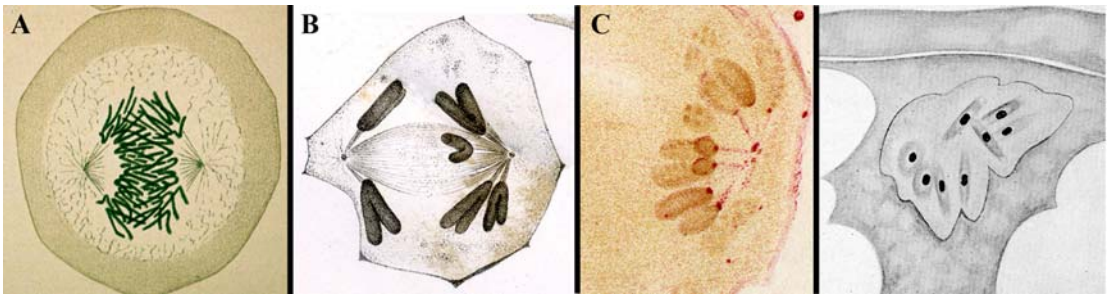

Fig. 1. A) Flemming's 1882 drawing of a newt cell in anaphase of mitosis. B) Druner's 1895 drawing showing K-fibers during anaphase in an insect spermatocyte; C) Metzner's 1894 drawing of kinetochores during anaphase; D) Hughes-Schrader's 1924 depiction of prometaphase in *Achroschismus wheeleri* oocytes. Note that each chromosome appears to be organizing its own mini-spindle within an intact nuclear envelope.

Electron microscopic (EM) studies in the early to mid 1960's, starting with that of Harris (Harris, 1961), revealed that the spindle and its associated K-fibers were composed primarily of *microtubules* (MTs). Subsequent EM work on the kinetochore in mammals led to the view that this assembly is structured as a "tri-laminar" disk in which its associated MTs (K-MTs) are embedded in an "outer" thin, circular electron-dense plate. This plate is separated from an inner chromosome-associated dense layer by an electron lucent clear zone (Brinkley and Stubblefield, 1966; Jokelainen, 1967; Roos, 1973) (Fig. 2A). Jokelainen (Jokelainen, 1967) emphasized that "the outward surface of the outer kinetochore layer is consistently covered by a *corona* of low density material that is practically devoid of cytoplasmic particulate structures".

(Although the kinetochore is often referred to as an organelle, because it lacks a surrounding membrane it is really a macromolecular assembly like the chromosome).

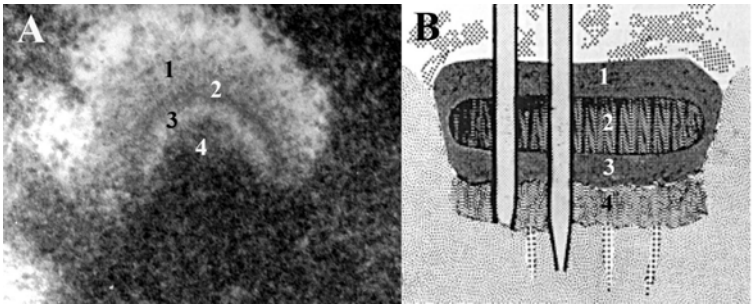

Fig. 2. The mammalian kinetochore. A) An unattached kinetochore from a newt cell viewed in section after conventional fixation and embedding. Note extensive corona material (1) and its tri-laminar structure (2-4). B) Jokelainen's (1967) drawing of a kinetochore in a fetal rat cell. C) A PtK1 kinetochore viewed in section after high pressure freezing and freeze substitution. (B is from P. Jokelainen. J. Ultrastr. Res., 19:19-44, 1967; C is courtesy of Dr. B.F. McEwen, Wadsworth Center).

The multiple functional roles of the kinetochore, and the distribution of its associated proteins, are still modeled exclusively in the context of this tri-laminar structure (Fig. 2B). However, this appearance is likely *a fixation artifact* caused by shrinkage of the chromosome away from the kinetochore during the dehydration steps used in conventional EM embedding. When viewed after high pressure freezing and freeze-substitution, which minimizes structural changes, the kinetochore in mammals appears as a 50-75 nm thick mat of light-staining fibrous material connected directly to the more densely staining surface of the centromeric heterochromatin (McEwen et al., 1998) (Fig. 2C). This mat is surrounded on its cytoplasmic surface by a 100-150 nm wide corona that contains a loose network of light-staining, thin (~9 nm dia) fibers that exclude ribosomes and other particles. As a rule, the number of MTs that bind to a kinetochore as its K-fiber matures is set by its surface area, with kinetochores in mammals binding 20-40 MTs (Rieder, 1982).

