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Evolutionary Advantages of Cell Specialization: Save and Protect DNA

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As the transcribed regions of nuclear DNA are in a more open state, they are liable to be damaged by naturally or environmentally produced mutagens. Cell specializations make it possible to express fewer genes in individual cells of multicellular organisms, thus protecting genes from the damage of mutagens. We propose that this might account for the advantage of cell specialization, as an alternative to the traditional conception that cell specializations result in increased efficiency. The most efficient cell specialization to protect DNA is in the segregation of germ cell(s) and somatic cell(s). But in optimal environments, such specialization is expected to reduce the rate of reproduction, which might counteract its advantage of protecting DNA.

1. In DNA, Saving is Protecting

As DNA is the target of numerous physical and chemical factors, cellular DNA is continually subject to endogenous and environmentally induced structural alterations. Such alterations can manifest themselves as mutations, recombinations and rearrangements, gross chromosomal abnormalities, etc. Although DNA structural alterations are raw materials upon which natural selection acts, in most cases, the alteration rates are obviously much higher than those needed for evolutionary progress, because organisms have developed so many DNA repair systems to reduce them (Sancar & Sancar, 1988). We wondered if organisms have alternate strategies to eliminate the DNA structural alterations, because, clearly, repair can only make the damaged DNA resemble its original state as far as is possible. In addition, the more accurate the repair, the greater its cost.

There are two ways to maintain the original state of an object. One is to repair its damages as accurately as possible; the other is to store it carefully. And if possible, avoid using it. Above all, a glass in use is liable to be broken.

Eukaryotic cells have their nuclear DNA wrapped around histones and folded into highly ordered structures. The folding appears to be hierarchical, with several levels required to achieve the highest degree of condensation found in transcriptionally inactive regions (Kornberg & Lorch, 1992). The tight package of DNA in the nucleus might be expected to render the DNA inaccessible to most of its interactive factors. Here we do not use the word mutagens or DNA-damaging agents because the accessibility of transcription related proteins and/or transcription-repair coupling factors would also be blocked simultaneously. When DNA fulfils its functions and expresses the stored genetic information, alterations occur in conformation and as a result they become more accessible. (Gross & Gerrard, 1988; Morse & Simpson, 1988; Kornberg & Lorch, 1992). Consequently, primary DNA damage rates and transcriptional activity might be closely associated. However, organisms have developed transcription-coupled repair systems to preferentially repair the transcribed genes (Drapkin *et al*., 1994; Selby & Sancar, 1994). Are the net alteration rates (primary DNA damages

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minus those being repaired) of transcriptional active genes higher than those of inactive genes? Under normal conditions, instead of the mutation inductive experimental conditions (the latter is used in the studies on the transcription-coupled repair system), the net DNA structural alteration rates of transcriptionally active genes are expected to be higher than those of the inactive genes. Datta & Jinks-Robertson (1995) found a stimulatory effect of high levels of transcription on spontaneous mutation rates, where the spontaneous mutations could be attributed to *in vivo* free radicals, background radiation, etc. In addition, the somatic hypermutation of immunoglobulin genes has been demonstrated to be associated with transcription initiation (Peters & Storb, 1996). Ironically, the transcription–repair coupling pathway has been proposed to be one source of spontaneous mutations (Hanawalt, 1994; Datta & Jinks-Robertson, 1995).

2. Effects of Cell Specialization on DNA

It is worth noting that organisms may have made use of the characteristics just mentioned to fulfil certain functions, e.g. to increase the somatic mutation rate of the immunoglobulin variable region sequence (Betz *et al*., 1994; Maizels, 1995; Peters & Storb, 1996). The alternate strategy to eliminate DNA structural alterations is to reduce the transcriptional time in genes, in order to leave them in an inactive state for more time. Post-transcriptional regulation of gene expression can accomplish this to some extent. Increasing the longevity of mRNAs, proteins or enzymes can partially reduce the transcriptional time of genes. Cooperation between cells makes it possible for each cell to express fewer genes, which is obviously an effective way to protect DNA. In multicellular organisms, individual cells become specialized in fulfiling different functions, they rely on other cells in the organism's body to do some of the things they are unable to do. These specializations have obviously given multicellular organisms great evolutionary advantages, for there are so many multicellular organisms on earth. Here, we propose a hypothesis, that cell specialization in multicellular organisms, is not all an evolutionary advantage.

