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AP-2-null cells disrupt morphogenesis of the eye, face, and limbs in chimeric mice

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ABSTRACT **The homozygous disruption of the mouse AP-2 gene yields a complex and lethal phenotype that results from defective development of the neural tube, head, and body wall. The severe and pleiotropic developmental abnormalities observed in the knockout mouse suggested that AP-2 may regulate several morphogenic pathways. To uncouple the individual developmental mechanisms that are dependent on AP-2, we have now analyzed chimeric mice composed of both wild-type and AP-2-null cells. The phenotypes obtained from these chimeras indicate that there is an independent requirement for AP-2 in the formation of the neural tube, body wall, and craniofacial skeleton. In addition, these studies reveal that AP-2 exerts a major influence on eye formation, which is a critical new role for AP-2 that was masked previously in the knockout mice. Furthermore, we also have uncovered an unexpected influence of AP-2 on limb pattern formation; this influence is typified by major limb duplications. The range of phenotypes observed in the chimeras displays a significant overlap with those caused by teratogenic levels of retinoic acid, strongly suggesting that AP-2 is an important component of the mechanism of action of this morphogen.**

The transcription factor AP-2, also known as AP-2α, is a retinoic acid (RA)-responsive gene that is expressed in tissues undergoing complex morphogenic changes during vertebrate embryogenesis (1–4). In both the mouse and chick, AP-2 RNA and protein are first detected at the initial stages of neural crest cell formation, and the expression of AP-2 continues when these cells enter their migratory phase (2, 3). At later stages of embryogenesis, AP-2 expression is concentrated in the developing epidermis, facial prominences, branchial arches, and the limb bud progress zone.

A functional AP-2 gene is vital for normal mammalian embryogenesis: mice that contain a homozygous disruption of the AP-2 gene die perinatally and exhibit severe developmental defects (5, 6). The earliest gross morphological alteration observed [embryonic day 9.0 (E9.0)] is a complete failure of cranial neural tube closure (5, 6). By E12.5, the exposed cranial neural tissue expands to cover the entire head so that normal cephalic morphology is disrupted. The exencephaly is accompanied by major alterations in the craniofacial skeleton, such that many bones of the achordal skeleton are missing or deformed. In the trunk region, the absence of AP-2 disrupts two additional morphogenic events. First, the ventral body wall fails to close, resulting in thoracoabdominoschisis. Second, many AP-2-null mice have forelimb phocomelia, which is characterized by loss of the radius.

Given the severe and pleiotropic abnormalities displayed by the knockout (KO) mice, we reasoned that multiple independent developmental programs are probably regulated by AP-2. Therefore, we adopted an approach that enabled independent developmental defects to be obtained in isolation. This strategy relied on the generation of chimeric mice composed of both wild-type (wt) and AP-2-null cells; when a phenotype results from the interaction of multiple independent developmental defects, an individual chimera will display particular aspects of the pathology depending on the distribution of the AP-2-null cells. A second consideration behind the generation of chimeras was to determine whether the severity of the KO phenotype concealed more subtle requirements for AP-2 in later developmental processes. In particular, we reasoned that the exencephaly might obscure a need for AP-2 in other morphogenic events shaping the head. The presence of appropriate populations of wt cells in a chimera might rescue such early defects and allow the influence of AP-2-null cells to be studied subsequently in embryogenesis. Below, we present the data obtained from this analysis and discuss our findings in relation to the original AP-2-null phenotype. These studies demonstrate that AP-2 is required for at least five major independent morphogenic processes during mammalian embryogenesis and strongly support a role for AP-2 in the etiology of human congenital defects.

MATERIALS AND METHODS

Targeting Vector Construction. A hygromycin-based targeting vector, pHygKO (Fig. 1), was used to disrupt the remaining AP-2 allele in an embryonic stem (ES) cell line heterozygous for AP-2 (6). The pHygKO plasmid was identical with the neomycin cassette used previously (6), except that the *neo* gene was replaced with a 6.4-kb *Xho*I fragment derived from the plasmid pGKβH (a gift of Harris Jacobs, Yale University, New Haven, CT). The insertion of pGKβH sequences replaced a critical region of the AP-2 dimerization domain with both a phosphoglycerate kinase promoter/hygromycin resistance gene fusion and a phosphoglycerate kinase promoter/*lacZ* gene. The pHygKO construct was linearized in vector sequences with *Bsp*HI prior to electroporation.

