cular aspects of differentiation in plants and pointed out that research on genetically induced nuclear male sterility and apomixis will be of considerable importance for plant breeding. H. Y. Mohan Ram described how the generally accepted patterns of embryo development have become modified in members of Podostemaceae. S. Mahadevan summarized the current understanding of differentiation and growth patterns in the non-chlorophyllous, leafless and rootless parasitic twiner *Cuscuta*. Sipra Guha Mukherjee reviewed the biochemical studies on the transition of cells from the proliferative to differentiative phase.

Usha Vijayaraghavan and J. P. Khurana summarized the attractive features of *Arabidopsis* and illustrated how this genetically amendable system is being employed to isolate developmental mutants and to clone the genes regulating flowering and photomorphogenesis. A. K. Tyagi discussed the expression of chloroplast and nuclear genes encoding for thylakoid proteins. R. Maheshwari and V. Nanjundiah reviewed the current ideas in the fields of differentiation and morphogenesis in fungi.

Pre-proposals invited from mid-career and young scientists were also presented and discussed in detail with the experts helping in focusing the ideas into meaningful projects.

The following general recommendations were made:

- Research on all aspects of development and differentiation is needed in a variety of plant species irrespective of their immediate application.
- Organizing of training workshops for teaching new technologies to young scientists.
- Research extension facilities to scientists who have developed model systems.
- Promote production of equipment and biochemicals indigenously.

The following aspects were recommended as focal points for intensive studies:

1. Development, differentiation and transformation studies including isolation, characterization and expression of genes controlling developmental patterns, development of somatic and pollen embryos, production of synthetic seeds, land-to-lab transfer technology, regeneration of forage crops, tree species and scientifically interesting or endangered plants and transfer of species declared recalcitrant for organogenesis.
2. Biochemical changes and gene expression including study of form and patterns, identification of genes regulating development, characterization of regulatory proteins and RFLP mapping of selected species.
3. Signal perception and transduction including characterization of receptors for hormone and other stimuli, regulation of hormone levels, transport pathways, role of various components in signal transduction and processes such as flowering, pathogenesis, thermogenesis, etc., and endogenous rhythms and clocks in plants and their molecular basis.

Decisions on the pre-proposals have been taken separately and communicated to the scientists. A comprehensive report on the brainstorming session giving detailed recommendations has been brought out by the Department of Science & Technology and can be obtained on request.

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RESEARCH NEWS

Imprintor gene identified

Vani Brahmachari

'Imprinting' was a term used by Helen Crouse to describe the selective elimination of paternal chromosomes that occurs in the dipteran insect *Sciara*. Since *Sciara* appears to have the ability to distinguish between homologous chromosomes on the basis of their parental origin, Crouse thought that the phenomenon indicated a memory effect: whence the word imprinting (originally used by ethologists to describe the long-lasting effect on behaviour of a brief exposure to a stimulus early in life). In the early seventies Spencer Brown and Sharat Chandra extended the concept of genomic imprinting to mammalian genetics while discussing the inactivation of one of the two X-chromosomes in each somatic cell of a mammalian female, as also the selective inactivation of paternal chromosomes in male coccids. Brown and Chandra also raised the possibility of autosomal imprinting in mammals and, on theoretical grounds made a distinction between genes which are sensitive to parental origin and those which actually exhibit differential behaviour such as inactivation. Selective activation or inactivation dependent on the sex of parental origin is also known to act at the level of single genes. In most experiments dealing with imprinting it is assumed that imprinting leads to functional inactivation of genes. Around 10% of the mouse genome is now known to be affected by parental imprinting in this sense. Genomic imprinting has recently assumed significance because of its occurrence in several human genetic disorders such as the juvenile forms of Huntington's disease, Prader-Willi and Angelman deletion syndromes and in Wilm's tumour, a childhood tumour of the kidney. In order to account for a number of experimental results, it has been postulated that there exist *imprintor* loci in addition to the *imprinted* loci. The first genetic evidence for the existence of an 'imprintor' gene has now been reported (Jiři Forejt and Soňa Gregorova, *Cell*, 1992, **70**, 443–450). The discovery of an imprintor locus is a significant step towards our understanding of the control

of genomic imprinting. The experiments exploited available strain differences in the mouse *t*-complex. Interest in the *t*-complex, dates back to the discovery in the 1930s of a dominant mutant allele *T* associated with a short-tailed phenotype. One of the alleles at this locus is designated T^{hp} for *h*air *p*in tail phenotype. There are several loci mapping in the *t*-complex which lead to embryonic lethality. One such locus is *Tme*, *T*-associated maternal effect locus. The T^{hp} mutation is due to a large deletion induced by X-irradiation extending into the *Tme* locus. The gene(s) responsible for causing the mutant phenotype are differently modified, or imprinted, probably during maternal and paternal gametogenesis. Imprinting is inferred from the observation that in a cross involving only wild-type alleles, the paternal locus is selectively rendered non-functional in the offspring. Therefore in the progeny resulting from the mating of a $T^{hp}/+$ female and a normal male no short-tailed (ST) individuals are recovered, there being no functional wild-type allele to complement the lethal effect of T^{hp}. On the other hand, when the mother is $+/+$ and the father $T^{hp}/+$, 50% of the progeny are genetically $T^{hp}/+$, survive and are ST.

