may be related: is mirroring of familiar actions also involved in learning by copying, and can any non-human animal learn by copying? It is not impossible that the two kinds of imitation, whose differing functions hint at separate evolutionary histories, rely on quite unrelated brain mechanisms. Learning by copying involves hierarchical construction of a behavioural program [9], just as does linguistic syntax, so a common origin is possible, uniquely on the human line of evolution. But it is tempting (and parsimonious) to relate the powerful properties of the mirror neuron system to the perceptual deciphering required in learning by copying.

Some theorists view the imitative learning of a 2 year old child as simply an extension of neonatal imitation [17]: as social mirroring develops, it enables learning by copying. This simple scheme leaves unexplained why macaque monkeys do not follow the same developmental path, and seem unable to learn by imitation. Alternatively, it has been suggested that imitative learning co-opts the perceptual decomposition power of the mirror neuron system, evolved originally in response to social needs, for a new purpose [10]. In animals that are able to construct new behavioural routines by hierarchical planning, then the sequence of actions picked out by the successive firing of mirror neurons becomes far more useful, as the basis for constructing a novel, complex skill.

Monkeys and very young children lack such hierarchical constructional ability, so their imitation is restricted to social mirroring. Although learning by copying has proved difficult to study experimentally in animals, observational evidence implies that great apes learn their elaborate feeding skills by imitation [18]. If so, then the evolutionary origins of syntactical skill may lie earlier than the advent of language itself, in the feeding needs of our ancient ancestors and their flexible co-option of an existing neural system [19].

References

1. Galef, B.G. (1988). Imitation in animals: History, definitions, and interpretation of data from the psychological laboratory. In Social Learning: Psychological and Biological Perspectives. T. Zentall and B.G. Galef, Jnr., Eds. (Erlbaum: Hillsdale, New Jersey.), pp. 3-28.
2. Meltzoff, A.N., and Moore, M.K. (1983). Newborn infants imitate adult facial gestures. Child Dev. *54*, 702–709.
3. Chartrand, T.L., and Bargh, J.A. (1999). The Chameleon effect: The perception-behavior link and social interaction. J. Pers. Soc. Psychol. *76*, 893–910.
4. Meltzoff, A.N., and Prinz, W., eds. (2002). The imitative mind: development, evolution, and brain bases. (Cambridge University Press: Cambridge.)
5. Hurley, S., and Chater, N. (2004). Perspectives on imitation: from mirror neurons to memes. (Cambridge MA: MIT Press.)
6. Rizzolatti, G., Fadiga, L., Fogassi, L., and Gallese, V. (1996). Premotor cortex and the recognition of motor actions. Brain Res. *3*, 131–141.
7. Gallese, V., Fadiga, L., Fogassi, L., and Rizzolatti, G. (1996). Action recognition in the premotor cortex. Brain *119*, 593–609.
8. Visalberghi, E., and Fragaszy, D.M. (1990). Do monkeys ape? In Language and Intelligence in Monkeys and Apes. S.T. Parker and K.R. Gibson, Eds. (Cambridge University Press: Cambridge.), pp. 247-273.
9. Byrne, R.W., and Russon, A.E. (1998). Learning by imitation: a hierarchical approach. Behav. Brain Sci. *21*, 667–721.
10. Byrne, R.W. (2003). Imitation as behaviour parsing. Phil. Trans. Roy. Soc. Lond. (B) *358*, 529–536.
11. Byrne, R.W. (1995). The thinking ape: evolutionary origins of intelligence. (Oxford: Oxford University Press.)
12. Paukner, A., Anderson, J.R., Borelli, E. , Visalberghi, E., and Ferrari, P.F. (2004). Macaques (*Macaca nemestrina*) recognize when they are being imitated. Biology Letters, DOI: 10.1098/rsbl.2004.0291.
13. Rizzolatti, G., Fadiga, L., Fogassi, L., and Gallese, V. (2002). From mirror neurons to imitation: facts and speculations. In The imitative mind: development, evolution, and brain bases. A. Meltzoff and W. Prinz, Eds. (Cambridge University Press: Cambridge.), pp. 247-266.
14. Whiten, A., Custance, D.M., Gomez, J.-C., Teixidor, P., and Bard, K.A. (1996). Imitative learning of artificial fruit processing in children (*Homo sapiens*) and chimpanzees (*Pan troglodytes*). J. Comp. Psychol. *110,* 3–14.
15. Tomasello, M., Savage-Rumbaugh, E.S., and Kruger, A.C. (1993). Imitative learning of actions on objects by children, chimpanzees, and enculturated chimpanzees. Child Dev. *64*, 1688–1705.
16. Custance, D., Whiten, A., and Fredman, T. (1999). Social learning of an artificial fruit task in capuchin monkeys (*Cebus apella*). J. Comp. Psychol. *113*, 13–23.
17. Meltzoff, A.N. (2002). Elements of a developmental theory of imitation. In The imitative mind. A.N. Meltzoff and W. Prinz, eds. (Cambridge University Press: Cambridge.), pp. 19-41.
18. Byrne, R.W. (2005). Detecting, understanding, and explaining animal imitation. In Perspectives on imitation: from mirror neurons to memes. S. Hurley and N. Chater, eds. (MIT Press: Cambridge, MA.), pp. 255-282.
19. Byrne, R.W. Parsing behaviour. A mundane origin for an extraordinary ability? In The Roots of Human Sociality (in press). S. Levinson and N. Enfield, eds. (Berg: Oxford.)

