Of particular interest, degrees of relatedness within a huddle may differ for genes of maternal and paternal origin. For example, in a multiple-paternity litter, more individuals will share genes of maternal origin than will share genes of paternal origin. Therefore, maternally expressed imprinted genes are predicted to promote higher contributions to communal heating than the level favored by paternally expressed imprinted genes (Figure 1) [9].

Brown Fat and Genomic Imprinting

Young mammals generate heat by non-shivering thermogenesis in brown adipocytes [10]. At least three imprinted loci influence this process in mice. Two paternally expressed loci, *Pref1/Dlk1* and *Necdin* [11,12], reduce the size of the 'furnace' by inhibiting differentiation of preadipocytes into brown adipocytes [13]. The third imprinted locus, *GNAS*, encodes the G-protein α stimulatory subunit (Gαs) that initiates the cellular events that activate thermogenesis downstream of β-adrenergic receptors [10]. Both maternally and paternally derived *GNAS* alleles produce Gαs in most tissues, but in brown adipose tissue the maternally derived allele is expressed preferentially [14]. By contrast, the paternally derived *GNAS* allele produces the XLαs protein, which antagonizes the effects of Gαs in brown adipose tissue [15]. Thus, *GNAS* produces both a maternally expressed promoter and a paternally expressed inhibitor of non-shivering thermogenesis. This is the pattern that would be predicted if matrilineal relatedness exceeds patrilineal relatedness within huddles. Future studies will test whether this pattern is maintained at other imprinted loci.

The evolution of cooperation has been a major area of theoretical and empirical research in evolutionary biology, but with a perceived need to exploit new study systems for testing theoretical models [16]. Social thermogenesis has certain advantages for studying the stability and breakdown of cooperation. Huddles are spatially localized, and fitness-related variables, such as temperature, body weight or milk consumption, are easily measured. Moreover, pharmacological and genetic interventions are available to adjust how much particular individuals contribute to the collective good.

References

1. Willis, C.K.R., and Brigham, R.M. (2007). Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. Behav. Ecol. Sociobiol. *62*, 97–108.
2. Gilbert, C., Le Maho, Y., Perret, M., and Ancel, A. (2007). Body temperature changes induced by huddling in breeding male emperor penguins. Am. J. Physiol. *292*, R176–R185.
3. Gilbert, C., Blanc, S., Giroud, S., Trabalon, M., Le Maho, Y., Perret, M., and Ancel, A. (2007). Role of huddling on the energetic of growth in a newborn altricial mammal. Am. J. Physiol. *293*, R867–R876.
4. Ancel, A., Visser, H., Handrich, Y., Masman, D., and Le Maho, Y. (1997). Energy savings in huddling penguins. Nature *385*, 304–305.
5. Alberts, J.R. (1978). Huddling by rat pups: group behavioral mechanisms of temperature regulation and energy conservation. J. Comp. Physiol. Psychol. *92*, 231–245.
6. Alberts, J.R. (2007). Huddling by rat pups: ontogeny of individual and group behavior. Devel. Psychobiol. *49*, 22–32.
7. Sokoloff, G., and Blumberg, M.S. (2001). Competition and cooperation among huddling infant rats. Devel. Psychobiol. *39*, 65–75.
8. Arnold, W. (1990). The evolution of marmot sociality: II. Costs and benefits of joint hibernation. Behav. Ecol. Sociobiol. *27*, 239–246.
9. Haig, D. (2004). Genomic imprinting and kinship: how good is the evidence? Annu. Rev. Genet. *38*, 553–585.
10. Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. Physiol. Rev. *84*, 277–359.
11. da Rocha, S.T., Tevendale, M., Knowles, E., Takada, S., Watkins, M., and Ferguson-Smith, A.C. (2007). Restricted co-expression of Dlk1 and the reciprocally imprinted non-coding RNA, Gtl2: implications for cis-acting control. Dev. Biol. *306*, 810–823.
12. Bittel, D.C., Kibiryeva, N., McNulty, S.G., Driscoll, D.G., Butler, M.G., and White, R.A. (2007). Whole genome microarray analysis of gene expression in an imprinting center deletion mouse model of Prader-Willi syndrome. Am. J. Med. Genet. *143A*, 422–429.
13. Tseng, Y.-H., Butte, A.J., Kokkotou, E., Yechoor, V.K., Taniguchi, C.M., Kriauciunas, K.M., Cypess, A.M., Niinobe, M., Yoshikawa, K., Patti, M.E., *et al.* (2005). Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necdin. Nat. Cell Biol. *7*, 601–611.
14. Yu, S., Yu, D., Lee, E., Eckhaus, M., Lee, R., Corria, Z., Accili, D., Westphal, H., and Weinstein, L.S. (1998). Variable and tissue-specific hormone resistance in heterotrimeric Gs protein α-subunit (Gsα) knockout mice is due to tissue-specific imprinting of the Gsα gene. Proc. Natl. Acad. Sci. USA *95*, 8715–8720.
15. Plagge, A., Gordon, E., Dean, W., Boiani, R., Cinti, S., Peters, J., and Kelsey, G. (2004). The imprinted signaling protein XLαs is required for postnatal adaptation to feeding. Nat. Genet. *36*, 818–826.
16. West, S.A., Griffin, A.S., and Gardner, A. (2007). Evolutionary explanations for cooperation. Curr. Biol. *17*, R661–R672.