The development of MT subunit tagging and live cell fluorescence imaging in the 1980's confirmed Inoue's conclusion that K-fibers are highly dynamic structures in which their constituent K-MTs exhibit a coordinated behavior (Cassimeris et al., 1988; Gorbsky et al., 1987; Mitchison et al., 1986);(Mitchison, 1989). Also, during

this decade the first non-axonemal MT motor proteins were discovered (Vale et al., 1985) as were the first antibodies to the kinetochore/centromere complex (Moroi et al., 1981);(Brenner et al., 1981). After these discoveries research on how chromosomes move focused on a search for spindle and kinetochore associated molecular motors and how K-fiber MTs (K-MTs) form and function.

Several excellent reviews have recently been published on the kinetochore and its role in spindle assembly, chromosome motion, and mitotic progression (Biggins and Walczak, 2003; Kline-Smith et al., 2005; Maiato et al., 2004b; Rogers et al., 2005; Wadsworth and Khodjakov, 2004). None of these, however, focus on the formation of K-fibers, on which important new data has recently become available. What follows below is a brief chronological summary and an evaluation of the more seminal discoveries that have led to our current view of how K-fibers form in vertebrate somatic cells.

Kinetochore fiber formation: the early years (1911 – 1980)

An early notion for how K-fibers form was outlined by E.B. Wilson in 1911 (Wilson, 1911), and was based on the work of Hermann, Van Beneden, and others. During the early stages of mitosis in animals, each spindle pole is defined by a centrosome and its associated radial or "astral" arrays of fibers (i.e., MTs). Wilson concluded that mantle-fibers (K-fibers) "are essentially a part of the asters, *i.e.*, are those astral rays that come into connection with the chromosomes" (pg 315). This simple idea remained relatively unchallenged until 1924 when Hughes-Schrader reported that each meiotic tetrad in *Acroschismus wheeleri* oocytes appears to organize its own mini-spindle, all of which then coalesce into a single bipolar spindle (Fig. 1D; see (Rieder and Nowogrodzki, 1983). In the first edition of his book, Schrader (Schrader, 1944) interpreted this and similar data on coccid spermatocytes to mean that K-fibers can arise "chiefly or entirely through the activity of the kinetochore alone" (pg 32).

The notion that K-fibers grow from the kinetochore garnered strong subsequent support from Dietz's (Dietz, 1966) phase-contrast observations on live crane fly spermatocytes. He noted that functional K-fibers and bipolar spindles form in these cells even when the two astral MT arrays (centrosomes) are physically inhibited from separating. Around this time, Forer (Forer, 1965), investigating the same material with polarized LM, reported that holes generated in K-fibers with a UV microbeam move poleward at rates similar to those of anaphase chromosomes. An Occam's razor interpretation of these findings was that K-fibers form by growing from the kinetochore, i.e., each kinetochore generates its associated MTs. This interpretation gained experimental support in the mid-1970's when many labs reported that kinetochores on isolated mammalian chromosomes nucleate MTs in the presence of tubulin (e.g., (McGill and Brinkley, 1975; Telzer et al., 1975). Ris and Witt [(Ris and Witt, 1981);(Witt et al., 1981)] even extended this finding to chromosomes *in-situ*. Using serial section EM, they discovered that when Chinese