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Chromosome Segregation: Seeing Is Believing

For chromosome segregation in mitosis, each centromere directs assembly of a complex, proteinaceous structure — the kinetochore, which connects the chromosome to microtubules of the mitotic spindle. A recent study has provided important new insights into the mechanism by which kinetochores capture spindle microtubules.

Kerry Bloom

The mechanisms that contribute to accurate chromosome segregation are manifold and complex. The players have been identified from early genetic mapping studies, cytological observations of autoimmune patients and genetic screens in model organisms. Centromeres are responsible for directing the assembly of a complex proteinaceous structure, the kinetochore. The kinetochore provides the linkage between the chromosome and microtubules of the mitotic spindle. How the kinetochore engages the microtubule, promotes the complex oscillatory dance by which replicated chromosomes attain correct attachment, yet maintain the ability to correct errors has been the subject of intensive study.

This is a complex process, but the idea that we might be able to dissect it by genetic and molecular analysis was given a

boost when it was realised that a 'point centromere' in budding yeast [1] — a chromosome with a single microtubule–chromosome attachment — exhibits similar oscillatory behavior as the 20-30 microtubule–chromosome attachments in a mammalian cell [2]. This conjecture has been realized in a *tour de force* of experimental biology reported recently by Tanaka *et al*. [3]. Their success has come from the judicious use of a combination of approaches: high resolution digital microscopy; fusion of multiple copies of the green fluorescent protein (GFP) to key kinetochore components; and a simple genetic switch to regulate centromere activity and therefore the segregation properties of a single chromosome.

The yeast kinetochore is composed of more than 70 proteins organized into discrete subcomplexes [4,5]. The breakthrough in monitoring chromosome movement came from the use of an indirect labeling strategy employing integrated copies of lac operator and lac-repressor-GFP fusion proteins to visualize the operator at specific chromosomal loci in live cells.

In metaphase of mitosis, the centromeres of replicated sister chromatids are bi-oriented on the spindle, and appear as two foci. The kinetochore microtubules are dynamic, resulting in constant oscillatory centromere movements. Mechanisms involving tension-dependent microtubule rescue act to preserve the average separation of 0.6 μm (~1/2 total spindle length) between separated centromeres. Centromere oscillations continue, allowing all 16 chromosomes to become bi-oriented — the metaphase configuration — before anaphase ensues.