Cell Culture. ES cells were cultured by using standard procedures (7) on a layer of γ-irradiated feeder fibroblasts in DMEM (Mediatech, Herndon, VA) containing 15% fetal bovine serum (HyClone) and supplemented with 1,000 units/ml leukemia inhibitory factor (Life Technologies, Gaithersburg, MD). Following electroporation, clones were selected in the presence of 200 mg/ml G418 (Life Technologies), 100 units/ml hygromycin (Calbiochem), and 2 mM ganciclovir (Syntex, Palo Alto, CA) on a layer of feeder-free extracellular matrix (8). ES cell clones that had undergone a second homologous recombination were identified by Southern blotting (Fig. 1). Clones that contained a nonhomologous insertion

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Abbreviations: KO, knockout; wt, wild type; RA, retinoic acid; RAR, retinoic acid receptor; ES, embryonic stem; β-gal, β-galactosidase; E, embryonic day.

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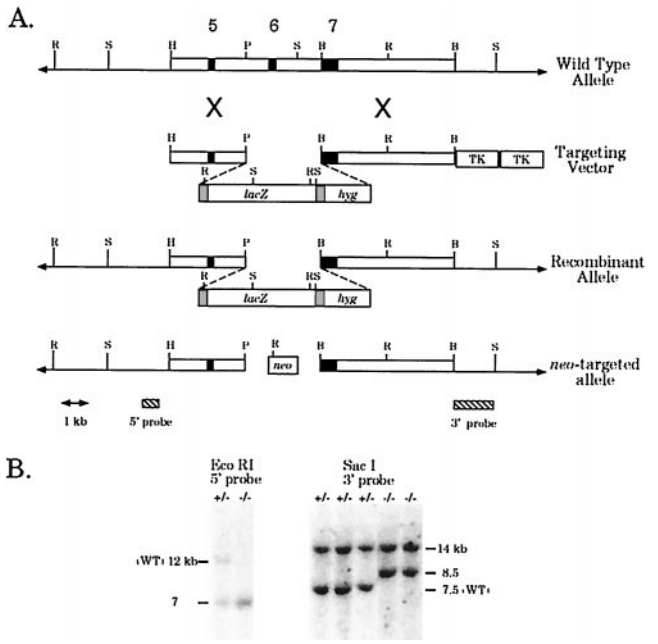

FIG. 1. Targeting of both alleles of the AP-2 gene. (*A*) Targeting strategy. The wt AP-2 gene (*Top*) and original *neo*-targeted disruption (6) (*Bottom*) are shown along with the pHygKO vector and the recombinant allele derived from this targeting construct (*Middle*). Exons 5, 6, and 7 are indicated by black boxes; TK, thymidine kinase gene; restriction enzyme sites (R, *Eco*RI; S, *Sac*I; H, *Hin*dIII; P, *Pst*I; B, *Bgl*II) and locations of 5′ and 3′ external probes are also indicated. (*B*) (*Left*) Southern blot of *Eco*RI-digested DNA from heterozygous ES cell line (+/−) and AP-2-null line TN63 (−/−) probed with 5′ fragment. In TN63, the remaining 12-kb wt fragment is converted to 7 kb by homologous recombination. (*Right*) Southern blot of *Sac*I-digested DNA from heterozygous ES cell lines (+/−) and AP-2-null lines TN91 and TN95 (−/−) by using the 3′ probe. The 14-kb *neo*-disrupted allele is constant; the 7.5-kb wt fragment is converted to 8.5 kb by homologous recombination.

of the *hyg* gene elsewhere in the genome and still retained one wt allele of AP-2 were selected as heterozygous controls.

