found in the yeast Bro1 and mammalian Alix proteins, which are recruited to a complex involved in the sorting of cargo into these inward budding profiles [19] — has a structure that resembles a 'boomerang' with a convex face that contains a highly positively charged region that may assist binding to acidic phospholipids [20]. With the characterisation of the ability of the IM domain to generate negative membrane curvature through the properties of its convex surface, it would certainly be of interest to establish whether the convex surface of the Bro1 domain is also able to generate and/or scaffold negative curvature within the endocytic network.

References

1. Zimmerberg, J., and Kozlov, M.M. (2006). How proteins produce cellular membrane curvature. Nat. Rev. Mol. Cell Biol. *7*, 9–19.
2. Farsad, K., Ringstad, N., Takei, K., Floyd, S.R., Rose, K., and De Camilli, P. (2001). Generation of high curvature membranes mediated by direct endophilin bilayer interactions. J. Cell Biol. *155*, 193–200.
3. Ford, M.G., Pearse, B.M., Higgins, M.K., Vallis, Y., Praefcke, G.J., Evans, P.R., and McMahon, H.T. (2002). Curvature of clathrin-coated pits driven by epsin. Nature *419*, 361–366.
4. Lee, M.C., Orci, L., Hamamoto, S., Futai, E., Ravazzola, M., and Schekman, R. (2005). Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. Cell *122*, 605–617.
5. Peter, B.J., Kent, H.M., Mills, I.G., Vallis, Y., Butler, P.J., Evans, P.R., and McMahon, H.T. (2004). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science *303*, 495–499.
6. Gallop, J.L., Jao, C.C., Kent, H.M., Butler, P.J.G., Evans, P.R., Langen, R., and McMahon, H.T. (2006). Mechanism of endophilin N-BAR domain-mediated membrane curvature. EMBO J. *25*, 2898–2910.
7. Itoh, T., and De Camilli, P. (2006). BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane-cytosol interfaces and membrane curvature. Biochim. Biophys. Acta *1761*, 897–912.
8. Mattila, P.K., Pykalainen, A., Saarikangas, J., Paavilainen, V.O., Vihinen, H., Jokitalo, E., and Lappalainen, P. (2007). Missing-in-metastasis and IRSp53 deform $PI(4,5)P_2$-rich membranes by an inverse BAR domain-like mechanism. J. Cell Biol. *176*, 953–964.
9. Millard, T.H., Bompard, G., Heung, M.Y., Dafforn, T.R., Scott, D.J., Machesky, L.M., and Futterer, K. (2005). Structural basis of filopodia formation induced by the IRSp53/MIM homology domain of human IRSp53. EMBO J. *24*, 240–250.