The major unsolved problems concern the mechanisms that lead to the metaphase configuration and how metaphase is maintained. The initial events in centromere attachment have been directly visualized, and the key players in these processes have been identified [3].

Tanaka *et al*. [3] were able to visualize these events because they found conditions where most of the centromeres were already aligned, save for one malpositioned centromere. Yeast centromeres can be inactivated in *cis* by transcription of a proximal promoter [6]. In a still somewhat mysterious process — probably involving the targeted degradation of centromere components — the promoter inactivates the microtubule binding function of the centromere. By arresting cells in mitosis, the bulk of the chromosomes are bi-oriented, while the chromosome with an inactive centromere is detached.

The last centromere is activated on switching to conditions that repress its proximal promoter, and the initial events of centromere attachment of this 'lost' chromosome can then be directly visualized. The initial attachments were seen to proceed via interactions between the kinetochore and the side of the microtubule [3]. This behavior is similar to lateral attachments observed in tissue culture [7,8] for the initial encounters between mammalian chromosomes and microtubules.

Initial Encounters of the Lateral Kind

Initial kinetochore attachment with the microtubule lattice is facilitated by mechanisms that favor microtubule growth. This growth is stimulated by a class of microtubule plus-end binding proteins, known as +TIPs, and the small GTPase Ran. One of these microtubule binding proteins, Stu2 (XMAP215/ch-TOG) had previously been found to regulate microtubule dynamics [9,10]. In the absence of Stu2, Tanaka *et al.* [3] found a reduced density of nuclear microtubules and decreased efficiency of attachment between an unattached chromosome and microtubule.

In the absence of core kinetochore components — Ndc10, Ndc80, Mtw1, Ctf19 — there was reduced efficiency of kinetochore–microtubule encounters, but a normal frequency of nuclear microtubule extension. Thus the 'global' regulation of microtubule dynamics by Stu2p, +TIPs and Ran are critical for ensuring the length and number of microtubules sufficient for kinetochore capture.

Once a lateral attachment has been formed, the chromosome migrates toward the pole. Interestingly, the rate of chromosome movement (0.5–2.0 μm min^{-1}) is slower than that of microtubule depolymerization (2–3 μm min^{-1}), so what keeps the microtubule from depolymerizing through the site of chromosome attachment? In an astounding observation, Tanaka *et al.* [3] found that Stu2 migrates to the plus-end of an attached microtubule. Stu2 is localized to microtubules and centromeres that have not yet been captured. Upon capture, Stu2 appears at the microtubule plus-end, where it prevents depolymerization. The inference is that Stu2 mediates kinetochore-dependent microtubule rescue.

One possibility is that Stu2 on the recently attached kinetochore migrates from the kinetochore to the microtubule plus-end. In this way, the kinetochore regulates the stability of the microtubule to which it is attached. The technique of fluorescence recovery after photobleaching (FRAP) applied to centromere-bound Stu2 should show whether Stu2 released from the attached kinetochore migrates to the plus-end of a microtubule. Kinetochores have been shown to exert local control of the dynamics of attached microtubules [10]. Perhaps the initial lateral interaction results in a conformational change that signals kinetochore bound +TIPs to disperse.

This finding raises an additional consideration for understanding spatial regulatory networks. Various lines of evidence have indicated that Stu2 promotes microtubule growth or shortening [10–13]. The finding that Stu2 coming from kinetochores promotes microtubule rescue [3], while Stu2 coming from elsewhere may promote microtubule shortening

