**How to write a report? - Some guidelines**

1. **Introduction**

The main purpose of the introduction is to provide reason and background for the paper/report. In the introduction, you should guide the reader from a general/theoretical description of the topic to the very specific question or hypothesis you are aiming to investigate. The part of the general description should be rather concise, whereas the specific question should be addressed in more detail (keep in mind, not many people have a good knowledge of your topic).

The different steps for the introduction:

* Step 1: Establishing a research territory

By indicating that the general research area is of importance

And by introducing and reviewing previous publications in the area that are relevant to the study

* Step 2: Establishing a niche

By raising a question of a potential gap in the previous research, generating a hypothesis, or indicating the need to extend previous work

* Step 3: Occupying the niche

By outlining the purpose of the presented research

And by announcing the experimental procedure and general findings

Example:

The mammalian intestine is densely colonized by microorganisms, collectively referred to as microbiota (Ley et al., 2008). The microbiota feature a network of metabolic activities facilitating efficient breakdown of complex diet- and host-derived carbohydrates to short-chain fatty acids (SCFAs), hydrogen (H2), and carbon dioxide (Fischbach and Sonnenburg, 2011; Flint et al., 2008). Microbial fermentation products are subsequently consumed by crossfeeding secondary fermenters, absorbed by the host, or released into the environment. Gut ecosystem invasion is defined herein as the initial growth phase of a pathogen (or any other newcomer) in the host’s intestine. At this stage, the intestinal mucosa appears healthy, and the microbiota is (still) intact and limits nutrient availability. This prohibits growth of most newly arriving bacteria. Despite the scarce nutrient availability, enteropathogens can invade the gut ecosystem. Yet, the factors enabling ‘‘gut ecosystem invasion’’ by enteropathogens remain unclear.

The human food-borne pathogen *Salmonella* Typhimurium (*S.* Tm), a causative agent of diarrhea, can grow up in this nutrient-depleted environment to high numbers and cause disease. Animal experiments established that gut luminal pathogen densities must rise to 107–108 cfu per gram of stool before enteropathy is elicited (Ackermann et al., 2008; Barthel et al., 2003). As inoculum sizes as low as 103–105 bacteria suffice for causing diarrheal disease in humans (Food and Agriculture Organization of the United Nations, 2002), we speculated that *S.* Tm can grow initially in the face of an intact microbiota and a healthy gut. The mechanisms fostering *S.* Tm growth in this densely colonized niche are still enigmatic. Such mechanisms can be studied using ‘‘low complex microbiota’’ (LCM) mice, which are permissive for gut luminal *S.* Tm growth (Figure S1A available online; Stecher et al., 2010). LCM mice are ex-germ-free mice which had originally been colonized with strains of the ‘‘Altered Schaedler Flora’’ (Experimental Procedures, Figures S1A and S1E) and permit gut luminal colonization by inoculum sizes as small as 200 colonyforming units (Endt et al., 2010; Stecher et al., 2010). During the first 2 days, there are no signs of enteropathy, and the pathogen grows up to 106–108 cfu/g stool (gut ecosystem invasion). Mucosal inflammation is elicited at days 3–4 postinfection when the pathogen reaches a final density of 108–1010 cfu/g stool (Stecher and Hardt, 2011; Figure S1A). Thus, LCM mice should provide a unique model for analyzing all phases of host gut colonization, including gut ecosystem invasion.

1. **Methods**

The methods describe how the experiments were set up and performed and what kind of materials were used. This is the most formal part of the report, but it is a really important section, because it allows other people to reproduce your experiments. In general, you should keep the description of the experiments as simple (but accurate) as possible and you can use the same style to explain a setup of similar experiments.

Example:

Analysis of polymyxin B resistance. The equivalent of 1 OD600 unit/ml of exponentially growing cultures from serovar Typhimurium wild-type strain SL1344 or the serovar Typhimurium Δ*wbaP* strain (SKI12) was spun down, resuspended in 150 μl cold sterile 1× phosphate-buffered saline (PBS), and diluted 5 × 106-fold before use. For the assay, 45 μl of the diluted cultures was mixed with 5 μl of polymyxin B (final concentration, 1 μg/ml; Sigma) or 5 μl PBS and incubated for 1 h at 37°C under slight agitation. After addition of 80 μl LB agar, bacteria were plated on LB agar plates containing streptomycin. The survival efficiency was calculated by dividing the number of CFU of the peptide-treated culture by the number of CFU of untreated culture and multiplying by 100. The assay was performed in triplicate for two independent experiments, and data are shown as means ± standard deviations.

