also, many sex chromosome mosaics have been detected. Thus genetic mosaics have been observed in numerous cases and there are diverse ways such as chromosome loss, mitotic recombination, chromosome inactivation, mutations causing chromosome loss, nuclear or cell transplantation by which they could be generated.

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Prolactin inhibiting factor has structural motif common to developmental-gene regulators

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Many DNA-binding proteins that regulate transcription of developmental genes have been shown to contain a helix–loop–helix structural motif. This characteristic domain is involved in the interaction with DNA. We show here that human prolactin release-inhibiting factor, the 56-residue polypeptide contained within the C-terminal portion of luteinizing hormone-releasing hormone precursor, has striking structural homology with negative regulators of developmental-gene transcription. Two 14-residue helices showing strong amphipathic character are linked through an intervening sequence with distinct loop characteristics. The typical sequence pattern representing coiled-coil folding is also evident within these helices. We therefore propose that prolactin secretion is regulated by prolactin release-inhibiting factor (PIF) at the transcriptional level by a mechanism similar to the negative regulation of other developmental genes.

THE pituitary hormone prolactin is involved in regulation of a number of physiological processes. In mammals, it plays a critical role in development and maintenance of mammary glands. Secretion of prolactin is predominantly under inhibitory control of hypothalamic factors. Although dopamine has been known to inhibit prolactin secretion, it is not the only factor mediating tonic hypothalamic regulation of this hormone[1]. The existence of another factor, of peptidic nature, has subsequently been recognized. Nikolics *et al.*[2] have shown that a 56-residue polypeptide contained within the C-terminal sequence of the luteinizing hormone-releasing hormone (LHRH) precursor is a potent inhibitor of prolactin secretion. However, the mechanism by which this inhibitory control is brought about has not been established.

We deduced secondary-structure propensities in human, mouse and rat LHRH precursor sequences[3,4] using the algorithm of Garnier *et al.*[5] Further analysis of sequence regions with well-defined secondary-structure features by visual comparison allowed detection of signature residues associated with various known structural motifs[6,7]. Sequence alignment of PIF with other transcription-regulating proteins that have similar sequence features was achieved by matching the signature residues.

The tertiary-structure motif of PIF has been established by recognizing patterns in the amino-acid sequence. The predicted secondary structure of human LHRH precursor (Figure 1,*a*) suggests that PIF has two helical regions (residues 42–55 and 72–85) separated by an intervening non-helical region. The majority of the residues in the intervening region have higher propensity[8] for being in a loop. As expected, the signal sequence is predicted to be helical, whereas the LHRH sequence shows distinct turn preference. The secondary-structure profiles for rat and mouse LHRH precursors are also similar. It is evident from helical-wheel diagrams (Figure 1,*b*) that the two helices in PIF have very marked amphipathic character. One other distinctive feature was discovered in this sequence. The two helical regions, each 14 residues long, show a heptad repeat pattern such that the first and the fourth residue of each heptad are hydrophobic. Such a pattern of heptad repeats with characteristic hydrophobic signature residues has been analysed in many proteins, and is known to represent coiled-coil folding[9,10]. In PIF there are two heptad repeats within each helix (see Figure 2). The hydrophobic surfaces generated by the heptad repeat pattern may facilitate coiled-coil folding of the two helices. Thus the tertiary structure of PIF corresponds to a helix–loop–helix (HLH) motif with coiled-coil features.

The HLH motif has also been identified in a large number of mammalian and *Drosophila* proteins involved in growth control and differentiation[11,12]. In fact this DNA-binding/dimerization motif characterizes a large family of nuclear proteins implicated in developmental

