Maize Genetics Cooperation Newsletter vol 84 2010

BERGAMO, Italy

Unità di Ricerca per la Maiscoltura – CRA

Constitution and characterization of maize lines with down-regulation of *nfc102* gene expression

- - Altana, A; Battaglia, R; Michelotti, V; Locatelli, S; Lauria, M; Rossi, V

The *nfc102* gene encodes a WD-repeat protein belonging to the *Multicopy suppressor of IRA* (*MSI*) family, originally identified in yeast (Ruggieri et al., Proc. Natl. Acad. Sci. USA 86: 8778-8782, 1989). In maize, five genes of the *MSI* family have been identified and named *nfc* by the Plant Chromatin DB USA initiative (http://www.chromdb.org), because they display homology with one of the NURF complex component, where NURF is the Nucleosome Remodeling Factor: a multi-proteins complex that regulates transcription by catalyzing nucleosome sliding (Kwon and Wagner, Trends Genet. 23:403-412, 2007). On the basis of sequence homology with the five Arabidopsis *MSI* sequences, the maize *nfc* genes can be sub-divided in two separate groups (http://www.chromdb.org). The first group contains *nfc103*, *nfc104*, and *nfc108*, which are the putative orthologs of the three Arabidopsis *MSI1*-like genes involved in the epigenetic-mediated control of several aspects of the reproductive development (Hennig et al., Trends Cell Biol. 15:295-302, 2005; Jullien et al., PLoS Biol. 6:e194, 2008). Conversely, the maize *nfc101* and *nfc102* genes are closely related to the Arabidopsis *FVE* gene, which is a component of the autonomous pathway regulating the flowering time (Ausin et al., Nature Genet. 36:162-166, 2004; Kim et al., Nature Genet. 36:167-171, 2004). Recently it has been shown that *FVE*, together with other members of the autonomous pathway, has a more general role and acts in regulating the epigenome stability (Baurle and Dean PLoS ONE 3:e2733, 2008).

In maize the *nfc102* gene was previously named ZmRbAp1 because, similarly to the mammalian Retinoblastoma associated proteins RbAp46/48, exhibits the ability to physically interacts with the maize homolog of Retinoblastoma protein (ZmRBR; Rossi et al., Plant Mol. Biol. 51:401-413, 2003). Specifically, it was reported that ZmRbAp1/NFC102 protein associates with histones (Rossi et al., 2001 Mol. Gen. Genom. 265:576-584, 2001) and cooperates with ZmRBR and with maize Rpd3-type histone deacetylases (HDAs) to repress gene transcription (Rossi et al., Plant Mol. Biol. 51:401-413, 2003; Varotto et al., Plant Physiol. 133:606-617, 2003). These findings suggest a role for the ZmRBR/ZmRbAp1/HDA complex in controlling G1/S progression in maize, by directing changes in histone modification of specific targets to modulate their chromatin structure and transcription.

To functionally characterize the *nfc102* gene we have generated transgenic maize plants that constitutively expressed the antisense *nfc102* transcript (AS plants). The *nfc102* antisense transcription was driven by the constitutive maize ubiquitin promoter (Christensen et al., Plant Mol.

Biol. 18:675-689, 1992). The polyadenylation domain of the *nos* gene was inserted opposite to the ubiquitin promoter. The resulting cassette was cloned into a vector expressing the *phosphinothricin acetyl transferase* (*PAT*) gene, which allows to select for resistance to the herbicide gluphosinate. The final construct was used to transform protoplasts from maize suspension cells as described by Rossi et al., (Plant Cell, 19:1145-1162, 2007). Transgenic cell clones were sub-cultured in the presence of gluphosinate and callus lines were further screened for the presence of the transgene by PCR, using specific primer combinations. A total of 30 independent T0 transformants were obtained and on the basis of preliminary quantitative RT-PCR (qRT-PCR) analysis of *nfc102* down-regulation, seven plants were selected for further characterization. In addition a maize RNAi *nfc102* line (RNAi plant), with constitutive expression of the *nfc102* RNAi transgene, was obtained by the Maize Genetic Stock Center, where it was deposited by the Plant Chromatin DB USA initiative (http://www.chromdb.org; stock number: 3480.04). Regenerated T0 plants were first converted to the B73 recurrent parent by two sequential crosses to minimize mixed genetic background influence (T1 and T2 generations). After crosses to B73, plants heterozygous for the presence of the transgene were self pollinated to obtain the T3 generation. T3 plants showing resistance to gluphosinate were further self pollinated and 35 seeds from T4 single ears were germinated and screened for gluphosinate resistance and PCR positivity, in order to identify seeds heterozygous or homozygous for the presence of the transgene. The T4 homozygous plants were selfed to produce the T5 homozygous plants to be used for molecular analysis. DNA gel blot analysis showed that *nfc102* mutant plants contained one to four independent insertions of the transgene within the genome and that six AS and the RNAi mutant plants contain at least one intact transgene cassette. The mRNA level of *nfc102* and of its closest homolog *nfc101* was measured in leaves sampled at V2/V3 developmental stage and in tissue enriched in the shoot apical meristems collected at V6 stage by manual dissection. Total RNA was extracted from both tissues and the transcript level was assessed by means of real-time PCR analysis (for details of the method see Rossi et al., Plant Cell, 19:1145-1162, 2007). The results showed a statistically significant reduction of both *nfc102* and *nfc101* transcript in three AS and in the RNAi plants with respect to the wild-type B73 line. The fold changes spanned from two to four in AS plants, while a tenfold reduction was observed in the RNAi line. For phenotypic analysis the T4 heterozygous plants were selfed to produce T5 segregating plants, which were subsequently screened to identify ears with homozygous and wild-type seeds. At least 5 different homozygous and 5 different wild-type ears were used to constitute two pools of 200 seeds for each pool. These pools were planted for phenotypic observations. Preliminary phenotypic analysis indicated that down-regulation of *nfc102* and *nfc101* is associated with developmental defects such as delay in germination and in flowering time, reduction of seed size and plant height, etc. A more detailed analysis of various quantitative and morphological traits has been planned. Similarly, experiments for genome-wide identification of *nfc102* targets and for investigation of the effect of *nfc102* mutation on the epigenome are in currently progress.