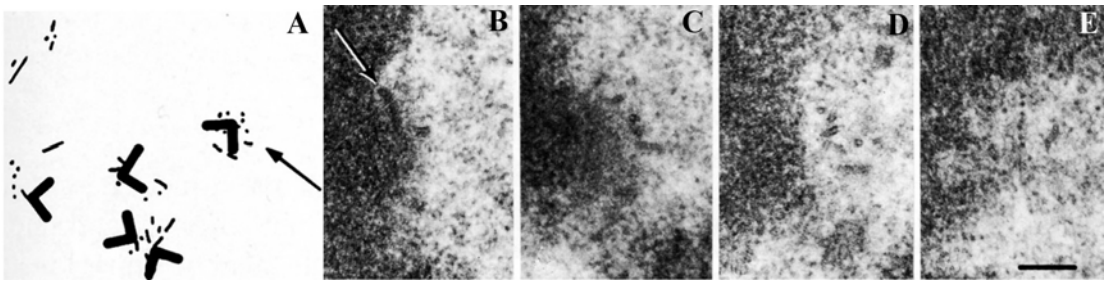

Fig. 3. Kinetochores nucleate microtubules during recovery from colcemid. A) Reconstruction from 33 serial 0.25 μM sections of several kinetochores (> = kinetochore outer disk, chevron pointing away from the chromosome) in a CHO cell fixed 15 into a recovery from a prolonged colcemid block. Note that numerous short microtubules appear first near each kinetochore. B-E) Serial sections through the kinetochore depicted by the arrow in A. Note the numerous short microtubules in the corona material. From P.L. Witt, H. Ris and G.G. Borisy. Chromosoma 81:483-505, 1980.

hamster ovary (CHO) or mouse cells were allowed to recover from prolonged (colcemid) treatments with drugs that inhibit MT assembly, numerous small MTs appeared first *within the kinetochore corona* (Fig. 3). Thus, by 1980 the idea that the kinetochore generates its associated MTs was, as noted by Pickett-Heaps and Tippit (Pickett-Heaps and Tippit, 1978), "virtually unquestioned in nearly every paper or

review on mitosis", and it formed "a basic building block upon which most models of mitosis (were) erected".

Kinetochore fiber formation: the middle years (1981-2000)

Despite the data that kinetochores nucleate MTs in cells recovering from drugs, in animals chromosomes exhibit a number of behaviors and features during spindle formation which suggest that this nucleation activity does not occur in untreated cells. For example, the closer a kinetochore is to a centrosome at nuclear envelope breakdown (NEB), the more rapidly it attaches to the spindle (Roos, 1976; Rieder and Alexander, 1991). During NEB in newt lung cells one or more chromosomes sometime become positioned well removed from the two asters (Fig. 4). Under this condition the attachment of these "lost" chromosomes to the spindle is delayed for hours (Rieder and Alexander, 1990), and throughout this delay there is no evidence of MT formation in the vicinity of the kinetochores. Furthermore, the formation of K-fibers on sister kinetochores in mammalian cells usually occurs asynchronously. As a result, cells in early prometaphase contain a variable number of monooriented chromosomes (Fig. 4) in which one kinetochores is attached to a spindle pole by a K-fiber while the other, which is positioned on the other side of the primary constriction and faces in the opposite direction, remains free of MTs (see e.g., (Mole-Bajer et al., 1975; Roos, 1973). As emphasized by Rieder and Borisy (Rieder and Borisy, 1981) these features are more consistent with the idea that K-fibers normally form in animals as kinetochores capture astral MTs growing from the centrosome.

Within the living cell, as in vitro, MTs are polarized structures that elongate by preferentially adding subunits onto their fast growing (plus) ends, which are distal to their site of nucleation. Thus, if the MTs used for construction of the K-fiber are nucleated by the kinetochore, their plus ends should be located near the spindle poles,

