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The two-domain hypothesis in Beckwith-Wiedemann syndrome

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Letter to the Editor

I was pleased to find that Maher and Reik, in their recent Perspective (1), agreed with our two-domain hypothesis, which we first proposed last year (2), for Beckwith-Wiedemann Syndrome (BWS), which causes prenatal overgrowth, midline birth defects, and cancer. According to this hypothesis, two separate domains of imprinted genes, on chromosomal band 11p15, are involved in BWS. (Imprinting involves epigenetic silencing of a specific parental allele, and loss of imprinting is a common cause of abnormal gene expression in cancer [reviewed in ref. 3].) One of these two domains, involving a novel antisense transcript within KVLQT1 and termed LIT1, was the subject of our report. We found that approximately half of BWS patients undergo loss of imprinting (LOI) of LIT1, compared with 20% of BWS patients showing LOI of IGF2, which is located approximately 500 kb telomeric to LIT1. Furthermore, LOI of LIT1 was independent of LOI of IGF2 when examined in the same patients (2). Moreover, we and our collaborators have observed no evidence of LOI of LIT1 in Wilms tumors (4), despite frequent LOI of IGF2 in embryonal tumors (5). Finally, our two-domain model was consistent with other observations from our laboratory that a group of genes lying between these two domains, including TSSC4 and TSSC6, are imprinted minimally or not at all (6). In Maher and Reik's […]

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I was pleased to find that Maher and Reik, in their recent Perspective (1), agreed with our two-domain hypothesis, which we first proposed last year (2), for Beckwith-Wiedemann Syndrome (BWS), which causes prenatal overgrowth, midline birth defects, and cancer. According to this hypothesis, two separate domains of imprinted genes, on chromosomal band 11p15, are involved in BWS. (Imprinting involves epigenetic silencing of a specific parental allele, and loss of imprinting is a common cause of abnormal gene expression in cancer [reviewed in ref. 3].) One of these two domains, involving a novel antisense transcript within K_VLQT1 and termed LIT1, was the subject of our report. We found that approximately half of BWS patients undergo loss of imprinting (LOI) of *LIT1*, compared with 20% of BWS patients showing LOI of *IGF2*, which is located approximately 500 kb telomeric to *LIT1*. Furthermore, LOI of *LIT1* was independent of LOI of *IGF2* when examined in the same patients (2). Moreover, we and our collaborators have observed no evidence of LOI of *LIT1* in Wilms tumors (4), despite frequent LOI of *IGF2* in embryonal tumors (5). Finally, our two-domain model was consistent with other observations from our laboratory that a group of genes lying between these two domains, including *TSSC4* and *TSSC6*, are imprinted minimally or not at all (6).

In Maher and Reik's excellent review, they proposed a model of enhancer competition between *LIT1* and $p57^{KIP2}$, similar to that known to exist between *H19* and *IGF2* in the more telomeric domain (1). We had also suggested in our article last year that $p57^{KIP2}$ might compete for a shared enhancer. However, I would like to point out an alternative to Maher and Reik's and our own earlier speculation about the possible location of such an enhancer, which was hypothesized to lie on the telomeric side of a 450-kb cluster of germline balanced chromosomal rearrangement breakpoints in BWS patients, termed BWSCR1.

Alternatively, this enhancer might lie on the centromeric side of *BWSCR1* and *LIT1*. *In the absence of data, we cannot favor one possibility over the other at this point.* However, if the alternative location were correct, one would have an elegant genomic structure of the two imprinted domains as mirror images of each other, with BWSCR1 in between them (Figure 1). Both the known *H19/IGF2* enhancer and the hypothetical $p57^{KIP2}$/*LIT1* enhancer would lie near one end of the entire 11p15 imprinted domain, and if one walked toward the center of the domain from either end, one would encounter, in this order: (a) the hypothetical enhancer for $p57^{KIP2}$/*LIT*, and the known enhancer for *H19*; (b) the $p57^{KIP2}$ gene, which is maternally expressed, and the *H19* gene, which is maternally expressed; (c) an as yet unidentified insulator centromeric to *LIT1*, and the known insulator centromeric to *H19*; (d) an approximately 100-kb interval; and (e) the paternally expressed *LIT1* gene, and the paternally expressed *IGF2* gene. The disadvantage of this alternative hypothesis is that it would leave unexplained the mechanism of BWS in the BWSCR1 rearrangement patients, although we should not overlook the possibility of a more centromeric enhancer.

