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Review

The immortal strand hypothesis: still non-randomly segregating opinions

Jane A. Wakeman[1,*], Abdelkrim Hmadcha[2], Bernat Soria[2] and Ramsay J. McFarlane[1,*]

[1] North West Cancer Research Fund Institute, College Natural Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK
[2] CABIMER, Avda. Americo Vespucio s/n, Cartuja 93 Scientific and Technological Park, E-41092 Seville, Spain

* Corresponding authors
e-mail: j.a.wakeman@bangor.ac.uk; r.macfarlane@bangor.ac.uk

Abstract

Cairns first suggested a mechanism for protecting the genomes of stem cells (SCs) from replicative errors some 40 years ago when he proposed the immortal strand hypothesis, which argued for the inheritance of a so-called immortal strand by an SC following asymmetric SC divisions. To date, the existence of immortal strands remains contentious with published evidence arguing in favour of and against the retention of an immortal strand by asymmetrically dividing SCs. The conflicting evidence is derived from a diverse array of studies on adult SC types and is predominantly based on following the fate of labelled DNA strands during asymmetric cell division events. Here, we review current data, highlighting limitations of such labelling techniques, and suggest how interpretation of such data may be improved in the future.

Keywords: asymmetric/symmetric chromosome segregation; immortal strand hypothesis; mutation/cancer avoidance; replicative errors; stem cells.

Introduction

Stem cells (SCs) of all types have a requirement to maintain their genomic integrity, whether they are embryonic, germ or tissue-specific in nature. The mechanisms employed to maintain this integrity may alter depending on the developmental stage, age and the type of divisions being undertaken by a particular SC. For example, embryonic stem (ES) cells, which undergo symmetrical divisions to give rise to functionally equivalent daughter cells, may utilise mechanisms to retain genomic integrity that treat both the 'old' template strand and the newly synthesised strand equally, each strand being of equal importance to the next generation of equivalent SCs. However, when an SC undergoes asymmetric divisions, resulting in an SC and a more differentiated cell type, a hierarchy of 'importance' is established in terms of maintaining healthy growth and homeostasis of the organism, whereby retaining the DNA coding integrity of the SC becomes relatively more 'important' (as it must undergo both self-renewal and give rise to differentiated progeny) compared with a committed differentiated daughter cell. It is this proposed protection of the SC genome during such asymmetric divisions that led Cairns to put forward the immortal strand hypothesis (ISH), resulting in an extensive experimental programme of testing that continues to divide scientific opinion.

Overview of the ISH

John Cairns originally put forward the ISH in 1975 to explain how multicellular organisms might protect the genomes of their SCs from replicative damage (considered to be one of the major causes of spontaneous mutations) (1). In particular, it was suggested that this would be highly applicable to SCs that are constantly renewing cells of a particular tissue with a high cell turnover such as epithelial cells; as such, it was proposed as a cancer avoidance mechanism, to protect against the accumulation of replicative errors in the SC genome over the lifetime of an individual.

DNA replication normally occurs in a semi-conservative manner, giving rise to pairs of sister chromatids at the end of each round of genome duplication, which then segregate to opposite poles during mitosis to be inherited by each of the daughter cells. It might reasonably be expected that the inheritance of the sister chromatids in the newly derived cells would occur through a random segregation process. However, each DNA strand within a chromatid is not necessarily the same, certainly in terms of age, as one strand is inherited from the immediate parent and the other is newly synthesised, and as such has the potential to carry newly incurred replication-mediated errors. To avoid the accumulation of replication-induced errors to the SC genome, the postulate suggests that during asymmetric SC divisions, the 'immortal' stem daughter cell would always receive the 'older' DNA strand of the two parental strands (for clarity, this may be referred to as the ancestral template or grandparent strand), whereas the differentiated daughter cell, destined for 'turnover' and therefore ultimate removal from the organism, would acquire the younger of the two parental strands (referred to as the parent strand, more likely to have accrued errors incurred through replication) (Figure 1). By extrapolation, this infers that during symmetrical divisions of SCs, equivalence of SC function