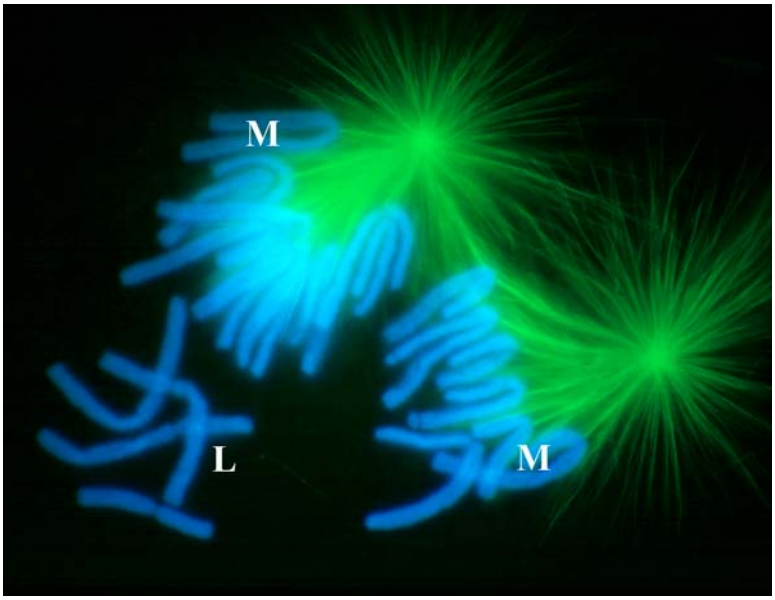

Fig. 4. An indirect immunofluorescence micrograph of an early prometaphase newt cell that contains several "lost" (L) and mono-oriented (M) chromosomes. In this cell the microtubules are green/yellow and the chromosomes are blue. Many of the mono-oriented chromosomes can clearly be seen to possess a single kinetochore fiber. Note the absence of microtubules in the vicinity of the lost chromosomes.

away from the kinetochore, and their minus ends should remain associated with the kinetochore. This was indeed reported to be the case for MTs nucleated by kinetochores on isolated chromosomes (Bergen et al., 1980). However, *in situ* MT polarity studies on whole cells by Euteneuer and McIntosh (Euteneuer and McIntosh, 1981) demonstrated that all MTs within a half-spindle, including K-MTs, possessed the same polarity *which was plus end away from the spindle pole.* This finding implied that the nucleation of MTs by kinetochores on isolated chromosomes is an *in vitro* artifact. By contrast, unlike MTs generated from kinetochores on isolated chromosomes, K-MTs nucleated in cells recovering from drug treatments have the correct polarity, i.e., their plus ends are at the kinetochore (Euteneuer et al., 1983). However, the physiological relevance of this finding remained questionable. Thus, by the mid 1980's the question of how K-fibers formed in vertebrates remained unresolved. Although it was evident that kinetochores could seed the formation of their own fibers in many gamete producing cells, and during recovery from drug

treatments, the relevance of this mechanism to normal untreated animal somatic cells had not been demonstrated.

Kinetic analyses by Mitchison and Kirschner in 1985 (Mitchison and Kirschner, 1985a) revealed that MTs nucleated in the presence of tubulin by kinetochores on isolated CHO chromosomes grow with complex kinetics. After an initial lag phase, they found that MTs "are continuously nucleated with both plus and minus ends distally localized", a feature that was "inconsistent with the formation of an ordered, homopolar kinetochore fiber in vivo". In a companion paper these same authors (Mitchison and Kirschner, 1985b) reported that kinetochores on isolated CHO chromosomes could also "*capture*" *preformed MTs and move them in an ATP dependent manner*. This study also noted that MT subunits were added in vitro to growing K-MTs *at the kinetochore*. Shortly thereafter tubulin microinjection approaches were used to extend this important *in vitro* conclusion to live cells (Mitchison et al., 1986).

Mitchison and Kirschner's discovery that kinetochores on isolated chromosomes can capture preformed MTs, combined with their earlier in-vitro finding that centrosome-nucleated MTs constantly grow and shrink at their plus ends (i.e., they exhibit dynamic instability; (Mitchison and Kirschner, 1984)), led to a "*search-and-capture*" model for K-fiber formation in animals. This scenario (Kirschner and Mitchison, 1986), similar to that suggested earlier by Wilson and later by others, envisions that K-fibers form in animal cells as kinetochores capture and stabilize astral MTs generated from the centrosome. This model was subsequently validated using live cell video-enhanced LM by Rieder and co-workers (Hayden et al., 1990; Rieder and Alexander, 1990). Their studies revealed that when an astral MT contacts an unattached kinetochore in newt cells, the kinetochore immediately attaches to the MT lattice and begins moving towards the pole *on the surface of the MT at a very high rate of speed*. Around this time cytoplasmic dynein was found to be a major component of unattached kinetochores (Pfarr et al., 1990). This motor molecule is the only kinetochore associated MT minus end motor identified to date; it

remains the most likely force-producing candidate for the rapid poleward motion exhibited by attaching kinetochores in many cell types.

The search-and-capture hypothesis rapidly gained widespread acceptance in part because it was supported by direct evidence, but also because it explained a number of behaviors (see above) seen during early prometaphase in animal somatic cells. Indeed, by the end of the 20th century the textbook consensus for how the kinetochore acquires its MTs during mitosis in animal cells was, and still is, that it captures MT plus ends growing from the spindle pole.