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Cytosine Methylation: Remaining Faithful

DNA methyltransferase-1 (DNMT1) has a higher specific activity on hemimethylated DNA than on unmethylated DNA, but this preference is too small to explain the faithful mitotic inheritance of genomic methylation patterns. New genetic studies in plants and mammals have identified a novel factor that increases the fidelity of maintenance methylation.

Steen K.T. Ooi and Timothy H. Bestor

In 1975 Art Riggs and Robin Holliday independently predicted the existence of DNA methyltransferases that would methylate only hemimethylated DNA, thereby rendering genomic methylation patterns subject to mitotic inheritance [1,2]. Wigler [3] later showed that methylation patterns were indeed subject to mitotic inheritance, and Groudine and colleagues [4] showed that this inheritance was stable for at least 80 cell doublings in a system that controlled for copy number and integration site effects. Eric Richards and colleagues showed remarkably stable mitotic and meiotic inheritance of CpG methylation patterns in *Arabidopsis thaliana* [5]. Faithful maintenance of methylation patterns is essential for the survival of differentiated cells and may be involved in diseases in which the perpetuation of aberrant DNA-methylation patterns may contribute to disorders of imprinted

gene expression and to carcinogenesis [6].

An enzyme that came to be called DNA methyltransferase-1 (DNMT1) was shown to prefer hemimethylated substrates [7] and this observation seemed to confirm the predictions of Riggs and Holliday. However, this preference depended on the nature of the sequence and was not more than 30-fold on hemimethylated poly d(CG) and was as low as 5-fold on hemimethylated DNA of random sequence [8]. This means that 3–20% of CpG sites would lose methylation in each cell division (if methylation of each CpG site were independent of the methylation status of other local CpG sites). The high *de novo* methylation activity of DNMT1 (much higher than that of the *de novo* methyltransferases DNMT3A and DNMT3B) [9], coupled with cellular expression levels that are higher than either *de novo* enzyme, would rapidly result in randomized genomic methylation patterns. Given that genomic methylation patterns are faithfully transmitted, additional factors must therefore stimulate the activity of DNMT1 at hemimethylated CpG sites and prevent it from methylating previously unmethylated sites. What are these factors?

Previous studies identified a replication focus targeting domain in DNMT-1 that coordinates maintenance methylation and DNA replication [10], and this domain is likely to increase the heritability of methylation patterns. Proliferating cell nuclear antigen (PCNA) was a candidate for augmenting this heritability [11], but human cells expressing DNMT1 that lacked the amino-terminal PCNA-binding domain showed only a minor reduction in the post-replicative rate of methylation [12]. An important advance came from a genetic screen in *Arabidopsis* that identified VARIANT IN METHYLATION 1 (VIM1) as being important for the maintenance of DNA methylation at centromeric repeats [13]. VIM1 contains a number of annotated domains, including a SET and RING associated (SRA) domain, which is able to bind to methylated CpG and CpNpG sites [14]. Woo *et al.* [13] noted the existence of a mammalian homologue of VIM1 named NP95 (also known as UHRF1 and ICBP90, among several other names).

As early as 2001 it was stated by Miura *et al.* [15], "…NP95 does not take a direct part in DNA replication as part of the DNA synthesizing machinery… but is presumably involved in other DNA replication-linked nuclear events". This proposed replication-linked role has now been elucidated in two recent studies [15,16] via a combination of genetic and biochemical approaches. *NP95*-null ES cells are found to be demethylated at interspersed repeats, tandem repeats and differentially methylated regions of imprinted loci, all of which are heavily methylated in normal cells. The *NP95* mutant essentially phenocopies the *Dnmt1* mutant both in ES cells and in mouse embryos [16].

