other. However, there are now doubts that UCP-2 and UCP-3 can mediate thermogenesis. An alternative explanation for why UCP-1–deficient mice are not obese is that brown adipose tissue and UCP-1 may not be quantitatively as important for DIT as is often assumed, even in small mammals.

Apart from brown adipose tissue, where else might the extra heat due to increased DIT be produced? Several other tissues and organs (such as liver, kidneys, heart, pancreas) are activated by the SNS in response to diet, but whether they contribute to DIT is unknown (see the figure). Even for skeletal muscle, the body's largest single tissue, evidence for SNS-mediated thermogenesis remains sketchy. SNS activity in the skeletal muscle of rats is unresponsive to starvation and overfeeding, and the addition of norepinephrine to mouse skeletal muscle ex vivo does not stimulate thermogenesis (*8*). In adult humans (where brown adipose tissue is scarce or quiescent), infusion of norepinephrine increases the resting metabolic rate, but there is no detectable increase in thermogenesis in forearm skeletal muscle (*9*). Nonetheless, in adult humans as in rodents, SNS activity can be modulated by short-term under- or overnutrition, as judged by measurements of norepinephrine spillover in blood and urine (*5*). Furthermore, low SNS activity has been shown to be a risk factor for weight gain in Pima Indians (*10*). But the central issue of whether subtle variations in DIT—which over months and years may lead to obesity in some humans but weight maintenance in others—reflect variations in SNS activity still remains to be firmly established in humans.

Our slow progress in understanding human variability in DIT calls into question its adaptive role in guarding against weight gain. In terms of natural selection, it may not be obvious why DIT, a process that "wastes" food energy, should have evolved. One explanation may be that DIT is poorly recruited when individuals are feeding on well-balanced diets, but readily recruited when diets are low in essential nutrients (*3*). According to the late Michael Stock, DIT probably conferred an evolutionary advantage of "homeostatic waste" because it enabled individuals to overeat relatively large quantities of poor-quality food to obtain essential nutrients without the deposition of excess, nonessential energy as fat. Excessive weight gain would be a hindrance to optimal locomotion, hunting capabilities, and the ability to fight or flee. Stock's legacy to this field lies in his proposal that DIT may have evolved as a means of regulating the metabolic supply of essential nutrients (proteins, minerals, vitamins) with only a secondary role in regulating energy balance and body weight (*3*). Indeed, in human subjects who were overfed normal and low-protein diets for 4 weeks, the relatively small individual differences in DIT that accompanied a balanced normal-protein diet were amplified on the protein-deficient diet (*11*). Consequently, short-term overfeeding on low-protein diets could provide a very sensitive method for discriminating between those who are metabolically predisposed to leanness or to fatness. Given the potent impact of protein deficiency on the activation of the SNS and thermogenesis in rodents (*3*, *5*, *11*), it remains to be seen whether low-protein diets can be used to unmask the genetic and metabolic basis of human susceptibility to obesity. Such experiments are likely to pinpoint activation of βAR signaling and SNS activity as important determinants of variations in DIT and resistance to obesity in humans.

It is almost 222 years since Lavoisier asserted that "Life is a combustion." Now, we must simulate the appropriate (unbalanced) dietary conditions under which DIT is recruited in order to understand the physiological and molecular mechanisms that enable the fire of life to "burn brighter in some than in others" (*12*).

References

1. D. S. Miller *et al.*, *Am. J. Clin. Nutr.* **20**, 1223 (1967).
2. C. Bouchard *et al.*, *N. Engl. J. Med.* **322**, 1477 (1990).
3. M. J. Stock, *Int. J. Obes.* **23**, 1105 (1999).
4. E. S. Bachman *et al.*, *Science* **297**, 843 (2002).
5. L. Landsberg *et al.*, *Am. J. Physiol.* **247**, E181 (1984)
6. N. J. Rothwell, M. J. Stock, *Nature* **281**, 31 (1979).
7. M. E. Harper, J. Himms-Hagen, *Biochim. Biophys. Acta* **1504**, 159 (2001).
8. A. G. Dulloo *et al.*, *Int. J. Obes.* **26** (suppl. 2), 46 (2002).
9. A. V. Kurpad *et al.*, *Am. J. Physiol.* **266**, E877 (1994).
10. P. A. Tataranni *et al.*, *Obes. Res.* **5**, 341 (1997).
11. A. G. Dulloo, J. Jacquet, *Int. J. Obes.* **23**, 1118 (1999).
12. A. G. Dulloo, D. S. Miller, *World Rev. Nutr. Diet.* **50**, 9 (1987).