Generation and Analysis of Chimeric Mice. C57BL/6 and Swiss–Webster mice were purchased from Taconic Farms. ES cells were microinjected into 3.5 days postcoitum C57BL/6 blastocysts by using standard techniques (7). Injected embryos were transferred into pseudopregnant Swiss–Webster females and analyzed subsequently at several developmental timepoints. Similar phenotypes were obtained in chimeras derived from three independent AP-2-null ES cell clones. ES cells contributed significantly to the chimeras (up to ≈90%) as shown by β-galactosidase (β-gal) activity, isoenzyme analysis, and coat color determination. We had originally designed the *lacZ* insertion to be an independent marker of cell autonomy. However, a comparison between the distribution of β-gal staining and glucose-6-phosphate isomerase isozyme analysis of isolated tissues revealed that the *lacZ* gene was not uniformly expressed in the chimeras. In particular, we found that β-gal staining was limited to cell types that normally express AP-2, such as the ectoderm and facial mesenchyme. In contrast, tissues such as the liver, which do not normally express AP-2, did not contain any cells staining for β-gal activity; however, these tissues were extensively populated by AP-2-null cells as judged by glucose-6-phosphate isomerase isozyme analysis (T.N., A.P., and T.W., unpublished observations). These findings suggest that the phosphoglycerate kinase promoter, which is embedded within the AP-2 gene locus, is influenced by the tissue-specific components of the AP-2 enhancer sequences or by the chromatin context of the AP-2 gene. Although we could not use *lacZ* expression to determine the cell types responsible for a particular defect, we nevertheless found that populations of AP-2-null cells were always associated with the site of pathology in a given chimera (for example, Fig. 2; and data not shown).

Glucose-6-phosphate isomerase analysis of adult mouse tissues and whole-mount β-gal staining of 0.2% paraformaldehyde-fixed embryos were performed by standard procedures (7). For cryosections, embryos were fixed in 0.2% paraformaldehyde and embedded in OCT medium (Tissue-Tek). Subsequently, sections were stained for β-gal activity (7) and counterstained with nuclear fast red. For paraffin sections, animals were fixed in 4% paraformaldehyde prior to embedding and then stained with hematoxylin and eosin. Skeletal staining with alcian blue and alizarin red was as described previously (9).

RESULTS AND DISCUSSION

Generation of Chimeras. To identify the individual developmental programs affected by the absence of a functional AP-2 gene, chimeric mice derived from either AP-$2^{+/-}$ or AP-$2^{-/-}$ ES cells were examined between E9.5 and birth for any overt developmental defects. Of the 60 mice generated from the heterozygous +/− ES cells, only two showed any defects, and these defects were both minor herniations associated with the umbilicus (data not shown). In contrast, 88 of 240 embryos generated from AP-$2^{-/-}$ cells showed gross developmental abnormalities (≈37%; Table 1). The extent of the pathology in the chimeras varied from undetectable to an almost complete recapitulation of the AP-2-null phenotype (compare Fig. 2 *A* and *B*). Overall, the developmental defects observed in the chimeras could be grouped into five major categories affecting critical morphogenic events: formation of the body wall, face, neural tube, eye, and limbs (Figs. 2–5). The individual pattern formation defects were frequently seen in various combinations, but each of these five categories of congenital abnormality also were observed in isolation (Table 1), demonstrating that AP-2 is independently required for each of these developmental processes. Below, we discuss these individual developmental abnormalities and their relationship to the AP-2-null phenotype.

Body Wall Closure Defects. The analysis of chimeric animals demonstrated that an intact AP-2 gene is specifically required for ventral body wall closure (Fig. 2 *C* and *D*). Chimeric animals had a high incidence of ventral body wall closure defects (67% of the mutants) that varied in their location and

Table 1. Phenotypes of chimeric mice

	No. observed	%
Phenotype		
Body wall closure defects	59	67%
Midline	35	
Neck/behind ears	11	
Midline + neck/behind ears	13	
Exencephaly	20	23%
Eye defects	34	39%
Eye missing/abnormal	6	
Eyelid missing	28	
Craniofacial defects	39	44%
Limb defects	17	19%
Polydactyly/polysyndactyly	15	
Extra forepaw	1	
Extra forelimb	1	
Isolated phenotypes		
Exencephaly only	3	
Body wall only	14	
Craniofacial only	5	
Eye only	12	
Limb only	5	

severity (Table 1). In some embryos, the schisis only affected the abdominal region, whereas in others the defect extended through both the abdomen and thorax (Fig. 2*C*). Openings in the body wall also occurred as holes or nicks behind the ears or as slits from ear to ear. A combination of throat and ventral defects (Fig. 2*D*) occasionally produced the severe schisis observed in the AP-2 KO animals (5, 6). The failure of ventral closure is associated with the transition from a stratified keratinized epithelium on the dorsal surface of the embryo to only a thin peridermal covering that begins at the lateral margins of the body wall (Fig. 2*E*).