10. Lee, S.H., Kerff, F., Chereau, D., Ferron, F., Klug, A., and Dominguez, R. (2007). Structural basis for the actin-binding function of Missing-in-metastasis. Structure *15*, 145–155.
11. Casal, E., Federici, L., Zhang, W., Fernandez-Recio, J., Priego, E.-M., Miguel, R.N., DuHadaway, J.B., Prendergast, G.C., Luisi, B.F., and Laue, E.D. (2006). The crystal structure of the BAR domain from human Bin1/Amphiphysin II and its implications for molecular recognition. Biochemistry *45*, 12917–12928.
12. Suetsugu, S., Kurisu, S., Oikawa, T., Yamazaki, D., Oda, A., and Takenawa, T. (2006). Optimization of WAVE2 complex-induced actin polymerization by membrane-bound IRSp53, PIP_3, and Rac. J. Cell Biol. *173*, 571–585.
13. Suetsugu, S., Murayama, K., Sakamoto, A., Hanawa-Suetsugu, K., Seto, A., Oikawa, T., Mishima, C., Shirouzu, M., Takenawa, T., and Yokoyama, S. (2006). The RAC binding domain/IRSp53-MIM homology domain of IRSp53 induces RAC-dependent membrane deformation. J. Biol. Chem. *281*, 35347–35358.
14. Yamagishi, A., Masuda, M., Ohki, T., Onishi, H., and Mochizuki, N. (2004). A novel actin bundling/filopodium-forming domain conserved in insulin receptor tyrosine kinase substrate p53 and missing in metastasis protein. J. Biol. Chem. *279*, 14929–14936.
15. Dawson, J.C., Legg, J.A., and Machesky, L.M. (2006). BAR domain proteins: a role in tubulation, scission and actin assembly in clathrin-mediated endocytosis. Trends Cell Biol. *16*, 493–498.
16. Merrifield, C.J., Qualmann, B., Kessels, M.M., and Almers, W. (2004). Neural Wiskott Aldrich Syndrome Protein (N-WASP) and the Arp2/3 complex are recruited to sites of clathrin-mediated endocytosis in cultured fibroblasts. Eur. J. Cell Biol. *83*, 13–18.
17. Habermann, B. (2004). The BAR-domain family of protein: a case of bending and binding? EMBO Rep. *5*, 250–255.
18. Disanza, A., Mantoani, S., Hertzog, M., Ggerboth, S., Frittoli, E., Steffeb, A., Berhoerster, K., Kreienkamp, H.-J., Milanesi, F., Di Fiore, P.P., *et al*. (2006). Regulation of cell shape by Cdc42 is mediated by the synergic actin-bundling activity of the Eps8-IRSp53 complex. Nat. Cell Biol. *8*, 1337–1347.
19. Slagsvold, T., Pattni, K., Malerod, L., and Stenmark, H. (2006). Endosomal and non-endosomal functions of ESCRT proteins. Trends Cell Biol. *16*, 317–326.
20. Kim, J., Sitaraman, S., Hierro, A., Beach, B.M., Odorizzi, G., and Hurley, J.H. (2005). Structural basis for endosomal targeting by the Bro1 domains. Dev. Cell *8*, 937–947.

