This study investigated prospective secondary mathematics teachers’ understanding and use of mathematical definitions in three content areas: set theory, linear algebra and geometry. Four secondary mathematics education students were individually interviewed four times to collect data. The focus of the first interview was on their perceptions of mathematics, mathematical definitions and proof. The other three interviews focused on their understanding of a given definition, use of definitions in doing proofs and assessment of the validity of a given proof that follows a direct application of a definition in the three content areas. The results of the study suggested that there might be a relation between students’ perceptions of mathematics and proof, and their approaches to proof production. In addition to this, it has been observed that students’ previous knowledge can inhibit their understanding of a given definition or using the definition to construct a proof. Lastly, students’ familiarity with the content may not be an indicator of successful proof production within that area. (Received September 17, 2013)