For convenience, let us consider such hypothetical organisms that have two types: unicellular and bicellular. For survival and reproduction, these organisms have to fulfil five functions—A, B, C, D and E, each of which is accomplished by one set of genes—a, b, c, d and e, respectively. The capital letters represent the amounts of work needed to fulfil the corresponding functions required for one cell. The individual cell of a unicellular organism has to express all the genes and fulfil all the functions sooner or later. The small letters represent the transcriptional time of the gene set per generation in a unicellular organism. In bicellular organisms, specialization has occurred between the two cells. Suppose one cell (α) expresses gene set a, b and c and fulfils functions A, B and C, while the other cell (β) expresses gene set c, d and e and fulfils functions C, D and E. In addition, suppose the net alteration rate increment of one set of genes that results from per unit time transcription is m. From the above discussion, it can be seen that $m > 0$. In unicellular organisms, an individual cell has to accomplish the work of $\mathrm{A} + \mathrm{B} + \mathrm{C} + \mathrm{D} + \mathrm{E}$ and suffer the total DNA alterations (referred to as the part that results from transcription, the same below) of $m(a + b + c + d + e)$ in one generation. In bicellular organisms, cell α and cell β have to accomplish the work of $2A + 2B + C$ and $C + 2D + 2E$, respectively. Because of the existence of post-transcriptional regulation of gene expression, gene set a is not required to be transcribed for the time $2a$ in cell α, a certain proportion x ($0 \leq x \leq 1$) of transcriptional time might be reduced, and similarly, for gene set b, d and e. Thus, to fulfil all its functions, one bicellular organism has to suffer the total DNA alterations of $2m(a + b + c + d + e) - xm(a + b + d + e)$ in one generation. Then the average DNA alterations of one cell in bicellular organisms are $m(a + b + c + d + e) - \frac{1}{2}xm(a + b + d + e)$ per generation. Compared with the unicellular organisms, the DNA alterations are reduced $\frac{1}{2}xm(a + b + d + e)$ in one specialized cell per generation. The reduction ratio (M_r) is $\frac{1}{2}xm(a + b + d + e)/m(a + b + c + d + e) = \frac{1}{2}x[(a + b + d + e)/(a + b + c + d + e)]$. The independent variate x is positively related with the efficiency of post-transcriptional regulation of gene expression, while $(a + b + d + e)/(a + b + c + d + e)$ is the reflection of the extent of cell specialization (E_s). Thus, for a specialized bicellular organism, the proportion of DNA alteration reduced by cell specialization is $M_r = \frac{1}{2}xE_s$.

The conspicuous specialization of multicellular organisms—segregation of germ cells and somatic cells, had resulted in the segregation of reproductive functions and vegetative functions. As the function of germ cells is to transmit genetic information, only a few genes are required to be expressed to maintain cell vitality and fulfil cell division. The specialization of germ cells has developed so far in some higher organisms, in that intracellular components that are needed for (gametogenesis and) the division of fertilized eggs may also be imported from surrounding cells or other somatic cells. For instance, in the

chicken, vitellogenin (the major component of yolk) is synthesized in liver cells and transported to oocyte by the bloodstream (Loomis, 1986). In some invertebrates, macromolecules and even ribosomes of oocyte are imported from the nurse cells through the inter-cellular bridges (Browder, 1984). Most genes in germ cells are inactive, so as discussed above, these genes have a lower chance of getting damaged. Perhaps the segregation of germ cells and somatic cells is the most conspicuously efficient specialization in protecting DNA.

With the increase of complexity that results from cell specialization, more and more genes must be depressed, which, as a consequence, must enhance the frequency of inadvertent gene expression. This gives a more reasonable answer to the question: Why did DNA methylation (a noise reduction mechanism) (Bird, 1995) extensively spread in vertebrate genomes, but scarcely in invertebrate genomes? Repression of inadvertent gene expression is important for the increase of gene number (or biological complexity), while a low mutation rate is also required for the evolution of large genomes. Which is the limitation (Hurst, 1995)? Our theory of cell specialization might bridge the two. The establishment of noise reduction has smoothed the way for cell specialization, while cell specializations have made it possible to reduce mutation rate without increasing the cost of DNA repair, as a result, the limitation of gene number increase is relieved.