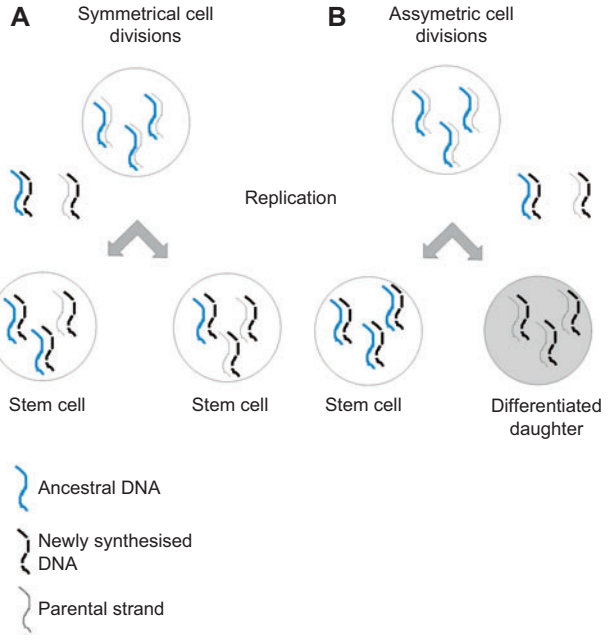

Figure 1 Random and non-random segregation patterns of sister chromatids in symmetric and asymmetric divisions of SCs.
Chromosome replication occurs in a semi-conservative manner to give rise to sister chromatids, and each chromatid then segregates to opposite poles to be inherited by the new daughter cells. In general, the inheritance of chromatids by the nascent daughter cells is thought to be a random process. However, each pair of DNA strands within a chromatid is not equal with one strand being inherited from the parent cell and one strand being newly synthesised. During symmetrical divisions of SCs, evidence suggests that there is random distribution of chromosomes between two functionally equivalent stem daughter cells (A). However, when asymmetric division of daughter cells occurs, there is evidence of non-random distribution of chromosomes during homeostasis of some tissues, with the stem daughter retaining the 'old' DNA from the template strands (ancestral or grandparental DNA) and the more differentiated daughter containing the 'newer' DNA (parental) (B).

would dictate that there is random distribution of chromosomes between two equivalent daughter cells.

Cairns' hypothesis proposes that there are specific requirements and constraints on SCs if they are to employ an immortal strand genome protection mechanism, as summarised below:

- The so-called immortal SCs would be at the base of a hierarchical structure of progressively more differentiated progeny and as such may be expected to be fewer in number within a specific hierarchical grouping. This relatively small number of SCs would employ strand protection mechanisms to minimise the risk of accumulating mutations over the lifetime of an individual. In general, defining SC hierarchies and their number in various tissues remains a major problem in confirming or refuting this hypothesis.
- Tissue-specific multi-potent SCs would be predicted to divide more infrequently compared with more rapidly dividing daughter/progenitor cells (that would have a more limited capacity for self-renewal). As a result of fewer divisions, the SCs would have a reduced mutation risk during genome duplication possibly having more time to complete DNA replication. As will be discussed later, however, recent evidence suggests that quiescence in some SC types is actually associated with the accumulation of DNA damage rather than protecting against the occurrence of DNA damage.
- Accumulation of DNA replication errors would be avoided specifically within the SC population through non-random segregation of the template DNA strand during asymmetric SC divisions, such that the 'older', template strand (grandparental/ancestral) would pass to the 'immortal' SC and the more recently replicated strand (parental) would pass to the 'mortal', differentiated daughter cell (Figure 1). During semi-conservative replication of DNA and symmetric SC divisions, it is assumed that due to equivalence of function of SCs, the 'old' and 'new' strands would be randomly segregated at least up to the time point immediately before an asymmetric cell division (unless following the fertilisation event an 'original' strand is marked for the germline; however, to date, there is no evidence to support this).
- Sister chromatids might be expected to carry different 'marks' functionally linked to the centromere, to direct non-random chromosome segregation in a co-ordinated manner for all chromosomes within the dividing cell.
- One of the most important pre-requisites for preservation of an 'immortal strand' is that the sister chromatids must not (or very rarely) undergo inter-sister chromatid 'exchanges' (ISCE) and DNA repair by this pathway would not be permitted for the ISH to hold, as such events would lead to shuffling of the immortal DNA strand, potentially separating immortal regions from strand segregation control mechanisms at the kinetochore, where the microtubules of the mitotic spindle form attachments to the chromatids through the centromeres. Instead, according to the ISH of Cairns, any immortal SCs incurring damage would be liable to apoptosis (2). As discussed later, however, recent evidence suggests that some SC genomes do undergo repair, albeit error-prone in nature, and in undergoing such repair actually avoid apoptosis with the effect of extending the regenerative capacity of the SCs.