PERSPECTIVES: DEVELOPMENT

Riding the Crest of the Wnt Signaling Wave

Paul Trainor and Robb Krumlauf

The neural crest or "fourth germ layer" of the vertebrate embryo is one of the defining characteristics of evolution and is synonymous with the transition of invertebrates to vertebrates (*1*). The neural crest—which gives rise to the peripheral nervous system, facial skeleton, and melanocytes—is generated at the interface between the surface ectoderm and neural plate of the embryo (see the figure on the next page). The interaction between the surface ectoderm (which gives rise to the epidermis) and the neural plate (which gives rise to the central nervous system) is essential for formation of the neural crest (*2*). Arising from the transition of epithelial cells to mesenchyme, neural crest cells are a pluripotent, migratory population that differentiate into an enormous array of cell types, tissues, and organs (*3*). The multistep process of neural crest development is an excellent model system with which to investigate the events of early embryogenesis, including induction, signaling, migration, differentiation, and patterning. Diverse experimental systems including those of frog and chick have helped to elucidate the complex processes that govern the formation and migration of neural crest cells [reviewed in (*2*)]. Now, on page 848 of this issue, Bronner-Fraser and colleagues (*4*) working in chick embryos disclose that Wnt signaling, and Wnt6 signaling in particular, is crucial for neural crest induction.

Given that the neural crest is highly conserved among vertebrates, why has there been so much trouble in identifying the key molecules that induce its formation? Part of the difficulty is that neural crest formation is intimately associated with the induction of neural tissue itself, and many of the same signals—such as bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and Wnts—have been implicated in the induction of both structures (*2*, *5*). In addition, there are major differences among species regarding the timing of nervous tissue induction; for example, in the chick but not the frog, neural crest seems to be specified before the massive cell migrations that drive the formation of the three embryonic germ layers (gastrulation) (*5*). This makes it extremely difficult to distinguish between primary and secondary events in neural plate and neural crest induction and to determine the extent to which they are coupled and rely on common versus distinct signals.

Although experimental models of neural tissue induction yield controversial re-

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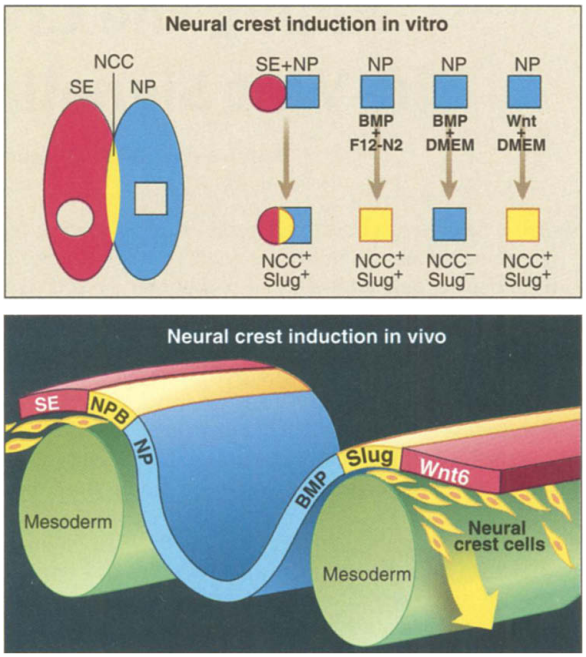
sults, a common feature appears to be a requirement for down-regulation of BMP signaling in the neural plate, together with an increase in BMP signaling in the surface ectoderm (*2, 5*). Attenuation of BMP signaling in the neural plate seems to be accomplished differently depending on the species. For example, direct BMP antagonists—such as noggin, chordin, or follistatin—operate in the frog embryo, whereas FGF or Wnt signaling seems to repress BMP signals in the chick embryo (*5*). These different models raise an interesting possibility with respect to neural crest induction. If high and low levels of BMP signaling help to define non-neural and neural fates, respectively, perhaps intermediate levels of BMP signaling at the interface between the surface ectoderm and neural plate mediate a neural crest fate. In explant cultures of chick embryo neural plate tissue, intermediate levels of BMP signaling do not seem to be sufficient to induce neural crest markers, and therefore other factors in addition to BMPs must be required.

