Review Article

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Regulatory Mechanisms of Asymmetric/Symmetric Cell Division and Quiescence in the Primitive Stem/Progenitor Cell Lineage of Entamoeba

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ABSTRACT

Entamoebae are anaerobe pathogenic eukaryotes capable of asymmetric cell division, self renewal, mitotic cell cycle arrest and differentiation. Key regulator of amoebic growth and differentiation is the ambient oxygen. Host's intestine and oxygen consuming cultures (OCB cultures) are low oxygen micro-environments favouring Entamoeba's asymmetric cell fate. In contrast, axenic cultures and extraintestinal oxygen pressure favour symmetric cell fate, identical progeny and logarithmic growth. In OCB cultures one of both daughter cells is the self-renewing cell and the other enters a state of G1 arrest (G0 quiescence) or differentiates terminally forming a tetranucleated cyst. Quiescent G0 cells have dual potential for proliferation and differentiation similar to the cycling cells prior RP commitment. Entry, maintenance and exit of quiescence are controlled by a network of intrinsic mechanisms including oxygen sensing and oxygen signalling pathways. Strong hypoxic conditions near anoxia abolish asymmetric cell division and asymmetric cell fate; the progeny consisting of identical daughter cells (ISH cells) arrest at the G2 check point. This post replicative state of quiescence is temporarily. Hypoxia decrease leads ISH cells back into asymmetric proliferation. Increased oxygen pressure as occurring in upper intestinal gradient zones (≤ 5.5% O_2) favours terminal differentiation. Oxygen contents above 5.5% as occurring in axenic cultures replace asymmetric by symmetric cell division.

Keywords

Entamoeba invadens, E. histolytica, Oxygen gradients, Oxygen sensing, Hypoxia.

Abbreviations

OCB: Oxygen Consuming Bacteria, p-SRL, s-SRL, t-SRL: Primary,Secondary and Tertiary Cell Lines, ISH: Identical Strong Hypoxic Cell, ILH: Identical Low Hypoxic Cell, MAS: Mitotic Arrested Secondary Cell, MAT:Mitotic Arrested Tertiary Cell, MA/td: Mitotic Arrested Trans-differentiated Cell, ATD: Autonomous Terminal Differentiation, ITD: Induced Terminal Differentiation, MD: Maternal Cell Fate Determinant, CdK: Cyclin-dependent Kinase, CKI: Cyclin Dependent Kinase Inhibitor, HIF: Hypoxia Inducible Factor, P4H: Prolyl 4 Hydroxylase, PHI: Prolyl Hydroxylase Inhibitor, PHD: Prolyl Hydroxylase Domain, Skp1: S-phase Kinase Associated Protein.

Introduction

Over the past 50 years, *Entamoeba* researchers have used more and more axenic methods to culture pathogenic amoebae such as *E. histolytica* and *E. invadens*. It was assumed that germ free cultures improve amoebic growth and cell cycle progression and provide accurate material for genetic and molecular biological studies. In our opinion however, axenic media are incomplete culture media depriving amoebae of many physiochemical stimuli provided by intestinal microbiota.

For this reason, we introduced in the early 1970s culture media enriched by bacterial metabolites and grew *E. invadens* in culture sediments with non proliferating oxygen consuming bacteria (OCB) [1]. Metabolically repressed OCB sediments mimic the intra-intestinal micro-environment and help to decipher the primitive stem/ progenitor cell lineage of *Entamoeba* [1-3].

Both culture philosophies lead to contradictory effects. While OCB

cultures are more hypoxic, axenic cultures are more oxygenic. After culture inoculation OCB sediments rapidly consume the oxygen supply in the vicinity of amoebae generating transversal and longitudinal oxygen gradients, similar to the oxygen gradients of the intestine stretching from ≤ 0.1% to 5.5% O_2. In contrast, the isotonic medium used in axenic *E. histolytica* cultures has 6.42% O_2* and the oxygen pressure remains relatively constant during the logarithmic growth phase.