Because chimeras exhibited a high incidence of body wall closure defects that varied in their axial position, we postulate that AP-2 is normally required early in development throughout the margins of the lateral body wall. Indeed, the thoracoabdominoschisis observed in the absence of AP-2 is one of the most extensive body wall defects identified thus far. A more limited gastroschisis has been observed in mice containing a targeted disruption of either the *BMP-1* or MARCKS genes (10, 11). Similarly, mutation of genes in the *hoxb* gene cluster also can cause abnormal body wall closure and associated sternal defects (12, 13). Given the greater severity of the AP-2-null phenotype, the AP-2 protein may act upstream of these other genes to coordinate ventral closure.

Cephalic Defects. The phenotypes of the chimeras indicated that the exencephaly that would disrupt many of the normal tissue–tissue interactions regulating formation of the head is not the sole mechanism responsible for the AP-2-null cephalic phenotype. Instead, it is apparent that AP-2 has a direct role in regulating three major developmental pathways shaping the head: neural tube closure, craniofacial morphogenesis, and eye formation (Table 1, Figs. 2–4). The disruption of these three independent processes together contributes to the overall appearance of the AP-2 KO mouse head.

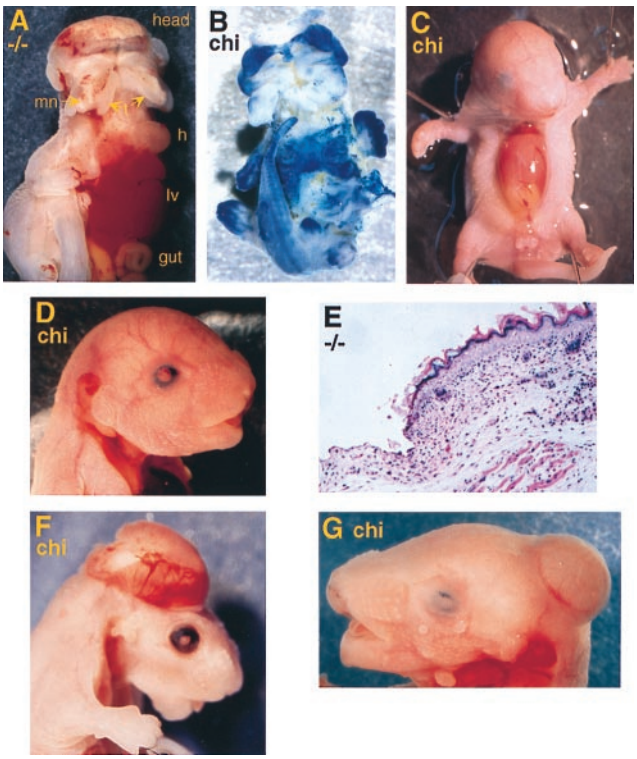

FIG. 2. Neural tube and body wall defects in AP-2 chimeric mice. (*A*) Newborn AP-2-null mouse. mn, mandible; t, tongue; h, heart; lv, liver. (*B*) E13.5 chimeric mouse with similar defects to AP-2-null animal. The *lacZ* gene present in one of the disrupted AP-2 alleles enables a component of the AP-2-null ES cell contribution to be detected by β-gal activity. (*C* and *D*) Failure of body wall closure in E18.5 AP-2 chimeric mice. In *D*, the schisis extends behind the ear pinna. (*E*) Transition of body wall at the lateral margins of an AP-2-null newborn pup from multilayered epidermis to thin peridermal covering. (*F* and *G*) E18.5 chimeras exhibiting cranial neural tube closure defects.

Several chimeras were obtained that exhibited only exencephaly. This finding was predicted because abnormal neural tube formation is the first gross morphological alteration observed in AP-2-null mice. In chimeras with exencephaly, the defects in cranial neural tube closure varied in position and severity along the anterioposterior axis of the head (Fig. 2 *B*, *F*, and *G*). These data suggest that appropriate neural tube closure requires AP-2 activity over the entire length of the cranial neural tube. This hypothesis is consistent with the high levels of AP-2 expression detected throughout the ectoderm adjacent to the lateral margins of the neural plate during embryogenesis (2, 3). The phenotypes observed in the exencephalic chimeras confirm that the AP-2 gene is an integral component of the regulatory hierarchy governing normal neurulation.

