The flawless distribution of chromosomes to the daughter cells in mitosis depends in part on unlikely sources – microtubule instability and chance events.

Equal chromosome distribution results from the regular arrangement of microtubules shown in Fig. 1. The result is attachment of the partner chromosomes to opposite spindle poles and their movement to opposite poles in anaphase, so that each daughter cell will contain one copy of each chromosome. Attachment probably arises from the capture of spindle microtubules by kinetochores. Capture is facilitated by a novel form of instability called 'dynamic instability', which is driven by GTP hydrolysis or at least leads to GTP hydrolysis. Thus, energy may be used to purchase lability rather than stable bonds (for reviews see Kirschner & Mitchison, 1986; Cassimeris et al. 1987).

The key feature of dynamic instability is a remarkable state in which some microtubules shorten rapidly, while others continue to grow. Microtubules are continually nucleated at each spindle pole, replacing others that have disassembled. The ends of growing microtubules extend in all directions from the poles and, on contacting a kinetochore, may be captured. Kinetochores capture microtubules efficiently in vitro (Mitchison & Kirschner, 1985), and in cells, microtubules appear at kinetochores with impressive speed and at a wide variety of angles (reviewed by Nicklas, 1988a). Thus, dynamic instability promotes the chance encounter of microtubule ends with a kinetochore and an efficient kinetochore promotes the probability of capture at each encounter. The expected result is the attachment of every chromosome to the spindle by facilitated chance.

Moreover, captured microtubules are stabilized, which suggests an appealingly simple mechanism for generating overall spindle morphology (Kirschner & Mitchison, 1986). The selective persistence of those microtubules that by chance extended toward chromosomes would inevitably generate a directed array of microtubules. Kirschner & Mitchison (1986) suggest that 'morphogenesis by selective stabilization' may be a general principle of cell organization.

A price is paid for the role of chance in chromosome attachment to the spindle, however. The very features that promote attachment promote errors in attachment. A generous supply of microtubule ends from many directions and a permissive kinetochore make likely the capture of microtubules from both spindle poles by a single kinetochore. In fact, such mal-arrangements are commonly seen early in mitosis (e.g. Church & Lin, 1982). Given a role of chance, such errors are inevitable. Interestingly, chance is also involved in correcting the errors.

If the faulty arrangement shown in Fig. 2 persisted, one daughter cell would receive two copies of the chromosome and the other daughter would receive none. But in living cells, inappropriate arrangements of kinetochore microtubules do not persist. Instead, faulty arrangements are unstable, and repeatedly mutate until the one microtubule arrangement that

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**Fig. 1.** The arrangement of kinetochore microtubules (thin lines) necessary for equal chromosome distribution. Kinetochores are depicted as black ovals and the arrows indicate the direction of spindle forces. A pair of partner chromosomes in meiosis is shown; the same principles apply to a pair of sister chromatids in somatic mitosis.

**Fig. 2.** An inappropriate configuration; both chromosomes would be distributed to the same pole if it persisted. Graphic conventions the same as in Fig. 1.
leads to equal chromosome distribution is hit upon; only that arrangement is stable (Dietz, 1958; for a review see Nicklas, 1985). Tension is the probable basis of the difference in stability. The arrows in the figures show the direction of the forces produced by the spindle; tension is present in the appropriate orientation but absent in the inappropriate one. Direct experimental evidence that tension stabilizes microtubule arrangements was obtained by pulling upward on chromosomes like those in Fig. 2 with a micromanipulation needle; the tension thus produced made the inappropriate orientation as stable as the appropriate one, leading to distribution of both chromosomes to the same pole (Nicklas, 1985).

Thus, order in mitosis comes from variation and selection: because of variation, the genetically appropriate microtubule array eventually arises; because of selection, it alone persists. What are the sources of change and how does force stabilize the selected microtubule arrangement? These are largely questions for the future, but we know enough to be sure the answers will be interesting.