* https://www.ysi.com/File%20Library/Documents/Technical%20Notes/DO-Oxygen-Solubility-Table.pdf

Cultures sediments containing 5mg OCB (*Aerobacter aerogenes*) soon reach low/mid hypoxic ranges while triple dose sediments of 15mg bacteria reach strong hypoxia [3,4]. On the other side, culture homogenization and transfer into subcultures re-oxygenates the amoebae. On can say OCB cultures mimic *in vitro* intestinal life conditions while axenic cultures offer rather extra-intestinal O_2 contents (Figure 1). The back and forth between more and less hypoxia trigger signals and stimuli that control self renewal, quiescence and differentiation of *E. invadens* [2].

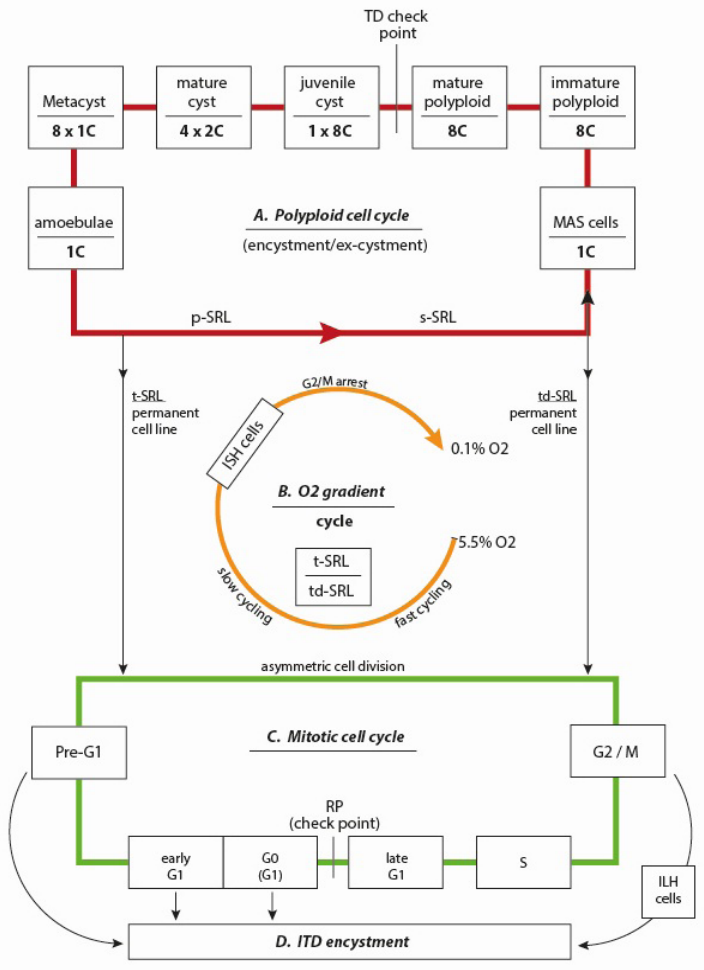

Figure 1: The intestinal life cycle of *Entamoeba invadens*, its intrasystemic cell lines and the dual potential of amoebic cells for self-renewing and differentiation.

Concerning the ambient O_2 supply there is a certain similarity between OCB culture sediments and the stem cell niche of mammalian stem cells. The bone marrow (BM) niche has deep perisinusoidal zones of low oxygen tensions (<1%) and arteriolar zones up to 7% near the blood vessels [5-7]. The different oxygen zones influence directly proliferation and hematopoietic stem cell behavior. Consequently, HSC cells residing in the BM niche can be quiescent, proliferative or differentiating, according to the signals of the niche and the intrinsic mechanisms of stem cells [8]. Under strong hypoxic conditions of about 1.5% O_2 HSCs enter a dormant cell state [9] while oxygen concentrations below 5% O_2 promote rapid proliferation, leading HSCs toward specific differentiation [10,11]. Many of the cell responses to hypoxia are mediated through changes in gene expression [9]. Intracellular hypoxia may be visualized by pimonidazole, a hypoxia sensitive marker.