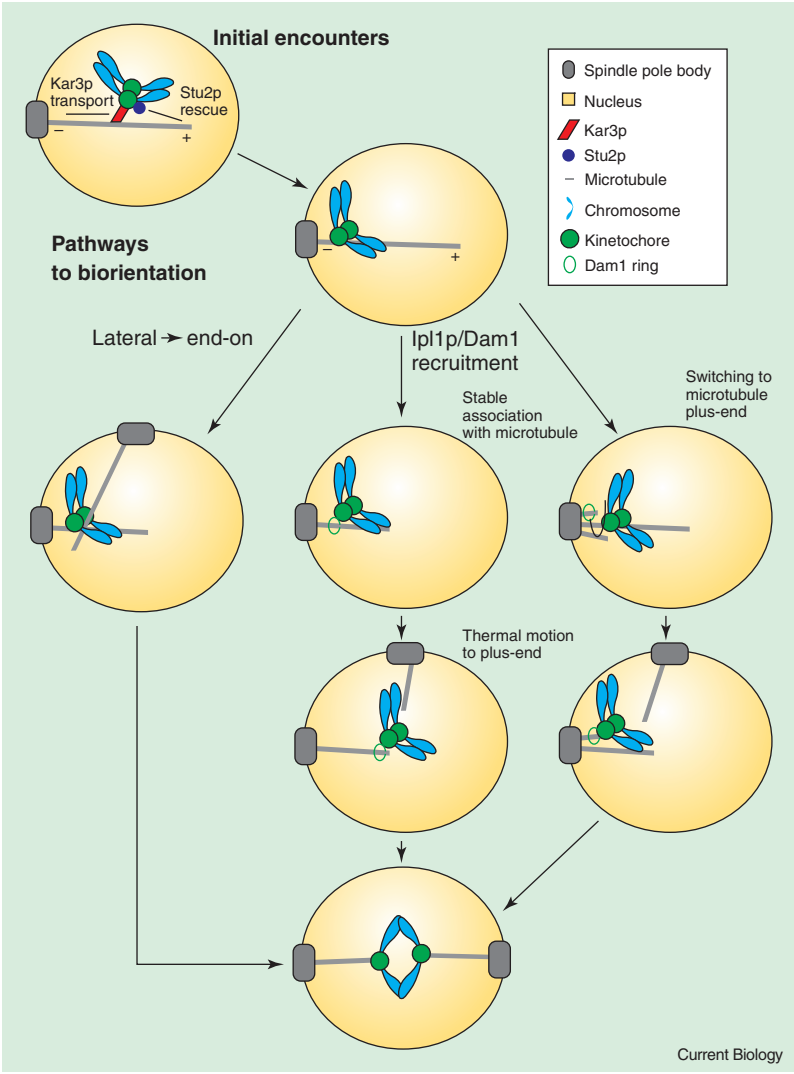

Figure 1. Chromosome capture and bi-orientation.

A kinetochore encounters the lattice of a microtubule and gets transported to the spindle pole via the minus-end directed kinesin Kar3 (top). Stu2 is recruited to microtubule plus-ends to promote rescue. To achieve bi-orientation, both sister kinetochores must attach to a microtubule. Once one kinetochore is attached, its sister may encounter a microtubule from the opposite spindle pole (lateral → end-on) and as the poles separate, the attachment matures into an end-on attachment (bottom). Alternatively, an attached kinetochore may recruit the Ipl1 kinase and/or the Dam1 ring (Ipl1/Dam1 recruitment), where thermal motion of the Dam1 ring favors end-on attachments and subsequently sister kinetochore attachment. A variation of the Ipl1/Dam1 recruitment model (Ipl1/Dam1 recruitment, far right) is that kinetochores 'switch' to microtubule plus-ends that may be highly concentrated at the pole, followed by sister kinetochore attachment.

(or the release of shortening factors [14]) indicates that perhaps the history of a protein's location is important: not only is there spatial segregation of function, but spatial 'memory'.

Lateral Transport to the Pole

The minus-end directed kinesin Kar3 contributes to poleward chromosome movement: mutational disruption of the ATP hydrolysis site in Kar3 leads to a significant increase in 'pauses' along the way. This cannot be the only mechanism of chromosome movement, however, as while *kar3* mutant cells are sick, they are still viable, and chromosome translocation can be observed in *kar3* mutants.

