Mutations in factors that have not been associated with *Shh* signaling also can lead to anterior limb defects. For example, patients with Holt–Oram syndrome (HOS) and Okihiro syndrome (OS), which are caused by mutations of *TBX5* and the *Spalt* family zinc finger transcription factor *SALL4*, respectively, show anterior forelimb defects, including loss of a thumb or a triphalangeal digit and/or hypoplasia of the radius (29–36). Loss of function of promyelocytic leukemia zinc finger gene (*Plzf*) function in both human patients and mouse mutants also results in similar limb AP patterning defects (37–39). Interactions among the myriad of required factors in limb AP patterning and their relationships to *Shh* signaling and other signaling pathways remain incompletely understood.

In this study, we demonstrate that *Hox5* genes perform a novel function in limb AP patterning. Loss of function of *Hox5* paralogous genes (*Hoxa5*, *Hoxb5*, and *Hoxc5*), an anterior set of *Hox* genes not belonging to the *AbdB*-related *Hox* group, results in defects in anterior forelimb patterning that closely resemble some point mutations in the ZRS in both mice and humans. Early pattering of the anterior and posterior limb compartments is not disrupted in these mutants; however, the limb defects in *Hox5* mutants are associated with ectopic *Shh* expression in the anterior forelimb buds, and we provide molecular and genetic evidence indicating that *Hox5* interacts with *Plzf* to restrict *Shh* expression and pattern the anterior forelimb.

Results

Inactivation of *Hox5* Paralogous Group Genes Results in Anterior Forelimb Defects. Single mutants for *Hoxa5*, *Hoxb5*, and *Hoxc5* (the three mammalian *Hox5* paralogous group genes) have been generated previously (40–42). Although loss of *Hoxa5* function results in a smaller scapula (43), no limb patterning abnormalities have been reported for any of the three *Hox5* single mutants despite the expression of these genes in the developing forelimb and hindlimb (42, 43) (Fig. S1). Compound mutants deficient for any combination of as many as five of the six *Hox5* alleles did not exhibit limb defects (Fig. 1 *A* and *I*). Only when all six *Hox5* alleles were mutated were defects in the anterior forelimb skeletal elements observed (Fig. 1 *A–H*). The humerus of *Hox5* triple mutants was variably affected (Fig. 1 *B* and *C*), the radius was truncated or lost (Fig. 1 *B* and *C*), and digit 1 was often missing or transformed into a triphalangeal digit, with the distal portion of digit 2 occasionally bifurcated (Fig. 1 *E–G*). Hindlimb development was not affected in *Hox5* mutant mice, even though *Hox5* was expressed at early hindlimb bud stages (Fig. 1 *J* and *K*).

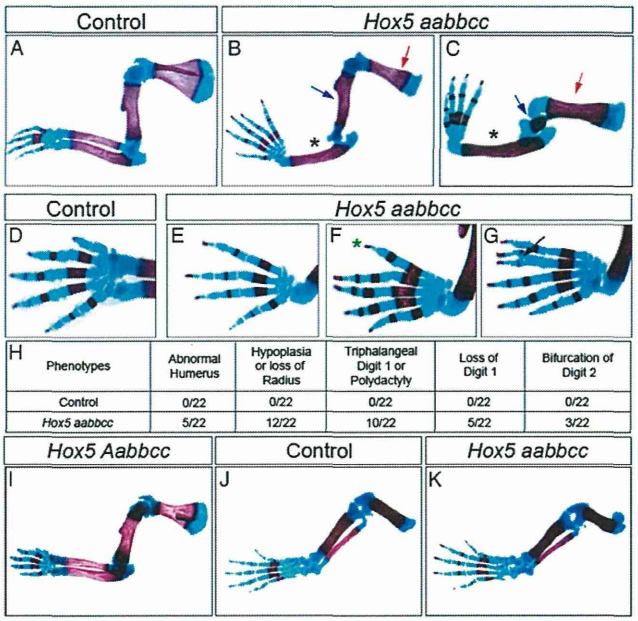

Fig. 1. Loss of function of *Hox5* paralogous genes results in anterior forelimb defects. (*A–G*) Skeletal analysis of control and *Hox5* triple-mutant forelimbs at E18.5. The scapula is reduced in *Hox5* triple mutants compared with controls, as observed for *Hoxa5* single mutants (*A–C*, red arrows). The stylopod is reduced or truncated only in embryos with a phenotype in the radius (*B* and *C*, blue arrow). The radius of mutant forelimbs is missing or severely truncated (*B* and *C*, black asterisk). (*D–G*) The most anterior digit develops abnormally in *Hox5* mutants compared with controls. Digit 1 is often missing (*E*) or triphalangeal (*F* and *G*, green asterisk). Less frequently, *Hox5* mutants also have a bifurcated digit 2 (*G*, black arrow). Digit phenotypes do not correlate with the severity of stylopod/zeugopod defects. (*H*) Table summarizing forelimb phenotypes of *Hox5* mutant forelimbs. (*I*) Compound mutants deficient for as many as five of the six *Hox5* alleles do not exhibit limb defects. (*J* and *K*) Hindlimb development is not affected in *Hox5* mutants.

***Shh* Is Ectopically Activated in *Hox5* Mutant Forelimb Buds.** Given the clear disruption of AP limb patterning and the importance of *Shh* in this process, we examined *Shh* expression in *Hox5* mutants. *Shh* expression expanded anteriorly in early forelimb buds of *Hox5* mutants, and ectopic *Shh* expression was observed in anterior domains in some instances (Fig. 2 *A–E*). The expression of downstream factors *Ptch1* and *Gli1* was consistently anteriorized in *Hox5* triple-mutant embryos (Fig. 2 *F*, *G*, *J*, and *K*). *Fgf4* expression in the AER extended anteriorly compared with controls (Fig. 2 *H* and *L*), consistent with anteriorized *Shh* expression, whereas *Fgf8* was expressed normally in the AER (Fig. 2 *I* and *M*).

Because *Shh* expression is disrupted at early stages, we examined AP patterning regulators upstream of *Shh* signaling. In somite-matched *Hox5* mutants and controls, there were no observable differences in the expression of *Gli3* (Fig. 3 *A*, *C*, *F*, and *H*) or *Hand2* (Fig. 3 *B*, *D*, *G*, and *I*) at any stage examined. *Alx4*, another early regulator of anterior limb patterning, was expressed normally in *Hox5* mutants (Fig. 3 *E* and *J*).