Craniofacial Morphogenesis. Two-thirds of all craniofacial abnormalities occurred independently of any neural tube closure defect. The craniofacial defects could be visualized both at the level of gross morphology and at the level of skeletal staining and typically involved structures derived from the frontonasal process (Figs. 3 *A–F* and 4*A*). Malformations included cleft lip with or without cleft palate and pronounced mandibular and maxillary dysmorphology. Examination of heterozygous animals provided further support for the role of AP-2 in face formation (Fig. 4 *G* and *H*). Several heterozygotes (4% penetrance) displayed an abnormal curvature of the upper snout that is associated with dental malocclusion, suggesting that even the loss of a single allele of AP-2 can cause inappropriate craniofacial morphogenesis.

The severe facial clefting, hypoplastic development of the jaws, and drastic facial dysmorphology observed in the chimeras are consistent with inappropriate development of the facial prominences, which are major sites of AP-2 expression during embryogenesis (2). The facial prominences are composed of neural crest-derived mesenchyme encased in an overlying epithelium. Normal craniofacial morphogenesis relies on the growth and fusion of these structures between E10 and E12 of mouse development (14). Our data suggest that inappropriate levels of AP-2 in the maxillary and mandibular prominences lead to hypoplastic development and dysmorphology of these facial primordia. Similarly, midline clefting of the primary palate in AP-2-null chimeras would be caused by defects in the morphogenesis and fusion of the frontonasal prominence and lateral nasal prominences. The craniofacial pathologies observed in AP-2 KO and chimeric mice are reminiscent of defects associated with homeobox and retinoic acid receptor (RAR) gene mutations (15–18). These data suggest that AP-2, RAR, and homeobox genes form part of a regulatory network of transcription factors that shape craniofacial development.

Studies in the chicken also have indicated a potential link between AP-2 expression and craniofacial morphogenesis. The addition of RA to the nasal pit of a developing chicken embryo causes hypoplastic growth of the facial prominences that results in facial clefting (3). These morphogenic changes are preceded by a rapid down-regulation of AP-2 expression, specifically in the frontonasal mass and lateral nasal prominences, tissues that are most sensitive to the presence of RA. Taken together with our findings, these data suggest that AP-2 may be an integral component of the pathway by which RA induces teratogenic alterations in craniofacial morphology. The AP-2 gene also has been linked to craniofacial defects in humans. Heterozygous deletions encompassing the distal end of chromosome 6, where *TFAP2A* is located, are associated with a variety of craniofacial deformities (19, 20). Moreover, chromosomal breakpoints defining the orofacial clefting type I syndrome have been mapped in the vicinity of the AP-2 gene (19). The precise nature of the mutations associated with these breakpoints has not been characterized. However, given the

