(ii) Entrust the National Commission with the responsibility of coordinating and enforcing a Precautionary Package for the safe and beneficial use of GMOs comprising the following major components.
- Bioethics: Ethical codes for experimentation and field testing.
- Biosafety: National and international protocols.
- Biosurveillance: Policies for introduction of technologies (e.g. 'terminator') and assessment of environmental and social impact.
- Food safety: Toxic or allergenic effects as well as wholesomeness.
- Consumer choice: Compulsory labelling.
- Public information: Transparency, information empowerment.

The National Commission on GMOs may establish four standing committees, each chaired by an appropriate commission member to pay speedy and integrated attention to issues such as the following:
- Bioethics and biosurveillance.
- Biosafety (national guidelines and international protocol under CBD).
- Biodiversity and food safety.
- Public education, understanding and participation.

This proposed National Commission on GMOs should have an autonomous status and can be serviced either by the Ministry of Environment and Forests or DBT or ICAR. Since the role of the National Commission is not limited only to biosafety issues, its small professional secretariat, though linked for budgetary purposes to an existing Ministry or Department should have complete autonomy coupled with accountability. The Commission, for this purpose, should be a statutory body.

The Government of India has already in place a 3-tier regulatory structure for ensuring the safe-handling of GMOs. The proposed National Commission is designed *to streamline and strengthen and not to supplant* ongoing activities. The present regulatory structures will all report to the National Commission on GMOs, which will provide overall policy guidance and oversight, within the framework of the National Environment Protection Act.

The National Commission on GMOs should prepare an annual report on the work done during the year in the country and present it to Parliament.

(iii) State Governments may set up similar bodies at the State level in order to provide a channel for the speedy and effective implementation of approved protocols, regulations and guidelines. The State level Genetic Engineering Board should not only have regulatory functions but also promotional and educational functions, including the sponsorship of Biotechnology Parks for providing opportunities to young men and women for remunerative self-employment. The State Boards can prepare an annual report for being placed before the respective State Legislatures.

(iv) At the village level, Panchayat Biotechnology Committees may be set up to monitor field experiments with GMOs and assess their impact on the economic well being of rural families and on the ecological health of the area.

Thus, an organizational structure which extends from the village to the national capital will help not only to promote public understanding of the opportunities and implications of the emerging biological century, but also to allay public fears and apprehensions.

While the above institutional structures are important at the governmental level, every research institution and commercial company, whether public or private, should have its own voluntary code of conduct based on the recommendations of their inhouse Bioethics and Biosafety Committees. Government laws, self-regulation, education and public understanding will all be necessary to ensure that genomics and molecular breeding become powerful instruments for building an environmentally and socially sustainable food security system.

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RESEARCH NEWS

Telomere dynamism and developmental connection of differential telomerase activation in cell proliferation and replicative senescence

U. C. Lavania, Seshu Lavania and Y. Vimala

Role of telomere erosion in cell ageing and its prevention by forcing the expression of telomerase is of topical interest[1–5]. Axiomatically, the various cell types in eukaryotic organisms maintain a differential but dynamic control of such regulation *in situ* – a precise knowledge of which has value to underpin growth and differentiation for prospective utilization.

Telomeres are specialized essential elements at chromosome ends of eukaryotic chromosomes (Figure 1) that remain associated with the nuclear matrix in the interphase nuclear organization[6,7] and facilitate separation of chromosomes at anaphase[8]. They are thought to function as buffers against end-to-end chromosome fusion and protection from exonuclease degradation[9–11]. Telomeres of most eukaryotes

are composed of short G-rich, randomly repeated sequences, wherein G-rich strand is extended over the C-rich strand and forms a single-stranded overhang. The single-stranded overhang imposes the so called 'end replication problem' because the conventional DNA replication mechanisms cannot completely replicate telomeres in the given situation[11]. This would consecutively lead to telomere shortening and thereby degeneratively affecting the cell life span. Telomerase – a specialized reverse transcriptase, by using its own RNA subunit as the template mitigates the 'end replication problem' and facilitates maintenance of telomere length that may be necessary for cell proliferation. However, the telomerase activation is developmentally connected in a cell cycle dependent manner which may give way to either telomere shortening, stability or even elongation to meet the developmental objectives in the evolutionary hierarchy. The problem is analysed in respect to germline progression, organogenesis, somatic tissues *in vivo* and *in vitro vis-à-vis* developmental regulation of telomerase in cell proliferation and replicative senescence.