Variation — sources of change
In the configuration shown in Fig. 2, the first requirement for useful change is the association of one kinetochore or the other with a microtubule running toward the other pole. Notice how improbably this seems. Both kinetochores face downward, and hence they are in just the wrong position to capture microtubule ends extending from the upper pole. Several possible sources of microtubules extending upward need to be explored in the future, but dynamic microtubule instability is likely to play an important role by providing a continuous supply of microtubule ends probing in the vicinity of the mal-oriented kinetochores.

We know something about what happens after the appearance of one or two microtubules associated with the other pole (Nicklas, 1985). First, the motors associated with a single microtubule extending in the appropriate direction are stable as the chromosome. As movement proceeds, the kinetochore faces more directly toward the opposite pole and the source of microtubules in that direction. Hence conversion to the arrangement shown in Fig. 1 is made more likely. The potent effect of a single microtubule on later events heightens the significance of rare chance encounters and of chance in general. Second, a new sort of instability plays a role in reorientation. Kinetochore microtubules may be stable, but their anchorage to the spindle apparently is not: in unstable arrangements, kinetochore microtubules often appear to have come loose at their poleward ends (Nicklas, 1985). The molecular basis of this anchorage instability is an interesting problem for the future.

Selection of variants
How does tension stabilize appropriate arrays of microtubules? This is a prime question for further work. A particularly intriguing possibility will serve to illustrate the novel mechanisms that may be involved.

Mechanical force has thermodynamic effects on microtubule assembly and stability (Hill & Kirschner, 1982). Calculations suggest that a tension force of $10^{-6}$ dyne per microtubule would suffice to enhance stability greatly (Hill & Kirschner, 1982; Nicklas, 1988b; for an alternative view, see Buxbaum & Heidemann, unpublished). Recently, the forces acting on single microtubules in mitosis have been measured (for a review see Nicklas, 1988b). A value of $3 \times 10^{-6}$ dyne per microtubule was measured in the experiments in which chromosomes in the configuration in Fig. 2 were stretched with a microneedle to stabilize the microtubule array. Values lower than $10^{-6}$ were sometimes measured in configurations stabilized by the spindle (i.e. chromosomes in the orientation shown in Fig. 1), but clearly the spindle can and does produce forces in the range calculated to have a substantial thermodynamic effect on microtubule stability. Further work is needed to determine directly the effect of forces on microtubule assembly and to explore alternative molecular explanations for the stabilizing effects of tension.

Finally, it is interesting to contrast chromosome distribution with DNA replication. Accuracy in both processes depends on highly effective ways of correcting inevitable errors. The way errors are corrected is fundamentally different in the two processes. For chromosome distribution, there is no specific correction process, only quasi-random change until a favoured, more stable state is reached, by whatever path. In sharp contrast, correction of errors in DNA replication follows a specific path made possible in bacteria by a polymerase/nuclease complex that can remove as well as add nucleotides. As replication proceeds, any incorrect nucleotide is detected, excised, and the correct one is added. This reads like a prescription for precision and indeed it is. Why has no similarly exact prescription for the detection and correction of mistakes in mitosis evolved? The evident answer is scale: the errant structures in mitosis probably are simply too large for enzymes to encompass. A large enzyme complex might be able to examine a single microtubule–kinetochore junction for any error, but in the configuration shown in Fig. 2, for instance, each junction is perfectly normal. The error involves microtubules that lie many micrometres apart and extend toward the same rather than to opposite poles. It would require enzyme complexes very much larger than any known to grapple with so large a structure. Hence, faultless chromosome distribution must be left, in part, to chance. The evolutionary achievements that make chance so effective are no less remarkable than
the enzymes of DNA repair. The first is an energy-consuming form of microtubule instability that enhances the probability of stumbling on the proper orientation. The second achievement is a mechanism in which the normal mitotic forces are elegantly used to stabilize the proper orientation as well as to separate the chromosomes.

References