Cord blood cells such as CD34+ cells divide at least once in nearly anoxic conditions (0.1% O_2) and half of the progeny enter G0 quiescence [12]. Stemness, G0 preservation, self renewal and commitment are regulated by a variety of signals: 0.1% oxygen allows survival of CD34+ cells but does not favors their amplification.

Oxygen is the key regulator both in human stem cells and *Entamoeba's* cell lineage [3,4]. Oxygen gradients stretching from 5.5% to <0.1% offer amoebic cells a multitude of stimuli and regulators for proliferation and differentiation [2,3]. Similar to mammalian stem and progenitor cells in the stem cell niche, t-SRL/ td-SRL cells of *E. invadens* switch, in strong hypoxic conditions, to an amplified identical ISH cell population that arrests in a state of G2 quiescence. When hypoxia is decreased by OCB depletion, ISH cells finished the cell cycle and divided by asymmetric cell division to non identical daughter cells that promote G0 quiescence and differentiation [13].

Totipotent amoebulae of *E. invadens* cellularized in low oxygen environments find conditions to express stemness. They give rise to a primary stem cell line (p-SRL) capable of proliferation and asymmetric division. While the stem cell line p-SRL resides in low/mid hypoxic sediments, the progenitor cell line s-SRL producing ATD cysts (Figure 1) arises and proliferates in more oxygenic sediments of the upper intestinal gradient zones. In contrast to the short living p-SRL and s-SRL, the permanent cell lines t-SRL/td-SRL are ubiquitous cell lines colonizing all hypoxic and oxygenic zones of the intestinal oxygen gradient [3]. The "immortal" cell lines t-SRL/td-SRL proliferate both in the upper oxygenic zones of the gradient by fast cycling (cell cycle duration: ≤ 6 hrs) and in the mid hypoxic zones by slow cycling (≥ 24 hrs). Cell line conversion is controlled by environmental signals: while oxygenic cues convert the p-SRL to the s-SRL cell line, low/mid hypoxic stimuli convert the p-SRL into the t-SRL and trans-differentiate the s-SRL into the td-SRL [3].

Asymmetric vs. symmetric cell division

Asymmetric cell division, cell cycle arrest, quiescence and terminal differentiation are hallmarks of the intestinal amoebic life.

In the past, asymmetric cell division was sporadically observed in cultures with proliferating bacteria [14], but it remained obscure and less understood. It was not observed in axenic cultures were amoebae divide symmetrically. In contrast to axenic and mixed polyxenic cultures, non-proliferating OCB sediments induce amoebae to asymmetric cell division, quiescence and autonomous terminal differentiation. In OCB cultures *E. invadens* proliferate rarely by complete self renewal and symmetric cell division. This occurs only in conditions of extreme hypoxia prior G2 arrest.

Cultures	Cell division (D2 cell fate)	Cell cycle	Cell types/ cell lines	O_2
OCB cultures; (intestinal amoebic life)	symmetric	G2/M arrest	ISH cells (t-SRL/td-SRL)	≤ 0.1% strong hypoxic
	asymmetric (D2 cell quiescence)	slow cycling (~24 hrs)	t-SRL /td-SRL	mid hypoxia
		fast cycling (~6 hrs)	t-SRL /td-SRL	low hypoxia
	asymmetric (D2 cells form ATD cysts)	fast cycling (~6 hrs)	s-SRL	upper oxygenic zones (≤ 5.5% O_2)
Axenic cultures (extraintestinal amoebic life)	symmetric	fast cycling (~6 hrs)	s-SRL /t-SRL (analogous to syngenic A, B cell lines)*	≤ 6.42%

Table 1: Symmetric and asymmetric cell fate of Entamoeba invadens and E. histolytica as occurred in OCB and axenic cultures. s-SRL, t-SRL, td-SRL, secondary, tertiary and transdifferentiated cell lines of E. invadens; A and B: syngenic cell lines* of *E. histolytica* [3]. D2 cells/ quiescent cells are cells produced by the t-SRL that exit temporarily mitotic cell cycle; D2 cells that form ATD cysts are cells produced by the s-SRL that exit definitively mitotic cell cycle as committed ATD-precursor cells.

