Abstract  During development of the limb Shh plays a key role as a mediator of zone of polarizing activity (ZPA). However, the molecular mechanisms by which Shh directs anterior/posterior patterning in the limb remain unknown. Members of the Gli gene family encode zinc-finger transcription factors and represent likely candidates for being regulators of Shh target genes. In this review we would like to summarize the current knowledge on expression and function of Gli genes in limb development.

Key words  Gli · Polydactyly · Mutations · Shh · Limb development

Positioning and patterning of the vertebrate limb is largely carried out by intercellular interactions (for review, see Johnson and Tabin 1997; Schwabe et al. 1998). In the developing limb the zone of polarizing activity (ZPA) defines a signaling center in the posterior part of the limb bud that is necessary for anterior-posterior (A/P) patterning. Transplantation of the ZPA into anterior locations of the limb bud leads to mirror image duplications (Saunders and Gasseling 1968). The patterning defects that result from ZPA transplants can be mimicked by misexpression of Sonic hedgehog (Shh) (Echelard et al. 1993; Riddle et al. 1993; Chang et al. 1994). Further support for the idea that Shh mediates ZPA activity has been obtained from the analysis of several polydactylous mouse mutants, which show an ectopic Shh expression domain in the anterior part of the limb bud (Chan et al. 1995; Büscher et al. 1997; Masuya et al. 1995).

Despite its well-defined role as the key signaling molecule of the ZPA, the molecular mechanisms by which Shh achieves patterning along the A/P axis remain unresolved. For example, it is unclear whether Shh functions as a diffusible morphogen or whether it acts exclusively by local mechanisms. Resolution of this important question requires the definition of the Shh-signaling pathway and the isolation of genes whose transcription is directly regulated by Shh. The realization that members of the Gli family of zinc-finger transcription factors play a role in converting the Shh signal into transcriptional responses therefore provided an important step towards achieving this goal. This family consists of three members, Gli1 to Gli3, which are closely related to each other, containing seven regions of similarity (53–100% of identity) including the zinc-finger region (88% identity) (Ruppert et al. 1990). All proteins bind to the same so-called GLI-binding site, a 9-bp motif selected for binding to the GLI1 protein in vitro (Kinzler and Vogelstein 1990). This interaction is mediated by the highly conserved zinc-finger domain as has been demonstrated by crystal structure analysis (Pavletich and Pabo 1993). In addition to being related to each other, Gli genes are also highly conserved in evolution. Homologues have been identified in all vertebrate species analyzed so far and are also present in nonvertebrates (Orenic et al. 1990). Importantly, the Drosophila homologue, cubitus interruptus (ci), has been implicated to play a major role in mediating hh signals during development.

Based on these findings and on the fact that certain phenotypic alterations in mice and humans are associated with mutations in Gli3, Gli genes are of much interest to human geneticists and developmental biologists. This review summarizes important recent advances relevant to our understanding of Gli gene function during development obtained from genetic and biochemical analyses. We also wish to compare these findings with studies that address roles for Gli genes in patterning the neural tube. Finally, we would like to integrate this information into a model of Gli gene function during limb development in response to Shh activation.
Expression of Gli genes during development

A first indication of potential functions of Gli genes in limb development can be assessed from their expression patterns. Gli genes are expressed in a highly dynamic but evolutionary conserved manner during limb development (Marigo et al. 1996b; Mo et al. 1997; Platt et al. 1997; Büscher and Rüther 1998). In the following we describe representatively the results from in situ hybridization analysis in the mouse. At all stages Gli gene expression is confined to the mesenchyme whereas no transcripts can be detected in the ectoderm. At 9.5 days postcoitum (dpc) Gli2 and Gli3 expression spans the entire anterior-posterior (A/P) axis of the newly formed limb bud while Gli1 transcripts are confined to the posterior half. During subsequent development a refinement of these patterns occurs which correlates with an expanded and increased Shh expression in the posterior part of the limb bud. At 10.5 dpc Gli2 and Gli3 transcription remains confined to the anterior limb bud while expression of these genes is excluded from the posterior part. Also, Gli1 expression fades from the most posterior domain. Finally, at 11.5 dpc the limb bud can be subdivided into three distinct domains along the A/P axis in respect to Gli gene expression. A posterior area which corresponds to the ZPA and Shh expression is devoid of any Gli transcripts and is surrounded by a Gli1-positive but Gli3-negative domain. The anterior part of the limb bud expresses Gli2 and Gli3. Therefore, Gli genes seem to prepattern the embryonic limb bud along its A/P axis.

