

A protein folding and RNA transport based model for eukaryotic gene regulation

Nascent polypeptide folding¹ and RNA trafficking² are areas of great significance in cell function. Here I present a hypothetical eukaryotic gene regulatory mechanism where the two cellular activities interact with each other through molecular chaperones and bring about translational regulation. Although a theoretical consideration, most of the steps involved are known cellular events.

From transcription of mRNA in the nucleus to its subsequent translation in the cytoplasm, the process of gene expression requires concerted and coordinated activities of a host of molecular machines. It is tempting to speculate that if the growing polypeptide chain is somehow able to communicate with the translating mRNA, the message so generated at the site of translation would have the potential to flow in the reverse direction to the site of transcription; this can add to the cells' repertoire of gene regulatory mechanisms.

The sequence of events in this model would be more or less like this: accumulation of large amounts of protein beyond a certain threshold level (protein crowding) → nascent polypeptide destabilization → molecular chaperone-nascent polypeptide-RNA transporting (localizing) protein [RT(L)P] interaction → mRNA masking accompanied by translational inhibition and RT(L)P tethering → failure of RT(L)P nucleocytoplasmic shuttling → inhibition of fresh nuclear mRNA export → inhibition of pre-mRNA processing → inhibition of mRNA turnover from the site of transcription → inhibition of transcription.

Once the excess protein turns over from the site of translation, the following feedback may ensue: protein uncrowding → loss of molecular chaperone-nascent polypeptide-RT(L)P interaction → mRNA unmasking → resumption of translation and availability of RT(L)P for fresh nuclear mRNA export resulting in normal pre-mRNA processing, and mRNA turnover from transcriptional site would then resume and the gene in question would start transcribing.

The autoregulatory model described above is based on the premise that masking a partially translated mRNA from further translation would inhibit its further localization in cytoplasm, thereby affecting its transport, processing, turnover from transcriptional site and synthesis of RNA in the nucleus, in that order. Likewise, mRNA unmasking would help resume the information flow from DNA to RNA to polypeptide. The specificity of RT(L)P in transcript localization would, of course, be important in this model. In other words, the above model requires the mRNA to be asymmetrically localized, and is therefore a model for mRNA-specific translational regulation. However, the same model when involves general RNA transporting, not mRNA-specific localizing, factor(s) could however also serve for a general translational regulation as described below.

During heat-shock (stress) response expression of normal (non heat shock) genes is regulated in such a way that onset of heat shock results in inhibition of their transcription as well as translation, and once the stress period is over, the normal synthetic activities of RNA as well as of proteins are resumed. It is possible that expression of some of the normal genes is regulated according to the above model, i.e. the stress would result in the destabilization of nascent polypeptides and the rest of the negative-feedback pathway would then follow, resulting in translational as well as transcriptional inhibition. Once the stress is discontinued, the molecular chaperones will dissociate from the complex, resulting in resumption of normal synthetic activities. Thus, for the heat shock model to work, the nascent polypeptide to molecular chaperone ratio might be important.

The hypothetical autoregulatory model described above is simple and accommodative of alternative views. Gene expression can be regulated at transcriptional, post-transcriptional, translational, and post-translational levels. The present model has the potential for regulation at all such levels, transcription, pre-mRNA processing,

RNA turnover from the site of transcription, nuclear RNA export, cytoplasmic RNA accumulation, translation and protein turnover. However, the common requirement for regulation at all these levels is the nascent polypeptide – molecular chaperone – RT(L)P interaction.

Protein crowding, heat shock and several other stress conditions are known to result in protein destabilization (aggregation, unfolding and misfolding)³. Co-operation of translational machinery and heat-shock proteins in protein synthesis⁴⁻⁶ as well as the involvement of molecular chaperones in nascent polypeptide stabilization^{1,3} are well-documented events. Molecular chaperones, which include heat-shock proteins, are a class of proteins that mediate protein-protein interaction and participate in diverse activities such as synthesis, folding assembly, disassembly, translocation, and degradation of proteins^{7,8}. Similarly, heat shock proteins are also believed to interact with mRNA and play a role in their protection, localization, transport and degradation⁹⁻¹⁶. They are also known to bind actin filaments¹⁷, a part of cytoskeleton known to participate in RNA transport. Keeping the above in view, it is perhaps not unreasonable to think of some kind of molecular chaperone-RT(L)P interaction.