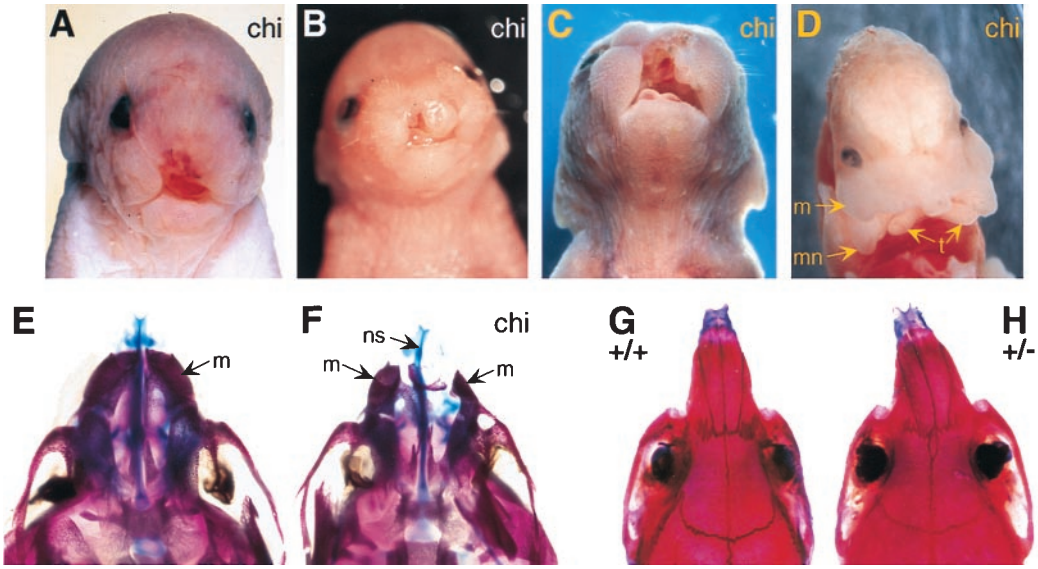

FIG. 3. Craniofacial defects. (*A–C*) E18.5 chimeras exhibiting craniofacial dysmorphology including orofacial clefting. (*A* and *B*) Frontal view; (*C*) View from the underside of the mandible illustrating cleft palate. (*D*) E13.5 chimeric mouse with craniofacial defects, most notably midline clefting of tongue and mandible. m, maxilla; mn, mandible; t, tongue. (*E* and *F*) Skeletal staining of E18.5 chimeric embryos. The mandibles were removed before photography. (*E*) Upper jaw of embryo with normal facial structures. (*F*) Upper jaw of chimeric embryo with cleft palate. ns, nasal septum. (*G* and *H*) Skeletal staining of adult mice. (*G*) wt; (*H*) AP-2 heterozygote exhibiting curvature of snout.

phenotype of the chimeric mice, it is feasible that this human craniofacial abnormality is caused by AP-2 gene alterations.

Eye Development. We also obtained chimeric embryos in which the only visible alteration was a defect in eye morphology (Fig. 4). The most frequent phenotype observed was an open eyelid, in which the epithelium that normally covers the eye from E17 onwards was absent (Fig. 4*A*). More strikingly, several chimeric mice were derived in which no eye was visible externally (Fig. 4 *B–F*). Gross morphological examination failed to reveal any recognizable ocular structures in some of these instances (data not shown); in other chimeras the eye was

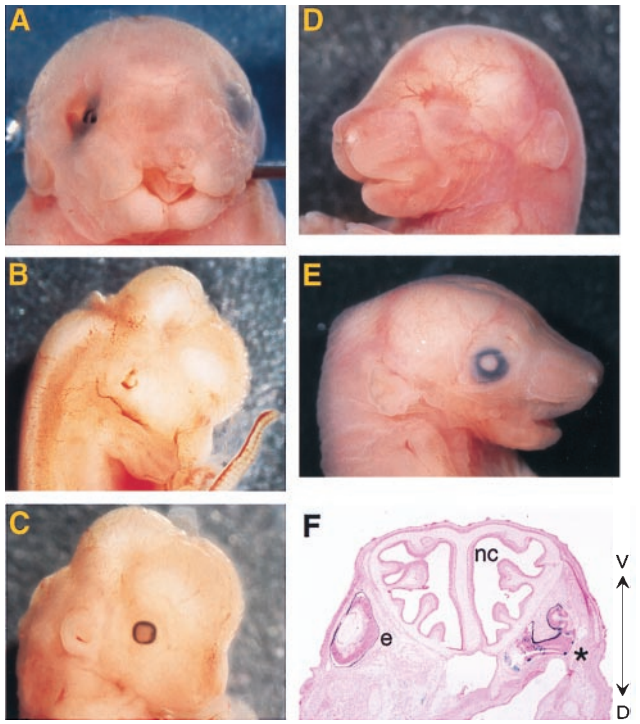

FIG. 4. Eye defects in AP-2 chimeric mice. (*A*) E18.5 chimera lacking the right eyelid (compare with normal fused left eyelid). (*B*) Anopthalmic E13.5 chimera (compare with normal littermate in *C*). (*D–F*) E16.5 chimera with an embedded, dysmorphic eye. The left eye is not readily apparent (*D*), in contrast to the right eye (*E*). (N.B. The photograph in *E* was taken after fixation). A transverse section (*F*) of this embryo reveals both the normal eye (e) and an embedded, dysmorphic eye (*). The nasal cavity (nc) and the dorsoventral polarity are marked.