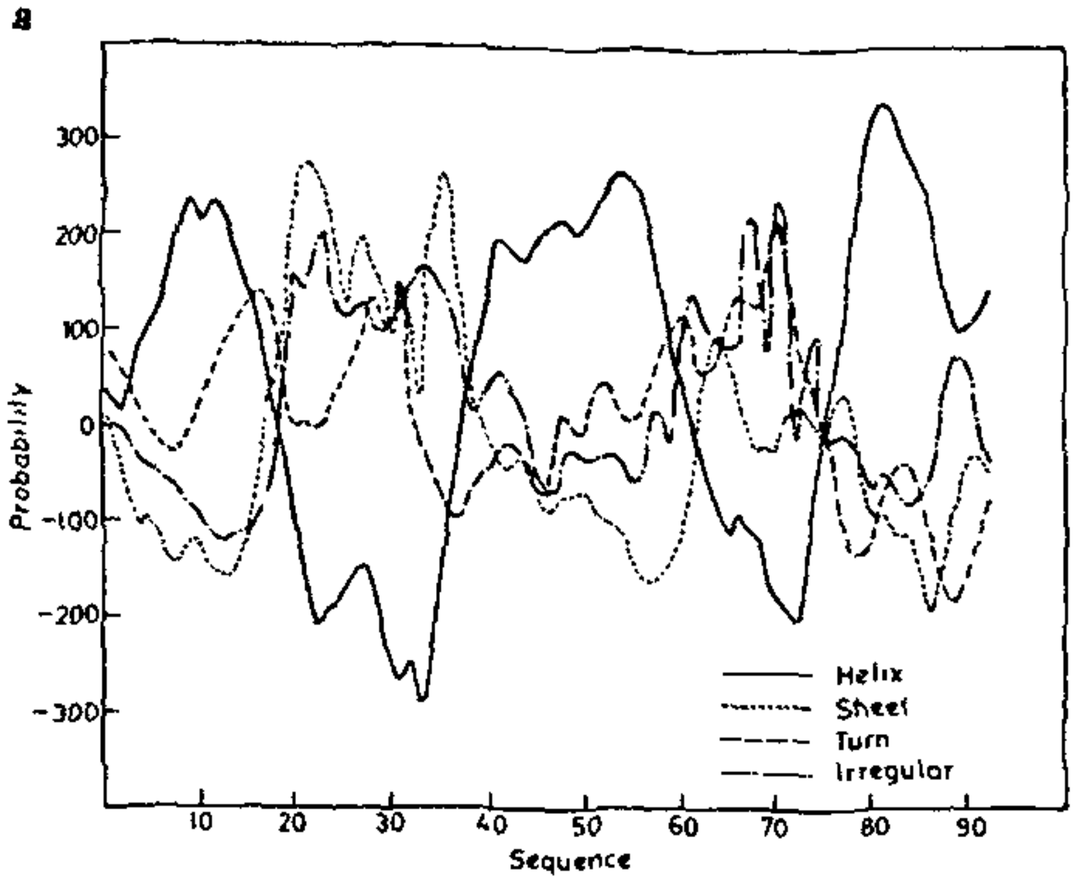

Figure 1. Structural analysis of PIF based on amino-acid sequence. *a*, Secondary-structure propensities in human LHRH-precursor sequence. The precursor includes the signal peptide (residues 1–23), LHRH (24–33) and PIF (37–92). The probability scores are on an arbitrary scale. Patterns of predicted secondary structure for rat[6] and mouse[7] precursor sequences are similar. *b*, Helical-wheel diagrams for human LHRH-precursor residues 42–55 (helix I) and 72–85 (helix II), which correspond to the two predicted helices in the PIF sequence. Both helices show distinct amphipathicity, with polar and hydrophobic residues. The block encloses the hydrophobic residues of the two helices.