Besides the cellular level, functional specialization has also occurred at the subcellular level of the multinucleate ciliates. Each ciliate contains at least one germ line nucleus (micronucleus), which is used for sexual exchange of DNA, and a somatic nucleus (macronucleus) for the production of RNA to support vegetative cell growth and cell proliferation. The DNA in the micronucleus occurs in uniformly and densely packed chromatin, in contrast with the DNA in the macronucleus, which occurs in many chromatin bodies dispersed in the nucleoplasm (Prescott, 1994). This suggests that ideas concerned with the protection of germ DNA might naturally arise in the minds of biologists even if they have not noticed the relation between transcription and mutation rate.

3. Traditional Conception

The genus *Volvox* which has a *bona fide* segregation of germ line and soma, is an excellent example of cell specialization. Its benefit of specialization had been demonstrated: colonies produce a large bulk of smaller offspring than do single cells of a similar size. According to traditional theory, the evolutionary advantages of cell specialization are manifested in increased efficiency (Szathmáry & Maynard Smith, 1995). It seems unwise to reach this conclusion just because they reproduce more vital offsprings. There exist at least two alternate possibilities. First, after cell specialization, the efficiency is increased, and as a consequence, organisms could produce more offsprings. Second, there are no differences in the quantities produced in reproduction between specialized and non-specialized vital organisms, but qualitative difference do exist in offspring, e.g. the non-specialized will reproduce a larger proportion of defective offspring (with decreased possibility of survival and/or reproduction) because their genes have a higher alteration rate, which is a result of transcription. This may also indicate that specialized organisms reproduce more offsprings than non-specialized organisms over a longer period of time.

It should be noted that task specialization and coordination are two independent processes, there is no reason to believe that task specialization is always accompanied by good coordination. If different specialized parts of an organism could be well coordinated, efficiency would be increased and the organism would survive. Conversely, if they could not be well coordinated, efficiency would be decreased and the organism is more likely to be eliminated. Most mutations after long-term natural selection are advantageous, however, primary mutations are comprised of at least three classes: deleterious, neutral and advantageous. Primary specializations might also be comprised of three classes: increasing efficiency, decreasing efficiency and unaltering efficiency, but all these specializations can result in saving and protecting genes, which is undoubtedly advantageous. The increased efficiency just corresponds to those specializations that enable organisms to survive in natural selection, but the organisms that have survived with cell specializations do not necessarily have increased efficiency, because those specializations which have little effect on efficiency are also selectively advantageous. Once accurate measurements for efficiency of biological processes have been established, the question of whether selected organism have unaltered efficiency specializations, will give a final answer.

4. Effects of Cell Specialization on Fitness

Since cell specialization is a way to protect DNA and, at least, it has no invariable negative effect on the efficiency of biological process, why have not all organisms taken advantage of this? Are there any

disadvantages that might counteract cell specialization for some organisms, or for some special environments?

Here we discuss cell specialization in the light of fitness. The two fundamental components of fitness are reproduction and survival. A simple measure of fitness (F) may be obtained from the lifetime sum of age-specific fecundity (b_x) and age-specific survivorship (l_x): $F = \Sigma l_x b_x$. The optimal life history strategy among a collection of possible alternatives is the one(s) that produce the highest value of fitness (Silvertown, 1982). Because the life histories of organisms at the unicellular–multicellular transition are very simple, the measure of their fitness may be simplified: $F = lb$. For convenience, we roughly classify cell specializations into: specialization I which do not involve the division of reproductive function and vegetative function, and specialization II which is the segregation of somatic cell(s) and germ cell(s). All cell specializations can increase survivorship by protecting DNA and, specialization II, although there is no definite evidence available, can increase survivorship to a higher extent than specialization I. For an organism with only specialization I, all cells can produce offspring at a rate at least not lower than those of non-specialized cells, in other words, specialization I is not likely to reduce fecundity. Thus, specialization I would always increase fitness.