1. **Results**

In the results, the research findings are presented and accompanied by various figures and tables. It provides the reader with the data, without putting them into a larger context. An important function of the results section is to draw small conclusions from your experiments at the end of the paragraph. These conclusions should not end in a detailed discussion of your results (this is part of the discussion section), but rather look like a summary, what you learned from your experiment, and what was your motivation to move on to do the next experiment. In general, the text reports the data and directs the reader to the figures or tables that present the data.

The results are organized as the following:

* Title that explains the purpose or the main question
* Methods are briefly mentioned that are used to answer the question
* Results are mentioned
* Simple explanation/conclusion can be mentioned
* Figures, and individual sub-parts of figures, as well as supplementary figures, must appear in the order in which they are mentioned in the text. Supplementary figures and data will have their own numbering system (check the journal guidelines, but typically "Figure 1" for the first main figure and "Figure S1", for the first supplementary figure etc.)

Example:

**Hydrogen consumption by S. Tm is only required during the Initial phase of gut ecosystem invasion**

In order to verify the role of hydrogenases during gut infection, we constructed site-directed mutants (Figure S2B; Supplemental Experimental Procedures). In competitive infections, the *hyb* mutant (*S.* Tmhyb; hybBCAhypO, which lacks all structural genes of the hyb hydrogenase) displayed a pronounced growth defect compared to the isogenic wild-type strain (~100-fold; p < 0.05; Figure 2). This was corrobated by *hyb* expression in the gut lumen (Figure S2D). Interestingly, the growth defect of *S.* Tmhyb was restricted to the first day of the experiment when pathogen loads were still low (≤ 108 cfu/g stool) and no signs of mucosal inflammation were observed (Figures 2B–2D). Thereafter, the competitive index did not drop any further (Figure 2A). These data indicate that S. Tm requires *hyb* only in the initial phase of gut ecosystem invasion, but not at later stages of the infection, and that this initial stage (days 0–1) is mechanistically distinct.

Further experiments excluded major contributions of two alternative H2-consuming hydrogenases encoded in the *S.* Tm genome (Figure S2B; Supplemental Experimental Procedures). Disrupting the two alternative hydrogenases yielded no defects in gut ecosystem invasion, and the hydrogenase triple mutant (*S.* Tmhyd3) displayed the same *in vivo* growth defect as did *S.* Tmhyb (Figures S3A and S3B). Thus, while *hyb* is necessary for robust pathogen growth in the host’s intestine, the other two hydrogenases contribute little. This was further supported by complementation (Figure S3B). Furthermore, the gut ecosystem invasion defect of the hydrogenase mutant was independent of the inoculum size and also observed upon gavage of 5 x 103 cfu (data not shown; standard inoculum size = 5 x 107 cfu; Experimental Procedures). Finally, in vitro experiments in anaerobic broth culture verified that the growth defect of *S*. Tmhyd3 was only observed in the presence of H2, but not in its absence (Figures S4A and S4B). In conclusion, these data confirmed the pivotal importance of *hyb* for H2-dependent *S.* Tm growth. Our initial data suggested that the *hyb* hydrogenase may fuel pathogen growth during gut ecosystem invasion, i.e., the first 24 hr p.i. (Figure 2A). At this stage the pathogen grows in the face of the resident microbiota (which presumably still produces H2) and overt inflammation is not yet triggered (Figures S1A and 2B–2D). To further substantiate the need for hydrogenases in the noninflamed gut, we performed competition experiments in the avirulent strain background. The isogenic *S.* Tm mutant (*S.* Tmavir; DinvGDsseD; Supplemental Experimental Procedures) colonizes the gut but remains ‘‘locked’’ in gut ecosystem invasion phase of the infection, as it lacks two key virulence factors and therefore cannot elicit overt mucosal inflammation (Hapfelmeier et al., 2005; Stecher et al., 2007). To this end, we constructed a hydrogenase-deficient mutant in the *S.* Tmavir background (*S.* Tmavir hyd3). First, we tested this strain’s capacity to grow up in the gut of LCM mice. In competitive infections, *S.* Tmavir hyd3 displayed a pronounced colonization defect on day 1 p.i. but no further decrease from day 1 to day 4 p.i. (Figure 3). These results were strikingly similar to those obtained in the wild-type *S.* Tm strain background (compare Figure 2A and Figure 3A) and verified that hydrogenases are indeed only required during gut ecosystem invasion, whether inflammation is triggered or not. Accordingly, intravenous infection experiments confirmed that hydrogenases are not needed for growth at systemic sites (Figure S3C). This further supported the notion that gut ecosystem invasion is a distinct step in host intestinal colonization, which prepares the ground for subsequent stages of the infection.