Kinetochore fiber formation: the current view

Although it provides a solid conceptual framework for how K-fibers and spindles form, the random nature of the search-and-capture process is not consistent with the kinetics of K-fiber formation in animal cells. While it is predicted that this mechanism would take only minutes to capture a kinetochore (Hill, 1985);(Holy and Leibler, 1994), hours would be required to capture all 96 kinetochores in a human cell (Wollman et al., 2005)—which is far too long. In addition, it does not explain how all captured kinetochore become saturated with 20-40 MTs in span of just 15-20 minutes (McEwen et al., 1997; Wollman et al., 2005). Clearly, other processes must be occurring to facilitate K-fiber formation and maturation. Also, as originally formulated, search-and-capture does not explain how K-fibers form in cells lacking centrosomes (and thus asters), including all plants and many animal oocytes (e.g., (Heald et al., 1997; Karsenti et al., 1984; Szollosi et al., 1972). In these cells another mechanism must exist for generating K-MTs.

Our knowledge of how K-Fibers form in cells lacking centrosomes is based on to two decades of investigating the assembly of meiotic spindles in *Xenopus* oocyte extracts. In these extracts spindles “self-assemble” around chromatin in the absence of centrosomes (Heald et al., 1997). The process is initiated by the random nucleation of MTs in the vicinity of the chromosomes after NEB. The nucleation of these MTs results from the production of a Ran-GTP gradient around the chromosomes by

chromatin bound RCC1, a Ran-GTP exchange factor. The enhanced production of Ran-GTP near chromosomes, which occurs during mitosis in response to the CDK1-mediated phosphorylation of RCC1, frees TPX2 from its carrier (Gruss and Vernos, 2004);(Li and Zheng, 2004). TPX2 is a MT-associated protein intimately involved in chromatin-associated MT nucleation. After MTs are generated near the chromosomes they then elongate and are "sorted" into a bipolar array with the proper MT polarity, by various MT-associated motor molecules, some of which are bound to the chromosomes (Walczak et al., 1998).

Before 1980 the prevailing idea was that the mechanism responsible for K-fiber formation (and chromosome motion) was highly conserved, and was the same in all cells. However, the work in *Xenopus* (and in yeast) led to the view that multiple "redundant" mechanisms often exist within a cell to affect important processes. This appreciation prompted the question of whether the "self-assembly" pathway for K-fiber formation, used by plants and many animal oocytes, also exists in cells that normally use centrosomes to form their spindles and K-fibers (Hyman and Karsenti, 1998). The answer to this question required the development of approaches for removing centrosomes from animal somatic cells entering mitosis. The results of these studies revealed the answer to be "yes": in the absence of centrosomes and their associated astral MT arrays animal somatic cells form functional bipolar spindles with relatively normal kinetics via a self-assembly process, whether they be derived from flies (Debec et al., 1995) or mammals (Hinchcliffe et al., 2001; Khodjakov et al., 2000).

Given that many (if not all) animal cells are capable of building functional spindles without centrosomes, it became important to determine if the presence of centrosomes inhibits or depresses the self-assembly pathway or if it is intrinsic and always working in the background. This question was difficult to address because in the presence of centrosomes the density of astral MTs in the vicinity of the chromosomes after NEB is extremely high. Yet, by imaging rat kangaroo (PtK1) cells expressing GFP-tagged tubulin, Khodjakov and colleagues (Khodjakov et al., 2003)

were able to show that K-fibers often form without a direct connection to a centrosome in cells recovering from monastrol treatment as well as in untreated cells. It was unclear, however, whether the MTs for constructing these K-fibers were spontaneously nucleated in the cytoplasm (and captured by the kinetochores), nucleated directly at the kinetochores, or derived from the correction of a syntelic attachment (in which both sister kinetochores initially become attached to a single pole).