Misexpression of *HoxD* genes results in preaxial polydactyly phenotypes bearing some resemblance to *Hox5* mutants (12, 13, 16, 44), and ectopic or anteriorized *Shh* expression also leads to coincident anteriorization of *Hoxd10-13* (45). Expression of *Hoxd10-13* genes in *Hox5* mutants was shifted anteriorly in *Hox5* mutant forelimbs at embryonic day (E) 10.5 (Fig. 3 *K–R*), consistent with misregulation of *Shh*. It is important to note that *HoxD* genes are not linked to the *HoxA*, *HoxB*, or *HoxC* clusters, and thus these effects cannot be due to *cis* effects from the targeted mutations introduced into the *Hox5* alleles.

***Plzf* Is a Potential Coregulator of *Shh* Repression.** Several additional regulators of anterior limb patterning have been identified in human disease syndromes as well as in mouse mutants. Forelimb defects similar to those seen in *Hox5* mutants have been identified in patients with HOS caused by *Tbx5* mutations (32–36), and *Hox* genes have been reported to be capable of driving *Tbx5* expression (46), OS caused by *Sall4* mutations (29, 30), Townes–Brocks syndrome caused by *Sall1* mutations (31, 47) and Saethre–Chotzen syndrome caused by *Twist1* mutations, which also show limb phenotypes in mutant mice (48–50). Mutations of both the human and mouse limb enhancer of *Shh*, ZRS (7, 18, 19, 24), and human multiple congenital anomaly/mental retardation syndromes caused by mutations of *Plzf*, as well as loss-of-function mutations in *Plzf* in mice (37, 39), lead to similar phenotypes. Based on these similarities, we investigated the expression of *Tbx5*, *Sall1*, *Sall4*, *Twist1*, and *Plzf* in our *Hox5* mutants. We found no change in the expression of any of these genes in *Hox5* mutant forelimbs (Figs. S2 *A–L* and S3 *A–D*).

To test whether these factors might act in parallel in the same pathway as *Hox5* and affect *Shh* expression, we examined *Shh*

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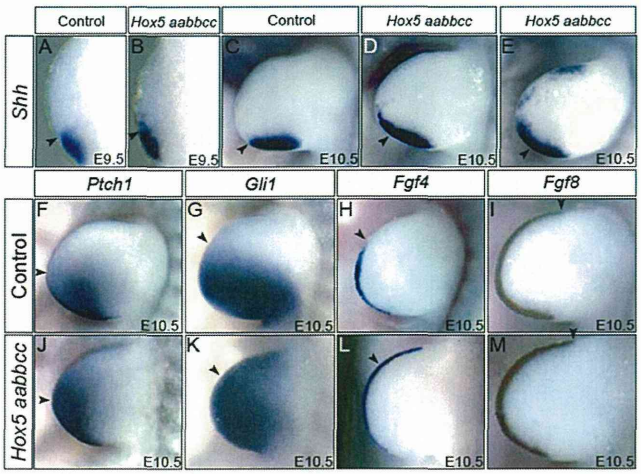


Fig. 2. *Shh* signaling is disrupted in *Hox5* mutant forelimbs. (*A–E*) *Shh* expression is anteriorized in *Hox5* mutant forelimbs. At E9.5, *Hox5* mutant forelimbs display slightly anteriorized *Shh* expression compared with controls (*A* and *B*). Anteriorization of *Shh* expression is observed by E10.5 in *Hox5* mutant forelimbs compared with controls (*C–E*), and ectopic *Shh* expression appears in anterior regions of some mutant forelimbs (*E*). (*F–M*) Expression of *Ptch1* and *Gli1* is consistently shifted anteriorly in *Hox5* mutants at E10.5 compared with controls (*F, J, G,* and *K*). Fgf4 expression is also anteriorized in *Hox5* mutant forelimbs compared with controls (*H* and *L*), whereas *Fgf8* expression is unchanged (*I* and *M*). Black arrowheads in each panel mark the WT anterior boundary of expression for each probe.

expression in *Tbx5* heterozygotes with or without loss of *Sall4* function, as well as in *Plzf* mutant embryos. Consistent with previous reports that *Shh* expression is not altered with loss of *Tbx5* function (51), we found that *Shh* was not altered in *Tbx5* or *Sall4* heterozygous mutants or compound *Tbx5*/*Sall4* heterozygous mutants (Fig. S3 *I–L*); however, *Plzf* mutants showed a small but reproducible anterior shift in *Shh* expression (Fig. 4 *A* and *B*). In addition, *Shh* transcripts were increased by ~50% in *Plzf* mutant limbs (Fig. 4*C*). To confirm changes in *Shh* expression, we examined the expression of *Ptch1* and *Gli1,* factors immediately downstream of *Shh.* In *Plzf* mutants, *Ptch1* and *Gli1* were consistently anteriorized and ectopically expressed (Fig. 4 *D–G*), demonstrating that *Shh* expression is affected downstream of *Plzf* in the developing limb.

Hox5 Proteins Interact with *Plzf* and Can Regulate *Shh* Expression Through the ZRS in Vitro. *Plzf* mouse mutants have been found to have similar forelimb phenotypes as *Hox5* mutant mice, although with low penetrance (37). The mice used in the present study demonstrated a similar phenotype (Fig. 5 *A* and *B*), but with 100% penetrance in the forelimb (52). Heterozygous embryos had no limb phenotype (Fig. 5*C*). Having already demonstrated no changes in *Plzf* expression in our *Hox5* mutants, we investigated the possibility that *Hox5* acts downstream of *Plzf* in forelimb AP patterning, but found normal *Hox5* expression levels in *Plzf* mutant forelimbs (Fig. S3 *E–G*).

To test whether Hox5 and Plzf proteins are capable of interacting to regulate downstream limb target genes, we examined potential physical interactions between these proteins in vitro. In cells cotransfected with epitope-tagged Hoxa5, Hoxb5, or Hoxc5 protein in combination with tagged Plzf, we found coprecipitation of all three Hox5 proteins with Plzf protein (Fig. 4*H* and Fig. S3*H*), consistent with the possibility that these proteins interact to regulate downstream targets.

