Among these retinoid-dependent genes are included for example genes for fibroblastoid growth factors 8 and 10 (Niederreither et al., 1999; Vermot et al., 2000), for Sonic hedgehog (*Shh*) and *Shh*-pathway genes (Power et al., 1999; Stratford et al., 1999), for bone morphogenetic proteins (Francis et al., 1994; Dupé et al., 1999; Niswander et al., 2000; Pizette and Niswander, 2000), and homeobox genes, namely 5' members of *Hoxd* and *Hoxa* gene clusters, which are downstream mediators of the *Shh* signal and are required for limb skeletal patterning (Small and Potter, 1993; Yokouchi et al.,1995; Carpenter et al., 1997; Goff and Tabin, 1997; Knezevic et al., 1997; Mackem and Knezevic, 1999). Some of the genes coding for bone-specific proteins (osteocalcin) and for enzymes (alkaline phosphatase) are down-regulated by RA (Cohen-Tanugi and Forest, 1998). The gene for tissue transglutaminase, which participates in apoptosis, was induced by RA (Jiang and Kochhar, 1992), and RAREs were found in the promoter of this gene (Nagy et al., 1996).

Some of the genetic factors interacting with RA in our experiments may be included among genes regulated by RA. It follows from the study of apoptosis in limb buds of rat foetuses differing in the *Lx* genotype and in the genetic background that the mutant *Lx* allele manifests itself in the phenotype, among others, by means of apoptosis (Sedmera et al., 1998). While on day 13 of development no differences were found among ND, PD and OD limb buds, on day 14 in OD hind limb buds, an apoptotic zone was found in preaxial mesenchyme, which in ND limb buds was present one day later and in PD limb buds was attenuated. It is in correlation with the present results of RA-*Lx* interaction on day 13 of development, which suggest the possibility that RA interacts with genes participating in apoptosis.

Besides the *Lx* allele, all RA-inducible genes present in the genetic background may play a role in the response to RA teratogenic influence. BN modifying genes may function as normal morphogenes, but excess of RA, the influence of the *Lx* mutant allele, or both these factors can change their expression, and consequently a malformation develops. From this it follows that searching for the polymorphism of the above-mentioned genes between BN and SHR is a promising way for uncovering the major morphogenetic steps involved in realization of the PLS phenotype. In next experiments, we will direct our attention to interstrain polymorphism of modifying genes of the genetic background, which were shown to participate in the expression of mutation or in interaction of the mutant allele with teratogenes. The system of rat congenic, double-congenic and recombinant inbred strains provides genetically defined material for this study.

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References

Ahuja, H. S., James, W., Zakeri, Z. (1997) Rescue of the limb deformity in hammertoe mutant mice by retinoic acid-induced cell death. *Dev. Dyn.* **208**, 466-481.

Alles, A. J., Sulik, K. K. (1989) Retinoic-acid-induced limb-reduction defects: perturbation of zones of programmed cell death as a pathogenetic mechanism. *Teratology* **40**, 163-171.

Bílá, V., Kašpárek, R., Kršiaková, M., Křen, V., Křenová, D., Otová, D., Panczak, A., Sladká, M. (1982) Inbred and congenic strains of the Norway rat. *Rat News Lett.* **9**, 45-47.

Bílá, V., Křen, V. (1996) The teratogenic action of retinoic acid in rat congenic and recombinant inbred strains. *Folia Biol. (Praha)* **42**, 167-173.

Carpenter, E. M., Goddard, J. M., Davis, A. P., Nguyen, T. P., Capecchi, M. R. (1997) Targeted disruption of Hoxd-10 affects mouse hindlimb development. *Development* **124**, 4505-4514.

Cash, D. E., Bock, C. B., Schughart, K., Linney, E., Underhill, T. M. (1997) Retinoic acid receptor alpha function in vertebrate limb skeletogenesis: a modulator of chondrogenesis. *J. Cell. Biol.* **136**, 445-457.

Chambon, P. (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J.* **10**, 940-954.

Cohen-Tanugi, A., Forest, N. (1998) Retinoic acid suppresses the osteogenic differentiation capacity of murine osteoblast-like 3/A/1D-1M cell cultures. *Differentiation* **63**, 115-123.

Cohn, M. J., Bright, P. E. (1999) Molecular control of vertebrate limb development, evolution and congenital malformations. *Cell Tissue Res.* **296**, 3-17.

Dupé, V., Ghyselinck, N. B., Thomazy, V., Nagy, L., Davies, P. J. A., Chambon, P., Mark, M. (1999) Essential roles of retinoic acid signaling in interdigital apoptosis and control of BMP-7 expression in mouse autopods. *Dev. Biol.* **208**, 30-43.

Francis, P. H., Richardson, M. K., Brickell, P. M., Tickle, C. (1994) Bone morphogenetic proteins and a signalling pathway that controls patterning in the developing chick limb. *Development* **120**, 209-218.

Goff, D. J., Tabin, C. J. (1997) Analysis of Hoxd-13 and Hoxd-11 misexpression in chick limb buds reveals that Hox genes affect both bone condensation and growth. *Development* **124**, 627-636.

Inouye, M. (1976) Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S. *Congenital Anomal.* **16**, 171-173.

Jiang, H., Kochhar, D. M. (1992) Induction of tissue transglutaminase and apoptosis by retinoic acid in the limb bud. *Teratology* **46**, 333-340.

Jiang, H., Soprano, D. R., Li, S. W., Soprano, K. J., Penner, J. D., Gyda, M. 3rd, Kochhar, D. M. (1995) Modulation of limb bud chondrogenesis by retinoic acid and retinoic acid receptors. *Int. J. Dev. Biol.* **39**, 617-627.

Kistler, A. (1981) Teratogenesis of retinoic acid in rats: susceptible stages and suppression of retinoic acid-induced limb malformations by cycloheximide. *Teratology* **23**, 25-31.

Knezevic, V., De Santo, R., Schughart, K., Huffstadt, U., Chiang, C., Mahon, K. A., Mackem, S. (1997) Hoxd-12 differentially affects preaxial and postaxial chondrogenic branches in the limb and regulates Sonic hedgehog in a positive feedback loop. *Development* **124**, 4523-4536.

Kochhar, D. M. (1973) Limb development in mouse embryos. I. Analysis of teratogenic effects of retinoic acid. *Teratology* **7**, 289-298.