Since the publication of the ISH, making an assessment of support for or against the existence of an 'immortal' strand is somewhat hard to balance or give credence to, owing to the different approaches taken [these have predominantly been indirect approaches except for the study of Falconer and co-workers (3)] and the different cell systems studied. As such, the development of novel, more directed tools to study various aspects of the ISH would be hugely beneficial.

Support and evidence for the ISH

There were several lines of evidence that prompted Cairns to propose the ISH. These included the observation of non-random template strand segregation in mouse embryonic

fibroblasts, which was demonstrated to be dependent on the age of the DNA template strand (using radioactive labelling techniques) (4). Selective strand segregation was also shown in *Escherichia coli*, where, under certain growth conditions, the progeny of chromosomes segregated in a manner that again distinguished between the age of the chromatids. It was suggested that this non-random segregation might be due to a physical association between the oldest DNA strand and the membrane. Since this first report of non-random segregation in *E. coli*, further studies in this organism have demonstrated that sister chromosomes may non-randomly segregate on the basis of leading and lagging strands, which are differentially partitioned following DNA replication (5).

These early reports prompted further studies that were set up specifically to observe non-random chromosome segregation patterns in the context of the ISH. Some of the earliest studies were carried out in mouse intestinal epithelium cells using double-strand labelling techniques [[^{3}H]-Td was used to label the primary strand followed by 5-bromo-2′-deoxyuridine (BrdU) labelling, which was incorporated into the newly synthesised second strand in irradiated crypts; if an immortal strand is retained, as predicted by the hypothesis, then the SC will retain its label in the primary strand, whereas the newly labelled 'second' strand will lose its label on the second division]. These studies showed that nearly all the [^{3}H]-Td-labelled cells rapidly incorporated the second BrdU label, showing that [^{3}H]-Td-labelled DNA cells were actively dividing [and were not 'label-retaining cells' (LRCs) due to their being slow cycling]. The BrdU label was lost from these cells, leaving the older, [^{3}H]-Td-labelled strands; this pattern of label distribution was indicative of non-random segregation of strands (6, 7). However, as commonly encountered in interpreting such experiments, one cannot be certain as to the origins and identities of the cells being addressed in these studies and, due to limitations in the specific identification of SCs, it is possible that these observations extend to cells outside the SC compartment. These studies were recently brought up to date by Quyn and co-workers (8), who demonstrated that the older, label-retaining DNA strand (labelled with the nucleotide analogue, 5-ethynyl-2′-deoxyuridine (EdU), following SC ablation in mouse) was preferentially (not exclusively) held at the basal side of dividing SCs (defined now as Lgr5+ and Bmi1+4+) in the intestinal crypt. This bias of non-random chromatid segregation was associated with spindle orientation relative to the crypt axis. These SC studies were carried out in comparison with the transit-amplifying population of differentiated progenitor cells and have the benefit of employing definitive SC markers within the intestinal crypt compartment (compared with earlier studies) and also use an approach that negates the effects of differences in rates of cell division. It was proposed that after a stochastic cell division event, the niche in which either new daughter cell finds itself directs the fate of that cell, and it is not the initial division event that drives the asymmetry of segregation [a similar situation is observed in the *Drosophila melanogaster* testis (9)]. At the very simplest level, the observations made by Quyn and co-workers may be interpreted as being consistent with Cairns' ISH being a cancer avoidance mechanism, where disruption in asymmetric chromosome segregation and cell division leads to an increase in cancer (10). (In contrast to these studies, other groups have more recently reported evidence of random chromosome segregation in intestinal SCs as outlined in the section below.)