From a mechanistic viewpoint, one might expect that the transmission of methylation patterns should involve recognition of the newly synthesized hemimethylated substrate. NP95 appears to be capable of doing this, although the presence of a ubiquitin-like domain at the amino terminus leaves open the possibility that ubiquitylation may also be involved in replication-focus targeting. Use of a DNA-labelling approach and ES cells lacking either DNMT1 or all three mammalian DNA methyltransferases, Sharif and colleagues [17] showed by immunofluorescence that NP95 has a strong preference for newly replicated, hemimethylated DNA. In combination with the experiments of Bostick and colleagues [18] (who examined the binding of the NP95 SRA domain to methylated and unmethylated oligonucleotides), this indicates the importance of recognition and binding of hemimethylated DNA. The presence of methylation at only CpG dinucleotides has clearly driven the evolution of the NP95 SRA domain. Under Bostick and colleagues' assay conditions, there was no binding of NP95 to hemimethylated CpNpG or CpNpN sites, two sequences that are unmethylated in mammalian DNA, but can be methylated in flowering plants.

Although a number of factors have been reported to interact with the amino terminus of DNMT1, the biological functions of the numerous sequence motifs located within this region have long been unclear. ES cells that express only a catalytically inactive version of DNMT1 caused by a single conservative substitution at the active site recapitulate the phenotypes caused by DNMT1 deficiency [19], which suggests that the transcriptional repressor activity attributed to DNMT1 plays a very minor biological role. The finding that null alleles of *NP95* largely phenocopy null alleles of *Dnmt1* provides further evidence that the essential function of DNMT1 is primarily or exclusively the methylation of DNA.

By deletion analysis Bostick and colleagues [18] showed that the amino-terminal region encompassing the RFT domain of DNMT1 is necessary for association with NP95, via

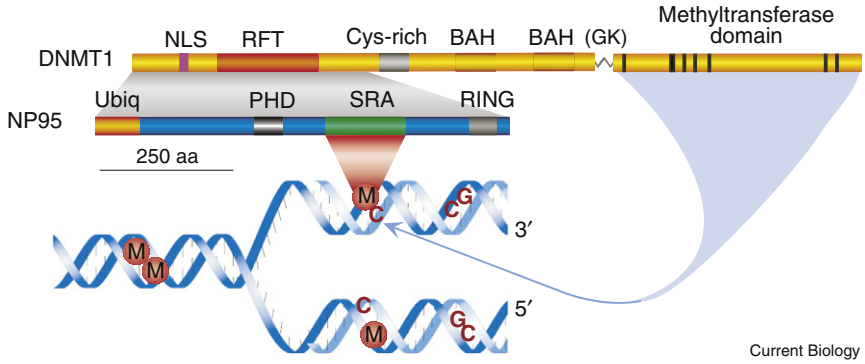

Figure 1. Domain structure of DNMT1 and NP95 and the mechanism through which they mediate the maintenance of DNA methylation profiles.

Following DNA replication, methylated CpGs are hemimethylated and NP95 is recruited via the recognition and binding of hemimethylated sites by the SRA domain. DNMT1, in turn, is recruited as a result of an interaction between its amino-terminal domain (which contains the RFT motif) and NP95, possibly involving the NP95 PHD domain. DNMT1 methyltransferase activity subsequently restores symmetrical CpG methylation, thereby maintaining the parental methylation pattern. The RFT domain is a replication focus targeting motif; the functions of the Cys-rich and Brahma adjacent homology (BAH) motifs are not understood. NLS, nuclear localization sequence; GK, Glycine and Lysine repeat region; Ubiq, ubiquitin-like domain; RING, Really Interesting New Gene.

a region that included the NP95 plant homeodomain (PHD) region (Figure 1). It is interesting that, in their assay, they also observe NP95 binding to part of the DNMT1 carboxy-terminal methyltransferase domain, although it is unclear whether this interaction is mediated through the NP95 PHD domain or another domain. NP95 (under various names) had been previously identified as a protein involved in cell-cycle progression, sensitivity to genotoxins, and DNA replication, and it remains to be seen whether these effects are related to changes in genomic methylation patterns or reflect some other function of NP95.

NP95 adds to a growing list of factors that have been genetically determined to be involved in the establishment or maintenance of genomic methylation in mammals but are not DNA methyltransferases. Cells or mouse embryos deficient in DNMT3L, MILI, MIWI2, CGBP, Lsh or both Suv39h1 and Suv39h2 all display some degree of demethylation in one or more sequence compartments. With the exception of DNMT3L, which has been shown to be involved in establishment of genomic methylation patterns, it remains unclear whether these other factors play roles in establishment and/or maintenance, and the mechanisms through which they function remain to be determined. These new studies on NP95 not only represent the identification of a factor involved in the maintenance of global methylation patterns, but also might reconcile the limited dependence of DNMT1 on hemimethylated substrates with the faithful mitotic inheritance of genomic methylation patterns.