Most assays for neural crest induction are based on activation of the early marker Slug. This protein, a zinc finger transcription factor belonging to the Snail superfamily, is important for multiple steps in neural crest development (*6–8*). Current experimental evidence suggests that neural crest induction may involve a two-step process, with intermediate levels of BMP signaling initiating the first step, and FGFs, retinoic acid, or Wnts providing the signal for the second step (*2, 9*). Chick embryo tissue recombination experiments in vitro illustrate the importance of interactions between the surface ectoderm and neural plate, and are beginning to reveal the timing and source of signals that induce the neural crest (*10–13*). During the early BMP-insensitive phase of neural crest induction, neural crest cells expressing Slug can be induced within the neural plate by signals from the surface ectoderm (see the figure) (*10*). However, during later phases of neural crest induction (when BMP signaling is evident in the dorsal neural tube) the appearance of Slug-positive neural crest cells is BMP-dependent (see the figure) (*11, 12*). The critical question is whether these BMP signals alone are enough to induce neural crest formation.

The new work from the Bronner-Fraser laboratory takes us one step closer to the prize with the demonstration that Wnt6 is both necessary and sufficient for instructing neural crest cell formation in the chick embryo (*4*). The advantage of the avian system is that specific "naïve" tissues can be removed in isolation and then their responses to exogenous signals can be assessed in a culture dish. The investigators set up naïve neural plate explants from chick embryo and cultured them in vitro. They found that Wnt6 was able to induce Slug and other neural crest markers in the explants, whereas BMPs could not unless the explants were supplemented with a richer culture medium containing additional factors (see the figure) (*4*). Conversely, blocking Wnt signaling both in the cultured explants and in the whole avian embryo inhibited expression of the Slug and HNK1 neural crest markers. This analysis also revealed that Wnt6 is expressed specifically in the surface ectoderm at the time of neural crest induction, making Wnt6 the prime candidate for the ectodermal signal that induces neural crest formation.



Seductive induction. Induction of neural crest cells in avian embryos. **(Bottom)** The induction of neural crest cells (NCC; yellow) at the border of the neural plate (NP; blue) and the surface ectoderm (SE; red) can be followed by expression of the Slug protein (yellow). Neural crest induction depends on contact-mediated interactions between the SE and NP. **(Top)** Explants of chick embryo NP tissue, cultured in rich medium (F12-N2) together with BMPs, express the Slug neural crest marker. In chemically defined medium without additives (DMEM), BMPs cannot induce expression of Slug, whereas Wnts can. In vitro, Wnt6 is expressed in the SE, which is adjacent to the NP. (Bottom) In response to Wnt signals from the SE, neural crest cells generated at the NP border (NPB) detach from the neural plate and migrate underneath the ectoderm and over the surface of the mesoderm (green) to their new location in the branchial arches. Here, they give rise to the peripheral nervous system, facial skeleton, and melanocytes.

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Perhaps it is easier to show the inducing capabilities of Wnts in chick embryos because, relative to the frog system. there is a greater temporal separation between neural plate induction and neural crest induction. The new findings, however, are consistent with the neural crest phenotypes obtained in frog embryo experiments in which Wnt pathway components were either overexpressed or blocked. Although in the chick FGF signaling can induce neural crest formation when BMP signaling is blocked, this induction is probably mediated by Wnts because dominant-negative Wnts that antagonize normal Wnt signaling block induction. These findings also tie in with observations in the frog embryo that Wnt signaling components, such as Lef and β-catenin, directly bind to the Slug protein (*14*). This puts Wnt signaling at the very top of the cascade that induces neural crest formation during embryogenesis of the chick and frog.