The protein-crowding model assumes that various mRNAs involved in such regulation are asymmetrically localized in the cytoplasm and that their exact localization requires specific localizing protein/factor(s). The accumulation of particular mRNAs at specific sites in the cytoplasm is now believed to be a general phenomenon occurring in all polarized cell types. Other than germ cells and early embryos, it also occurs in a host of somatic cell types, including muscle, epithelia, fibroblasts, neurons, oligodendrocytes etc.^{2,9,18-20}. The evidence that transcripts are localized by vectorial export from the nucleus¹⁸ further supports the model. In addition, the known involvement of cytoskeleton in RNA localization/transport^{2,9,18-22} and the association of mRNA-cytoskeleton

complex with translational machinery²⁰⁻²³ are in consonance with the proposed model. The 3' untranslated region (UTR) of the mRNA is known to function in RNA transport/localization^{2,19-21,24,25}. This evidence, in conjunction with others that 3' UTR-binding proteins associate with polyribosomes²⁶, that 3' UTR can regulate translation^{2,27,28}, and that 3' UTR-binding proteins display homology with the heat-shock proteins²⁵ support the present hypothesis.

In order to make the model work, the transcribed RNA must associate with some protein(s) first and then be transported to the site of translation. Further, the protein involved must also be able to go back to the transcription site, pick up another RNA molecule and come back again to the site of translation. And the cycle should go on. That such events indeed occur have now become increasingly clear^{2,29-31} by co-transcriptional binding of heterogeneous ribonucleoprotein particles (hnRNPs) and various other proteins to 3' UTR and the role of these proteins in transport of RNA from the site of transcription to that of translation. Moreover, it is also known that the RNA-transporting proteins shuttle between the transcription sites of RNA and the sites of protein synthesis^{2,32-35}.

The model also assumes that alteration in RNA turnover from the site of synthesis would affect pre-mRNA processing which, in turn, would influence transcription. Nucleus is highly organized and dynamic with respect to functions of gene transcription, pre-mRNA processing, RNA turnover from transcriptional site and nuclear RNA export. Co-transcriptional pre-mRNA processing as well as coupled RNA turnover; pre-mRNA processing are all known events³⁶⁻³⁸. Not only that, RNA polymerase II has also been shown to be involved in mRNA processing^{39,40}. All these, along with the existence of a communication system between RNA polymerase II and 3' UTR processing factors integrating different nuclear processes, are in agreement with the proposed model.

The present hypothesis describes a gene-regulatory mechanism by which translational inhibition and activation can bring about repression and induction, respectively, of RNA localization,

nuclear export, turnover, processing and transcription in the given order. The focal point in this regulatory pathway is the unmasking of mRNA, i.e. formation of nascent polypeptide-molecular chaperone-RT(L)P complex to transiently sequester RNA into translationally inert ribonucleoprotein (mRNP) particles. Several examples of 3' UTR-based and transacting-factor-mediated translational regulation in metabolic and developmental control are known⁴¹⁻⁴⁴. The feature which is unique in the model proposed here, is the involvement of molecular chaperones. As molecular chaperones are ubiquitous proteins occurring constitutively, developmentally as well as under stress conditions, the model can possibly serve for global gene regulation. In addition, owing to the involvement of 3' UTR in the regulatory pathways, the model also has the advantage of being applicable to transcriptional regulation. 3' UTR can be mRNA specific, and thus capable of spatially controlling translation.

While in the heat shock model, the normal mRNA and protein synthetic activities are inhibited due to a general unavailability of RNA transporters, the protein-crowding model provides an autogenous regulatory pathway where excess protein inhibits further synthesis of RNA due to a shortage of poly(A)⁺ RNA-specific localizing factor(s). In case of threonyl-tRNA synthase, the protein binds to its own mRNA. This binding competes with that of the ribosome and thus inhibits translation initiation⁴⁵. Similarly, thymidylate synthase also binds to its own mRNA and brings about translational repression⁴⁶. Several other examples of autoregulation, based on direct protein-mRNA interaction, are also known. The autoregulatory mechanism described here, however, envisages a complex interaction among the various proteins and the mRNA.

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ABHAY SHARMA

Central Institute of Medicinal and
Aromatic Plants,
Lucknow 226 015, India

Dose-response relationship in the microbial suppression of *Sclerotium rolfii* by *Trichoderma pseudokoningii*, strain MTCC 3011

Sclerotium rolfii is a devastating plant pathogen infecting over 500 species of plants¹. It can infect seeds, seedlings, mature plants in the field, and cause diseases of fresh vegetables and rhizomes while in storage and during transit². In recent years, the biological control as well as the management of this pathogen under field conditions³, using its antagonistic fungi, *Trichoderma* spp., has emerged as a viable alternative to chemical fungicides. Recently, we have isolated a strain (MTCC 3011) of *T. pseudokoningii* from sclerotium of *S. rolfii*⁴. This strain was found to be highly effective in suppressing the growth of *S. rolfii* on ginger rhizome, and on several vegetables (beet, carrot, bitter melon, elephant-foot yam, etc.) while in storage^{4,5}. Under other species of *Trichoderma* (*T. viride*, *T. harzianum*, *T. virens*, *T. koningii*), *T. pseudokoningii* has been rarely reported in literature as a biocontrol agent⁶. Hence, there is a need for a thorough characterization of this species in order to use it as an effective biocontrol agent of plant diseases. In this paper, we report on the effect of the type of inoculum and inoculum density of *T. pseudokoningii* for its efficacy as a microbial-suppressive agent of *S. rolfii*. We also propose the possible mechanism of biocontrol in this system on the basis of inferences from the present results as well as our previous observations.