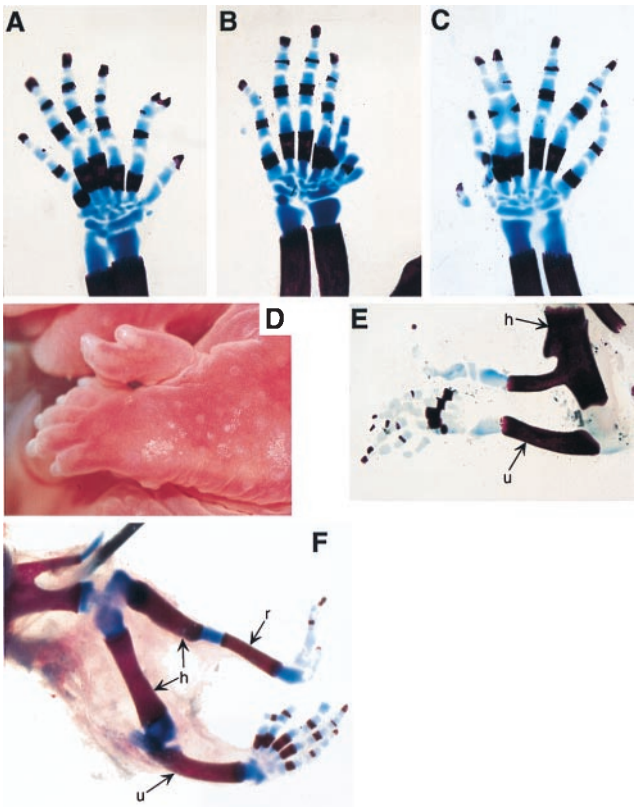

FIG. 5. Limb defects. (*A–C*) Polydactyly and polysyndactyly in forelimbs of E18.5 AP-2 chimeras. (*D*) Extra forepaw in E18.5 chimera. (*E*) Skeletal staining of forelimb shown in *D*. (*F*) Duplication of the humerus (h), resulting in an additional forelimb.

embedded within the head (Fig. 4 *D–F*). The ocular defects observed indicate that severe disruptions have occurred in the normal tissue/tissue interactions responsible for eye formation (21, 22), which is consistent with the expression of AP-2 in several ocular tissues during normal embryogenesis (ref. 2; J.W.-M. and T. W., unpublished observations). Further studies will be required to pinpoint the particular cell types that require AP-2 for normal eye formation; nevertheless, our findings conclusively demonstrate a new role for AP-2 as a component of the gene network controlling ocular development. The micropthalmia and anopthalmia observed in the KO and chimeric animals are reminiscent of those caused by mutations in the *BMP-7*, *Pax-6*, and *Otx2* genes (21, 23). Similarly, manipulations of RAR and *msx* gene expression also can lead to comparable defects in eye development (16, 24). These findings again suggest that there is a regulatory connection between AP-2, RARs, and homeobox genes.

Limbs. Limb defects had been recognized previously in the AP-2-null mice, where the lack of a radius was observed at a high penetrance (5, 6). Chimeras exhibited far more striking forelimb defects, indicating that the presence of AP-2-null cells had a major impact on limb pattern formation. Limb defects were observed in ≈20% of the mutant chimeras and were confined to the forelimb in all cases. The most common defects observed consisted of extra digits (polydactyly) or branched digits (syndactyly or polysyndactyly; Fig. 5 *A–C*). We also obtained chimeras that displayed more radical duplications of the proximal/distal axis of the forelimb (Fig. 5 *D–F*). In one chimera, two forepaws were observed on the same limb; both of these structures appeared to maintain the same anterior/posterior polarity (Fig. 5*D*). Skeletal staining of this chimera revealed what may represent either a branch in the humerus or an inappropriate fusion between the humerus and zeugopod (Fig. 5*E*). In a second instance, there was an extra forelimb on one side of the body axis associated with a duplication of the humerus (Fig. 5*F*).

The extra limb phenotypes observed in the chimeras have been documented in chickens, frogs, and *Drosophila* but are not commonly seen in mammals (25–28). Expression of AP-2 occurs in the limb bud progress zone during embryogenesis (2). Therefore, one possible mechanism for the action of AP-2 is that it is involved in specifying regional identity in the forelimb. In this instance, the absence of AP-2 might specifically disrupt the formation of the radius in the KO mice and complicate other pattern formation mechanisms in chimeric animals. An alternative possibility that is not mutually exclusive is that the expression of AP-2 in the limb bud progress zone may control cell proliferation in this region in response to signals from the overlying apical ectodermal ridge. Under these circumstances, the absence of a radius in the AP-2-null mice might arise if there were a temporary decrease in the number of cells being produced by the progress zone. Indeed, it has been found that after the progress zone moves more distally, the expression of AP-2 persists in the anterior part of the developing limb bud in which the radius is forming (29). This observation may explain why the zeugopod defects in KO mice are limited to the radius. Alternatively, it may indicate that other AP-2 family members present in the limb bud (29) can compensate for the absence of this transcription factor in some limb pattern formation processes.

