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A precise size-estimate for the small RNA products arising from Neurospora crassa Dicer activity

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A precise size-estimate for the small RNA products arising from Neurospora crassa Dicer activity

Abstract

Neurospora crassa cell-free extracts prepared from strains containing one or both functional Dicer genes, but not from a strain lacking functional Dicer genes, converts radiolabeled double-strand RNA (dsRNA) in an energy-dependent manner into short RNAs with an estimated size of ~25-nt (Catalanotto et al. 2004). A smaller nucleolytic digestion product was also produced in an energy-dependent manner from either dsRNA or single-stranded RNA. Here we obtained more precise sizes for these products by electrophoresis of samples on a long (40-cm) denaturing DNA sequencing gel (20% polyacrylamide/7M urea).

A precise size-estimate for the small RNA products arising from *Neurospora crassa* Dicer activity.

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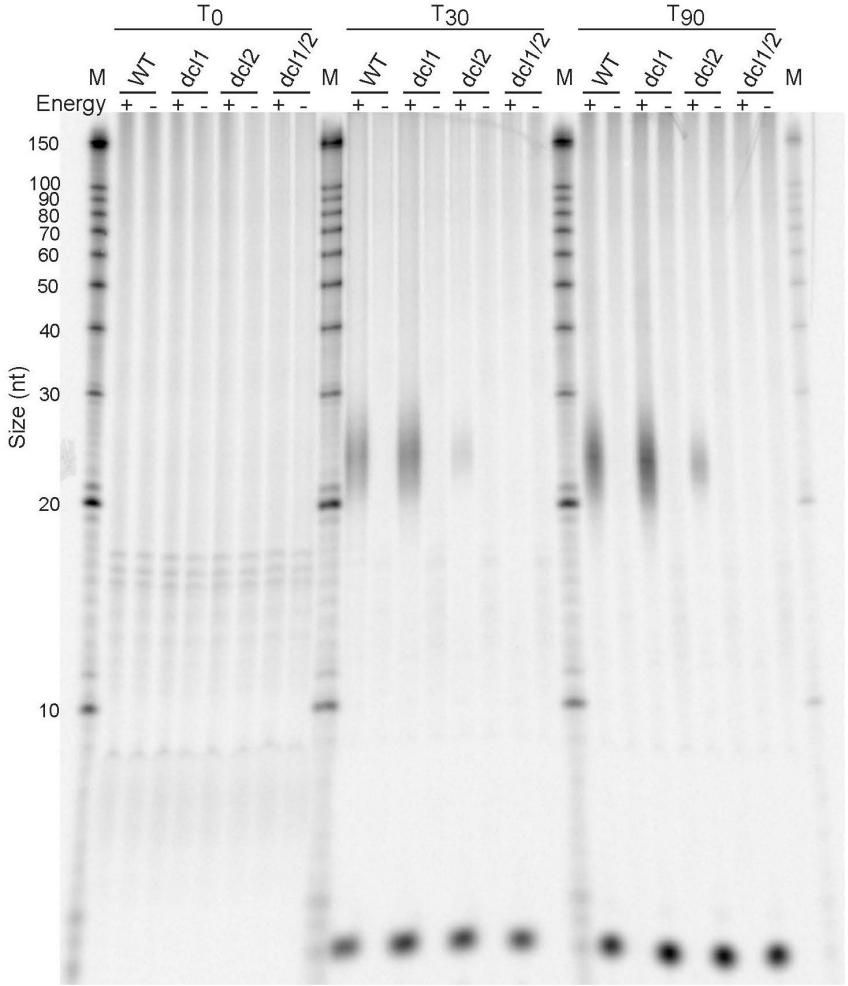

Fig. 1. Dicer activity in *N.crassa* single and double mutants for *dcl-1* and *dcl-2*. Analysis of WT, *dcl-1*, *dcl-2* and *dcl-1*/*dcl-2* strains for Dicer activity was accomplished as described (Catalanotto et al. 2004). *N. crassa* cell-free extracts were incubated with radiolabeled dsRNA for 0, 30, or 90 minutes (T_0, T_{30}, T_{90}) in the presence or absence of an energy regenerating system as indicated, and the RNA was examined by denaturing gel electrophoresis on a denaturing 20% polyacrylamide sequencing gel. Decade RNA Markers (Ambion) labeled with ^{32}P were used as size standards (lanes marked M).

The data (Fig. 1) show the Dicer- and energy-dependent products obtained from the dsRNA substrate were clustered in a region indicating the majority of species had sizes between 21-26 nt, with most approximately 23-nt in length. This is

consistent with results from other organisms (Agrawal *et al.* 2003). An additional very small degradation product was produced in an energy-dependent but Dicer-independent manner. This product, but not the ~21-26 nt products, was also obtained from single strand RNA (data not shown). Apparently, this small product migrated anomalously (suggesting a size of approximately ~16-nt) in the shorter gels containing a lower-percentage of polyacrylamide that were used previously (Catalanotto *et al.* 2004). Thus, the results in Fig. 1 indicate that, from input dsRNA, *N. crassa* extracts with Dicer activity produced RNAs of the size expected to function as small interfering RNA. *N. crassa* extracts did not contain other activities that processed input RNA into other large oligonucleotide products.

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