THE CONCEPT OF PRE-EMBRYONIC GENES

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ABSTRACT. The evolutionary origin of pre-embryonic genes can be conceived as having arisen from pre-embryonic type organisms such as yeast. Control mechanisms of yeast were explored conceptually emphasizing heterochromatization and epigenetic mechanisms. It was argued, by using the carcinogen-induced tRNA methylase system in liver cells, that experiments showing a similar gene-induced activity in yeasts, could only be explained if both were sets being controlled by the same or similar mechanisms. By virtue of the fact that carcinogens induce embryonic gene activities, one can then say that carcinogens induce a similar set of pre-embryonic genes in yeasts.

KEY WORDS. Embryonic genes, evolution, yeast, tRNA methylase activity, carcinogens, Heterochromatin spread, histone code, Ubiquitin-conjugation enzymes, Ethionine, enhancer mechanisms.

INTRODUCTION

The control of the direction of the evolutionary vector towards metazoan complexity from single cell simplicity is not understood. However, one may derive scenarios to account for the history of this process. For example, a mutational gene change in membrane structural proteins could give new adhesive properties resulting in systems like colonial Volvox. This progression would allow for a further division of labor. Such metazoans with their simple incremental modifications would then be selected upon by natural selection for any survival attributes—thus modern metazoan evolution. Obviously, many such scenarios can be devised.

Eventually, metazoans, from annelids and their ancestors to vertebrates, acquired the ability to differentiate embryonic type cells for their reproductive schemes. This paper is devoted to the concept of pre-embryonic genes. Teleologically speaking, metazoans appear to use genes found in prokyarotes for embryonic purposes. The acquisition of such genes requires that they be strictly controlled in order for the adult metazoan phenotype to be maintained. In fact, losses of control over such embryonic-type genes, e.g., growth factor genes, apparently lead to the neoplastic state.

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One of the potential processes for controlling embryonic genes is heterochromatization mechanisms. In the fertilized ovum the embryonic genes that are in a repressed state are required to become derepressed to act during differentiation stages. This process could be via alterations in heterochromatized segments involving embryonic genes. These temporal processes would be achieved by epigenetic changes by such modifications as acetylations, phosphorylations and methylations of chromatin proteins.

The evolution of these controllable genes (embryonic type) must mean that the control mechanisms, themselves, had to evolve in concert with this subset of genes that differ from housekeeping genes or the genes that eventually become derepressed and comprise the adult set of genes for a given cell type.

Thus one is confronted with the evolution of heterochromatin with its various states and controlling processes.

CONTROL ASPECTS OF HETEROCHROMATIN IN YEAST

Evolutionary mechanisms of regulatory systems utilize undoubtedly classical natural selection processes that act by random variation. The characteristics of these first pre-heterochromatin segments might be some simplified version of the heterochromatin of a vertebrate. Since yeast can be considered as pre-animal and pre-embryonic type organisms, an examination of yeast heterochromatin should be informative.

Yeast chromatin contain heterochromatic segments that are controlled by targeted mechanisms, i.e., histone acetyltransferases and deacetylases affecting specific promoter regions via DNA sequence specificity. There are five specific histone deacetylases that have been well defined of his type. But there are acetylation enzymes that can also modify histones of large chromatin domains.

It has been determined that unacetylated lysine 16 of histone 4 is required for heterochromatin in the telomeric region and acetylation of this residue causes a barrier to any heterochromatin spread. (Kurdistani *et al.* 2003). Methylation of histone H3 is associated with higher order chromatin structure and H3-Lys 9-methyl is connected with 'silent' chromatin and H3-Lys 4-methyl is connected with 'active' chromatin. It has also been determined that two ubiquitin-conjugation enzymes regulate heterochromatin silencing in yeast (Choi *et al.* 2002).

Nakayama (Nakayama *et al*. 2001) have described a protein that methylates lysine 9 of histone H3 at heterochromatin-associated regions and have suggested that there is a "histone code" that is established by certain sequential histone modifications and that this is required for any epigenetic inheritance of heterochromatin assemblies.