Asymmetric cell division as observed in OCB cultures

Asymmetric cell division leads to non identical progeny: one of two daughter cells (D1 cell) is the self renewing undifferentiated stem or progenitor cell. It continues the mitotic cell cycle and divides again by asymmetric cell division. The other daughter cell (D2 cell) enters a state of G0 quiescence or progresses, through endoreplication to terminal differentiation. From the four types of mitotic arrested cells observed in OCB cultures (MAP, MAS, MAT and MA/td cells) only MAS cells form ATD cysts by autonomous terminal differentiation. Quiescent G0 cells (MAT and MA/td cells) maintain dual cell fate potential and are capable for self renewal and/ or further differentiation (Table 1).

When passaged in subcultures, MAT and MA/td cells exit quiescence and continue proliferation. Before passaging, culture sediments were dispersed in the liquid medium and bubbled by aeration. For a short period of time amoebae were exposed to extraintestinal oxygen contents (>5.5% O_2). The increased oxygen supply, forces G0 cells to exit quiescence and re-enter mitotic cell cycle. It takes a certain period of time until the young OCB sediments achieve intestinal oxygen values again (<5.5% O_2). 3-4 hrs after culture start low oxygen.

In contrast, MAS cells are precursor cells committed for terminal differentiation. In nutrient rich cultures with the standard OCB dose (≤ 5mg) they enter the developmental cell cycle of endopolyploidisation and depolyploidisation (encystment-excystment cycle). Committed MAS cells make 8 genome copies and go through a phase of maturation before transiting the terminal differentiation checkpoint TD [3,4]. To form ATD cysts MAS cells need the upper intestinal oxygen gradient zones of ≤ 5.5% O_2. Increasing hypoxia slows down endopolyploidisation but also polyploid cell maturation and TD check point passage.

MAT and MA/td cells have terminal differentiation potential. In vitro they form ITD cysts when transferred in nutrient free hypoosmotic media and sediments with the triple OCB dose (15 mg) [3]. Starvation reduce the oxygen consuming capacity of OCB sediments.

Amplifying and terminal symmetric cell divisions

Symmetric cell divisions are rare in OCB cultures, they occur only in unfavorable growth conditions. One is the amplifying strong hypoxic cell division observed in strong hypoxic OCB cultures and the other is the terminal symmetric cell division observed in encystment media. In strong hypoxic cultures containing the triple OCB dose of 15 mg A. aerogenes symmetric cell division amplifies the t-SRL/td-SRL cell lines giving rise to identical daughter cells that arrest in a state of G2 (ISH/G2 cells). When hypoxia decreases, the amplified cell line respectively ISH/G2 cells continue cell cycle progression and divide by asymmetric cell division to non identical daughter cells. When transferred in nutrient-free hypo-osmotic media the arrested ISH/G2 cells go through a terminal symmetric cell division producing low hypoxic daughter cells (identical ILH/preG1cells) as precursor cells of ITD encystment.

Cycling G2/M cells can be also manipulated into terminal symmetric cell division. When transferred in nutrient-free hypoosmotic media enriched with the triple OCB dose, G2/M cells finish the terminal symmetric cell division giving rise to two identical ILH/preG1 cells that form ITD cysts. In contrast, self renewing G1 cells prior RP (early G1 cells) encyst directly.