If *Hox5* and *Plzf* function together to repress *Shh* expression anteriorly and affect AP patterning of the forelimb, then we would expect these genes to interact in vivo. *Hox5* mutants demonstrate no forelimb defects unless all six alleles of the three *Hox5* genes are mutated (compare Fig. 1 *A–G* and Fig. 5*D*). *Plzf* mutants exhibited forelimb phenotypes only with loss of both alleles, whereas heterozygous animals had no phenotype (Fig. 5 *A–C*). Compound mutants heterozygous for *Plzf* combined with either one or two mutant *Hox5* alleles did not exhibit any forelimb defects. Embryos heterozygous for *Plzf* plus three *Hox5* mutant alleles resulted in preaxial limb skeletal defects in only 1 of 18 forelimbs (Fig. 5*G*). Compound mutants heterozygous for *Plzf* plus four *Hox5* mutant alleles showed more severe forelimb defects with higher penetrance (Fig. 5 *E* and *G*). Forelimb defects were further exacerbated in compound mutants heterozygous for *Plzf* plus five *Hox5* mutant alleles, with 12 of 14 forelimbs demonstrating anterior forelimb defects (Fig. 5 *F* and *G*). These findings indicate that strong genetic interactions occur between *Hox5* and *Plzf* in vivo, supporting the hypothesis that these proteins cooperatively regulate forelimb AP patterning events.

If *Hox5* and *Plzf* coordinately regulate *Shh* expression, then the phenotypes observed in the compound mutants should result in changes in *Shh* pathway expression. Obvious anteriorization of *Shh* expression was observed in *Plzf*/*Hox5* compound mutants (Fig. 5 *H* and *I*), similar to that seen in both *Hox5* triple mutants and *Plzf* mutants, but not in *Plzf* heterozygotes or in compound *Hox5* mutants carrying up to five mutant alleles. *Gli1* and *Ptch1* expression also was anteriorized in the forelimb of the compound *Hox5*/*Plzf* mutants (Fig. 5 *J–M*), but not in control WT embryos, *Plzf* heterozygotes, or compound *Hox5* mutants harboring up to five mutant alleles. These genetic data further support the assertion that *Hox5* and *Plzf* cooperatively repress

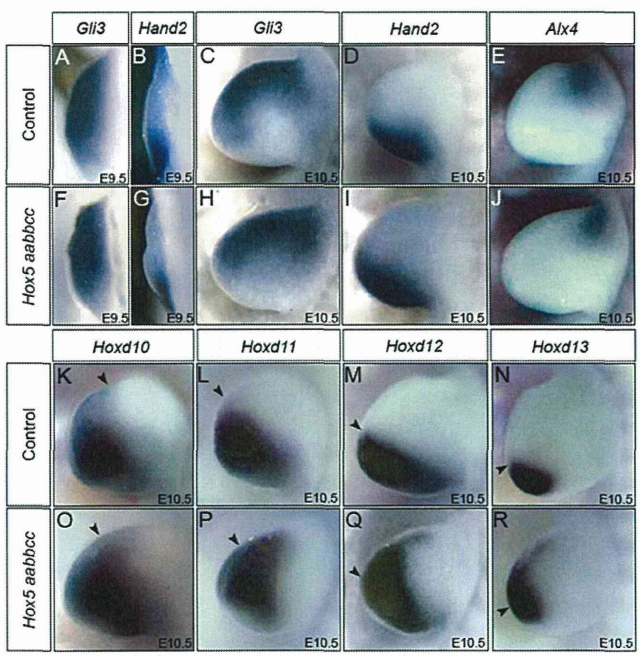


Fig. 3. Early limb patterning pathways are not disrupted, but posterior *HoxD* gene expression is anteriorized in *Hox5* mutants. (*A–J*) Early AP patterning factors are not disrupted in *Hox5* mutants. The expression of *Gli3* is not altered at E9.5 (*A* and *F*) or E10.5 (*C* and *H*). *Hand2* is expressed normally in *Hox5* mutant forelimbs at E9.5 (*B* and *G*) and E10.5 (*D* and *I*). *Alx4* expression in *Hox5* mutants is also comparable to that in controls at E10.5 (*E* and *J*). (*K–R*) The expression limits of posterior *HoxD* genes are anteriorized in E10.5 *Hox5* mutant forelimbs. *Hoxd10* (*K* and *O*), *Hoxd11* (*L* and *P*), *Hoxd12* (*M* and *Q*), and *Hoxd13* (*N* and *R*) are all expressed more anteriorly in *Hox5* mutant forelimb buds (*O–R*) compared with controls (*K–N*). Black arrowheads mark the WT anterior boundary of expression for each probe.

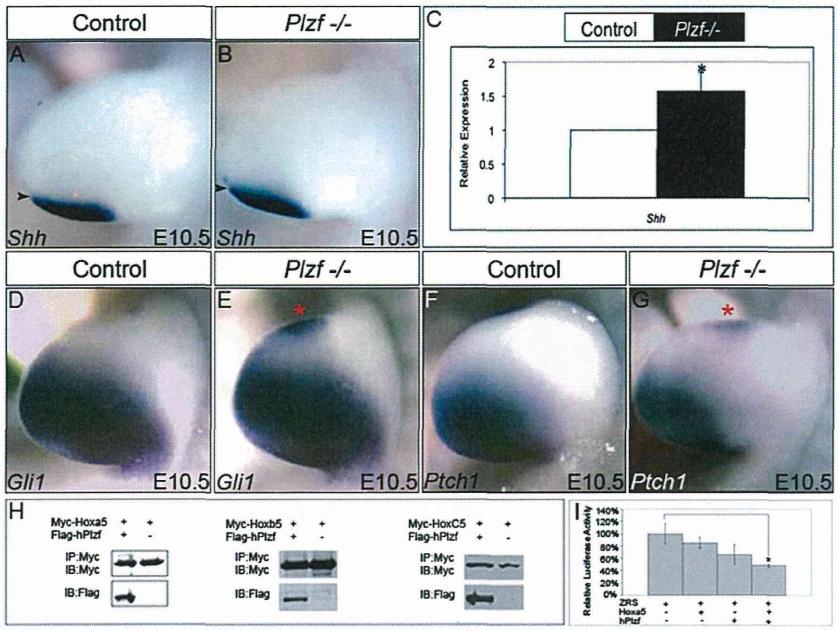
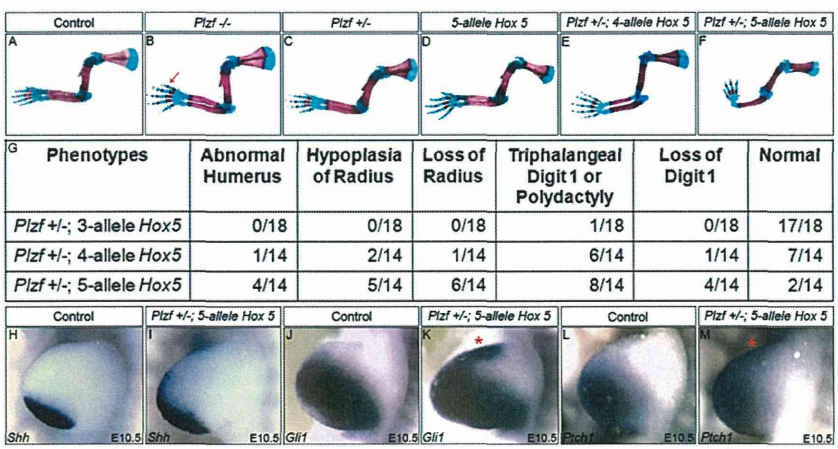