The chimeric analysis produced very striking forelimb duplications that are not seen in AP-2 KO animals. Even in the complete absence of AP-2, ≈20% of the KO mice still developed forelimbs with a normal morphology, whereas the remainder were distinguished only by the absence of a radius (6). Thus, the limb duplications observed in chimeras must arise from inappropriate interactions between groups of wt and AP-2-null cells. Alterations in the pattern formation of appendages caused by the interaction of different fields of gene expression have been documented in both vertebrate and *Drosophila* systems (26, 28). Therefore, the interaction between fields of AP-$2^{+/+}$ and AP-$2^{-/-}$ cells also may be involved in establishing compartments responsible for mammalian forelimb pattern formation. One possible mechanism for this phenomenon is that AP-$2^{+/+}$ and AP-$2^{-/-}$ cells in the progress zone have different rates of proliferation. The juxtaposition of these fields of differential growth potential could lead to the development of new limb and digit fields. In support of this hypothesis, the expression of AP-2 in the progress zone is very sensitive to the presence of the overlying apical ectodermal ridge (3). In the chick, removal of the apical ectodermal ridge causes a rapid loss of AP-2 expression in the limb bud mesenchyme considerably before any subsequent truncation of limb outgrowth occurs. The effects of apical ectodermal ridge loss on AP-2 expression and limb bud morphology can be rescued by the application of fibroblast growth factor 4. Intriguingly, ectopic expression of fibroblast growth factor 4 during embryogenesis also can lead to limb duplications (25, 28). Taken together, these data suggest that AP-2 acts as one of the downstream effector molecules that control the growth and patterning of the forelimb in response to the fibroblast growth factor signal transduction pathway.

CONCLUSIONS

We have used AP-2 chimeric mice to separate and identify the various morphogenic events that are affected by the absence of AP-2 during embryogenesis. This study has revealed important new roles for AP-2 in embryogenesis that were masked by the severe phenotype of the KO mouse. In total, we have identified five major morphogenic pathways that are disrupted in chimeras containing AP-2-null cells: formation of the neural tube, face, eye, body wall, and limbs. When combined, the interaction of these independent defects results in the severe phenotype of the AP-2-null mouse. Future analysis of the tissues that require AP-2 function for each of these developmental programs will yield significant insight into the cellular mechanisms regulating these morphogenic events. In this regard, three common mechanistic features link the developmental pathways that are dependent on AP-2.

The first connection between several of these pathways is the neural crest, which is a major site of AP-2 expression during embryogenesis (2). Formation of the neural tube, cranial ganglia, eye, and craniofacial skeleton all involve a contribution from cranial neural crest cells (30). Therefore, aberrant cranial neural crest cell function could influence several of the developmental mechanisms that are disrupted in the absence of AP-2. Trunk neural crest cell derivatives do not seem to be as dependent on the presence of a functional AP-2 gene, because melanocytes, adrenal glands, and spinal ganglia appear essentially normal in AP-2 KO and/or chimeric animals (T.N. and T.W., unpublished observations; refs. 5 and 6).

The second mechanistic linkage between AP-2-dependent systems is the commonality of inductive tissue interactions. AP-2 expression is frequently associated with developmental programs that involve an epithelial–mesenchymal interaction or transition, such as formation of the neural crest, body wall, limbs, and face. Thus, there may be a common set of genes through which AP-2 exerts its influence on morphogenesis. The AP-2 protein has been implicated in the transcriptional regulation of cell adhesion molecules and matrix metalloproteinases, which may coordinate cell/cell communication and cell movement in these developmental processes (31–34). AP-2 also has been linked with genes involved in cell growth and cell cycle regulation; therefore, inappropriate expression of this transcription factor also might lead to the observed changes in cell proliferation and apoptosis (35–37).

The third notable feature that connects developmental pathways which depend on AP-2 is the sensitivity of these pathways to various teratogens, especially RA (38, 39). Aber-

rant levels of RA during human and rodent embryogenesis can result in a wide variety of congenital malformations, and RA treatment early in mouse embryogenesis leads to a range of phenotypes that is similar to those observed in our chimeric animals (27, 39). These data suggest that RA and AP-2 may be influencing many of the same developmental processes during embryogenesis. In further support of this hypothesis, the AP-2-null phenotypes also are reminiscent of the congenital abnormalities observed with various combinations of RAR gene disruptions (16). Because AP-2 expression also is responsive to the presence of RA (1, 3, 4), these findings indicate that AP-2 may be an integral component of the response of an organism to RA signaling.

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