Non-random DNA segregation has been identified in the germ SCs of female adult *D. melanogaster* ovaries. In these studies, DNA was labelled during the larval growth stages and the early-labelled DNA strands were subsequently traced in label retention studies. This revealed non-random segregation of DNA in the germ SCs undergoing asymmetric divisions, compared with the differentiated daughters, which, in contrast, appear to segregate their DNA randomly. The non-random DNA segregation appeared to be dependent on age of the DNA strands (11). Interestingly, these results might suggest a mechanism whereby the cell differentiation process is linked to the asymmetric segregation of chromosomes. While this study has the benefit of unequivocally identifying an SC population and supports the ISH, it cannot be certain that all the chromosomes segregated non-randomly (and as such, may be consistent with other alternative hypotheses such as template DNA strand segregation, discussed in a later section).

Studies by Conboy and co-workers (12), again using indirect sequential labelling techniques, demonstrated that muscle SCs segregate DNA non-randomly. This was observed at the initial asymmetric SC division event; however, segregation also continued to occur non-randomly in the differentiated daughter cells in a manner whereby the least differentiated daughter cell was shown to inherit the oldest DNA. In accordance with this, Shinen and co-workers also demonstrated non-random segregation of chromosomes in adult muscle SCs (13).

Further examples of asymmetric template strand DNA segregation were observed during *in vitro* studies in adult neural SCs (14) and in mouse mammary epithelial SCs, which were shown to retain the parental strand during asymmetric divisions of SCs (15). This latter study, which was carried out *in vitro* and *in vivo*, showed that the differentiated daughter cells do not maintain a parental template DNA strand.

Studies of cultured murine embryonic fibroblasts by Merok and co-workers (16) demonstrated that cells only undergo non-random chromatid segregation when induced to divide asymmetrically, as is the case for adult SCs, and that in a population of symmetrically dividing SCs the template DNA was randomly segregated between daughters. Furthermore, they were able to demonstrate that p53 was involved in controlling the switch to asymmetric cell division using a p53 null cell line stably transfected with wild-type p53 under the control of a metal-responsive promoter.

The first evidence for non-random segregation of template strand DNA in cancer cells was recently described for a sub-population of human lung cancer cells (17). The tumour environment was shown to be important in regulating the occurrence of non-random segregation of the template DNA strand such that co-segregation occurred within a sub-population of cells that are positive for a marker carried by cells that display the so-called cancer SC characteristics. Interestingly, LRCs were also demonstrated in pre-malignant

breast epithelial cells and these were also shown to non-randomly segregate their template DNA following asymmetric cell division events (18).

In addition to points raised here, the reader is referred to two additional, excellent texts; first, an essay by T. Rando [(19); see also (20)] presenting evidence and arguments in support of the ISH and second, a 'Correspondence' describing potential mechanisms that may exist to drive asymmetric template strand segregation, an area outside the scope of this text (21).

Evidence and arguments against the ISH

To date, many of the arguments presented against the ISH are based on limitations of experimental approaches used to address this hypothesis and while agreeing with the existence of non-random chromatid segregation in some populations of cells, such objections are offered with alternative explanations for experimental observations. Lansdorp (22) describes such objections to evidence presented in favour of the ISH, including concerns about

- the lack of suitable markers for the unequivocal identification of SCs and subsequent ill definition of the fraction of cells undergoing asymmetric segregation
- the inability to firmly identify whether all the chromosomes or just a sub-population of chromosomes segregate asymmetrically (in a cell that has been identified as undergoing asymmetric template strand segregation)
- why the protection of one strand is beneficial when both strands are subject to mutation errors and when both strands carry genetic information that is transcribed.