References

1. Riggs, A.D. (1975). X inactivation, differentiation, and DNA methylation. Cytogenet. Cell Genet. *14*, 9–25.
2. Holliday, R., and Pugh, J.E. (1975). DNA modification mechanisms and gene activity during development. Science *187*, 226–232.
3. Wigler, M.H. (1981). The inheritance of methylation patterns in vertebrates. Cell *24*, 285–286.
4. Lorincz, M.C., Schubeler, D., Goeke, S.C., Walters, M., Groudine, M., and Martin, D.I. (2000). Dynamic analysis of proviral induction and de novo methylation: Implications for a histone deacetylase-independent, methylation density-dependent mechanism of transcriptional repression. Mol. Cell. Biol. *20*, 842–850.
5. Vongs, A., Kakutani, T., Martienssen, R.A., and Richards, E.J. (1993). Arabidopsis thaliana DNA methylation mutants. Science *260*, 1926–1928.
6. Goll, M.G., and Bestor, T.H. (2005). Eukaryotic cytosine methyltransferases. Annu. Rev. Biochem. *74*, 481–514.
7. Bestor, T.H., and Ingram, V.M. (1983). Two DNA methyltransferases from murine erythroleukemia cells: Purification, sequence specificity, and mode of interaction with DNA. Proc. Natl. Acad. Sci. USA *80*, 5559–5563.
8. Yoder, J.A., Soman, N.S., Verdine, G.L., and Bestor, T.H. (1997). DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. J. Mol. Biol. *270*, 385–395.
9. Okano, M., Xie, S., and Li, E. (1998). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat. Genet. *19*, 219–220.
10. Leonhardt, H., Page, A.W., Weier, H.U., and Bestor, T.H. (1992). A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell *71*, 865–873.
11. Chuang, L.S., Ian, H.I., Koh, T.W., Ng, H.H., Xu, G., and Li, B.F. (1997). Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science *277*, 1996–2000.
12. Spada, F., Haemmer, A., Kuch, D., Rothbauer, U., Schermelleh, L., Kremmer, E., Carell, T., Langst, G., and Leonhardt, H. (2007). DNMT1 but not its interaction with the replication machinery is required for maintenance of DNA methylation in human cells. J. Cell. Biol. *176*, 565–571.
13. Woo, H.R., Pontes, O., Pikaard, C.S., and Richards, E.J. (2007). VIM1, a methylcytosine-binding protein required for centromeric heterochromatinization. Genes. Dev. *21*, 267–277.
14. Unoki, M., Nishidate, T., and Nakamura, Y. (2004). ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. Oncogene *23*, 7601–7610.
15. Miura, M., Watanabe, H., Sasaki, T., Tatsumi, K., and Muto, M. (2001). Dynamic changes in subnuclear NP95 location during the cell cycle and its spatial relationship with DNA replication foci. Exp. Cell Res. *263*, 202–208.
16. Li, E., Bestor, T.H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell *69*, 915–926.
17. Sharif, J., Muto, M., Takebayashi, S.I., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K., *et al*. (2007). The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature *450*, 908–912.
18. Bostick, M., Kim, J.K., Esteve, P.O., Clark, A., Pradhan, S., and Jacobsen, S.E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science *317*, 1760–1764.
19. Damelin, M., and Bestor, T.H. (2007). Biological functions of DNA methyltransferase 1 require its methyltransferase activity. Mol. Cell. Biol. *27*, 3891–3899.

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Active Sensing: Matching Motor and Sensory Space

A recent study has shown that, unusually, both the sensory and motor capabilities of an electric fish are omnidirectional. This matching of motor and sensory spaces helps the fish to hunt prey efficiently — particularly important given their energetically costly active sensory system.

Stefan Schuster

Bats, electric fish and head-shaking locusts have one thing in common: they all invest energy into probing actions that help them to obtain useful information about their surroundings from sensory feedback. In some animals, the energy invested in such 'active sensing' can be substantial, so that best possible use should be made of the investment. A recent study shows how this is done in hunting electric fish that use a particularly costly active sensory system. Using a combined behavioral and computational approach, Snyder *et al.* [1] were able to determine the precise shapes of the volume of surrounding space a hunting electric fish can probe for the presence of its prey and of the motor space in which the fish can actually maneuver to make a catch.

The black ghost knifefish (*Apteronotus albifrons*) studied by Snyder *et al.* [1] is an amazing creature. It can move elegantly in a wide variety of body orientations and can rapidly switch from one mode of moving to another. Its major propulsion system is its ventral ribbon fin, which runs over almost the full body [1–3]. To probe its nocturnal environment, the fish sends a current across its skin which continuously oscillates at about 1000 cycles per second. With a large number of electroreceptors, tuned to this high frequency [4], the fish monitors how the self-generated current spreads over the fish's surface.