The disease-control potential of *T. pseudokoningii* for *S. rolfii* was studied using a bioassay described by us earlier⁴. Briefly, one side of sliced ginger

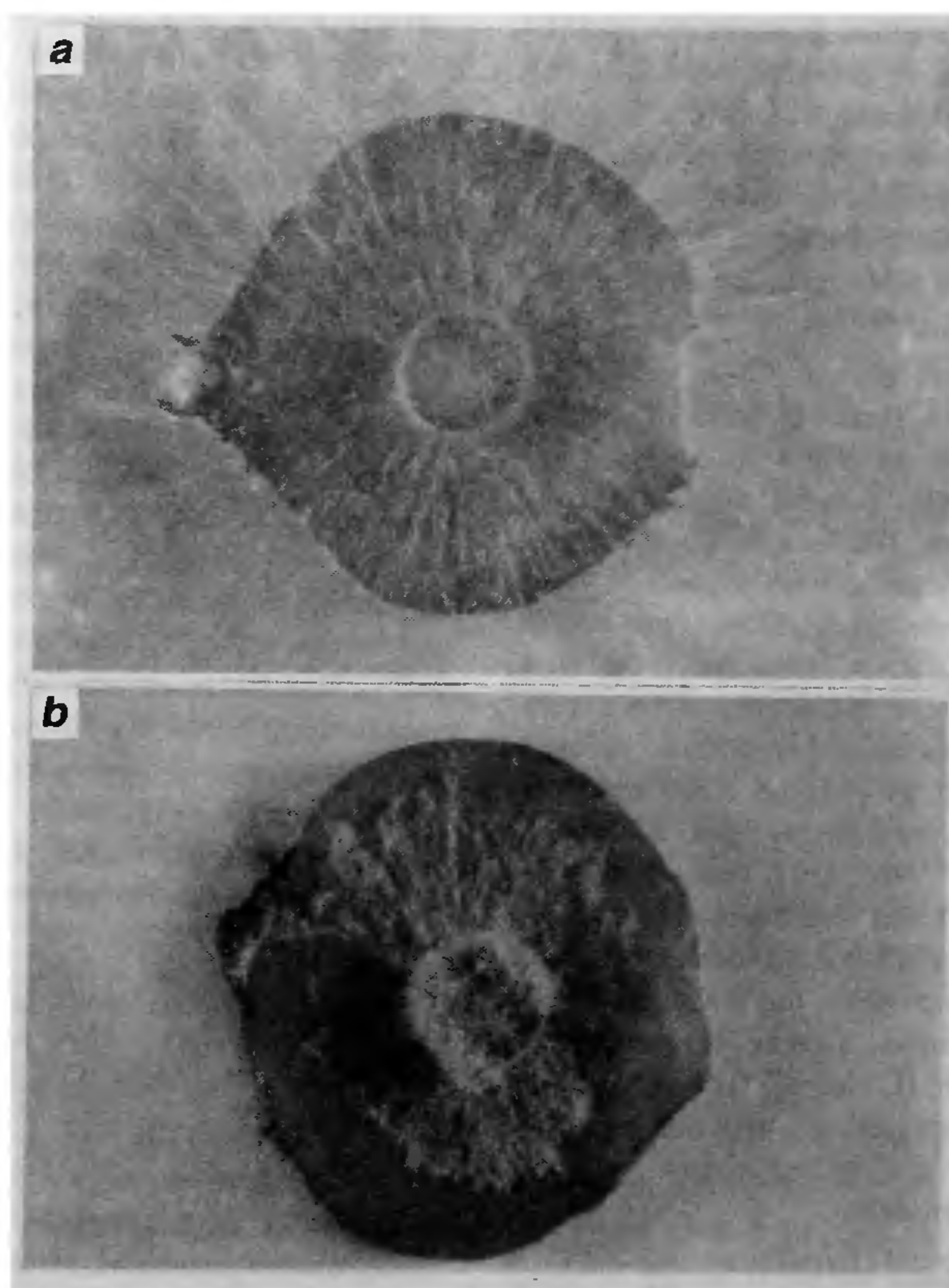


Figure 1. Overgrowth of *T. pseudokoningii* on *S. rolfii* after 5 days of incubation. *a*, Growth of *S. rolfii* on sliced ginger rhizome not inoculated with *T. pseudokoningii*; *b*, *S. rolfii* growth being overgrown by *T. pseudokoningii* on sliced ginger rhizome inoculated with *T. pseudokoningii* (0.05 mg ml⁻¹ mycelial suspension).