While these points raise concerns, doubts are compounded by the existence of experimental evidence that is not able to demonstrate asymmetric segregation of template strand DNA in certain cell types. For example, LRCs could not be demonstrated during *Caenorhabditis elegans* development following BrdU labelling of sperm or oocyte DNA (23). Furthermore, when the fate of labelled paternal DNA strands was followed during early cell divisions of mouse embryogenesis, there was no evidence to suggest that the paternal DNA strands segregated together and segregation appeared to follow a random pattern (24).

More recently, studies carried out with multipotent epidermal basal SCs demonstrated that these SCs predominantly undergo random segregation of their chromosomes (25). Furthermore, the DNA of hair follicle SCs was shown to adopt a predominantly random distribution during cell divisions (26). Similarly, studies in mouse hematopoietic SCs (HSCs) using labelling techniques to track LRCs show no evidence for non-random chromosome segregation and resulted in the suggestion that HSCs do not use asymmetric segregation of chromosomes as a mutation avoidance mechanism. This study also concluded that the BrdU label was not specifically confined to the SC pool (27).

In contrast to studies carried out in adult neural SCs, mouse embryonic neocortical precursor cells were shown to randomly segregate template strand DNA during cell divisions, demonstrating differences between adult and embryonic neural SCs (28).

Great progress in addressing issues of template strand segregation was made when Lansdorp's group demonstrated the first direct evidence of non-random chromatid segregation, by specific marking of the Watson and Crick strands of the sister chromatids, in a sub-set of colonic epithelia. However, such asymmetry of segregation was observed both in the SC region and in cells extending outside this region; the mechanism for this non-random segregation remains unclarified (3).

Recent studies in male germline SCs in *D. melonogaster* have shown that chromosome strands segregate randomly in these cells. Male germline SCs in this species have the advantage of being unequivocally identified and they always undergo asymmetric cell divisions, thereby avoiding major ambiguities that generally interfere with the interpretation of many such studies (29).

Adult pluripotent SCs of the flatworm *Macrostomum lignano* were also shown to undergo random chromosome segregation, and LRCs in this study were actually shown to be a consequence of quiescent cells (30). Imposing a state of quiescence on a cell has itself been proposed as a mechanism to protect against replicative errors; however, as discussed later, this is not always the case.

Finally, in contrast to the report by Quyn and co-workers (8) (who demonstrated a high incidence of non-random template strand segregation in asymmetrically dividing colonic SCs), a recent report by Schepers and co-workers (31) demonstrated that mouse intestinal SCs (marked as Lgr5+) randomly segregate chromosomes at all locations along the crypt. Moreover, it has previously been shown that the Lgr5+ SCs do not divide asymmetrically but instead divide symmetrically, with each cell division being dependent on extrinsic niche signals to determine the outcome of the SC division (32). Furthermore, it was also shown that the cycle time of the intestinal SCs was just 21.5 h (more rapid than might have been predicted by the ISH), thereby potentially further challenging the idea that genome integrity in the SC population might be maintained by the protection of an immortal strand. In agreement with this, other recent studies in mouse intestinal epithelial cells have also described random segregation patterns of chromosomes in the SC compartments of both intact and regenerating crypts. These studies also benefitted from the unequivocal identification of the SC compartment (being the Lgr5+ crypt base columnar cells and Bmi1+ labelled cells of the +4 position) (33, 34). It is possible that the asymmetric cell divisions that show a bias for non-random template strand DNA segregation in the study by Quyn and co-workers might represent a different type of SC division event that occurs following post-irradiation regeneration of the crypt, and which is still yet to be defined.

Alternative models of non-random chromatid segregation

Other explanations have been offered for the observations and results obtained from experiments that have set out to address

the ISH, and also to take into account evidence associated with this hypothesis.