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Color Vision: Mice See Hue Too

A transgenic mouse has been generated with three cone types, instead of the normal murine two. Remarkably, some of these mice use the extra cone to make trichromatic color discriminations similar to those that are the basis of human color vision.

Bevil R. Conway

Normal mice have just two cone pigments, unlike humans who usually have three. Our extra cone colors the world in a way that mice can only dream of… until now [1]. A few years ago, two groups [2,3] independently generated a transgenic mouse that expresses the human red retinal pigment, in addition to the native mouse green and blue pigments, giving the mouse a trichromatic retina. The burning question has been whether these mice use the extra pigment to measure differences in spectral distribution — do they see in red-green color? Many scientists, including me, would probably have guessed not, because color vision involves all sorts of specialized neural circuits, both in the retina and in the cortex [4,5], that would seem to require more than a single genetic switch to invent. But Jacobs *et al.* [1] have now shown with careful psychophysical experiments that a fraction of these transgenic mice are indeed trichromatic.

Color vision comes about by a comparison of the relative activities of different cone types, a calculation typified by cells with cone-opponent receptive fields [4,5]. In addition to short-wave sensitive cones, mammals typically have one additional cone type sensitive to longer wavelengths. Somewhere around 30–40 million

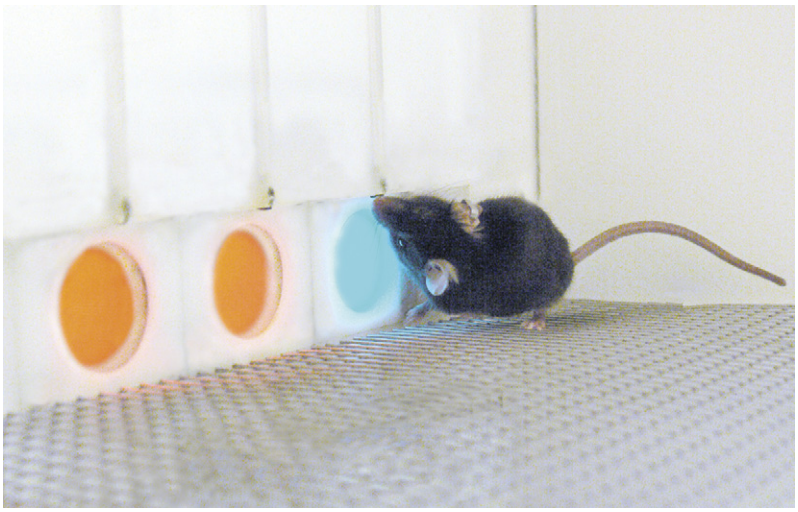

Figure 1. Transgenic mouse performing a red-green color discrimination task. Photo: Kris Krogh.

years ago, the X-linked gene for this cone's pigment was duplicated in the Old World primates, producing the precursors to our present day red and green pigment genes. These genes are 98% identical [6], yielding pigments with peak sensitivities separated by only 30 nm. Although this is a small fraction of the ~380–700 nm range of the visible spectrum, it is sufficient to introduce a new dimension in color, enabling us to see greens, reds and yellows — colors indistinguishable to many color-blind people (those who lack the red or green pigment gene) and to almost all other mammals besides Old World primates.

New World monkeys, such as squirrel monkeys, have color vision that is intermediate between most other mammals and Old World trichromats. New World monkeys have only one X-linked pigment gene, but because this gene shows allelic variation, females can sometimes carry two different genes, one on each of their X chromosomes. X-inactivation randomly shuts off one or the other X chromosome in each cone, so the retinas of female New World monkeys can be trichromatic. Quite remarkably, these special females have red-green color vision [7]. As with the mice, one wonders how this is possible, if color vision requires elaborate neural circuitry to keep the cone signals distinct. One popular idea is that the color computations take advantage of neural machinery already present in the monkeys, machinery that evolved for a different purpose — high-resolution form vision. High acuity vision depends on retinal neurons with center-surround antagonistic receptive fields, called midget cells. A given midget cell in the fovea derives its high acuity by sampling the activity of just a single cone cell, and by comparing this to the activity of surrounding cones. By introducing a new cone type, midget cells could become color cells, almost by accident, comparing the spectral sensitivity of the pure central cone with the average of neighboring cones.

Using knock-in genetic engineering, Jacobs *et al.* [1] have effectively created a mouse with a retina like that of a New World monkey, with one very important difference: mice do not have well-developed form vision, and do not have midget cells. Mice do not already have the hardware that many assume is a critical first step to the evolution of color vision. Despite this, three of the five transgenic heterozygote female mice tested by the authors could discriminate reds from greens, showing color matches remarkably similar to humans (Figure 1).

This result shows how powerful a single genetic mutation can be in generating a potential behavioral advantage. But how is color vision mediated in these mice? Both normal and transgenic mice are capable of brightness discriminations. Brightness is a primitive form of color: both color and brightness are cues to surfaces; and in primates, brightness and color are processed in the same sub-compartment of primary visual cortex, the cytochrome oxidase blobs. (Cats, incidentally, can make brightness discriminations and have cytochrome oxidase blobs, yet lack red-green color vision.) One possibility is that color vision in the transgenic mice piggybacks on brightness calculations. But it remains a mystery how this is, or could be, implemented at the neural level. Do normal mice have dedicated brightness-detecting retinal ganglion cells, which become co-opted to handle red-green color? Color (and brightness) vision is of relatively low acuity, so the retina would not require many of them. Regardless of the mechanism, the presence of red-green color vision in an animal that lacks midget cells resurrects the off-beat idea that Old World primates do not depend on the midget system for red-green color either [8], but rather on some as yet unidentified coarse-grained non-midget red-green cell analogous to the primate blue-yellow retinal cell, which would expose the apparent color-coding of midget cells as a red herring.