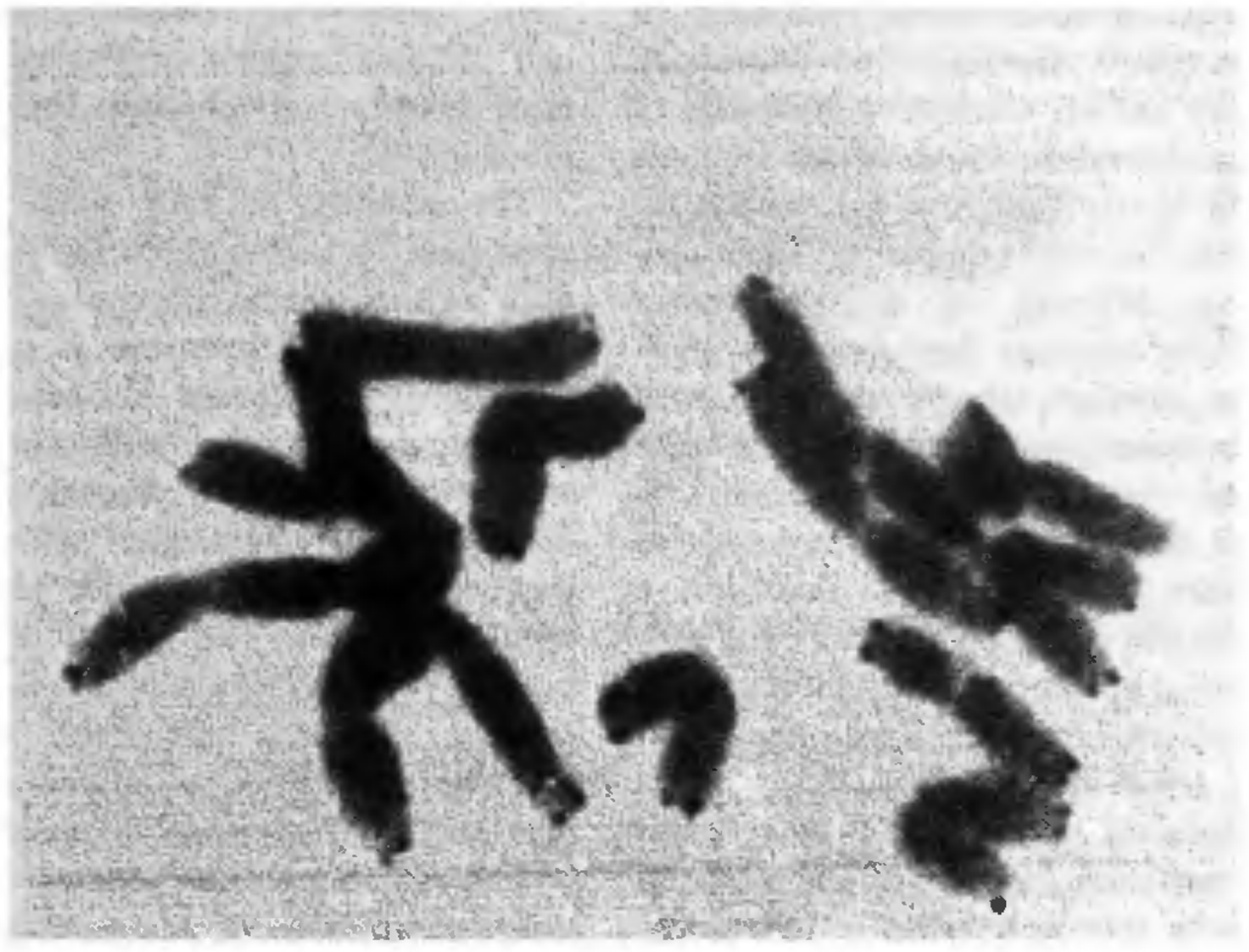

Figure 1. Fluorescent *in situ* localization of telomeric motifs on *Hordeum vulgare* chromosomes.