An alternative suggestion was presented to explain non-random chromosome segregation, the 'silent sister hypothesis' (SSH), whereby the cell retains an epigenetic mark for the purpose of cell fate determination rather than being driven by an avoidance mechanism to prevent the accumulation of replicative errors over time (22). This hypothesis suggests that epigenetic marking underpins the selection of chromatids that are actively expressing SC genes; the other 'non-SC expressing' chromatid is then inherited by the differentiating daughter during asymmetric cell divisions, the so-called silent chromatid.

Template DNA strand segregation (TDSS) is an alternative term, used to refer to individual chromatid strands rather than 'immortal strands' [the reader is referred to ref. (35) for an excellent review], and is based on the same assumption as the ISH, that all segregating chromatids would be equivalently distributed following cell division.

A similar, and perhaps not unrelated observation, is the selective segregation of a single chromosome in a cell type-dependent manner. The term somatic strand-specific imprinting and selective chromatid segregation (SSIS) was coined to define this particular segregation pattern, being distinct from TDSS. SSIS describes the non-random segregation of a single chromosome in a manner where both 'old' Watson strands of a replicated homologous pair of chromatids, are retained in one cell and both 'old' Crick strands are inherited by the other daughter cell (36–38). It is possible that SSIS and TDSS work through similar mechanisms; however, the mechanisms driving either type of non-random strand segregation are currently unclear.

Why have advances in addressing ISH been so slow? LRC assays, labelling techniques and shortfalls in data interpretation

One of the main reasons that the ISH is still open to debate is the limitation of approaches available to address this issue. The ISH supports the notion of non-random segregation of chromosomes following asymmetric cell division events, and as already mentioned makes an assumption that SCs might cycle more slowly than proliferating amplifying cells. These properties have been the basis of experimental approaches used to study the segregation patterns of chromosomes. Classically, two indirect approaches have been employed for labelling and tracking DNA using halogenated nucleotide analogues (such as BrdU) in pulse chase experiments. (i) The label retention assay: interpretation of results from this assay must take into account the cycle time of the SCs vs. proliferative daughter cells and whether segregation of chromatids is random or non-random. In this approach, a pulse of label is given to the population of adult, symmetrically dividing SCs, before the (proposed) specification of an immortal strand resulting in labelling of the 'immortal/parental' strands of the SC. Following onset of asymmetric cell division, the label is 'chased', resulting in label retention in the SC (LRC) if non-random segregation of chromosomes has occurred (this will be independent of the cycling time of the SCs) and the more differentiated daughter cell will acquire the newly synthesised, unlabelled DNA. The problem with interpretation of these observations is that studies use the term 'LRCs' to identify an assumed population of slow-cycling SCs. In this concept, label would be taken up by all dividing cells but the slow-cycling SCs would retain label compared with their rapidly dividing daughter cells where the label would soon be diluted out. This latter concept is based on the assumption of random segregation of chromatids to the daughter cells.

Problems arise in interpretation of results as not all SCs cycle at similar rates, even within a single tissue. For example, mouse adult HSCs are able to reversibly switch between states of dormancy (where they retain the potential for self-renewal) to a state of active self-renewal during homeostasis or in response to stress (39–41). Defining SCs and their heterogeneity can also be problematic as even within a defined population of SCs they may display heterogeneity at different points in their cell cycle (as seen in mouse marrow SCs) (42). Current evidence for intestinal SCs, which serves as one of the most actively cycling and regenerating tissues of the body, indicates the existence of two populations of SCs, one of which is a long-term quiescent population (and may be activated in response to injury) and the other is actively cycling and may be involved in regeneration of the tissue (43–45). In addition, cell cycle times of intestinal SCs have also proven to be heterogeneous (between 1 and 4 days) (46). Likewise, the mammalian epidermis is known to house different populations of SCs occupying various locations and also having variable cycling times, being either active or quiescent (47). Interpretation of results from label-retaining assays has been further complicated by recent evidence from mouse epithelial progenitors, which has suggested that the decision to self-renew or to commit to differentiate is determined by random choice with stochastic cell cycle times. This 'randomness' has implications for the interpretation of results from label-retaining assays (48). Recent evidence for the gut also suggests that homeostasis in this tissue is maintained by the random choice of SCs to undergo self-renewal or differentiation (32). (ii) The second approach for studying non-random strand segregation is the label release/exclusion assay. In this approach, the newly synthesised DNA is labelled in a pulse given to asymmetrically dividing cells followed by a chase and label loss that is analysed after two cell divisions.