Fig. 4. Hox5 and Plzf cooperatively mediate repression of *Shh* expression via ZRS. (*A–G*) The *Shh* signaling pathway is disrupted in *Plzf* mutant forelimbs similar to that in *Hox5* mutant forelimbs. The expression of *Shh* is anteriorized in *Plzf* mutant forelimb buds compared with controls (*A* and *B*; black arrowheads mark the WT anterior boundary), and qRT-PCR analysis demonstrates an increase in *Shh* levels in *Plzf* mutants (*C*; asterisk indicates significant differences from controls; $P < 0.05$). Ectopic, anteriorized *Gli1* and *Ptch1* expression is observed in all *Plzf* mutant forelimbs at E10.5, confirming anteriorized *Shh* activity (*D–G*; red asterisks mark ectopic anterior expression). (*H*) Coimmunoprecipitation assays from cell lysates cotransfected with Myc-tagged Hox5 proteins and Flag-tagged Plzf protein. Immunoprecipitation with anti-Myc (Hox) antibodies and immunoblotting (IB) for anti-Flag (Plzf) results in coimmunoprecipitation (IP) of Myc-tagged Hoxa5, Hoxb5, and Hoxc5 with Plzf. (*I*) ZRS-driven luciferase reporter activity trends downward when cotransfected with Hox5 protein expression constructs or with the Plzf protein expression construct; however, cotransfections of the ZRS reporter with both Hox5 and Plzf proteins result in statistically significant down-regulation of expression from this reporter.

anterior *Shh* expression during forelimb development to influence forelimb AP patterning.

To provide evidence supporting possible direct regulation at the ZRS, we examined the ability of transfected Hox5 proteins and Plzf to regulate reporter expression through the ZRS enhancer. Transfection of any of the three Hox5 proteins or Plzf alone with the ZRS reporter did not result in any statistically significant change in expression, although the levels trended downward; however, transfection of any of the Hox5 proteins and Plzf together resulted in a statistically significant down-regulation of baseline expression, consistent with direct regulation of *Shh* expression through the ZRS enhancer (Fig. 4*I*). We made multiple



Phenotypes	Abnormal Humerus	Hypoplasia of Radius	Loss of Radius	Triphalangeal Digit 1 or Polydactyly	Loss of Digit 1	Normal
Plzf +/-; 3-allele *Hox5*	0/18	0/18	0/18	1/18	0/18	17/18
Plzf +/-; 4-allele *Hox5*	1/14	2/14	1/14	6/14	1/14	7/14
Plzf +/-; 5-allele *Hox5*	4/14	5/14	6/14	8/14	4/14	2/14

Fig. 5. *Hox5* and *Plzf* genetically interact to pattern the anterior limb and *Shh* expression in vivo. (*A–G*) Skeletal preparations from E18.5 *Plzf*, 5-allele *Hox5*, and compound *Hox5;Plzf* mutants. *Plzf* heterozygotes and embryos with up to five mutant *Hox5* alleles (*Hox5Aabbcc*) are indistinguishable from controls (*C* and *D*), whereas *Plzf* mutants exhibit preaxial defects with 100% penetrance in our background (*B*; arrow denotes triphalangeal digit 1). Compound mutants heterozygous for *Plzf* plus three mutant *Hox5* alleles rarely display a phenotype (*G*), but *Plzf* heterozygotes with four or five mutant *Hox5* alleles display increases in the penetrance of anterior limb defects (*E–G*), demonstrating strong genetic interaction between *Hox5* and *Plzf*. (*H–M*) The *Shh* pathway is derepressed in *Hox5;Plzf* compound mutants. *Shh* (*H* and *I*), *Gli1* (*J* and *K*), and *Ptch1* (*L* and *M*) are ectopically expressed in *Plzf* $^{+/-}$*;Hox5* 5-allele compound mutants compared with controls. Controls include WT, Plzf heterozygous, and compound Hox5 mutants with five or fewer mutant alleles; red asterisks denote anteriorized expression.

attempts to perform ChIP of both Plzf and Hox5 at the ZRS promoter in vivo. Despite the successful use of the antibodies at other enhancers, the very high level of background (control antibody) binding at the ZRS and the potentially small numbers of responding cells in the limb bud precluded conclusive evidence of binding of these proteins in vivo (Fig. S4 and *SI Materials and Methods*).

Discussion

In this study, we show a unique and unexpected role for *Hox5* genes in limb AP patterning. A myriad of genetic studies have defined important roles for *HoxA* and *HoxD* group 9–13 genes in forelimb development (9–14). Recently, we reported that *Hox9* genes from *HoxB* and *HoxC* complex, along with *Hoxa9* and *Hoxd9*, are also required to define the posterior forelimb compartment (15). However, previous loss-of-function studies have provided no evidence that anterior, non-*AbdB Hox* genes participate in patterning limb skeletal elements. Here we report limb phenotypes resulting from loss of *Hox5* paralogous gene function, further demonstrating pivotal roles for *HoxB* and *HoxC* complex genes in forelimb AP patterning, with *Hoxa5*, *Hoxb5*, and *Hoxc5* controlling anterior forelimb patterning.

The limb defects in our *Hox5* mutants are restricted to forelimbs, with no defects in hindlimb development observed. The limb defects in quadruple *Hox9* mutants are also restricted to the forelimbs (15). Taken together, our findings highlight significant differences in how anterior and posterior limb compartments are established in forelimbs and hindlimbs. This is surprising, considering the downstream factors currently known to play critical roles in AP patterning (i.e., *Hand2*, *Gli3*, and *Shh*) function similarly in both forelimbs and hindlimbs. Our findings from both loss of *Hox9* paralog function (15) and the present study suggest that early axial *Hox* expression in the lateral plate mesoderm controls the establishment of the anterior (*Hox5*) and posterior (*Hox9*) compartments of the forelimb. None of the numerous combinations of posterior *Hox* loss-of-function mutants reported to date (9–14) are known to lead to hindlimb AP patterning defects analogous to those reported for loss of *Hox9* or *Hox5* paralogs. Itou et al. (53) recently demonstrated that LIM-homeodomain factor *Islet1* is a critical regulator of *Hand2* expression and the posterior compartment in hindlimbs. How the hindlimb anterior compartment is established remains to be discovered.