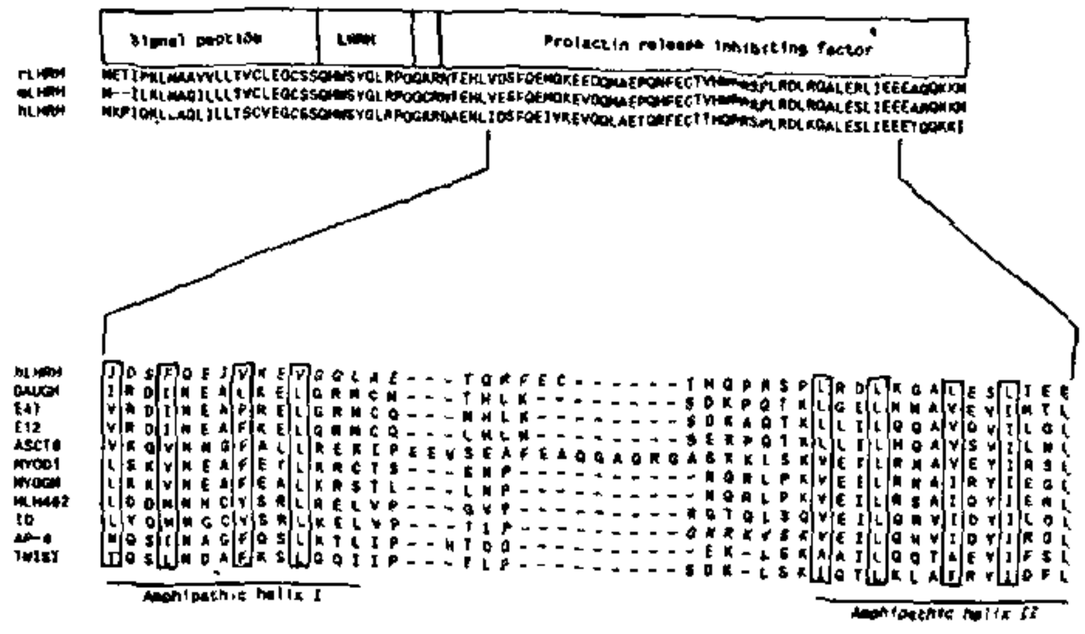

Figure 2. Homology of PIF with helix–loop–helix transcription factors. The PIF peptide (residues 37–92) of the human LHRH precursor[6] (hLHRH) has been aligned with *Drosophila* daughterless (DAUGH)[17], human kappa-immunoglobulin enhancer regulating factors *E47* and *E12* (ref. 12), achaete-scute complex T8 (ASCT8)[13,14], *muscle differentiation factors MyoD1 (ref. 28) and* mygenin (MYOGN)[19], HLH462 (ref. 24), Id[23], AP-4 (ref. 29) and TWIST[15] (see text). The first and the fourth residues of each of the two heptad repeats in both amphipathic helices of all the sequences are highlighted. The upper panel shows alignment of rat (rLHRH) and mouse (mLHRH) with the human (hLHRH) homologue; the three have high homology (68–70% identical residues) between them.

control of gene expression. For example, the *Drosophila* proteins achaete-scute complex[13,14], twist[15] and daughterless[16], which are involved in differentiation of the peripheral nervous system, have distinct features of the HLH motif. The protein factors MyoD1 (ref. 17), Myf-5 (ref. 18) and Myogenin[19], which are important in muscle differentiation, also possess the HLH folding domain. Other proteins in this family include the human kappa-immunoglobin enhancer regulating factors E12 and E47 (ref. 12), as well as the product of *lyl-1* (ref. 20), a gene found at the breakpoint of a chromosome translocation. In addition to the HLH motif all these tissue-specific as well as ubiquitous activators of developmental-gene transcription have a characteristic sequence region of predominantly basic residues on the N-terminal side of the HLH region

This stretch, of about 15–20 residues containing five or more lysines and arginines, facilitates direct binding to DNA through electrostatic interactions. A group of developmental-gene transcription-regulating factors with inhibitory effect have also been predicted to include the HLH motif. Extramacrochaete, a negative regulator of sensory-organ development[21,22], Id[23], an antagonist regulator of muscle differentiation, and HLH462 (ref. 24), an Id-related protein coded by a growth factor-inducible gene, belong to this group. Whereas HLH folding is common to both activators and inhibitors (negative regulators) of transcription, only the former possess a predominantly basic sequence adjacent to the N-terminal of the HLH region, which results in the basic-helix–loop–helix (bHLH) motif. For the regulatory function, homomeric or heteromeric dimerization leading to a structurally stable four-helix bundle is essential. Although the HLH domain is directly involved in the dimerization interaction, its involvement in binding to DNA may be indirect.

Sequence alignment of PIF with proteins known to be involved in developmental-gene transcriptional control through dimerization and subsequent sequence-specific binding to DNA (Figure 2) suggests striking homology of PIF with the HLH region of the majority of these proteins. Besides the conserved hydrophobic character of residues at the first and fourth positions in the heptad repeats, there are a large number of homologous residues in the helices and in the loop region. Further, as in the case of negative regulators, the predominantly basic stretch of amino acids adjacent to the N-terminal of the HLH region is absent in PIF.

On the basis of the structural homology of the LHRH-associated hypothalamic peptide with factors controlling transcription of genes involved in diverse developmental processes, we propose that the 56-residue peptide is also involved in development-related transcriptional regulation. It has been demonstrated that genetically expressed LHRH-associated peptide inhibits prolactin secretion. It was earlier shown that prolactin secretion, which is predominantly under inhibitory control, is regulated by hypophysiotropic hormones[1]. In addition, protein complexes similar to the E-box-binding transcription regulators with the HLH structural motif have recently been located in prolactin-secreting endocrine cell lines of pituitary origin[2-6]. Therefore it is indeed possible that negatively regulated prolactin secretion is controlled at the transcriptional level by PIF through a mechanism that involves dimerization with a bHLH protein and subsequent DNA binding.

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Publications received

Books received by the journal in 1991 but not reviewed are listed. The list is intended to serve as both a notice of the books and an acknowledgement of receipt.

Advances in Composite Materials: Proceedings of the International Conference on Advances in Composite Materials: P. Ramakrishnan (ed.). Oxford & IBH Publishing Co., New Delhi. 1991. 679 pages. Price not known.

Alchemy and Metallic Medicines in Ayurveda. Dash. Concept Publishing Co., New Delhi. 1986. 247 pages. Rs 160/$32.

Canal Irrigation Management: Problem of Time and Use Relationship. Lakshmi Shukla and Ram Kumar Gurjar. Agricole Publishing Academy, New Delhi. 1991. 111 pages. Rs 125/$25.

Carpentry and Joinery: Equipment Planning Guide. Oxford & IBH Publishing Co., New Delhi. 1991. 131 pages. Rs 170.

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Contribution to Indian Enthnobotany. S. K. Jain (ed.). Scientific Publishers, Jodhpur. 1991, 341 pages. Rs 275/$50.

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