In both of these labelling approaches, the potentially toxic effects of BrdU must be considered when used over extended growth periods (49). In particular, BrdU incorporation into DNA may cause alterations in protein-DNA binding interactions, increase the radiosensitivity of the DNA (50), induce sister chromatid exchanges (51) and alter the cell cycle time of SCs; this latter point might imply that label-retaining assays may be fundamentally flawed, as label may be retained because some cells are slow cycling and not because of asymmetric strand segregation (52).

Recent advances in the study of chromosome segregation have been achieved by Falconer and co-workers (3) who were able to distinguish and resolve single sister chromatids

through a direct labelling technique known as chromosome orientation fluorescence *in situ* hybridisation. Using this technique, this group were able to follow the segregation patterns of parental 'Watson' and 'Crick' strands in different cell types. Random segregation patterns were observed in pairs of cultured cells of lung fibroblasts and ES cells in contrast to that observed in a sub-set of mitotically active cells (outside the SC zone) in the colonic crypt, which were observed to undergo non-random segregation of chromatids in a large proportion of cells (but not all cells). It is hoped that such direct labelling techniques may allow significant advances in the information gained from these studies (3, 22); however, this approach still relies on a BrdU labelling step, which, as mentioned previously, may affect the cellular responses.

Opinion

As discussed, there are evidence-based arguments for and against the existence of an immortal strand genome protection mechanism in different cell types. Most of the evidence for or against the ISH is derived from experiments aimed at confirming or refuting the occurrence of non-random template strand segregation, and as discussed, until recently these have all used indirect labelling approaches with all the associated shortcomings. One of the main concerns addressed by the ISH was the avoidance of replication-induced errors, considered to be one of the most prevalent causes of spontaneous mutations to occur in cells. However, as already mentioned, damage can occur in SCs in other ways, including oxidative damage (due to metabolic bi-products; these may be reduced in level in slow-cycling cells), chromosomal breakage, chemical modifications such as alkylation, nicks and cross-links (inter/intra strand), all of which could result in non-replication-induced errors that would be maintained in the immortal/template strand if not repaired with high fidelity. The diverse systems of study, types and ages of SCs and types of experimental damage administered make it difficult to generalise about repair mechanisms, especially when the total extent of data is fairly limited; however, taking into account these many considerations, it might be inferred that if an immortal strand is preserved, then it is only a mechanism that guards against the predominant form of errors, i.e., DNA replication-induced errors, and is not a mechanism for the maintenance of an absolutely unaltered (immortal) thread of genetic information. In accord with SCs responding to other types of DNA damage, incurred at pre-replication stages, there is evidence that HSCs utilise the error-prone, non-homologous end joining (NHEJ) repair pathway to repair damage incurred at early stages of the cell cycle, and that such cells avoid apoptosis to preserve the longer-term regenerative capacity of SCs (53–55). Bulge SCs in the epidermis have also been shown to be proficient for NHEJ repair (56), and the rapidity at which this repair is carried out appears to be an important factor in determining whether apoptosis will be induced following the damage (57). Furthermore, NHEJ pathways have also been shown to be involved in the maintenance of the crypt SC population in mice (58). Thus, pre-replication SCs do undergo repair of non-replication-induced damage, predominantly through NHEJ. It may be noteworthy that the types of SCs shown to undergo NHEJ repair also undergo random segregation of template strand DNA following asymmetric divisions of SCs. While this may be co-incidental (as only relatively few studies have been carried out; HSCs, bulge SCs, crypt SCs), it is worth speculating that SC types undergoing error-prone NHEJ to repair pre-replication damage might not undergo asymmetric template strand segregation as the 'immortal' nature of the strand may now effectively have been changed. It will be interesting to determine whether some tissue-specific SC types do not undergo NHEJ, and whether this property is correlated with non-random template strand segregation.