And where does this leave us? With numerous questions still to be answered! For example, which signaling pathways are activated in naïve neural tissue explants, and which are still active during neural crest induction? Do dominant-negative Wnts block BMP signaling in explants cultured in richer media containing additional factors? Is the ability of Wnt6 to induce neural crest cells in explants affected by inhibitors of BMP or FGF? This experiment would demonstrate whether Wnts control BMP signaling, or whether synergy between these two pathways is required for neural crest formation. That the surface ectoderm and neural plate must interact before neural crest can be induced suggests that perhaps together they provide an appropriate interface for BMP and Wnt6. Besides the Wnts and BMPs themselves, there seems to be an ever-increasing array of cofactors, inhibitors, and modulators of Wnt and BMP signaling that might influence neural crest induction.

The expectation is that mechanisms for neural crest induction should be conserved among species. However, even the new results do not reveal a model of neural crest induction that accounts for data from all species. One explanation for the differences among species may be the timing and nature of the culture assays used to assess neural crest induction. Another possibility is that there are genuine mechanistic differences in signaling and inductive processes among species. Continuing discrepancies highlight just how little we understand neural crest induction, even after

decades of intensive research. We need a broader array of molecular markers to dissect earlier and later steps of neural crest induction, as well as a better understanding of the signals seen by the neural plate that pave the way for participation by the surface ectoderm.

References

1. B. K. Hall, *The Neural Crest in Development and Evolution* (Springer-Verlag, New York, 1999).
2. A. K. Knecht, M. Bronner-Fraser, *Nature Rev. Genet.* **3**, 453 (2002).
3. N. Le Douarin, C. Kalcheim, *The Neural Crest*, J. Bard, P. Barlow, D. Kirk, Eds. (Cambridge Univ. Press, Cambridge, 1999).
4. M. I. García-Castro, C. Marcelle, M. Bronner-Fraser, *Science* **297**, 848 (2002); published online 13 June 2002 (10.1126/science.1070824).
5. C. D. Stern, *Nature Rev. Neurosci.* **2**, 92 (2001).
6. M. Manzanares, A. Locascio, M. A. Nieto, *Trends Genet.* **17**, 178 (2001).
7. M. Sefton, S. Sanchez, M. A. Nieto, *Development* **125**, 3111 (1998).
8. M. A. Nieto, M. G. Sargent, D. G. Wilkinson, J. Cooke, *Science* **264**, 835 (1994).
9. C. LaBonne, M. Bronner-Fraser, *J. Neurobiol.* **36**, 175 (1998).
10. M. A. Selleck, M. Bronner-Fraser, *Development* **121**, 525 (1995).
11. K. F. Liem Jr., G. Tremml, T. M. Jessell, *Cell* **91**, 127 (1997).
12. K. F. Liem Jr., G. Tremml, H. Roelink, T. M. Jessell, *Cell* **82**, 969 (1995).
13. M. E. Dickinson, M. A. Selleck, A. P. McMahon, M. Bronner-Fraser, *Development* **121**, 2099 (1995).
14. J. Vallin *et al.*, *J. Biol. Chem.* **276**, 30350 (2001).

PERSPECTIVES: GEOPHYSICS

Redistributing Earth's Mass

Anny Cazenave and R. Steven Nerem

Like a pumpkin, Earth is a bit wider around the equator than the meridian. This slight oblateness (by about 0.3%) results from axial rotation and large-scale mantle convection (*1*). If the dynamic oblateness J_2 decreases with time, then mass must have been redistributed from equatorial regions to the high latitudes, and inversely. But relative to the mass of Earth, any such mass distribution is likely to be very small.

Changes in J_2 were first measured 20 years ago by Yoder *et al.* (*2*), who used satellite laser ranging to show that it was decreasing linearly by 3×10^{-11} per year. Several investigators later confirmed his observation (*3*). Changes in J_2 with time have now been monitored for more than 25 years with satellite laser ranging. On page 831 of this issue, Cox and Chao (*4*) show that, contrary to expectation, in recent years J_2 has started to increase.

The earlier decreasing trend in J_2 meant that Earth was becoming less oblate. This observation can be largely explained by postglacial rebound—the viscous relaxation of Earth's mantle that began when polar ice caps started to melt at the end of the last glaciation 18,000 years ago. Postglacial rebound still continues today. Seasonal oscillations of J_2 have also been observed. They are caused by the redistribution of air mass in the atmosphere and of water mass among atmosphere, oceans, and continental water reservoirs (*5, 6*).