Telomere shortening and replicative senescence

(TTAGGG)n is a charactersitic repeat that is added to the end points of chromosomes to constitute the telomeres (plant telomeric repeats generally comprise of 5'-TTTAGGG-3'). Such telomeric motifs have been found to be conserved across taxa. However, maintenance of telomeres requires a dynamic process involving competitive degradation and elongation. Conventional DNA replication mechanisms cannot replicate telomere completely as they yield one blunt DNA end, and the other end having a 3' overhang. Further, a C-strand specific exonuclease activity converts blunt DNA ends into ends with G-rich 3' overhangs in a cell cycle-dependent manner[12,13]. Thus the telomeric DNA in eukaryotic cells is progressively lost through successive generations on account of both incomplete replication and strand-specific exonuclease activity[11–13]. Therefore, unless this loss of telomeric DNA is counterbalanced, the telomeres would continue to shorten with each generational doubling. In fact, it has been observed that when normal human cells are cultured *in vitro*, their telomeres gradually shorten with every division cycle, and they are able to pass only through a limited number of cell divisions to the extent when telomeres erode to a critical size. This indicates that sufficiently short telomeres may provide a signal for replicative senescence. Such observations have led to the telomeric hypothesis which suggests that telomeres may serve as a mitotic clock, by which cells count their divisions by the extent of telomere shortening[14,15].

Telomere maintenance and telomerase

To provide developmental continuity to the growing tissues and the germline the loss of telomeric sequences enforced by 'end replication problem' is counterbalanced by a specialized enzyme termed telomerase. Telomerase is a reverse transcriptase that facilitates repair of telomeric repeats using its own RNA component as a template and compensates for the inability of DNA polymerase to replicate 5' ends of linear DNA molecules[16,17]. This view is reinforced by the experiments depicting correlated response between lack of telomerase activity and progressive reduction in telomeric length in the mouse germline having telomerase RNA gene deleted[18], and also avoidance of senescence in the human retinal pigmented epithelial cells in presence of ectopic expression of the catalytic subunit of the telomerase holoenzyme[1]. Such observations obviously point towards establishment of causal relationship between telomere shortening and cellular replicative senescence[19].

Dynamism in telomere replication and developmental connection of telomerase activation

Telomere maintenance is a dynamic process consisting of competitive degradation (end-replication problem and strand-specific exonuclease activity) and elongation (telomerase and telomerase-independent mechanisms) of telomeric DNA, and is connected with telomerase activation in dividing cells. In single cell organisms, human germline cells, and in plants, the telomere elongation and degradation mechanisms are balanced so that overall telomere length remains constant. However, the balance in telomere length regulation may be perturbed during *in vitro* dedifferentiation[10]. By contrast, telomeres shorten with cellular ageing in human

somatic tissues suggesting that telomere degradation mechanisms dominate in such cells[11]. Detection of telomerase activity and its correlation with rate of cell proliferation could be the simplest means to ascertain that telomerase facilitates telomere replication. There may be four different cell division centres involving telomere degradation or replication activity: (i) somatic cells with determinate growth pattern, e.g. human somatic tissue, (ii) cells that are predicted to be immortal *in vivo*, such as germline cells, (iii) cells involved in growth and development, (iv) cells with unlimited proliferation capacity, such as tumour/virus-induced transformed cell lines, and *in vitro* cultures.

Telomeres shorten during human cell differentiation, and this decrease in telomere size in somatic cells was determined to be ~50 bp per cell division. The telomeric decrease is thought to be on account of absence of telomerase activity in such cells. However, telomerase is constitutively expressed in germline cells. This facilitates their balanced telomere replication and stabilization, sustaining their continuous proliferation. Similarly tumour- or virus-transformed cell lines that possess unlimited proliferation capacity exhibit high telomerase activity[15,20]. A corollary to differential telomerase activity as observed in somatic and germline human cells could also be traced in plants. Telomerase activity seems to be developmentally regulated in plants. By comparing the terminal restriction fragment (TRF) lengths in pollen grains, seedlings and leaves of the dioecious plant *Melandrium album*, which possesses relatively short telomeres, it has been observed that telomere lengths do not change during plant ontogenesis, although the various tissues exhibit differential rate of telomerase activity – the meristematic zones depicted higher telomerase activity than maturing ones[10]. This means that telomerase activity is connected with cell proliferation, and stable maintenance of telomere length is connected with telomerase activation *per se* in dividing cells[4,10]. Whereas telomere lengths are maintained stably during plant development, a substantial increase to the tune of three-fold in TRF size was observed during cell dedifferentiation and growth in long-term plant callus cultures[10,21], and in some immortalized cell lines *in vitro*[22]. Such TRF elongation in plant cell cultures appears due to nonregulated synthesis of telomeric repeats by telomerase[10].