However it is not the same case for cell specialization II. For the above hypothetical organisms, suppose α is the germ cell and β is the somatic cell. In addition, we introduce two other cells: γ and δ, which are free unicellular organisms, or two producible cells of a bicellular organism. Cell α just divides, it entirely relies on cell β. Meanwhile cell β cannot divide, but it can support the division of cell α to the doubled rate compared with cell γ or δ growing in the same environment. We start the study of fecundity with cell cycle because fecundities of these simple organisms depend on their division rates. The normal eukaryotic cell cycle consists of M (mitotic) phase, a G_1 (the first gap) phase, a S (DNA synthetic) phase and a G_2 (the second gap) phase. The M and S phases are always essential for the division. The preparation (e.g. transcription of specific genes) for mitosis and DNA synthesis is carried out in G_1 and G_2 phases. It is obvious that preparation is easy for organisms growing in an optimal environment, but difficult for organisms growing adverse environemnt. So the G ($G_1 + G_2$) phase of the organisms in optimal an environment may be expected to be shorter than that of the organisms in adverse environment. There are four cases of eukaryotic cell cycles according to the environmental qualities.

Case (1). In an optimal environment, G approaches the limit zero and the division rate is only limited by $M + S$, then no matter how much cell β can provide for cell α, the division rate of cell α equals that of cell γ or δ. Thus the fecundity has been reduced by 50% after cell specialization II: $b_s = \frac{1}{2}b_n$ (b_s is the fecundity of the bicellular organisms with specialization II while b_n is the fecundity of non-specialized organisms or of those organisms with only specialization I).

Case (2). In a suitable (but not optimal) environment, $0 < G < S + M$. In the time cell α needs to synthesize its DNA and divide, cell β can make enough preparation for the next division of cell α, so cell α can divide continually. $2(S + M)$ is needed for cell α to divide twice while $G + S + M$ is needed for cell γ and δ to divide twice, and because $S + M > G$, the total production rate of cell α and cell β is still lower than that of cell γ and cell δ. Thus $\frac{1}{2}b_n < b_s < b_n$.

Case (3). In a moderate environment, $G = S + M$, then, with the help of cell β, cell α can divide twice as fast as cell γ and cell δ: $b_s = b_n$.

Case (4). In an adverse environment, $G > S + M$. In the time that cell α is dividing and synthesizing its DNA, cell β cannot make enough preparation for the next division of cell α. Cell α has to take a gap phase in its cell cycle. In this case, if cell α can do something in its gap phase, its division rate equals the total rate of cell γ and cell δ: $b_s = b_n$. But if cell α 'rests' in its gap phase (as the oocyte of *Drosophila*) (Browder, 1984), a bicellular organism (with cell specialization II) can increase only one effective Δt [compared with case (3)] while cell γ and cell δ combined can increase two effective Δt for the preparation of the next division. The former would produce less offspring than the latter in a certain period of time: $\frac{1}{2}b_n < b_s < b_n$.

In summary, cell specialization II decreases fecundity to some extent (from 0 to 50% according to the environmental qualities). The other component of fitness (survivorship) of all organisms changes from 0 to 100% with the variation of environments. Although we do not know the exact value of the survivorship increase by specialization II, there is no doubt that cell specialization II cannot increase survivorship up to 100% to counteract the decreased fecundity in an optimal environment where survivorship of non-specialized organisms is above 50% (e.g. 84%). It may be concluded that natural selection would not always favor cell specialization II.

5. Specialization of Somatic Cells

The above discussions and speculations are mainly concerned with specialized cells that transmit genetic

information. Could the principle be applied to further specializations of somatic cells of multicellular organisms, which are a much more universal phenomena? Plant somatic cells retain the totipotency to produce new individual plants in special cases like the shed stonecrop leaves. More genes protected by cell specialization would ensure the somatic cells produce more vital individual plants. But the somatic cells of animals cannot produce new animals even in laboratory. The ability to regenerate lost tissues and organs, according to Bullough (1967), can be divided into two main categories. The first, which was termed major regeneration, involves the replacement of whole body regions (as in planarians) or whole limbs (as in amphibians). This process depends on dedifferentiation and subsequent redifferentiation of adult tissue cells. The protection of unexpressed genes of specialized somatic cells is thus to maintain the ability of major regeneration. Unfortunately, the power of major regeneration has been reduced in complex animals, e.g. in birds and mammals it is impossible. If the specialized somatic cells are unable to transform into other types of specialized cells or non-specialized cells, their unexpressed genes are not potentially to be expressed genes, so the protection of unexpressed genes that results from cell specialization seems unnecessary. This does not mean that further specialization of somatic cells is unimaginable. The fact that most mutations are not advantageous has not retarded the increment of new variations in organisms. This is also true for cell specialization. The occurrence of further specializations of somatic cells might be analogous to the process of mutation-selection.

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