1. **Discussion**

The discussion section aims at reflecting your experimental work in a bigger context, whereby you can draw your conclusions. This is usually done through a series of points, which refer to statements made in the introduction. Future research is also frequently mentioned. In contrast to the Results section in which you explain each experiment individually, the discussion section serves the purpose to integrate the results of all experiments you did. The discussion should be short and sharp! Make clear and simple statements, because the reader needs to get that point.

The discussion follows a series of steps:

* Step 1: make a reference to the main purpose or hypothesis of the study (e.g. "The aim of this study was to investigate...")
* Step 2: write a brief summary of the most important findings, and whether (or not) they agree with the original hypothesis
* Step 3: discuss possible explanations for the findings and compare them to other investigations/publications (do they support your explanation or do they argue against your hypothesis?). Thereby, it is important to cite other publications to strengthen your arguments.
* Step 4: you can state some limitations of the study
* Step 5: explain some potential implications of the study by which the whole content gets more interesting
* Step 6: end your report with an open question or a small statement what needs be addressed in the future

Example:

Our findings establish gut ecosystem invasion as a critical step of the orogastric *S.* Tm infection. During this initial phase of the infection, pathogen growth in the gut relies at least in part on metabolites provided by the microbiota. This differs markedly from the interactions observed later (i.e., during expansion/maintenance), when the host’s mucosal immune response fuels pathogen growth and suppresses the microbiota (Kaiser et al., 2012; Winter et al., 2013). Thus, colonization of the host’s gut comprises different phases featuring distinct sets of positive and negative interactions. The interactions between the pathogen, the microbiota, and the host are clearly more complex than previously anticipated.

Gut ecosystem invasion by S. Tm relies on H2. This is true for mice harboring two different microbiotas of reduced complexity (LCM mice used throughout most of this study; VLCM mice used in Figures S4C and S4D) or animals with a normal SPF microbiota, alike (Figures 4D–4F and 5B). In contrast, intravenous infections did not yield any evidence for H2-dependent pathogen growth at systemic sites (Figure S3C). At first sight, this seems to be in conflict with earlier work in the oral infection model for typhoid fever (Maier et al., 2004). Upon oral infection, hydrogenase mutants of *S.* Typhimurium ATCC14028 failed to colonize the livers and spleens. Our data may suggest that this attenuation was attributable at least in part to defective growth in the gut, before the bacteria had actually disseminated to systemic sites. This hypothesis would be in line with hydrogenase expression of ATCC14028 in the murine ileum (Zbell et al., 2008). However, we cannot formally exclude that ATCC14028 differs from the SL1344 strain used in our study in being capable of utilizing H2 in liver and spleen. Such strain-specific differences may affect the adaptation to new hosts. Clearly, S. Tm SL1344 requires H2 only for gut colonization, but not at systemic sites (Figure S3C). This provides a striking example for a central intermediate of microbiota metabolism fueling pathogen growth at a site occupied by a dense commensal community. Due to the conserved nature of the metabolic network of the gut microbiota, this metabolite will likely be available in any host animal as well as in humans. Thus, H2 could be regarded as an ‘‘Achilles’ heel’’ of microbiota metabolism which can be exploited by S. Tm for gut ecosystem invasion, etc.

