The Genetics of Fasciation

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Fasciation is a relatively rare plant deformity, which leads to the flattening of the stem bud by elongation, perpendicularly to the direction of growth of the flower. It can be caused by a range of different genetic factors such as inefficient repair of double stranded breaks, mutations and hormonal imbalances induced by fungal pathogens. The mutation of the breast cancer 2 gene (BRCA2), also present in plants, which is involved in double stranded break repair mechanisms, has been implicated in the progression of fasciation. Radiation has also been seen to induce an effect by increasing the rate of mutation. Gain of function mutations in the Arabidopsis thaliana (Arabidopsis) Fasciated Stem 4 gene (AtFAS4) during certain periods of development can lead to a fasciated stem and also the loss of function mutations in the Maintenance of Meristem genes (MAINS). Main mutants induce fasciation by causing premature differentiation of the meristematic stem cells or cell death by the loss of stem cell maintenance. Rhodococcus Fascians (R.fascians) releases methylated cytokinins (MeCKs) which mimic plant hormonal activity leading to fasciation in plants. Cytokinins induce fasciation in plants by regulating genes which control shoot apical meristematic activity such as the CLAVATA 1 gene (CLV1). Phytopathogens which induce fasciation in plants promote their own proliferation by decreasing host fitness. This is done by producing phytohormones which lead to unusual development of floral organs. They can also alter the main primary carbon metabolism which leads to increased disease establishment.

Introduction

Fasciation is the elongation of the meristem tip which grows perpendicularly to the direction of growth of the plant. The tip which is usually concentrated at a particular point becomes elongated, flattened and cylindrical in shape (White, 1948). Fasciation is a relatively rare condition and can be caused by many different genetic factors such as double stranded breaks (Abe *et al*., 2009), point mutations in apical meristematic genes (Wenig *et al*., 2013), by phytopathogen infections e.g. *R. fascians* (Radhika *et al*., 2015) or by different types of radiation such as gamma radiation (Abe *et al*., 2009). Figure 1 shows wildtype daisies vs ones which are fasciated (this photograph went "viral" as it was falsely suspected to be attributed to radiation released from the Fukushima plant disaster). Fasciation has many contributing factors as will be discussed in this review along with the mechanisms of overcoming plant barriers to infection and issues which are unclear in the literature, requiring future research.

Fig 1. *Photograph of three fasciated daisies on the left vs two normal daisies on the right (Photograph courtesy of Kaido (2015). This photograph was taken 65 miles from the Fukushima plant (Harrington, 2015) and went viral because it erupted fears that these daisies were due to the radiation from the plant. However, the atmospheric dose of radiation (0.5 μSv/h at 1m above the ground) was not significantly higher than background levels and hence there was no evidence of an effect.*

What are the Genetic causes of Fasciation?

Fasciation can be caused by various different types of alterations in DNA including DNA damage and mutations. The most potent of these alterations are double stranded breaks (DSBs) as if not repaired, can lead to cell death

and if not repaired correctly, can lead to the formation of many more different types of mutations such as deletions, translocations or even fusions within the DNA. These DSBs are inefficiently repaired in plants containing what is known as a 'BRCA2 mutation' which have also been demonstrated to induce fasciation (Abe *et al.*, 2009). This mutation is more commonly known in humans where it is associated with increased risk of breast cancer (Wooster *et al.*, 1994), but also present in higher plants. In vertebrates the BRCA2 gene is involved in repair of double stranded breaks in two ways. The first and the most error prone mechanism used is known as non-homologous end joining (NHEJ). This is a pathway which involves the ligation of the break ends of the DNA without using the homologous chromosome as a template It is used at the beginning of cell division before the identical copies of the homologous chromosomes have been formed and made available for the second, less error prone mechanism, known as Homologous Recombination (HR). HR uses the homologous chromosome as a template for repair of the double stranded break and occurs towards the end of cell division (Abe *et al.*, 2009).

To identify the role of BRCA like genes in plants in DNA repair, transposon insertion mutants of the AtBRCA2a and AtBRCA2b genes were identified and characterised. The proteins encoded by AtBRCA type a and type b genes were found to be 94.5% identical (Abe *et al.*, 2009) and therefore hypothesized to be most likely the result of a recent genome duplication (Siaud *et al.*, 2004). It was thus believed that the two genes would perform a similar role or have redundant functions. To test this redundancy, genetic crosses were performed between homozygous single mutants to obtain double mutants. Previous studies in vertebrates showed that double mutants were more sensitive to genotoxic agents such as gamma radiation and cis-platin (an interchelating agent) and this was tested in Arabidopsis. It was shown as a result of these experiments that double mutants showed altered cell cycle progression. This suggested that there was inefficient repair of the double strand breaks in the double mutants which led to the disorganisation of cell cycle progression in apical meristems. In addition it was also found that mutants deficient in HR showed fasciation phenotypes and were also more sensitive to genotoxic agents.

One such example is the MRE11 gene. It was shown by Bundock and Hooykas (2002) that one of these mutants mre11-1 was hypersensitive to the alkylating agent Methyl Methane Sulfonate and also displays fasciation. However mutants deficient in NHEJ did not display this hypersensitivity to genotoxic agents and fasciation phenotype (Abe *et al.*, 2009). Examples of such mutants include: ku70 shown by Riha *et al.* (2002), ku80 shown by Friesner and Britt (2003); Gallego *et al.* (2003); West *et al.* (2002) and ligIV shown by Friesner and Britt (2003) and van Attikum (2003) . Indeed, it was confirmed that abnormal phyllotaxy (unusual arrangement of leaves around the stem) and stem fasciation were not increased in the ku80 mutant (WS ecotype background;(West *et al.*, 2002)) compared to the wild type, with or without

c-irradiation (short wavelength Ultra Violet germicidal radiation used to kill microbes by disrupting nucleic acids and breaking their DNA). Thus in contrast to the Homologous Recombination repair pathway, inefficient repair of DSB via NHEJ does not seem to induce disorganisation of apical meristem cells. As such the fasciation phenotype observed in atbrca2 single and double mutants may be linked to c-irradiation (Abe *et al.*, 2009).

Mutations in genes involved in specifying organ number such as the CLV3 gene leads to fasciation. CLV3 encodes a supposed ligand for a transmembrane receptor kinase, known as CLVI. Mutations in the CLV3 gene leads to an increase in the accumulation of undifferentiated stem cells in the stem tip (Clark *et al.*, 1996). It was also reported in another study that a CLV3 RNAi construct induced by dexamthamethasone also shows stem fasciation (Reddy and Meyerowitz, 2005). Many gene regulatory networks can be perturbed using this technique. The dexamthamethasone inducible RNAi construct enables the downregulation of CLV3 at will. Reports like this indicate that plant Stem Apical Meristem (SAM) developmental programmes can be changed by outside factors during development. This has been demonstrated using c-irradiation and will also be highlighted by discussing *R. fascians* which mimics plant hormonal activity.

Phytopathogens such as *R. fascians* produce phytohormones which alter plant development to facilitate their own development in the host plant. *R. fascians* is an aerobic, gram positive pathogenic bacterium which induces leafy gall disease and fasciation in plants. Leafy gall disease is the outgrowth of plant tissues which is analogous to benign tumours or warts in humans (Stes *et al.*, 2013). In order to achieve this *R. fascians* contains a *fas* locus. A *fas* locus is an operon which contains genes which are homologous to genes required for cytokinin (CK) biosynthesis and metabolism (Stes *et al.*, 2011). The phytopathogen produces methylated cytokinins (MeCKs) which inhibit root growth, which is a sign of CK action. MeCKs were however retained longer in the plant and showed signs of enhanced biological stability as they were not good substrates for oxidases and dehydrogenases. The MeCKs were shown to mimic cytokinins and play a role in plant-pathogen interaction (Radhika *et al.*, 2015).

Methylated cytokinins from the fungal pathogen R.fascians mimic plant hormonal activity leading to the induction of fasciation in plants by regulating genes which control shoot apical meristematic activity, such as the CLV1 gene discussed. When a cytokinin such as N6 -benzylaminopurine (BAP) is applied to the shoot apical meristem of Arabidopsis thaliana, floral organ number is increased, which is reminiscent of the CLV1 mutant phenotype (Radhika *et al.*, 2015). Transcriptome analysis reveals that exogenous cytokinin treatment of plants significantly reduces expression of CLV1, which a gene is encoding a receptor like kinase, which is involved in stem cell maintenance in shoot and floral apical meristems (Nikolaev *et al.*, 2007). Time course RTPCR of the BAP

treated plant transcript levels showed a decline and subsequent recovery of CLV1 and a concurrent increase in WUSCHEL (WUS). This is consistent with our knowledge that CLV1 suppresses WUS. WUS encodes a homeodomain which is known to be linked to shoot meristem proliferation. The fact that floral development is altered at the same time at which transcript levels of CLV1 and WUS change suggests that cytokinins regulate flower development through genes controlling shoot apical meristem activity (Radhika *et al*., 2015).

Gain-of-function mutations in the AtFAS4 gene are also shown to result in a fasciated stem. These gain-of-function mutations were introduced by inserting an overexpression construct of ATFAS4 into Arabidopsis. The analysis of the ATFAS4 gene by Pogorelko *et al.* (2008) showed that this gene is expressed at very low levels, if at all in the wildtype whereas it is constitutively expressed in the overexpression construct and highly expressed in the mutant plant. When they analysed the protein amino acid sequence, they located the presence of helicase domains on the N-terminal domain of the protein which are involved in DNA denaturation, which could possibly lead to the destruction of genes involved in apical stem cell maintenance, thus leading to symptoms of fasciation or leaf gall disease. These helicases can also lead to the activation of DNA and RNA substrates leading to fasciation. This is supported by the fact that a large quantity of peptides are made by ATFAS4 mutant DNA and also by their short life span, suggesting that they are only around during certain short periods of time and during certain environmental conditions such as hormonal exposure, such as what is expected during the development of the plant.

Loss-of-function mutations such as in MAIN gene, which encodes a protein DNA binding domains in transcription factors, can lead to altered stem cells which perform different functions, which leads to premature cell death (Wenig *et al*., 2013). MAIN encodes a nuclear localisation protein. Malfunction of MAIN leads to roots which are visibly shorter, leaves which are misshapen, reduced fertility and partial fasciation of stems as seen in Figure 2. These mutants become more sensitive to genotoxic agents which leads to the expression of genes involved in DNA repair, which may also be mutated in fasciated plants. The increased sensitivity was shown by analysing the expression of DNA repair genes (Deveaux *et al*., 2000; Lafarge and Montané, 2003; Zhu *et al*., 2011). Dead cells along with mutant DNA accumulate in the meristems of plants. MAIN maintains stem cell genome integrity. Descendant MAIN mutants have a disorganised shoot apical meristem (SAM) and show a fasciated phenotype.

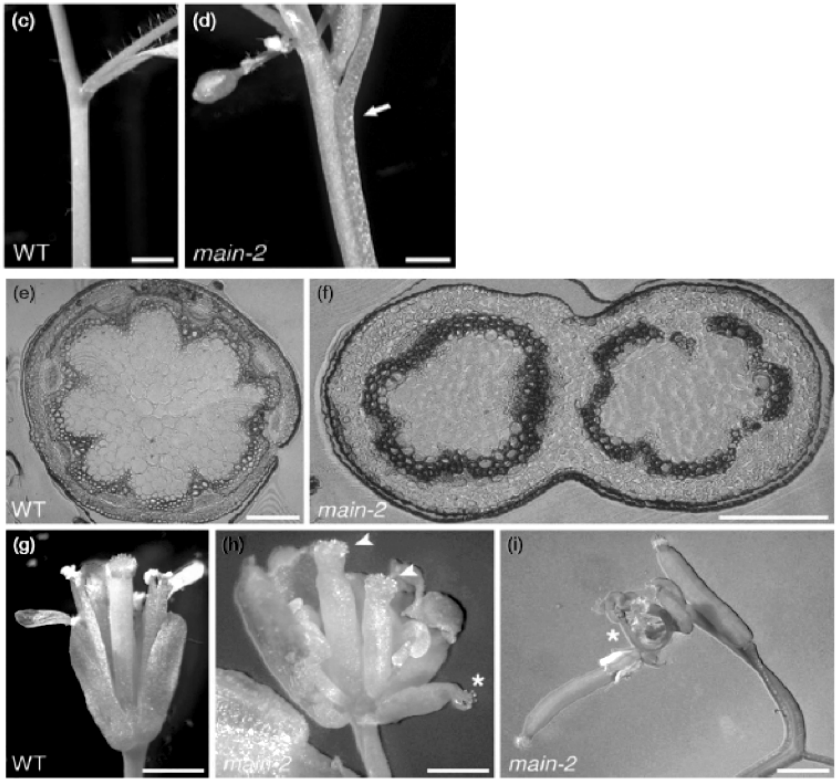

Figure 2. *Wildtype Arabidopsis vs Main mutants showing cell patterning defects in the cells in which MAIN is usually expressed (Root apical meristems and SAMs). Mutant MAIN phenotypes shown in Arabidopsis vs wildtype (g). Fasciation was observable in stems of both main mutants, but not in the wildtypes (WT). Cross section of main 2 mutant stem, indicated by arrow, in (d) is shown in (f). Cross section of wildtype stem shown in (c) is displayed in (e). (h) Shows how some of the main plants had more than one ovary, as shown by the arrows. The asterisk in (h) and (i) show how some main mutants have lost organ identity, which include papillae in the place where sepals should be. Fused flowers and pedicels are shown in (i) Scale bars 20 lm (e,f), 500 lm (h), 1 mm in (g) and 2 mm (all other images). Figure from Wenig et al. (2013).*

How is the immune response of the plant overcome by the pathogen?

For *R. fascians* and other pathogens involved in causing fasciation in plants, Cytokinin (CK) production is thought to be critical for virulence. *R. fascians* carries genes for CK homologue biosynthesis on a *fas* locus, as described previously. Since these genes are encoded on the *fas* operon, it is thought that these genes are essential for the pathogen-plant interaction. These genes are carried within a plasmid in the bacterium (Jacobsen *et al*., 1996). Phytopathogens promote their own virulence within the plant by producing phytohormones which lead to unusual development of floral organs. They can also alter the main primary carbon metabolism which leads to increased disease establishment (Robert-Seilaniantz *et al*., 2007). Bacterial pathogens such as the gram positive *R. fascians* develop gall structures (Sakakibara, 2006) which provides a better nutrient source environment for the bacterium.

Cytokinins (CKs) are detected by sensory Histidine kinases in Arabidopsis (arabidopsis his kinase 2 (AHK2) to AHK4. Once the cytokinins are detected, the sensory histidine kinases transfer a phosphoryl group from one Histidine kinase to another. This leads to the activation of direct target genes, e.g. type A ARABIDOPSIS RESPONSE REGULATOR (ARR) genes (Kieber and Schaller, 2010). Infection of Arabidopsis plants by *R. fascians* activates type-A ARR5 expression which leads to an increased expression of AHK3 and AHK4. This causes mitotic cell divisions which leads to infected cells being arrested in a meristematic state which establishes a nutrient-rich niche (Stes *et al*., 2011). This leads to the formation of a gall type structure. As the infection progresses, ISOPENTENYLTRANSFERASE (IPT) genes associated with cytokinin synthesis are turned off. However expression of all CYTOKININ OXYGENASE/DEHYDROGENASE (CKX) genes are highly upregulated in affected tissues (Depuydt *et al*., 2008).

FAS4 encodes an IPT which is involved in CK biosynthesis. The encoded IPT catalyses the rate limiting step of CK biosynthesis. This is vital for virulence (Stes *et al*., 2013). There are an additional 2 methyl transferase genes upstream of this FAS4 gene. The function of these is however unknown. Even though FAS genes are present in *R. fascians*, not many CKs have been detected compared to other bacteria which produce the leafy gall phenotype, such as *Agrobacterium tumefaciens* (Goethals *et al*., 2001). The virulence of *R. fascians* is thought to not be caused by CKs alone, as the leaf gall phenotype it produces is not caused by any one cytokinin in the bacteria containing the FAS genes (Goethals *et al*., 2001). CK analogues used by *R. fascians* to induce an effect are known as methylated cytokinins (Radhika *et al*., 2015). MeCKs are produced using two methyltransferases and the action of the AtFAS4 gene. MeCKs show CK-like activity and have a higher stability. Their higher stability suggests a role in co-ordinating efficient pathogenesis (Radhika *et al*., 2015).

Unclear in literature?

Despite several CKs having been isolated from R. fascian culture filtrates. There has of yet been no clear correlation with pathogenesis detected. This is due mainly to the low concentration of bacterial CKs (Eason *et al.*, 1996). It is thought that many CKs work together to produce an effect. When accumulated locally (Pertry *et al.*, 2009). No virulence-associated CK analogues have been identified that could contribute to the infection symptoms.

Conclusion

Fasciation is a relatively rare condition of plants which leads to the flattening and elongation of the stem tip. This disease can be induced by a wide variety of different factors such as double stranded breaks of DNA and mutations in critical genes involved in the development and maintenance of the plant, with cytokinin analogues released by fungal pathogens also being implicated. Different forms of radiation can also increase the rate of mutation in DNA imparing developmental, maintenance and repair pathways, thus inducing fasciation. The study of fasciation is fascinating as it provides insight into plant developmental mechanisms and how they can be manipulated in order to prevent such defects. This could lead to a minimization of major crop losses to affected plants.

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