Axenic cultures miss asymmetric cell division

Isotonic media such as the TYI-S-33 medium introduced by Diamond et al in 1978 [15], and largely used in recent years have extra-intestinal oxygen contents of about 6.42% O_2* when incubated at 37°C for *E. histolytica* and 7.71%* when kept at 26°C for *E. invadens*. Both pO_2 values are above the intestinal oxygen limit of 5.5% O_2. In contrast to OCB cultures which consume rapidly oxygen excesses (culturing amoebae in intra-intestinal oxygen concentrations) axenic cultures maintain relatively constant extra-intestinal oxygen content. This is particularly true for early axenic cultures of the logarithmic growth phase.

*https://www.ysi.com/File%20Library/Documents/Technical%20Notes/DO-Oxygen-Solubility-Table.pdf

In axenic cultures *E. histolytica* give rise to subpopulations of apparently identical cells. However, axenic populations are not homogenous. They consist of a minor subpopulation of about 10-15% capable of endopolyploidisation [16], that represents probably the complete self renewing s-SRL cell line and a dominant subpopulation representing rather the t-SRL/td-SRL lines. Both subpopulations proliferate by fast cycling (5.5-6 hrs) similarly with *E. invadens* grown in the upper O_2 zones of OCB cultures. Mitoticly arrested cells (GO cells) and precursor cells for ATD encystment are lacking in axenic cultures and the s-SRL can not produce cysts. Missing ATD cyst formation, axenic cultures lack p-SRL and the td-SRL cell line also.

Amoebae grown in axenic culture maintain the potential of terminal differentiation, but only E. invadens cells can survive and differentiate in hypo-osmotic non nutrient media to ITD cysts. According to Eichinger [17], up to 95% of axenic grown E. invadens may be induced to encyst. However, we know that only cells prior the RP checkpoint (preG1 and early G1 cells) may be induced to form cysts directly, and it is very unlikely that 95% of the asynchronous axenic grown cells are all pre-RP cells. We think that a multitude of cysts are derived from the progeny of G2/M cells that divide in encystment media after cell transfer.

Axenic grown *E. histolytica* do not survive when transferred in diluted culture media. On the other hand, previous studies confirm *E. histolytica's* encystment potential in xenic cultures with proliferating bacterial commensals [18]. Encystment may be promoted by intestinal microflora originally isolated with amoebic samples [17], or by so-called NRS substitution flora [18,19]. In monoxenic cultures started by the Dobel - Laidlow protocol E. histolytica needs about 72 hrs to produces cysts. In OCB cultures of *E. invadens* cyclic encystment occurs in cultures younger as 30 hrs [1,2]. There are five to six generations of MAS precursor cells that encyst autonomously during this time period. When culture sediments become too hypoxic, encystment stops.

Regulatory mechanisms of daughter cell fate

Surveillance

Cell cycle progression is controlled by interdependent regulatory transition networks that bring proliferating cells to a state competent for duplication and division. This "surveillance mechanism" ensures that cells will not progress to the next phase of the cell cycle before events of the preceding phase have been completed. If this is not the case regulatory key check points decide that "immature" cells remain arrested. Cells arrested at the G1 checkpoint enter the state of G0 quiescence. Quiescence is a state of growth cessation, however, an actively maintained state capable to preserve stemness. It allows rapid activation of self renewal and/or differentiation [20]. Molecular regulators of cell cycle progression, intrinsic molecular switches and multiple transcription factors control quiescence entry, maintenance and exit [21]. Low/mid hypoxic environments containing less as 5.5% O_2 favors quiescence [13].

Quiescent cells have a characteristic molecular signature and transcript profile. The gene signature is common for several quiescent cell types. It reveals downregulation of genes encoding cyclins A2, B1, E2 that are involved in DNA replication and cell cycle progression [22-24]. Conversely, genes that are upregulated in quiescent stem cells includes genes encoding signalling molecules for transcriptional regulation and cell fate decision [20].