The receptive fields of mouse retinal ganglion cells are large, having centers that pool responses of many cones. The cone mosaic of the transgenic mice is patchy, in a pattern that varies from animal to animal, just as it does from person to person. The retinas of color-sighted transgenic mice have not been studied in detail, leaving open the possibility that the transgenic mice that develop color vision have cone patches of sizes that match those of ganglion cell receptive-field centers, producing color-responsive retinal neurons through the same mechanism proposed for midget cells.

Jacobs *et al.* [1] suggest that it is the mice with balanced cone ratios that develop color. Balanced cone ratios presumably sculpt patch size, although in humans there is little or no correlation between cone ratios and color vision ([9], but see [10]). Moreover, although the patchiness increases the variability in cone ratios that feed different ganglion cells, of the few dozen cells tested (in transgenic mice in which behavior was not tested), none were cone opponent [2]. This is why many believed these mice would not be able to see red-green color.

Leaving aside the possibility that an as yet uncharacterized type of ganglion cell is responsible for red-green color (in mouse and/or primate), the task of comparing cone signals would seem to be taken up by stages of visual processing subsequent to the retina. Primate primary visual cortex contains specialized cone-opponent cells; perhaps the cortex of the transgenic mice develops them, too, enabled by the same plasticity that produces visual receptive fields in auditory cortex when retinal signals are forced to crosswire [11]. Color receptive fields in macaque are push-pull, a complex structure: the center of a red-ON cell, for example, is not only excited by the activity of red cones and suppressed by the activity of green cones, but also excited by a decrease in activity of green cones and suppressed by a decrease in activity of red cones [12]. Such a receptive field structure could be constructed from midget cells, or hypothetical non-midget red-green cells, or even non-cone-opponent ganglion cells that simply have different cone ratios, like those found in the retinas of the transgenic mice.

Even if a specific cone ratio is necessary, it might not be sufficient for the mice to develop color. Some transgenic mice might not assign any behavioral relevance to the incoming signals and therefore not develop the appropriate neural architecture. As Hubel and Wiesel showed in their ocular dominance plasticity experiments, the brain will disregard the input from a perfectly healthy eye if the neural signals coming from that eye don't make sense to the brain (for example, if the ocular muscles are cut, whacking the eyes out of alignment). A more subtle example in which the brain edits incoming signals is shown by the color abilities of native speakers of languages that do not distinguish certain colors, like blue and green. Speakers of these languages have impoverished color discrimination within the relevant part of the spectrum ([13] but see [14]), despite the fact that all humans have essentially the same genetics for color. Yet another example: many women have four different cone pigments, yet do not seem to take advantage of the additional color information [15]. One could test the importance of development (and learning) in the case of the transgenic mice, by raising them in environments where the color cues provide significant advantages.

The results reported by Jacobs *et al.* [1] demonstrate the tremendous plasticity of the cortex to respond to incoming signals. Surprising at first, such plasticity is probably the rule rather than the exception. Within some limitations, the brain accommodates novel signals that are the consequence of any number of simple interventions, so long as the interventions happen early enough in development — genetic interventions, as in this case, or physical interventions, as in the case of introducing an extra eye in a frog [16] or forcing sensory neurons to send projections to the wrong bits of brain [11,17]. Synesthesia, brought about by a lack of neural pruning during development [18], also points to the powerful plasticity of the brain, as does the fact that color vision in people is largely unaffected by radically skewed cone ratios yet can be modified by experience [19]. These examples show that a basic feature of brain tissue is its ability to interpret almost any incoming signal, raising all sorts of exciting, if freaky, possibilities. A final tantalizing possibility: could we use these mice to uncover other genes important in establishing red-green color, by looking for differences in gene expression between color-seeing and non-color-seeing transgenic mice, or by interbreeding experiments followed by strong selection of mice that use the color signals, mimicking the co-evolution of fruit-color and primate trichromacy [20]?