Regardless of whether this alternative for the location of this theoretical enhancer, or a more telomeric location suggested earlier by us (2) and by Maher and Reik (1), is correct, the two-domain hypothesis that we first proposed (which is compatible with both) might also help to explain the apparent differences in the phenotype of BWS patients with altered imprinting of the two domains (M. deBaun et al., unpublished observations) or with $p57^{KIP2}$ mutations (7). *Note added in proof.* I appreciate Maher and Reik's description of the evolu-

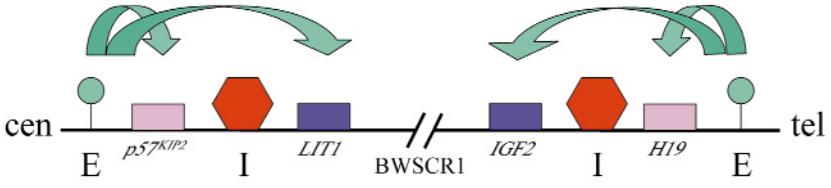

Figure 1
A model of a shared enhancer for $p57^{KIP2}$ and *LIT1* centromeric to BWSCR1 and *LIT1*. If the enhancer were centromeric to *LIT1*, then it would activate $p57^{KIP2}$ when an insulator between $p57^{KIP2}$ and *LIT1* is unmethylated, and it would not be affected by the BWSCR1 chromosomal rearrangements. The same enhancer would activate *LIT1* when the insulator was methylated. Note that the precise location of the enhancer is immaterial as long as it is centromeric to *LIT1*. E, enhancer (green); I, insulator (red); maternally expressed $p57^{KIP2}$ and *H19* (pink); paternally expressed *LIT1* and *IGF2* (blue); cen, toward the centromere; tel, toward the telomere. Arrows indicate alternate use of the enhancer.

tion of their independent thinking that led to the two-domain model in their *JCI* review. I emphasize my point that either location for a shared enhancer is possible with current data, but I did not suggest that *KvDMR1* is itself the insulator. Indeed, I think that unlikely, as we find that the sequence is not conserved in the mouse.

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The authors reply — A.P. Feinberg raises two questions: (a) the origin of the two-domain model, and (b) the organization of enhancers and insulators within chromosome 11p15.5. Our concept that two imprinting control centers exist within chromosome 11p15.5 was developed independently. In a series of reports, we established, first, that loss of imprinting of *IGF2* in Beckwith-Wiedemann syndrome (BWS) may be associated with *H19* hypermethylation and silencing, consistent with loss of function in a distal imprinting center (1); second, that a BWS-associated maternally inherited inversion with a breakpoint within *KCNQ1* was associated with an *H19*-independent loss of imprinting in *IGF2* (2); and, finally, that such *H19*-independent loss of *IGF2* imprinting is frequently found in sporadic cases of BWS that lack chromosomal rearrangements (3). The finding that epigenetic alterations at *KvDMR1* and *H19* appeared to be mutually exclusive provided us with confirmation of our concept (4).

With regard to the organization of imprinting elements within 11p15.5, we agree that it is possible that the *CDKN1C* ($p57^{KIP2}$) enhancer could be on the centromeric side, but we favor a telomeric location for several reasons. First, if the enhancer were centromeric, *CDKN1C* would need its own imprinting mechanism. This is less likely because (a) there is no differential methylation in the human (5); (b) a maternal germline imprint is required for activity of *cdkn1c* (6); (c) *cdkn1c* transgenes do not become imprinted (7); and (d) in Dnmt1-deficient mice, *cdkn1c* is biallelically expressed, but inspection of the gels shows that this could be a low-level expression from both alleles (8), corresponding to the low-level paternal expression in humans. Finally, and importantly, the organization suggested by A.P. Feinberg would require a closed boundary on the maternal chromosome and an open one on the paternal chromosome, but *KvDMR1* methylation is maternal (presumably indicating that the boundary is open, as with the *H19* upstream region).

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