Maternal determinants

The decision of whether or not daughter cells enter asymmetric cell fate and quiescence is deterministic, depending first of the mother cell which lays the basic molecular features of daughter cell fate and development.The deterministic theory states that the asymmetric daughter cell fate depends on the equal or unequal segregation of maternal cell fate determinants (MD). In *E. invadens* MD segregation hardly depends on the environmental oxygen supply. Accordingly, mother cells proliferating in extra-intestinal oxygen pressure (>5.5% O_2 content) have sufficient amounts of determinants and could segregate them equally in both daughter cells. The progeny that receive the same set of determinants are apparently identical and continue proliferation by logarithmic growth (complete self renewal).

In contrast, intestinal oxygen contents <5.5% O_2 - as occurring in OCB cultures - down regulate MD transcription; the amount of determinants produced by the mother cell is insufficient for equal MD segregation. One of the daughter cells (D1 cell) gets a sufficient amount of MD and continues cell cycle progression while its sister cell (D2 cell) gets an insufficient amount and stops as an "immature" cell at the G1check point. This cell enters temporarily a state of pre-replicative quiescence (G0/G1 quiescence) (Table 1). Strong hypoxia, nearly anoxia (oxygen content ≤ 0.1%) does not favor asymmetric cell division and G0 quiescence. The ubiquitous t-SRL and td-SRL cell lines of *E. invadens* divide in strong hypoxic OCB culture sediments by symmetric cell division forming ISH cells that arrest in post-replicative state of G2 quiescence.

In other words, transcriptional regulation in association with oxygen supply creates, in *E. invadens*, different stem and progenitor cell compartments: (i) amplified cell populations in a state of deeply G2 arrest that colonize strong hypoxic environments towards the region of lowest oxygen tension, (ii) asymmetric cell populations containing up to 90% quiescent cells (G0 cells) that conserve proliferation and differentiation potential and finally (iii) complete self renewing populations of apparently identical cells that divide symmetrically and proliferate logarithmically.

Cyclin-dependent kinases (CdK)

CdKs are a family of protein kinases regulating cell cycle, transcription and differentiation. They are present in all eukaryotes and their regulatory function has been evolutionarily conserved; there are not significant functional differences between yeast and human CdK genes. CdKs are relatively small proteins of relatively little activity. To become an active kinase they bind a regulatory protein called cyclin. Cyclin-CdK complexes regulate mitotic cell cycle progression and are also involved in postmitotic cellular processes such as transcription control and DNA repair [25-27].

CdKs were also identified in protists [28].

Current models for cell cycle exit and quiescence entry invoke mechanisms of mitotic cell cycle arrest respectively molecular repression of Cyclin/Cdk activity by cyclin dependent kinase inhibitors CKI [29-30]. According to Fujita et al. [30], the balance between the levels of CKI and cyclin E determines three distinct cell states: (i) terminally differentiated, (ii) quiescent and uncommitted and (iii) proliferating. The authors found complexes that block proliferation permitting differentiation of complexes that repress terminal differentiation maintaining stem cells in quiescent uncommitted cell state. Pathways linking quiescent exit to signal-dependent metabolic reprogramming were also described [31].

Oxygen sensors and functional mediators in mammalians and protists

Hypoxia inducible factor in human stem and progenitor cells

Notable progress has been made recently regarding signalling mechanisms and cell responses to microenvironmental oxygen variation in human hematopoietic stem cell (HSC) and progenitor cell (HPC) lineages. A family of oxygen-labile / hypoxia inducible transcription factors (HIFs) mediates many of the cellular processes of mammalian hematopoietic stem/progenitor cells. HIF-1 activity is controlled by oxygen dependant synthesis, accumulation and degradation of its α- subunit [32]. Oxygen concentrations above 2-3% rapidly degrade the oxygen-labile HIF-1α [33], while deep hypoxic niches (<0.1-2.0% O_2) leads to the stabilization and accumulation of HIFs molecules that induce hematopoietic cell quiescence [20,34]. When O_2 becomes limiting HIF-1α translocates to the nucleus, binds to specific intranuclear DNA (hypoxia-responsive elements) triggering the transactivation of the target genes and determination of the proteins expressed [35].

