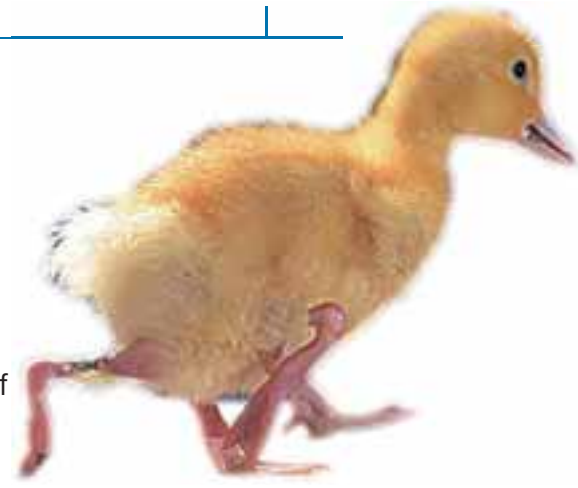

Limb morphogenesis in Cantabria, Spain

Digit-al Revolution

The digits of the vertebrate limb arise from a multi-step process during which the precursor cells take on one of three different fates. While the process itself is modulated by a network of cascades, Juan M. Hurlé in his 30 years of research, is close to zeroing on the master regulator all the way upstream.

When Gary Sobers, the former captain of the West-Indian cricket team, decided to get rid of his two extra fingers, one on each hand, little was known about the congenital physical anomaly of having supernumerary fingers or toes, a trait medically termed polydactyly. The digits of vertebrates, inappropriately called "appendages", have seen crucial morphological changes over evolutionary periods, transforming their ability from merely perching and clasping in birds to grasping and clenching in mammals. The number, structure and form of the vertebrate digits endow organisms with a prehensile strength and are acquired early during development as a result of a complex network of regulatory cascades that are strictly timed and ordered and which, when flawed, can lead to developmental disorders of the skeletal connective tissues, including polydactyly and syndactyly, the fusion of two or more digits.

Juan M. Hurlé (3rd from left) and his group

What determines when the differentiation of a limb is concluded? And what stops the limb from growing in late development? Just two of the many questions that intrigued Juan M. Hurlé in the late 1970s when he started his scientific career as an associate professor of Human Anatomy and Embryology at the University of Santander, Spain. For over three decades, Hurlé and his group have been involved in exciting research on cartilage differentiation in the vertebrate limb and they are now closing in on a master regulatory pathway that they propose to be the sole cell-fate determinant at the growing limb bud. As Juan Hurlé mulls over his past discoveries, he obliges *Lab Times* with a quick chat about his career.

How the story came about

The research groups of John Saunders and of John Fallon both in the United States and of Richard Hinchliffe in the UK, under whom Juan Hurlé completed his postdoctoral training in 1977, set the stage for more profound study on the developmental patterning of the vertebrate limb. Their work ascribed apoptotic cell death programmes to the 'sculpturing' of the shape of the limb into digits and inter-digital regions. With increasing evidence of apoptotic cell death programmes in the 'interdigital necrotic zones' (INZs) of the developing vertebrate limbs, Juan's focus turned to investigating the underlying molecular mechanisms that triggered apoptosis. Given that the size of the INZs co-related with the phenotype of the digits, Juan adopted a comparative approach to look at apoptotic programmes in a wide gamut of species in order to address the differences in digit patterns with or without interdigital webs or lateral membranous lobulations.

In the late nineties, the Hurlé group identified the first players in the regulation of the apoptotic programmes in the apical ectodermal ridge (AER) overlying the developing limb bud and in the mesodermal cells in the chick embryo. These were the bone morphogenetic proteins (BMPs) (*Development* 122:2349-57), members of the TGF-β superfamily, which were found to be extensively expressed in the limb buds and evoked the apoptotic signal. It came as a surprise when Juan's work revealed that interdigital induction of certain BMP receptor sub-types by local application of TGF-β actually inhibited apoptosis and eventually triggered the formation of an ectopic digit.