The next year Maiato and co-workers (Maiato et al., 2004a), working with flattened centrosome-containing *Drosophila* S2 cells expressing GFP-tagged α-tubulin, found that K-MTs routinely form and grow from unattached kinetochores that are not facing a centrosome (Fig. 5). As in PtK_1 cells, the growth of K-fibers from kinetochores in S2 cells was seen only after a lag period; furthermore, once initiated, each fiber grew as a linear track toward a random point within the cell. During the course of spindle assembly, the ends of these elongating K-fibers were then captured by astral MTs and transported towards the centrosome by a process requiring cytoplasmic dynein (Khodjakov et al., 2003; Maiato et al., 2004a)

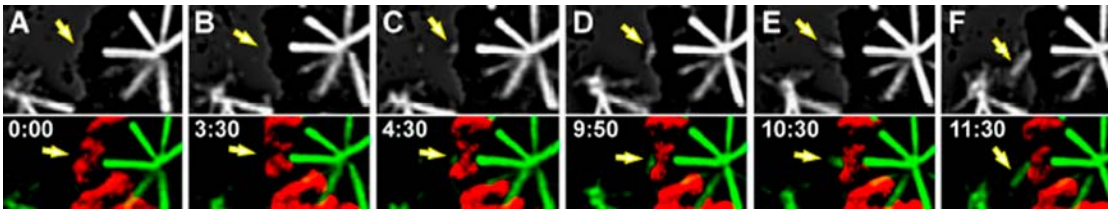

Fig. 5. Kinetochores nucleate microtubules in the presence of centrosomes. A-F) Selected frames from a fluorescence/DIC time-lapse recording of a *Drosophila* S2 cell stably expressing GFP/α-tubulin. The top part in each frame represents a deconvolved EGFP/α-tubulin fluorescence image while the bottom part is an overlay of tubulin fluorescence (green) and chromosomes (red). Yellow arrow notes the nucleation and progressive growth of microtubules from the unattached kinetochore that is not facing a spindle pole on a mono-oriented chromosome. From H. Maiato, C.L. Rieder and A. Khodjakov. J. Cell Biol., 167:831-840, 2004.

The demonstration that kinetochores can seed MT formation in untreated animal cells containing centrosomes has several interesting ramifications. As noted by Wadsworth and Khodjakov (Wadsworth and Khodjakov, 2004), and others (Gruss et al.,

2002);(Gadde and Heald, 2004)), it suggests that spindle assembly "proceeds by a generally conserved acentrosomal mechanism in all higher eukaryotes, regardless of the presence of a centrosome". In this view the real function of the centrosome during mitosis is to generate two astral MT arrays; the presence of these arrays are needed to "collect" preassembled chromosome-generated mini-spindles into a single common bipolar spindle, and also to properly position the spindle within the cell. Thus, since the discovery of K-fibers in the late 19th century, the two "dueling" notions of how they form has resolved into a draw. Schrader's original conclusion in 1944 is correct: K-Fibers can form via two pathways, *and in animals both work concurrently in the same cell.*

How do kinetochores seed what will be their own MTs and, once initiated, how do these MTs elongate? Although the molecular details remain incomplete, the first part of this question is better understood than the second. There is ample experimental evidence (see above) that a Ran-GTP gradient is established around sister kinetochores at NEB by RCC1 (aka CENP-D; see (Kingwell and Rattner, 1997) which is highly concentrated in the centromere. As this gradient forms it frees TPX2 from its importin-α binding partner. This then allows TPX2 to somehow seed the formation of short MT "stubs" in the kinetochore corona (Ris and Witt, 1981), where tubulin subunits are highly concentrated (Mitchison and Kirschner, 2005);(Pepper and Brinkley, 1977) This scenario explains the observation reported by Ris and colleagues (Witt et al., 1980), and later by Debrabander's group (De Brabander et al., 1981), that K-MTs first form between kinetochores that are in close proximity in cells recovering from colcemid treatment (or in untreated cells; Fig. 3). This is where the levels of Ran-GTP would be expected to be the highest.

Once nucleated in the kinetochore corona, short MTs begin to elongate by the addition of MT subunits onto their plus ends. This process may be facilitated by the elevated levels of tubulin in the kinetochore region. As the corona-associated MTs begin to elongate, their minus ends are pushed away from the kinetochore. This may result from the action of kinetochore-associated stationary MT plus end motors, like

CENP-E, that work along the MT lattice near its plus end (see (Maiato et al., 2004a). Alternatively, the polymerization process itself may provide the force for pushing the MT minus end away from the kinetochore. As noted by Ris and Witt (1981), the subsequent binding of these MTs to the outer plate (or kinetochore proper) "is a separate and subsequent step in kinetochore MT bundle formation". This could occur, *e.g.*, as the plus ends of TPX2-induced MTs become associated with Ndc80, a complex of kinetochore-associated proteins including Hec1 and Nuf2 that is required for the stable attachment of MT plus ends to the kinetochore.

In mature (metaphase) K-fibers, MT subunits are constantly inserted into K-MT plus ends at the kinetochore, while they are removed from the MT minus ends anchored in the polar region at the same rate. This property of the K-fiber is known as poleward MT subunit flux (Mitchison, 1989). Unlike other MT +TIP proteins (e.g., EB1, APC), CLASP's target and bind kinetochores in the absence of MTs. Recently Maiato and colleagues (Maiato et al., 2005) reported that knocking CLASPs down in S2 cells by RNAi does not inhibit K-fiber formation. However, it does prevent MT flux by inhibiting the incorporation of tubulin subunits into the MT plus ends at the kinetochore. This finding has an intriguing implication: it suggests that the growth of nascent K-fiber MTs from the kinetochore, and the subsequent maintenance of these MTs once the fiber matures, occur by different mechanisms: the former appears to be CLASP independent, while the latter is not. One explanation for this result is that as MTs nucleated at the kinetochore elongate to form the K-fiber, their MT plus ends are only loosely associated with the kinetochore/corona. Then, once the MT minus ends of the growing K-fiber become incorporated into a spindle pole, the fiber begins to generate a force on the kinetochore as it becomes anchored in the pole and MT flux is initiated. In turn, the resulting tension on the kinetochore may induce it to become more tightly associated with its associated MT plus ends. After this more stable association is established, the CLASP protein then becomes essential for the continued maintenance of the now fluxing fiber, which requires the continuous addition of subunits into the K-MT plus ends.

Given that kinetochores participate in forming their own K-MTs, why do many animal cells often contain unattached kinetochores for extended periods (see above and Fig.4). One possibility is that the ability of a kinetochore to generate its own MTs in the presence of a centrosome differs in different parts of the spindle. For example, mono-oriented chromosomes that undergo repeated oscillatory motions, as those associated with the periphery of the spindle often do, may not remain stationary long enough to generate enough Ran-GTP in the kinetochore region to seed MT assembly. The absence of MTs in the vicinity of "lost-chromosomes" in newt cells (Fig. 3) may be related to the fact that they are well removed from the forming spindle and its various factors that influence MT assembly. Also, these cells have been reported to contain very little free MT protein (i.e., to be "starved" for tubulin; see Ohnuki et al., 1976). Another question is why all of the growing K-MTs seeded by the kinetochore appear to elongate toward a common point in the cell. Does this occur because the capture sites in the kinetochore are rigid with respect to one another, so that the elongating MTs remain parallel as they grow; or is it because neighboring K-MTs rapidly become cross-linked to one another by structural (e.g., NuMA) or motor (e.g., Eg5, dynein) proteins as they begin to elongate?

Summary:

After 125 years of investigation, it is now clear that Schrader's 1944 conclusion was right: K-Fibers can form via two pathways. Furthermore, during prometaphase both of these pathways can work simultaneously in the same animal cell, and both involve the capture of MT plus ends by the kinetochore. In the first route components in the kinetochore corona trap and stabilize astral MTs that grow into their vicinity. This path prevails at NEB; with it the initiation of K-Fiber formation coincides with kinetochore orientation toward a pole, and attachment to the forming spindle. The second route occurs after a lag period, and involves the centromere-mediated assembly of short MT "stubs" in the kinetochore corona. These then begin to elongate by subunit addition onto their kinetochore-associated MT plus

ends, which pushes the MT minus ends away from the kinetochore. These MTs become incorporated into the forming K-fiber by laterally associating with other K-MTs, and/or the spindle pole, by a search and capture mechanism involving astral MTs and cytoplasmic dynein. The extent that this "self assembly" route contributes to K-fiber formation in the presence of a centrosome remains to be determined. It is likely to be significant since inhibiting the self-assembly pathway in HeLa cells by knocking down TPX2 leads to the production of spindles that appear depleted of K-fibers (Gruss et al., 2002);(Moore et al., 2002). In addition, the self-assembly route provides a straightforward explanation for why nascent K-fibers, generated from the capture of one or just several astral MTs, mature with a kinetics that exceeds that predicted from search and capture.

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