The stability of TRF sizes during plant development suggests the presence of a precise mechanism controlling telomere length. Telomerase is required for telomere synthesis, but telomerase activity *per se* probably does not control telomere length. Recent studies indicate that telomerase activity is regulated by double stranded telomere binding proteins. The human telomere binding protein TRF1 acts as a negative regulator of human telomerase. Overexpression of TRF1 in a telomerase expressing cell line leads to progressive telomerase shortening, whereas inhibition of TRF1 increases telomere length[23]. A protein-counting mechanism for telomere length regulation was described for yeast in which the Rap1p telomere-binding protein plays a key role[24]. Further, in an attempt to look for regulatory factors that link with telomere maintenance and function, a second enzyme named 'tankyrase' has now been discovered[25]. Tankyrase may serve as a possible partner to telomerase that enables telomerase to do its work. It is assumed that TRF1 normally sits on the telomeres, thereby blocking telomerase access to the chromosome ends. During or perhaps after DNA replication, tankyrase modifies TRF1 such that it comes off the DNA leaving the telomere, and enabling the telomerase to replace DNA lost during replication[25,26]. Proteins binding to telomere repeats have also been found in plants, and probably mechanisms similar to those described for humans and yeast could participate in telomere length regulation in plants, as well[10].

Rationale for developmental connectivity of telomerase re-activation

Whereas cellular differentiation is established during initial stages of development in animal systems, no true germline is set aside in early embryogenesis in plants where both vegetative and generative parts are derived from meristems during growth and differentiation. This implies that any change in the nuclear genome, including telomere shortening, if it occurs in the meristematic cells could be sexually transmitted in plants, whereas such changes encountered in animal cells other than the germlines would not affect the genetic stability of the progeny. The observations that telomeres are stably maintained during ontogenesis and the extent of telomerase activity correlated with cell proliferation requirement fits well the pattern of plant body formation, as well as prospective immortalization of germline cells in animals, and suggests strict control of telomere lengths during development. If such a control mechanism did not operate and the telomeres shortened during multiple cell divisions, resulting in the formation of gametes, this change would finally, after repeated sexual generations, lead to the complete loss of telomeres.

Avoidance of replicative sensescence via telomerase overexpression?

Expression of telomerase that allows cells to repair their telomeres can subvert the generational clock, thus avoiding senescence or crisis and achieve unlimited proliferation. In fact, it has been demonstrated that ectopic expression of hTERT, the catalytic subunit of the telomerase holoenzyme, enabled human cells that were destined to senesce to multiply indefinitely[1,27]. This promises to confer replicative immortality on cells without effecting other regulatory systems such as those that control differentiation[5]. However, it has now been indicated that it may not be so easy to avoid senescence, at least in certain cell types. The work with two human epithelial cell types – keratinocytes and mammary epithelial cells – indicates that immortalization of these cells requires expression of the hTERT genes and also inactivation of the retinoblastoma tumour–suppressor pathway[28]. Unfortunately, the latter affects differentiation programmes[29]. This may complicate effective realization of cell immortalization for which alternative solutions need to be searched. Curiously, with the discovery of tankyrase that controls whether telomere can do its job by removing telomere blocking protein, a way may be open to developing com-

pounds that would exploit tankyrase to control cell life span[25].

Notwithstanding, extreme correlation between telomerase expression and failure to undergo replicative senescence underlines potential usefulness of telomerase activity as a diagnostic and prognostic tool in cancer. Also, the *in situ* measurement of telomeric motifs through quantitative FISH can further facilitate such analysis. Further, identification of novel agents that activate or inhibit tankyrase may promise far reaching consequences for cell-based therapies/utility by extending their lives or halting uncontrolled cell proliferation through controlled intervention of telomerase activity.

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SCIENTIFIC CORRESPONDENCE

Characterization and utilization of iron-rich dry ash from an electric arc furnace

Fine drosses generated from various metallurgical operations present serious environmental problems on account of their large volume and difficulty in handling. A typical example is pulverized coal fly ash generated in thermal power stations which has attracted the worldwide attention of researchers and environmentalists alike[1–5]. A similar waste, rich in iron content, produced from steel mills has not received the attention it deserves. In fact, iron-rich ash is a significant waste product from several steel-making industries.

A case in point is the Wheel and Axle Plant, a Ministry of Railways concern, situated at Yelahanka in Bangalore, which employs a state-of-art technology for melting steel scrap in an ultrahigh frequency electric arc furnace. In this process, about 20 tonnes of scrap steel is melted at 1700°C along with 300 kg of graphite powder and 1.5 tonnes of limestone as flux. The final composition is maintained by adding ferrosilicon, ferromanganese, etc. The process generates about 3 tonnes of fine-sized dry ash per day.

From the environmental point of view, the ash generated in this plant poses some disposal problems as it may contain toxic heavy metals such as lead, chromium, manganese, copper, nickel and cobalt[6]. It was therefore thought worthwhile to characterize this material