References

1. Jacobs, G.H., Williams, G.A., Cahill, H., and Nathans, J. (2007). Emergence of novel color vision in mice engineered to express a human cone photopigment. Science *315*, 1723–1725.
2. Smallwood, P.M., Olveczky, B.P., Williams, G.L., Jacobs, G.H., Reese, B.E., Meister, M., and Nathans, J. (2003). Genetically engineered mice with an additional class of cone photoreceptors: implications for the evolution of color vision. Proc. Natl. Acad. Sci. USA *100*, 11706–11711.
3. Onishi, A., Hasegawa, J., Imai, H., Chisaka, O., Ueda, Y., Honda, Y., Tachibana, M., and Shichida, Y. (2005). Generation of knock-in mice carrying third cones with spectral sensitivity different from S and L cones. Zoolog. Sci. *22*, 1145–1156.
4. Dacey, D.M. (1996). Circuitry for color coding in the primate retina. Proc. Natl. Acad. Sci. USA *93*, 582–588.
5. Conway, B.R. (2001). Spatial structure of cone inputs to color cells in alert macaque primary visual cortex (V-1). J. Neurosci. *21*, 2768–2783.
6. Neitz, M., and Neitz, J. (2000). Molecular genetics of color vision and color vision defects. Arch. Ophthalmol. *118*, 691–700.
7. Jacobs, G.H. (1984). Within-species variations in visual capacity among squirrel monkeys (Saimiri sciureus): color vision. Vision Res. *24*, 1267–1277.
8. Hubel, D., and Livingstone, M. (1990). Color puzzles. Cold Spring Harb. Symp. Quant. Biol. *55*, 643–649.
9. Brainard, D.H., Roorda, A., Yamauchi, Y., Calderone, J.B., Metha, A., Neitz, M., Neitz, J., Williams, D.R., and Jacobs, G.H. (2000). Functional consequences of the relative numbers of L and M cones. J. Opt. Soc. Am. A Opt. Image. Sci. Vis. *17*, 607–614.
10. Hood, S.M., Mollon, J.D., Purves, L., and Jordan, G. (2006). Color discrimination in carriers of color deficiency. Vision Res. *46*, 2894–2900.
11. Roe, A.W., Pallas, S.L., Kwon, Y.H., and Sur, M. (1992). Visual projections routed to the auditory pathway in ferrets: receptive fields of visual neurons in primary auditory cortex. J. Neurosci. *12*, 3651–3664.
12. Conway, B.R., and Livingstone, M.S. (2006). Spatial and temporal properties of cone signals in alert macaque primary visual cortex. J. Neurosci. *26*, 10826–10846.
13. Davidoff, J., Davies, I., and Roberson, D. (1999). Colour categories in a stone-age tribe. Nature *398*, 203–204.
14. Lindsey, D.T., and Brown, A.M. (2002). Color naming and the phototoxic effects of sunlight on the eye. Psychol. Sci. *13*, 506–512.
15. Jordan, G., and Mollon, J.D. (1993). A study of women heterozygous for colour deficiencies. Vision Res. *33*, 1495–1508.
16. Constantine-Paton, M., and Law, M.I. (1978). Eye-specific termination bands in tecta of three-eyed frogs. Science *202*, 639–641.
17. Kahn, D.M., and Krubitzer, L. (2002). Massive cross-modal cortical plasticity and the emergence of a new cortical area

in developmentally blind mammals. Proc. Natl. Acad. Sci. USA *99*, 11429–11434.
18. Hubbard, E.M., and Ramachandran, V.S. (2005). Neurocognitive mechanisms of synesthesia. Neuron *48*, 509–520.
19. Neitz, J., Carroll, J., Yamauchi, Y., Neitz, M., and Williams, D.R. (2002). Color perception is mediated by a plastic neural mechanism that is adjustable in adults. Neuron *35*, 783–792.
20. Regan, B.C., Julliot, C., Simmen, B., Vienot, F., Charles-Dominique, P., and Mollon, J.D. (2001). Fruits, foliage and the evolution of primate colour vision. Phil. Trans. R. Soc. Lond. B *356*, 229–283.