Prolyl hydroxylation and HIF-1α over-stabilization

Prolyl 4 hydroxylases (P4H) and prolyl hydroxylated hypoxia-inducible complexes (HIF/P4Hs) - termed prolyl hydroxylase domain (PHD) - play the key role in the oxygenic regulation of cell physiologic network [36,37]. A number of prolyl hydroxylase inhibitors (PHI) and activators have been developed, clarifying this network functions. PHDs are involved in the oxygenic degradation of HIF-1α by regulating its hydroxylation. HIF-1ά degradation by PHI inhibiting PHD proteins increased the proportion of HSCs and immature quiescent HPCs, decreasing proliferation [33]. Hematopoietic stem cell quiescence is also inhibited in HIF-1ά deficient mice.

The HIF-1α stabilization level is important for HSC quiescence [38-40]. Authors consider that HSCs quiescence and their hypoxic cell cycle status are regulated in an HIF-1α dose dependent manner [34]. Prolyl hydroxylated HIFά complexes are successively degraded under increased O_2 conditions. Hermitte et al. [12] found that cord blood cells at 3.0% and 0.1% O_2 pressure were 1.5 and 2.5 times more in G0 quiescence than at 20% O_2. About 46.5% of the progeny – in our opinion D2 daughter cells - enter at 0.1% O_2 the quiescent state, as compared with about 8% at 20% O_2 content. Taken together, prolyl 4 hydroxylases (P4H) are the key cellular O_2 sensor and HIF-1ά is functional mediator for HSC/HPC quiescence although many researchers are not definitively convinced if HIF-1ά is a consequence of hypoxic environments or whether HIF-1α expression is regulated completely intrinsically [21].

It seems that specific hypoxic stem cell state could be regulated by additional specific mechanisms and not directly by environmental oxygen. Despite these doubts, HIF-1α conveys in mammalian stem cells important regulatory mechanisms for cell cycle quiescence being essential regulators of quiescence.

Oxygen sensing pathways in yeasts and protists: the Skp1 substrate

Analogous subunits are also observed in *Saccharomyces cerevisiae*, although yeasts are mediated by a completely different mechanism [32]. While the HIF-1α system is characteristic for metazoan - beginning with *Tricoplax*, the simplest animals known today [40], - prolyl 4 hydrolase (P4Hs) is an universal oxygen sensor conserved in all eukaryotes including protists. However, the substrates of P4H enzymes are different. Oxygen sensing mechanisms were studied in social amoebae such as *Dyctiostelium discoideum* (Dd), and several pathogenic protist such as *Toxoplasma gondii* (Tg) and different *Trypanosoma* species. All these protist eukaryotes are permanently exposed to variation of the ambient oxygen content, switching between more and less hypoxia by forced under- and overexpression of PhyA (P4H1) activity [41-43].

The sensing mechanism of *Dyctiostelium* consists of the S-phase kinase associated protein 1 (Skp1) hydroxilated by PhyA. Skp1 is the substrate and PhyA the functional mediator. PhyA overexpresion reduces Skp1 activity and O_2 is limiting for the hydroxylation of newly synthetizied Skp1. Skp1 contributes to multiple functions of development that vary in their dependence on hydroxylation: over-expression of Skp1 inhibits sporulation of *Dictyostelium*.