1. **Abstract**

The abstract is a summary (often less than 200 words) of the entire report. Usually, it contains information from all sections introduced before. Further, the abstract should be as clear and as attractive as possible, because it is used as a first measure of quality and novelty of the presented work. Furthermore, the abstract must stand for its own, i.e., an expert must be able to understand it without further information.

Steps of the abstract:

* Background information: in the beginning you should give the minimal information needed for an expert of the field to understand your story
* Purpose of the study: here you should explain in one to two sentences the reason, why you conducted the study
* Methods: just write the overall experimental approach, but not a list of all methods used in the study
* Results: only state the major findings
* Conclusion: e.g. "These results show…, indicate…, suggest that …"

Example:

The intestinal microbiota features intricate metabolic interactions involving the breakdown and reuse of host- and diet-derived nutrients. The competition for these resources can limit pathogen growth. Nevertheless, some enteropathogenic bacteria can invade this niche through mechanisms that remain

largely unclear. Using a mouse model for Salmonella diarrhea and a transposon mutant screen, we discovered that initial growth of *Salmonella* Typhimurium (*S.* Tm) in the unperturbed gut is powered by *S.* Tm *hyb* hydrogenase, which facilitates consumption of hydrogen (H2), a central intermediate of microbiota metabolism. In competitive infection experiments, a *hyb* mutant exhibited reduced growth early in infection compared to wild-type *S.* Tm, but these differences were lost upon antibiotic mediated disruption of the host microbiota. Additionally, introducing H2-consuming bacteria into the microbiota interfered with *hyb*-dependent *S.* Tm growth. Thus, H2 is an Achilles’ heel of microbiota metabolism that can be subverted by pathogens and might offer opportunities to prevent infection.

1. **Figure legends**

Often, figure legends can be treated as a hybrid between Methods and Results. But, whereas Methods often describe a procedure in general terms, the figure legend contains specific information as the amount, time, temperature etc. In addition, number the figures consecutively in the order of their first appearance in the text. The figure legend should be introduced by an informative heading and one should understand what is shown in the figure without reading the main text.

Structure of the figure legend:

* Title
* Method
* Result

Examples:

Figure 3. S. Tm Only Profits from H2 during the Initial, Noninflammatory Phase of Gut Ecosystem Invasion

(A) C. I. experiments were performed in five naive LCM mice to test in vivo fitness of S. Tmavir hyd3. ns, not significant (pR0.05), \*\*p < 0.01; Mann-Whitney U test.

(B) Pathological scores of the cecal mucosa at day 4 p.i. Cecal tissue sections from the competitive infection experiment shown in (A) were stained with HE and

scored for inflammation.

(C) Fecal loads of S. Tmavir hyd3 and S. Tmavir at day 1 and day 4 p.i. were determined by differential plating. \*p < 0.05, one-tailed Wilcoxon matched pairs signed

rank test on paired data (dashed lines).

See also Figure S3.

OR:

FIG. 3. The serovar Typhimurium \_*wbaP* strain (SKI12) is a potent inducer of colitis at day 2 p.i. Three groups of streptomycin-treated wild-type C57BL/6 mice (*n* \_ 5 per group) were infected for 2 days with the serovar Typhimurium wild-type strain (WT), \_*wbaP* strain (SKI12), or \_*invG* strain (total of 5 \_ 107 CFU intragastrically). Serovar Typhimurium colonization of cecum, MLNs, and spleen and cecal pathology were examined at day 2 p.i. (A to C) The number of serovar Typhimurium CFU in different organs (cecal content [A], MLNs [B], spleen [C]). Horizontal bars, median; dashed line, minimal detectable level; NS, not statistically significant (*P* \_ 0.05). (D) Cecal pathology and its quantification at day 2 p.i. PMN, polymorphonuclear leukocytes. (E) Thin sections (5 \_m) of cryoembedded cecal tissues were stained with H&E, as described in Materials and Methods. L, intestinal lumen; lp, lamina propria; S, submucosa. Scale bars, 200 \_m.