The precise function of Kar3 has been enigmatic, and the Kar3-dependent chromosome translocation observed by Tanaka *et al*. [3] is one of the clearest examples of its role in mitosis. Whether Kar3 is at the kinetochores, overlapping anti-parallel microtubules or microtubule plus-ends [15], or all of the above, is still not clear.

Docking and Bi-Orientation

Once a kinetochore has migrated to the pole, sister centromeres become randomized and bi-oriented by attachment to a microtubule from the opposite pole (Figure 1). Interestingly, this transition requires an additional set of proteins, the Dam1 complex and Ipl1 [1].

This is a clear case of different complexes being required for specific processes — lateral interactions versus bi-orientation — and raises the question of how the transition to bi-orientation is established. If the lateral interactions take place early in the formation of the spindle, microtubules from the adjacent spindle pole may interact with the sister kinetochore (see Figure 1 and [3]). As the poles separate, the lateral interactions may mature into end-on interactions. Alternatively, once Dam1 and Ipl1 are recruited, the biochemistry of these interactions may favor end-on interactions [16,17].

Maintaining Bi-Orientation and the Metaphase Configuration

Even when bi-oriented, sister kinetochores continue to oscillate, though they maintain an average position midway between the poles. A given kinetochore makes very few excursions to the opposite pole [18]. What are the mechanisms that give rise to metaphase and do they relate to the mechanisms described for initial encounters?

An important consequence of bi-orientation is the tension generated between replicated but unseparated sister chromatids. This tension may promote rescue of microtubule depolymerization. In this view, attached kinetochore microtubules depend on the stretch of chromatin between sister kinetochores for rescue [19], an effect antagonized by increased kinetochore

microtubule catastrophe at the spindle equator.

Local control of microtubule dynamics by the kinetochore could depend on a combination of a spatial cue, possibly provided by release of Stu2 from the kinetochore, and/or a mechanical tension-sensing cue. This type of regulation could result in highly dynamic kinetochore microtubules while orchestrating the organization of kinetochores into a metaphase configuration with remarkable fidelity to one spindle-half.

With the direct visualization of initial encounters and the components in hand, the prospect of understanding the molecular basis for the 'dance of the chromosomes' is on the horizon.