It is also interesting to note that although *Hox5* paralogs and *Hox9* paralogs control anterior and posterior patterning, respectively, *Hox9* paralogs are responsible for initiation of *Hand2*, whereas no disruption of early anterior/posterior compartment formation is observed in *Hox5* mutants (ref. 15 and this paper). In *Hox5* mutant limbs, the initial *Hand2*/*Gli3* pattern is normal, but downstream expression of *Shh* is affected, consistent with *Hox5* regulating *Shh* expression more directly. This finding is also consistent with previous reports demonstrating that numerous point mutations in the ZRS of mouse, humans, chickens, and cats result in ectopic *Shh* activity in anterior domains of the limb bud and result in phenotypes similar to those that we detected in *Hox5* mutant mice, including defects in the stylopod, anterior zeugopod, and digits (5–7, 17–28).

The findings reported here also reveal a role for *Plzf* in regulating *Shh* expression in limb AP patterning. We detected anteriorized *Shh* expression in *Plzf* mutants, in contrast to a previous report (37). The discrepancy may be related to the use of different *Plzf* mutant alleles; we observed 100% penetrance of forelimb defects in the mutants used in the present study, significantly higher than in the previously reported mutant allele (37). Our finding that *Shh* is regulated downstream of *Plzf* in limb AP patterning is further supported by changes in *Ptch1* and *Gli1* expression in addition to *Shh* expression.

Our genetic and molecular analyses of *Hox5* function in forelimb development support a model in which Hox5 proteins, interacting with Plzf, act as repressors of *Shh* expression. Among the many potential binding sites in the ZRS are several putative Hox- binding sites and at least one putative Plzf-binding site. The putative Plzf site is mutated in the "Cuban mutation," one of the human mutations with limb phenotypes similar to those in the mice reported here, including radial aplasia (7). There is also a report of three independent probands with anterior forelimb phenotypes (mostly triphalangeal thumbs) that harbor a mutation in one of the three putative Hox-binding sites (19).

The activity of Hox5 and Plzf likely combine with numerous other factors that bind to this enhancer to both activate and repress *Shh* expression. Several factors, including *Hand2* and *HoxD*, have been shown to activate *Shh* expression via the ZRS limb enhancer element (54, 55). *Etv4*/Etv5 and *Twist 1* have been shown to cooperate to restrict *Shh* expression to the posterior limb bud (50, 56). The ZRS is more than 700 bp long, and thus it is likely that a myriad of factors converge at this critical regulatory hub to direct proper expression of *Shh* in the developing vertebrate limb. A complete understanding of the factors that bind to these sites and how they interact remains to be delineated in future studies.

Materials and Methods

Mice and Whole-Mount in Situ Hybridization. All mouse mutant strains used in this study have been reported previously (41, 52). Control mice included both WT embryos and low-allele littermates from *Hox* and *Hox/Plzf* crosses. The results were identical, and thus we use the term "control" throughout for clarity. Mutant mouse strains, early skeletal preparations, and standard whole-mount in situ hybridization were as described previously (14, 41, 52). All in situ probes were prepared as described previously (15, 57, 58). All experiments were performed following protocols approved by the University of Michigan's Institutional Committee on the Use and Care of Animals.

Cell Culture, Transfections, Luciferase Assays, and Coimmunoprecipitation Assays. HEK293 or HEK293T cells were used and plated as described previously (59). Cell transfections were performed by $CaPO_4$ precipitation. Coimmunoprecipitation assays were performed as described previously (59). Hox5 and Plzf protein-coding sequences were amplified from their cDNAs using the primers listed in *SI Materials and Methods*, then subcloned into pCS2+MT or p3XFlag-CMV vectors (Sigma-Aldrich). Details of plasmid generation and reporter assays have been reported previously (59). The highly conserved ZRS core region was amplified using the primers listed in *SI Materials and Methods* and then subcloned into a pGL3 promoter vector (Promega). The Student *t* test was used to determine statistical significance. All experiments were repeated at least three times in independent experiments.

RNA Isolation and Quantitative RT-PCR. RNA was isolated from mouse limbs with the Qiagen RNeasy Micro Kit. Quantitative RT-PCR (qRT-PCR) was carried out using Roche FastStart SYBR Green Master Mix. Primer sequences have been described previously (51). Relative expression values were calculated as $2^{-\Delta\Delta Ct}$, and values of controls were normalized to 1. GAPDH served as an internal control for normalization in all qRT-PCR experiments, and the Student *t* test was used to determine statistical significance ($P < 0.05$). All experiments were repeated at least three times.

ACKNOWLEDGMENTS. We thank Drs. Benoit Bruneau, Xin Sun, and Licia Selleri for the in situ hybridization probes. This work was supported by National Institutes of Health Grants AR057018 and AR061402 (to D.M.W.) and National Center for Research Resources Grant UL1RR024986 (to S.M.H.).

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概論

エピジェネティクスで組織可塑性を理解する

竹内　純

受精し１つの命が生まれ，組込まれた遺伝プログラムに従って形作りを行っていくうえで，組織特異的に発現する転写因子群を中心としたgeneticなアプローチのみでは説明がつかない現象が明らかになってきた．それは，器官形成後，各々の臓器は環境変化に適応（adaptation）すべく機能を変化させ，再構築（remodel）すべく形も変化させ，双方をくり返して生命活動の維持をしているからである．また逆に，発生過程においても成体組織においても外界のシグナルや転写因子の作用に簡単に応答しないようなシステムも存在する．このような能力を高める因子がエピジェネティック因子群である．本特集では，このような疑問をエピジェネティック因子に着目することから理解をめざす研究を紹介したい．