After replication, repair of the highly toxic strand breaks in DNA would be most likely to occur through homologous recombination (this has been shown to occur in mouse and human ES cells) (59–61), in which process, partner choice for repairing damaged DNA would be critical for preserving a stable genome. Of relevance to this, and an area of the ISH that is still very much unresolved, is whether ISCE would be permitted in the DNA of adult SCs and their progeny. According to the ISH, ISCE events would be inhibited in the immortal strand to be inherited by the SC daughter following an asymmetric division event to preserve the integrity of this strand. To date, very few experiments have been carried out to demonstrate the levels of ISCE events in SCs, and all approaches have involved indirect labelling methods with halogenated nucleotides, which, as already mentioned, themselves induce ISCE among other changes (62, 63). The development of a genetic assay system to determine the frequency of ISCE events in SCs would be of great benefit in answering this unresolved question of the ISH.

It is likely that the accumulation of damaged DNA in SCs over time is handled in different ways and may be a balance between incurring damage through repair and maintaining regenerative capacity (20, 57). Furthermore, while a tissue-specific SC may avoid replicative errors through employing cell states such as quiescence, this in itself may result in the implementation of error-prone repair processes such as NHEJ being used by the SCs, as seen in HSCs. The occurrence of error-prone, NHEJ repair processes and avoidance of apoptosis is not necessarily in direct agreement with preservation of an immortal strand; however, as mentioned previously, little is known about mutation avoidance mechanisms in SCs and it is possible that these vary in adult SCs vs. ESCs and with age and in response to different types of damage.

Outlook

As new markers are identified for tissue-specific SCs, it is expected that the fate of labelled DNA strands might be better linked with a particular type of cell, be it either SC or differentiated. The development of tissue-specific adult SC markers for the unequivocal evaluation of SC populations is a necessary development that would improve understanding in this field and recent advances made in this area have been reviewed in ref. (45). However, advances in identification of

SCs in the intestinal crypts (marked by Lgr5) have not managed to address this question definitively, and conflicting reports of random vs. non-random chromosome segregation have still come out in this area.

Certainly, indirect labelling techniques have hindered progress in this field in terms of following the fate of a labelled strand after an asymmetric division of an SC; however, it is envisaged that new direct labelling techniques will allow progress to be made on this front. Furthermore, the development of totally new approaches such as genetic marker systems to follow ISCE in SCs would be of great benefit to confirming or refuting this part of the ISH, as it would permit one of the central requirements of the ISH to be directly tested.

Highlights and key conclusions

- During asymmetric divisions of SCs, and following semi-conservative replication of DNA, the older 'immortal', grandparental DNA strand is passed to the SC whereas the newly formed daughter cell inherits the new DNA strand. This is the basis of the ISH, proposed as a mechanism to protect against errors incurred during replication, the major source of toxic damage to the cell.
- Asymmetric segregation of chromosomes would have no benefit in a functionally equivalent and symmetrically dividing population of SCs, nor would it be of benefit in a population of asymmetrically dividing SCs if this had been preceded by error-prone repair of DNA, which would potentially destroy 'immortal' regions of DNA/chromosomes. It would be interesting to determine if the ability of SCs to undergo specific types of repair is linked to the ability to undergo asymmetric strand segregation.
- At this stage in this field, there is little known about mutation avoidance mechanisms in adult SCs vs. ESCs and how these might vary with age and in response to different types of damage, and indeed how this might correlate with replication damage avoidance mechanisms as proposed in the ISH.

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