Cox and Chao (*4*) report satellite laser ranging data to numerous satellites from 1979 to 2001. For most of the past two decades, J_2 has been steadily decreasing. But in early 1998 it suddenly started to increase substantially [see figure 2 of (*4*)], indicating a large-scale mass redistribution from high latitudes to the equatorial regions.

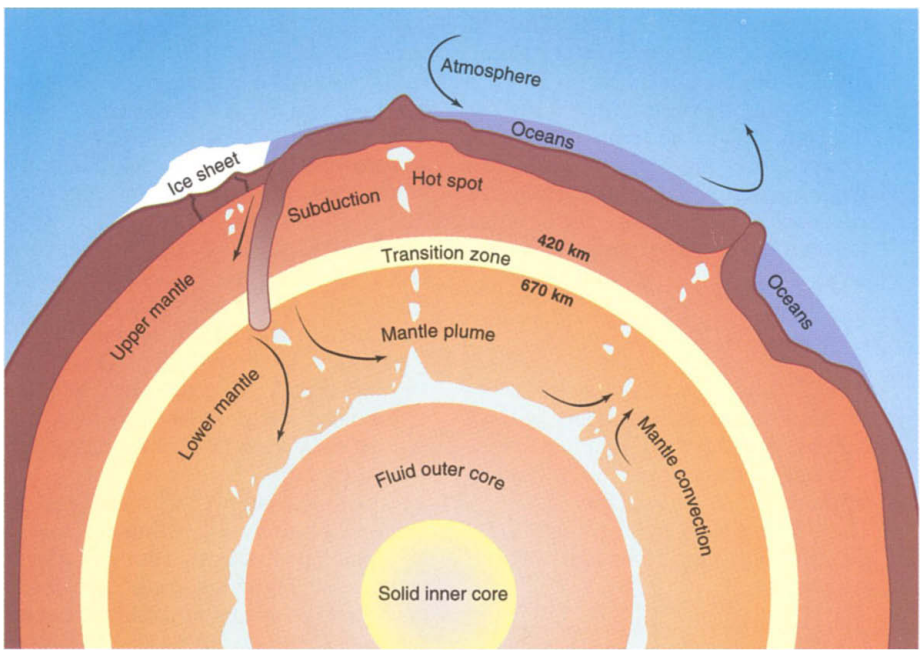
Cox and Chao discuss several mechanisms that might explain these observations: melting of the polar ice caps, melting of the Alpine glaciers, or melting of Arctic sea ice. According to current knowledge (*7*), however, none of these can explain the observations. Ice cap melting should indeed lead to a rise in the global mean sea level, but the observed sea level rise since 1992 (*8*) is incompatible with the amount of ice melting required to explain the observed J_2 change (*4*) [even if the observed rise is attributed entirely to ice melting, which is not the case (*9*)].

What, then, is causing the change? Cox and Chao apparently rule out the atmosphere as a possible source. There remain two potential candidates: Earth's fluid outer core and the oceans (see the figure).

A sudden change in material flow at the top of the fluid outer core, as evidenced by geomagnetic "jerks" (changes in the trend of the secular variation of the geomagnetic field), could produce a non-negligible change in J_2. As pointed out by Cox and Chao (*4*), a jerk around 1999 suspected from geomagnetic observations (*10*) was recently confirmed from updated data (*11*). Thus, one cannot rule out that redistribution of mass inside the core before the observed jerk may have contributed to the observed change in J_2.

Large-scale mass redistribution in the oceans remains a serious candidate. Figure 2 of (*4*) shows other fluctuations in J_2 (for example, from 1980 to 1983 and from 1989 to 1992), although they are smaller than the fluctuation that began in 1998. Hence, what may at first appear to be a sudden single event (or a change in the trend direction) may rather be a recurrent interannual or decadal fluctuation of varying intensity.

The recent J_2 change occurred in late 1997 to early 1998, at the time of the strongest El Niño event this century. The El Niño–Southern Oscillation and its decadal modulation are primarily associated with



Schematic representation of the Earth system inside which mass redistribution may occur.

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