Later in limb development, at 12.5 dpc, a second phase of Gli gene expression becomes obvious. Gli1 transcripts are found in the condensing mesenchyme while the surrounding mesenchyme expresses Gli2 and Gli3, with the latter being expressed at higher levels in the autopods.

In addition, Gli gene expression can be found in a number of developing tissues including the central nervous system (Hui et al. 1994; Lee et al. 1997; Sasaki et al. 1997; Ding et al. 1998; Ruiz i Altaba 1998). In the posterior part of 8.5-dpc mouse embryos, the three Gli genes are expressed throughout the neural plate except that Gli3 expression is absent from the ventral midline (Lee et al. 1997). In more anterior and thus more mature regions of the neural tube these expression patterns are maintained. Transcripts are, however, excluded from the floor plate. Concomitantly with Shh expression in the floor plate Gli gene expression becomes further restricted. At 9.5 dpc, Gli1 transcripts are found in the ventricular zone (VZ) of the neural tube adjacent to the Shh domain, while Gli2 and Gli3 are expressed in the dorsal VZ. Gli2 also shows low levels of expression in the ventral VZ (Ruiz i Altaba 1998). These patterns of expression are maintained throughout development of the spinal cord. Frog embryos show a very similar distribution of Gli transcripts, except for the prospective floor plate, which expresses Gli1 (Lee et al. 1997).

Expression of Gli genes in different embryonic tissues therefore seems to follow similar rules. For example, once a source of Shh has been established, Gli genes are never expressed in these cells. Gli1 transcripts can usually be found adjacent to embryonic sources of Shh consistent with a role as a transcriptional mediator of the Shh signal. In contrast, Gli3 is expressed in cells situated opposite these signaling centers, suggesting that its function might be to oppose this signal and to limit its range. Gli gene expression thereby subdivides a given tissue into distinct domains.

Mutations of GLI3 in human syndromes

While these expression patterns are suggestive of an important role of Gli genes in pattern formation in the developing limb, molecular analyses of human polydactyly syndromes have provided first functional support for this idea. Greig cephalopolysyndactyly syndrome (GCPS) represents a rare autosomal dominant disorder which affects limb and craniofacial development (Greig 1926). Patients are characterized by polydactyly of the hands and feet. Most strikingly, preaxial polydactyly of the feet is found. The hallux is usually duplicated and the first two or three toes appear to be syndactylous. While the thumbs are broadened and in rare cases duplicated, syndactyly of fingers 3–5 also occurs. Also, a postaxial nubbin of tissue on the ulnar border of the fifth finger can be present. In addition, several craniofacial defects including macrocephaly, a broad nasal root and bridge and in rare cases hydrocephaly and ear anomalies have been described (Greig 1926; Gollop and Fontes 1985).

Cytogenetic analyses revealed that GCPS is linked to chromosome 7p13 (Tommerup and Nielsen 1983; Krüger et al. 1989; Brueton et al. 1988; Vortkamp et al. 1991b; Wagner et al. 1990). The identification of three translocation breakpoints, either within or immediately adjacent to the GLI3 gene, indicated an involvement of this gene in the development of GCPS (Vortkamp et al. 1991a). Two of the three GCPS translocations localize upstream of or within the zinc-finger region (Fig. 1). The third translocation, which resides 10 kb downstream of the GLI3-coding region, may exert a negative influence on GLI3 expression (Vortkamp et al. 1991a, 1995). The identification of two female GCPS patients with point mutations in the GLI3-coding region has lent direct support to GLI3 being the causative gene of GCPS. In the first case the GLI3 protein is truncated within the first zinc-finger [amino acid (aa) 496 – numbered according to Ruppert et al. 1990] eliminating the DNA-binding domain and thereby probably creating a nonfunctional protein. The second mutation results in the exchange of a Pro to Ser residue (aa 707) (Fig. 1), thereby possibly destroying a putative phosphorylation site for serine/threonine kinases (Wild et al. 1997). Alternatively, the stability of the mutated protein might be affected. Due to the typical GCPS appearance of these patients, it is assumed although not yet shown that the putative GLI3 protein is nonfunctional.

Mutations of GLI3 have been implicated in two other human developmental disorders. Pallister-Hall syndrome (PHS) was first described in 1980 in six severely affected newborns. PHS is a rare autosomal dominant disorder including hypothalamic hamartoma and central, sometimes