Thus, BMPs were found to not only induce massive cell death in the undifferentiated mesoderm but also exert a chondrogenic effect and hence produce a tendency to growth and differentiation in the prechondrogenic mesenchyme; the immediate local effect of the signal, however, seemed to be modulated by the upstream TGF-βs. TGF-β thus emerged as a key component of a signalling cascade that determined the fate of the cells in the developing limb bud and could bring about either cell death (apoptosis) or cartilage differentiation (chondrogenesis).

A fatal decision

The two possible fates of the cells of the developing limb bud, apoptosis or chondrogenesis, were derived largely from experiments *in vivo*. In addition, the Hurlé group has recently demonstrated the effect of TGF-βs in phenotypic re-programming in chick micromass cultures of undifferentiated mesodermal cells. Their findings (*J Biol Chem* 284(43):29988-96) attribute TGF-βs to a lineage switch of cartilage progenitors.

They observe that treatment of chondrocyte cultures with TGF-βs transforms the cells into a fibrous tissue lamina. "As we get deeper into our studies, we find that the system is actually a lot more complex than we thought," admits Juan. The crucial cell fate determinant TGF-β, indeed, exerts more than one phenotypic effect. He elaborates, "We now find that TGF-β signalling can direct the precursor 'chondrogenic' cells to

assume any of three different fates namely, cartilage formation, apoptotic cell death and fibrosis or tendon formation." In other words, the limb phenotype is not a direct read-out of the presence or absence of the signal but depends on the developmental stage at which the TGF-β signal is delivered to the cells and is, in fact, partly influenced by a "cross-talk" with other regulatory cascades.

No more tensed tendons

For their study of limb morphogenesis, Juan and colleagues have employed many species among avian, amphibian and mammalian systems. The duck and chick have, however, been most appropriate as models for species with inter-digital webs and the lack of them, respectively. Besides the ease of surgical manipulations in the chick and duck embryos, the two species serve as structurally different models for their study in terms of limb morphology. "There are some disadvantages, too," explains Juan. "The chick for example, is not a good model for genetics." Unlike mice mutants, maintenance of chick mutants and their breeding is often difficult and time-consuming. For instance, the absence of wings in the *wingless* chick mutants presents problems with reproduction in the mutant birds. "In essence, studies in birds need to be, and have been in our work, complemented with genetics in mice," concludes Juan.

As a professor of Human Anatomy in the Faculty of Medicine, University of Cantabria, Spain, Juan Hurlé leads a high-spirited group, which is at a sophisticated level of research. Their work has gained them a foothold in Medicine as their recent results hold significant therapeutic implications and are likely to contribute a great deal in the treatment of skeletal connective tissue injury.

The discovery of the downstream effectors of TGF-β signalling in the lineage switch of cartilage precursors to tendons has opened new portals in the treatment of tendon ailments and the Hurlé group has now embarked on the endeavour of re-programming human stem cells to form fibrous tissue. Their work holds prospects in the treatment of tendinopathy and of tendon injuries associated with ageing. Juan agrees that severe tendon injuries have been hard to treat and now proposes the use of their *in vitro* system to start with their experiments right away. He then intends to switch to surgical approaches *in vivo*. The strategy is familiar to Juan after a collaborative project in the 1970s, when he performed human umbilical cord stem cell transplants into the developing chick heart and monitored their implantation and subsequent differentiation into myocardial tissue by following the genetic markers on stem cells.

At a higer level

Juan's closing remark is an implicit exhibit of his fascination for science. "The study of signals that direct the formation of digits was initiated twenty years ago and I wish to complete it at a higher level of complexity in the next years," he states in an ambitious tone. With his colleagues on board, Juan Hurlé is game to set sail on his new and promising "medical" venture.

MADHUVANTHI KANNAN

ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER