From chloroplast to nucleus and back - the role of stromulae in keeping in touch

Hello, little Mr. Protein! Are you happy in your tiny compartment in the chloroplast? Your green gardens of thylakoids, your wealthy house supplied by gentle Sun energy? There is a danger coming! The Sun glares you down! The Orcs of reactive oxygen species want to destroy your fields! Some rearrangements of your Shire have to be done to protect your people, and someone needs to pass the information to the Mighty King, to send an army (or a supply). This might be the life story of a messenger protein, that – like Bilbo Baggins the Hobbit, is sent to the nucleus, to inform that stress is there. Or, maybe they are not heroes, but regular postman staff? Maybe there is no One Ring to Rule them all, not a Fellowship, send in a high emergency to uncertain roads, but just a post-office well-established route?

Therefore, when we are trying to understand communication between chloroplast and nucleus, so-called retrograde signaling, do we have a case of Gandalf, calling the chosen hobbit to accompany the dwarfs in the travel to Lonely Mountain, or do we rather observe dwarfs, coming every day to their mines, singing heigh-ho, heigh-ho, it's way from chloroplast we go? To be sure, we need first to understand, how the connection between chloroplast and nucleus function. One of the possible routes, a really physical one, is called stromulae. They are tubular extensions of chloroplast membranes, that point to other cellular structures, mostly the nucleus, and they are observed to be formed after the attack of pathogen, but also under regular illumination. Although they do not contain chlorophyll, so under normal conditions they are transparent, there is a way to visualize them, using fluorescent protein, combined with chloroplast targeting peptide. The same way we may tag a protein, that is transferred via stromulae to the nucleus. Our messenger candidates are two proteins of the CryDASH family, AtPhr2 and AtPhr3. They were postulated to work as cryptochromes or photolyases, however, recent results deny this thesis. We believe that they are rather sensors of particular chloroplast conditions (redox state, chlorophyll decomposition), maybe – but not necessarily - including the presence of illumination together with mentioned factors. We already observed that AtPhr2 may be found in stromulae, therefore having a significant chance to get to the nucleus that way. We also selected potential conditions, that may cause AtPhr3 to do the same.

Having such a system, we may then correlate the presence of stromulae with a protein messenger movement. Therefore, we will identify what lures a messenger to a nucleus, if its location correlates with the presence of other factors, such as pH or redox state. We will show how plant age is correlated with stromulae formation tendency, with a special focus on early plant life, when chloroplasts are just formed from prolamellar bodies, and the chloroplasts really need to be in touch with the nucleus. Later, we will check if stress factors (drought, high light intensity, cold, and heat stress) also influence the messenger journey. We will identify if the messengers travel alone or form a bigger team; the last is greatly expected and would mean, that the final effect depends on multiple co-existing characteristics of the environment. Last but not least, we will attempt to identify the messenger's place of action in the nucleus. That way, we will identify the Ring, that motivates our Protein the Hobbit to move, the Fellowship he travels with, and the target of his journey.

We will work using model plants, *Arabidopsis thaliana* and *Nicotiana sp*., and their stable and transient transformants. Additionally, we will use an in vitro model of stromule formation created by isolated chloroplasts. Although these are just models, the results will be of importance for a general understanding of retrograde signaling, and therefore – the performance of chloroplasts and photosynthesis. This, finally translates into understanding how to improve crop biomass production, which is crucial for our human survival. So, no more talking, time to work! heigh-ho, heigh-ho, it's the lab from home we go…. heigh-ho, heigh-ho…