1 遺伝子機能に多様性をもたらす因子

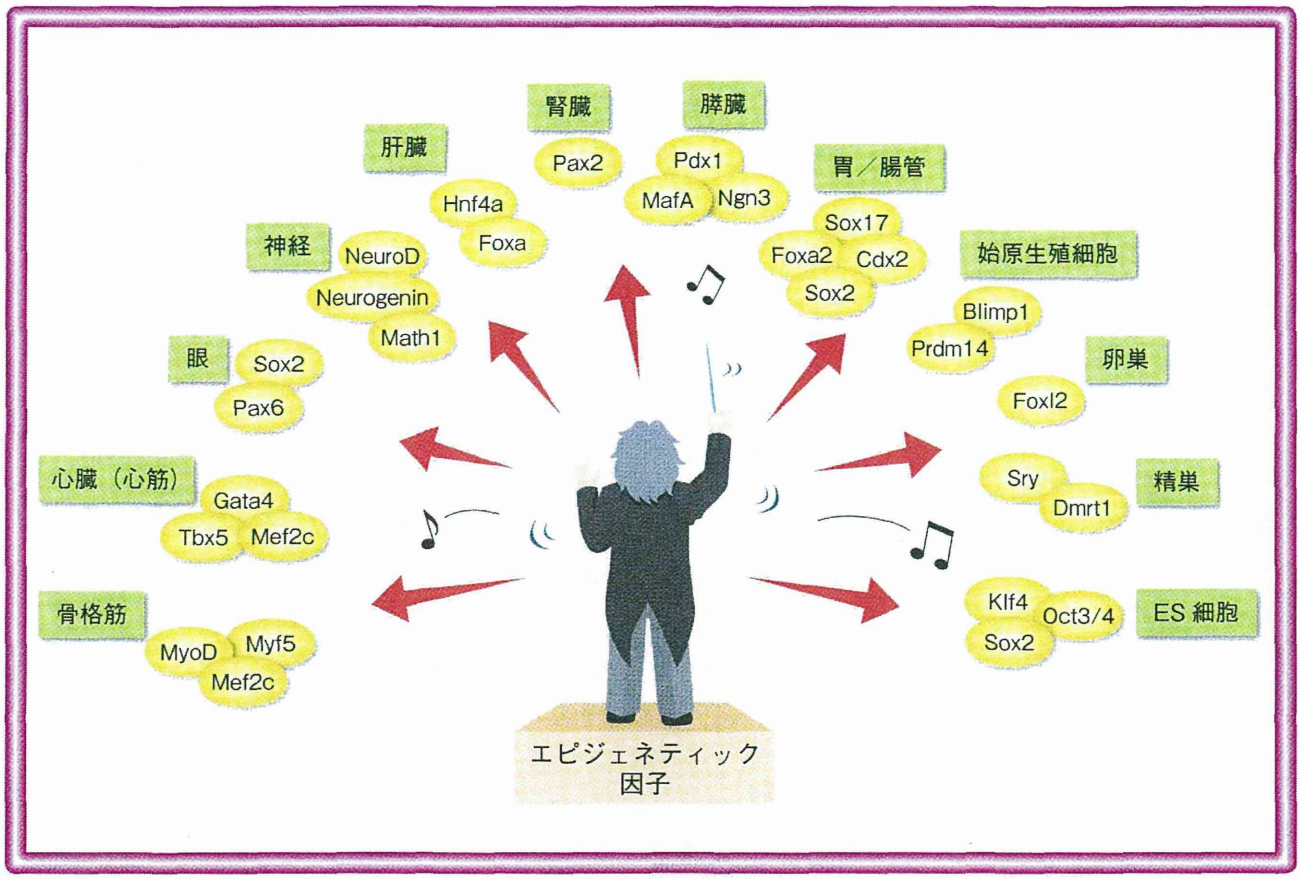
❶ 発生過程に不可欠なプログラム因子としてのエピジェネティック因子

われわれの身体は，運動，感覚，循環，呼吸，消化，生殖などさまざまな機能を司る器官から構成されている．各々の器官には，脳，心臓，肝臓，腎臓，など専門の機能をもった臓器が含まれ，これらが連携をとりつつ成り立っている．概念図１のように各器官の形成には，似たような遺伝子が使われている．例えば，心筋も骨格筋もMef2cを利用していることが見て取れる．同じ中胚葉由来，かつ，階層的な意味で利用しているといわれればそうかもしれない．では，Sox2はどうだろう？ 眼，腸管，そしてES細胞でも重要な遺伝子である．もう少し体系的に見ると，Sox遺伝子群，Pax遺伝子群，Fox遺伝子群は多くの細胞分化において重要因子として見受けられる．もちろん，パラログ間での機能的な差異がないとは言い切れないが，細胞に特異性をもたせ，固有な組織を形成するためには，適切なタイミングでの遺伝子発現制御，RNA修飾，翻訳後修飾が行われなければならない．この調節を担い，遺伝子の機能に多様性をもたせているのがエピジェネティック因子（エピ因子）である．

❷ エピ因子はオーケストラのコンダクター
―心臓や肝臓，精子の特異性はどうやって生まれるのか？

１つの受精卵から機能形態の異なる臓器ができるまでには，さまざまな因子が関わってい

Understanding tissue plasticity by epigenetic factors
Jun K. Takeuchi：Department of Biological Science, Graduate School of Science, The University of Tokyo/ Institute of Molecular and Cellular Biosciences, The University of Tokyo/ "Understanding Life by iPS Cells Technology", PRESTO, JST（東京大学大学院理学系研究科生物科学専攻／東京大学分子細胞生物学研究所／JSTさきがけ「iPS細胞と生命機能」）



概念図1　エピジェネティック因子はオーケストラのコンダクターである
エピジェネティック因子はオーケストラのコンダクター（指揮者）のように，各臓器・器官分化に関わる因子の機能を時空間的に統率する

る．マスター因子という解釈は個々の研究者によって考えが異なると思われるが，それでも転写調節因子群を中心とする各細胞・組織の分化に重要な因子が少しずつ明らかにされてきている．概念図1は各臓器・器官分化に深く関わる因子を示したが，かつて骨格筋分化のマスター因子と称されたMyoDでさえ，単独では骨格筋への分化誘導は難しい．それに加えてMyoDの働ける環境をつくり出すためのエピ因子の1つ，クロマチンリモデリング因子群であるChd2やBrg1の存在が重要である[1]．心臓誘導では転写因子Tbx5とGata4の他にクロマチンリモデリング因子Baf60cの存在が重要である（中村らの稿）[2]．神経発生においてもメチル化因子の絶妙なバランスによって，ニューロンになるかアストロサイトになるかの分化決定がなされる（上薗らの稿）[3]．もちろん分化誘導にはさまざまな組合わせパターンがあるだろうし，概念図1で取り上げた組合わせがすべてではない．だからこそ興味深い．そして，事実，個々の細胞分化・運命決定にはエピ因子の存在は欠かせない．転写因子は実行隊，オーケストラに例えるなら演奏者であるのに対して，エピ因子は演奏者たちの能力を上手に引き出し，独特の音色を出し，ハーモニーを奏でる指揮者のようである．

❸ 細胞未分化性はどのように維持されているのであろうか？

一方，細胞分化が抑制されているES細胞や胚性前駆細胞・体性幹細胞の未分化性はどのように保たれているのであろうか？ これには，分化を司る遺伝子群の抑制と未分化性を維持す