A specific gene (epe 1) has been shown to enhance position effect variegation at heterochromatic domains. Thus it is concluded that the epe 1

protein counteracts heterochromatization. Furthermore, heterochromatin assemblage requires sequential modifications of histone H3 amino-terminal tail and histone deacetylases cooperate with Clr4 histone methyltransferases in establishing a "histone code" for the Swi6 localization to heterochromatic loci (Ayoub *et al*. 2003). Theoretical considerations of position effect variegation has been explored in connection with carcinogenic mechanisms involving derepression of embryonic genes by the writer (Hancock 1982).

Combining the control of heterochromatin by pre-embryonic type organisms with the requirement of down-regulating some potential pre-embryonic genes, e.g., those required for budding, could be used for a theoretical model for the evolution of control mechanisms for vertebrate embryonic genes.

The genome size of human as compared with yeasts is about 200 fold larger and the number of genes is 80 000 as compared to approximately 6 000 yeast genes. But it is the control mechanisms that have evolved for these genes that has apparently allowed for greater complexity.

For example, the bacteriophage lamda repressor does not act at a distance as contrasted with eukaryotic DNA protein promoter factors which interact via distant enhancers. It is believed that this arrangement allows for temporal expression during ontogeny. A specific example of this is Myo D, which is involved in the embryonic development of vertebrate muscle. Myo D forms heterodimers with another factor that binds to enhancer regions which control specific muscle type genes, e.g., actin and myosin. Theoretical interpretations of enhancer mechanisms called 'Key Theory' involving carcinogenesis and embryonic genes has been developed by the writer (Hancock 1989).

A UNIQUE APPROACH IN IDENTIFYING PRE-EMBRYONIC GENES

If metazoan embryonic genes evolved from pre-embryonic genes of non-metazoans, then examples of these genes could be present in yeasts. But how would one recognize such a pre-embryonic gene. One approach would be to demonstrate a similar function between yeasts and mammals. For example, possibly those gene expressions involved in budding would be likely candidates for those genes involved in rapid vertebrate embryonic cell division.

Another completely different approach is the reaction of embryonic genes to carcinogens. Ethionine is a hepatocarcinogen to rats and it was demonstrated to induce an embryonic type protein, alpha-fetoprotein (Hancock *et al*. 1976).

t-RNA methylase activity was found to be increased in embryonic mouse liver as compared with adult liver (Hancock *et al*. 1967). Ethionine induces tRNA methylase activity (Hancock 1968). It therefore was hypothesized

that carcinogens create the embryonic situation with respect to the status of tRNA methylation.

EVIDENCE FOR EMBRYONIC GENES AS EVOLVING FROM PRE-EMBRYONIC GENES

If yeast cells are cultured with known hepatocarcinogens, namely ethionine or dimethylaminoazobenzene or their comparable non-carcinogens, methionine or aminoazobenzene respectively, there is a dramatic increase in the tRNA methylase activity of the carcinogen-treated cells (Hancock 1972).

The biophilosophical question becomes: By what means do yeast cells, which are not capable of becoming neoplastic, perceive an operational difference that is effective in mammalian liver cells? The only way this can be made logical is as follows. If the genes, in this case, those for expressing tRNA methylases, are the same genes, with the same control mechanisms present in vertebrate liver, having thus evolved as such, then one should expect a similar reaction between the two organisms-yeast and rats. Furthermore, one should consider the possibility that the control mechanisms for this set of genes, being the family of tRNA methylases, are similar even though the specific tRNA methylases are not.

Thus one might conclude that certain pre-embryonic genes in yeasts could represent a subset of embryonic type genes in vertebrates that carcinogens are capable of derepressing which establishes the embryonic phenotype so recognizable in neoplastic cells. In fact, the first (within 72 hours of treatment) biochemical change detectable in ethionine hepatocarcinogenesis is the derepression of an embryonic gene (alpha-fetoprotein) (Hancock et al. 1976). Although it is highly unlikely that alpha-fetoprotein per se has anything to do directly with carcinogenesis, it may represent a process critical to carcinogenesis that involves other embryonic genes.

Using broad generalizations and projections, the biophilosophical implications these relationships seem to present are that by the time yeasts had evolved, major genic patterns were established that allowed for metazoan evolution to be efficacious.

SUMMARY

Using carcinogens that are capable of inducing embryonic gene activities in vertebrates, one can induce similar activities in organisms not capable of embryonic type cells, suggesting that such activities are due to, in evolutionary terms, pre-embryonic genes.

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