Forejt and Gregorova's strategy was to search for an imprinting-related polymorphism at the *Tme* locus by looking for ST progeny in crosses involving $T^{hp}/+$ females and $+/+$ males from different inbred strains. The idea was that the survival of such progeny would imply that there was a gene that abolished the imprinting effect. Forejt and Gregorova crossed hairpin-tailed females ($T^{hp}/+$) of laboratory inbred strains of *Mus musculus domesticus* (C57B1/10, C3H/Di and BALB/C) with wild type males from different inbred strains of *Mus m. musculus* (PWD, PNK and PNB). If the *Tme* locus is paternally imprinted no ST progeny are expected to survive, as we have seen. On the other hand if the *Tme* locus is not imprinted in males, ST progeny should be recovered in crosses with hairpin tailed females. The finding was that the ST progeny could be recovered in crosses between males from the three *M. m. musculus* strains and $T^{hp}/+$ females, therefore the *Tme* locus was not paternally imprinted in these cases.

In a test cross between F_1 hybrid males (derived from a cross between C3H, the laboratory strain and PWD *M. m. musculus*) and $T^{hp}/+$ females, viable ST progeny were obtained. The origin of *Tme* locus in the progeny could be distinguished by the difference in the hybridization pattern of restriction endonuclease-digested DNA from the C3H and PWD strains with probes specific to chromosome 17 (on which the *t*-complex is located). An analysis of ST progeny from the test cross indicated that some of them inherited the *Tme* allele from PWD strain and others from the C3H strain. This shows that *Tme* locus and the putative imprintor locus, designated *Imp*-1, segregate independently. The probable genotypes of the parents in the test cross are: the F_1 male is $\frac{+^{m}Imp\text{-}1^{m}}{+^{d}Imp\text{-}1^{d}}$ and the female $\frac{+^{d}Imp\text{-}1^{d}}{+^{d}Imp\text{-}1^{d}}$ where m represents the *M. m. musculus* allele and d represents the *M. m. domesticus* allele (laboratory strain). Apparently $Imp\text{-}1^{d}$ causes imprinting while $Imp\text{-}1^{m}$ allele does not. Only a small proportion of the progeny of the test cross were short-tailed (Table 1). This means that the non-imprinted allele of *Tme* was present only in about 1/2 of the male gametes produced and shows that imprintor acts during gametogenesis. Further Forejt and Gregorova have shown that the putative imprintor acts during gametogenesis by crosses in which the $Imp\text{-}1^{m}$ allele (the non-imprintor) is derived from the female parent (that is T^{hp} $Imp\text{-}1^{m}$ female) and where no ST progeny are obtained. The proportion of ST mice obtained in the test cross shows that there is no straightforward dominance–recessive relationship between the two alleles, m and d at the imprintor locus, *Imp*-1.

Table 1. Back cross between F_1 male and T^{hp} female

Parental genotype	Gamete haplotype	Progeny genotype	Phenotype
Male $\frac{+^{m}Imp\text{-}1^{d}}{+^{d}Imp\text{-}1^{m}}$	$+^{ia}Imp\text{-}1^{d}$	$\frac{+^{ia}Imp\text{-}1^{d}}{T^{hp}Imp\text{-}1^{d}}$	Lethal
	$+^{a}Imp\text{-}1^{m}$	$\frac{+^{ia}Imp\text{-}1^{d}}{+^{a}Imp\text{-}1^{d}}$	Normal
Female $\frac{T^{hp}Imp\text{-}1^{d}}{+^{d}Imp\text{-}1^{d}}$	$T^{hp}Imp\text{-}1^{d}$	$\frac{+^{a}Imp\text{-}1^{m}}{T^{hp}Imp\text{-}1^{d}}$	Short-tailed
	$+^{a}Imp\text{-}1^{d}$	$\frac{+^{a}Imp\text{-}1^{m}}{+^{a}Imp\text{-}1^{d}}$	Normal

ia, imprinted and inactivated; a, remains active; d, *M. m. domesticus* allele; m, *M. m. musculus* allele.

The results obtained can be interpreted in more than one way.

(i) The imprintor acts at the haploid nuclear stage and therefore only 1/2 the gametes—those that contain $Imp\text{-}1^{m}$ and the + allele at *Tme*—bear the non-imprinted *Tme* locus.

(ii) Alternatively, the imprintor acts in the diploid stage but the amount of *Imp*-1 product is limiting, being dependent on the number of copies of the gene. Therefore it can imprint only one of the two alleles at the *Tme* locus, conceivably on a random basis.

The above two possibilities may be distinguished if one can analyse the segregation pattern of the *Imp*-1 locus itself in the ST individuals from the test cross. If (i) is true ST progeny in the test cross would bear only the $Imp\text{-}1^{m}$ allele whereas in case (ii) ST progeny can have either $Imp\text{-}1^{m}$ or $Imp\text{-}1^{d}$ allele.

Yet, another locus on chromosome 17, insulin-like growth factor 2 receptor (Igf2r), shows paternal imprinting. Interestingly, Igf2r is found to be imprinted in *M. m. musculus* where the *Tme* locus is not imprinted. This shows that imprinting is locus-specific even within short chromosomal regions of about 3 Mb. If one thinks of methylation as a mechanism of imprinting, one would expect locus-specific methylases or methylation mechanisms in systems like the mouse. Global mechanisms of methylation will not be able to explain these findings.

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