る遺伝子群の恒常的な活性化という双方向の制御が関与する．

前者の観点では，標的遺伝子の転写を抑制するポリコーム複合体やヒストン脱メチル化酵素，mRNAの転写・翻訳制御に関与するsmall RNA，lncRNA（long non-coding RNA）といった機能性RNAなどが重要になる．なかでもポリコーム複合体は，ES細胞に特徴的なbivalentなエピジェネティックマークの形成など，胚発生過程のさまざまな局面で機能している．ショウジョウバエの前後軸形成においてポリコーム複合体が重要であることはよく知られているが（遠藤・古関の稿），神経幹細胞の未分化性維持と分化方向決定にもポリコーム複合体のRing1a/bが深く関わっている[4]．さらに最近の研究により，ES細胞のself-renewal機能[5]，TS細胞維持と栄養膜巨細胞分化制御機構における，新たなポリコーム複合体の機能が明らかになってきた（遠藤・古関の稿）．

一方，後者の未分化性の維持におけるクロマチンマークの関与であるが，transdifferentiationに必須の因子が細胞種間で異なるように，エピ因子の関与も細胞種間で異なると考えられる．例えばH3K79メチル化は，ES細胞の未分化性維持には必須ではなく，むしろ分化因子としての性質を有する一方で，精子幹細胞においては幹細胞性の維持に必須である（牧野・岡田の稿）．エピ因子の標的特異性に関する知見は乏しいが，転写因子とエピ因子の協調を考えるうえで，今後掘り下げていくべき課題の1つである．

❹ 染色体へのマーキングによる遺伝子量の調整？

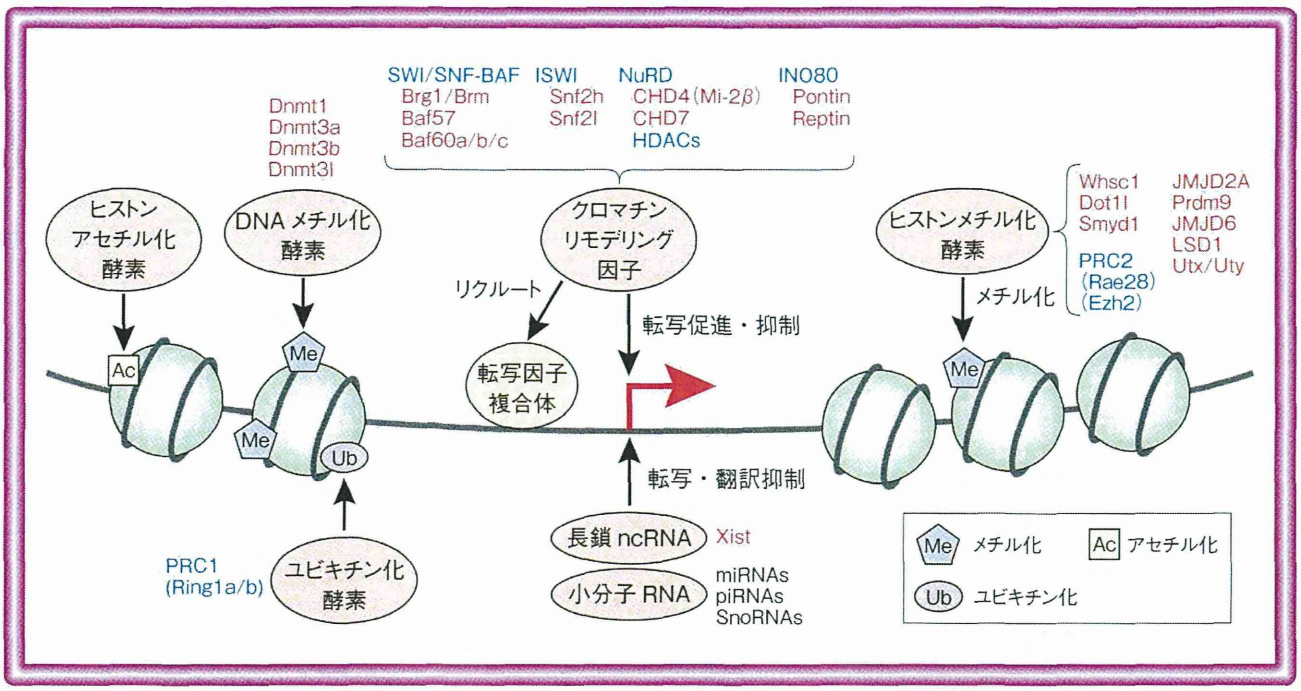
体内で分化した組織や細胞においては，それぞれに特徴的な遺伝子発現が行われている．では発現する遺伝子の量は，どのように調整されているのだろうか？ 先ほど述べた機能性RNA分子はその一端を担う．ncRNAであるXistは，雌にある2本のX染色体のうち1本を不活性化することでX染色体から転写される遺伝子量を調整している（酒田・佐渡の稿）．またインプリント遺伝子（2909ページの用語解説参照）の発現調節やトランスポゾンの抑制にも，DNAメチル化およびヒストンメチル化の関与が知られている．miRNA分子の1つmiR-1は心発生過程において転写因子であるHand2の転写後調節を行い，タンパク質の量を調節することで心筋増殖を抑制し心筋量を調節している[6]．さらに最近の研究では，ヒストンメチル化酵素Prdm9が遺伝子配列を読み取って染色体組換えを制御するという例も報告されている（牧野・岡田の稿）．

❺ 疾患発症と重篤化はいかにして生じるのか？

細胞運命決定のみならず，エピ因子は疾患責任遺伝子としてもふるまい，疾患が重篤化する際にも，エピ因子の発現量が関わっていることが報告されている．なかでもがん化とエピ因子の作用機序に関しての研究は解析が非常に進んでおり，エピ因子を標的とした創薬（＝エピドラッグ）開発も盛んに行われている．本編では，エピ因子のなかでもクロマチンリモデリング因子とヒストン修飾因子に変異が生じた際に，発生過程に起こる症状をいくつか概説したい（中村らの稿，牧野・岡田の稿）．心筋症のタイプとクロマチンリモデリング因子であるBrg1の発現量との明確な相関性は，今後の臨床応用に新たな知見を示したといえる[7][8]．一方遺伝性疾患は，従来生殖細胞のゲノム変異に起因するとされてきたが，近年の研究で，親ゲノムの一過性のエピジェネティック変化が子孫に伝達する可能性が示唆されており（牧野・岡田の稿），今後のさらなる調査・解析が待たれるところである．