References

1. Biggins, S., and Walczak, C.E. (2003). Captivating capture: how microtubules attach to kinetochores. Curr. Biol. *13*, R449–R460.
2. Pearson, C.G., Maddox, P.S., Salmon, E.D., and Bloom, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. *152*, 1255–1266.
3. Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E.K., Prescott, A.R., Antony, C., and Tanaka, T.U. (2005). Molecular mechanisms of kinetochore capture by spindle microtubules. Nature *434*, 987–994.
4. Westermann, S., Cheeseman, I.M., Anderson, S., Yates, J.R., 3rd, Drubin, D.G., and Barnes, G. (2003). Architecture of the budding yeast kinetochore reveals a conserved molecular core. J. Cell Biol. *163*, 215–222.
5. De Wulf P., McAinsh A.D., Sorger P.K.. (2003). Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev. *17*, 2902–2921.
6. Hill, A., and Bloom, K. (1987). Genetic manipulation of centromere function. Mol. Cell. Biol. *7*, 2397–2405.
7. Merdes, A., and De Mey, J. (1990). The mechanism of kinetochore-spindle attachment and polewards movement analyzed in PtK2 cells at the prophase-prometaphase transition. Eur. J. Cell Biol. *53*, 313–325.
8. Rieder, C.L., and Alexander, S.P. (1990). Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. J. Cell Biol. *110*, 81–95.
9. Kosco, K.A., Pearson, C.G., Maddox, P.S., Wang, P.J., Adams, I.R., Salmon, E.D., Bloom, K., and Huffaker, T.C. (2001). Control of microtubule dynamics by Stu2p is essential for spindle orientation and metaphase chromosome alignment in yeast. Mol. Biol. Cell *12*, 2870–2880.
10. Pearson C.G., Maddox P.S., Zarzar T.R., Salmon E.D., and Bloom, K. (2003). Yeast kinetochores do not stabilize Stu2p-dependent spindle microtubule dynamics. Mol. Biol. Cell. *14*, 4181–4195
11. Severin, F., Habermann, B., Huffaker, T., and Hyman, T. (2001). Stu2 promotes mitotic spindle elongation in anaphase. J. Cell Biol. *153*, 435–442.
12. Shirasu-Hiza, M., Coughlin, P., and Mitchison, T. (2003). Identification of XMAP215 as a microtubule-destabilizing factor in Xenopus egg extract by biochemical purification. J. Cell Biol. *161*, 349–358.
13. van Breugel, M., Drechsel, D., and Hyman, A. (2003). Stu2p, the budding yeast member of the conserved Dis1/XMAP215 family of microtubule-associated proteins is a plus end-binding microtubule destabilizer. J. Cell Biol. *161*, 359–369.
14. Usui, T., Maekawa, H., Pereira, G., and Schiebel, E. (2003). The XMAP215 homologue Stu2 at yeast spindle pole bodies regulates microtubule dynamics and anchorage. EMBO J. *22*, 4779–4793.
15. Maddox, P.S., Stemple, J.K., Satterwhite, L., Salmon, E.D., and Bloom, K. (2003). The minus end-directed motor Kar3 is required for coupling dynamic microtubule plus ends to the cortical shmoo tip in budding yeast. Curr. Biol. *13*, 1423–1428.
16. Westermann, S., Avila-Sakar, A., Wang, H.W., Niederstrasser, H., Wong, J., Drubin, D.G., Nogales, E., and Barnes, G. (2005). Formation of a dynamic kinetochore- microtubule interface through assembly of the Dam1 ring complex. Mol. Cell *17*, 277–290.
17. Miranda J.J., De Wulf P., Sorger P.K., and Harrison S.C. (2005). The yeast DASH complex forms closed rings on microtubules. Nat. Struct. Mol. Biol., *12*, 138–143.
18. Pearson, C.G., Yeh, E., Gardner, M., Odde, D., Salmon, E.D., and Bloom, K. (2004). Stable kinetochore-microtubule attachment constrains centromere positioning in metaphase. Curr. Biol. *14*, 1962–1967.
19. Gardner, M.K., Pearson, C.G., Sprague, B.L., Bloom, K., Salmon, E.D., and Odde, D.J. (2005). Tension-dependent regulation of kinetochore microtubule dynamics can explain metaphase congression in yeast. Mol. Biol. Cell, Jun 1 [epub ahead of print].

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Auditory Plasticity: Vocal Output Shapes Auditory Cortex

Studies in humans and songbirds have revealed a close link between vocal output and hearing. Now experiments in marmosets have shown that self-generated vocalizations can modulate the activity of neurons in the auditory cortex and even remodel their response properties.

Andrew J. King

The auditory areas of the brain are potentially responsive not only to sounds arising from external sources but also to self-generated sounds. This raises the question of what effect an animal's own vocalizations have on central auditory processing. While studies in a range of species have shown that activity in the auditory system is modulated during vocal production, a recent report [1] has revealed that changes in vocal output can induce a much longer-term change in the way in which cortical neurons process these complex sounds.

Hearing Self-Generated Sounds

It clearly makes sense that our perception of external sounds should not be masked by the additional acoustical input provided by one's own voice. By attenuating the input to the cochlea, contraction of the middle ear muscles prior to vocalizing — the middle ear reflex — helps to reduce the impact of self-produced sounds. Moreover, studies in humans [2,3] have shown that cortical responses evoked during speaking are smaller than those generated during passive listening to the same sounds played over headphones. This is also supported by the results of electrophysiological recordings in other species [4–6], which have shown that an animal's own vocalizations can inhibit the responses of auditory neurons.

Despite these suppressive effects, an ability to detect self-produced sounds appears to be critical for the control of vocal output. This has been demonstrated in humans [7–9] and other species [10–12] by the compensatory changes in vocal production that result when