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Mitosis: Springtime for Chromatin

When a eukaryotic cell divides, tension builds at centromeres as spindle forces pull chromosomes toward opposite poles during metaphase. New data show that centromeric chromatin stretches in response to these forces, revealing a mechanical role for chromatin packaging in mitosis.

Lawrence C. Myers and Duane A. Compton

DNA replication yields two identical sister strands, chromatids, which remain associated through cohesion until they separate in mitosis and partition to daughter cells. The microtubule-based mitotic spindle generates force for chromosome segregation. The accuracy of chromosome segregation relies on the attachment of each sister chromatid to spindle microtubules from opposite poles of the spindle (bi-orientation). Centromere-associated structures called kinetochores mechanically link spindle microtubules to chromosomes, permitting force from microtubule-dependent motor proteins — kinesins and dynein — and microtubule polymer disassembly to displace chromosomes. Spindles single-mindedly generate poleward force on kinetochore-bound microtubules throughout all phases of mitosis [1]. That is advantageous in anaphase, where it separates chromatids without equivocation. In metaphase, however, that single-minded behavior causes bi-oriented chromosomes to experience poleward force toward opposite poles simultaneously. The resulting tug-of-war generates tension on centromeres that increases the separation between sister kinetochores on each chromosome. Microtubule-dependent stretching of sister kinetochores has been observed for many years [1]; however, the compliant element of the chromosome or kinetochore was not known. Data reported recently in *Current Biology* [2] indicate that centromeric chromatin stretches in response to spindle force, suggesting an active role for chromatin packaging in mitosis.

Centromeres in budding yeast are defined by a unique 125 base-pair DNA sequence [3]. A nucleosome containing the histone H3 variant Cse4p (CENP-A in mammals) forms on this DNA, and works with other centromere-specific DNA binding proteins to recruit kinetochore components to create the microtubule-attachment site on each chromosome. This centromeric DNA and specialized nucleosome are surrounded by a precisely positioned array of nucleosomes [4]. The strategic placement of nucleosomes suggests a role for chromatin packaging in mitosis, and Bouck and Bloom [2] set out to test that idea by examining mitotic spindles in cells with reduced histone densities. They extinguished histone H3 or H4 expression in G1 phase yeast cells using a regulatable promoter and examined cells in the ensuing mitosis. Reducing histone density did not inhibit bipolar spindle assembly in most cells and chromosomes established and maintained bipolar attachments to spindle microtubules. However, both spindle length (pole-to-pole) and the distance between sister kinetochore clusters increased in cells with fewer histones. These size increases were not caused by reductions in cohesin recruitment, but appeared to be caused by spindle forces, because inactivation of either Cin8p or Kip1p kinesin motors led to a significant reduction in both spindle size and sister kinetochore spacing. Importantly, kinetochore clusters in histone-depleted cells continued to oscillate, indicating that spindle and kinetochore dynamics were not adversely affected by reductions in histone density.

Shortening of sister kinetochore separation in the *Δcin8* and *Δkip1* mutant cells suggests that an elastic element in chromatin resists these microtubule-based motors which provide an outward force. Although an inelastic barrier could set a maximum distance for sister kinetochore separation, it would not be expected to provide a force that shortens separation upon decreasing the outward force. As a starting point for the interpretation, chromatin is modeled as a simple spring that obeys Hooke's Law, $F_s = -kX$, which states that the force exerted by the spring, F_s, is proportional to the distance stretched, X, and a spring constant k. The distance between metaphase sister kinetochores is proposed to be established when a mechanical equilibrium is reached between outward force generators and inward force generators, such as chromatin. On the basis of this model, one possibility is that the chromatin based spring constant decreases upon histone depletion. A second possibility is that chromatin rest length — the total length of DNA available to be stretched outward without appreciable resistance — increases upon histone depletion. Because no significant difference in the