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

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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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![Image](https://example.com/image1.png)

**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 (T₀, T₃₀, T₉₀) 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 ³²P 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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