It was found that Skp1 pathway genes of *Dictyostelium* (Dd-Skp1 genes) are also conserved in *Toxoplasma* [44]. Bacterially expressed Tg-PhA protein can prolyl hydroxilates both *Toxoplasma* and *Dictyostelium* Skp1s [45], and Tg-PhyA is 40% similar in sequence to Dd-PhyA. Interestingly, the loss of the Tg-PhyA gene does reduce the growth of *Toxoplasma* at atmospheric O_2. The same growth defect is intensified at 0.5% O_2. It seems that lacking an O_2 sensor *Toxoplasma* is unable to accommodates to low oxygen contents. In biochemical analysis Tg-PhyA activity revealed a high affinity for O_2 [45] which was in contrast to Dd-PhyA and the PHDs of Metazoa, that have apparent low affinities for oxygen. Thus, only low O_2 levels – like those encountered in the intestine – would be expected to impact Tg-PhyA activity. Authors suppose that related O_2 and metabolite sensing may play a role in the wide range of O_2 tensions encountered by the parasite [45].

Genetic analyses indicate that the prolyl-hydroxylation/ glycosylation pathway PhyA/Skp1/Gnt1 which was originally

discovered in *Dictyostelium* is present in several amoebozoa such as *Acanthamoeba*; However, it is lacking in *Trypanosoma* and Entamoeba. The O_2 sensor of *Trypanosoma* seems to be JBP1/2 [46] while the oxygen sensor of *Entamoeba* is yet unknown.

Conclusion

Oxygen is the key regulator of *Entamoeba's* life cycle development. Intestinal oxygenic pressure below 5.5% O_2 content as occurring in OCB cultures control asymmetric cell fate, G0 quiescence and autonomous terminal differentiation, while extra-intestinal oxygen pressure above 5.5% O_2 as occurring in axenic cultures leads to symmetric cell fate and logarithmic cell growth. OCB culture sediments are responsible for ambient oxygen depletion assuring extrinsic stimuli for intestinal amoebic development. Culture aeration and passaging re-oxygenates amoebic microenvironment forcing quiescence exit. Subsequent oxygen depletion by passaging in new OCB sediments re-starts asymmetric cell fate of *E. invadens*. In contrast to OCB cultures, axenic media do not support asymmetric cell fate; all cells are proliferating by complete self renewal. All identical daughter cells borne in axenic cultures are mature for RP commitment, G1/S transition, and symmetric cell fate. Complete self renewal occurs as the active repression of genetic programs that promote cell differentiation [47]. Asymmetric cell fate is deterministic. It depends of maternal determinants segregated asymmetrically in both daughter cells. In low oxygenic conditions (< 5.5% O_2) a complex of genes and pathways regulates asymmetric cell fate. While the undifferentiated self renewing state is controlled by cell cycle regulators that repress the expression of genes promoting quiescence and differentiation, other factors inactivate or repress genes promoting the dual differentiation/ proliferation potential of quiescent cells. Oxygen signals are transmitted as a series of molecular events, which result ultimately in the different phenotype of amoebic cells. Symmetric or asymmetric cell fate is finally decided by an opposite mechanism of prolyl hydroxylation on different substrates such as HIF-1α and Skp1. The universal eukaryotic oxygen sensor is prolyl 4 hydroxylase (PhyA). Pathogens such as *Entamoeba* that are permanently exposed to variation of the ambient oxygen content convert between a state of substrate over-stabilization and substrate degradation. Microenvironmental oxygen content and hypoxia determine the under- or overexpression of prolyl hydroxilases activity. Via prolyl hydrolysation of appropriate substrates and functional mediators such as Skp1, this regulatory mechanism of prolyl-hydroxylation and glycosylation (PhyA/Skp1/Gnt1) controls multiple functions in quiescence, development and differentiation dependent on the prolyl hydroxylation rate. In mammalian stem cells prolyl hydroxylated complexes could be degraded under increased O_2 conditions. When O_2 becomes limiting, PhyA complexes accumulate, translocating to the nucleus, where they activate target gene expression. Quiescence and asymmetric cell fate are regulated in dose- dependent manner.

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