❻ エピゲノム研究をどのように応用していくのか？

山中3因子によるiPS細胞の樹立をはじめとして，日本ではリプログラム研究が精力的に行



概念図２　エピジェネティック因子群による遺伝子発現・翻訳の制御

われており，世界的な影響力の強い研究領域である[9)~11)]．これらの結果は非常に有用で，細胞の可塑性，可逆性を見事に証明した研究であるが，課題点は分化転換能が低効率であるところであろう．この効果を上げる因子としてエピ因子が考えられる．

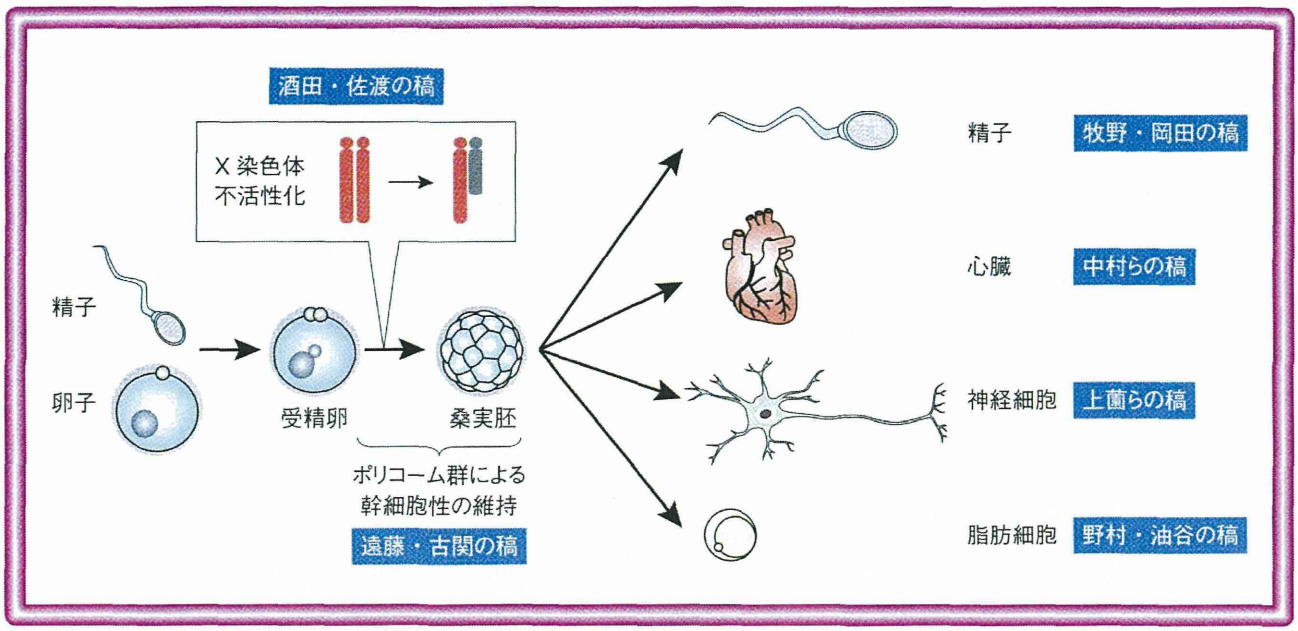
神経系は発生過程における細胞分化研究がいち早く進んだ器官系である．発生過程の詳細が理解されることによって，エピ因子を用いた組織の機能回復に向けた研究も発展してきている（上薗らの稿）．これからの研究はエピ因子をツールの１つとして認知していくことであろう．エピ因子と転写因子は演奏者と指揮者，一人ひとりが奏でる音色から１つの音楽が創り出せるように，お互いの想いが一致すると生命科学だけでなく，医療の面でもわれわれの生活においても素晴らしい相乗効果をもたらすと思われる．

❼ ゲノム上での変化を可視化する？

発生過程においてゲノム上におけるエピ因子と，エピ因子によるヒストン修飾・ＤＮＡ修飾は，本当に刻々と変化しているのだろうか？ 各々のステージにおける細胞種依存的なエピ因子の機能機序が明らかになっていくに従って，時系列的なゲノム上での変化に着目する研究の重要性が増してきた．本特集でも，今後発生とエピジェネティク作用とを結ぶ研究の中核となると考えられるエピゲノム変化の検出法を取り上げた（野村・油谷の稿）．

2 エピジェネティック因子の種類と機能

概念図２に示すとおり，転写因子を取り巻くように，非常に多くのエピ因子が存在する．詳細な機能は各説をご覧いただきたい．興味深いことは，次頁に示すような各エピ因子が単独で機能しているのではなく，相乗的な作用で標的遺伝子の抑制や亢進を担っていることである．また，転写産物はその後の多様な修飾を受けることにより，時間軸での多様性が生まれる．



概念図３　発生の各段階に関わるエピジェネティクス
１つの受精卵が多様な細胞・組織へと分化する段階において，さまざまなエピジェネティック制御が行われる

① ヒストン修飾酵素
② DNA 修飾酵素
③ クロマチンリモデリング酵素
④ RNA 分子
⑤ ユビキチン化，SUMO 化，リン酸化修飾

3 発生学におけるエピジェネティクス研究とは？—細胞の個性を決めるしくみに迫る

筆者は小学２年生の時にカエル卵の発生する様子を観察して以来，発生学の魅力に取り憑かれた．発生学の醍醐味は，なんといっても胚が劇的にその形を変えていくところにある．形作りを知るために細胞を知り，細胞を知るために遺伝子の機能解析を行うというように研究の中心が移行した．本概論で述べてきたが，20 世紀末に多くの形態形成遺伝子が単離され，その機能の精巧さが明らかになるにつれて，形作りはより複雑なネットワーク機構によって成り立っていることが理解されてきた．この複雑性を理解，つまり，発生学を理解するうえで，エピ因子の理解は欠かせない．エピジェネティック因子群は細胞の個性を決定し，細胞の維持に深く関わる因子なのである．

本特集では，細胞・組織が個性を獲得し形作りに関わる重要なエピ因子を６つのトピックスに絞り，紹介する（概念図３）．ページ数に限りがあり，細胞の数だけ，疾患の数だけ，組織・臓器の数だけ存在するエピ因子の機能を紹介できなかった点，およびDNA メチル化酵素の機能，ncRNA 分子の多様な機能，翻訳後修飾因子群について取り上げることができなかったことが残念である．本特集を読んで興味をもたれた方は，別視点からのエピ因子についてぜひ理解深めていただきたい．